*LIVER ACETYL COA CARBOXYLASE: INSIGHT INTO THE MECHANISM OF ACTIVATION BY TRICARBOXYLIC ACIDS AND ACETYL COA**

By Elena Ryder,† Carlo Gregolin,‡ Huei-Che Chang, and M. Daniel Lane§

DEPARTMENT OF BIOCHEMISTRY, NEW YORK UNIVERSITY SCHOOL OF MEDICINE

Communicated by Severo Ochoa, March 24, 1967

Recent investigations in this laboratory have shown[1, 2] that the isocitrate- (or citrate-) activated form of liver acetyl CoA carboxylase (E.C. 6.4.1.2) is a large protein structure having a molecular weight of about four million. Electron microscopic examination of the carboxylase in the presence of isocitrate reveals[1] that it has a filamentous structure with dimensions of 80–100 Å by up to 5000 Å. Reversible dissociation to inactive protomeric subunits (molecular weight 409,000) occurs[2] at low enzyme concentration in the carboxylation assay reaction mixture in the absence of isocitrate or at higher enzyme concentration in the presence of 0.5 *M* NaCl at pH 8.0. Reconstitution to the active filamentous form can be accomplished either by addition of isocitrate (or citrate) or by removal of NaCl and introduction of isocitrate by dialysis.

The carboxylation of acetyl CoA catalyzed by liver acetyl CoA carboxylase involves the following two partial reactions (reactions 1 and 2):

$$\text{ATP} + \text{HCO}_3^- + \text{Enz} \overset{\text{Mg}^{2+}}{\rightleftharpoons} \text{Enz-CO}_2^- + \text{ADP} + \text{P}_i \quad (1)$$

$$\text{Enz-CO}_2^- + \text{acetyl CoA} \rightleftharpoons \text{Enz} + \text{malonyl CoA} \quad (2)$$

$$\text{Over-all: ATP} + \text{HCO}_3^- + \text{acetyl CoA} \overset{\text{Mg}^{2+}}{\rightleftharpoons} \text{ADP} + \text{P}_i + \text{malonyl-CoA}. \quad (3)$$

The occurrence of these reactions has been well documented by appropriate exchange reactions,[3] by direct demonstration of enzyme-$C^{14}O_2^-$ formation[4, 5] (from enzyme, ATP, $HC^{14}O_3^-$, and Mg^{2+} or enzyme and 3-C^{14}-malonyl CoA), and by carboxyl transfer from enzyme-$C^{14}O_2^-$ to acetyl CoA to form malonyl CoA.[4, 5] It has been observed by Matsuhashi *et al.*[3] and confirmed in our laboratory that $P^{32}{}_i$-ATP exchange (reaction 1) and C^{14}-acetyl CoA-malonyl CoA exchange (reaction 2) as well as the over-all carboxylation and decarboxylation (reaction 3) exhibit essentially an absolute requirement for isocitrate (or citrate). Moreover, as shown below, isocitrate activates an acetyl CoA-dependent decarboxylation of malonyl CoA and the decarboxylation of enzyme-CO_2^-. Although certain gross structural changes[1, 2, 6] of acetyl CoA carboxylase associated with isocitrate (or citrate) activation have been characterized, the mechanism by which these changes are translated into increased catalytic activity has not been clarified. The present investigation was undertaken to gain further insight into the activation mechanism.

Experimental Procedure.—Acetyl CoA carboxylase was prepared in homogeneous form from chicken liver and acetyl CoA carboxylation (C^{14}-bicarbonate fixation assay), and C^{14}-acetyl CoA-malonyl CoA exchange rates were determined as previously described.[1, 2] Protein was determined according to the method of Lowry *et al.*[7]

