Biosynthesis of Fatty Acids

I. STUDIES WITH ENZYMES OBTAINED FROM LIVER

Roscoe O. Brady, Roy M. Bradley, and Eberhard G. Trams

From the Laboratory of Neurochemistry, National Institute of Neurological Diseases and Blindness, National Institutes of Health, United States Public Health Service, Department of Health, Education and Welfare, Bethesda, Maryland

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The requirement for bicarbonate in the incubation medium for the conversion of octanoic acid to stearic acid by slices of liver tissue was reported some 10 years ago (1). Furthermore, the inclusion of bicarbonate in the homogenizing medium permitted the preparation of a consistently active enzyme system from pigeon liver which catalyzed the conversion of acetate or acetyl coenzyme A to long chain fatty acids (2). The significance of these observations was not fully realized until the complete dependence of fatty acid synthesis on the presence of CO2 was demonstrated soon thereafter (7, 9, 10), and the participation of this reactant in fatty acid synthesis was fully confirmed (11-13).

The finding that HC4O3 was not incorporated into long chain fatty acids by enzyme systems which require the presence of CO2 (3, 4) suggested that acetyl-CoA was first carboxylated to malonyl-CoA. Accordingly, malonyl-CoA was synthesized and was found to be a required precursor for the synthesis of long chain fatty acids in an enzyme system obtained from pigeon liver tissue (8). The enzymatic carboxylation of acetyl-CoA was demonstrated soon thereafter (7, 9, 10), and the participation of this reactant in fatty acid synthesis was fully confirmed (11-13).

The original demonstrations of the conversion of malonyl-CoA to long chain fatty acids (8) were performed with crude enzyme preparations obtained from pigeon liver which catalyzed the reduction of acetyl-CoA by TPNH to acetaldehyde. This finding led to the tentative proposal that elongation of the carbon chain of fatty acids might occur by reduction of acetyl-CoA derivatives to the respective aldehydes, followed by condensation between the carbonyl carbon of the aldehyde with the methylene carbon of malonyl-CoA (8). Experiments with purified enzyme preparations indicate that such a mechanism is probably incorrect. It is the purpose of the present communications to report the results obtained with purified enzyme preparations which afford a clearer insight into the nature and the sequence of reactions that occur in the biosynthesis of long chain fatty acids.

EXPERIMENTAL PROCEDURE

Materials

Preparation of Substrates—Malonyl-1,3-Cl*-CoA was synthesized from labeled malonic acid via the monothiophenyl ester of malonic acid (14). After purification of the product by paper chromatography, malonyl-C14-CoA of 96% purity was obtained with an over-all yield of 18 to 27%.

Malonic acid-1,3-C14 was obtained from Isotopes Specialties Company. Other labeled short chain fatty acids, DPNH, TPNH, CoA, and hexokinase were purchased from commercial sources. Octenoyl-CoA was a gift from Dr. Feodor Lynen. Glucose-1-H3 was generously supplied by Dr. John M. Lowenstein. Glucose 6-phosphate dehydrogenase was prepared according to Kornberg (18).

Methods

Microdecarboxylation Procedure—The decarboxylation of long chain fatty acids was accomplished via the Schmidt reaction by adapting the method of Phares (19) to a suitable micro scale. The radioactive samples obtained from the incubation mixtures were evaporated to dryness from ethanol in an even layer on the bottom of pharmaceutical multiple dose vials which had been modified by sealing glass wells to the center of the botton (20). Then 50 mg of sodium azide were added and the flasks closed with a self-sealing rubber stopper which was held in place by an aluminum cap. A mixture of 1 part of fuming H2SO4 and 3 parts of concentrated H2SO4, 0.5 ml, was injected into the main compartment. The mixture was heated with gentle shaking for 1 hour at 70°, chilled in an ice bath, and 0.3 ml of a 1 M solution of the free base of p-(diisobutylcresoxyethoxyethyl) dibenzylammonium chloride (Hyamine) in methanol (21) was injected into the center well. Absorption of the C14O2 was carried out with gentle shaking at 37° for 35 minutes. The Hyamine solution was transferred to the appropriate toluene-phosphor solution for radioactivity determination with a liquid scintillation spectrometer. The recovery of C14O2 from palmitic acid-1-C14 ranged from 93 to 105% in 10 experiments.

