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Biology Department, Brandeis University, Waltham, Mass. (U.S.A.) HAROLD P. KLEIN*

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* Present address: Exobiology Division, NASA, Moffett Field, Calif. (U.S.A.).

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Inhibition of citrate formation by long-chain acyl thioesters of coenzyme A as a possible control mechanism in fatty acid biosynthesis

Various workers[1–3] have reported that the carboxylation of acetyl-coenzyme A, yielding malonyl-CoA, is the rate-limiting step in fatty acid synthesis. Citrate or isocitrate are required for this synthesis in liver extracts[2,4], and it has now been shown by LYNEN and coworkers[5] that these, or related acids, are absolutely required by rat-liver acetyl-CoA carboxylase (acetyl-CoA:CO_2 ligase, EC 6.4.1.2). The activity of the carboxylase from other tissues is also stimulated[6]. Thus citrate and isocitrate may play a vital role in the regulation of fatty acid synthesis.

Lipogenesis is impaired in livers from fasted or diabetic rats, in which, although oxaloacetate levels are normal[7,8] and acetyl-CoA may be elevated[9], citrate is much diminished in amount[10], suggesting that the activity of citrate synthase (citrate oxaloacetate-lyase (CoA-acetylating), EC 4.1.3.7, formerly known as citrate condensing enzyme) may be reduced. Liver contains much less of this enzyme than does, for example, heart muscle[11].

It has been found[12] that livers from normally fed rats contain 30–60 mμmoles of long-chain acyl-CoA per gram wet weight, but that fasting increases this to 80–180 mμmoles/g. Refeeding fasted animals with fat causes a further increase, while sugar refeeding causes a rapid drop to values below the controls. Fig. 1 shows that palmitoyl-CoA, in a concentration range comparable to these tissue levels of long-chain acyl-CoA, is a profound inhibitor of citrate synthase. Free coenzyme A and palmitate do not cause this inhibition, which is not relieved by an increase in acetyl-CoA concentration. It thus appears possible that the increase in long-chain acyl-CoA which occurs in some conditions may, by reducing citrate formation, result in the inhibition of acetyl-

CoA carboxylase and hence of fatty acid synthesis; this would be an example of a feedback regulation system. Inhibition in liver of citrate synthase and the carboxylase may divert acetyl-CoA towards acetoacetate formation. It has also been found[12] that palmitoyl-CoA inhibits the "synthetase" step of fatty acid synthesis, namely the reductive condensation of two-carbon units from malonyl-CoA with acetyl-CoA as primer.

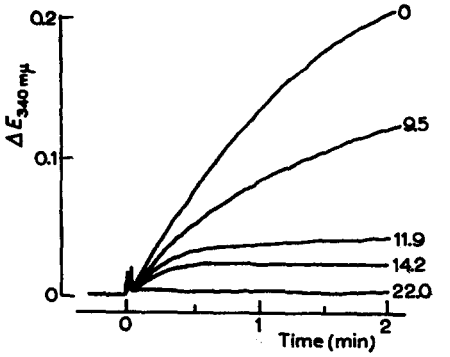

Fig. 1. Inhibition of citrate synthase by palmitoyl-CoA. Citrate synthase activity was measured by following NAD reduction in a coupled system containing: Tris–HCl buffer (pH 7.8), 45 mM; L-malate, 25 mM; NAD, 0.25 mM; acetyl-CoA, 0.09 mM; excess purified malate dehydrogenase (EC 1.1.1.37). Numbers indicate mμmoles of palmitoyl-CoA per ml of reaction mixture. The reaction was started by addition of 5.8 μg of crystalline citrate synthase. Final volume 2 ml; temperature 25°; light path 1 cm.

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Department of Biochemistry, University of Cambridge, Cambridge (Great Britain) P. K. TUBBS

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