SYNTHESIS OF BRANCHED-CHAIN AND ODD-NUMBERED FATTY ACIDS FROM MALONYL-CoA

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Recent studies on the mechanism of long chain fatty acid biosynthesis by soluble enzyme preparations have indicated that malonyl-CoA is an intermediate in the conversion of acetyl-CoA to palmitate (Wakil, 1958; Brady, 1958; Lynen, 1959). The principle compound produced by these fatty acid synthesizing systems is palmitate, although small amounts of shorter and longer chain fatty acids have been identified.

Long chain fatty acid synthesis has been studied in soluble enzyme systems derived from rat tissues by measuring the incorporation of radioactivity from 2-C^{14}-malonyl-CoA (Vagelos, 1960) into n-hexane-extractable fatty acids. Enzyme preparations were tested from liver, brain, intestinal mucosa, and epididymal adipose tissue. The enzyme system from epididymal adipose tissue had the highest specific activity by far. This enzyme has been purified about 50-fold by ammonium sulfate precipitation, calcium phosphate gel adsorption, alumina C_Y gel adsorption, and DEAE cellulose column chromatography (Martin, Horning and Vagelos). The synthesis of fatty acids by this enzyme requires acetyl-CoA and TPNH in addition to malonyl-CoA (Table I), as reported in

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Table 1

Long Chain Fatty Acid Synthesis from Malonyl-2-C^{14}-CoA By Rat Adipose Tissue Enzyme

Component omitted	C.p.m/mg. protein
None	23,060
Acetyl-CoA	3,620
DPNH	26,950
TPNH	1,090
TPNH + DPNH	608

Complete system contained 100 µmoles of KPO_4 buffer, pH 7.0, 0.1 µmole of 2-C^{14}-malonyl-CoA (66,000 cpm), 0.1 µmole of acetyl-CoA, 1.0 µmole of TPNH, 1.0 µmole of DPNH, 2 µmoles of 2-mercaptoethanol and 0.5 mg. of enzyme in a total volume of 2.0 ml. Incubated at 30° for one hour under helium.

other systems (Wakil and Ganguly, 1959; Ganguly, 1960), and it is enhanced by the presence of mercaptans. When the enzyme was incubated with 2-C^{14}-malonyl-CoA, acetyl-CoA, and TPNH, the principle product was palmitate (Table II) although myristate and stearate were identified in smaller amounts. The possibility that this enzyme might synthesize branched-chain and odd-numbered fatty acids when branched-chain and odd-numbered fatty acyl-CoA derivatives were substituted for acetyl-CoA was tested.

Propionyl-CoA, isobutyryl-CoA, isocaproyl-CoA, isovaleryl-CoA, and α-methylbutyryl-CoA were synthesized by the method of Wieland and Rueff (Wieland and Rueff, 1953). After the standard incubation (Table II) the reactions were stopped by the addition of sulfuric acid and ethanol. The long chain fatty acids were extracted from the acidified incubation mixtures with three thirty-ml portions of redistilled n-hexane. The combined hexane extracts were washed with water and concentrated to a small volume by evaporation for counting and gas chromatography. The radioactive fatty acids were tentatively identified by gas-liquid

Table II

Long Chain Fatty Acids Synthesized from 2-C^{14}-Malonyl-CoA and an Acyl-CoA Acceptor

Acyl-CoA Acceptor	μMoles incubated	% of 2-C^{14}-malonyl-CoA incorporated	Fatty acid synthesized*
Acetyl-CoA	0.20	63-75	C-16 (c-14, C-18)
Isocaproyl-CoA	0.24	55	iso-C-16 (iso-C-14, C-16)
Isobutyryl-CoA	0.24	64	iso-C-16 (iso-C-14 iso-C-18, C-16)
Isovaleryl-CoA	0.24	55	iso-C-15, iso-C-17 (C-16)
α-Methyl-butyryl-CoA	0.24	65	anteiso-C-15, anteiso-C-17 (C-16)
Propionyl-CoA	0.20	71	C-15 (C-13, C-17)

*Major product as indicated; minor products listed in parenthesis were present in less than 15% level.

The complete system contained potassium phosphate buffer (pH 7.0) 100 μmoles; 2-C^{14}-malonyl-CoA, 0.38-40 μmoles (sp. act. 1 μc/μmole); TPNH, 0.5 μmoles; β-mercaptoethanol, 2.5 μmoles; enzyme, 0.5 mg., an acyl-CoA acceptor as listed. The final volume was adjusted to 2 ml. with water. After incubating at 30° C for one and one-half hours under helium the reaction was stopped by adjusting the pH to 1 with sulfuric acid and adding two ml. of ethanol.

chromatography along with authentic carrier fatty acids. The radioactivity of the effluent peaks was measured by trapping the material in a tube containing anthracene crystals coated with D.C. silicone oil 550 according to the method developed by Karmen and Tritch (Karmen and Tritch, 1960). To aid in identifying the the radioactive products (8-10γ of fatty acid were synthesized in each incubation) 40γ of an appropriate fatty acid series was added as carrier.

