The formation of cholic acid from 3α,7α-dihydroxycoprostane in the rat

The two main bile acids in the rat are cholic acid and chenodeoxycholic acid, and both are formed from cholesterol[1,2,3,4]. However, chenodeoxycholic acid is not a precursor of cholic acid since it has been shown that labelled chenodeoxycholic acid does not give rise to cholic acid, but to two other as yet unidentified compounds, one slightly more and one less polar than cholic acid[2,5].

These, and other results obtained in this laboratory have led to the suggestion that the nucleus of the bile acids is elaborated before the degradation of the side chain is completed[6]. If this is the case, rat liver might be able to hydroxylate 3α, 7α-dihydroxycoprostane or its corresponding intermediate with partially degraded side chain in the 12α-position. In the former case the product would be 3α, 7α, 12α-trihydroxycoprostane, a compound already known to be rapidly degraded to cholic acid in the rat[7]. To test this theory, we have prepared tritium-labelled 3α, 7α-dihydroxycoprostane by anodic coupling of tritium-labelled chenodeoxycholic acid and *iso*valeric acid according to a procedure described earlier[7,8]. The labelled chenodeoxycholic acid was prepared by platinum-catalyzed exchange with tritiated water essentially according to TRENNER *et al.*[9] and purified by chromatography[10]. The 3α, 7α-dihydroxycoprostane (m.p. 83–85) had a specific activity of ~$3 \cdot 10^5$ c.p.m./mg when counted in a Tracerlab gas-flow counter.

An emulsion of 4.7 mg of the 3α, 7α-dihydroxycoprostane was prepared in 2 ml of saline containing 0.1% sodium oleate and injected intraperitoneally into a rat with a bile fistula. About 50% of the activity was excreted during the following five days. To obtain more labelled material another rat was injected with 4 mg daily for four days. The free bile acids obtained after alkaline hydrolysis were chromatographed according to NORMAN[11]. About 40% of the activity was eluted in the three bands shown in Fig. 1.

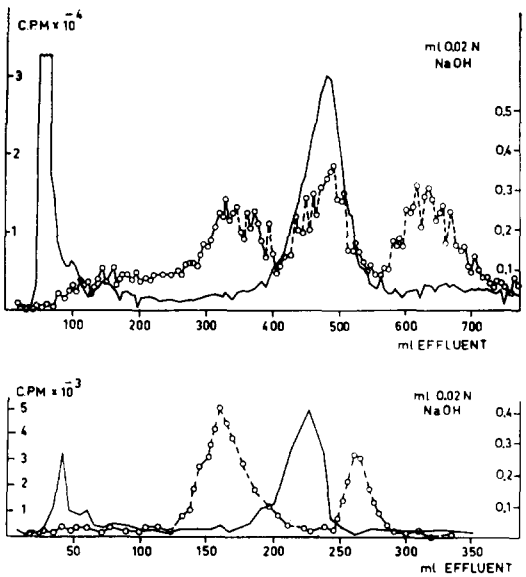

Fig. 1. Upper curve. Chromatography of acid from hydrolysed bile excreted after administration of tritium-labelled 3α, 7α-dihydroxycoprostanic acid. Column: 18 g hydrophobic kieselguhr. Moving phase: methanol/water 50:50. Stationary phase: *iso*octanol/chloroform 1:1. ---- titration curve. O--O activity curve (direct plating). Lower curve. Chromatography of acids from hydrolysed bile excreted after administration of ^{14}C-labelled chenodeoxycholic acid. Column: 9 g hydrophobic kieselguhr. Phases: Same as above. Redrawn from BERGSTRÖM AND SJÖVALL[2].

The second peak coincided with the titration peak of cholic acid. This material was rechromatographed and then diluted with cholic acid and recrystallized five times. From each crystallization material was taken for activity determination by the method of GLASCOCK[12]. The specific activity was found to remain constant.

The first and third peak in Fig. 1 upper curve correspond to those obtained when chenodeoxycholic acid had been administered (*cf.* Fig. 1 lower curve).

In the experiment shown in Fig. 1 about 55% of the activity remained in the column. This material was eluted and found to consist mainly of chenodeoxycholic acid, that had thus been formed by direct degradation of the side chain without any further change on the nucleus.

The results obtained thus show that the structure of the side chain has a pronounced influence on the course of the hydroxylation and strengthen the hypothesis referred to above[6]. The reactions discussed are summarized in the formulas.

Studies are now being made on the metabolism of other possible intermediates in the conversion of cholesterol to cholic acid such as 7α-hydroxycholesterol and 3α, 7α-dihydroxycoprostanic acid.

A rate-determining step in the conversion of cholesterol to cholic acid might be reactions on the nucleus, as both 3α, 7α, 12α-trihydroxycoprostane and 3α, 7α-dihydroxycoprostane are degraded to bile acids much more rapidly than cholesterol.

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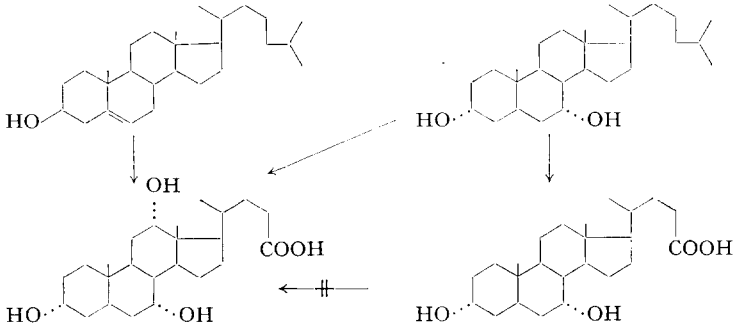

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The distribution of radioactivity in monkey serum lipids following feeding of triolein-^{3}H

BERGSTRÖM *et al.*[1] have recently reported on the distribution of triolein in the rat following the feeding of triolein-^{14}C. The partition of this triglyceride in the serum of monkeys has been measured following a feeding[2] of triolein-^{3}H and is the basis of this brief report.

Triolein-^{3}H (0.75 g, 16.9 μc/g) was administered by stomach tube to two male Java monkeys weighing 6 pounds. The animals were placed in individual cages and bled at intervals starting at 2 hours after feeding and continuing for 3 and 7 days respectively. The serum obtained after bleeding was deproteinized with methylal-methanol 4:1[3] and chromatographed on a silicic acid column following the method of FILLERUP AND MEAD[4]. In the first case sterol ester, neutral fat, sterol, phospho-lipid and fatty acid fractions were collected and assayed for radioactivity. In the second case only the neutral fat and fatty acid fractions were counted. Tritium assay was carried out using the liquid scintillation counting technique employing 2,5-diphenyloxazole in toluene[5] and using an LP-2 double channel liquid phosphor counter (Technical Measurement Corp., New Haven, Conn.)*. All counts were carried out for 30–60 minutes, with the total sample being assayed each time. Under the conditions used, the background was 47 counts per minute. The data are given in Tables I and II.

Feces from Monkey No. 1 were collected at 24, 48 and 72 hours; these samples contained 0.2, 0.5 and 0.4% of the administered counts, respectively. From our data it is apparent that most of the triolein activity fed was present in the neutral fat and fatty acid fractions of the serum lipids, with peak uptake occurring between 2 and 6 hours after feeding. The specific activity values for the fatty acid fractions are open to question because the amounts of fatty acid are minute and a small weighing error could result in a large specific activity error.