SQUALENE AS A PRECURSOR OF CHOLESTEROL IN LIVER*

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Many compounds have been implicated as capable of serving as precursors for cholesterol, and of these the most intriguing is undoubtedly squalene. In 1926, and again in 1937, Channon (1, 2) reported that the feeding of squalene to rats resulted in an increased cholesterol content of the liver. These observations led Srere (3), in 1949, in this laboratory, to study the conversion of acetate-C\(^{14}\) to cholesterol in liver slices prepared from rats fed a diet containing 1 per cent squalene. The livers from the squalene-fed rats showed a marked depression in their incorporation of the C\(^{14}\) into cholesterol but not into CO\(_2\). Since such a depression is compatible with the idea that the fed squalene had diluted a metabolic pool in the path of conversion of acetate to cholesterol, we next undertook the synthesis of C\(^{14}\)-labeled squalene, and this synthesis was reported elsewhere (4). Surprisingly enough, practically no C\(^{14}\)-cholesterol was recovered in the liver when the labeled squalene was administered to rats or when the labeled compound was incubated with liver slices in a bicarbonate medium. Langdon and Bloch, however, have reported that the C\(^{14}\) of biologically prepared C\(^{14}\)-squalene can be incorporated into cholesterol in vivo (5). An explanation of the failure of the synthetic squalene to be incorporated into cholesterol was suggested by examination of the infra-red spectra of the natural and synthetic compounds (6). It was found that the synthetic material, as well as the natural squalene regenerated from the solid hexahydrochloride, differed from the naturally occurring material in the types of carbon-carbon double bonds. In the present investigation, we have compared the effects of feeding natural squalene and regenerated squalene upon hepatic cholesterogenesis from added acetate-C\(^{14}\).

EXPERIMENTAL

Three groups of Long-Evans female rats were used. The first group was fed an adequate stock diet. The second was fed the stock diet to which had been added 3 per cent natural squalene.\(^1\) The third group received the stock diet mixed with 3 per cent regenerated squalene; i.e., natural

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\(^1\) Obtained from the Distillation Products, Inc., Rochester, New York.
Squalene which had been passed through the solid hexahydrochloride and regenerated by the method of Heilbron et al. (7). The infra-red spectrum of this regenerated squalene was identical with those reported earlier for regenerated natural and synthetic C\textsuperscript{14}-squalene (8). We are indebted to Dr. N. K. Freeman for this measurement.

The diets were fed for 9 days, and the animals were then sacrificed. Their livers were rapidly excised and sliced, and 500 mg. portions of each were incubated with carboxyl-labeled sodium acetate, as described in a previous report (9). The determinations of C\textsuperscript{14}O\textsubscript{2}, fatty acid-C\textsuperscript{14}, and cholesterol-C\textsuperscript{14} have also been described elsewhere (10).

**Table I**

**Effect of Squalene Feeding on Cholesterol Synthesis from Acetate in Liver**

Long-Evans female rats (180 to 260 gm.) were fed a standard laboratory ration, with additions as noted below. All the animals were fed for 9 days. On this day they were sacrificed, and their livers were sliced and incubated at 37.5° for 3 hours with carboxyl-labeled acetate.

<table>
<thead>
<tr>
<th>Rat No</th>
<th>Diet</th>
<th>CO\textsubscript{2}</th>
<th>Fatty acids</th>
<th>Cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Stock</td>
<td>30.8</td>
<td>17.2</td>
<td>2.36</td>
</tr>
<tr>
<td>2</td>
<td>&quot;</td>
<td>43.0</td>
<td>19.7</td>
<td>2.57</td>
</tr>
<tr>
<td>3</td>
<td>&quot;</td>
<td>40.8</td>
<td>10.3</td>
<td>1.49</td>
</tr>
<tr>
<td>4</td>
<td>&quot; + 3% natural squalene</td>
<td>45.6</td>
<td>15.8</td>
<td>0.065</td>
</tr>
<tr>
<td>5</td>
<td>&quot; + 3% &quot;</td>
<td>38.6</td>
<td>23.5</td>
<td>0.136</td>
</tr>
<tr>
<td>6</td>
<td>&quot; + 3% &quot;</td>
<td>43.0</td>
<td>14.3</td>
<td>0.033</td>
</tr>
<tr>
<td>7</td>
<td>&quot; + 3% regenerated squalene</td>
<td>38.6</td>
<td>12.0</td>
<td>1.06</td>
</tr>
<tr>
<td>8</td>
<td>&quot; + 3% &quot;</td>
<td>36.8</td>
<td>14.0</td>
<td>1.24</td>
</tr>
<tr>
<td>9</td>
<td>&quot; + 3% &quot;</td>
<td>38.6</td>
<td>16.4</td>
<td>5.75</td>
</tr>
</tbody>
</table>

**Results**

The incorporation of the added acetate-C\textsuperscript{14} into cholesterol by the livers of the three groups of rats is shown in Table I. From 1.5 to 2.6 per cent of the C\textsuperscript{14} was recovered as cholesterol in the experiments with the rats fed only the stock diet. The feeding of a diet containing 3 per cent natural squalene resulted in a pronounced reduction in the recovery of the added acetate-C\textsuperscript{14} in cholesterol. Interestingly enough, the same dietary content of regenerated squalene had no such effect. The recoveries of C\textsuperscript{14}O\textsubscript{2} and fatty acid-C\textsuperscript{14} were unaffected by the ingestion of either type of squalene. It is this observation which suggests that the reduced cholesterol-C\textsuperscript{14} values found in the experiments with the rats fed the natural squalene diet are the result of dilution of a cholesterol precursor pool rather than of a liver poison. It is also apparent that regenerated squalene does not interfere with the incorporation of acetate carbon into cholesterol.
DISCUSSION

It is shown in the present study that a difference exists in the biological behavior of natural and of regenerated squalene. If it is assumed that the reduced incorporation of acetate-C\textsuperscript{14} into cholesterol observed in the livers of rats fed natural squalene results from dilution of a precursor pool, it may then be inferred that the natural, but not the regenerated, form of squalene is a precursor of cholesterol.

Since it was shown by Dauben et al. (8) that natural squalene is a homogeneous compound containing only trisubstituted ethylene bonds and, further, that the regenerated material contains as much as 40 per cent of unsymmetrically disubstituted carbon-carbon bonds, it is feasible to explain our findings on this structural detail. Thus the biosynthesis of cholesterol from squalene is a very stereospecific reaction, as indeed might be expected from the postulated ring closure mechanism of Robinson (11).

SUMMARY

1. The effect of squalene feeding upon the incorporation of the C\textsuperscript{14} of acetate-1-C\textsuperscript{14} into cholesterol by liver slices was studied.
2. The previous feeding of natural squalene for 9 days resulted in a marked reduction in the conversion of the added C\textsuperscript{14} to cholesterol.
3. No reduction in the cholesterol-C\textsuperscript{14} recoveries was observed in the experiments with rats that had been fed regenerated squalene.
4. The findings are taken to mean that the natural squalene is a cholesterol precursor, while the regenerated material is not.

BIBLIOGRAPHY

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