ON THE METABOLISM OF $\Delta^{4,5}$-CHOLESTENONE*

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While it has been demonstrated that cholesterol is synthesized in animal tissues from molecules of small size (1), notably acetic acid (2), the search for compounds which might be intermediates in the acetate-sterol conversion has so far been unsuccessful. The possibility that cholesterol arises from $\Delta^{4,5}$-cholesteneone has been investigated by Anchel and Schoenheimer (3) with inconclusive results, since the labeled cholesteneone used in their experiments contained deuterium in labile positons only. On the other hand, good evidence exists that cholesteneone plays a rôle in reactions of cholesterol metabolism which lead to the formation of saturated sterols. The direct reduction of cholesterol could conceivably afford two of the epimeric dihydrosterols, namely coprosterol and dihydrocholesterol, but the change of steric configuration associated with the formation of epicoprosterol (4) requires an intermediate which lacks asymmetry at carbon atom 3. Moreover, in analogy to the chemical reduction of cholesterol, which leads to the formation of dihydrocholesterol but not of coprosterol, it has been suggested by various investigators that the fecal sterols do not arise directly from cholesterol but by way of the unsaturated ketone cholesteneone as the common intermediate. Cholesteneone has not been isolated from animal tissues, but Rosenheim and Webster (5) succeeded in isolating the compound in considerable quantities from feces of rats and dogs. With the aid of partially labeled cholesteneone Schoenheimer and collaborators have demonstrated its conversion into coprosterol in man and dog (6). These results, while strongly indicating that cholesterol is converted to fecal sterol by way of cholesteneone, failed to reveal whether these processes occurred in the tissues or whether they were the result of bacterial action in the intestinal tract. The belief that coprosterol formation takes place following secretion of cholesterol into the gut has been widely held (7) in spite of the fact that this conversion has not been unequivocally demonstrated with isolated intestinal contents.

In the present experiment the rôle of cholesteneone in cholesterol metabolism has been investigated further by feeding to rats labeled cholesteneone which contained deuterium uniformly distributed over the entire molecule.

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This compound was obtained as a by-product (8) of the platinum-catalyzed exchange reaction employed for the preparation of deuteriocholesterol (9). Administration of deuteriocholestenone to rats and subsequent isolation of the sterols from tissues and excreta gave the results shown in Table I. In accord with the findings of Schoenheimer and collaborators (3, 6) the fecal sterols following deuteriocholestenone feeding were found to contain high concentrations of deuterium, showing that in the rat also coprosterol may arise by way of the unsaturated ketone. The principal result of the present investigation is the appearance in the tissue of a sterol with a high isotope concentration. The digitonin-precipitable sterols from liver and carcass showed a significant deuterium concentration but were found on fractionation to consist of two components of widely different isotope content. Cholesterol,

### Table I

**Deuterium Concentrations in Sterols after Feeding Deuteriocholestenone Containing 5.65 Atom Per Cent Excess D**

The results are expressed in atom per cent excess D.

<table>
<thead>
<tr>
<th>Sterols isolated from</th>
<th>Experiment I</th>
<th>Experiment II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total sterols</td>
<td>Cholesterol</td>
</tr>
<tr>
<td>Blood</td>
<td>1.05</td>
<td>0.19</td>
</tr>
<tr>
<td>Liver</td>
<td>1.11, 1.14*</td>
<td>0.036, 0.048</td>
</tr>
<tr>
<td>Carcass</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin</td>
<td>0.72</td>
<td></td>
</tr>
<tr>
<td>Feces</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* After reprecipitation of the digitonide in the presence of 60 mg. of non-isotopic cholestenone, the isotope concentration was 1.08 per cent D.

which comprises the bulk of the mixture, was separated by way of the dibromides and was shown to contain small but significant deuterium concentrations. On the other hand, the saturated sterol, which was separated in one case from the bromination mixture as the digitonide, and, in a second experiment, after oxidation to cholestanone, had an isotope concentration at least one-half that of the cholestenone fed. The identity of this saturated sterol could not be established by direct isolation because of lack of sufficient material but good evidence has been obtained to show that it is mainly dihydrocholesterol. This is described in detail in the experimental part below. Dihydrocholesterol in small amounts is known to be present normally in animal tissues, while coprosterol has not been isolated from any source but feces. Moreover, the finding that the fecal sterols had an isotope content less than one-third that of the saturated sterol isolated from the tis-
sues makes it unlikely that the high isotope content of the tissue sterols was due to coprosterol rather than to dihydrocholesterol. The normal dihydrocholesterol content of tissues is not higher than 1 to 2 per cent (10), but in the present experiment, when cholestenone was fed, the content of saturated sterols, particularly in the liver, must have been considerably higher. In this connection it is of interest that Diels, after feeding cholestenone to guinea pigs, noted a very low melting point for the tissue cholesterol (11). In the light of the present findings this depression may be attributed to admixture with larger than normal amounts of dihydrocholesterol. Thus on the basis of the experiment reported here it may be suggested that the small quantities of dihydrocholesterol which are ordinarily found in tissues arise from cholesterol not by direct hydrogenation but by way of cholestenone.

According to Schoenheimer (10) dihydrocholesterol cannot be absorbed from the gastrointestinal tract and its presence in the tissues therefore indicates that it is formed in the internal organs. The labeled cholestenone fed in the present experiment must have been readily absorbed from the intestine. This conclusion is supported by the data which show that only 5 per cent of the administered isotope was recovered from the feces. The intestinal absorption of cholesterol is known to be a highly selective process and the ability of cholestenone to pass through the intestinal wall distinguishes this ketone from most compounds which are chemically related to cholesterol.

