Bile Acids and Steroids

LXXXIII. ON THE INTERCONVERSION OF CHOLIC AND DEOXYCHOLIC ACID IN THE RAT

SVEN LINDSTEDT* AND BENGT SAMUELSSON*

From the Department of Physiological Chemistry, University of Lund, Lund, Sweden

(Received for publication, March 4, 1959)

Cholic acid and chenodeoxycholic acid are the main bile acids in rat bile, occurring in the approximate proportion of 8:2 (1). The occurrence of minor amounts of metabolites of cholic and chenodeoxycholic acid, formed by the action of intestinal microorganisms and liver enzymes, has recently been reported (2, 3).

The transformation of labeled cholic acid during the enterohepatic circulation has been studied by Norman and Sjövall (2) who isolated labeled deoxycholic acid and 7-ketodeoxycholic acid from the feces. These two acids were evidently formed by the action of intestinal microorganisms on cholic acid. Furthermore, labeled 7-ketodeoxycholic acid was found to give rise to deoxycholic acid in the intestinal tract.

The deoxycholic acid formed in the intestine is absorbed and hydroxylated at the 7α-position to cholic acid (4). This hydroxylation reaction has so far not been found in other species and explains the relative absence (1 to 2 per cent) of deoxycholic acid from rat bile.

The mechanism of the elimination of the 7α-hydroxyl group has been studied in the rabbit (5). In this animal 7-ketodeoxycholic acid was only of minor importance as an intermediate, and explains the relative absence (1 to 2 per cent) of deoxycholic acid from rat bile.

The present work was carried out in order to study the reaction mechanisms in the sequence

\[
\text{Cholic acid} \rightarrow \text{deoxycholic acid} \rightarrow \text{cholic acid}
\]

and to obtain some information of the relative speed of these reactions.

EXPERIMENTAL

Cholic Acid-7β-H3, 24-C14—The doubly labeled cholic acid was prepared as described in the preceding paper (5), and a stock solution was made containing approximately 0.5 μc. of 7β-H3 and 0.3 μc. of 24-C14 per mg. of cholic acid.

Animal Experiments—White male rats, 200 to 250 gm., of the institute stock were used. The diet consisted mainly of bread and oats. Cannulation of the bile duct was performed as described earlier (6).

In some experiments the action of the intestinal microorganisms on cholic acid was studied, and the effect of the liver enzymes on the metabolites formed had to be excluded. The acid as sodium salt in water solution was therefore injected with a fine needle into the cecum of the rat, immediately after a bile fistula had been made. The feces were collected in ethanol.

The germ-free rats used were reared under germ-free conditions according to the technique of Gustafsson (7). The sodium salt of cholic acid-7β-H3, 24-C14 in water solution was autoclaved at 120° for 20 minutes and given orally to the animals. A bile fistula was made after 7 days and the bile collected during the following 2 hours was used.

Isolation and Chromatographic Separation—The bile and feces were collected, hydrolyzed, extracted, and chromatographed as described earlier (8, 9). The following phase systems were used:

<table>
<thead>
<tr>
<th>System</th>
<th>Moving phase</th>
<th>Stationary phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>150 ml. of methanol</td>
<td>15 ml. of isooctanol</td>
</tr>
<tr>
<td>F</td>
<td>165 ml. of methanol</td>
<td>45 ml. of chloroform</td>
</tr>
<tr>
<td></td>
<td>150 ml. of distilled water</td>
<td>15 ml. of chloroform</td>
</tr>
<tr>
<td></td>
<td>150 ml. of distilled water</td>
<td>5 ml. of heptane</td>
</tr>
</tbody>
</table>

Four ml. of the stationary phase were used per 4.5 gm. of hydrophobic Super-Cel. All the chromatograms were run at a constant temperature of +23°.

Isotope Determination—Tritium and C14 activity in the doubly labeled acids administered and in the isolated bile acids were determined by gas phase counting as described previously (5).

