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Mitochondrial Stimulation of Fatty Acid Biosynthesis*

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The original observation of Brady and Gurin (1) and Dituri and Gurin (2) that citrate stimulated fatty acid biosynthesis has been confirmed and extended by many other workers (3–8). Brady, Mamoon, and Stadtman (4) showed that the stimulation of fatty acid synthesis by citrate was due in part to the generation of reduced nicotinamide adenine dinucleotide phosphate (NADPH) by *cis*-aconitase and isocitrate dehydrogenase present in the enzyme preparation. They could partially replace the citrate effect by adding NADPH or other NADPH-generating systems. The replacement did not enhance biosynthesis as much as citrate, indicating that citrate probably played still another role in this biosynthetic process.

In 1962, Martin and Vagelos (9, 10) showed that citrate specifically stimulated the acetyl coenzyme A carboxylase reaction in rat adipose tissue preparations. This reaction had been reported to be the rate-limiting step for fatty acid synthesis in a number of tissues (11, 12). Similar results were obtained by Waite and Wakil (13), who showed that isocitrate stimulated the catalytic activity of acetyl-CoA carboxylase from chicken liver. It would seem likely, therefore, that in addition to acting as a source of NADPH, part of the stimulatory effect of citrate observed with soluble crude enzyme systems was due to the activating effect of citrate on the carboxylating enzyme. It should be noted, however, that the concentration of citrate needed for maximal fatty acid synthesis and for maximal carboxylation activation is very high, about 10 μmoles per ml. We have reported a third role of citrate in fatty acid biosynthesis, that of a carbon source via the citrate cleavage enzyme (8).

Recently Wakil *et al.* (14, 15) reported the elongation of fatty acids in a mitochondrial system by the successive addition of acetyl-CoA to intermediate chain length fatty acyl-CoAs (C_{10} to C_{16}). Similar results were reported by Stumpf and Barber (16) in mitochondria derived from avocado mesocarp. In addition to these chain elongation observations, Hülsmann (17, 18) has reported on the biosynthesis of fatty acids in rat or rabbit heart sarcosomes. The amount of fatty acid synthesis *de novo* in mitochondria, in all cases, is significantly lower than that observed with soluble enzyme systems. Moreover, even the combined rate of chain elongation, desaturation, and synthesis *de novo* is considerably lower than the rate of synthesis obtained with soluble enzyme systems.

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The purpose of this paper is to report on the effect of mitochondria added to a cytoplasmic fatty acid-forming enzyme system. We find that the stimulatory effect of a high concentration of citrate can be replaced by motochondria, indicating that the mitochondria probably are involved in cellular fatty acid synthesis. Mitochondria could replace several cofactor requirements. When mitochondria were used to replace citrate, the composition of the synthesized fatty acids was greatly changed. The results indicate that for a proper understanding of cellular fatty acid synthesis and its control, the mitochondrial fatty acid-synthesizing system should be studied in combination with the cytoplasmic system.

EXPERIMENTAL PROCEDURE

Preparation of Mitochondria—The pigeon liver mitochondria were prepared by the method of Hogeboom and Schneider (19).

Livers obtained fresh from decapitated pigeons were cleaned of all adhering fats. They were cut into small pieces and suspended in 10 volumes of 0.25 M sucrose solution. The minced tissue was then homogenized by four strokes of a pestle in a motor-driven Potter-Elvehjem homogenizer, with care to keep the temperature no higher than 4°. The crude homogenate was centrifuged at 750 to 850 $\times$ *g* for 10 minutes in a model PR-2 International centrifuge with a multispeed attachment. The precipitate, which contained most of the unbroken cells and nuclear debris, was discarded, and the supernatant suspension obtained from this centrifugation was recentrifuged at 800 to 900 $\times$ *g* for 10 minutes. The supernatant obtained from this centrifugation was centrifuged for 10 minutes at 8000 $\times$ *g*, and the precipitate obtained at this stage was resuspended in 40 ml of 0.25 M sucrose (about one-fifth the original volume), very lightly homogenized once, and then centrifuged again at 800 $\times$ *g* for 10 minutes. The supernatant suspension obtained at this stage was centrifuged once again at 8000 $\times$ *g* for 10 minutes. The precipitated mitochondria were suspended in 40 ml of 0.25 M sucrose solution and reprecipitated by centrifugation at 8000 $\times$ *g* for 10 minutes. The last operation was repeated once more, and the mitochondria thus obtained were taken into an equal volume of the same sucrose solution. Mitochondria prepared in this way were used for all experiments with the reconstructed system.

