THE BIOLOGICAL CONVERSION OF CHOLESTEROL TO
CHOLIC ACID*

BY KONRAD BLOCH, BENJAMIN N. BERG, AND D. RITTENBERG

(From the Departments of Biochemistry and Surgery, College of Physicians and
Surgeons, Columbia University, New York)

(Received for publication, June 14, 1943)

It has recently been demonstrated (1) that acetate is utilized for the
in vivo synthesis of cholesterol, but the mechanism by which steroids are
formed by animal tissues is unknown. No evidence is available as to
whether bile acids and steroid hormones arise independently by a similar
condensation of small molecules or whether they are products of cholesterol
metabolism.

Of the three types of cyclopentenophenanthrene derivatives, cholesterol
is the most abundant in the animal organism and, in contrast to bile acids
and hormones, is found in all tissues and body fluids. Cholesterol is con-
tinuously being synthesized from small molecules and the total amount of
sterol in the animal body appears to remain rather constant under normal
dietary conditions. Data on the rate of bile acid production in the intact
animal are not available, as accurate determinations of the relative propor-
tions which are reabsorbed or excreted in the feces are not feasible. The
balance type of experiments could, therefore, hardly be expected to provide
an answer as to the biological relationship between sterols and bile acids.
Attempts to correlate cholesterol supply and bile acid production in ani-
mals with fistulas have been unsuccessful (2, 3) and experiments of this type
have never been carried out with intact animals.

It was thought that cholesterol labeled with deuterium should be of aid
in throwing light on the relationship between cholesterol and bile acids;
appearance of deuterio cholic acid after its administration would constitute
clear evidence of direct conversion. This substance, prepared by the
method described in the foregoing article (4), was administered to a dog
previously cholecystonephrostomized by the procedure of Kapsinow (5).
Since sterol absorption after oral administration was uncertain under the
experimental conditions, the deuterio cholesterol was given by intravenous
infusion.

Cholic acid isolated from urine collected during the experimental period
was analyzed for deuterium. After careful purification, the absence of
deuterio cholesterol as a contaminant was demonstrated by the “washing

* This work was carried out with the aid of a grant from the Josiah Macy, Jr.,
Foundation.
out” procedure (6), with ordinary cholesterol. It is evident from the data in Table I that bile acid had been formed from cholesterol.

3 days after the last administration of deuterio cholesterol, the dog was exsanguinated and cholesterol was isolated from the blood and various organs. The isotope concentrations found in these samples of cholesterol are given in Table II.

The feces were collected during the experimental period. Total sterols were isolated from 24 hour specimens. The isotope concentrations found in these samples are given in Table III.

**TABLE I**
Atom Per Cent Excess Deuterium in Cholic Acid and Bile Cholesterol after Intravenous Injection of Deuterio Cholesterol (4.16 Atom Per Cent Excess Deuterium)

<table>
<thead>
<tr>
<th>Sample No</th>
<th>Days after 1st injection</th>
<th>Cholic acid</th>
<th>Bile cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1, 2</td>
<td>0.17</td>
<td>0.13</td>
</tr>
<tr>
<td>II</td>
<td>4</td>
<td>0.24</td>
<td>0.50</td>
</tr>
<tr>
<td>III</td>
<td>5, 6</td>
<td>0.16</td>
<td>0.29</td>
</tr>
</tbody>
</table>

**TABLE II**
Atom Per Cent Excess Deuterium in Tissue Cholesterol after Intravenous Injection of Deuterio Cholesterol (4.16 Atom Per Cent Excess Deuterium)

