THE ACETYL CoA-MALONYL CoA CONDENSATION REACTION IN RAT LIVER DURING STARVATION AND FAT-FREE REFEEDING

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It has been postulated that the CO_2 which is evolved during fatty acid synthesis from malonyl CoA occurs during a concerted "condensation-decarboxylation" reaction preceeding the TPNH reductive steps (Vagelos, 1959; Vagelos and Alberts, 1960b). One cycle may be presented by the following equations:

$$(1)\quad RCH_2COSCoA + HOOCCH_2COSCoA \rightleftharpoons [RCH_2COCH_2CO^-] + CO_2 + [2\ CoA]$$

$$(2)\quad [RCH_2COCH_2CO^-] + 2\ TPNH + 2H^+ \rightleftharpoons [RCH_2CH_2CH_2CO^-] + 2TPN^+ + H_2O$$

Lynen (1961) and Alberts and Vagelos (1961) have provided evidence for an enzyme-bound β-keto acyl intermediate in the first step. Other investigators have some indication that a free intermediate exists (Steberl, Wasson and Porter, 1960; Pujari, 1961; Bressler and Wakil, 1961; Wakil, 1961). An acyl CoA-dependent net production of CO_2 from malonyl CoA can be demonstrated in purified preparations from Clostridium kluyveri (Vagelos and Alberts, 1960a), from rat brain (Brady, 1960), from pigeon liver (Pujari, 1961) and from rat liver (Hubbard, Allmann, McLain, and Gibson, 1961).

We have previously reported that the specific activity and yield of the enzyme system that catalyzes the synthesis of long chain fatty acids from

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malonyl CoA is markedly depressed in liver from starved rats (Gibson and Hubbard, 1960). This activity returns to normal levels after refeeding the starved animal with a balanced stock diet. However, levels up to 10 times normal are observed if a fat-free diet is employed (Hubbard, Allmann, McLain and Gibson, 1961; Gibson, Allmann and Lingeman, 1961). It was therefore of interest to determine whether or not the wide fluctuations in the activity of the fatty acid synthesizing system observed in these nutritional states are also reflected in the condensation-decarboxylation reaction between malonyl CoA and acetyl CoA.

As a control in the present study, the rate of fatty acid synthesis was measured in the presence of TPNH, malonyl CoA and acetyl CoA in three ways: (1) CO_2 evolution from 1, 3-C^{14}-malonyl CoA, (2) TPNH oxidation, and (3) incorporation of 2-C^{14}-malonyl CoA into fatty acids. As expected, all three rate values were similarly depressed in starvation and greatly elevated after fat-free refeeding.

By measuring the evolution of $C^{14}O_2$ from 1, 3-C^{14}-malonyl CoA in the presence of acetyl CoA but in the absence of TPNH, the condensation-decarboxylation reaction as shown in equation 1 was followed. In Figure 1, fatty acid synthesis from malonyl CoA is compared with the condensation-decarboxylation reaction. In both systems, the specific enzyme activities are decreased in starvation and greatly increased in fat-free refeeding. Although the magnitude of the rate is much less, the condensation-decarboxylation activity varies in the same direction as overall fatty acid synthesis.

In Figure 2, the complete fatty acid synthesizing system is compared with the condensation-decarboxylation reaction in livers from rats which had been starved and then refed a fat-free diet for the hours indicated on the abscissa. The condensation-decarboxylation activity (third curve from top) increased on refeeding a fat-free diet in the same manner as the fatty acid synthesizing activity (first and second curves). The ratio of specific activity values remained relatively constant during the emergence of new enzyme activity. These findings are consistent with the postulate

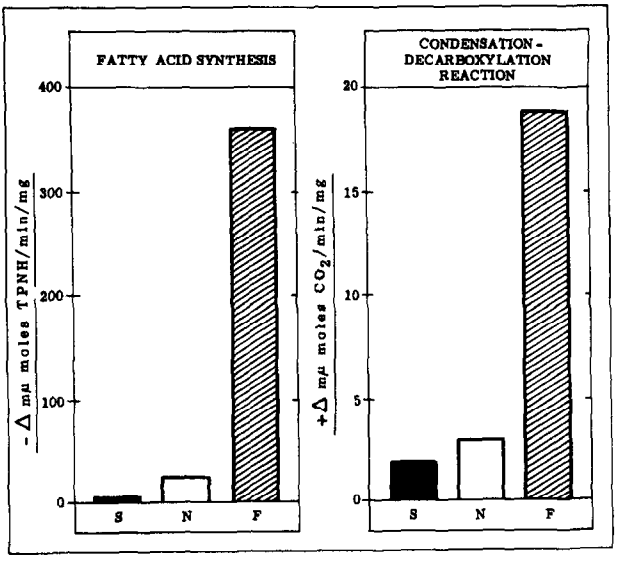

Figure 1. The specific enzyme activity of fatty acid synthesis (left side) and of the condensation-decarboxylation reaction (right side) are measured in soluble liver fractions obtained from rats starved for 48 hours (S), from starved rats refed a fat-free diet for 48 hours (F) and from controls continuously fed a balanced stock diet (N).