unless otherwise specified and can be converted to refractometrically determined protein by multiplying by a factor[2] of 1.24. Unlabeled malonyl CoA was prepared according to the method of Trams and Brady[8] and purified by *O*-(diethylaminoethyl) cellulose (DEAE-cellulose) chromatography using a linear LiCl gradient (0.01–0.25 *M*) containing 3×10^{-3} *M* HCl. 3-C^{14}-malonyl CoA (specific activity, 6.4×10^6 cpm per μmole) was enzymatically synthesized from acetyl CoA and $HC^{14}O_3^-$ in the presence of acetyl CoA carboxylase and the other components of the carboxylase assay system. After inactivation of the carboxylase, the unreacted ATP was removed by incubation with glucose and hexokinase to avoid partial coelution with malonyl CoA in subsequent chromatography. The labeled malonyl CoA was then chromatographically purified as described above. Avidin was obtained from Worthington Biochemical Corp. and was found to bind 12 μg of (+)-biotin per mg when assayed by the dye-displacement technique described by Green.[9] All other preparations and methods not described herein were as previously reported.[1, 2]

Malonyl CoA decarboxylation reaction: The rate of malonyl CoA decarboxylation was determined using a basic reaction mixture which contained the following components (in μmoles, unless specified): Tris (Cl^-) buffer, pH 7.5, 30; 3-C^{14}-malonyl CoA (specific activity, 6.4×10^6 cpm per μmole), 0.02; acetyl CoA, 0.03; potassium DL-isocitrate, 5; glutathione (GSH), 1.5; ethylenediaminetetraacetate (EDTA), 0.1; and acetyl CoA carboxylase, 4–12 μg, in a total volume of 0.5 ml. The reaction was initiated by the addition of enzyme, allowed to proceed for ten minutes at 25°, and was terminated by addition of 0.1 ml of 6 *N* HCl. An aliquot was taken to dryness in a scintillation counting vial at 85° for 30 minutes, water and scintillator were added, and residual acid-stable C^{14}-activity was determined using a liquid scintillation spectrometer.

Preparation of enzyme-$C^{14}O_2^-$: Enzyme-$C^{14}O_2^-$ was prepared by incubating acetyl CoA carboxylase (1–2 mg of protein) with the following components (in μmoles): Tris (Cl^-) buffer, pH 7.5, 70; ATP, 2.5; $MgCl_2$, 10; $NaHC^{14}O_3$ (specific activity, 20 μc per μmole), 10; potassium DL-isocitrate, 2; and GSH, 4, in a total volume of 1.2 ml for one minute at 37°. After the addition of 30 μmoles of potassium EDTA (pH 7), the mixture was applied to a 2- × 25-cm Sephadex G-25 column equilibrated with 0.01 *M* Tris, pH 8.2 (at 4°) containing 0.1 mM EDTA and 5 mM 2-mercaptoethanol. The column was eluted (at 4°) with the same buffer and the eluate fractions containing enzyme-$C^{14}O_2^-$ were pooled. The enzyme was carboxylated to the extent of 95–100 per cent as calculated from the stoichiometric relationship between protein content and alkali-stable radioactivity. The efficiency of "-$C^{14}O_2^-$" transfer from enzyme-$C^{14}O_2^-$ to acetyl CoA (to form 3-C^{14}-malonyl CoA) in the presence of isocitrate and acetyl CoA at room temperature ranged from 45 to 55 per cent. Incomplete transfer is attributable to the rather rapid rate of decarboxylation of enzyme-$C^{14}O_2^-$ in the presence of acetyl CoA and isocitrate (see *Results*).

Results.—Two classes of activator effects are expected in allosteric systems,[10] namely, V_m or K_m effects, which bring about variations in catalytic activity or affinity for substrate (or cofactor). The effect of isocitrate activation of the chicken liver acetyl CoA carboxylase on the V_m or K_m values for acetyl CoA, ATP-Mg, and HCO_3^- was investigated.[11] In each case isocitrate activation was associated with

an increased maximum velocity. While isocitrate activation has no apparent effect on the K_m values for acetyl CoA, deviations from linearity in Lineweaver-Burk analysis render uncertain the effect of isocitrate on the K_m values for ATP-Mg and HCO_3^-. Our observations with the chicken liver carboxylase which does not require preincubation with isocitrate for activation are in basic agreement with those of Numa *et al.*[12] who studied the "cold-labile" rat liver carboxylase which has a preincubation requirement.

