THE ESTERIFICATION OF CHOLESTEROL WITH PALMITIC ACID BY RAT LIVER HOMOGENATES*†

BY SUPRAVAT MUKHERJEE, GEORGE KUNITAKE, AND ROSLYN B. ALPIN-SLATER

(From the Department of Biochemistry and Nutrition, University of Southern California School of Medicine, Los Angeles, California)

(Received for publication, March 15, 1957)

Although the enzymes involved in the synthesis and hydrolysis of cholesterol esters of fatty acids have been the subject of a number of communications (1-7), the sites and mechanisms of these reactions have not been definitely established. It has been suggested by Schramm and Wolff (8) that fatty acids are esterified in the intestinal lumen before absorption into the cells of the mucosa and that fatty acids are carried from the blood into tissue cells as cholesterol esters. The reported occurrence of a serum cholesterol esterase (9) has led to the suggestion that this enzyme may be involved in the maintenance of the cholesterol ester level of blood.

Cholesterol esterases of pancreatic origin have been shown to have the required specificity and activity for the synthesis and hydrolysis of long chain fatty acid esters of cholesterol (10-12). The presence of a cholesterol-esterifying system in liver has also been demonstrated by several investigators (13-17). According to a recent report by Swell and coworkers (18), the activity in liver is due to a liver esterase which acts only on short chain fatty acid esters (e.g. butyrate); these esters do not occur normally in any appreciable amount in the animal body.

It is apparent from the existing literature that cholesterol esterases occur in a number of tissues, but typical preparations of the enzymes have extremely low activity. Systems were often incubated for a period of 2 to 3 days to demonstrate appreciable synthesis or hydrolysis of the ester. The possibility exists that this low activity may indicate that the enzymatic reaction requires certain cofactors for the synthesis of fatty acid esters.

In the present investigation it has been possible to verify the presence of an enzyme in rat liver which can bring about the synthesis of a cholesterol ester of a long chain fatty acid (palmitic acid). This enzymatic

* This work was supported by research grants from The Best Foods, Inc., Bayonne, New Jersey, and from the National Heart Institute, National Institutes of Health, United States Department of Health, Education, and Welfare.

† Contribution No. 427 from the Department of Biochemistry and Nutrition, University of Southern California.
esterification requires the presence of adenosine triphosphate (ATP) and coenzyme A (CoA). On the basis of this requirement, and by analogy with the mechanism of ATP activation of acetate (19), it is proposed that the over-all reaction is a result of two distinct steps: (1) the activation of the fatty acids by ATP to form the acyl CoA derivative

\[
\text{RCOOH} + \text{CoA-SH} \xrightarrow{\text{ATP}} \text{RC-S-CoA} + \text{H}_2\text{O}
\]

and (2) the esterification reaction

\[
\text{RC-S-CoA} + \text{HO-R'} \rightarrow \text{RC-O-R'} + \text{CoA-SH}
\]

**EXPERIMENTAL**

*Materials*—To study the esterification of palmitic acid with cholesterol both 4-C\(^{14}\)-cholesterol plus sodium palmitate and non-labeled cholesterol plus the sodium salt of 1-C\(^{14}\)-palmitic acid have been used.

The cholesterol suspensions were prepared from an acetone solution by a slight modification of the method employed by Nieft and Deuel (15). Both unlabeled and labeled (1-C\(^{14}\)) palmitoyl-S-CoA were enzymatically prepared by employing a lyophilized “residue fraction” of guinea pig liver according to the method of Kornberg and Pricer (20). The rat liver homogenates were prepared from normal adult male rats (weight 250 to 350 gm.) in a Potter-Elvehjem type homogenizer with approximately 4 ml. of 0.5 \(\text{M}\) phosphate buffer (pH 7.2) per gm.

The complete system used in the experimental incubation mixture (described in Table I) was incubated at 38\(^\circ\) with constant shaking for 3 hours.

*Isolation of Cholesterol Esters*—At the end of the 3 hour reaction period, 10 mg. of cholesterol palmitate were added to the incubation mixture as a carrier; the whole solution was extracted twice with 15 ml. portions of hot CHCl\(_3\). The extracted lipid material was dissolved in CCl\(_4\) and repeatedly washed with aqueous ammoniacal ethanol to remove unchanged palmitate and palmitoyl-S-CoA. The solvent was evaporated, and the cholesterol esters were separated from the total extracted lipid on a silicic acid column by a modification of a procedure of Fillerup and Mead (21) developed in this laboratory (22). The cholesterol ester fraction was extracted with 1 per cent ether in \(n\)-pentane. The isolated cholesterol palmitate was rechromatographed on another prewashed silicic acid column and reextracted, and the radioactivity of the isolated ester fraction was measured by direct plating and counting of either the whole material or a
suitable aliquot thereof. The counting equipment was a Nuclear auto-
scaler and gas flow counter.

