LOCALIZATION OF THE STIMULATING EFFECT OF ISOCITRATE ON FATTY ACID SYNTHESIS BY RAT LIVER HOMOGENATE FRACTIONS*

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The stimulating effect of citrate, isocitrate and aconitate on fatty acid synthesis by liver homogenate fractions is well established. Previous reports from our laboratory (Abraham et al., 1960a, 1960b) and others (Brady and Gurin, 1952; Porter et al., 1957) have shown that, in addition to TPNH generation and bicarbonate production, a further mechanism must be involved in the isocitrate effect on lipogenesis. The exact nature of the additional mechanism has not been clarified (Lynen, 1961). Some experiments bearing on it are presented here. The findings recorded in Table I demonstrate that the unknown mechanism is localized at the level of conversion of acetyl-CoA to malonyl-CoA. Replacement of glucose-6-phosphate and its dehydrogenase by isocitrate and its dehydrogenase for TPNH generation resulted in a threefold increase in fatty acid synthesis by the supernatant fraction alone, and a more than tenfold increase when the microsomal fraction was added to the supernatant fraction. This was the case with acetate and acetyl-CoA as substrates. (It should be noted that the potential TPNH generation was the same with glucose-6-phosphate or isocitrate.) On the other

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hand, the conversion of malonyl-CoA to fatty acids by either the supernatant or the supernatant plus microsome system did not show this isocitrate effect.

TABLE I

EFFECT OF TPNH GENERATING SYSTEMS ON THE CONVERSION OF ACETATE-1-C^{14}, ACETYL-1-C^{14}-CoA AND MALONYL-1,3-C^{14}-CoA TO FATTY ACIDS BY HOMOGENATE FRACTIONS PREPARED FROM NORMAL RAT LIVER

24 μmoles of glycylglycine-KOH buffer (pH 7.5), 1 μmole of $KHCO_3$, 7 μmoles of $MgCl_2$, 0.1 μmole of $MnCl_2$, 6 μmoles of reduced glutathione (K^+ salt), 4.8 μmoles of ATP (K^+ salt), 0.05 μmole of TPN and either 2 μmoles of glucose-6-phosphate (Na^+ salt) or 4 μmoles of d,l potassium isocitrate were incubated with either a) 0.5 μmole potassium acetate-1-C^{14} (1.7×10^5 CPM) plus 0.01 μmole CoA or b) 0.3 μmole acetyl-1-C^{14}-CoA (4.1×10^4 CPM) or c) 0.15 μmole malonyl-1,3-C^{14}-CoA (3.2×10^4 CPM) plus 0.03 μmole acetyl-CoA, in the presence of the homogenate fractions indicated below, for 30 minutes at 37° with air as the gas phase. In the experiments with microsomes alone, purified glucose-6-phosphate dehydrogenase or isocitric dehydrogenase was added to the appropriate incubation mixture so that the TPNH production was the same as in the experiments with the supernatant fractions. Final volume of each incubation mixture was 0.4 ml. The fatty acids were isolated and assayed for C^{14}-activity as described in (Abraham et al., 1961b).

Homogenate fraction		Labeled substrate	mμmoles of fatty acids synthesized per mg protein per 30 minutes in the presence of:	
mg supernatant protein	mg microsome protein		Glucose-6-phosphate	Isocitrate
3.0	0	Acetate-1-C^{14}	0.10	0.30
3.0	0	Acetyl-1-C^{14}-CoA	0.10	0.30
3.0	0	Malonyl-1,3-C^{14}-CoA	19.0	21.9
3.0	0.6	Acetate-1-C^{14}	0.20	4.0
3.0	0.6	Acetyl-1-C^{14}-CoA	0.36	4.0
3.0	0.6	Malonyl-1,3-C^{14}-CoA	18.6	20.0
0	1.5	Acetate-1-C^{14}	0	0
0	1.5	Acetyl-1-C^{14}-CoA	0	0
0	1.5	Malonyl-1,3-C^{14}-CoA	4.8	4.8

Table I also shows that the recently described rat liver microsomal system capable of fatty acid synthesis from malonyl-CoA but not from acetate or acetyl-CoA (Abraham et al., 1961a) responds equally to glucose-6-phosphate and isocitrate in the presence of their respective dehydrogenases and TPN. It is of interest to note that the fatty acid synthesizing system contained in the supernatant or in the supernatant plus microsome system is at least four times more active than the microsome system when malonyl-CoA is the substrate.

Since, in the presence of isocitrate, the rate of conversion of malonyl-CoA to fatty acids is about 100 times that of the conversion of either acetate or acetyl-CoA to fatty acids, these new experiments may be interpreted as indicating that a transcarboxylase reaction (Abraham et al., 1960a; Hülsmann, 1960; Numa et al., 1961), such as

acetyl-CoA + oxalosuccinate $\longrightarrow$ malonyl-CoA + α ketoglutarate,

is involved in the conversion of acetyl-CoA to fatty acids by the rat liver supernatant and by the supernatant plus microsomal system. However, several types of experiments designed to demonstrate such a transcarboxylase reaction were not successful.

With our relatively crude rat liver fatty acid synthesizing systems, we have observed that ATP is a necessary component for conversion of malonyl-CoA to fatty acids (Table II). This finding is, of course, surprising because, on theoretical grounds, the synthesis of palmitate or palmityl-CoA from malonyl-CoA should not require ATP (Lynen, 1961; Bressler and Wakil, 1961). The exact nature of the lipids synthesized by these liver systems is now under investigation.

TABLE II

EFFECT OF ATP ON CONVERSION OF MALONYL-1, 3-C^{14}-CoA TO FATTY ACIDS BY SUPERNATANT FRACTION AND MICROSOMES PREPARED FROM NORMAL RAT LIVER HOMOGENATES

56 mμmoles of malonyl-1, 3-C^{14}-CoA (2.7 x 10^4 CPM) plus 11 mμmoles of acetyl-CoA were added to each incubation mixture. Other experimental details and incubation conditions are described in Table I.

Homogenate fraction		ATP added	Incorporation of C^{14} from malonyl-1, 3-C^{14}-CoA into fatty acids in presence of:	
mg supernatant protein	mg microsome protein		Glucose-6-phosphate	Isocitrate
		μmoles	CPM	CPM
1.0	0	0	350	730
1.0	0	4.8	2230	2160
0	3.4	0	180	210
0	3.4	4.8	1760	1670

REFERENCES

Abraham, S., Chaikoff, I. L., Bortz, W. M., Klein, H. P., and Den, H., Nature, 192, 1287 (1961a).

Abraham, S., Matthes, K. J., and Chaikoff, I. L., J. Biol. Chem., 235, 2551 (1960a).

Abraham, S., Matthes, K. J., and Chaikoff, I. L., Biochem. Biophys. Res. Comm., 3, 646 (1960b).

Abraham, S., Matthes, K. J., and Chaikoff, I. L., Biochim. Biophys. Acta, 49, 268 (1961b).

Brady, R. O., and Gurin, S., J. Biol. Chem., 199, 421 (1952).

Bressler, R., and Wakil, S. J., J. Biol. Chem., 236, 1643 (1961).

Hülsmann, W. C., Biochim. Biophys. Acta, 45, 623 (1960).

Lynen, F., Fed. Proc., 20, 941 (1961).

Numa, S., Matsuhashi, M., and Lynen, F., Biochem. Z., 334, 203 (1961).

Porter, J. W., Wakil, S. J., Tietz, A., Jacob, M. I., and Gibson, D. M., Biochim. Biophys. Acta, 25, 35 (1957).