THE PATHWAYS OF SYNTHESIS OF FATTY ACIDS BY MITOCHONDRIA*

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Mitochondria obtained from mammalian liver are capable of incorporating acetyl CoA into long chain fatty acids. Some of the characteristics of synthesis of fatty acids within mitochondria have been reported (1,2). The purpose of this communication is to present evidence for the existence of several pathways of synthesis within mitochondria and to indicate the important role of mitochondria in the synthesis and alteration of unsaturated fatty acids.

Methods: Mitochondria were obtained from rat liver by centrifugation at 10,000 x g in 0.25 M sucrose after the tissue was homogenized. A soluble enzyme extract was prepared from beef liver mitochondrial acetone powder by extraction with 0.1 M KPO_4 buffer at pH 7.4.

The complete reaction mixture was composed of 1.0 mg. mitochondrial protein, 100 mμM acetyl-1-C^{14} CoA (2600 c.p.m. per mμmole), 2.0 μM DPNH, 2.0 μM TPNH, 4.0 μM ATP, and 30 μM potassium phosphate buffer (pH 6.5). After incubation at 38° for one hour, the reaction mixture was saponified, acidified, and extracted four times with pentane. Radioactivity of an aliquot of the extracted lipids was measured. The products were decarboxy-

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lated using a modification of the Phares procedure as suggested by Brady (3). The fatty acids were methylated and separated by gas-liquid chromatography with collection of the effluent gas and measurement of radioactivity of the collected fractions. The identity of the fatty acids was determined by cochromatography with known standards and comparison with known relative positions (4). Further confirmation of the identity was achieved by separation of hydroxy-fatty acids on silicic acid columns and separation of saturated and unsaturated methyl esters by formation of the mecuric acetate adduct (5). Unsaturated esters were documented by hydrogenation and rechromatography.

Results and Discussion: The requirements for incorporation of acetyl-1-C^{14} CoA into fatty acids may be seen in Table I.

TABLE I

System	Incorporation mμM	Ratio of radioactivity in carboxyl carbon to total radioactivity
Complete	1.4	1:8.3
" -less ATP	0.84	1:6
" -less ATP + oleyl CoA	2.2	1:2
" -less DPNH	0.32	1:7
" -less TPNH	0.47	1:4

The relative requirement for ATP may be obviated by the addition of a fatty acyl CoA. The ratio of radioactivity in the carboxyl carbon to the total radioactivity is presented in the last column. The ratio of 1:8.3 in the complete system is comparable to a ratio of 1:9 observed on

decarboxylation of the products of non-mitochondrial synthesis. The mechanism of synthesis in this latter system has been shown to be due to successive condensation of acetyl-1-C^{14} CoA with labeling of the odd carbons of the products, C_{16} and C_{18} acids. The theoretical ratio would be between 1:8 and 1:9 depending on the relative quantities of each product. On addition of oleyl CoA, 50 percent of the radioactivity was present in the carboxyl carbon suggesting an addition of the labeled acetyl CoA to the oleyl CoA rather than the more uniform labeling present in the complete system. Similar results were obtained with other long chain acyl CoA's. To test further the concept that chain elongation occurs in the presence of an acyl CoA and to identify the products, acyl-1-C^{14} CoA derivatives of various long chain fatty acids (C_{14} CoA, C_{16} CoA, C_{18} CoA) were synthesized and reacted with unlabeled acetyl CoA in the system previously outlined. In each instance it was found that 33 percent to 63 percent of the radioactivity in the product acids was present in fatty acids two carbons longer than the substrate (e.g. myristyl-1-C^{14} CoA plus acetyl CoA yielded 46 percent of the radioactivity in C_{16}, 17 percent in C_{18}, 11 percent in C_{20}, 4 percent in C_{22}, and less than 1 percent in unsaturated fatty acids.) This process of elongation was not inhibited by the addition of avidin.

The effect of addition of avidin to a solubilized extract of mitochondria is presented in Table II.