The distribution of the radioisotope in the cholesterol and fatty acid
of the ester was determined by hydrolyzing the cholesterol ester with
0.1 N sodium ethylate and isolating the cholesterol and fatty acid by
standard extraction procedures. Each fraction was further purified on
separate silicic acid columns before being plated for measurement of
radioactivity.

RESULTS AND DISCUSSION

In Table I is shown the incorporation of labeled cholesterol or labeled
palmitic acid into cholesterol palmitate in the presence and absence of
certain cofactors. In the absence of CoA and ATP, but with palmitoyl-S-
CoA in the incubation mixture (Series I, Experiments 2 and 3), there is
practically no change in the amount of incorporation of radioactivity in
the cholesterol ester fraction from what is observed with the complete
system containing CoA and ATP (Experiment 1). However, in the
absence of palmitoyl-S-CoA (Experiments 4 and 5), there is a 60 per cent
decrease in incorporation. In the absence of CoA, ATP, and palmitoyl-
S-CoA (Experiments 6 and 7), the esterification of cholesterol is reduced
to approximately 5 per cent of that occurring in the complete system.
The small amount of activity observed here is probably due to the action
of small amounts of endogenous CoA present in the system which can
form limited amounts of palmitoyl-S-CoA with the palmitic acid in the
incubation medium.

The experiments in Series II provide additional evidence in support
of the theory that esterification of cholesterol with fatty acids proceeds
through the intermediary formation of acyl CoA derivatives. In the
absence of CoA, ATP, and sodium palmitate, with 1 μmole of labeled
palmitoyl-S-CoA, considerable esterification is observed (Experiments 8,
9, 10, and 11). In fact, the incorporation of radioactivity is similar to
that of the complete system in which 1-C¹⁴-cholesterol was used as the
labeled material (Experiments 1 and 2).

In the experiments in Series III both the sodium palmitate and the
palmitoyl-S-CoA were labeled with C¹⁴. This double source of radio-
activity probably accounts for the higher radioactivity obtained in the
cholesterol ester fraction of the complete system in Experiments 12, 13,
and 14. Again, in the absence of palmitoyl-S-CoA, CoA, and ATP
(Experiment 19), the esterification obtained is negligible, in confirmation
of Experiments 6 and 7. When CoA and ATP are added again into the
system, approximately a 7-fold increase in esterification is noted (Experi-
ments 15, 16, 17, and 18), similar to that observed in Experiments 4
TABLE I

Esterification of Cholesterol with Palmitic Acid

<table>
<thead>
<tr>
<th>Incubation mixture</th>
<th>Experiment No.</th>
<th>Total Cholesterol (c.p.m.)*</th>
<th>Palmitic acid fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Series I. With 1-C\textsuperscript{14}†-cholesterol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complete system</td>
<td>1</td>
<td>2008</td>
<td>2000</td>
</tr>
<tr>
<td>&quot; less CoA, ATP</td>
<td>2</td>
<td>1872</td>
<td>1668</td>
</tr>
<tr>
<td>&quot; &quot; &quot; palmitoyl-S-CoA</td>
<td>3</td>
<td>2150</td>
<td>2085</td>
</tr>
<tr>
<td>&quot; &quot; &quot; CoA, ATP, palmitoyl-S-CoA</td>
<td>4</td>
<td>825</td>
<td>800</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>800</td>
<td>796</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>160</td>
<td>159</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>95</td>
<td>92</td>
</tr>
<tr>
<td>Series II. With palmitoyl-S-CoA labeled with 1-C\textsuperscript{14}-palmitic acid‡</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complete system less CoA, ATP, Na palmitate</td>
<td>8</td>
<td>2510</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>2560</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>2840</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>2150</td>
<td>62</td>
</tr>
<tr>
<td>Series III. With Na 1-C\textsuperscript{14}-palmitate§ and palmitoyl-S-CoA labeled with 1-C\textsuperscript{14}-palmitate¶</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complete system</td>
<td>12</td>
<td>3150</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>3010</td>
<td>122</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>3550</td>
<td>132</td>
</tr>
<tr>
<td>Complete system less palmitoyl-S-CoA</td>
<td>15</td>
<td>897</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>800</td>
<td>not determined</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>920</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>873</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td>Complete system less CoA, ATP, palmitoyl-S-CoA</td>
<td>19</td>
<td>133</td>
<td>&quot; &quot;</td>
</tr>
</tbody>
</table>

