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On the Metabolism of Lithocholic Acid in Chicken and Rabbit

Bile Acids and Steroids 167

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The metabolism of lithocholic acid-24-^{14}C was studied in chickens and rabbits with bile fistulas. In the chicken, 5—10 % of administered lithocholic acid was transformed into more polar products including 3α,6β-dihydroxy-5β-cholanoic acid, and the remainder was excreted as unchanged lithocholic acid. In the rabbit, lithocholic acid was excreted unchanged in bile, and no conversion into more polar products was observed.

Lithocholic acid (3α-hydroxy-5β-cholanoic acid) is present in small amounts in the bile of many species, *e.g.* man, rabbit, and chicken and is a major constituent of the fecal bile acid fraction in the aminals so far examined.[1–8] The metabolic origin of lithocholic acid is well established.[8–10] In all species studied it is formed from chenodeoxycholic acid by the action of intestinal microorganisms during the enterohepatic circulation of bile. Lithocholic acid possesses several interesting metabolic effects. Intramuscular administration of lithocholic acid to man produces a febrile reaction of 8—12 hours' duration.[11] Holsti,[12] Stolk,[13] and Leveille *et al.*[14,15] have shown that oral administration of lithocholic acid may cause cirrhosis of the liver. Some animals including chicken and rabbit are much more susceptible to this effect than others, which difference might depend on the capacity of the liver to metabolize lithocholic acid or on the production in the liver of metabolites having these harmful effects. In the rat, which is comparatively resistant, lithocholic acid is extensively metabolized in the liver into a number of more polar products including chenodeoxycholic acid, 3α,6β-dihydroxy-5β-cholanoic acid, and 3α,6β,7β-trihydroxy-5β-cholanoic acid.[16,17] In man, only 2—3 % of intravenously administered lithocholic acid is hydroxylated and the remainder is excreted as taurolithocholic acid and glycolithocholic acid.[18] After oral administration of lithocholic acid-24-^{14}C to man, several less polar metabolites were excreted in bile in addition to unchanged lithocholic acid.[19] About half of the labeled acids were conjugated with two unidentified compounds and the remainder with taurine and glycine.[19] The metabolism of lithocholic acid has not been

examined in any other species known to be susceptible to administration of lithocholic acid. To obtain further information on the possible correlation between the effects of lithocholic acid and its metabolism in the liver, the fate of lithocholic acid-24-^{14}C has been studied in chicken and rabbit.

EXPERIMENTAL

Lithocholic acid-24-^{14}C (8 μC/mg) was prepared as described by Bergström, Rottenberg and Voltz [20] and was a generous gift of Dr. A. Norman.

Male rabbits weighing 2 to 3 kg and male white Leghorn chickens 6 to 8 weeks old were used. The common bile duct of the rabbits was cannulated as described previously [21] and the loss of fluid through the fistula was compensated by subcutaneous administration of 15 ml of saline solution every two hours. The sodium salt of the labeled acid was dissolved in 1 ml of saline and was injected into the marginal vein of the ear 4 h after operation. The chickens were anaesthetized by intravenous administration of mebumal and the right hepatic duct was cannulated with a polyethylene tube. The left hepatic duct which drains the small left liver lobe was left intact. One hour after the operation the labeled acid was injected into a brachial vein.

Bile was collected in hourly portions. Bile was hydrolyzed with M potassium hydroxide in 50 % aqueous ethanol in sealed steel tubes at 110°C for 12 h. The hydrolysis mixture was acidified with hydrochloric acid and extracted with ether. The ether extract was washed with water until neutral and evaporated to dryness. In some experiments the bile was only acidified and extracted with ether. In one experiment lithocholic acid-24-^{14}C was injected intravenously into an intact chicken. After 24 h the chicken was sacrificed and the gallbladder and the intestines were removed. The bile was hydrolyzed as above. The intestines together with the intestinal contents were refluxed twice with a 5 % solution of potassium hydroxide in ethanol. The ethanol extracts were combined and most of the ethanol was evaporated. The aqueous phase was acidified and extracted with ether.

