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).

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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.

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tion of the 7α-H^3 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 killed after one and a half days and deoxycholic acid was isolated from the feces by chromatography with phase system F.

The band of radioactive deoxycholic acid was diluted with inactive deoxycholic acid and crystallized from acetic acid-water. The specific activity of successive samples remained constant.

The results from the determinations of H^3 and C^14 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-H^3, 24-C^14 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 H^3 and C^14 content in this acid (Table III) showed that almost all of the tritium label was lost during the hydroxylation in the liver.

TABLE I

<table>
<thead>
<tr>
<th>Duration of enterohepatic circulation</th>
<th>C^14 (cpm/mg)</th>
<th>H^3 (cpm/mg)</th>
<th>H^3/C^14</th>
<th>Per cent (per cent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Administered</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isolated from rats with preformed bile fistula</td>
<td>424</td>
<td>460</td>
<td>1.11</td>
<td>100</td>
</tr>
<tr>
<td>Isolated from rats with intact enterohepatic circulation</td>
<td>318</td>
<td>286</td>
<td>0.85</td>
<td>77</td>
</tr>
<tr>
<td>Isolated from germ-free rats with intact enterohepatic circulation</td>
<td>314</td>
<td>256</td>
<td>0.80</td>
<td>72</td>
</tr>
</tbody>
</table>

TABLE II

<table>
<thead>
<tr>
<th>Duration of enterohepatic circulation</th>
<th>C^14 (cpm/mg)</th>
<th>H^3 (cpm/mg)</th>
<th>H^3/C^14</th>
<th>Per cent (per cent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Administered cholic acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deoxycholic acid isolated from feces</td>
<td>297</td>
<td>294</td>
<td>1.10</td>
<td>100</td>
</tr>
<tr>
<td>Rat XVII</td>
<td>304</td>
<td>301</td>
<td>0.98</td>
<td>89</td>
</tr>
<tr>
<td>Rat XVIII</td>
<td>236</td>
<td>221</td>
<td>0.05</td>
<td>4.8</td>
</tr>
</tbody>
</table>

TABLE III

<table>
<thead>
<tr>
<th>Determination of H^3 and C^14 in cholic acid after administration of deoxycholic acid-7α-H^3-24-C^14</th>
</tr>
</thead>
<tbody>
<tr>
<td>C^14 (cpm/mg)</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>Administered deoxycholic acid, Rat XVII</td>
</tr>
<tr>
<td>Cholic acid isolated from bile Rat XIX</td>
</tr>
<tr>
<td>Rat XX</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 7α-hydrogen occurs in the conversion of cholic acid into deoxycholic acid and suggest that a shift from the 7α- to the 7β-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 C^14 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.
CHOLIC ACID
Small intestine, liver
bile ducts

K_{AB} \quad K_{BA}

COECUM

K_{BC}

CHOLIC ACID

DEOXYCHOLIC ACID

COECUM

K_{BE} \quad K_{CE}

EXCRETED

With the use of this model the following general set of equations\(^1\) may be worked out. For tritium:

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

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

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

For C\(^14\):

\[
\frac{dA}{dt} = - K_{AB}A + K_{BA}B + K_{CA}C \tag{20}
\]

\[
\frac{dB}{dt} = K_{AB}A - (K_{BA} + K_{BC} + K_{BE})B \tag{21}
\]

\[
\frac{dC}{dt} = K_{BC}B - (K_{CA} + K_{CE})C \tag{22}
\]

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 Sjöqvall (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, 3\(\alpha\), 7\(\beta\) and 12\(\alpha\)-tri-hydroxycholanic acid, 12-keto-cholesterolic 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 Sjöqvall, small amounts of cholic acid and 3\(\alpha\), 7\(\beta\), 12\(\alpha\)-trihydroxycholanic 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 \((K_{BE} = K_{CE}; K_{BA} = K_{CA})\). If a tracer dose of cholic acid 7-\(\beta\)-H\(^3\), 24-C\(^14\) is introduced into pool A at zero time, 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.)

\[
\frac{H^3}{C^{14}} = \frac{IP_{A1} (\mu_1 - \lambda_1)(\mu_1 + 2K_{BC})}{IP_{A2} (\mu_1 - \mu_2)(\lambda_1 + \lambda_2)} \tag{42}
\]

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) \(\mu_1 = -0.55, K_{AB} = 0.8, K_{BC} = 1.8, K_{BE} = 0.9, K_{BB} = 0.9\), and \(\mu_1 = -1.2, K_{AB} = 2.2, K_{BC} = 5.2, K_{BE} = 4.3, K_{BB} = 0.9\); 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 \(\mu_1 = -0.75\), \(K_{AB} = 1.24, K_{BC} = 2.9, K_{BE} = 2.0\), and \(K_{BB} = 0.9\); that is, \(\delta 1\) 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 approximative 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 taurocholate 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. Sjöqvall, 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 Sjövall.