Rat liver mitochondria were prepared essentially in the same way. The yield of mitochondria per g of liver was higher in the case of rat liver.

For some experiments, pigeon liver mitochondria were ruptured by sonication in a Branson high intensity sonifier (Branson Instrument Corporation, Stamford, Connecticut). Mitochondria suspended in 8 ml of 0.25 M sucrose solution were subjected to sonic vibrations for 1 minute at a current flow of 8.0 amperes.

TABLE I

Stimulation of fatty acid synthesis by mitochondria

To the tubes in Experiment A were added 3 μmoles of acetate-2-^{14}C (1.5×10^5 c.p.m.), 5 μmoles of magnesium fructose diphosphate, and 0.6 ml of soluble supernatant extract (20 mg of protein) in a total volume of 0.9 ml. The amounts of citrate or mitochondrial protein added are indicated. The tubes in Experiment B contained a total volume of 0.7 ml: 3 μmoles of acetate-2-^{14}C (1.5×10^5 c.p.m.), 0.2 μmole of $MnCl_2$, 2.0 μmoles of $MgCl_2$, 5 μmoles of Na_2ATP, 0.015 μmole of CoA, 0.6 μmole of NAD, 0.2 μmole of NADP, 20 μmoles of $KHCO_3$, 10 μmoles of cysteine, 30 μmoles of potassium phosphate buffer (pH 6.5), and charcoal-treated soluble enzyme (5 mg of protein). Time of incubation was 1 hour at 37°. Each of the flasks was flushed thoroughly with a 95% O_2-5% CO_2 mixture for 3 minutes before they were tightly stoppered. Results are expressed as millimicromoles of acetate-^{14}C incorporated per hour per mg of supernatant protein (except in those experiments containing mitochondria only, when the results are expressed per mg of mitochondrial protein).

Reaction contents	Fatty acid synthesis
Experiment A	
Supernatant	2.0
+ Citrate (10 μmoles)	26.1
+ Mitochondria (3.6 mg)	14.9
+ Mitochondria (11.0 mg)	19.6
Mitochondria (3.6 mg)	2.1
Experiment B	
Fortified supernatant	0.17
+ Citrate (10 μmoles)	32.5
+ Mitochondria (6.0 mg)	22.1
Mitochondria (6.0 mg)	1.2

TABLE II

Properties of mitochondria in reconstructed system

The complete system included 3 μmoles of acetate-2-^{14}C (1.5×10^5 c.p.m.), 0.2 μmole of $MnCl_2$, 2.0 μmoles of $MgCl_2$, 5 μmoles of Na_2ATP, 0.015 μmole of CoA, 0.6 μmole of NAD, 0.2 μmole of NADP, 20 μmoles of $KHCO_3$, 10 μmoles of cysteine, 30 μmoles of potassium phosphate buffer (pH 6.5), and 5 mg of soluble enzymes in a total volume of 0.65 ml. In the reconstructed system, the reaction was started by adding the soluble enzymes. Other additions or any change in addition are mentioned in the table. Time of incubation was 1 hour at 37°. The flasks, unless otherwise indicated, were flushed the same way as in Table I. Results are expressed as millimicromoles of acetate-^{14}C incorporated per hour per mg of supernatant protein.

Reaction system	Fatty acid synthesis
Complete	1.3
+ Citrate (10 μmoles)	40.4
+ Mitochondria (5.2 mg)	28.8
+ Mitochondria (heated)	1.4
+ Mitochondria (sonicated)	2.4
+ Mitochondria (N_2 atmosphere)	5.6
+ Dinitrophenol (10^{-3} M) + citrate (10 μmoles)	22.8
+ Mitochondria + dinitrophenol (10^{-3} M)	4.1

The temperature was kept below 10° by putting the mitochondrial suspension in a salt-ice bath.