The residues of the ether extracts were chromatographed with phase system F 1 or F 2 [22] using Hostalene GW (Farbwerke Hoechst, Germany) or hydrophobic Hyflo SuperCel (Johns Manville and Co., USA) as support for the stationary phase. The effluent fractions were titrated with a 0.05 M solution of potassium hydroxide in methanol. Radioactivity was measured with a methane gas-flow counter. When required, the material in the chromatographic fractions was hydrolyzed as described above for bile or by refluxing in a 5 % solution of potassium hydroxide in methanol.

Thin layer chromatography of free bile acids was performed with phase systems described by Eneroth [23] and of conjugated bile acids with those described by Gänshirt *et al.*[24]

RESULTS

Metabolism of lithocholic acid-24-^{14}C in the chicken. After intravenous injection of 3 μC of lithocholic acid into bile fistula chickens about 50 % of administered isotope was excreted in the first 2 h portion of bile. During the following 2 h an additional 20 % of administered isotope was excreted. It should be pointed out that only the right hepatic duct was cannulated. Chromatography of the hydrolyzed bile with phase system F 2 revealed that the labeled material excreted in bile consisted predominantly of unchanged lithocholic acid which was established by crystallization to constant specific activity after addition of unlabeled lithocholic acid. About 5 % of the radioactivity was eluted before lithocholic acid with an elution volume characteristic of 3α,6α-dihydroxy-5β-cholanoic acid and 3α,6β-dihydroxy-5β-cholanoic acid which do not separate with this phase system. Thin layer chromatography

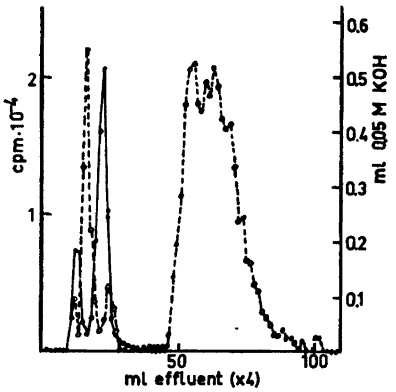

Fig. 1. Chromatogram of hydrolyzed bile from chicken injected intravenously with lithocholic acid-24-^{14}C before removal of the gallbladder. Column, 18 g of hydrophobic Hyflo SuperCel. Phase system F 2. Broken line, radioactivity; solid line, titration values.

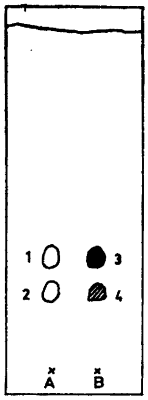

Fig. 2. Autoradiogram of the more polar products isolated from the effluent of the chromatogram shown in Fig. 1. Mobile phase S 11.[23] A: Reference compounds: 1, 3α,6β-dihydroxy-5β-cholanoic acid; 2, 3α,6α-dihydroxy-5β-cholanoic acid. B: Labeled material: 3, intense spot; 4, clearly visible spot.

of this labeled material indicated that it consisted predominantly of 3α,6β-dihydroxy-5β-cholanoic acid. In an attempt to obtain more of this material for further identification an experiment was performed with an intact chicken. Twenty-four hours after the administration of lithocholic acid the animal was sacrificed. Chromatography of the hydrolyzed bile with phase system F 2 (*cf.* Fig. 1) showed that about 10 % of the radioactivity was eluted before lithocholic acid. This more polar material was chromatographed with phase system F 1 together with unlabeled 3α,6α-dihydroxy-5β-cholanoic and 3α,6β-dihydroxy-5β-cholanoic acids and was found to be eluted together with these acids. Further evidence for the chromatographic identity of the labeled material with these two acids was obtained by thin layer chromatography in which 3α,6α-dihydroxy-5β-cholanoic acid is completely separated from 3α,6β-dihydroxy-5β-cholanoic acid (Fig. 2). Chromatography of the extract of the intestines and the intestinal contents showed that about 30 % of the labeled material consisted of these two 6-hydroxylated acids and that the remainder was unchanged lithocholic acid.