<table>
<thead>
<tr>
<th>Organ</th>
<th>D₂ excess</th>
<th>Organ</th>
<th>D₂ excess</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red blood cells</td>
<td>0.31</td>
<td>Pancreas</td>
<td>0.25</td>
</tr>
<tr>
<td>Plasma</td>
<td>0.33</td>
<td>Adrenals</td>
<td>0.30</td>
</tr>
<tr>
<td>Liver</td>
<td>0.71</td>
<td>Omentum</td>
<td>0.20</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.31</td>
<td>Testis</td>
<td>0.15</td>
</tr>
<tr>
<td>Lungs</td>
<td>2.00</td>
<td>Brain</td>
<td>0.00</td>
</tr>
<tr>
<td>Heart</td>
<td>0.39</td>
<td>Spinal cord</td>
<td>0.00</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.46</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**TABLE III**
Atom Per Cent Excess Deuterium in Fecal Sterols Excreted after Intravenous Injection of Deuterio Cholesterol

<table>
<thead>
<tr>
<th>Days after 1st injection</th>
<th>Excess D₂ in fecal sterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.00</td>
</tr>
<tr>
<td>2</td>
<td>0.04</td>
</tr>
<tr>
<td>4</td>
<td>0.13</td>
</tr>
<tr>
<td>6</td>
<td>0.17</td>
</tr>
</tbody>
</table>
EXPERIMENTAL

In a male adult dog weighing 10 kilos an anastomosis of the gallbladder to the pelvis of the right kidney was established and the common duct doubly ligated and divided. The dog was kept on a diet of raw beef. 2 weeks following the operation, the animal received daily by intravenous infusion an emulsion of 1 gm. of deuterio cholesterol containing 4.16 per cent D₂ for 3 consecutive days. The ring system of this cholesterol contained 3.66 atom per cent excess D and the side chain 4.93 atom per cent excess D (4). 1 gm. of sterol and 1 gm. of lecithin were suspended in 100 ml. of 0.05 M phosphate buffer, pH 7.3, and emulsified with the aid of a Waring blendor. Homogeneous, filtrable emulsions were thus obtained. 100 ml. of emulsion were administered by infusion into the femoral vein in the course of 30 minutes. The dog was placed in a metabolism cage and kept for 3 days following the last injection, when it was killed. Urine and feces were collected during the experimental period.

Isolation of Cholic Acid—Urine of 48 hour periods were pooled, acidified with dilute sulfuric acid, and extracted continuously with ether for 24 hours. The ether extracts were brought to dryness and the residue was saponified by being heated in 20 per cent aqueous NaOH solution for 24 hours on the steam bath. After exhaustive extraction with ether to remove unsaponifiable material, the alkaline solution was acidified and the mixture of bile acids and fatty acids was taken up in ether. The ether was distilled off, the residue was dissolved in dilute ammonia, and saturated barium hydroxide solution added until no more precipitation occurred. The barium salts of desoxycholic acid and fatty acids were removed by filtration, and the cholic acid was extracted with ether from the acidified filtrate. The ethereal solution was dried and concentrated, when cholic acid crystallized out. In order to eliminate any possible contamination with deuterio cholesterol, the cholic acid was redissolved in ether and twice the weight of ordinary non-isotopic cholesterol was added. The ether solution was then extracted with dilute sodium carbonate and the alkaline solution acidified. The precipitated cholic acid was taken up in ether, and the ether solution was dried and brought to a small volume. Cholic acid crystallized from the solution.

Of the three cholic acid samples isolated from the dog urine, Samples I and III were treated in this manner, whereas Sample II was not “washed out.”

The analytical data for the cholic acid samples were:

Sample I—C 70.5, H 9.5, m.p. 198°
Sample II—M.p. 190°
Sample III—C 71.0, H 9.6, m.p. 199°
Calculated. C 70.6, H 9.8
The deuterium concentrations found in the cholic acids are listed in Table 1.

Isolation of Cholesterol from Urine — The ether extracts obtained from the alkaline hydrolysate of the initial urine extracts were brought to dryness, taken up in alcohol, and precipitated with digitonin. Sterol Samples I, II, and III were extracted from the same runs as cholic acid Samples I, II, III. Total amounts isolated from urine were, Sample I, 37 mg. of sterol; Sample II, 15 mg. of sterol; Sample III, 42 mg. of sterol. These samples are referred to as bile cholesterol and their deuterium concentrations are given in Table I.