The fact that $P^{32}{}_i$-ATP exchange (reaction 1) which has no acetyl CoA requirement, that C^{14}-acetyl CoA-malonyl CoA exchange (reaction 2) and the malonyl CoA decarboxylation reaction which have no ATP, Mg^{2+}, nor HCO_3^- requirement, and that the enzyme-CO_2^- decarboxylation which has no other requirements exhibit isocitrate activation, supports the view that a V_m rather than K_m effect is of primary kinetic importance in isocitrate activation. Investigation of two of these reactions, namely the acetyl CoA-dependent decarboxylation of malonyl CoA and the decarboxylation of enzyme-CO_2^-, permitted the study of minimal reaction sequences responsive to isocitrate. These investigations are described in the next sections.

Activation of malonyl CoA decarboxylation by isocitrate and acetyl CoA: In the course of an investigation of the C^{14}-acetyl CoA-malonyl CoA exchange, it was observed that acetyl CoA carboxylase catalyzes an isocitrate- and acetyl CoA-dependent decarboxylation of malonyl CoA. The rate of this reaction (0.09 μ-mole per minute per mg of refractometrically determined protein at 25°) is approximately 2.5 and 10 per cent as rapid as the over-all carboxylation (reaction 3) and C-^{14}-acetyl CoA-malonyl CoA exchange reactions, respectively. As illustrated in Figure 1, carboxylase by itself supports only a slow rate of malonyl CoA decarboxylation, which is essentially unaffected by acetyl CoA, but is increased somewhat by addition of isocitrate. A marked activation (seven- to ten-fold) of the carboxylase-catalyzed decarboxylation results when both isocitrate (or citrate) and acetyl CoA are present simultaneously. A synergism between isocitrate and acetyl CoA is evident from the autocatalytic character of the kinetics of decarboxylation in the presence of isocitrate (Fig. 1). That this is due to the generation of acetyl CoA from the decarboxylation reaction *per se* is demonstrated by the disappearance of "autocatalysis" and reversion to zero-order kinetics when an acetyl CoA-trapping system (arsenate and phosphotransacetylase or oxaloacetate and citrate condensing enzyme) is added. The K_m values for citrate ($K_m = 7 \times 10^{-5}$ M) and DL-isocitrate ($K_m = 3 \times 10^{-4}$ M) in this reaction are considerably lower than in the over-all forward reaction (K_m's = 3 to 6×10^{-3} M). It was

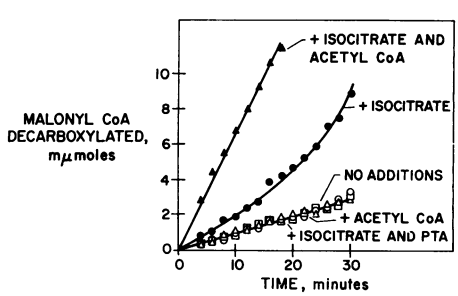

FIG. 1.—Kinetics of malonyl CoA decarboxylation. The decarboxylation of 3-C^{14}-malonyl CoA was determined as described under *Experimental Procedure* except that each reaction mixture contained 2.5 μmoles of potassium arsenate and 8.8 μg of acetyl CoA carboxylase (specific activity, approximately 8 units per mg) in a final volume of 0.5 ml. Variable additions were: potassium DL-isocitrate, 5 μmoles; acetyl CoA, 0.03 μmole; and crystalline phosphotransacetylase (PTA), 5 μg (specific activity, 1200 units per mg).

pointed out earlier[2] that K_m values for the tricarboxylic acid activator are dependent upon the presence and concentrations of other reaction mixture components (particularly malonyl CoA and ATP-Mg) which promote dissociation of the carboxylase. The relatively low K_m for citrate under these conditions is probably attributable to the low malonyl CoA concentration and, as will be shown later, to the presence of acetyl CoA. The K_m values for acetyl CoA and malonyl CoA in this reaction are 3×10^{-6} M and 1.7×10^{-5} M, respectively. Like all of the other acetyl CoA carboxylase-catalyzed reactions studied in our laboratory, malonyl CoA decarboxylation is avidin-sensitive, is not activated by tricarballylate, and is activated by malonate to the extent of 35–50 per cent that by comparable concentrations of isocitrate (or citrate.)