RESULTS

Loss of 7β-H3 in Cholic Acid-7β-H3, 24-C14 during Enterohepatic Circulation—Cholic acid-7β-H3, 24-C14, 1 mg., was injected intraperitoneally into rats, and after different time intervals (0 to 7 days) a bile fistula was made. The bile was collected for 2 hours and the cholic acid isolated by chromatography with phase system C. Determinations of the ratio of isotopes in the administered and isolated cholic acids were made, and the amount of tritium retained was calculated. From the results shown in Table I it is obvious that a progressive loss of the tritium label occurs with time as the acid takes part in the enterohepatic circulation.

In order to prove that there is no loss of the 7β-H3 label in cholic acid during the liver passage, the doubly labeled acid was administered to two germ-free rats and allowed to take part in the enterohepatic circulation for 7 days, at the end of which time the tritium retention in the normal rat was less than 5 per cent. As seen in Table III, the ratio of tritium to C14 remains constant in the cholic acid in the germ-free rats.

Retention of 7β-H3 in Intestinal Formation of Deoxycholic Acid from Cholic Acid-7β-H3, 24-C14—In order to determine the reten-
tion of the 7β-H³ in the step, cholic acid → deoxycholic acid, the rehydroxylation of deoxycholic acid in the liver had to be excluded. This was accomplished by injecting the doubly labeled cholic acid into the cecum of bile fistula rats. The rats were

| TABLE I |
| Determination of H³ and C¹⁴ in administered cholic acid and in cholic acid isolated from rats after different times of enterohepatic circulation |

<table>
<thead>
<tr>
<th>Cholic acid</th>
<th>Duration of enterohepatic circulation</th>
<th>C¹⁴</th>
<th>H³</th>
<th>H³/C¹⁴</th>
<th>HP retained</th>
</tr>
</thead>
<tbody>
<tr>
<td>Administered</td>
<td>days</td>
<td>c.p.m./mg</td>
<td>c.p.m./mg</td>
<td>Mean</td>
<td>Mean</td>
</tr>
<tr>
<td>0</td>
<td>208</td>
<td>293</td>
<td>75</td>
<td>302</td>
<td>100</td>
</tr>
<tr>
<td>1</td>
<td>280</td>
<td>285</td>
<td>233</td>
<td>227</td>
<td>0.80</td>
</tr>
<tr>
<td>2</td>
<td>318</td>
<td>316</td>
<td>283</td>
<td>269</td>
<td>0.85</td>
</tr>
<tr>
<td>4</td>
<td>500</td>
<td>503</td>
<td>112</td>
<td>108</td>
<td>0.18</td>
</tr>
<tr>
<td>7</td>
<td>500</td>
<td>503</td>
<td>112</td>
<td>108</td>
<td>0.18</td>
</tr>
<tr>
<td>Isolated from rats with intact enterohepatic circulation</td>
<td>2</td>
<td>318</td>
<td>316</td>
<td>283</td>
<td>269</td>
</tr>
<tr>
<td>4</td>
<td>500</td>
<td>503</td>
<td>112</td>
<td>108</td>
<td>0.18</td>
</tr>
<tr>
<td>7</td>
<td>500</td>
<td>503</td>
<td>112</td>
<td>108</td>
<td>0.18</td>
</tr>
<tr>
<td>Isolated from germ-free rats with intact enterohepatic circulation</td>
<td>2</td>
<td>318</td>
<td>316</td>
<td>283</td>
<td>269</td>
</tr>
<tr>
<td>4</td>
<td>500</td>
<td>503</td>
<td>112</td>
<td>108</td>
<td>0.18</td>
</tr>
</tbody>
</table>

The results from the determinations of H³ and C¹⁴ in this compound (Table II) showed that about 85 per cent of the original tritium label is retained.

