On the Turnover and Excretory Products of Cholic and Chenodeoxycholic Acid in Man*

BILE ACIDS AND STEROIDS 134

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The main bile acids in human bile are cholic, chenodeoxycholic, and deoxycholic acids, which are present as conjugates with taurine or glycine. Cholic and chenodeoxycholic acids are the "primary" bile acids formed from cholesterol in the liver, whereas deoxycholic acid is a product of microbial dehydroxylation of cholic acid in the intestine during the enterohepatic circulation of bile (1). Intestinal microorganisms also cause a dehydroxylation of chenodeoxycholic acid yielding lithocholic acid (2). However, lithocholic acid is reabsorbed only to a limited extent and is therefore practically absent from bile.

In addition to these dehydroxylations, the bile acids are further modified by intestinal microorganisms and are excreted in feces as a complex mixture of predominantly free bile acids (1-4). Several of the bile acids present in human feces have been identified. Carey and Watson (5) isolated deoxycholic acid and Heftmann et al. (6) described the isolation of lithocholic, isolithocholic, and 12-ketolithocholic acids. Recently, Rosenfeld and Hellman (7) reported that deoxycholic and lithocholic acids were the main bile acids in human feces.

Gas chromatographic analyses of methylated extracts of human feces revealed, however, the presence of several additional compounds with retention times similar to those of bile acid methyl esters, and a more extensive study of the nature and origin of the fecal bile acids therefore seemed of importance, especially in view of the investigations currently conducted in this laboratory on the influence of diet and hormones on the turnover and excretion of bile acids in man.

According to current concepts, cholic and chenodeoxycholic acids are not metabolically interconvertible in man (1, 2), and it should therefore be possible to establish the origin of the different fecal bile acids from either cholic or chenodeoxycholic acid by administering at the same time these two acids labeled with tritium and determining the turnover of these two acids in the same individual and give information on the isotope equilibrium attained in the fecal bile acids. Although the turnover of cholic acid has been determined in a number of subjects under different physiological conditions (8-10), no data are available concerning the turnover of chenodeoxycholic acid in man, and no simultaneous determination of the turnover of different bile acids has been made in any species.

The present communication reports on the turnover of cholic and chenodeoxycholic acid in two subjects given a mixture of randomly tritium-labeled cholic acid and 24-C14-chenodeoxycholic acid. The main bile acids in feces have been isolated and characterized.

EXPERIMENTAL PROCEDURE

All melting points are uncorrected.

Labeled Compounds—Cholic acid-H3 was prepared by the method of Wilzbach (11, 12) and purified by repeated chromatography on phase system C1. Dilution with unlabeled cholic acid gave material with a specific activity of 40 mc per mg. Chenodeoxycholic acid-24-C14 was prepared according to Bergström, Rottenberg, and Volta (13) and had a specific activity of 8 mc per mg.

Reference Compounds—Islithocholic, lithocholic, and 12-ketolithocholic acid methyl esters, and deoxycholic acid were material used in earlier studies in this series of investigations. The synthesis of methyl 3β,12α-di hydroxy-5β-cholanate has recently been described (14). Methyl 3β-hydroxy-12-keto-5β-cholanate was prepared, in principle, as described by Kyogoku (15) although the reduction was made on methyl 3,12-diketo-5β-cholanate. The reaction mixture was chromatographed on a column of aluminum oxide (Woelm, Eche wege, West Germany), Grade III. The methyl 3β-hydroxy-12-keto-5β-cholanate obtained had a melting point of 129-130°, reported (15) 117-118°. The material with m.p. 129-130° was completely homogeneous in thin layer and gas chromatography. Saponification of a sample of the methyl ester gave 3β-hydroxy-12-keto-5β-cholanic acid with m.p. 218-219°, reported (15) 218-220°. Thus, the methyl 3β-hydroxy-12-keto-5β-cholanate prepared is not contaminated with any methyl 12-ketolithocholate. When present as free acids, these two compounds can form mixed crystals (15); this is easily avoided, however, by suitable chromatographic procedures. In this connection it should be mentioned that the methyl 12-ketolithocholate used as reference compound was synthesized by K. Hollström and S. Lindstedt, to be published.
as described by Bergström and Hacelwood (16). It might also be mentioned that a crude preparation of methyl 3β-hydroxy-12-keto-5β-cholanate described by Press, Grandjean, and Reichstein (17) had melting point of 120-126° (corrected).

**Isotope Determinations**—The content of C\textsuperscript{4} and H\textsuperscript{3} in the isolated compounds was determined by the method of Glasscock (18) or by liquid scintillation spectrometry in a Packard Tri-Carb spectrometer.