The isocitrate- and acetyl CoA-activated decarboxylation of malonyl CoA can be described by the following minimal reaction sequence which is consistent with our experimental findings:

$$\begin{array}{ccccccc} & & & & \overset{CO_2^-}{|} & & \overset{CO_2^-}{|} \\ & (1') & & (2') & & (3') & \\ E + \text{M-CoA} & \rightleftharpoons & E(\text{M-CoA}) & \rightleftharpoons & E\ (\text{A-CoA}) & \rightleftharpoons & E + \text{A-CoA}. \\ & & & & \downarrow\ (4') & & \downarrow\ (5') \\ & & & & E(\text{A-CoA}) + CO_2 & & E + CO_2 \end{array}$$

E represents the enzyme; M-CoA, malonyl CoA; and A-CoA, acetyl CoA. C^{14}-acetyl CoA-malonyl CoA exchange, which involves only the reversible reactions 1′, 2′, and 3′, is considerably faster than the decarboxylation of malonyl CoA under comparable conditions either in the presence or in the absence of isocitrate. Therefore, either reaction 4′ or reaction 5′, both of which involve the decarboxylation of a "carboxylated" enzyme species, must be rate-limiting in malonyl CoA decarboxylation. Since removal of acetyl CoA by a trapping system (e.g., arsenate and phosphotransacetylase or oxaloacetate and citrate-condensing enzyme) drastically reduces the rate of malonyl CoA decarboxylation, it is apparent that $\overset{CO_2^-}{|}\!E$ (A-CoA) undergoes decarboxylation (reaction 4′) more rapidly than $\overset{CO_2^-}{|}\!E$ (reaction 5′). More direct evidence from studies on the decarboxylation of enzyme-CO_2^- *per se* which are described in the next section, supports the contention that both isocitrate and acetyl CoA affect the reactivity of the labile carboxyl group of enzyme-CO_2^- and hence its susceptibility to decarboxylation.

Activation of the decarboxylation of enzyme-CO_2^- by isocitrate and acetyl CoA: The above-mentioned investigations on the decarboxylation of malonyl CoA indicated that isocitrate and acetyl CoA activate the rate-limiting step, i.e., the decarboxylation of enzyme-CO_2^-. These findings were corroborated in a more direct manner by following the rate of decarboxylation of enzyme-$C^{14}O_2^-$ (1′-N-C^{14}-carboxybiotinyl enzyme) *per se* in the presence and absence of isocitrate and/or acetyl CoA. The results of ten experiments revealed that isocitrate at a concentration (10 mM) which maximally activates the over-all carboxylation reaction, also activates the decarboxylation of enzyme-CO_2^- (at pH 7.5 and temperatures ranging from 11 to 25°). This is reflected in the shortening of the half life of enzyme-CO_2^-

at 25° from 20 minutes to 6–7 minutes and corresponds to a three-fold increase in the first-order decarboxylation rate (see Fig. 2). An even greater increase (about eight-fold) in the "apparent" decarboxylation rate is promoted by low concentrations (5 μM) of acetyl CoA. When both isocitrate and acetyl CoA are present, the rate of enzyme-CO_2^- decarboxylation is increased by at least 24-fold as shown in Figure 2. The latter rate actually exceeds the upper reliability limit for this method. It should be noted that in the experiments in which acetyl CoA was added (5 μM), the equilibrium described by reactions 1′, 2′, and 3′ in the preceding section would be rapidly established. Hence, the method used to follow the decay of "-$C^{14}O_2^-$" in this case measures the sum of the rates of decay of enzyme-$C^{14}O_2^-$ and 3-C^{14}-malonyl CoA. This leads to an underestimation of the rate of decarboxylation of enzyme-$C^{14}O_2^-$ since the concentration of the rate-determining species, i.e., enzyme-CO_2^-, would be lower (calculated to be approximately 50% lower initially)[13] in the presence of acetyl CoA than in its absence. It is is apparent from the experiments described that the simultaneous presence of isocitrate and acetyl CoA renders enzyme-CO_2^- more susceptible to decarboxylation, presumably due to conformational changes in the carboxylase which enhance the reactivity of the 1′-N-carboxyl group of carboxybiotinyl-enzyme. Evidence indicating that conformational changes occur in the vicinity of the biotinyl prosthetic group of the carboxylase is presented in the next section.