Loss of Tritium Label in Deoxycholic Acid-7-H³, 24-C¹⁴ during 7α-Hydroxylation to Cholic Acid—The deoxycholic acid which had been isolated from the feces after injection of doubly labeled cholic acid into the cecum was administered to two rats with a functioning bile fistula. About 50 per cent of the administered deoxycholic acid was 7α-hydroxylated to cholic acid, which was isolated by chromatography with phase system C and crystallized from ethyl acetate after dilution with inactive cholic acid. Determinations of the H³ and C¹⁴ content in this acid (Table III) showed that almost all of the tritium label was lost during the hydroxylation in the liver.

| TABLE II |
| Determination of H³ and C¹⁴ in deoxycholic acid in feces |

<table>
<thead>
<tr>
<th>Cholic acid</th>
<th>C¹⁴</th>
<th>H³</th>
<th>HP/C¹⁴</th>
<th>H³ retained</th>
</tr>
</thead>
<tbody>
<tr>
<td>Administered cholic acid</td>
<td>1.10</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deoxycholic acid isolated from feces</td>
<td>0.98</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat XVII</td>
<td>315</td>
<td>17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat XVIII</td>
<td>320</td>
<td>13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat XIX</td>
<td>286</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat XX</td>
<td>285</td>
<td>11</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DISCUSSION

Reaction Mechanism—The experiments in which deoxycholic acid was isolated from feces and readministered clearly showed that only a small loss of the 7β-hydrogen occurs in the conversion of cholic acid into deoxycholic acid and suggest that a shift from the 7β- to the 7α-position takes place. These results therefore confirm the findings obtained in the preceding paper (5), in which the reaction has been discussed.

Rate of Formation of Deoxycholic Acid—The fact that cholic acid and deoxycholic acid are converted into each other should obviously be taken into account in studies of bile acid turnover in the rat. Such studies have been carried out with the use of cholic acid-24 C¹⁴ at a time when it was assumed that the isotope did not re-enter the cholic acid pool (10). We have now tried to devise a model that is more suitable for discussions of the quantitative aspects of the cholic-deoxycholic acid metabolism in the rat and which could make it possible to estimate the amount of deoxycholic acid formed per day.
With the use of this model the following general set of equations\(^1\) may be worked out. For tritium:

\[
\begin{align*}
\frac{dA}{dt} &= -K_{AB}A + K_{BA}B \\
\frac{dB}{dt} &= K_{AB}A - (K_{BA} + K_{BC} + K_{BE})B \\
\frac{dC}{dt} &= K_{BC}B - (K_{CA} + K_{CE})C
\end{align*}
\]

For C\(^{14}\):

\[
\begin{align*}
\frac{dA}{dt} &= -K_{AB}A + K_{BA}B + K_{CA}C \\
\frac{dB}{dt} &= K_{AB}A - (K_{BA} + K_{BC} + K_{BE})B \\
\frac{dC}{dt} &= K_{BC}B - (K_{CA} + K_{CE})C
\end{align*}
\]

From studies with labeled cholic acid (2) it is known that the conversion to bacterial metabolites starts in the large intestine, i.e., the cecum, so it seems justified to consider the cholic acid as being divided between two compartments. The distribution of the bile acids in these compartments shows large individual variations owing to differences in nutritional status of the animals and to the nature of the intestinal microorganisms. In eight animals Norman and Sjövall (2) found 20 to 52 per cent of the total radioactivity in the large intestine after feeding of labeled cholic acid. In the calculations below it is assumed that 30 per cent of the total bile acids is located in the large intestine.