**Chromatographic Procedures**—Reversed phase partition chromatography was performed with phase systems reported by Norman and Sjövall (19). For aluminum oxide chromatography, Woelm aluminum oxide, suitably graded according to Brockmann and Schodder (20), and redistilled solvents were used. Gas chromatography was performed as recently described (21). Thin layer chromatography was performed with phase systems described by Eneroth (22).

**Human Experiments**—Two healthy human subjects were given a standardized diet with 40% of the calories as butter fat.\textsuperscript{2} This diet was based on only four different meals (veal, chicken, cod, or plaice with rice or mashed potatoes prepared with butter fat) of equal caloric composition which had been prepared in large batches and frozen until used. The subjects ate each day two of these meals, which constituted their predominant caloric intake. At zero time they were given an oral dose of 8 μg of 24-C\textsuperscript{4}-deoxycholic acid and 8 μg of tritium-labeled cholic acid. Bile samples were collected by duodenal intubation four times over a 10-day period. The gall bladder was stimulated to contraction by an intravenous dose of ephedrine (Cecelin, Vitrum Pharmaceutical Company, Sweden).

**Analysis of Bile Samples**—Cholic acid was isolated from hydrolyzed bile by chromatography on phase system C1 (19) and crystallized from ethyl acetate. The ethanol eluate of the previously column containing deoxycholic and chenodeoxycholic acids was rechromatographed on phase system F1 (19). The total amount of each of the two acids in the dihydroxycholanic acid fraction obtained was then determined by gas chromatography. The total amount of C\textsuperscript{4} and H\textsuperscript{3} in the dihydroxycholanic acid fraction was determined after combustion, and the specific activity of deoxycholic acid (H\textsuperscript{3}-labeled) could be calculated. The chromatographic fractions were analyzed by subjecting a small aliquot to gas chromatography. In most cases the respective acids had to be purified by additional chromatography on aluminum oxide, Grade III.

**RESULTS**

**Turnover of Cholic and Chenodeoxycholic Acids**—Fig. 1 shows semilogarithmic plots of the specific activity of the cholic and chenodeoxycholic acids from the bile from which the half-lives of the respective acids were calculated (8). From the extrapolated zero time values, the dilution factors of the administered doses were obtained and used for calculating the size of the miscible pools of the two acids. Figures for half-lives and pool sizes are given in Table I together with the daily production calculated from the relation:

\[
P = \frac{P}{A} = \text{daily production (=turnover)}; \text{grams per day} \quad A = \text{pool size; grams}
\]

\[
k = \frac{A}{t_1} = \text{rate constant} \left(= \frac{0.693}{t_1}\right) ; \text{days}^{-1}
\]

\[
t_1 = \text{half-life; days}
\]

**Isolation and Identification of Fecal Bile Acids**—As described in "Experimental Procedure," the partially purified fecal extracts were methylated. The weight of these extracts varied from 1.7 to 4.2 g.

Before chromatography on aluminum oxide, it was desirable to obtain a gas chromatogram of the crude mixture. However, owing to the presence of interfering material, this was found difficult and an aliquot of the total extract was therefore purified by an additional chromatography on silicic acid, eluting with ether and ether-acetone, 1:1. These fractions were separately analyzed by gas chromatography. Fig. 2 shows a chromatogram obtained of the ether fraction. The acetone-ether fraction which would contain cholic acid or any bile acid of similar polarity or both did not give any peak with retention time compatible with such acids.

The four fecal extracts (Fd I, Fd II, Tl I, Tl II) were chromatographed under identical conditions with a 50-fold excess of aluminum oxide. The patterns of elution of the different compounds were very similar in the four cases. Table II summarizes the result of one such chromatogram (Extract Tl I).

The following compounds could be isolated.

**Methyl Isolithocholate**—Fractions 3 to 5 were combined and crystallized twice from ether-petroleum ether, yielding 36.2 mg, m.p. 114°, reported (23) 115-116°. Mixed melting point with authentic material of m.p. 115° was 114-115°.

