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; Deu 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 synth[illegible] measured.* For determination of [illegible]yl [illegible], the dialyzed 0-30% ammonium sulfate [illegible] the H.S.S. [illegible].

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 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 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.

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

*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.

to inhibit fatty acid synthesis [illegible] (Wakil and Ghosh, 1958; Tubbs and Garland, 1963; Bortz and Lynen, [illegible]). This hypothesis was tested in our system by incubating palmityl-CoA with H.S.S. plus cofactors in the presence and absence of L-α-glycerophosphate. Table III illustrates that, although palmityl-CoA severely inhibited fatty acid formation, L-α-glycerophosphate failed to reverse this inhibition. It would appear, therefore, that the mechanism of stimulation by L-α-glycerophosphate in these preparations is not by the reversal of acyl-CoA inhibition. This conclusion is supported by the recent report of Kuhn and Lynen (1965) that, in baker's yeast, the enzyme required for phosphatidic acid synthesis is located exclusively in a particulate fraction of the cell. Table III also shows that palmityl-CoA inhibits the synthesis of nonsaponifiable lipids in addition to fatty acids. Furthermore, the incorporation of malonyl CoA into fatty acids was also found to be impaired by low concentrations of palmityl-CoA, although the incorporation of acetate into fatty acids is more sensitive to palmityl-CoA. Our results thus suggest that inhibition by palmityl-CoA may be relatively nonspecific. In this connection, it has been found that the inhibition of the citrate condensing enzyme by palmityl-CoA (Wieland and Weiss, 1963; Tubbs, P. K., 1963) is apparently caused by nonspecific binding of the acyl-CoA to the enzyme (Srere, personal communication).

Rate studies carried out with 1-C^{14}-acetate, 1-C^{14}-acetyl-CoA, and 1,3-C^{14}-malonyl-CoA demonstrated that the preparations employed here incorporate malonyl-CoA into fatty acids approximately 10 times faster than either acetate or acetyl-CoA. This finding suggested that the stimulation of fatty acid synthesis from acetate by citrate or

L-α-glycerophosphate might be at [illegible] action. Accordingly, these compounds were [illegible] effect on the incorporation of bicarbonate into [illegible].

Table IV contains the results of [illegible] in which citrate, fructose-1.6-diphosphate and L-α-[illegible]glycerophosphate were each found to stimulate the carboxylation of acetyl-CoA, thus supporting the contention noted above. Figure 1 contains scans for radioactivity in the products formed during this experiment, and shows that in each case the incorporated radioactivity travels as a single peak with the R_f of malonyl-CoA.

Summary. Long-chain fatty acid synthesis was demonstrated in high-speed supernatants from yeast. Citrate, glucose-6-phosphate, fructose-1.6-diphosphate, and L-α-glycerophosphate were found to stimulate fatty acid synthesis several-fold. The site of stimulation appears to be the carboxylation of acetyl-CoA. The mechanism of citrate stimulation is not known. Palmityl-CoA inhibited both fatty acid and nonsaponifiable lipid synthesis. However, L-α-glycerophosphate failed to reverse the inhibition, suggesting that glycerophosphate stimulation of fatty acid synthesis is not merely a reversal of acyl-CoA inhibition.

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TABLE I

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

Citrate (mM)	Acetate Incorporation (mμmoles/mg)
1.0	200
2.5	[illegible]
5.0	[illegible]
10.0	[illegible]
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.

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.

TABLE III

Effect of Palmityl-CoA and L-α-glycerophosphate on Lipid Synthesis

ADDITIONS	[illegible]	INCORPORATION (cpm/moles)		
	[illegible]	EXPERIMENT II		
	FATTY ACIDS	NON-SAP.	FATTY ACIDS	NON-SAP.
NONE	31	15	37	36
L-α-GLYCEROPHOSPHATE				
10 mM ...	90	11	74	27
25 mM ...	-	-	103	18
PALMITYL-CoA				
0.2 mM ...	3	7	3	15
0.6 mM ...	1	< 1	1	2
2.0 mM ...	< 1	< 1	-	-
0.2 mM PALMITYL-CoA				
+10 mM L-α-GLYCEROPHOSPHATE	2	4	1	7
+25 mM L-α-GLYCEROPHOSPHATE	-	-	2	2

Experiment I

Incubation mixtures contained in a final volume of 0.5 ml the following: 6 mg dialyzed H.S.S. protein, 13 μmoles potassium phosphate buffer (pH 6.5), 1.3 μmoles ATP, 5.5 μmoles creatine phosphate, 2.2 units creatine phosphokinase, 0.026 μmoles CoA, 1.5 μmoles $MnCl_2$, 1 μmoles NADPH, 30 μmoles HCO_3^-, and 0.5 μmole 1-C^{14}-acetate (2×10^6 cpm/μmole). Samples were incubated for 1 hr.

Experiment II

Same incubation mixture as Experiment I except that 1 μmole of 1-C^{14}-acetate (2×10^6 cpm/μmole) was used.