The complete system contained 10 μmoles of ATP, 25 μmoles of CoA, 60 μmoles of glutathione, 60 μmoles of MgCl\textsubscript{2}, 100 μmoles of NaF, 1000 μmoles of potassium phosphate buffer (pH 7.2), 40 μmoles of sodium palmitate, 1 μ mole of palmitoyl-S-CoA, 1 ml. of cholesterol suspension (1 mg. per ml.), 2 ml. of liver homogenate, and water to a total volume of 8 ml.

* Counts per minute per dish at infinite thinness.
† 1.23 × 10\textsuperscript{5} c.p.m.
‡ 1.0 × 10\textsuperscript{6} c.p.m.
§ 1.1 × 10\textsuperscript{5} c.p.m.
¶ 1.18 × 10\textsuperscript{6} c.p.m.

and 5. It is postulated that this activity is due to the activation of the palmitic acid in the incubation mixture by these cofactors.
When boiled enzyme was used in place of fresh rat liver homogenate, there was practically no synthesis, as was evidenced by the low activity obtained in cholesterol ester fractions (23, 20, and 42 c.p.m.) isolated from the medium.

The data on the distribution of radioactivity in the cholesterol and fatty acid fractions isolated after the hydrolysis of the esters show that, whereas none of the activity appears in the fatty acid when labeled cholesterol is used in the synthesis (Table I, Series I), a small amount of activity is always associated with the cholesterol fraction when unlabeled cholesterol and 1-C¹⁴-palmitate are employed (Table I, Series II and III). This might be explained as a result of the biosynthesis of small amounts of radioactive cholesterol from the 2-carbon fragments obtained in the breakdown of 1-C¹⁴-palmitate. However, most of the activity in the ester appears in the fatty acid when 1-C¹⁴-palmitate was used and in the cholesterol fractions when labeled cholesterol was employed in the synthesis.

It is therefore concluded that esterification of fatty acids by rat liver cholesterol esterase requires the cofactors CoA and ATP; no significant esterification is observed in the absence of these substances when an otherwise complete system, containing cholesterol and palmitic acid, is incubated. The esterification probably takes place in at least two steps, the first being the formation of the acyl CoA derivative which then combines with the sterol to form the ester. The rate of esterification is much enhanced if acyl CoA derivatives are supplied to the medium. It is possible that the inability of Swell et al. (18) to demonstrate activity of an enzyme for the esterification of cholesterol and higher fatty acids may very well be due to the omission of cofactors necessary for this reaction.

Similar mechanisms have been proposed for the esterification of α-glycerophosphate (20) and recently by Weiss and Kennedy (23) for the formation of triglycerides from diglycerides.

**SUMMARY**

1. The presence of an enzyme in the rat liver capable of esterifying cholesterol with long chain fatty acids has been demonstrated with labeled cholesterol and labeled fatty acids.
2. The esterification of 1-C¹⁴-palmitic acid with cholesterol (or palmitic acid with 4-C¹⁴-cholesterol) requires the presence of the cofactors co-enzyme A (CoA) and adenosine triphosphate (ATP).
3. A mechanism for this esterification is presented. The esterification is thought to proceed in two steps: (1) the activation of the fatty acids by ATP to form the acyl CoA derivative and (2) a transesterification reaction in which the fatty acid exchanges its CoA group for the hydroxyl of cholesterol to form the cholesterol ester.
The authors are indebted to Dr. Bernard Axelrod and Dr. Paul Saltman for their advice in this investigation.

**BIBLIOGRAPHY**

THE ESTERIFICATION OF
CHOLESTEROL WITH PALMITIC ACID
BY RAT LIVER HOMOGENATES
Supravit Mukherjee, George Kunitake and
Roslyn B. Alfin-Slater


Access the most updated version of this article at
http://www.jbc.org/content/230/1/91.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/230/1/91.citation.full.html#ref-list-1