The proportion between cholic acid and deoxycholic acid in the large intestine also shows large variations. From chromatograms of the fecal bile acids collected in this study we have estimated the cholic and deoxycholic acid pools in the large intestine to be of about equal size, and in this simplified system no corrections have been made for the other bile acids in the large intestine, consisting of 7-ketodeoxycholic acid, 3α, 7β, 12α-trihydroxycholic acid, 12-ketolithocholic acid, and very small amounts of unidentified microbial metabolites. The deoxycholic acid formation occurs to a small extent with an intermediate formation of 7-ketodeoxycholic acid, a fact that has no influence on the validity of these calculations. According to preliminary results obtained on germ-free rats by Norman and Sjövall,\(^2\) small amounts of cholic acid and 3α, 7β, 12α-trihydroxycholic acid may be formed from 7-ketodeoxycholic acid during the enterohepatic circulation, whereas no reduction of the 7-keto acid can be observed after one passage through the liver in a bile fistula rat. However, this reconversion of 7-ketodeoxycholic acid into cholic acid is too small to affect these calculations significantly.

To solve the kinetic equations one should ideally follow the specific activities of the labeled bile acids in the different pools; this however, does not seem possible for practical reasons. To get an approximation we have therefore assumed that the bile acids are excreted and reabsorbed from the large intestine in proportion to their concentration (\(K_{BE} = K_{CE}; K_{BA} = K_{EA}\)). If a tracer dose of cholic acid 7-β-H\(^3\), 24-C\(^{14}\) is introduced into pool A, the following expression is obtained for the ratio between H\(^3\) and C\(^{14}\) of cholic acid in pool A. (The derivation of this equation is shown in the appendix on page 2029.)

\[
\frac{[\text{H}^3]}{[\text{C}^{14}]} = \frac{I_{A1}(\lambda_1 - \lambda_2)(\mu_1 + 2K_{BC})}{C_{A1}(\mu_1 - \mu_2)(\lambda_1 + \lambda_2)}
\]

The straight lines which represent Equation 42 have been drawn to fit the relative minimal and maximal values for the H\(^3\) retention (Fig. 1) \(\mu_1 = -0.55, K_{AB} = 0.8, K_{BC} = 1.8, K_{BA} = 0.9, K_{BE} = 0.9\), and \(\mu_1 = -1.2, K_{AB} = 2.2, K_{BC} = 5.2, K_{BA} = 4.3, K_{BE} = 0.9\), which means that in this series from 32 to 92 per cent of the total amount of cholic acid in the body is converted into deoxycholic acid per day. The parameter for Equation 42, calculated by the method of least squares, gives \(\mu_1 = -0.75, K_{AB} = 1.24, K_{BC} = 2.9, K_{BA} = 2.0\), and \(K_{BE} = 0.9\); that is, 51 per cent of the total amount of cholic acid is converted into deoxycholic acid per day.

On the basis of the rate constants obtained in this work (calculated from the line with \(\mu_1 = -0.75\), Fig. 1) and the pool sizes, which are known from earlier investigations, some very approximate quantitative information on the enterohepatic circulation of the bile acids in the rat may be obtained. The rate of formation of bile acids in the liver has been found by Bergström and Danielsson (11) to be regulated by the amount of bile acids that reach the liver via the portal blood. A supply of 120 mg. of sodium taurochenodeoxycholate to the liver per day in a 200-gm. bile fistula rat inhibited the cholic acid synthesis

---

\(^1\) The equations are numbered according to their appearance in the Appendix at the end of the article.

\(^2\) A. Norman and J. Sjövall, to be published.
(40 mg. per day) to a level corresponding to that found for the intact animal (about 4 mg. per day). A similar figure for the supply of bile acids to the liver in the rat (14 mg. of cholic acid per day) was obtained by Olivecrona and Sjöwall,3 based on determinations of the bile acid concentration in the portal blood and correlation of these values with the portal blood flow.