Degradation of Tritiated Fatty Acids—Stepwise degradation of palmitic acid was carried out by modifying the procedure described by Anker (22). The critical step for the present investigations was the oxidation of the long chain alcohol containing 1 carbon atom less than the parent acid. It was found that the chromic acid oxidation method developed by Pattison et al. (23) effected the quantitative oxidation of the alcohol to the respective acid. Accordingly, 5 to 7 mg of the recovered alcohol were taken up in 0.2 ml of glacial acetic acid to which was added...
The products obtained in the first degradation sequence of palmitic acid were then extracted 2 times with 2 ml of perchloric acid. The mixture was allowed to stand overnight at room temperature. It was then extracted 2 times with 2 ml of petroleum ether. A representative determination of the conversion of palmitic acid to the original acidic residue. The mixture was made alkaline with 0.6 ml of 9 N NaOH. The final volume was 1 ml. The water was recovered by distillation and the radioactivity was determined after dissolving the water in 15 ml of the naphthalene-dioxane scintillation mixture described by Werbin et al. (24). The completeness of the degradative procedure was confirmed by gas chromatography of the methyl esters of the reaction products. The authors are indebted to Dr. Charles Sweeney of the National Heart Institute for these analyses. A representative determination of the conversion of palmitic acid to pentadecanoic acid is shown in Fig. 1.

RESULTS

Preparation of Enzyme—The enzymes required for the biosynthesis of long chain fatty acids are found in the supernatant solution of cell-free preparations of liver tissue centrifuged at 100,000 × g (23, 26). The initial specific activity was considerably greater if the supernatant solution from suitably homogenized tissue was used rather than the suspension obtained with a Waring Blender. Accordingly, enzymes were isolated from rat liver tissue which was disrupted in 2 volumes of 0.1 m potassium phosphate buffer (pH 7.0) with an all glass TenBroeck homogenizer. The heavier particles were sedimented by centrifugation at 25,000 × g for 30 minutes and the supernatant suspension was further centrifuged at 100,000 × g for 1 hour.

Solid ammonium sulfate was added to the 100,000 × g supernatant solution, and the protein precipitating between 30 and 40% saturation was redissolved in 0.05 M potassium phosphate (pH 7.0). The enzyme was then treated with calcium phosphate gel with a gel to protein ratio of 3:1. The gel was washed with water, 0.01, and 0.02 M potassium phosphate solutions (pH 7.0), and the fatty acid-synthesizing enzymes were eluted with 0.05 M potassium phosphate solution containing 0.001 M reduced glutathione. The eluate was diluted with an equal volume of water, and an aliquot of alumina gel C17 was added equal to the amount of protein. The fatty acid-synthesizing enzymes remained in the supernatant solution and were subsequently adsorbed on alumina gel C17 by the addition of another similar aliquot of gel. The fatty acid-synthesizing enzymes were eluted with 0.05 M potassium phosphate (pH 7.0) containing 0.001 M reduced glutathione. The amount of fatty acid synthesized by these preparations was proportional to the amount of enzyme employed in the incubation mixtures. The enrichment achieved with the use of these procedures was 580-fold over the initial soluble supernatant solution. The purification obtained in the individual steps and the respective yields are summarized in Table I.

Properties of Purified Enzyme—The ratio of absorbancy of the enzyme solution at 280 μg to that at 260 μg was 1.5. The enzymatic activity was considerably stabilized by the addition of reduced glutathione. The enzyme was activated by the addition of 2-mercaptoethanol and particularly by the dithiol, 2,3-dimercaptopropanol (27). Catalytic activity in the frozen state diminished by a factor of 10% per day. The preparation was free of glutathione reductase, but contained a variable amount of β-ketothiolase. The most highly purified fraction still exhibited strong malonyl-CoA decarboxylase activity.

The product of the enzymatic synthesis was identified by gas chromatography of the methyl esters obtained from the petroleum ether extracts of the acidified reaction mixtures. The petroleum ether was evaporated to dryness and the residue was refluxed for 30 minutes with 1 ml of 2% H2SO4 in methanol. The mixture was cooled, diluted with 1 ml of water, and extracted 3 times with 2 ml of n-hexane. The hexane solution was dried over anhydrous Na2SO4 and subsequently concentrated to 0.1 ml. The conditions of gas chromatography were the same as described in Fig. 1. The separated methyl esters were recovered and counted. Under these conditions, 81 to 87% of the radioactivity was found in methyl palmitate in confirmation of the observations of Porter and Tietz (28). The authors wish to thank Dr. Arthur Kamen and his associates in the National Heart Institute for the gas chromatography determinations.

