

The Transformation of Deoxycholic Acid into Allodeoxycholic Acid in the Rat

Bile Acids and Steroids 174*

ANDERS KALLNER

Department of Chemistry, Karolinska Institutet, Stockholm, Sweden

Deoxycholic acid was shown to be transformed into allodeoxycholic acid during the enterohepatic circulation of bile in the rat. After intracecal administration of 3β - ^3H - 24 - ^{14}C -deoxycholic acid, the deoxycholic acid as well as the allodeoxycholic acid isolated from bile and feces had lost most of the tritium label. It is suggested that in the intact rat deoxycholic acid is rapidly equilibrated with 12α -hydroxy-3-keto- 5β -cholanoic acid.

The main primary bile acids in the rat are cholic acid and chenodeoxycholic acid. During the enterohepatic circulation of bile these acids are partly metabolized by intestinal microorganisms, the main metabolites formed being deoxycholic acid and lithocholic acid, respectively.¹

The natural occurrence of bile acids having the A/B rings in *trans* configuration, has been demonstrated by several authors.²⁻⁴ Karavolas *et al.*⁵ showed that in the rat allocholic acid was the major bile acid metabolite of intracardially administered cholestanol. Hofmann and Mosbach⁶ found that allodeoxycholic acid was the main constituent of gallstones formed in rabbits fed a diet containing 1 % cholestanol. The mechanism of formation of allodeoxycholic acid from cholestanol was not established. Danielsson *et al.*⁷ demonstrated the presence of allodeoxycholic acid in bile and feces of rabbits

* In accordance with the recommendation of the IUPAC, the following systematic names are given for the compounds referred to in the text: cholic acid, $3\alpha,7\alpha,12\alpha$ -trihydroxy- 5β -cholanoic acid; allocholic acid, $3\alpha,7\alpha,12\alpha$ -trihydroxy- 5α -cholanoic acid; deoxycholic acid, $3\alpha,12\alpha$ -dihydroxy- 5β -cholanoic acid; allodeoxycholic acid, $3\alpha,12\alpha$ -dihydroxy- 5α -cholanoic acid; chenodeoxycholic acid, $3\alpha,7\alpha$ -dihydroxy- 5β -cholanoic acid; allochenodeoxycholic acid, $3\alpha,7\alpha$ -dihydroxy- 5α -cholanoic acid; lithocholic acid, 3α -hydroxy- 5β -cholanoic acid; cholestanol, 5α -cholestan- 3α -ol.

The following abbreviations are used: TLC, thin layer chromatography; PPO, 2,5-diphenyloxazole; dimethyl-POPOP, 1,4-bis-2-(4-methyl-5-phenyloxazolyl)-benzene; dpm, disintegrations per min.

and obtained evidence indicating that under these conditions allodeoxycholic acid is formed mainly from deoxycholic acid, probably by the action of intestinal micro-organisms.

The present communication reports on the formation of allodeoxycholic acid from deoxycholic acid in the rat.

EXPERIMENTAL

24-¹⁴C-Deoxycholic acid. This acid was prepared as described by Bergström *et al.*⁸ and had a specific activity of 50×10^6 dpm per mg. When chromatographed on TLC in phase system S 12 (see below) only one radioactive spot was detected by autoradiography⁹ and this spot coincided with a reference sample of deoxycholic acid run simultaneously.

3 β -³H-Deoxycholic acid. Sodium borohydride was tritiated at 350° for 6 h¹⁰ in the apparatus described by Bergström and Lindstedt.¹¹ Methyl 12 α -hydroxy-3-keto-5 β -cholanoate, 10 mg, was treated with 10 mg of tritium-labeled sodium borohydride in 15 ml methanol for 1 h at room temperature. The reaction mixture was acidified with 1 M sulfuric acid and extracted with ether. The resulting mixture of 3 α - and 3 β -hydroxy-epimers was hydrolyzed by refluxing in 2 M methanolic potassium hydroxide for 6 h. The free acids were isolated by ether extraction of the acidified hydrolysis mixture. The ether extract was washed with water until neutral and evaporated to dryness. The residue was purified by reversed phase partition chromatography with the phase system described below giving a partial separation of the epimers (Fig. 1). The later fractions of the radioactivity peak were combined, diluted with 10 mg of inactive deoxycholic acid and crystallized from acetic acid-water to constant specific activity (24×10^6 dpm per mg).