Rat and Pigeon Heart Sarcosomes—The heart mitochondria from pigeon and rat were prepared basically by the procedure of Holton *et al.* (20). The heart tissue, obtained fresh from the animal, was taken into 9 volumes of 0.25 M sucrose solution. It was cut into small pieces and then thoroughly minced. The homogenization was done in a motor-driven Potter-Elvehjem homogenizer. Four strokes of the pestle were necessary for a good homogenization. The temperature was maintained below 4°. The nuclei and cell debris were removed by centrifugation at $800 \times g$ for 3 minutes, and the mitochondria were collected by centrifuging the suspension at $7000 \times g$ for 10 minutes. The time for centrifugation at $800 \times g$ was reduced to prevent an excessive loss of sarcosomes. The number of washings employed was otherwise the same as that used in the preparation of liver mitochondria.

The mitochondrial preparations were occasionally checked for their functional integrity by the enzymatic method of ADP-dependent oxidative phosphorylation employed by Chance and Williams (21).

Other Methods—The preparation of supernatant enzyme and isolation of the fatty acids were carried out by the methods reported earlier (22). Fatty acids were methylated according to the procedure of Radin, Hajra, and Akahori (23), and their gas chromatography was performed according to Hajra and Radin (24).

RESULTS

Replacement of Citrate by Mitochondria—Results in Table I show that mitochondria can replace the citrate requirement for fatty acid synthesis. Experiment A shows the stimulatory effect of two different levels of mitochondria on the crude soluble enzyme system. In Experiment B, most of the nucleotide components required for fatty acid synthesis were removed from the enzyme solution by repeated charcoal treatment, and were then added back in known quantities. In both cases, mitochondria were an effective replacement for citrate. In some experiments, the total amount of fatty acid synthesized with mitochondrial stimulation exceeded the level of synthesis in the presence of citrate. In general, however, the amount of synthesis in the presence of mitochondria was 60 to 80% of citrate-stimulated fatty acid synthesis.

The combination of soluble enzymes and the mitochondria will be called the "reconstructed" system in the remainder of this paper.

Properties of Mitochondria Necessary for Stimulation—When heated mitochondria were added to soluble supernatant fraction, no significant increase in fatty acid synthesis was observed (Table II). When the reconstructed system was placed in a nitrogen atmosphere, the level of fatty acid synthesis dropped to one-fifth of the normal level. As can be seen from Table II, no stimulation of fatty acid synthesis could be observed when sonically treated mitochondria replaced citrate.

The last three lines of Table II show the effect of 2,4-dinitrophenol, a typical uncoupling reagent for oxidative phosphorylation, on the reconstructed system. The total amount of fatty acid synthesis in the reconstructed system in the presence of dinitrophenol was considerably lower than the amount of synthesis in the control tube. The soluble enzyme system in the presence of 2,4-dinitrophenol showed somewhat lower synthesis of fatty acids. The reason for this inhibition is not known. Structural and functional integrity of the mitochondria therefore seems to be an essential condition for this stimulation of fatty acid synthesis.

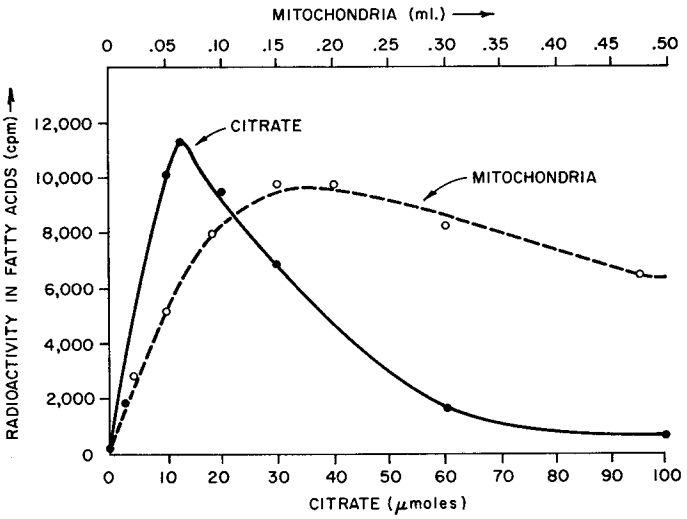

FIG. 1. Each tube had the same composition as given for the complete system in Table II. In addition, for the citrate stimulation curve (●——●), they contained an increasing concentration of citrate, and for the mitochondria stimulation curve (○- - -○), an increasing concentration of mitochondria. Conditions of the experiment are the same as given in Table II.