Accessibility of the biotinyl prosthetic group to avidin in the activated carboxylase; correlation to the state of carboxylase aggregation: Avidin, the specific biotin-binding protein from egg white, forms extremely tight stoichiometric complexes with biotin[14, 15] ($K_D = 10^{-15}$ M) and the biotinyl moiety of enzymes.[16] The rate of inactivation of acetyl CoA carboxylase by avidin would be expected to be influenced by changes in the environment of the biotinyl prosthetic group. Evidence is presented in Table 1 that isocitrate, which induces large conformational changes in the carboxylase, markedly protects the enzyme from inactivation by avidin. Partial protection from avidin by acetyl CoA is also evident. Even more striking, however, is the nearly complete protection that results when both isocitrate and acetyl CoA are present. The effect appears synergistic since more than additive protection results; in other experiments with different avidin and carboxylase

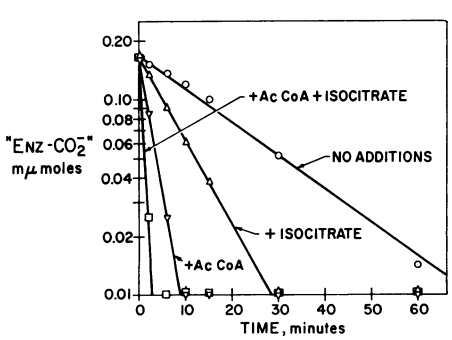

FIG. 2.—Kinetics of "enzyme-CO_2^-" decarboxylation. Enzyme-$C^{14}O_2^-$ (150 μg, 0.37 mμmole), prepared as described in *Experimental Procedure* and prewarmed at 25° for 2 min was incubated at 25° in a reaction mixture containing (in μmole): Tris (Cl^-), pH 7.5, 84, GSH, 4; and EDTA, 0.13 in a total volume of 1.4 ml. Potassium DL-isocitrate, 14 μmoles, and acetyl CoA, 7 mμmoles, were added as shown in the fig. At each time indicated, a 0.2-ml aliquot was withdrawn and added to 0.2 ml of "transfer" reaction mixture containing 4 μmoles of potassium DL-isocitrate and 0.075 μmole of acetyl CoA. One min at room temperature was allowed for "-$C^{14}O_2^-$" transfer to acetyl CoA, after which 0.1 ml of 6 N HCl was added. cid-stable C^{14}-activity (as 3-C^{14}-malonyl CoA) was then determined as described in *Experimental Procedure*. The ordinate in the fig., i.e., mμmoles of "Enz-$C^{14}O_2^-$", represents acid-stable C^{14}-activity as malonyl CoA after the transfer reaction (see text for explanation).

TABLE 1

SUSCEPTIBILITY OF ACETYL CoA CARBOXYLASE TO INACTIVATION BY AVIDIN

Additions to preincubation	Carboxylase activity after 20 min preincubation (units × 10^3)	Additions to preincubation	Carboxylase activity after 20 min preincubation (units × 10^3)
None	64	Citr + avidin	46
i-Citr	66	Mal + avidin	12
Avidin	2	Tricarb + avidin	2
i-Citr + avidin	44	Citr + Ac CoA + avidin	61
Ac CoA + avidin	10	Mal + Ac CoA + avidin	27
i-Citr + Ac CoA + avidin	66	Tricarb + Ac CoA + avidin	9
i-Citr + Mal CoA + avidin	1		