The following results were obtained.

If the cholic acid pool in the small intestine, liver, and bile ducts circulates 12 times per day\(^{3}\) about 10 per cent \((K_{AB/A}/12)\cdot 100\) 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 \((K_{AB/A})\) of which 50 per cent daily is converted into deoxycholic acid \((K_{BC/B})\), 30 per cent is excreted in the form of various microbial transformation products per day \((K_{BC/B} + (K_{CB/C})\)) 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 \((K_{BA/B}) + (K_{CA/C})\). 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-\(7\beta\)-H\(^3\), \(24\)-C\(^{14}\) has been administered to rats and the amount of \(H^3\) and \(C^{14}\) 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 \(7\alpha\)-hydroxylated to cholic acid in the liver. With the aid of cholic acid-\(7\beta\)-H\(^3\), \(24\)-C\(^{14}\) 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 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-\(7\beta\)-H\(^3\), \(24\)-C\(^{14}\) 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 \(7\alpha\)-hydroxylation to cholic acid in the liver; this suggests a shift of the \(H^3\)-label from the \(7\beta\) to the \(7\alpha\)-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 \(7\alpha\)-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 Jonsson 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 2026.

For tritium:

\[
\frac{dA}{dt} = -K_{BA}A + K_{AB}B
\]

\[
\frac{dB}{dt} = K_{BA}A - (K_{CA} + K_{BC} + K_{BA})B
\]

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

\[
K_{AB} = K_{CA}; \quad K_{BA} = K_{CA}; \quad K_{BC} = K_{CA} + K_{CB}
\]

\[A \cdot 70\text{ per cent}, B \cdot 15\text{ per cent, and } C \cdot 15\text{ per cent (for explanation see the text).}
\]

\[
\frac{dA}{dt} = -K_{BA}A + K_{AB}B
\]

\[
\frac{dB}{dt} = K_{BA}A - 2K_{BC}B
\]

\[
\frac{dC}{dt} = K_{BC}B - K_{BC}C
\]

\[A = x \text{ and } B = y, \frac{dz}{dt} = x', \frac{dx'}{dt} = x'', \frac{dy}{dt} = y', \frac{dy'}{dt} = y''.
\]

\[
x' = -\frac{1}{2}K_{BC}z + K_{BA}y
\]

\[
y' = \frac{1}{2}K_{BC}z - 2K_{BC}y
\]

\[x'' + y'' = K_{BC}z - 2K_{BC}y
\]

\[
y'' = -\frac{3}{2}K_{BC}z - 2K_{BC}y
\]

\[y'' + K_{BC}(z'' - 2K_{BC}y' = 0
\]

The roots \((\mu_1 \text{ and } \mu_2)\) are:

\[
\mu_1 \text{ and } \mu_2 = -\frac{1}{4}K_{BC} \pm \frac{1}{4} \sqrt{12K_{BC} + 8K_{BA}K_{BC}}
\]

and the solution of Equation 11 is:

\[
y = L(e^{\mu_1 t} + L(e^{\mu_2 t})
\]

\[t = 0, y = 0 \text{ and } L = -L = L
\]

\[y = L(e^{\mu_1 t} - e^{\mu_2 t})
\]

\[y' = L(\mu_1 e^{\mu_1 t} - \mu_2 e^{\mu_2 t})
\]