TABLE III

Replacement of various cofactors by mitochondria

The complete systems in Experiments A and B are the same as described for the complete system in Table II. The conditions of the experiment were also the same as in Table II. The complete system for Experiment C included 20 μmoles of (DL)-potassium isocitrate instead of 10 μmoles of potassium citrate. The concentration of other added substances and the conditions of incubation were the same as in Experiments A and B. The complete system in Experiment D was the same as in Experiment A. In this case, however, the soluble enzyme system was prepared by homogenization in a medium that was exactly the same for other preparations except that it contained no $KHCO_3$. Results are expressed as millimicromoles of acetate-^{14}C incorporated per hour per mg of supernatant protein.

Reaction system	Fatty acid synthesis
Experiment A	
Complete	27.0
− Citrate	2.8
− Citrate + mitochondria (3.6 mg)	16.9
− ATP	1.8
− ATP + mitochondria (3.6 mg)	30.3
Experiment B	
Complete	18.9
− ATP	1.7
− ATP + mitochondria (4.0 mg)	16.4
− NADP − NAD	8.1
− NADP − NAD + mitochondria (4.0 mg)	15.5
Experiment C	
Complete	17.1
− Isocitrate	0.6
− Isocitrate + mitochondria (5 mg)	14.0
− CoA	4.2
− CoA + mitochondria	14.6
Experiment D	
Complete	19.2
− CO_2	0.5
− CO_2 + mitochondria (5 mg)	11.6

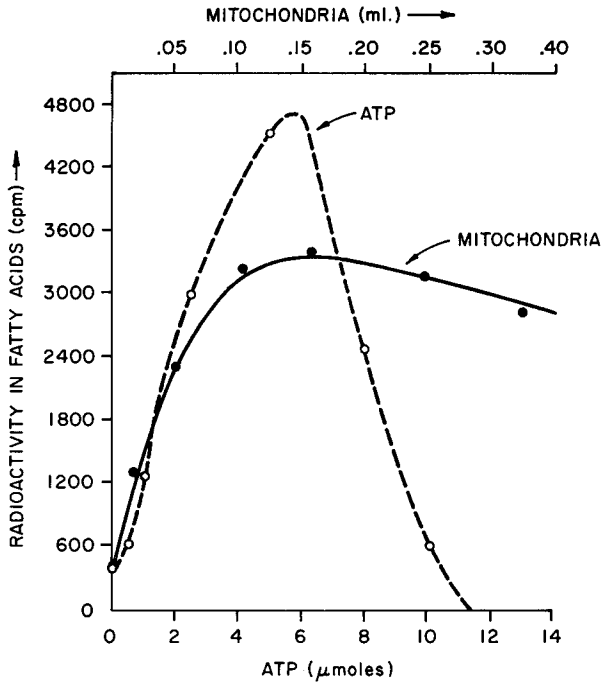

FIG. 2. Each tube contained 3 μmoles of acetate-2-^{14}C (1.5×10^5 c.p.m.), 10 μmoles of potassium citrate, 0.2 μmole of $MnCl_2$, 2.0 μmoles of $MgCl_2$, 0.015 μmole of CoA, 0.6 μmole of NAD, 0.2 μmole of NADP, 20 μmoles of $KHCO_3$, 10 μmoles of cysteine, 30 μmoles of potassium phosphate buffer (pH 6.5), and 5 mg of soluble enzyme in a total volume of 0.65 ml. In addition, some tubes contained varying concentrations of ATP (○- - -○) and others contained varying concentrations of mitochondria (●——●). The conditions of the experiment are the same as given in Table II.

