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On the Metabolism of Bile Acids in the Guinea Pig

Bile Acids and Steroids 86

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The catabolism of 4-^{14}C-cholesterol to bile acids has been studied in intact and bile fistulated guinea pigs. The only bile acid formed from cholesterol in the liver is chenodeoxycholic acid. During the enterohepatic circulation chenodeoxycholic acid is oxidized to 7-keto-lithocholic acid by the action of intestinal microorganisms. The 7-keto-lithocholic acid thus formed is reduced in the liver to chenodeoxycholic acid, as was shown by injection of 24-^{14}C-7-keto-lithocholic acid to intact and bile fistulated guinea pigs.

The bile of guinea pig is reported to contain chenodeoxycholic acid (3α, 7α-dihydroxycholanic acid) and 7-keto-lithocholic acid (3α-hydroxy, 7-keto-cholanic acid), present as conjugates with taurine [1]. The important part played by intestinal microorganisms in the metabolism of bile acids during their enterohepatic circulation has recently become apparent by work in this laboratory. Lindstedt and Sjövall [2] found, that deoxycholic acid, which makes up 90 % of the bile acids in gall-bladder bile of rabbit, is formed from cholic acid by the action of bacteria in the intestine. The same reaction has been shown also to occur in man [3], and in rat [4], that was also observed by Portman [5]. In the rat, however, the deoxycholic acid is rapidly rehydroxylated to cholic acid in the liver [6]. In the pig, hyodeoxycholic acid (3α,6α-dihydroxy-cholanic acid) is a microbial product of hyocholic acid (3α,6α,7α-trihydroxy-cholanic acid) [7]. In all above mentioned cases the intestinal microorganisms remove a 7α-hydroxyl from a trihydroxylated bile acid, whether the position of the hydroxyls is 3α,6α,7α or 3α,7α,12α. This reaction has been further studied *in vivo* by means of doubly labeled cholic acid (7β-tritio, 24-^{14}C-cholic acid) [8,9], and it has been found, that the removal of the 7α-hydroxyl occurs with retention of tritium that, however, apparently is shifted during this process from the 7β to the 7α position.

Norman and Sjövall [4] have recently made a detailed study of the metabolism of bile acids in the rat during their passage through the intestinal tract, and they have shown, that among microbially formed products of cholic acid

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small amounts of ketonic acids, mainly 3α,12α-dihydroxy -7-ketocholanic acid, are also absorbed from the large intestine and excreted in the bile.

Thus in the rat intestinal microorganisms are responsible for the removal of the 7α-hydroxyl from cholic acid and also for the oxidation of this group to a ketone. The latter reaction does not seem to be a quantitatively important intermediate step in the formation of deoxycholic acid, as the 7β-tritium in cholic acid is retained to a high extent in the deoxycholic acid.

In view of above mentioned data and considering that the guinea pig is reported to have no trihydroxylated bile acid, but only chenodeoxycholic and 7-keto-lithocholic acid in its bile, it was of interest to study the formation of bile acids in this species and the changes, if any, of the bile acids during their enterohepatic circulation.

EXPERIMENTAL

Male guinea pigs of the Danish State Serum Institute strain, weighing approx. 300 g, were used. Bile fistulas were prepared as described for rats [10].

Procedures for analysis of bile. Conjugated bile acids were extracted from acidified bile with *n*-butanol. Conjugates were split by hydrolysis with N sodium hydroxide at 120° for 8—12 h in a sealed steel tube, and the free bile acids were extracted with ether from the acidified reaction mixture. The bile acids were separated with reversed phase partition chromatography using phase system C for conjugated acids [11] and F for free acids [12].

Phase systems

	Moving phase	Stationary phase
C	150 ml methanol 150 ml water	15 ml chloroform 15 ml *iso*octanol
F	165 ml methanol 135 ml water	45 ml chloroform 5 ml heptane

Hostalene (Farbwerke Hoechst, G.m.b.H., West-Germany), prepared as earlier described [7], was used as supporting material for the stationary phase. Columns were prepared as described by Norman [11], using 3 ml of stationary phase per 4.5 g of Hostalene. Chromatograms were run at constant temperature of 23°. The effluent was titrated with 0.02 N methanolic sodium hydroxide, and suitable aliquots were assayed for radioactivity, which was determined with an automatic end-window counter, Frieseke und Hoepfner, West-Germany.

Labeled compounds. 4-^{14}C-cholesterol was obtained from the Radiochemical Centre, Amersham, England. Specific activity: $\sim$ 50 μC/mg. 24-^{14}C-7-ketolithocholic acid was prepared from 24-^{14}C-chenodeoxycholic acid according to the procedure described by Samuelsson [13]. Specific activity: $\sim$1 μC/mg.

RESULTS AND DISCUSSION

The formation of bile acids from cholesterol and the metabolism of these in guinea pig was studied by analyses of labeled products in gall-bladder and fistula bile after intraperitoneal injection of 4-^{14}C-cholesterol and of 24-^{14}C-7-keto-lithocholic acid. The 4-^{14}C-cholesterol in doses of 10—50 μC (0.2—1 mg) was injected as an emulsion with 1 % bovine serum albumin in saline. 1—5 days after administration of the isotope, a bile fistula was prepared and bile collected for 12—24 h or the animal was sacrificed and the gall-bladder removed.