No contamination of the crystallized material with 3 β ,12 α -dihydroxy-5 β -cholanoic acid could be detected on TLC, which would have revealed the presence of 1–2 % of this acid.

3 β -³H-24-¹⁴C-Deoxycholic acid. The doubly labeled deoxycholic acid was prepared by mixing 3 β -³H-deoxycholic acid and 24-¹⁴C-deoxycholic acid in a ³H/¹⁴C ratio of 4 to 1. A small amount of the mixture was oxidized, after methylation, with aluminium-tri-*t*-butoxide as described by Jones, Webb and Smith.¹² The oxidation mixture was chromatographed on a column of aluminium oxide, grade III (Woelm, Eschwege, Germany⁷). The fractions containing methyl 12 α -hydroxy-3-keto-5 β -cholanoate were combined, diluted with inactive material and crystallized. The compound was shown to be practically devoid of tritium (Tables 1 and 2).

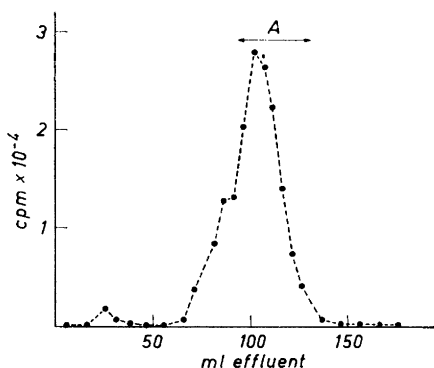


Fig. 1. Chromatogram of 3 α - and 3 β -hydroxy epimers obtained after NaBT₄-reduction of methyl 12 α -hydroxy-3-keto-5 β -cholanoate. Column 9 g of hydrophobic Hyflo Super-Cel. Phase system as described in the text. A indicates the fractions containing mainly deoxycholic acid.

Table 1. Retention of ^3H in bile acids after intraperitoneal administration of $3\beta\text{-}^3\text{H}\text{-}24\text{-}^{14}\text{C}$ -deoxycholic acid.

	^3H cpm	^{14}C cpm	$^3\text{H}/^{14}\text{C}$	Per cent ^3H retained
Administered $3\beta\text{-}^3\text{H}\text{-}24\text{-}^{14}\text{C}$ -Deoxycholic acid	56 600	13 100	4.1	100
Oxidation product: Methyl 12 α -hydroxy-3-keto-5 β -cholanoate	30	4 000	<0.01	0
Deoxycholic acid	16 300	4 260	3.8	93
Cholic acid	10 100	2 950	3.4	83

Chromatographic procedures. Reversed phase partition chromatography was performed as described by Norman and Sjövall¹ with Hyflo Super-Cel as support. The solvent system used consisted of 157.5 ml of methanol and 142.5 ml of water as mobile phase and 45 ml of chloroform and 5 ml of heptane as stationary phase. Thin layer chromatography was done with Silica Gel G (Merck A.G., West Germany) and the plates were developed with solvent system S 12.¹³ The samples to be analyzed were dissolved in a small amount of acetone and 50–150 μl were applied to the layer in a 5–15 cm long band. Deoxycholic acid and allodeoxycholic acid were run simultaneously as reference compounds.

After development, the plates were sprayed with water. The zones containing the bile acids were scraped off with a spatula and extracted on a sintered glass filter funnel with 2 % acetic acid in ether.¹⁴

Table 2. Retention of ^3H in bile acids after intracecal administration of $3\beta\text{-}^3\text{H}\text{-}24\text{-}^{14}\text{C}$ -deoxycholic acid.

