Biosynthesis of fatty acids by soluble enzyme fractions*

The synthesis of long-chain fatty acids from acetate in a soluble extract of pigeon liver was first reported by BRADY AND GURIN in 1952[4]. The activity of this extract or of a charcoal-treated extract was stimulated by addition of citrate, ATP**, CoA, Mg^{++} and DPN[4,5]. In 1955 POPJÁK AND TIETZ[6] showed that an extract of mammary gland clarified by high-speed centrifugation catalyzed the synthesis of fatty acids from acetate when supplemented with ATP, CoA, α-ketoglutarate and DPN. Later HELE AND POPJÁK[7] developed a spectrophotometric assay for fatty acid synthesis based on the oxidation of DPNH in the presence of acetate, ATP, and CoA. Recently LANGDON[8] has implicated TPN as a requirement for fatty acid synthesis carried out in an extract of rat liver.

The present communication deals with the reconstruction of fatty acid synthesis with a system of three separate enzyme fractions from pigeon liver (any one of which is inactive when tested alone) and seven essential cofactors. Under optimal conditions one μmole of acetate is incorporated into long-chain fatty acids per mg of protein in two hours at 38°.

In the routine assay system (carboxy-^{14}C) acetate is incubated under nitrogen with the enzyme system and cofactors at 38°. The mixture is then saponified, acidified and extracted with pentane. Aliquots are taken from the pentane fraction for plating and counting.

The centrifugate (100,000 *g*) of a pigeon liver homogenate[4] (Waring blendor) is the starting point for the preparation of three active ammonium sulfate fractions: A, 0–25% saturation; B, 25–40% and C, 50–65%. The 40–50% saturation fraction is discarded. After dialysis, A is purified further by treatment with calcium phosphate gel while B and C are subfractionated with ethanol at low temperatures. The specific enzyme activity of the purified system is 50-fold greater than the original unfractionated extract. The rate of fatty acid synthesis is a linear function of the concentration of each of the three fractions within a restricted range. Details of the assay procedure and methods of purification will be published in full[9].

The reconstructed system synthesizes a mixture of long-chain fatty acids which in a typical experiment consist of 65% palmitic, 22% myristic, 12% lauric and traces of stearic and decanoic acids[10]. The long-chain fatty acids were identified by R_F values in a paper chromatographic system[11] (acetic acid-kerosene) and their relative concentrations were determined by measuring the radioactivity of the appropriate spots. Another paper chromatographic system[12] (ammonia-butanol) was employed for the separation of short-chain acids from long-chain acids. When palmitic acid (synthesized by the above enzyme system from (carboxy-^{14}C) acetate) is decarboxylated[13], the barium carbonate obtained has 1.7 times the specific radioactivity (per carbon atom) as the whole fatty acid molecule—a finding consistent with the assumption of successive condensations of acetate units[14,15].

A component study[16] for the reconstructed system is shown in Table I. ATP, CoA, DPNH, *iso*citrate, manganese and GSH are absolute requirements whereas activity persists in the absence of TPN. Lipoic acid stimulates synthesis in less purified enzyme systems but is apparently without effect in the more purified system. Similarly, glucose-1-phosphate and DPN are absolute requirements when relatively crude enzyme fractions are employed but become fully replaceable with DPNH in the purified enzyme system.

*iso*Citrate may be replaced only by components which can generate *iso*citrate, *e.g.* citrate plus aconitase or a mixture of α-ketoglutarate, TPNH and CO_2 (synthesis of *iso*citrate by reversal of the oxidative decarboxylation of *iso*citrate). TPNH alone does not replace *iso*citrate. The optimal concentration for CoA is relatively low (0.02 to 0.05 μmoles/ml). Higher levels inhibit synthesis almost completely. While the presence of GSH is mandatory for synthesis other SH compounds such as cysteine or 2,3-dimercapto-1-propanol (BAL) can replace GSH almost completely. Preincubation of the enzyme fractions for 15 minutes at 38° with cysteine (in the absence of cofactors and acetate) abolishes the lag phase which is usually observed during the first 15 to 30 minutes.

Recently, a reconstructed system of enzymes has been prepared from chicken liver which shows virtually the same properties as the pigeon liver system[17]. From the standpoint of a ready supply of material and of ease in processing, the chicken liver preparation is the one of choice.

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** The following abbreviations are used: ATP, adenosine triphosphate; CoA, coenzyme A; DPN and DPNH, oxidized and reduced diphosphopyridine nucleotide; TPN and TPNH, oxidized and reduced triphosphopyridine nucleotide; GSH, glutathione.

In fraction C the presence of the four enzymes which can convert acetate to crotonyl CoA (according to the reactions which have been shown to apply in fatty acid oxidation[18]) is readily demonstrable by various lines of evidence such as the acetyl CoA-dependent oxidation of DPNH[19] (with formation principally of crotonyl CoA and β-hydroxybutyryl CoA). Whether this group of enzymes is involved in fatty acid synthesis has yet to be decided. There are two reactions catalyzed by the pigeon liver system which have not been demonstrated with the purified enzymes of the fatty acid oxidation sequence[20]. (1) In fraction C a DPNH-dependent condensation of acetyl CoA with a higher acyl CoA resulting in the formation of the corresponding β-hydroxy and β-enoyl acyl CoA and (2) in fraction B, a TPNH-dependent reduction of crotonyl CoA to butyryl CoA[8].

As yet there is no unambiguous direct or circumstantial evidence that fatty acid synthesis proceeds by the reversal of the oxidative pathway. Indeed it has been difficult to effect appreciable incorporation of labelled acetyl CoA[4] or butyryl CoA into the long-chain acids. Although there are several features which suggest that the synthetic pathway may be the reverse of oxidation of fatty acids[7], acyl CoA derivatives of longer chain length than butyrate have not been formed by incubating acetyl CoA with the purified enzymes of the fatty acid oxidation sequence[21].

TABLE I

COMPONENTS OF FATTY ACID SYNTHESIS

Reagent omitted	*μmoles acetate incorporated*	*Reagent omitted*	*μmoles acetate incorporated*
None	0.20	Mn^{++}	0.02
ATP	0.00	GSH	0.00
CoA	0.01	TPN	0.10
DPNH	0.03	Lipoic	0.10
*iso*Citrate	0.00	Mg^{++}	0.26

The complete system which contained all components listed plus acetate (2.0 μmoles), phosphate buffer (pH 6.5) and enzyme (pigeon liver fractions) in a volume of 0.5 ml was incubated for two hours at 38°.

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DAVID M. GIBSON
MIRIAM I. JACOB*
JOHN W. PORTER*
ALISA TIETZ*
SALIH J. WAKIL

Institute for Enzyme Research, University of Wisconsin, Madison, Wis. (U.S.A.)

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* Postdoctoral Trainee of the National Heart Institute, National Institutes of Health.