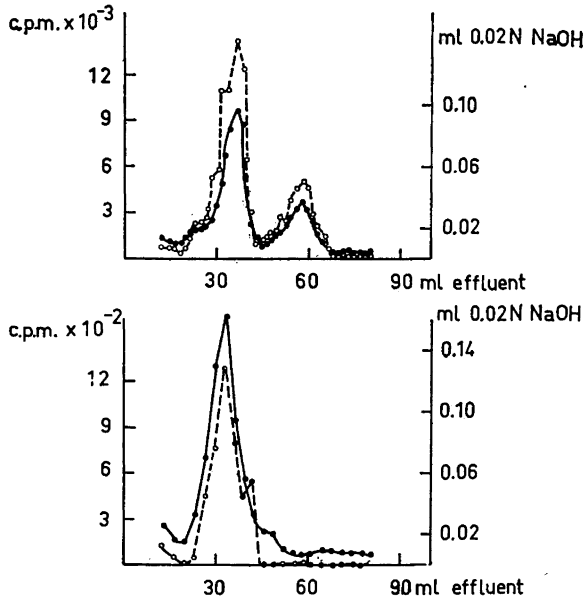

Fig. 1. Chromatogram of hydrolyzed bile collected from bile fistula between 0 and 4 h (upper curve) and between 8 and 20 h (lower curve) after fistulation. The animal had been injected with 50 μC 4-^{14}C-cholesterol 5 days before operation. Columns: 4.5 g Hostalene. Phase system F. Solid line: titration. Broken line: radioactivity.

The unhydrolyzed bile acids were chromatographed with phase system C, and it was found, that bladder bile and fistula bile collected 0—8 h after fistulation contained mainly glycine conjugates, about 80 % of total, the rest being taurine conjugates. Fistula bile collected later than 8 h after fistulation had a considerable part and sometimes all of the bile acids conjugated with taurine.

When the bile acids were chromatographed after hydrolysis on phase system F, it was found, that there appeared one or two radioactive titration peaks, depending on the time the bile samples were collected. Bile collected from bladder, and fistula bile collected during the first 4—6 h after fistulation contained two labeled acids, appearing in the chromatogram at the place for chenodeoxycholic and 7-keto-lithocholic acid, respectively, as is shown in the upper curve of Fig. 1. Bile collected later than 6 h after fistulation contained only one labeled acid with chromatographic mobility as chenodeoxycholic acid, *cf.* lower curve, Fig. 1. The identity of the labeled compounds with chenodeoxycholic, 7-keto-lithocholic and chenodeoxycholic acid, respectively, was established by co-crystallization with unlabeled material, the specific activity remaining constant through four crystallizations with a final weight of crystals about one quarter the original weight (chenodeoxycholic acid was crystallized from methanol/ethyl acetate twice and ethyl acetate twice; 7-keto-lithocholic acid from ethyl acetate, methanol/water, acetone/water and ethyl acetate).

Bladder bile and fistula bile collected 0—3 h after fistulation, when the preformed bile acids are being excreted, contained approximately the same

proportion of chenodeoxycholic to 7-keto-lithocholic acid, 3:1, whether the cholesterol had been injected 1 or 5 days before collection of bile, indicating that the microbial formation of 7-keto-lithocholic acid from chenodeoxycholic acid occurs rapidly and then reaches an equilibrium, which conceivably could be maintained by a continuous reduction of the 7-ketone to a 7α-hydroxyl in the liver. To test this, 24-^{14}C-7-keto-lithocholic acid in doses of 0.3 μC (0.3 mg) was injected intraperitoneally into intact and bile fistulated guinea pigs. The labeled products in bile were analyzed as described above.

Already after 1 day of enterohepatic circulation 73 % of the isotope in the bile was present as chenodeoxycholic acid; the remainder consisted of unchanged 7-keto-lithocholic acid. About the same distribution of isotope (83 % in chenodeoxycholic acid) was found in bile from an animal, in which the labeled 7-keto-lithocholic acid had taken part in the enterohepatic circulation for 3 days. When the 7-keto-lithocholic acid was injected into bile fistulated guinea pigs, the isotope appeared in bile rapidly, and after 4 h 70—80 % of the injected amount had been excreted. About 50 % of the isotope was present as chenodeoxycholic acid and the remainder as unchanged 7-keto-lithocholic acid.

Thus, the guinea pig liver efficiently reduces 7-keto-lithocholic acid to chenodeoxycholic acid. In this connection it is interesting, that the rat liver reduces 7-keto-lithocholic acid mainly to the 7β-epimer, ursodeoxycholic acid[13]. These experiments do not exclude the possibility, that the reduction of 7-keto-lithocholic acid to chenodeoxycholic acid is effected also by intestinal microorganisms, but this does not seem entirely likely in view of results obtained in the rat, where the microbial action on the bile acid nucleus mainly is oxidative or consists of dehydroxylations [4].

The results obtained in this investigation seem to establish, that the guinea pig, as the only species so far known, does not catabolize cholesterol in the liver to a trihydroxylated bile acid, but only to chenodeoxycholic acid. During the enterohepatic circulation of bile this acid is transformed by microbial action into 7-keto-lithocholic acid, which in turn is reduced in the liver to chenodeoxycholic acid.

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