If the cholic acid pool in the small intestine, liver, and bile ducts circulates 12 times per day8) about 10 per cent (KAB : A + 12A) of this pool is lost to the large intestine in each circulation. Of the total amount of cholic acid that is transported to the large intestine per day (KAB : A) of which 50 per cent daily is converted into deoxycholic acid (KBC : B), 30 per cent is excreted in the form of various microbial transformation products per day (KBB : B) + (KCB : C), and 70 per cent is absorbed from the large intestine and transported to the liver in the form of cholic acid, deoxycholic acid, and their metabolites (KBA : B) + (KCA : C). If the total cholic acid pool (A + B) in a 200 gm. rat amounts to approximately 12 mg.4 (in all these calculations chenodeoxycholic acid and its metabolites are disregarded since they constitute only a minor component of the bile acids present in the rat (12)) about 120 mg. of cholic acid are absorbed from the small intestine, and 12.2 mg. are transported to the large intestine per day. From this site about 8.4 mg. of bile acids are absorbed and 3.8 mg. are excreted in the feces per day.

SUMMARY

Cholic acid-7β-H3, 24-C14 has been administered to rats and the amount of H3 and C14 determined in the isolated cholic acid after different time intervals. Deoxycholic acid is formed from cholic acid in the large intestine during the enterohepatic circulation by the action of the intestinal microorganisms, but is subsequently 7α-hydroxylated to cholic acid in the liver. With the aid of cholic acid-7β-H3, 24-C14 the reaction mechanism for the deoxycholic acid formation in the intestine has been studied, and the localization of the hydrogen isotope in the isolated deoxycholic acid has been determined through hydroxylation to cholic acid. Taking advantage of the fact that the tritium label in the isolated deoxycholic acid is specifically lost in the hydroxylation reaction, it has been possible to construct a model, by which the amount of deoxycholic acid formed during the enterohepatic circulation may be determined. Furthermore, some approximate quantitative information has been obtained for the enterohepatic circulation of the bile acids. The following results were obtained.

1. The tritium label in cholic acid-7β-H3, 24-C14 was almost completely retained in the molecule during the conversion to deoxycholic acid by the microorganisms in the large intestine.

2. The tritium label in the isolated deoxycholic acid was lost in the 7α-hydroxylation to cholic acid in the liver; this suggests a shift of the H3-label from the 7β- to the 7α-position.

3. With the proposed model for the metabolism of cholic acid in the rat, it was found that about 50 per cent of the total cholic acid pool is converted into deoxycholic acid per day during the enterohepatic circulation; the greater part of this acid is subsequently 7α-hydroxylated to cholic acid in the liver.

4. Approximate values for the continuous loss of bile acids from the bile acid pool present in the small intestine, liver, and bile ducts to the pool in the large intestine are given. The rate of absorption of bile acids from the large intestine in the intact rat was found to be much slower than that reported for the absorption process in the small intestine, and figures for the total amount of bile acids that are absorbed from these two sites are given.

Acknowledgments—We are very grateful to Dr. B. Gustafsson, Department of Histology, Lund, who gave us the opportunity to carry out experiments with the germ-free animals. The technical assistance of Miss Irene Lindell and Mr. Sven Jönsson is gratefully acknowledged. This work is part of investigations supported by the National Institutes of Health, United States Public Health Service (Grant H 2842) and by Statens Medicinska Forskningsråd, Sweden.

APPENDIX

The following equations may be derived from the model on page 2028.

For tritium:

\[
dA/dt = -K_{AB}A + K_{BA}B \tag{1}
\]

\[
dB/dt = K_{AB}A - (K_{BA} + K_{BC} + K_{BE})B \tag{2}
\]

\[
dC/dt = K_{BC}B - (K_{CA} + K_{CB})C \tag{3}
\]

\[
A = x \quad B = y, \quad dx/dt = x', \quad dy/dt = y' \quad y' = K_{BC}z + K_{BA}y \tag{7}
\]

\[
y' = K_{BC}z - 2 K_{BC}y \tag{8}
\]

\[
x' = y' = K_{BA}y - 2 K_{BC}y \tag{9}
\]

\[
y'' = K_{BC}x' - 2 K_{BC}y' \tag{10}
\]