Acetyl CoA carboxylase (12 μg) was preincubated at 0° in a toal volume of 0.5 ml in the presence of 50 μmoles of Tris (Cl^-), pH 7.5; 5 μmoles of GSH; and 0.1 μmole of EDTA, with the following variable additions: 100 μg of avidin, 5 μmoles of di- or tricarboxylic acid, 0.1 μmole of acetyl CoA, and 0.1 μmole of malonyl CoA. After 20 min aliquots were withdrawn and assayed by the standard carboxylation assay procedure except that 25 μg of (+)-biotin were added to the reaction mixture.

Abbreviations: i-Citr, DL-isocitrate; Ac CoA, acetyl CoA; Mal CoA, malonyl CoA; Citr, citrate; Mal, malonate; and Tricarb, tricarballylate.

concentrations, this effect is more evident. Although the experiment illustrated in Table 1 was conducted at 0°, essentially the same pattern was obtained at 25°. Citrate is as active as isocitrate, malonate is only partially active, and tricarballylate is completely inactive in preventing inactivation of the carboxylase by avidin. A similar pattern of activation of the carboxylase-catalyzed carboxylation reaction is observed for these di- and tricarboxylic acids. It has been established[2] that a competitive kinetic relationship exists between malonyl CoA and isocitrate and also between malonyl CoA and acetyl CoA. Malonyl CoA which promotes[2] the dissociation of the polymeric to the protomeric form of the carboxylase also increases (Table 1) its susceptibility to inactivation by avidin. ATP-Mg (HCO_3^-) has an effect similar to that of malonyl CoA. These results indicate that the biotin prosthetic group is more susceptible to avidin in the protomeric than in the polymeric form of the enzyme and that a conformational change at the active site is probably associated with the transition between these forms.

The possibility that the protective effect of acetyl CoA is associated with an aggregation of the carboxylase was investigated by the sucrose-density-gradient centrifugation technique. It was evident that acetyl CoA alone causes a limited, but reproducible, increase in the sedimentation velocity of the carboxylase. Furthermore, in the presence of a comparatively low citrate concentration (0.1 mM), acetyl CoA increases the sedimentation velocity to a value nearly equivalent to that attained by a saturating level of citrate (10 mM). This suggests that acetyl CoA, which is capable of supporting only a limited degree of aggregation of the carboxylase, intensifies the aggregational action of a less than saturating citrate concentration.

Discussion and Summary.—Chicken liver acetyl CoA carboxylase has previously been shown[1, 2] to exist in several forms, as a 409,000-molecular-weight protomeric unit and as polymeric forms ranging from four to eight million molecular weight. Preliminary investigations[11] indicate that the protomeric unit is composed of four polypeptide chains of approximately 100,000 molecular weight. Isocitrate promotes the aggregation of the inactive protomeric unit to yield a catalytically active polymeric form (four million molecular weight). The present investigation shows that isocitrate (or citrate) is a positive allosteric effector of the V_m type according to the classification of Monod *et al.*[10]

