FACTORS AFFECTING FATTY ACID SYNTHESIS IN CELL-FREE PREPARATIONS FROM SACCHAROMYCES CEREVISIAE*

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In earlier work from this laboratory, it was shown that fatty acid synthesis in yeast preparations is strongly influenced by carbon dioxide (Klein, 1957) and by a particulate fraction sedimentable from postmitochondrial supernatants (Klein and Booher, 1956; Klein, 1960; Abraham et al., 1961; Den and Klein, 1961; Klein, 1963). Working with animal extracts, other workers have emphasized that citrate and other biochemical intermediates can exert profound effects on fatty acid formation, and several hypotheses based upon such interactions have been formulated (Vagelos et al., 1963; Tubbs and Garland, 1963; Bortz and Lynen, 1963; Vagelos, 1964; Howard and Lowenstein, 1964).

It is the purpose of this report to indicate that yeast preparations are also subject to controlling influences by certain intermediates of the oxidative and fermentative metabolism of glucose.

Saccharomyces cerevisiae, strain LK2G12, was grown and aerated as previously described (Klein, 1957) and disrupted in an Aminco French pressure cell (Klein, 1963). The high-speed supernatant (H.S.S.) obtained after centrifugation at 85,000 × G for 60 min. was dialyzed overnight against 0.1 M potassium phosphate buffer, pH 6.5, containing 0.5 mM reduced glutathione. This preparation was used in all

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experiments in which the synthesis of long-chain fatty acids was measured.* For determination of acetyl-CoA carboxylase activity, the dialyzed 0-30% ammonium sulfate fraction of the H.S.S. was used.

Citrate, which is known to stimulate fatty acid synthesis in animal preparations (Vagelos, 1964), profoundly increased the rate of incorporation of acetate into fatty acids (Table I). That citrate does

TABLE I

Effect of Citrate on the Incorporation of 1-C^{14}-Acetate Into Long-Chain Fatty Acids

Citrate (mM)	Acetate Incorporation (mμmoles/hr)
1.0	200
2.5	284
5.0	296
10.0	352
25.0	992
50.0	1772

Incubation mixture contained in a final volume of 2 ml the following: 20 mg supernatnat protein, 150 μmoles potassium phosphate buffer (pH 6.5), 2 μmoles 1-C^{14}-acetate (9.6×10^5 cpm), 5 μmoles ATP, 20 μmoles creatine phosphate, 0.5 mg creatine phosphokinase, 0.5 μmoles $NADP^+$, 20 μmoles glucose-6-phosphate,* 0.1 μmole CoA, 120 μmoles HCO_3^-, and 5 μmoles $MnCl_2$. Samples were incubated for 15, 30, and 60 min and the rates of incorporation were estimated from the linear portion of the curves.

*Other experiments have shown that the glucose-6-phosphate keeps the NADP in a reduced form throughout the incubation period.

not stimulate by chelation of metals is probable since versene, over a concentration range of 0.02 mM to 2 mM (where it became inhibitory), did not increase the rate of fatty acid formation. Isocitrate affected

*Methods for isolating major lipid fractions are similar to those used previously (Klein, 1957, 1960), except that following hydrolysis of the incubated suspensions, the hydrolysates were first acidified and extracted with petroleum ether to remove both fatty acids and nonsaponifiable lipids. Analysis by gas chromatography indicated that all the radioactive fatty acids extracted in this manner were 12-18 carbons long, chain lengths of 16 and 18 accounting for 75-80% of the products.

the yeast preparation in a manner analogous to citrate, while α-keto-glutarate had no effect. As is the case for the compounds referred to below, citrate and isocitrate did not stimulate the synthesis of nonsaponifiable lipids.

L-α-glycerophosphate was also very effective in stimulating fatty acid synthesis (Table II). Concentrations of the order of 1 mM are evidently optimal in this stimulation. Other related compounds that were found to stimulate in this manner are glucose-6-phosphate and fructose-1.6-diphosphate, both of which might be converted to α-glycero-phosphate in these preparations.

TABLE II

Effect of L-α-Glycerophosphate on the Incorporation of 1-C^{14}-Acetate Into Long-Chain Fatty Acids and Nonsaponifiable Lipids

L-α-Glycerophosphate (mM)	Acetate Incorporation (mμmoles)	
	Fatty Acids	Nonsaponifiables
0	156	104
0.8	532	122
4.0	625	83
8.0	645	82
16.0	711	62

Incubation mixture contained in a final volume of 2.0 ml the following: 20 mg H.S.S., 150 μmoles potassium phosphate buffer (pH 6.5), 2 μmoles 1-C^{14}-acetate (2×10^6 cpm), 5 μmoles ATP, 20 μmoles creatine phosphate, 0.5 mg creatine phosphokinase, 4 μmoles NADPH, 0.1 μmole CoA, 120 μmoles HCO_3^-, 5 μmoles $MnCl_2$; and where indicated, L-α-glycerophosphate. Samples were incubated for 15 min. Acetate incorporation is linear for at least 30 min. under these conditions.

Stimulation by L-α-glycerophosphate has been observed by Howard and Lowenstein (1964) in a rat liver system containing supernatant and microsomes. On the basis of their observations, these authors suggested that this compound may act by removing inhibitory amounts of acyl-CoA via glyceride synthesis, since long-chain acyl-CoA compounds were shown to inhibit fatty acid synthesis in animal systems (Porter and Long, 1958; Tubbs and Garland, 1963; Bortz and Lynen, 1963). This hypothesis was