The observation that crude extracts of pigeon liver catalyzed the reduction of acetyl-CoA with TPNH prompted an examination of the preparations obtained from rat liver for the presence of a reaction of this nature. In the course of purifying the enzyme system from rat liver, specific enzymes for both DPN and TPN were found which catalyzed the CoA-dependent oxidation of acetaldehyde (Fig. 2). The DPN-specific CoA-dependent dehydrogenase was eluted from the Ca3(P04)2 gel by washing with an equal volume of 0.1 M potassium phosphate solution (pH 7.0). The DPN-specific CoA-dependent aldehyde dehydrogenase was found in the fourth eluate upon repeated washing of the gel with 0.1 M phosphate solution. Neither of these enzymes was present in the more highly purified fatty acid-synthesizing fractions. These findings may explain why...
acetaldehyde was not utilized for fatty acid synthesis in highly purified enzyme preparations (12).

Nucleotide Specificity—TPNH is the preferred source of hydrogen atoms. The activity obtained with DPNH varied from 35 to 60% of that observed with TPNH. Pyridine nucleotide transhydrogenase activity was not observed. The most highly purified enzyme preparation catalyzed a TPNH-specific reduction of acetacetyl-CoA to β-hydroxybutyryl-CoA. Crotonyl-CoA (29), octenoyl-CoA (30), and β-hydroxybutyryl-CoA were not reduced in the presence of either nucleotide.

Effect of Inhibitors—The enzyme was completely inhibited by the addition of 1 X 10^-4 M p-hydroxymercuribenzoate. Experiments have been reported elsewhere (27) which indicated that complete inhibition was obtained by blocking closely juxtaposed enzyme sulfhydryl groups with low concentrations of arsenite. Effects have been reported elsewhere (27) which indicated that complete inhibition was obtained by blocking closely juxtaposed enzyme sulfhydryl groups with low concentrations of arsenite. The addition of 0.016 M semicarbazide caused a 15% inhibition of fatty acid synthesis.

Effect of Short Chain Acyl-CoA Derivatives—The conversion of malonyl-CoA to long chain fatty acids was markedly stimulated by the addition of acetyl-, propionyl-, or butyryl-CoA (Table II). Wakil and Ganguly (12) have observed a similar effect of acetyl-CoA. Increasing the length of the carbon chain of the acyl-CoA derivatives beyond 4 carbon atoms caused a progressive diminution of this conversion.

Wakil and Ganguly (12) have presented evidence for the conversion of acetyl-, butyryl-, and octanoyl-CoA to palmitic acid. We have confirmed these observations and have found that labeled hexanoyl-CoA was also a substrate for these reactions, thereby offering substantial evidence for the sequential addition of 2-carbon units. In order to determine precisely the sequence of the condensing reactions, the distribution of radioactivity in the recovered long chain fatty acids was examined by chemical decarboxylation of the products of the reaction. The results presented in Table III demonstrate that the synthesis of palmitic acid occurred by the condensation of 1 molecule of acetyl-CoA with the methylene carbon atom of malonyl-CoA and that subsequently 6 more molecules of malonyl-CoA were added. The finding that the carboxyl carbon of the labeled reaction product contained one-seventh of the radioactivity occurring during the transfer of H3 from glucose to TPN or in the medium.

\[ \text{Radioactivity in recovered fatty acids} = \frac{1}{7} \times \text{radioactivity transferred from glucose to TPN} \]

Malonyl-CoA Decarboxylase—All of the fractions obtained which catalyzed the biosynthesis of palmitic acid exhibited marked malonyl-CoA decarboxylase activity. The addition of short chain acyl-CoA derivatives suppressed the decarboxylation of malonyl-CoA (Table IV). Kinetic studies indicated that the inhibition of the decarboxylation of malonyl CoA by the various acyl-CoA derivatives was of the noncompetitive type. A plot of the effect of butyryl-CoA on the decarboxylation of malonyl-CoA is shown in Fig. 3. The Michaelis constant for malonyl-CoA is 2.8 X 10^-4 M, and the KI for butyryl-CoA is 4.3 X 10^-4 M. Although malonyl-CoA decarboxylase activity was 82% inhibited by 8 X 10^-4 M p-hydroxymercurobenzoate, neither the decarboxylation of malonyl-CoA nor the suppression of decarboxylase activity by acyl-CoA derivatives was affected by the addition of 5 X 10^-3 M sodium arsenite to the medium.