The mechanism by which isocitrate promotes catalytic activity has been explored by studying malonyl CoA and enzyme-CO_2^- decarboxylation since these constitute minimal reactions which are uncomplicated by a requirement for ATP, ADP, P_i, HCO_3^-, or Mg^{2+}. While these decarboxylations are comparatively slow with respect to the over-all acetyl CoA carboxylation reaction, variations in their rates are indicative of the reactivity of the labile-carboxy group of enzyme-CO_2^-. It has been found that isocitrate activates the decarboxylation of malonyl CoA and also shortens the half life of enzyme-CO_2^-. In addition to isocitrate, the decarboxylation of malonyl CoA exhibits a nearly absolute requirement for acetyl CoA. Furthermore, acetyl CoA alone markedly activates the decarboxylation of enzyme-CO_2^- ($\rightarrow$ enzyme + CO_2) and in combination with isocitrate leads to at least a 24-fold increase in the rate of this decarboxylation. This strongly suggests that both isocitrate and acetyl CoA increase the reactivity of the labile carboxy group of the carboxylated enzyme. These results are interpretable in terms of a mechanism in which it is visualized that the conformational change induced by isocitrate brings a proton-donating group into closer proximity with the ureido carbonyl of the biotinyl prosthetic group. Protonation of the ureido carbonyl oxygen would lead to polarization of the N-carboxy C-N bond. This promotes labilization of the N-carboxy group and accounts for the activation of both decarboxylation and transfer to acetyl CoA. Since acetyl CoA causes a limited, but reproducible, aggregation of the carboxylase it may, like isocitrate, lead to conformational changes which have the same net effect on the relationship of the biotinyl moiety to adjacent groups. That activation by isocitrate and acetyl CoA is accompanied by conformational changes in the vicinity of the biotinyl prosthetic group is supported by the protection from avidin inactivation afforded by these activators. This suggests that in the presence of isocitrate (or citrate) and acetyl CoA the biotinyl prosthetic group is less accessible to avidin possibly because of greater shielding by neighboring groups.

The authors wish to express their appreciation to Dr. Severo Ochoa for his encouragement during the course of this investigation and to Miss Inge Grumm for her skillful technical assistance.

* This investigation was supported by research grant AM-09116 from the National Institutes of Health, United States Public Health Service and was also aided by a grant from the Jane Coffin Childs Memorial Fund for Medical Research.

† Supported by the Instituto Venezolano de Investigaciones Cientificas and Facultad de Medicina, Universidad del Zulia, Venezuela.

‡ Fellow of the Jane Coffin Childs Memorial Fund for Medical Research, on leave of absence from the University of Padua, Padua, Italy.

§ Career Development Awardee (United States Public Health Service research career program award, K3-AM-18487).

[1] Gregolin, C., E. Ryder, A. K. Kleinschmidt, R. C. Warner, and M. D. Lane, these PROCEEDINGS, **56**, 148 (1966).

[2] Gregolin, C., E. Ryder, R. C. Warner, A. K. Kleinschmidt, and M. D. Lane, these PROCEEDINGS, **56**, 1751 (1966).

[3] Matsuhashi, M., S. Matsuhashi, and F. Lynen, *Biochem. Z.*, **340**, 263 (1964).

[4] Waite, M., and S. J. Wakil, *J. Biol. Chem.*, **237**, 2750 (1962).

[5] Numa, S., E. Ringelmann, and F. Lynen, *Biochem. Z.*, **340**, 228 (1964).

[6] Numa, S., E. Ringelmann, and B. Riedel, *Biochem. Biophys. Res. Commun.*, **24**, 750 (1966).

[7] Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).

[8] Trams, E., and R. O. Brady, *J. Am. Chem. Soc.*, **82**, 2972 (1960).

[9] Green, N. M., *Biochem. J.*, **94**, 23C (1965).

[10] Monod, J., J. Wyman, and J. P. Changeux, *J. Mol. Biol.*, **12**, 88 (1965).

[11] Ryder, E., C. Gregolin, H. C. Chang, and M. D. Lane, unpublished observations to be reported elsewhere.

[12] Numa, S., E. Ringelmann, and F. Lynen, *Biochem. Z.*, **343**, 243 (1965).

[13] Calculated for the initial equilibrium established upon addition of acetyl CoA, enzyme + malonyl CoA $\rightleftharpoons$ enzyme-CO_2^- + acetyl CoA, using a K′ of 35 determined experimentally for a similar reaction by Wood, H. G., H. Lochmüller, C. Riepertinger, and F. Lynen, *Biochem. Z.*, **337**, 247 (1963).

[14] Melamed, M. D., and N. M. Green, *Biochem. J.*, **89**, 591 (1963).

[15] Green, N. M., *Biochem. J.*, **89**, 599 (1963).

[16] Kaziro, Y., and S. Ochoa, in *Advances in Enzymology*, ed. F. F. Nord (New York: Interscience Publishers, 1964), vol. 26, p. 283.