	^3H cpm	^{14}C cpm	$^3\text{H}/^{14}\text{C}$	Per cent ^3H retained	Source
Administered $3\beta\text{-}^3\text{H}\text{-}24\text{-}^{14}\text{C}$ -Deoxycholic acid	163 000	19 500	8.3	100	
Oxidation product: Methyl 12 α - hydroxy-3-keto-5 β -cholanoate	50	5 887	<0.01	0	
Allodeoxycholic acid	765	1 211	0.63	7	feces
Allodeoxycholic acid	230	1 380	0.17	2	bile
Deoxycholic acid	393	2 967	0.13	2	feces
Deoxycholic acid	3 300	1 783	1.9	23	bile
Cholic acid	56 300	61 340	0.92	11	bile

The recovery of radioactivity by this method was 50 to 70 %. Repeated extractions, with this or other solvents, *e.g.* methanol and acetone, did not improve the yields.

Animal experiments. White, male rats of the Danish State Serum Institute strain V. S., weighing about 250 g, were used. Bile fistulas were prepared in the usual manner. 3β - ^3H -24- ^{14}C -Deoxycholic acid, $5-20 \times 10^6$ dpm, weighing between 2.5 and 3.5 mg, was dissolved in 0.5 ml of 0.02 M potassium hydroxide and administered intracecally or intraperitoneally to bile fistula rats. The intracecal administration of the labeled acids was made during the operation whereas the intraperitoneal administration was made a few hours after the operation.

Analysis of bile. Bile was collected in ethanol during the first 24 h after administration. After filtration, the bile was treated with 1 M potassium hydroxide in 50 % methanol for 8 h at 120° in a closed steel tube. The free bile acids were extracted with ether after acidification of the hydrolysis mixture and subjected to reversed phase partition chromatography. The effluent was assayed for radioactivity by counting a small aliquot (0.05–0.1 ml) of each fraction in a gas-flow counter.

The fractions containing the trihydroxycholanoic acids were combined, diluted with cholic acid and methylated. The methyl ester was crystallized to constant specific activity from methanol.

The dihydroxycholanoic acid fraction, eluted between 70 and 110 ml of effluent from a 9 g column, was combined, 100 μg of deoxycholic acid and allodeoxycholic acid¹⁵ were added and the samples were further analyzed by thin layer chromatography. Any activity extracted from the allodeoxycholic acid zone was diluted with 5–10 mg of unlabeled allodeoxycholic acid, methylated with diazomethane in ether and crystallized to constant specific activity from methanol-water. The radioactive material present in the deoxycholic acid zone was crystallized from acetic acid-water or ethyl acetate after dilution with 5–10 mg of unlabeled deoxycholic acid.

Analysis of feces. The feces excreted during the first 24 h were collected and combined with the contents of the cecum, colon and rectum. The material was homogenized in a Waring Blender and extracted twice by refluxing in ethanol. After concentration the extracts were hydrolyzed and analyzed as described above.

Radioactivity assay. Radioactivity was determined with a methane gas-flow counter or with a Packard scintillation counter model 4322. The scintillation solution used contained 4 g of PPO and 0.050 g of dimethyl-POPOP per liter of toluene. The bile acids were methylated with diazomethane before adding the scintillation solution. Tritium-carbon ratios were determined by the discriminator-ratio method. Correction for quenching was made after calibration with internal standards of ^3H -toluene and ^{14}C -toluene.¹⁶ At optimal settings the efficiency for tritium was about 33 % and for ^{14}C about 65 %. Less than 0.01 % of the tritium activity occurred in the carbon channel, whereas about 16 % of the carbon activity occurred in the tritium channel.

RESULTS

After intraperitoneal administration of deoxycholic acid, 40 to 60 % of the radioactivity — as measured by the amount of ^{14}C — was excreted in bile during the first 24 h. The radioactivity was localized in the trihydroxy- and in the dihydroxycholanoic acid fractions as shown in Fig. 2. On autoradiography of the dihydroxycholanoic acid fraction after thin layer chromatography, only one radioactive spot was detected which corresponded to deoxycholic acid. The allodeoxycholic acid fraction did not contain any significant amounts of radioactivity. The deoxycholic acid isolated was found to have retained most of the tritium as is shown in Table 1. The same extent of retention of tritium was found in the cholic acid formed from the doubly labeled deoxycholic acid.

