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On the Metabolism of Bile Acids in the Mouse

Bile Acids and Steroids 87

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The catabolism of 4-^{14}C-cholesterol to bile acids in the mouse has been studied. Cholic acid and small amounts of chenodeoxycholic acid are formed. The presence of chenodeoxycholic acid in mouse bile is therefore postulated. The latter acid was partially transformed into two more polar compounds having the same chromatographic mobility as the 6β-hydroxylated acids (3α,6β,7α- and 3α,6β,7β-trihydroxycholanic acid) formed from chenodeoxycholic acid in the rat.

24-^{14}C-Deoxycholic acid was hydroxylated to cholic acid in the mouse.

Recent work on the effect of intestinal microorganisms on bile acids has demonstrated, that the primary bile acids, *i. e.* those formed in the liver, often are modified during their enterohepatic circulation by the action of bacteria. Thus, deoxycholic acid in man [1] and rabbit [2] is a microbial metabolite of cholic acid. The same reaction occurs in rat [3,4], but the deoxycholic acid formed is in this species rehydroxylated to cholic acid in the liver [5]. The removal of a 7α-hydroxyl from a primary trihydroxylated bile acid by the action of intestinal microorganisms also occurs in the pig [6], where hyocholic acid is transformed into hyodeoxycholic acid.

These findings, that were discussed in more detail in the preceding paper [7], seem to suggest a general scheme of bile acid metabolism, irrespective of species. To obtain additional experimental support for such a hypothesis, and as a part of a more general survey of bile acid metabolism in different species, it was of interest to investigate the formation and metabolism of bile acids in the mouse, that is reported to have cholic acid in its bile [8].

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EXPERIMENTAL

White male mice of the Danish State Serum Institute strain, weighing approx. 25 g, were used. The animals were sacrificed by cervical dislocation. Bile was recovered from the gall-bladder and from 50 % ethanol washes of the upper part of the small intestine.

The analytical procedures for conjugated and free bile acids were essentially the same as those described in the preceding paper [7]. 4-^{14}C-cholesterol was obtained from Radiochemical Centre, Amersham, England, and was administered as an emulsion with 1 % bovine serum albumin in saline. 24-^{14}C-Chenodeoxycholic and 24-^{14}C-deoxycholic acid were prepared according to Bergström *et al.*[9] and were generous gifts from Dr. Arne Norman.

RESULTS AND DISCUSSION

10 μC (0.2 mg) of 4-^{14}C-cholesterol were injected intraperitoneally into each of 8 mice, and 24, 48, 72 and 120 h after injection 2 animals were sacrificed and the bile was collected. Chromatography of the unhydrolyzed bile samples revealed that the bile acids were present in bile exclusively as taurine conjugates. After hydrolysis the bile acids were chromatographed with phase system F and in Fig. 1 is shown a chromatogram of the free bile acids from 2 mice injected 48 h before sacrifice. The radioactive material in the first peak was identified as cholic acid by isotope dilution. The identity of the radioactive material in the second peak with chenodeoxycholic acid was established in the same manner. Chromatograms of hydrolyzed bile from the mice injected with 4-^{14}C-cholesterol 24, 72 and 120 h before sacrifice did not differ from the above mentioned, except that the amount of chenodeoxycholic acid varied from a few percent to 10 %.

The catabolism of cholesterol to bile acids in the mouse thus follows the same path as in rat. In the latter animal chenodeoxycholic acid is further metabolized to two 6β-hydroxylated acids (3α,6β,7α- and 3α,6β,7β-trihydroxycholanic acid), recently identified by Doisy and coworkers [10,11], and cholic acid is transformed by the action of intestinal microorganisms into deoxycholic acid [3], which in turn is rapidly rehydroxylated to cholic acid in the liver [5].

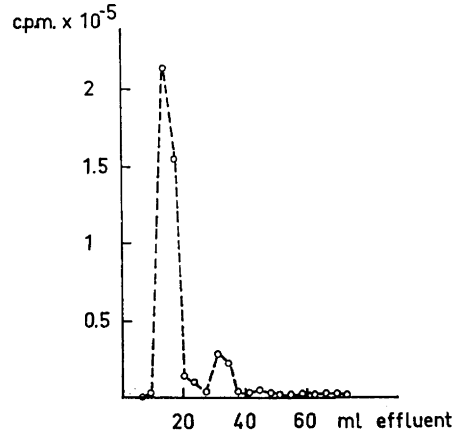

Fig. 1. Chromatogram of hydrolyzed bile collected 48 h after i. p. injection of 4-^{14}C-cholesterol. Column: 4.5 g Hostalene. Phase system F. Broken line: radioactivity.