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

\[L(\mu_1 e^{\mu_1 t} - \mu_2 e^{\mu_2 t}) = \frac{3}{2}K_{BC}z - 2K_{BC}L(e^{\mu_1 t} - e^{\mu_2 t})
\]

\[L(\mu_1 e^{\mu_1 t} - \mu_2 e^{\mu_2 t}) = \frac{3}{2}K_{BC}z - 2K_{BC}L(e^{\mu_1 t} - e^{\mu_2 t})
\]

\[x = \frac{3}{\mu_1 - \mu_2} [(\mu_1 + 2K_{BC})e^{\mu_1 t} - (\mu_2 + 2K_{BC})e^{\mu_2 t}]
\]

\[x = \frac{3}{\mu_1 - \mu_2} [(\mu_1 + 2K_{BC})e^{\mu_1 t} - (\mu_2 + 2K_{BC})e^{\mu_2 t}]
\]

\[x = \frac{3}{\mu_1 - \mu_2} [(\mu_1 + 2K_{BC})e^{\mu_1 t} - (\mu_2 + 2K_{BC})e^{\mu_2 t}]
\]

\[x = \frac{3}{\mu_1 - \mu_2} [(\mu_1 + 2K_{BC})e^{\mu_1 t} - (\mu_2 + 2K_{BC})e^{\mu_2 t}]
\]

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\]

\[x = \frac{3}{\mu_1 - \mu_2} [(\mu_1 + 2K_{BC})e^{\mu_1 t} - (\mu_2 + 2K_{BC})e^{\mu_2 t}]
\]

\[x = \frac{3}{\mu_1 - \mu_2} [(\mu_1 + 2K_{BC})e^{\mu_1 t} - (\mu_2 + 2K_{BC})e^{\mu_2 t}]
\]

\[x = \frac{3}{\mu_1 - \mu_2} [(\mu_1 + 2K_{BC})e^{\mu_1 t} - (\mu_2 + 2K_{BC})e^{\mu_2 t}]
\]

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\]

\[x = \frac{3}{\mu_1 - \mu_2} [(\mu_1 + 2K_{BC})e^{\mu_1 t} - (\mu_2 + 2K_{BC})e^{\mu_2 t}]
\]

\[x = \frac{3}{\mu_1 - \mu_2} [(\mu_1 + 2K_{BC})e^{\mu_1 t} - (\mu_2 + 2K_{BC})e^{\mu_2 t}]
\]
For C:
\[
\begin{align*}
\frac{dA}{dt} &= -K_{AB}A + K_{BC}B + K_{CA}C \\
\frac{dB}{dt} &= K_{AB}A - (K_{BA} + K_{BC} + K_{BB})B \\
\frac{dC}{dt} &= K_{BB}B - (K_{CB} + K_{CE})C \\
K_{BB} &= K_{CB}, K_{BA} = K_{CA}, \text{ and } K_{BC} = K_{CB} + K_{CE}
\end{align*}
\]

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

\[
\begin{align*}
\frac{dA}{dt} &= -\frac{1}{2}K_{BC}A + K_{BC}(B + C) \\
\frac{dB}{dt} &= \frac{1}{2}K_{BC}A - 2K_{BC}B \\
\frac{dC}{dt} &= K_{BC}B - K_{BC}C \\
\frac{d(B + C)}{dt} &= \frac{1}{2}K_{BC}A - K_{BC}(B + C)
\end{align*}
\]

\[A = x, B + C = y, \frac{dx}{dt} = x', \frac{dx'}{dt} = x'', \frac{dy}{dt} = y', \frac{dy'}{dt} = y''\]

\[x' = -\frac{1}{2}K_{BC}x + K_{BC}y \\
y' = \frac{1}{2}K_{BC}x - K_{BC}y \\
z' + y' = (K_{BA} - K_{BC})y \\
z' = (K_{BA} - K_{BC})\frac{y}{\lambda} \\
y'' - \frac{1}{2}K_{BC}z'' - K_{BC}y' \\
y'' + \frac{1}{2}K_{BC}z'' - \frac{1}{2}(K_{BA} - K_{BC})y = 0
\]

The roots (\(\lambda_1\) and \(\lambda_2\)) are:

\[\lambda_{1,2} = -\frac{1}{2}K_{BC} \pm \sqrt{\frac{1}{4}K_{BC}^2 + 21K_{BA}K_{BC}}\]

The solution of Equation 32 is:

\[y = L_0e^{\lambda_1t} + L_0e^{\lambda_2t}\]
Bile Acids and Steroids: LXXXIII. ON THE INTERCONVERSION OF
CHOLIC AND DEOXYCHOLIC ACID IN THE RAT
Sven Lindstedt and Bengt Samuelsson