**Methyl Lithocholate**—Fractions 8 to 13 were combined and crystallized from methanol-water, yielding 39.3 mg, m.p. 97°. After thorough drying, the melting point was 124°, reported (24)
FIG. 1. Semilogarithmic plots of specific activities of cholic and chenodeoxycholic acid in bile

TABLE I

<table>
<thead>
<tr>
<th>Case</th>
<th>Cholic acid</th>
<th>Chenodeoxycholic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( t_1 )</td>
<td>Pool size</td>
</tr>
<tr>
<td>Fd</td>
<td>4.0</td>
<td>1.19</td>
</tr>
<tr>
<td>Tl</td>
<td>2.0</td>
<td>0.54</td>
</tr>
</tbody>
</table>

Deoxycholic Acid—As previous experiments had shown that it was very difficult to obtain crystalline methyl deoxycholate, the fractions containing methyl deoxycholate were routinely saponified. Fractions 27 to 30 were saponified and extracted with ether after acidification, and the residue of the ether extract was crystallized from acetic acid-water, yielding 46.1 mg of deoxycholic acid, m.p. 172°, reported (27) 176 to 179° (corrected). Mixed melting point with authentic material of m.p. 172-174° was 171-173°.

Similar results were obtained with the other three fecal extracts. In all cases it was possible to isolate crystalline methyl 3β-hydroxy-12-keto-5α-cholanate. However, from chromatograms of Fractions Tl II and Fd II, no crystalline methyl 12-ketolithocholate and methyl 3β,12α-dihydroxy-5β-cholanate, respectively, could be isolated, although the presence of these two compounds in the extracts was indicated by gas chromatography. On gas chromatography, the isolated crystalline bile acid methyl esters gave single peaks with the same retention times as those of the authentic samples. In no case was there a significant depression of mixed melting points with authentic samples.

Isotope Content of Fecal Bile Acids—Table III gives the tritium and C\(^{14}\) specific activities of the crystalline bile acid methyl esters (deoxycholic acid determined as free acid) isolated from the four fecal portions. These data show that lithocholic and isolithocholic acids originate from chenodeoxycholic acid whereas deoxycholic, 12-ketolithocholic, 3β,12α-dihydroxy-5β-cholanic, and 3β-hydroxy-12-keto-5β-cholanic acids originate from cholic acid. In the cholic acid metabolites, varying amounts of C\(^{14}\) were
present. Excluding methyl 3β,12α-dihydroxy-5β-cholanate, the amount of C¹⁴ corresponds to the presence of less than 4% of material originating from chenodeoxycholic acid. In the case of all three samples of methyl 3β,12α-dihydroxy-5β-cholanate, the amount of C¹⁴ would correspond to 8 to 10% of such material. Thin layer chromatography of the original samples obtained from the aluminum oxide columns revealed the presence of minor impurities of which one had an Rf value similar to that of methyl 3β,7α-dihydroxy-5β-cholanate (14). This material could be removed from the main compound by preparative thin layer chromatography but was found not to be 3β,7α-dihydroxy-5β-cholanate as evidenced by the depression obtained in mixed melting point determination. The content of C¹⁴ in the samples of methyl 3β,12α-dihydroxy-5β-cholanate was decreased but not eliminated by preparative thin layer chromatography, and the amount of C¹⁴ in the samples after this purification step would correspond to the presence of 4, 6, and 10%, respectively, of material originating from chenodeoxycholic acid. The values given in Table III for methyl 3β,12α-dihydroxy-5β-cholanate are those obtained after preparative thin layer chromatography.

In all samples of methyl lithocholate and isolithocholate, gas phase counting as well as liquid scintillation spectrometry indicated the presence of tritium. Thin layer chromatography revealed the presence of very small amounts of impurities of unknown nature in six of the eight samples. The Rf values of most of these impurities did not correspond to those of known bile acid methyl esters. Whether the tritium figures obtained represent admixture of material originating from cholic acid or are due to experimental difficulties in measuring small amounts of tritium in samples of high C¹⁴ activity cannot be decided at present.

DISCUSSION

The turnover of cholic acid in man has been studied in a number of subjects (8-10). The turnover of chenodeoxycholic acid in man has not been examined previously, mainly because of the difficulty of isolating pure chenodeoxycholic acid from the mixture of deoxycholic and chenodeoxycholic acid present in bile. Experiments in rats have indicated that the half-lives of chenodeoxycholic and cholic acids are of the same order of magnitude (28). Determinations of the half-life of cholic acid in different human subjects have given values in the range of 1.4 to 6.5 days. Because of this individual variation, which can be expected to be found also for the half-life of chenodeoxycholic acid, it appears desirable to determine simultaneously the turnover of these two acids in the same subject. This can be achieved by the adminis-

