Incorporation of radioactive citrate into fatty acids

Brady and Gurin\(^1\) and Dituri and Gurin\(^2\) were the first to show that soluble enzymes of pigeon liver could synthesize long-chain fatty acids from acetate and that citrate stimulated this process. These results were confirmed and extended to a number of other tissues by other workers and purification of these systems has led to a rather detailed understanding of fatty acid synthesis\(^3,4\). One observation, however, that has never been adequately explained is that at low concentrations ATP, CoA and citrate stimulate \(^{14}\text{C}\) acetate incorporation into fatty acids but as their concentrations are increased, a decreased incorporation of \(^{14}\text{C}\) acetate occurs.

We believe these results can be explained by the presence in these systems of the citrate-cleavage enzyme\(^5\) which catalyzes the following reaction:

\[
\text{Citrate} + \text{ATP} + \text{CoA} \rightleftharpoons \text{Acetyl-CoA} + \text{oxaloacetate} + \text{ADP} + \text{P}_1
\]

Fig. 1. Each tube contained 0.6 ml enzyme (45 mg protein, pH 7.4), 10 \(\mu\)moles magnesium fructose 1,6-diphosphate, either 2 \(\mu\)moles sodium \(^{2-14}\text{C}\) acetate (96,000 counts/min) or 10 \(\mu\)moles potassium \(^{1,5-14}\text{C}_2\) citrate (270,000 counts/min) in a total volume of 0.78 ml. Tubes were gassed with \(\text{N}_2\) and incubated at 37° for 1 h. The soluble enzyme was prepared by centrifugation of a 1:1.5 extract of pigeon liver for 2 h at 100,000 \(\times\) g. The homogenization medium was 8.5 ml \(1\ M\ \text{K}_2\text{HPO}_4, 0.9\ ml\ 1\ M\ \text{KH}_2\text{PO}_4, 9\ ml\ 1\ M\ \text{KHCO}_3\) diluted to 100 ml. Fatty acids were isolated by copper-lime precipitation, washed, acidified and extracted with pentane. ○—○, acetate; ●—●, citrate.

When pigeon-liver extracts are incubated with \(^{2-14}\text{C}\) acetate only a small incorporation of \(^{14}\text{C}\) into fatty acids is seen (Fig. 1). After an initial stimulation of synthesis, probably partially due to TPNH formation, increasing citrate cleavage occurs and the continuing production of non-radioactive acetyl-CoA reduces the specific radioactivity of acetyl-CoA formed from acetate with a concomitant reduction of radioactivity in the fatty acid fraction. A similar increase in citrate cleavage to acetyl-CoA might well take place with increasing ATP and CoA concentrations and in a similar manner explain the decreasing incorporation of radioactive acetate into fatty acids observed with high concentrations of these compounds.

The results in Fig. 1 also show that radioactivity from \([1,5^{-14}C]\)citrate is incorporated into fatty acids. Evidence that citrate is being used for fatty acid synthesis via acetyl-CoA is provided by the results which show a decrease in counts in fatty acids from \([^{14}C]\)citrate with increasing amounts of non-radioactive acetate. Additional support for this mechanism is gathered from the fact that distribution of radioactivity in fatty acids analyzed by gas chromatography from labeled citrate is similar to that obtained from labeled acetate\(^6\). Fig. 1 indicates that 610 \(\mu\)moles of acetate are incorporated in the presence of 10 \(\mu\)moles of citrate, and also shows that under these conditions 290 \(\mu\)moles of citrate are being used for fatty acid synthesis. A total of at least 900 \(\mu\)moles of acetyl-CoA incorporation is thus indicated.

Does the cytoplasmic cleavage of citrate represent an important extramitochondrial source of acetyl-CoA? The localization of 70% of the cellular citrate in mitochondria is probably a reflection of the distribution of citrate-forming and citrate-utilizing enzymes\(^7\); citrate-condensing activity is high\(^8\) in the mitochondria where acetyl-CoA formation occurs; and aconitate hydratase (EC 4.2.1.3)\(^9\), TPN-specific isocitrate dehydrogenase (EC 1.1.1.42)\(^7\) and citrate-cleavage enzyme occur in the cytoplasm. Cytoplasmic citrate is oxidized with difficulty by mitochondria\(^10\) whereas \(\alpha\)-ketoglutarate, the product of its extra-mitochondrial oxidation, readily enters these organelles to be oxidized\(^7\).

It is thus possible to outline the following scheme:

![Diagram showing metabolic pathways]

The movement of citrate out of the mitochondria allows for transfer of acetyl-CoA without a net flux of the coenzyme. Also, reducing power is made available to the cytoplasm without a net movement of pyridine nucleotides. Substrate shuttles similar to these have been proposed for the tricarboxylic acids, the \(\alpha\)-glycerophosphate system, and the \(\beta\)-hydroxybutyrate system (see ref. \(11\) for review).

Part of the citrate stimulation of fatty acid synthesis may be due to the formation of TPNH. Still, citrate is a better activator than any other TPNH-generating system\(^12\)

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and tritium from $[^3H]TPNH$ is not incorporated into fatty acids in the absence of citrate$^{13}$. Similarly isocitrate is necessary for fatty acid synthesis even in the presence of a TPNH-generating system$^{14}$.

Recently HÜLSMANN$^{15}$ has proposed that transcarboxylation from citrate or products of citrate metabolism to form malonyl-CoA is responsible for this stimulation. ABRAHAM et al.$^{16}$ have also suggested such a possibility and the data of NUMA et al.$^{14}$ would support the conclusion that the rate-limiting step of fatty acid synthesis is malonyl-CoA formation. Oxaloacetate, a product of the citrate-cleavage reaction could give rise to malonyl-CoA for fatty acid synthesis. The incorporation of labeled citrate into fatty acids does not clarify the mechanism by which low concentrations of citrate increase fatty acid synthesis from acetate. However, these results can explain the effects of high concentrations of citrate, ATP, and CoA on fatty acid synthesis.

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The effect of temperature during the venom digestion of heart particles on the solubilization of reduced diphosphopyridine nucleotide dehydrogenase

Previously we found that snake-venom digestion of the Keilin and Hartree preparation could liberate an enzyme capable of catalysing DPNH oxidation by several oxidants such as cytochrome c, 2,6-dichlorophenolindophenol and ferricyanide$^{1-4}$. Shortly after our reports$^{1, 2}$, RINGLER et al.$^{5}$ employed a similar method of digestion and purified