The values for fatty acid synthesis were calculated from the initial reaction velocity of malonyl CoA-dependent TPNH oxidation. Malonyl CoA (prepared by the method of Trams and Brady, 1960), 0.25 mM; TPNH, 0.25 mM; acetyl CoA (prepared from acetyl thiophenol according to Wieland, Koppe and Rueff, 1953) 0.25 mM; 2-mercaptoethanol, 2.5 mM; versene 0.1 mM; and potassium phosphate buffer (pH 6.5), 50 mM were incubated with 50-800 μg enzyme in a total volume of 400 μl at 38° in the spectrophotometer.

The condensation-decarboxylation reaction was measured as the rate of $C^{14}O_2$ evolution from a reaction mixture containing 1,3-C^{14} malonyl CoA and acetyl CoA, but no TPNH. 1,3-C^{14} malonyl CoA, 0.25 mM; acetyl CoA, 0.25 mM; 2-mercaptoethanol, 2.3 mM; versene, 0.9 mM; and sodium phosphate buffer (optimal pH 7.4), 95 mM were incubated with 1-50 μg enzyme in a total volume of 22 μl for 5 minutes at 38° in sealed tubes. At the end of the incubation the mixture was acidified and the $C^{14}O_2$ collected by diffusion into Hyamine solution for subsequent counting in the liquid scintillation spectrometer.

The 0-40% ammonium sulfate subfraction of liver supernatants was prepared as previously described (Gibson and Hubbard, 1960). The fat-free diet was obtained from Nutritional Biochemicals Corp.

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that the condensation-decarboxylation reaction as measured here is an integral part of the fatty acid synthesizing system and that it is limiting in the conversion of malonyl CoA to fatty acids.

The soluble liver fractions examined in this experiment catalyze to a lesser extent the decarboxylation of malonyl CoA in the absence of added acetyl CoA (Wakil 1961). Since acetyl CoA is formed by this process an

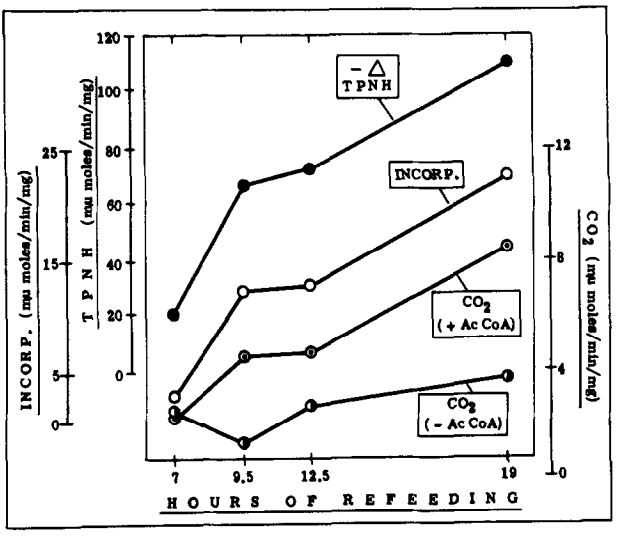

Figure 2. The specific enzyme activites for fatty acid synthesis and the condensation-decarboxylation reaction are compared in liver enzyme preparations from rats which have been starved for 3 days and then refed a fat-free diet for the time indicated on the abscissa of the graph. Reading from top to bottom the curves represent:
(1) "-ΔTPNH": The specific enzyme activity of fatty acid synthesis, determined from the observed initial reaction velocity of TPNH oxidation.
(2) "Incorp.": Fatty acid synthesis, measured from the rate of incorporation of 2-C^{14}-malonyl CoA into fatty acids. (Fatty acids were extracted into pentane from the acidified saponification mixture.)
(3) "CO_2 (+AcCoA)": The condensation-decarboxylation reaction measured as $C^{14}O_2$ evolution from an incubation system containing 1,3-C^{14} malonyl CoA and acetyl CoA. TPNH was omitted.
(4) "CO_2 (-AcCoA)": Decarboxylation of 1,3-C^{14} malonyl CoA measured in the absence of added acetyl CoA.

The conditions of incubation and the composition of the incubation mixtures are the same as cited in figure 1, except for the following: 2-C^{14}-malonyl CoA, 0.175 mM; TPNH, 0.160 mM; and acetyl CoA, 0.175 mM (fatty acid synthesizing system only).

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undetermined portion of the total CO_2 released is also the result of the condensation of malonyl CoA with generated acetyl CoA (bottom line of Figure 2). However, this decarboxylation activity does not correlate well with the activity of the complete system containing added acetyl CoA.

The 30-40% ammonium sulfate subfraction of rat liver supernatant which contains all of the malonyl CoA incorporating system also contains the entire yield of condensation-decarboxylation enzyme activity.

With the availability of highly active preparations from the livers of fat-free refed rats certain properties of this enzyme system could be examined.

Reduced CoA profoundly inhibited both the fatty acid synthesizing system and the condensation-decarboxylation reaction (50% inhibition range: 0.1 to 0.3 mM). As in earlier studies (Brady, 1960) sodium arsenite abolished both enzyme activities (100% inhibition at 2 mM). Employing the assay conditions of Vagelos and Alberts (1960b) the rat liver enzyme preparations did not catalyze the exchange of $C^{14}O_2$ with the free carboxyl group of malonyl CoA either in the presence of acetyl CoA or butyryl CoA.

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