ON THE CONVERSION OF PALMITIC ACID TO STEARIC ACID IN ANIMAL TISSUES*

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Tracer studies have revealed that acetate is an important building block for the synthesis of the fatty acids in animal tissues. Rittenberg and Bloch (1), by feeding labeled acetate to mice and rats, demonstrated that acetate is utilized in the formation of the higher fatty acids, and that in this process the carbon atoms of acetate are uniformly distributed along the fatty acid chain. Brady and Gurin (2), using rat liver slices, recently reported results which indicate that in vitro also acetate carbon becomes evenly distributed along the fatty acid chain.

The findings obtained in the present investigation show that under certain in vitro conditions and in the fasted animal the distribution pattern of acetate carbon in the higher fatty acids is markedly different from that observed in the earlier studies. This result permits certain conclusions to be made regarding the mechanism of chain elongation. The saturated fatty acids formed from carboxyl-labeled acetate either in vivo or in vitro were isolated and the isotope content of the total molecule, the carboxyl carbon, and the 2 terminal carbon atoms of the chain was determined. In one case, individual fatty acids were separated by fractional distillation of the methyl esters.

EXPERIMENTAL

Incubation Experiments (Experiments 2, 3, and 4)—Slices were cut by hand from livers of male rats of the Sprague-Dawley or Yale strain, weighing 60 to 100 gm. 1.5 gm. of liver slices were incubated in 12 ml. of Krebs' phosphate buffer (3), pH 7.4, which contained 3 mg. of 1-C14-sodium acetate. The flasks were shaken at 37° for 6 hours in oxygen. Eight or more flasks were combined for isolation of the fatty acids.

Feeding Experiments—In Experiment 1 the saturated fatty acids were isolated from the liver of a 200 gm. male Sprague-Dawley rat which on each of 7 days had been given 1 mm of 1-C14-sodium acetate (25.99 atom

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per cent excess C\textsuperscript{13} per 100 gm. of rat weight, mixed with 12 gm. of a fat-free diet (4). In Experiment 5, a 200 gm. male Sprague-Dawley rat was fasted for 24 hours, given by stomach tube 1 mM of 1-C\textsuperscript{13}-sodium acetate dissolved in H\textsubscript{2}O, and sacrificed 6 hours later. The saturated fatty acids were isolated from the liver.

Separation and Degradation Procedures—After hydrolysis of the liver tissue in alcoholic KOH, the saturated fatty acids were separated by way of the lead soaps (5). Sufficient material for the degradations was obtained by adding normal palmitic acid to the labeled fatty acids. One portion of the fatty acid mixture was decarboxylated with iron powder by the method of Easterfield and Taylor (6), and another portion was oxidized with chromic acid to acetic acid (7). The acetic acid resulting from this oxidation is derived from the terminal 2 carbon atoms of the fatty acids, and was isolated as the silver salt. Duclaux constants were determined in some cases to confirm the purity of the acetic acid obtained by the oxidation.

90 mg. of the saturated fatty acids isolated from the incubation mixture of Experiment 4 were converted to the methyl esters with diazomethane. The esters were then mixed with 0.52 gm. of non-isotopic methyl palmitate and 3.91 gm. of non-isotopic methyl stearate and fractionated in vacuo on a Podbielniak column. The separated esters were hydrolyzed and recrystallized from aqueous alcohol or acetone. Stearic and palmitic acids had melting points of 69.4° and 60.6°, respectively. The distillation was not repeated because of the small amounts of material available, but it can be assumed that the palmitic acid was contaminated with stearic acid to a maximal extent of 5 per cent.

Isotope Analyses—All isolated compounds were converted to CO\textsubscript{2} in a micro combustion apparatus at 900°. The isotope analyses were carried out as previously described (8). The C\textsuperscript{14} values are expressed as counts per minute of an infinitely thick sample of BaCO\textsubscript{3} of constant area.

RESULTS AND DISCUSSION

Data of Rittenberg and Bloch and of Brady and Gurin (Table I) show that, under their experimental conditions, carboxyl labeled acetate gives rise to fatty acids in which the carboxyl carbon atom has approximately twice the isotope concentration of the total saturated fatty acid molecule. Experiment 1, carried out under the same conditions as those of Rittenberg and Bloch, gave similar results. These data are best explained by assuming that the carbon atoms of acetic acid are distributed uniformly along the chain with the tracer in every odd carbon atom of the fatty acid.

In contrast, in liver slices under the present conditions or in the fasted intact animal, an asymmetrical distribution of the isotope was found
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(Table I). In Experiments 2 and 3 the carboxyl carbon atom had a specific activity approximately 3 times that of the total saturated fatty acid molecule, and in Experiment 4 the ratio of the two isotope concentrations was about 7. After administration of acetate to a rat fasted for 24 hours (Experiment 5), the isotope distribution in the saturated fatty acids of the liver likewise indicated a high degree of asymmetry. Oxidation to the acetic acid, which is derived from the $\omega$ and $\omega-1$ carbon atoms of the saturated fatty acids isolated in Experiments 2 to 5, confirmed the fact that an unequal isotope distribution existed along the chain.