An alternative explanation for the present data, which has not been ruled out, is a reduction of cholestenone to cholestanone in the intestinal tract, absorption of this saturated ketone, and subsequent conversion to dihydrocholesterol in the tissues. This possibility is under investigation.

The results reported here do not contain direct evidence to indicate the site of the transformation which results in coprosterol. However, since cholestenone is absorbed from the intestinal tract and converted into dihydrocholesterol in the tissues, it is most likely that, as Rosenheim and Starling (12) have suggested, at least the initial step in the formation of coprosterol, namely the oxidation of cholesterol to cholestenone, takes place prior to secretion into the gut.

In their experiment with partially labeled cholestenone Anchel and Schoenheimer (3) found small isotope concentrations in the tissue cholesterol which had been purified by way of the dibromides. They were unable to decide whether or not cholestenone had been reduced to cholesterol because the cholestenone employed contained isotopic hydrogen in labile positions only. The results secured here with uniformly labeled cholestenone clearly show that the transformation of cholestenone to cholesterol occurs to a certain extent under biological conditions, but do not favor the view that cholestenone is an intermediate in the biosynthesis of cholesterol.
Deuterio-$\Delta^4,5$-cholestenone—This compound was isolated as a by-product of the platinum-catalyzed exchange reaction with cholesterol in $D_2O$-acetic acid mixtures. The total ketones from the exchange reaction were isolated as $p$-carboxyphenylhydrazones and the regenerated ketones separated by chromatographic adsorption on aluminum oxide (8). The melting point of the cholestenone isolated was 78-79° and was not depressed by admixture of authentic cholestenone. It contained 5.65 atom per cent excess deuterium. Since this material had been treated with strong alkali during isolation, the deuterium is present in stable positions only (8).

Feeding Experiments—In the two experiments which were carried out, one rat each, weighing 150 gm., received a normal stock diet and in addition 20 mg. of deuteriocholestenone per day for 3 days. The cholestenone was dissolved in Wesson oil and mixed with the diet. The animals were killed by exsanguination and the unsaponifiable material was isolated from organs and excreta by customary procedures. Total sterols were obtained by digitonin precipitation and subsequent decomposition of the digitonides by pyridine (13). Bromination of this fraction yielded dibromocholesterol, which was debrominated with sodium iodide in acetone (10).

For the separation of the saturated from the unsaturated sterols the total carcass sterols from Experiment II (0.14 gm.) were brominated in ethanol according to Schoenheimer (10). Cholesterol dibromide was filtered off and digitonin added to the filtrate. The digitonide which precipitated was filtered and decomposed by pyridine. The ether-soluble fraction yielded, after recrystallization from methanol, 3.0 mg. of a sterol, m.p. 110-115°. It contained 2.75 atom per cent excess D. From the deuterium concentrations of the total sterols, cholesterol, and dihydrocholesterol it can be calculated that of the total sterols in the carcass at least 4 per cent was saturated sterols.

Direct isolation of the saturated sterols from liver and blood was not possible with the small quantities available. However, good evidence for the identity of the labeled sterol with dihydrocholesterol is furnished by the following experiment. To the combined sterols (7 mg.) of liver and blood from Experiment II, which were obtained by way of the digitonides, were added 40 mg. of non-isotopic dihydrocholesterol. The mixture was oxidized with $CrO_3$ to yield cholestanone (14). This was purified by adsorption on alumina from petroleum ether solution and by elution with benzene (8). There were obtained 35 mg. of cholestanone, m.p. 128.5°, unchanged by admixture with authentic cholestanone. The sample contained 0.098 atom per cent excess D. In order to eliminate the possibility that contamination with coprostanone was responsible for the isotope content, the cholestanone was mixed with an equal amount of non-isotopic coprostanone and subjected
again to chromatographic separation. The isotope concentration in the reisolated cholestanone was not significantly depressed (0.090 per cent D). Therefore, the sterol fraction, which was subjected to chromic acid oxidation, could not have contained any appreciable quantities of labeled coprosterol. Moreover, since only dihydrocholesterol or epidihydrocholesterol would be expected to yield cholestanone on oxidation with chromic acid and since epidihydrocholesterol would not have been present in the digitonin-precipitable fraction, it is reasonably certain that the saturated sterol with the high isotope concentration was indeed dihydrocholesterol.

While it has not been possible to determine directly the isotope concentration of the dihydrocholesterol in liver and blood which was formed from labeled cholestenone, it can be assumed that the value lies between the isotope concentration of the deuteriocholestenone fed (5.65 per cent D) and that of the "dihydrocholesterol" fraction isolated from the carcass sterols (2.75 per cent D). On this basis it can be calculated that of the total sterols in liver and blood a minimum of 11 per cent was dihydrocholesterol.

The feces excreted during the feeding period in Experiment I were pooled and yielded 12 mg. of digitonin-precipitable sterols, containing 0.72 atom per cent excess D. The material in the unsaponifiable fraction which was not precipitated by digitonin (38 mg.) contained 0.16 per cent D. The total quantity of deuterium excreted in the feces is calculated to be 0.147 mg. of D, corresponding to 5 per cent of the quantity present in the administered cholestenone. The labeled cholestenone was therefore well absorbed.

**SUMMARY**

Cholestenone containing stably bound deuterium was fed to rats and the sterols isolated from various tissues and excreta. The labeled cholestenone was well absorbed.

The highest isotope concentration was found in the saturated tissue sterols. Evidence is presented that this is due to the formation of isotopic dihydrocholesterol. The isotope concentration of the cholesterol was considerably smaller but significant.

It is concluded that cholestenone can be converted to cholesterol but is not an intermediate in the total synthesis of cholesterol. It is suggested that the dihydrocholesterol normally found in animal tissues is formed from cholesterol by way of cholestenone.

**BIBLIOGRAPHY**

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