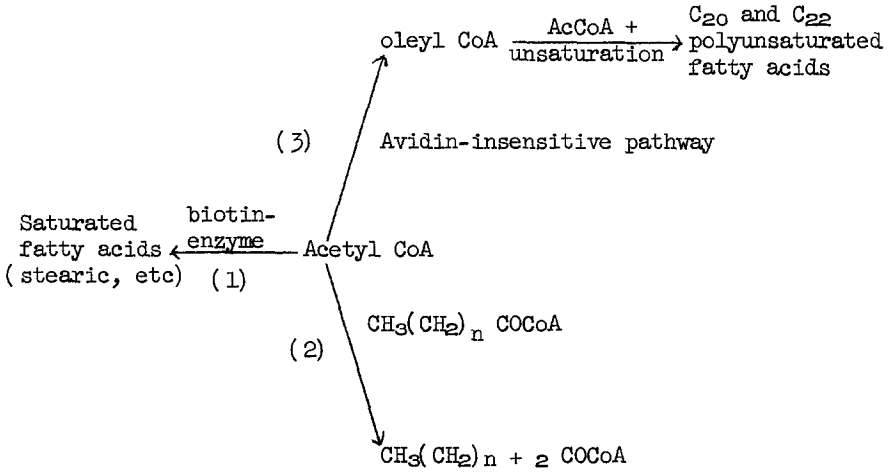
TABLE II

System	Incorporation	C^{14}-acetyl CoA incorporated into Product (cpm)						
		$<C_{16}$	C_{16}	C_{18}	Oleic	C_{20} unsat	C_{22} unsat	Hydroxy acids
Complete no avidin	3.4 mμM	716	678	725	237	1695	407	339
Complete plus 100 μg avidin	2.4 mμM	404	559	31	318	1722	525	267

Avidin decreased the total incorporation of acetyl CoA into long chain fatty acids but this decrease was primarily in the synthesis of stearic acid and shorter chain saturated fatty acids. There was no apparent decrease in the synthesis of oleic acid, C_{20} unsaturated, and C_{22} unsaturated fatty acids. The relative amount of these unsaturated products was increased in the presence of avidin inhibition. In concert with these observations, Barron and Stumpf (6) have recently demonstrated synthesis of oleic acid by particles from the avocado mesocarp and further indicated the relative insensitivity to avidin inhibition of this mechanism. These data indicate that while avidin inhibits a pathway for the synthesis of saturated fatty acids (particularly stearic acid), there is no alteration in the incorporation of acetyl CoA into unsaturated fatty acids.

The alteration of oleic acid by mitochondria presents a different and important facet of the process of elongation. Oleyl-1-C^{14} CoA (100 mμM, 4.2×10^5 cpm) was incubated with acetyl CoA under the conditions previously stated. The major products of this reaction were tentatively identified as C_{18} dienoic (4800 cpm), C_{20} dienoic (2100 cpm), C_{20} trienoic (1800 cpm), C_{22} dienoic (400 cpm), and C_{22} trienoic (200 cpm) plus hydroxy fatty acids (2000 cpm). These observations indicate that the mechanism of unsaturation and elongation of oleic acid proposed by Fulco and Mead (7) takes place within mitochondria in the sequence suggested: unsaturation of oleic acid to C_{18} dienoic followed by chain elongation and further unsaturation to C_{20} trienoic. Review of the products of acetyl-1-C^{14} CoA incubation revealed a similar pattern of polyunsaturated fatty acids. This suggests that the oleic acid synthesized in mitochondria may also undergo chain elongation and further unsaturation.

Summary: These observations have led us to propose the following scheme for the synthesis of fatty acids within mitochondria:



(1) Acetyl CoA may be incorporated into saturated fatty acids via an avidin-sensitive pathway presumably similar to the non-mitochondrial mechanism with malonyl CoA as an intermediate. Stearic acid is the primary product of this synthesis.

(2) Fatty acyl CoA's may combine with acetyl CoA's resulting in elongation of the original fatty acid by one or more two-carbon units.

(3) Oleic acid is produced by an avidin-insensitive pathway. The mechanism of this synthesis is unknown, but did not appear to be related to alteration of the saturated acyl CoA's examined thus far. The oleic acid thus formed may be altered by elongation and unsaturation to C_{20} and C_{22} polyunsaturated fatty acids.

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