In the liver slice experiments it will be seen that the smaller the uptake of $^{14}$C (Column 2, Table I), the greater was the asymmetry in the isotope distribution. Since the uptake of $^{14}$C-acetate into the fatty acids of a fasted rat is markedly lower than of a fed rat, a similar correlation seems to exist in vivo. The lower relative isotope concentration found in these experiments is presumably due to a decreased rate of fat synthesis, and it would follow therefore that an unequal distribution of acetate carbon in the fatty acids will result whenever the rate of fat synthesis is slow. The disagreement of these results with those of Brady and Gurin (2) may

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Acetate</th>
<th>R. I. C.*</th>
<th>Saturated fatty acids</th>
<th>Carboxyl CO$_2$</th>
<th>Terminal acetic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rittenberg and Bloch (1); fed animal, atom % excess C$^{14}$</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Brady and Gurin (2); liver slices, c.p.m. per mg. C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Fed animal, atom % excess C$^{14}$</td>
<td>0.16</td>
<td>0.29</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Slices, c.p.m.</td>
<td>2.7 x 10$^4$</td>
<td>0.48</td>
<td>120</td>
<td>380</td>
<td>80</td>
</tr>
<tr>
<td>3. &quot;        &quot;</td>
<td>1 x 10$^4$</td>
<td>0.37</td>
<td>90</td>
<td>290</td>
<td>60</td>
</tr>
<tr>
<td>4. &quot;        &quot;</td>
<td>1.6 x 10$^7$</td>
<td>0.019</td>
<td>50</td>
<td>365</td>
<td>25</td>
</tr>
<tr>
<td>5. Fasted animal, c.p.m.</td>
<td>5 x 10$^5$</td>
<td>0.016</td>
<td>13</td>
<td>78</td>
<td>8</td>
</tr>
</tbody>
</table>

*R Relative isotope concentration = (Column 3)/(Column 1) x 100. In Experiments 3, 4, and 5, the liver saturated fatty acids were diluted with non-isotopic palmitic acid before analysis and degradation. In these cases the isotope concentrations of the undiluted fatty acids were calculated to determine the R. I. C.

† The chromic acid used for the oxidation was found to be contaminated with acetic acid to the extent of 10 to 20 per cent of the yield. The true value would therefore be 0.11 or 0.12. All other oxidations were carried out with purified chromic acid solution.
be due to their use of bicarbonate buffer rather than phosphate buffer, which was employed in the present experiments. Bloch and Kramer (9) found that twice as much isotope from labeled acetate is incorporated into fatty acids in bicarbonate as in phosphate buffer, and it is probable therefore that the rate of synthesis of the fatty acids in the experiments of Brady and Gurin was greater than in the experiments reported here.

In order to determine the isotope distribution in individual components of the total saturated fatty acids, the fatty acid mixture of Experiment 4 was fractionally distilled, and the palmitic and stearic acid fractions were separately degraded. It may be seen (Table II) that almost the entire C\textsuperscript{14} content of the stearic acid can be accounted for by the radioactivity of the carboxyl carbon atom. The remaining C\textsuperscript{14} appears to be uniformly distributed along the chain (carbon atoms 3 to 18). Thus, the specific activity of stearic acid, when calculated from the isotope concentrations of the degradation products, \textit{i.e.}, CO\textsubscript{2} and CH\textsubscript{3}COOH, \((16 \times 4 + 305)/18 = 20.5\), is almost identical to that found for the total molecule, 20.

In contrast, the isotope concentrations in palmitic acid and its degradation products indicate only a very slight degree of asymmetry in the distribution of acetate carbon. The fact that the carboxyl carbon atom of the palmitic acid fraction contains somewhat more than twice the C\textsuperscript{14} concentration of the total molecule may be due to the admixture of a small quantity of unevenly labeled stearic acid.

On the basis of these data it appears that the unequal isotope concentrations found in the fatty acids under conditions which are unfavorable for fat synthesis are primarily due to the elongation of palmitic to stearic acid, and that under such circumstances the rate of this latter reaction decreases proportionally much less than the formation of fatty acids by total synthesis from 2-carbon units.

Stetten and Schoenheimer (10), using deuterium-labeled palmitic acid, demonstrated its direct conversion to stearic acid \textit{in vivo}. The results

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**Table II**

<table>
<thead>
<tr>
<th></th>
<th>Total fatty acid*</th>
<th>Carboxyl CO\textsubscript{2}</th>
<th>Terminal acetic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmitic</td>
<td>120</td>
<td>300</td>
<td>108</td>
</tr>
<tr>
<td>Stearic</td>
<td>20</td>
<td>305</td>
<td>4</td>
</tr>
</tbody>
</table>

\* The absolute isotope values of palmitic and stearic acids may not be compared, since the original concentrations of these acids in the liver fat were not known, and thus the two acids were diluted by carrier to an unknown extent.

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reported here show that this elongation involves condensation with acetate, or a 2-carbon particle derived from acetate, and that the addition takes place at the carboxyl end of palmitic acid. A similar lengthening of the carbon chain of some of the other fatty acids may also occur, but under the present experimental conditions these processes could not be demonstrated. The addition of a 2-carbon unit to the carboxyl group of a fatty acid or fatty acid precursor reported here for animal tissues is analogous to the reactions found by Barker, Kamen, and Bornstein in microorganisms (11). These authors in their study on caproic acid formation by Clostridium kluyveri showed that the carbon atoms of ethanol added to the carboxyl end of butyric acid to form the higher fatty acid.

The author is deeply grateful to Dr. Konrad Bloch for constant encouragement and valuable suggestions in the course of this work.

SUMMARY

1. Fatty acid synthesis from carboxyl-labeled acetate in liver was studied in a fasted rat and in rat liver slices. Acetate carbon was found in greater concentration in the carboxyl position than in the remainder of the saturated fatty acid chain.

2. Stearic acid, separated by the fractional distillation of the methyl esters of saturated fatty acids of rat liver after incubation with carboxyl-labeled acetate, contained almost all of the isotopic carbon in the carboxyl position. This finding demonstrates that chain elongation occurred by addition of 2 carbon atoms to the carboxyl end of palmitic acid.

BIBLIOGRAPHY

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