Table II

<table>
<thead>
<tr>
<th>Fractions</th>
<th>Solvent</th>
<th>Weight (mg)</th>
<th>Main compounds found in gas chromatography</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>H-B, 9:1</td>
<td>32</td>
<td>Compounds less polar than methyl isolithocholate</td>
</tr>
<tr>
<td>2</td>
<td>H-B, 9:1</td>
<td>142</td>
<td>Compounds less polar than methyl isolithocholate; methyl 3-keto-5β-cholanate; methyl isolithocholate</td>
</tr>
<tr>
<td>3-5</td>
<td>H-B, 9:1</td>
<td>98</td>
<td>Methyl isolithocholate</td>
</tr>
<tr>
<td>6-7</td>
<td>H-B, 7:3</td>
<td>31</td>
<td>Methyl lithocholate</td>
</tr>
<tr>
<td>8-9</td>
<td>H-B, 6:4</td>
<td>47</td>
<td>Methyl lithocholate</td>
</tr>
<tr>
<td>10-11</td>
<td>H-B, 5:5</td>
<td>40</td>
<td>Methyl lithocholate</td>
</tr>
<tr>
<td>12-13</td>
<td>H-B, 4:6</td>
<td>19</td>
<td>Methyl lithocholate and methyl 3β-hydroxy-12-keto-5β-cholanate</td>
</tr>
<tr>
<td>14</td>
<td>H-B, 3:7</td>
<td>17</td>
<td>Methyl 3β-hydroxy-12-keto-5β-cholanate</td>
</tr>
<tr>
<td>15-18</td>
<td>H-B, 2:8</td>
<td>30</td>
<td>Methyl 12-ketolithocholate</td>
</tr>
<tr>
<td>20-21</td>
<td>H-B, 1:9</td>
<td>34</td>
<td>Methyl 12-ketolithocholate, one compound emerging shortly after the above-mentioned and methyl 3β,12α-dihydroxy-5β-cholanate</td>
</tr>
<tr>
<td>23-23</td>
<td>B</td>
<td>36</td>
<td>Same as Fractions 20 to 21</td>
</tr>
<tr>
<td>24-25</td>
<td>B-E, 9:1</td>
<td>106</td>
<td>Methyl 3β,12α-dihydroxy-5β-cholanate</td>
</tr>
<tr>
<td>26</td>
<td>B-E, 9:1</td>
<td>70</td>
<td>Methyl deoxycholate and some methyl 3β,12α-dihydroxy 5β-cholanate</td>
</tr>
<tr>
<td>27-30</td>
<td>B-E, 8:2</td>
<td>154</td>
<td>Methyl deoxycholate</td>
</tr>
<tr>
<td>31-32</td>
<td>B-E, 7:5</td>
<td>36</td>
<td>No peaks on gas chromatography</td>
</tr>
<tr>
<td>33-34</td>
<td>B-E, 6:4</td>
<td>30</td>
<td>No peaks on gas chromatography</td>
</tr>
<tr>
<td>35-38</td>
<td>E</td>
<td>48</td>
<td>No peaks on gas chromatography</td>
</tr>
<tr>
<td>39-40</td>
<td>E-M, 9:1</td>
<td>14</td>
<td>No peaks on gas chromatography</td>
</tr>
</tbody>
</table>
TABLE III
Specific activities of fecal bile acids determined by gas phase counting according to Glascock (18).
Period I, 0 to 5 days; period II, 5 to 10 days.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Period</th>
<th>Case Fd</th>
<th>Case Tl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>c.p.m.</td>
<td>c.p.m.</td>
</tr>
<tr>
<td>Deoxycholic acid</td>
<td>I</td>
<td>22</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>47</td>
<td>14</td>
</tr>
<tr>
<td>Methyl 12-ketolithocholate</td>
<td>I</td>
<td>38</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>42</td>
<td>19</td>
</tr>
<tr>
<td>Methyl 3β,12α-dihydroxy-5β-cholanate</td>
<td>I</td>
<td>98</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>86</td>
<td>51</td>
</tr>
<tr>
<td>Methyl 3β-hydroxy-12-keto-5β-cholanate</td>
<td>I</td>
<td>30</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>81</td>
<td>42</td>
</tr>
<tr>
<td>Methyl lithocholate</td>
<td>I</td>
<td>1740</td>
<td>226</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>2165</td>
<td>1885</td>
</tr>
<tr>
<td>Methyl isolithocholate</td>
<td>I</td>
<td>1380</td>
<td>135</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>2175</td>
<td>1770</td>
</tr>
</tbody>
</table>

* Determined by liquid scintillation spectrometry and corrected to the same counting efficiency as that of gas phase counting.

The half-life and turnover of cholic acid found fall within the range previously encountered. The half-life of chenodeoxycholic acid was somewhat longer than that of cholic acid in both cases, and the turnover was calculated to be 1.5 and 2 times that of cholic acid. The same ratio should be found between the amount of chenodeoxycholic acid metabolites and the amount of cholic acid metabolites in feces. The ratios between these amounts, estimated from gas chromatograms of fecal extracts, were 1.5 and 1.4 in the two cases.