Isolation of Fecal Sterols — The feces collected during a 24 hour period were dehydrated by two acetone extractions and then extracted with ether continuously for 24 hours. The ether and acetone extracts were saponified with 10 per cent alcoholic KOH and an aliquot of the unsaponifiable material precipitated with digitonin. For deuterium analysis the sterol digitonides were burnt. The isotope concentrations of the sterols were calculated from that of the sterol digitonides and are given in Table III.

Cholesterol from Tissue Constituents — The tissues were saponified with 10 per cent potassium hydroxide in ethanol and cholesterol obtained from the unsaponifiable fraction in the usual fashion. Cholesterol was isolated either as the digitonide or, when sufficient material was available, recrystallized as the free compound until the melting point reached 147°. The isotope analyses of the cholesterol samples are given in Table II.

DISCUSSION

The concentrations of deuterium present in all three samples of isolated bile acid are significant and of the same order of magnitude as those of blood and "bile" cholesterol. Specifically, the cholic acid excreted during the last 48 hours before the killing of the animal contained one-half as much deuterium as the cholesterol excreted simultaneously and also one-half as much as the blood cholesterol immediately before the death of the dog. In contrast, the liver sterol contained 4 times as much isotope as this particular sample of cholic acid.

A conversion of cholesterol to cholic acid would necessitate shortening of the sterol side chain by 3 carbon atoms, introduction of hydroxyl groups at carbon atoms 7 and 12, saturation of the Δ5,6 double bond, and epimerization of the hydroxyl group at carbon atom 3.

Assuming that in the administered deuterio cholesterol all the hydrogen atoms of the iso-octyl side chain have the same average isotope concentration, i.e. 4.93 per cent, and that all carbon-bound hydrogen in the nucleus

1 Since the quantities of urinary sterol excreted by normal animals are insignificant, the sterol appearing in the urine under our experimental conditions may be assumed to be of biliary origin. It is therefore referred to as bile cholesterol.
contained an average of 3.66 per cent deuterium (4), it will be possible to calculate the isotope concentration of cholic acid if formed from cholesterol. This value will obviously depend on the chemical reactions taking place in the transformation, but in any case the cholic acid will of necessity contain less deuterium than the cholesterol from which it is formed. A minimum of 5, and possibly 6, normal hydrogen atoms will be introduced into the nucleus in the course of the following reactions. (1) Oxygen atoms, with normal hydrogen, are introduced at positions 7, 12, and 24 respectively, i.e. 3 normal H atoms. (2) Saturation of the Δ5,6 double bond in cholesterol involves uptake of 2 normal hydrogen atoms. (3) If the epimerization of the hydroxyl group at carbon atom 3 were to involve the intermediary formation of a 3-keto compound, at least 1 isotopic hydrogen atom would be replaced by a normal one. On the other hand, epimerization may be the result of a Walden inversion which could occur without loss of deuterium. A cholic acid formed as the result of these reactions could retain 8 of the hydrogen atoms (with 4.93 atom per cent D) originally present in the cholesterol side chain and 27 hydrogen atoms of the original nucleus containing 3.66 atom per cent D2, while at least 5 hydrogen atoms were derived from the normal hydrogen of the organism. Its deuterium content will then be at most 83 per cent of that of the cholesterol from which it was formed. Thus the cholic acid excreted during the last 48 hours of the experiment could not have contained more than 0.24 per cent D if derived from the circulating cholesterol (i.e. that of plasma, red cells, and bile) and not more than 0.59 per cent D if the liver cholesterol had served as the immediate precursor. The cholic acid actually contained 0.16 per cent D, i.e. respectively about two-thirds and one-quarter of these values. These values were arrived at by correcting only for those isotopic hydrogen atoms which must have been lost by reactions (1) and (2) (above). Additional loss of deuterium could be visualized if the introduction of hydroxyl groups at carbon atoms 7 and 12 involved either desaturation and subsequent hydrogenation or intermediary formation of 7,12-keto compounds as precursors of cholic acid.