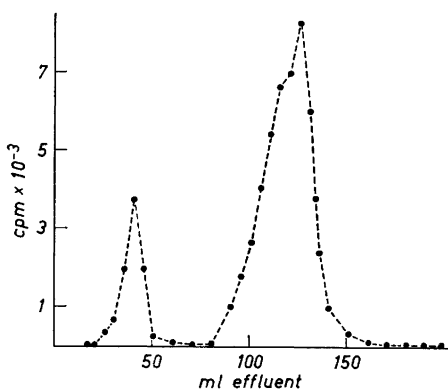


Fig. 2. Chromatogram of hydrolyzed bile obtained after intraperitoneal administration of $24\text{-}^{14}\text{C}$ -deoxycholic acid. Chromatographic conditions as in Fig. 1.

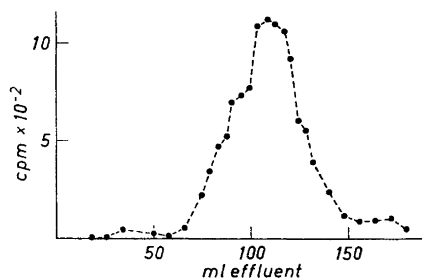


Fig. 3. Chromatogram of bile acids obtained from feces after intracecal administration of $24\text{-}^{14}\text{C}$ -deoxycholic acid. Chromatographic conditions as in Fig. 1.

When deoxycholic acid was administered intracecally, about half of the administered radioactivity was recovered from bile and feces produced during the first 24 h. The fecal extract contained 25 % of the radioactivity excreted. On reversed phase partition chromatography of the fecal acids only one radioactive peak was seen, appearing at the place characteristic of dihydroxycholanoic acids (Fig. 3), whereas in the chromatogram of the bile extract part of the radioactivity was present in the trihydroxycholanoic acid fraction. TLC of the dihydroxycholanoic acid fractions from feces and bile revealed the presence of radioactivity in the deoxycholic acid as well as in the allo-deoxycholic acid zone. The identity of the labeled material with deoxycholic and allodeoxycholic acid, respectively, was established by crystallization to constant specific activity after addition of unlabeled material. Determination of the amount of tritium retained showed that both compounds had lost most of the tritium label.

From 10 to 20 % of the radioactivity recovered from the preparative TLC of the dihydroxycholanoic acid fractions appeared in the allo-deoxycholic acid zone, whereas the remainder was present in the deoxycholic acid zone.

DISCUSSION

The present investigation has established that deoxycholic acid is converted into allo-deoxycholic acid in the rat. The reaction is probably catalyzed by enzymes present in the intestinal microorganisms. A similar pathway for the formation of allo-deoxycholic acid has been demonstrated previously in the rabbit.⁷ In addition, another pathway for the formation of allocholanoic acids exists in the rat as demonstrated by Karavolas *et al.*⁵ who isolated labeled allocholic acid and an acid tentatively identified as allochenodeoxycholic acid after administration of ^{14}C -cholestanol.

The deoxycholic acid present in the rat is produced through microbial dehydroxylation of cholic acid.¹ It is conceivable that allodeoxycholic acid could be formed from allocholic acid in an analogous manner. Work is in progress to establish whether or not such a pathway is present in the rat.

In the transformation of deoxycholic acid to allodeoxycholic acid 3-keto and Δ^4 -3-keto bile acids are probable intermediates. The finding that the deoxycholic acid isolated from bile and feces after intracecal administration of 3β -³H-24-¹⁴C-deoxycholic acid had lost most of the tritium indicates a rapid equilibrium between deoxycholic acid and a 3-keto bile acid in the intestine. Therefore, the loss of tritium observed in the allodeoxycholic acid provides no information concerning the mechanism of formation of allo-deoxycholic acid from deoxycholic acid.

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