Studies with TPNH—The incorporation of tritium from TPNH into the synthesized palmitic acid was investigated in an attempt to gain insight into the nature of the reductive process. The results of experiments comparing the conversion of malonyl-C14-CoA with the incorporation of radioactivity from TPNH into palmitic acid are shown in Table V. The results indicate that approximately one-tenth the amount of tritium was incorporated which would have been required if 2 hydrogen atoms were transferred for each molecule of malonyl-CoA converted to fatty acid. The results obtained in the second experiment in Table V indicate that the use of glucose-1-14H as the source of reduced TPN did not markedly depress the conversion of malonyl-C14-CoA to palmitic acid. These data suggest considerable isotopic discrimination against tritium. The experiments do not permit a decision between isotopic discrimination occurring during the transfer of H3 from glucose to TPN or in

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**Table I**

<table>
<thead>
<tr>
<th>Fraction No.</th>
<th>Volume (ml)</th>
<th>Total units</th>
<th>Protein (mg)</th>
<th>Specific activity (units/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Supernatant solution (100,000 x g)</td>
<td>100</td>
<td>480</td>
<td>7200</td>
<td>0.067</td>
</tr>
<tr>
<td>II. Ammonium sulfate, 0.30-0.40</td>
<td>15</td>
<td>400</td>
<td>1200</td>
<td>0.33</td>
</tr>
<tr>
<td>III. Ca(NO3)2, 0.05 M eluate</td>
<td>8</td>
<td>186</td>
<td>52</td>
<td>3.6</td>
</tr>
<tr>
<td>IV. Alumina gel CV supernatant solution</td>
<td>17</td>
<td>147</td>
<td>6.7</td>
<td>22</td>
</tr>
<tr>
<td>V. Alumina gel CV, 0.05 M eluate</td>
<td>8</td>
<td>93</td>
<td>2.4</td>
<td>39</td>
</tr>
</tbody>
</table>

**Fig. 2.** Demonstration of the CoA-dependent oxidation of acetaldehyde with diphaspho- or triphosphopyridine nucleotide.

The incubation mixtures contained 100 pmoles of potassium phosphate buffer (pH 8.0), 1 μmole of MgCl2, 0.5 μmole of MnCl2, and either 0.2 μmole of DPN and 1 mg of protein (Curve A), or 0.2 μmole of TPN and 0.6 mg of protein (Curve B). The reaction was initiated by the addition of 1 μmole of malonyl-CoA. Reduced CoA was added as indicated. The final volume was 1.0 ml, d = 1 cm, and the temperature was 22°C.
Table II  
Effect of added acyl-CoA derivatives on conversion of malonyl-C\textsuperscript{14}-CoA to long chain fatty acids

The conditions were the same as Table I except that unlabeled acyl-CoA derivatives were added as indicated. The enzyme obtained in fractionation Step III (0.5 mg of protein) was used in these experiments.

<table>
<thead>
<tr>
<th>Additions</th>
<th>Malonyl-C\textsuperscript{14}-CoA converted to fatty acid</th>
<th>Experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>None</td>
<td>19</td>
<td>22</td>
</tr>
<tr>
<td>Acetyl-CoA</td>
<td>34</td>
<td>40</td>
</tr>
<tr>
<td>Propionyl-CoA</td>
<td>40</td>
<td>45</td>
</tr>
<tr>
<td>Butyryl-CoA</td>
<td>52</td>
<td>53</td>
</tr>
<tr>
<td>Hexanoyl-CoA</td>
<td>16</td>
<td>19</td>
</tr>
<tr>
<td>Octanoyl-CoA</td>
<td>10</td>
<td>13</td>
</tr>
<tr>
<td>Palmitoyl-CoA</td>
<td>1.2</td>
<td>1.3</td>
</tr>
</tbody>
</table>

Table III  
Distribution of C\textsuperscript{14} in biosynthesized long chain fatty acids

The conditions were the same as in Table I except that unlabeled acetyl-CoA or acetyl-1-C\textsuperscript{14}-CoA (1 \mu per \mu mole) was used instead of butyryl-CoA as indicated. Enzyme Fraction III (0.5 mg of protein) was used in Experiment 1 and enzyme Fraction V (0.06 mg of protein) for Experiment 2. In order to calculate the amount of labeled acetyl-CoA converted to long chain fatty acids, the specific activity of the substrate was corrected for dilution with unlabeled acetyl-CoA which arose from the decarboxylation of malonyl-CoA in the respective preparations.