Comparison of Citrate and Mitochondrial Stimulation of Fatty Acid Synthesis—The results in Fig. 1 show the effect of increasing concentration of citrate on fatty acid synthesis as compared to the effect of increasing concentration of mitochondria. At higher concentrations, incorporation of radioactivity from acetate into fatty acids decreased considerably more rapidly in the case of citrate than in the case of mitochondria. The decrease in fatty acid synthesis observed with higher concentration of mitochondria was possibly due to the oxidation of synthesized fatty acids by mitochondria. The reduction of incorporation of ^{14}C-acetate into fatty acids at very high concentrations of citrate has been explained previously as being due to the increased production of nonradioactive acetyl-CoA from citrate as catalyzed by the citrate cleavage enzyme (22).

Replacement of Other Requirements—The need for bicarbonate and ATP was completely replaceable by mitochondria. The charcoal-treated soluble enzyme preparations probably contained NADP and CoA, since we could not regularly demonstrate a requirement for these substances. In some preparations, the addition of mitochondria in the absence of NADP and CoA brought the level of synthesis back to normal (Table III).

In Fig. 2 the effect of increasing ATP concentration is compared with the increasing mitochondria concentration. With ATP, a rather small range of ATP concentration (5×10^{-3} to 8×10^{-3} M) was observed where fatty acid synthesis could take place at an optimal level. When ATP was replaced by mitochondria, the shape of the curve was different from the shape of the ATP-stimulated curve. This difference was greatest at higher concentrations of ATP and mitochondria.

TABLE IV

Effect of mitochondria from different sources on fatty acid synthesis in soluble enzyme system

The complete system has the same composition as given for the complete system in Table II. The conditions of the experiment were the same as given in Table II. Results are expressed as millimicromoles of acetate-^{14}C incorporated per hour per mg of soluble protein (or mitochondria).

Components	Fatty acid synthesis
Complete system	0.15
+ Citrate	35.0
+ Pigeon liver mitochondria (4 mg)	22.3
Pigeon liver mitochondria (4 mg)	0.9
Complete system + rat liver mitochondria (4.2 mg)	5.9
Rat liver mitochondria (4.2 mg)	0.08
Complete system + pigeon heart sarcosomes (4.5 mg)	0.20
Pigeon heart sarcosomes (4.5 mg)	0.01
Complete system + rat heart sarcosomes (4.2 mg)	0.21
Rat heart sarcosomes (4.2 mg)	0.00

TABLE V

Distribution of radioactivity in fatty acids synthesized in reconstructed system

This table shows three different complete analyses for the fatty acids in the *n*-pentane extract from incubation mixtures containing the reconstructed fatty acid-synthesizing system. The contents of each tube are the same as given in Table II. Mitochondrial suspension (0.1 ml, approximately 3.5 mg of protein) was added as described in Table I. In the second column of the table are presented for comparison the results of an analysis of radioactivity in fatty acids synthesized in a citrate-stimulated system.

Chain length of fatty acid	Radioactivity in total fatty acids			
	Citrate-stimulated	Mitochondria stimulated		
	%	%	%	%
$<C_{14}$	0.36	0.50	0.49	2.1
C_{14}	22.0	7.2	19.1	15.0
C_{15}	1.0	0.42	1.6	
C_{16}	68.0	40.0	52.0	48.3
C_{17}	1.1	1.3	1.2	0.7
C_{18}	4.2	38.6	20.2	24.6
C_{20}	1.7	7.7	3.6	5.4
C_{18} and greater (total)	8.0	50.8	27.0	34.0

Effect of Mitochondria from Different Sources—If the mitochondria were needed essentially for the supply of cofactors and substrates, mitochondria from any source could be expected to stimulate fatty acid synthesis to more or less the same extent. The results of experiments designed to test this idea are given in Table IV. Mitochondria obtained from rat liver had a stimulatory effect, although the amount of synthesis was lower than that found with pigeon liver mitochondria. Pigeon and rat heart sarcosomes, on the other hand, had no stimulatory effect on fatty acid biosynthesis.

Composition of Fatty Acids Synthesized by Mitochondria-soluble Enzyme System—The distribution of radioactivity in the synthesized fatty acids in mitochondria-stimulated systems was determined by gas chromatographic analysis. Results of three such separate analyses are given in Table V. For convenience of comparison, a typical distribution of counts in fatty acids synthesized in a citrate-stimulated system is also tabulated. A large increase in the proportion of radioactivity in C_{18} and other longer chain fatty acids was observed in each of these cases. The proportion of radioactivity in C_{18} acids (which include both stearate and oleate) increased 4- to 6-fold. The total percentage of radioactivity in acids with 18 or more carbon atoms varied from 27 to 50 in the three different cases. The reason for such variation is not known, but the nature of the mitochondrial preparation might be an important factor. This significant change in the composition of the synthesized fatty acids clearly indicated an enzymatic role for the mitochondria in the combined system.