Cholesterol had evidently been deposited in the liver, since at the end of the experiment, 3 days after the last administration of deuterio cholesterol, the liver sterol still contained more than twice as much isotope as the circulating cholesterol. Thus the replacement of cholesterol present in the liver by newly formed cholesterol proceeds at a slow rate, indicating a low metabolic activity of the sterol deposited in the liver following the intravenous administration of deuterio compound. It cannot be decided

* Dilution of the bile acids newly formed by those originally present must have been insignificant, since bile had been excreted by the dog for 2 weeks before deuterio cholesterol was administered and ligation of the bile duct prevented reabsorption of bile acids.
whether the deposition of relatively inert liver cholesterol was due to the fact that cholesterol and not cholesterol ester was administered. The finding that the red blood cell cholesterol (which consists of free cholesterol only) and the plasma cholesterol (two-thirds of which is esterified) contain identical isotope concentrations seems to exclude this possibility.

The cholesterol excreted with the bile, having the same isotope value as that of the blood and only about 40 per cent of that of liver cholesterol, can hardly be of hepatic origin. The function of the liver with regard to bile cholesterol seems to be excretory only.

Similarly, the bile acids, though formed in the liver, could arise from the circulating cholesterol as the immediate precursor rather than from that stored in the liver. Our data on the relative isotope concentrations in circulating cholesterol and cholic acid do not belie such a concept, although they supply no proof for it.

The deuterium concentrations found in the cholesterol of various tissues of the animals represent the isotope content 3 days after the last administration of deuterio cholesterol. As can be seen from Table III, there is wide variation in the ability of different tissues to remove cholesterol from the circulating blood. The relative storage capacity is most pronounced in the lungs and liver. All other tissues, with the exception of brain and spinal cord, had incorporated cholesterol from the circulation, although to varying degrees. The absence of knowledge relating to the metabolic function of cholesterol precludes an interpretation of these data. However, the complete absence of deuterio cholesterol in brain and spinal cord is notable. It illustrates the lack or paucity of metabolic interchange between the sterol of the central nervous system and that of the blood. Slow rates of metabolism in brain have been observed previously by Waelsch, Sperry, and Stoyanoff (7), who demonstrated that cholesterol is synthesized very slowly, if at all, in the brain of adult rats. Cholesterol of the central nervous system seems to be the most inert of all tissue constituents which have as yet been studied; it is not regenerated and does not interchange with the dietary cholesterol at appreciable rates.

From the data in Table III it is evident that isotopic sterol had been secreted into the lumen of the intestine, although the experimental conditions prevented secretion of bile into the intestine. These findings confirm those of Sperry (8) who observed excretion of sterols in the feces of a dog with bile fistula kept on a sterol-free diet.

**SUMMARY**

1. Cholesterol containing 4.2 per cent deuterium was administered intravenously to a dog in which an anastomosis between the gallbladder and the pelvis of the kidney had been established.
2. Cholic acid isolated from the dog urine had an isotope concentration of the same order of magnitude as the blood or bile cholesterol, demonstrating the biological conversion of sterol to bile acid. Assuming that the circulating sterol provided the immediate precursor, it was calculated that a minimum of two-thirds of the cholic acid arose by degradation of cholesterol.

3. The distribution of the administered cholesterol was determined in various organs. The highest concentration was found in the lung, followed by the liver. All other organs, with the exception of the central nervous system, contain approximately the same concentrations as that of the blood. No deuterio cholesterol had been deposited in brain or spinal cord.

BIBLIOGRAPHY

THE BIOLOGICAL CONVERSION OF
CHOLESTEROL TO CHOLIC ACID
Konrad Bloch, Benjamin N. Berg and D. Rittenberg

J. Biol. Chem. 1943, 149:511-517.

Access the most updated version of this article at
http://www.jbc.org/content/149/2/511.citation

Alerts:
  • When this article is cited
  • When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at
http://www.jbc.org/content/149/2/511.citation.full.html#ref-list-1