### Table IV  
Effect of acyl-CoA derivatives on enzymatic decarboxylation of malonyl-1,3-C\textsuperscript{14}-CoA

The incubation mixtures contained 15 \mu moles of potassium phosphate buffer (pH 7.0), 0.6 \mu mole of 2-mercaptoethanol, 176 \mu moles of malonyl-1,3-C\textsuperscript{14}-CoA (140 c.p.m. per \mu mole) and enzyme Fraction IV (16 \mu g of protein), in a total volume of 0.19 ml. The incubation flasks were similar to those employed in the microdecarboxylation experiments and contained 0.25 ml of 1 M Hyamine in methanol in the center well. After incubation for 30 minutes at 37\degree, 1 \mu mole of KHCO\textsubscript{3} was added to the incubation mixture followed by 0.2 ml of 1 M H\textsubscript{2}SO\textsubscript{4}. The C\textsuperscript{14}O\textsubscript{2} was allowed to diffuse for 30 minutes at 38\degree with gentle shaking. The contents of the center well were transferred to a scintillation counting mixture for radioassay.

### DISCUSSION

Malonyl-CoA is an obligate intermediary compound for the biosynthesis of long chain fatty acids which is catalyzed by soluble enzymes obtained from rat and pigeon liver tissue. Evidence has been cited supporting a wide occurrence of this phenomenon in slices of liver tissue and in enzymes obtained from plant cells as well as yeast. The requirement for malonyl-CoA in fatty acid synthesis catalyzed by enzymes from mammary tissue has recently been described (31). Evidence presented in the following paper (32) indicates that the same requirement exists in enzymes obtained from brain. The dependency upon the presence of CO\textsubscript{2} is not restricted to experiments with the use of exogenous acetate as substrate for fatty acid formation. Chernick\textsuperscript{1} has observed that the addition of bicarbonate to an incubation medium with slices of rat liver tissue caused a 10-fold increase in lipogenesis from labeled glucose and a 6-fold increase from labeled fructose. The addition of bicarbonate had no appreciable effect on the amount of either labeled hexose that was oxidized to C\textsubscript{4}O\textsubscript{2}.

Decarboxylation of labeled palmitic acid synthesized from acetyl-C\textsuperscript{14}-CoA or malonyl-C\textsuperscript{14}-CoA indicated that 1 molecule of acetyl-CoA and 7 molecules of malonyl-CoA combine to form palmitic acid. The discovery that acetyl-CoA, butyryl-CoA (12), hexanoyl-CoA, and octanoyl-CoA (12) are incorpor-

\textsuperscript{1}S. S. Chernick, personal communication.
long chain fatty acids are formed by polymerization of molecules 0.6 pmole of 2-mercaptoethanol, enzyme Fraction IV (16 pg of cated in a final volume of 0.19 ml. The incubation time was 30 minutes at 37O.

malonyl-CoA (140 c.p.m. per mmole) as indicated, in the recovered C402. Curve B. The incubation conditions were the same as in Curve A except that the flasks contained 69 mmoles of unlabeled butyryl-CoA and varying concentrations of malonyl-C14-CoA.

sted into palmitic acid does not support the postulate (7) that long chain fatty acids are formed by polymerization of molecules of malonyl-CoA alone to yield a polyketo intermediate.

Trinit from TPNH3 is localized on alternate carbon atoms beginning with the &-carbon of palmitic acid. This finding is consistent with a direct transfer of hydrogen atoms observed by Marcus et al. (33) in the reduction of acetoacetyl pantetheine catalyzed by &-hydroxybutyryl dehydrogenase from pig heart. These investigators also concluded that the enzymatically trans-ferred hydrogen was found on the P-carbon atom of & hydroxybutyric acid. Experiments performed by these investigators with D2O indicated that deuterium was acquired from the medium and was probably localized on the a-carbon atom. The synthesis of fatty acids occurs through two reductive steps (32). Trinit could be incorporated during the reduction of a keto derivative as in the case of acetoacetyl-CoA and acetoacetyl pantetheine (33) or perhaps in the reduction of an &-unsaturated derivative. Hydrogen transferred from the reducing agent in the latter reaction would probably also appear on the &-carbon of the fatty acid (34). The present data do not dis-close in which reductive step(s) the tritium was introduced into the fatty acid. Recent experiments by D'Adamo et al., in which liver was perfused with lactic acid-H3, indicate the presence of tritium on the &-carbon atoms of recovered fatty acids. These findings are in complete accord with the present enzymatic studies, and the significance of these results is emphasized by the observations of Lowenstein et al. (35) that tritiated lactate is a very efficient source of H3 for fatty acid synthesis in rat liver slices.