The results of an experiment in which isocaproyl-CoA was substituted for acetyl-CoA are shown in Fig. 1. Carrier fatty acids included for gas chromatography were iso-C-16, C-16, iso-C-14, C-14, and C-12. The major radioactive product was

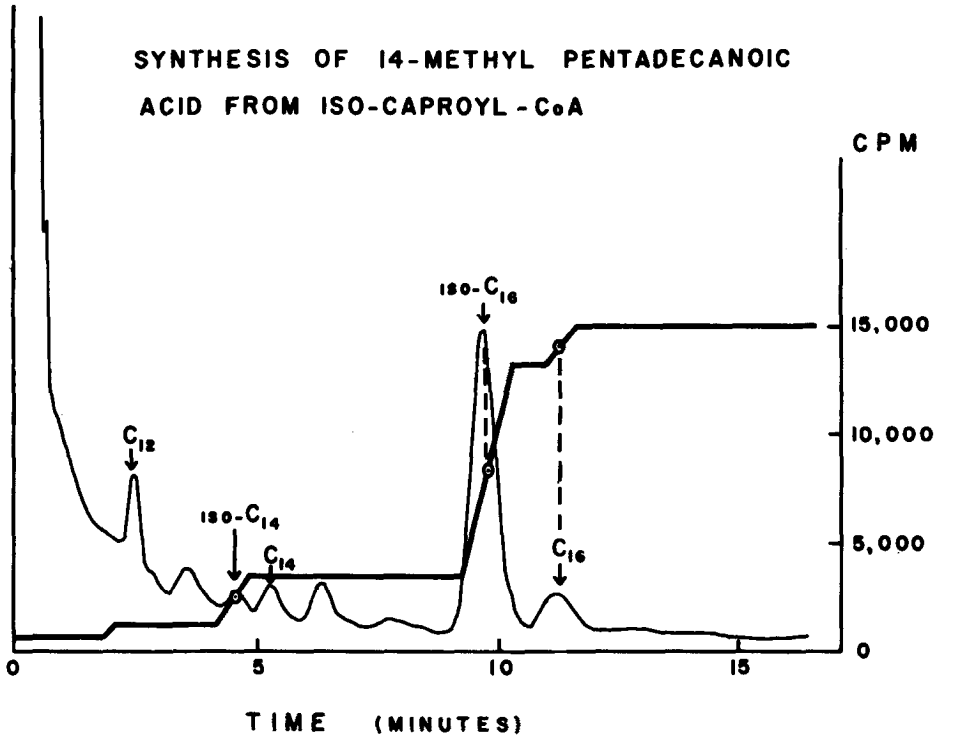

Fig. 1. Synthesis of fatty acids from isocaproyl-CoA and 2-C^{14}-malonyl-CoA under the experimental conditions in Table II.

The heavy line is the integral record of the radioactivity of the effluent peaks. An ethyleneglycol-succinate column was used at 155° C.

14-methylpentadecanoic acid (iso-C-16) although small amounts of iso-C-14 and C-16 were formed. Acetyl-CoA, formed by enzymatic decarboxylation of the malonyl-CoA, probably accounted for the small amount of palmitate that was produced. Similarly, when the acyl-CoA compound substituted for acetyl-CoA was isobutyryl-CoA, isovaleryl-CoA, α-methylbutyryl-CoA or propionyl-CoA, the major respective product(s) were iso-C-16, iso-C-15 and iso-C-17, anteiso-C-15 and anteiso-C-17, or C-15 (Table II). Since the positions of the methyl groups were deduced only on the basis of data from vapor phase chromatography, these assignments should be considered tentative (Woodford and van Gent, 1960).

It is clear that this enzyme preparation synthesized iso-,

anteiso-, and odd-numbered acids instead of the usual palmitate when branched-chain and odd-numbered fatty acyl compounds were substituted for acetyl-CoA. Furthermore, the extent of malonyl-CoA conversion to long chain fatty acids was essentially the same with all the different acyl-CoA compounds tested. The structure of the product was determined by the acyl-CoA acceptor. In every case only one acceptor unit entered the final product.

These results are in agreement with the findings of Wakil (Wakil and Ganguly, 1959) who found that only one fatty acyl-CoA compound is incorporated into the long chain fatty acid and that the remainder of the carbon chain is contributed by malonyl-CoA. The incorporation of these various CoA esters into long chain fatty acids by the rat adipose tissue enzyme suggests that such a reaction may account for the presence of these unusual fatty acids in mammalian tissue (Woodford and van Gent, 1960; Weitkamp, et al, 1947; Wheatley, 1957). The amino acids, valine, leucine and isoleucine could be the source of isobutyryl-CoA, isovaleryl-CoA and α-methylbutyryl-CoA respectively (Robinson, et al, 1957; Bachhawat, et al, 1955; Robinson, et al, 1956).

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