Metabolism of lithocholic acid-24-^{14}C in the rabbit. After intravenous administration of 8 μC of lithocholic acid to bile fistula rabbits about 70 % of administered isotope was excreted in the first hour portion of bile. During the following 3 h an additional 20 % of the administered isotope was excreted. Chromatography of the unhydrolyzed bile collected during the first 4 h after the administration of isotope showed that the radioactivity eluted from the column was distributed in three main peaks (*cf.* Fig. 3). The small front peak was identified as glycolithocholic acid by rechromatography with phase system

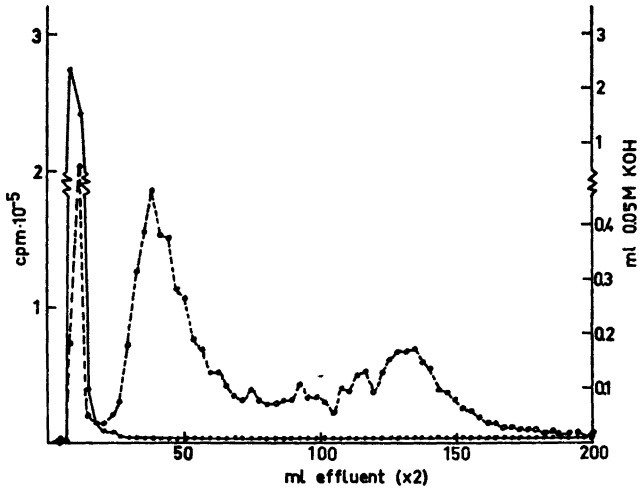

Fig. 3. Chromatogram of unhydrolyzed bile from bile fistula rabbit injected intravenously with lithocholic acid-24-^{14}C. Column, 9 g of Hostalene G. W. phase system F 1. Broken line, radioactivity; solid line, titration values.

F 1 and by thin layer chromatography. The radioactive material in the second peak was also identified as glycolithocholic acid. The identification included rechromatography as described above and also crystallization to constant specific activity after addition of authentic glycolithocholic acid. On rechromatography with phase system F 2 the radioactive material in the third peak of the chromatogram, shown in Fig. 3, appeared just after added lithocholic acid. After weak hydrolysis the labeled material was shown to be identical with glycolithocholic acid by rechromatography and by crystallization to constant specific activity after conversion to free lithocholic acid.

DISCUSSION

The results of the present investigation demonstrate that intravenously administered lithocholic acid is excreted as glycolithocholic acid and as a derivative of glycolithocholic acid in the bile fistula rabbit. In the bile fistula chicken all of the labeled material excreted in bile was conjugated with taurine. About 5 % of the administered lithocholic acid had been transformed into a compound that had the same properties in thin layer chromatography as 3α,6β-dihydroxy-5β-cholanoic acid. When lithocholic acid-24-^{14}C was allowed to circulate enterohepatically for 24 h about 10 % of the labeled products in bile were identified by chromatographic properties as 3α,6α-dihydroxy-5β-cholanoic acid and 3α,6β-dihydroxy-5β-cholanoic acid. Einarsson [25] has recently shown that in the rat 3α,6α-dihydroxy-5β-cholanoic acid is formed from 3α,6β-cholanoic acid during the entero-hepatic circulation of bile and it seems likely that the same metabolic transformation occurs in the chicken.

The low extent of hydroxylation of lithocholic acid in the chicken as well as in the rabbit lends support to the hypothesis that the degree of cirrhogenic and pyrogenic effects of lithocholic acid is correlated with the capacity of the liver to metabolize lithocholic acid into more polar products.

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