Our studies have revealed the presence of two CoA-dependent acetalddehyde dehydrogenases in liver tissue for which specific requirements for DPN or TPN have been found. These ob-

TABLE V

Comparison of incorporation of tritium from TPNH3 into palmitic acid with C14 from malonyl-C14-CoA

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Substrate</th>
<th>Labeled</th>
<th>Unlabeled</th>
<th>Incorporation of labeled substrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Malonyl-1-C14-CoA</td>
<td>34.4</td>
<td></td>
<td>C4/HP</td>
</tr>
<tr>
<td>2</td>
<td>Malonyl-1-C14-CoA + glucose-1-H3</td>
<td>30.3</td>
<td>6.39</td>
<td></td>
</tr>
</tbody>
</table>

* The radioactivity of the recovered fatty acids was assayed in the same sample. The values for carbon and tritium were determined by the difference of the energies of the emission spectra.

TABLE VI

Distribution of H3 in palmitic acid

<table>
<thead>
<tr>
<th>Alcohol</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
<th>Average</th>
<th>Carbon of original palmitic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-Pentadecanol</td>
<td>26.25%</td>
<td>55.55%</td>
<td>44%</td>
<td>0.40 α</td>
</tr>
<tr>
<td>1-Tetradecanol</td>
<td>13.33%</td>
<td>11.11%</td>
<td>12.2%</td>
<td>0.40 β</td>
</tr>
<tr>
<td>1-13-tridecanol</td>
<td>3.80%</td>
<td>0.1%</td>
<td>2.0%</td>
<td>0.40 γ</td>
</tr>
<tr>
<td>1-Dodecanol</td>
<td>16.66%</td>
<td>14.44%</td>
<td>15.55%</td>
<td>16.66 δ</td>
</tr>
</tbody>
</table>

a A. F. D'Adamo, Jr., D. Haft, and H. D. Hoberman, personal communication.

Observations indicate the presence of enzymes in mammalian tissue that catalyze a reaction similar to the DPN-specific dehydrogenase discovered by Burton and Stadtman (36) in extracts of Clostridium kluyveri. These findings complement the report of Berry and Stotz (37) that extracts of an acetone powder of rat brain tissue contain a CoA-dependent aldehyde dehydrogenase, although a pyridine nucleotide requirement was not reported by these authors. These results demonstrate the reversibility of the reduction of acetyl-CoA observed earlier in extracts of pigeon liver (8).

The most highly purified enzyme that we obtained from rat liver tissue catalyzed the vigorous decarboxylation of malonyl-CoA and contained varying amounts of P-ketothiolase. A detailed study of the nature of the condensing reaction was therefore not feasible with this preparation. An effort was made to obtain a fatty acid-synthesizing preparation free of these activities, and an enzyme was obtained from young rat brain tissue which fulfilled these requirements. An account of the investi-
gations undertaken with this preparation is contained in the companion communication (32).

SUMMARY

The biosynthesis of palmitic acid was investigated with an enzyme preparation from rat liver which has been purified 580-fold from the supernatant solution obtained by high speed centrifugation of cell-free suspensions of liver tissue.

Acetyl coenzyme A, 1 molecule, and malonyl coenzyme A, 7 molecules, are required for the synthesis of 1 molecule of palmitic acid.

Studies with Tritium-labeled reduced triphosphopyridine nucleotide indicate direct hydrogen transfer to alternate carbon atoms beginning with the C-carbon of palmitic acid.

The most highly purified fraction obtained in these experiments exhibited marked malonyl coenzyme A decarboxylase activity which was noncompetitively inhibited by short chain acyl coenzyme A derivatives.

Coenzyme A-dependent diphosphopyridine nucleotide-specific and triphosphopyridine nucleotide-specific acetaldehyde dehydrogenases have been demonstrated in extracts of rat liver tissue. The participation of these enzymes was not required for the synthesis of palmitic acid from acetyl coenzyme A and malonyl coenzyme A.

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