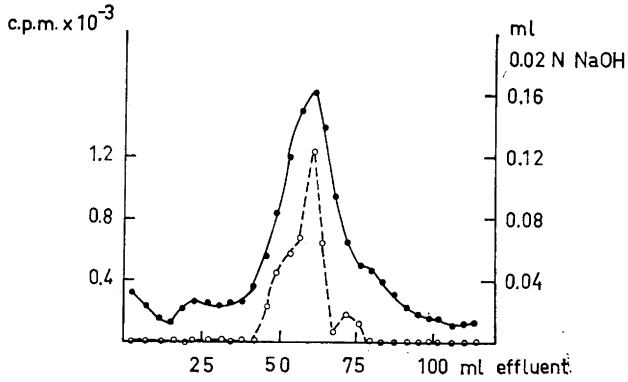

Fig. 2. Chromatogram of trihydroxycholanic acid fraction from bile collected 24 h after injection of 24-^{14}C-deoxycholic acid. Column: 4.5 g Hostalene. Phase system C. Solid line: titration. Broken line: radioactivity.

In order to provide further evidence for the similarity in the pattern of bile acid formation and metabolism in rat and in mouse, the metabolism of 24-^{14}C-chenodeoxycholic and 24-^{14}C-deoxycholic acid was studied in the mouse.

3 μC (0.3 mg) of 24-^{14}C-chenodeoxycholic acid were injected into each of 6 mice and another group of 6 mice received each 2 μC (0.3 mg) of 24-^{14}C-deoxycholic acid. 3, 24 and 72 h after injection, 2 animals in each group were sacrificed. The bile was hydrolyzed and chromatographed first on phase system F and then after addition of 5 mg unlabeled cholic acid on phase system C.

Bile collected 3 h after injection of either 24-^{14}C-deoxycholic or 24-^{14}C-chenodeoxycholic acid contained about 1/3 of the isotope in the trihydroxycholanic acid fraction, and the remainder was present as unchanged deoxy-

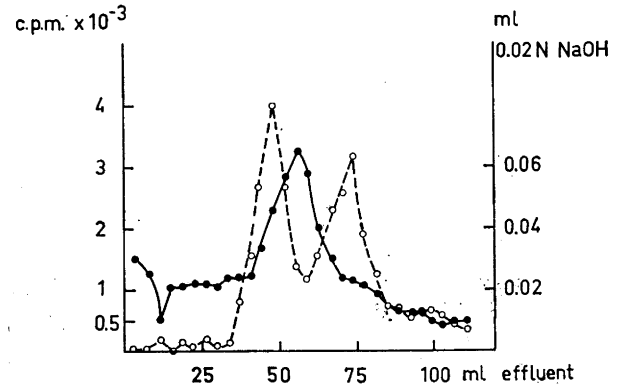

Fig. 3. Chromatogram of trihydroxycholanic acid fraction from bile collected 24 h after injection of 24-^{14}C-chenodeoxycholic acid. Column: 4.5 g Hostalene. Phase system C. Solid line: titration. Broken line: radioactivity.

cholic and chenodeoxycholic acid, respectively. The amount of isotope in the trihydroxycholanic acid band rose to 60—70 % in bile collected 24 h after injection and to 90—100 % after 72h. The trihydroxycholanic acid fractions were rechromatographed on phase system C. Fig. 2 shows the chromatogram of this fraction from animals injected 24 h before sacrifice with 24-^{14}C-deoxycholic acid. The radioactivity coincides with the titration peak of cholic acid. The identity of the isotope with cholic acid was confirmed by isotope dilution.

In the corresponding chromatogram of bile from animals injected with 24-^{14}C-chenodeoxycholic acid, the radioactivity is distributed in two peaks appearing before and after cholic acid, *cf.* Fig. 3. These two radioactive peaks have the same chromatographic mobility as the 6β-hydroxylated acids formed from chenodeoxycholic acid in the rat, 3α,6β,7α- and 3α,6β,7β-trihydroxycholanic acid [10,11].

The results obtained in this investigation indicate, that the bile acid formation and metabolism of the mouse is very similar to that of the rat.

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