Table IV

Effect of Citrate, Fructose-1.6-[illegible]osp[illegible] and L-α-Glycerophosphate on Acetyl-CoA Carbo[illegible]

Additions		Bicarbonate Incorporation (cpm)	
-		+ Acetyl-CoA	- Acetyl-CoA
None		14,[illegible]00	240
Citrate (60 mM)		240,000	240
Fructose-1.6-PO_4	(30 mM)	88,000	-
	(60 mM)	152,000	2000
L-α-Glycero-PO_4	(3 mM)	148,000	-

Incubation mixtures contained the following: 0-30% ammonium sulfate fraction (2 mg protein), 19 μmoles potassium phosphate buffer (pH 6.5), 2 μmoles ATP, 1 μmole $MnCl_2$, 5 μmoles creatine phosphate, 2 e.u. of creatine phosphokinase, 1 μmole acetyl-CoA where indicated, 20 μmoles of C^{14}-bicarbonate (1×10^6 cpm/μmole), and additions as indicated in the Table. The samples were incubated in a final volume of 0.4 ml for 90 min. at 25 C at which time excess dowex 50w-x8(H^+) was added to bring the pH to approximately 2. The mixtures were then centrifuged and an aliquot of the supernatant plated and counted.

Fig. 1. Nonvolatile radioac[illegible]ion. An aliquot of the products form[illegible] IV was chromatographed overnigh[illegible] buffer (Stadtman, 1957). Strip[illegible] radioactivity. (1) authentic [illegible]-C[illegible] incorporated in the presence of citrate (2) [illegible] incorporated in the presence of fructose-diPO_4 (4) radioactivity incorporated in the presence of glycero-PO_4.

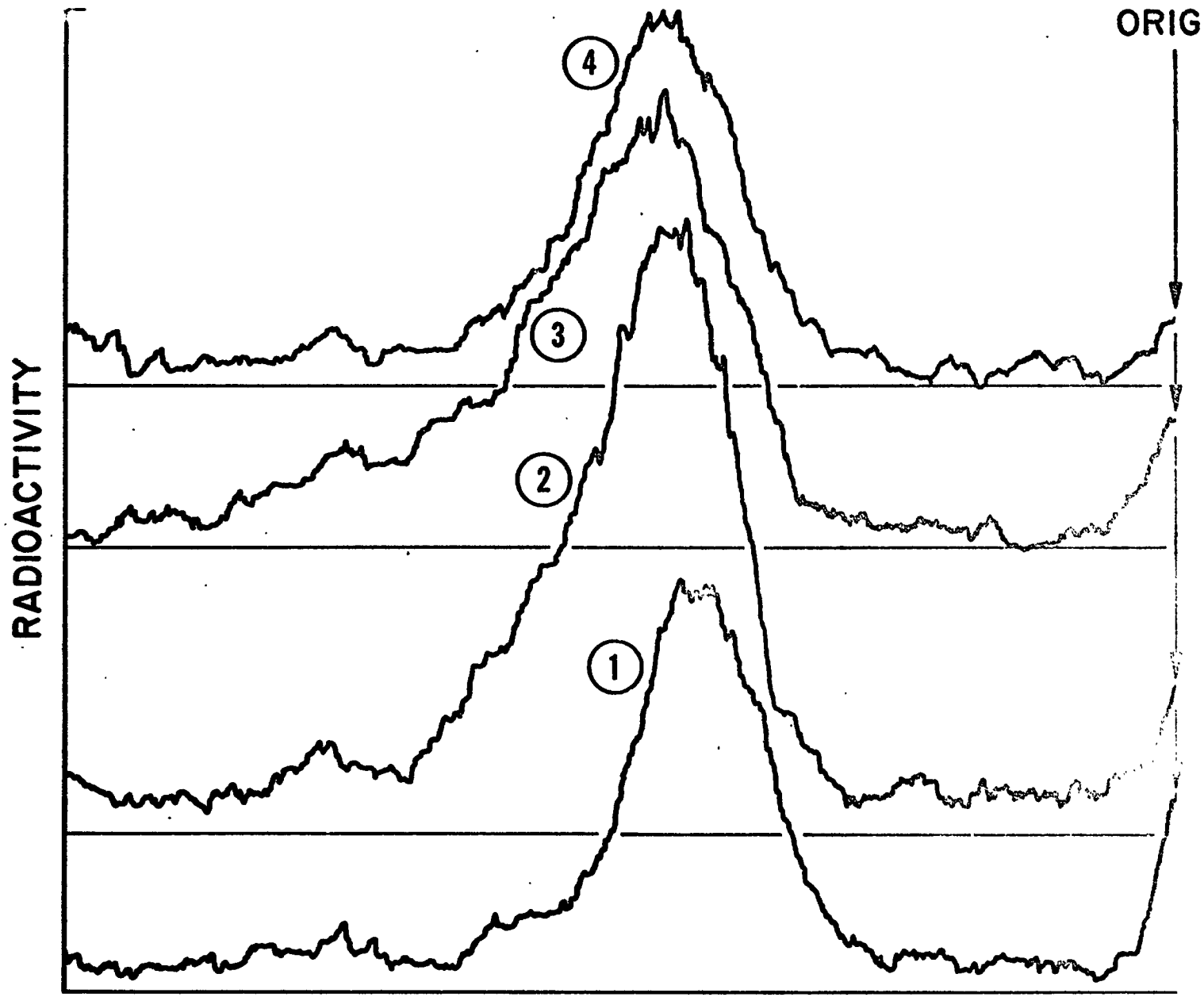

Fig. 1

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