Gas chromatography of partially purified fecal extracts showed the presence of six main components with retention times of known bile acid methyl esters (cf. Fig. 2). These components were present in similar proportions in the four extracts analyzed in this study. There is no indication of preferential losses of bile acids with the method used for extraction and purification and the same six main components have subsequently been found in feces from a number of subjects. It was possible to isolate and identify the following six bile acids corresponding to the six main peaks seen in the gas chromatograms (cf. Fig. 2): isolithocholic acid; lithocholic acid; 3β,12α-dihydroxy-5β-cholanic acid; deoxycholic acid; 3β-hydroxy-12-keto-5β-cholanic acid; and 12-ketolithocholic acid. (Excluding deoxycholic acid, all acids were isolated and identified as their methyl esters.) Previously, 3β-hydroxy-12-keto-5β-cholanic acid has not been found to occur naturally, and the presence of 3β,12α-dihydroxy-5β-cholanic acid in human feces has not been described earlier. Evidence for the presence of small amounts of other bile acids was obtained during the different chromatographic procedures. Thus, material with the properties of chenodeoxycholic acid was found in all four extracts (cf. Fig. 2). Furthermore, isolithocholic acid originates from chenodeoxycholic acid, most likely by way of lithocholic acid and 3-keto-5β-cholanic acid; 3β,12α-dihydroxy-5β-cholanic acid; 3β-hydroxy-12-keto-5β-cholanic acid; and 12-ketolithocholic acid. The presence of small amounts of tritium in the isolated isolithocholic and litho-
cholic acids and of C\textsuperscript{14} in the cholic acid metabolites cannot be explained from present knowledge of the metabolism of these acids and may possibly be ascribed to experimental error (cf. above under "Results"). In a recent experiment, the lithocholic and isolithocholic acids isolated from feces of a subject given 24-C\textsuperscript{14}-cholic acid were found to be unlabelled. Further work is needed, however, to clarify these points.

Previous work has shown that after feeding cholesterol, it takes 5 to 15 days (9, 10) until cholic and deoxycholic acid reach the same specific activity. If a strict product-precursor relationship exists, the specific activity of deoxycholic acid should then be higher than that of cholic acid. By the same reasoning, any further metabolites would have varying specific activities. Since chenodeoxycholic acid is the precursor of lithocholic acid (2), a similar relationship should exist between these acids. It was therefore of interest to compare the specific activities of the different fecal bile acids with each other and with those in the bile. Since the feces were collected in 5-day portions, no detailed kinetic analysis of the relationships between the different metabolites can be obtained. However, some general observations can be made regarding the interrelationships of the different bile acids in bile and feces. The determination of the specific activities of some fecal bile acid samples may be slightly incorrect owing to the presence of small amounts of impurities, but the magnitude of these errors is not such that it can invalidate the general conclusions that can be drawn from the present experiments. The mean specific activity of the biliary acids during each 5-day period was calculated from their specific activity time curves. These data are given in Fig. 3.

The relation between cholic and deoxycholic acids as well as that between chenodeoxycholic acid are such as would be expected from previous knowledge of the metabolic relations of these acids, i.e. the precursor with a higher specific activity in the first period and a lower specific activity in the second period. Deoxycholic acid and its further metabolites in the feces have almost the same specific activity and the same holds true for lithocholic and isolithocholic acids. In all periods but T\textsubscript{1} the specific activity of deoxycholic acid in the feces was about the same as the average in the bile.

**SUMMARY**

The turnover of cholic and chenodeoxycholic acid has been determined simultaneously in two human subjects by administration of tritium-labeled cholic and C\textsuperscript{14}-labeled chenodeoxycholic acid. In both cases, the daily production of chenodeoxycholic acid was somewhat larger than that of cholic acid.

The main bile acids in feces were isolithocholic, lithocholic, 3β-hydroxy-12-keto-5β-cholanic, 12-ketolithocholic, 3β,12α-dihydroxy-5β-cholanic, and deoxycholic acid.

The specific radioactivity of the fecal bile acids was determined to establish their formation from either cholic or chenodeoxycholic acid. Lithocholic and isolithocholic acid were derived from chenodeoxycholic acid; deoxycholic, 3β-hydroxy-12-keto-5β-cholanic, 12-keto-lithocholic, and 3β,12α-dihydroxy-5β-cholanic acid from cholic acid.

The metabolites of cholic acid in feces had almost the same specific activity. This was also the case with the specific activities of isolithocholic and lithocholic acid. The specific activities of the primary bile acids in bile differed from those of their fecal metabolites.

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