Table VI presents results of a detailed analysis of distribution of radioactivity in fatty acids synthesized under various conditions. The results in tube 4 show that the increase in radioactivity in C_{18} and higher acids is not due to the presence of palmitate from mitochondria on which chain elongation could take place. It seems that the change in composition of fatty acids synthesized is mediated by enzyme, since the addition of heated mitochondria along with citrate in a soluble enzyme system showed no change in the fatty acids synthesized (tube 5). The radioactivity in the fatty acids synthesized by the mitochondria alone was distributed in a manner similar to that of fatty acids synthesized in a reconstructed system. The total amount of fatty acid synthesized was, however, extremely low, showing the special nature of the reconstructed system. The distribution of radioactivity in the fatty acids synthesized in the presence of heated mitochondria alone could not be determined because of the low incorporation of radioactivity into the fatty acids.

Localization of Synthesized Fatty Acids—After a 1-hour period of incubation, the mitochondria were separated from the soluble portion by centrifugation, and each fraction was analyzed separately for its content of synthesized fatty acids. The results

TABLE VI

Distribution of radioactivity in fatty acids

The composition of the supernatant system is the same as that described for the complete system in Table II. The mitochondria used in tubes 3 and 5 were heated for 6 minutes at 80°. Where indicated, 10 μmoles of citrate were added. The conditions of the experiment were the same as that described in Table II. Fatty acid synthesis is expressed as millimicromoles of acetate-^{14}C incorporated per hour per mg of supernatant protein.

Tube	Fatty acid synthesis	Total radioactivity in fatty acids		
		Up to C_{15}	Up to C_{17}	C_{18} and greater
1. Supernatant	0.15	23.0	71.6	5.2
2. + Citrate	32.5	24.8	69.1	7.2
3. + Mitochondria (3.6 mg) (heated)	0.75			
4. + Citrate + palmitate (7.2 mg)	22.0	21.3	70.7	8.1
5. + Citrate + mitochondria (3.6 mg) (heated)	37.1	18.6	72.0	9.7
6. + Mitochondria (3.6 mg)	21.0	17.0	48.9	34.1
7. Mitochondria + citrate	1.8	12.1	52.3	35.0

TABLE VII

Localization and distribution of radioactivity in synthesized fatty acids in reconstructed system

The reconstructed system was the same as the complete system described in Table II. To each of these tubes was added 0.1 ml of mitochondrial suspension containing 3.8 mg of protein. The conditions of incubation were the same as described in Table I. After a 1-hour period of incubation, the mitochondria was separated from the soluble portion and each fraction was analyzed separately for its content of synthesized fatty acids. The analysis for the fatty acid composition was carried out on the samples obtained from Experiment B.

Fraction	Fatty acid synthesis*		Total radioactivity in fatty acids				
	A	B	C_{14}	C_{16}	C_{18}	C_{20}	$>C_{20}$
	mμmoles						
Supernatant	45.0	99.6	13.1	50.0	31.9	2.1	3.4
Mitochondria	162	592	6.4	39.1	43.1	7.3	3.1

* Total millimicromoles of acetate-^{14}C incorporated into fatty acids. A and B refer to separate experiments.

TABLE VIII

Effect of avidin on fatty acid synthesis in reconstructed system

The complete system has the same composition as that described for the complete system in Table II. The amounts of mitochondria, citrate, or avidin added are described in the table. The conditions of the experiment are the same as that described in Table I. Results are expressed as millimicromoles of acetate-^{14}C incorporated per hour per mg of protein.