The roots (μ1 and μ2) are:

\[
μ_1, μ_2 = -\frac{1}{2} K_{BC} ± \frac{1}{4} \sqrt{121 K_{BC}^2 + 84 K_{BA} K_{BC}} \tag{12}
\]

and the solution of Equation 11 is:

\[
y = L_1e^{μ_1t} + L_2e^{μ_2t} \tag{13}
\]

\[
t = 0, \quad y = 0 \quad \text{and} \quad L_1 = -L_2 = L \tag{14}
\]

\[
y' = L(μ_1e^{μ_1t} - μ_2e^{μ_2t}) \tag{15}
\]

If the value of y in Equation 14 is substituted for y in Equation 8 then:

\[
y' = \frac{1}{2} K_{BC}z - 2 K_{BC}L(e^{μ_1t} - e^{μ_2t}) \tag{16}
\]

\[
L(μ_1e^{μ_1t} - μ_2e^{μ_2t}) = \frac{1}{2} K_{BC}z - 2 K_{BC}L(e^{μ_1t} - e^{μ_2t}) \tag{17}
\]

at \( t = 0, L = \frac{1}{2} K_{BC} \quad x_0 = \frac{x_0}{μ_1 - μ_2} \tag{18}
\]

\[
x = \frac{x_0}{μ_1 - μ_2} [(μ_1 + 2 K_{BC})e^{μ_1t} - (μ_2 + 2 K_{BC})e^{μ_2t}] \tag{19}
\]

* T. Olivecrona and J. Sjöwall, to be published.

* S. Eriksson, to be published.
For C: 
\[
\begin{align*}
    \frac{dA}{dt} &= -K_{AB}A + K_{BA}B + K_{CA}C \\
    \frac{dB}{dt} &= K_{AB}A - (K_{BA} + K_{BC} + K_{BR})B \\
    \frac{dC}{dt} &= K_{BC}B - (K_{CA} + K_{CE})C \\
    K_{BB} &= K_{CB}, K_{BA} = K_{CA}, \text{ and } K_{BC} = K_{CA} + K_{CB}
\end{align*}
\]

\(A = 70\) per cent, \(B = 15\) per cent and \(C = 15\) per cent (see the text).

\[
\begin{align*}
    \frac{dA}{dt} &= -K_{BC}A + K_{BA}(B + C) \\
    \frac{dB}{dt} &= K_{BC}A - 2K_{BC}B \\
    \frac{dC}{dt} &= K_{BC}B - K_{BC}C \\
    \frac{d(B + C)}{dt} &= K_{BC}A - K_{BC}(B + C)
\end{align*}
\]

The solution of Equation 32 is:
\[
y = L(e^{\lambda_1 t} - e^{\lambda_2 t})
\]

where \(\lambda_1\) is known from earlier investigations to be equal to \(-0.30\) (10), \(\mu_1\) is chosen to fit the experimental data. The different \(K\) values as well as \(\lambda_2\) and \(\mu_2\) are then obtained from Equations 12 and 33.

For \(\mu_1 = -0.75\) and \(\lambda_1 = -0.30, \mu_2\) is \(-6.25, \lambda_2 = -3.82\) and \(K_{BC} = 2.88\). When these values are used in Equations (19) and (40) it appears that the second term within the brackets may be neglected already after one day. The ratio between \(H^2\) and \(C^1\) is then:
\[
\frac{H_{A4}}{C_{A4}} = \frac{H_{A4} (\mu_1 - \mu_2) ([\mu_1 + 2 K_{BC}] e^{\mu_1 t} - (\mu_1 + 2 K_{BC}) e^{\mu_2 t})}{C_{A4} (\mu_1 - \mu_2) ([\lambda_1 + K_{BC}] e^{\lambda_1 t} - (\lambda_1 + K_{BC}) e^{\lambda_2 t})}
\]

On a semilogarithmic paper, the straight line, which represents this simplified equation, intersects the ordinate at a value of \(H^2\)-retention that is higher than 100 per cent.

REFERENCES
