native acetyltransferase component of the enzyme complex contains at its C-terminus a valine residue that is partly removed by proteolysis during the isolation of fatty acid synthetase from yeast cells.

With respect to the genetics of the fatty acid synthetase complex from yeast, experiments by Schweizer *et al.* (1971) will be reviewed that suggest that several faggenes are linked and co-ordinatedly transcribed.

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Regulatory Mechanisms for Liver Acetyl-Coenzyme A Carboxylase

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Acetyl-CoA carboxylase plays a critical role in the control of fatty acid synthesis. Several lines of evidence indicate that the regulation at this carboxylation step is achieved by changes both in the quantity of the carboxylase and in the catalytic efficiency per enzyme molecule (see Numa *et al.*, 1970). It appears that the former mechanisms are more involved in the long-term control, whereas the latter mechanisms are more responsible for the short-term control.

Previous studies (Numa *et al.*, 1969; Majerus & Kilburn, 1969; Nakanishi & Numa, 1970) disclosed that the variations in the level of acetyl-CoA carboxylase activity in liver extracts derived from rats under different dietary and hormonal conditions are determined by changes in the quantity of the carboxylase protein. It was also shown that the increase or decrease in the carboxylase quantity in re-fed or alloxan-diabetic rats is due to differences in the rate of enzyme synthesis, whereas the decrease in the carboxylase quantity in starved animals is due both to diminished enzyme synthesis and to accelerated enzyme degradation. In the present work we have attempted to define the mechanisms underlying the increase in the level of liver acetyl-CoA carboxylase activity in genetically obese mice (C57BL/6J-*ob*). It was demonstrated that there were no qualitative differences between the enzymes derived from obese mice and non-obese mice (C57BL/6J), in terms of immunological and kinetic properties. Combined immunochemical and isotopic techniques revealed that the rate of enzyme synthesis per liver was 7.7-fold higher in obese mice than in non-obese mice. The rate constant of enzyme degradation was found to be 1.7-fold smaller in obese mice than in non-obese mice, the half-lives being 115h and 67h

respectively. These results indicate that the increase in the content of liver acetyl-CoA carboxylase in obese mice is due mainly to a rise in the rate of enzyme synthesis and in a minor degree to a decrease in the rate constant of enzyme degradation.

To investigate the mechanisms by which the catalytic efficiency of acetyl-CoA carboxylase molecules is controlled, we made kinetic studies on the reaction mechanism as well as on the allosteric activation by citrate. The rat liver enzyme purified to homogeneity were employed. Previous results of initial-velocity and product-inhibition studies (Hashimoto *et al.*, 1970; Hashimoto & Numa, 1971) indicated that the carboxylase reaction proceeds through the Bi Bi Uni Uni Ping Pong mechanism, the order of addition of substrates being ATP, HCO_3^- and acetyl-CoA in the forward reaction, and malonyl-CoA, P_i and ADP in the reverse reaction. It was also shown that the site of citrate action during the carboxylase catalysis lies predominantly on the carboxylated enzyme, the dissociation constant of the carboxybiotinyl-enzyme-citrate complex being 3–5 mM. In the present investigations the reaction mechanism was corroborated by analysis of the inhibition patterns obtained with kynurenate, which was found to be a novel dead-end inhibitor acting on the uncarboxylated enzyme. The site of citrate action during the carboxylase catalysis was confirmed by studies on initial rates of malonyl-CoA- ^{14}C acetyl-CoA exchange. On the other hand the dissociation constant of the uncarboxylated enzyme-citrate complex was estimated to be 3–14 μM in terms of ^{14}C citrate binding, protection against avidin inactivation, protection against trypsin activation and shift in the protomer-polymer equilibrium. This explains why the citrate effect on the uncarboxylated enzyme does not manifest itself during the carboxylase catalysis. These findings also indicate that the carboxylation of the biotinyl prosthetic group gives rise to a conformational change at the citrate site, resulting in a marked decrease in the affinity of the enzyme for the activator.

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