Reaction system	Fatty acids formed
Complete	
+ Citrate (10 μmoles)	26.0
+ Citrate + avidin (300 μg)	0.32
+ Mitochondria (4.0 mg)	16.7
+ Mitochondria (4.0 mg) + avidin (300 μg)	0.30
Mitochondria (4.0 mg)	0.82
Mitochondria (4.0 mg) + avidin (300 μg)	0.69

of two such incubations showed that nearly 80% of the total fatty acids synthesized are in the mitochondrial lipid fractions (Table VII). Table VII also shows that the distribution of radioactivity in fatty acids in the soluble portion also changed to resemble, in general, the over-all distribution seen in the reconstructed system.

Effect of Avidin on Reconstructed System—Recently, Harlan and Wakil (15) have pointed out the possible existence of an avidin-insensitive pathway for mitochondrial fatty acid synthesis. The quantity of fatty acid synthesized is definitely small (1.3 mμmoles per hour per mg of protein). The possible existence of such a pathway or some other avidin-insensitive pathway cannot be eliminated in the reconstructed system. Results of the effect of avidin on the combined mitochondria-soluble enzyme system are given in Table VIII. At levels of 300 μg, avidin inhibited fatty acid synthesis completely in both the citrate-stimulated and the reconstructed system. Interestingly, avidin did not have any effect on the synthesis of fatty acids by the mitochondria. A possible explanation might be the inability of avidin to penetrate the mitochondrial membrane barrier.

DISCUSSION

We have pointed out earlier (8) that since citrate serves both as a precursor for fatty acids and as a source of NADPH, the transport of citrate out of the mitochondria would be an important factor in fatty acid metabolism. Since citrate has also been shown (10) to affect the rate-limiting step in fatty acid biosynthesis, *i.e.* the acetyl-CoA carboxylation step, its concentration in the cytoplasm assumes even greater importance in the regulation of this biosynthetic pathway. A direct determination of the rate of citrate transport out of the mitochondria and the comparison of this rate with the rate of acetate and acetyl-CoA transport thus seems to be very important. Very little has been done on the mitochondrial transport of citrate, although in some mitochondria citrate seems to be incapable of penetrating the mitochondrial membrane from the outside (25). This, however, does not exclude the possibility that citrate moves out of the mitochondria rather freely. Preferential behavior of this kind has been noted in the transport of many compounds. We have discussed in earlier papers (8, 22) how the distribution of citrate-forming and citrate-utilizing enzymes in the cell leads us to believe that citrate is transported out of the mitochondria to the cytoplasm.

Experiments designed to test directly whether citrate generated in the mitochondria could be transported out have given equivocal results. The attempt to replace the requirement of citrate with mitochondrial suspension, on the other hand, seems to indicate that some such phenomenom may be occurring. As has been demonstrated, it is only the combination of the soluble proteins and citrate or functionally active mitochondria that gives a significant synthesis of fatty acids.

Initially, we believed that the mitochondria were exclusively supplying the cofactors, including citrate, necessary for fatty acid synthesis in the soluble system. The concentration of citrate required for maximal synthesis of fatty acids and for acetyl-CoA carboxylase activation is about 10 μmoles per ml. The normal concentration of citrate in cells and tissues is about 1.0 μmole per ml (26). The concentration achieved on the addition of mitochondria is probably only about 1.0 μmole per ml, thus emphasizing the difference between mitochondrial and citrate stimulation of fatty acid synthesis. Experiments in which varying concentrations of citrate, ATP, and mitochondria were used also clearly indicated a difference in pattern of stimulation of fatty acid synthesis. If the function of mitochondria were purely to supply citrate or ATP, one would expect the dilution curves to be very similar.

If the mitochondria were needed only for the supply of cofactors and energy, mitochondria from any source could be expected to stimulate the fatty acid synthesis to more or less the same extent. On the other hand, if mitochondrial enzymes were involved, mitochondria obtained from tissues which had a low fatty acid-synthesizing ability would be poorer stimulators for fatty acid synthesis. Heart mitochondria have been found to have a very high capacity for fatty acid oxidation (27). In contrast, the level of fatty acid synthesis in heart sarcosomes is very low. The absence of stimulation by the sarcosomes supported the idea that the liver mitochondria are performing some functions other than supplying cofactors.

The over-all change in the distribution of radioactivity in the

synthesized fatty acids in the reconstructed system indicates the participation of the mitochondrial enzymes. Failure of heated mitochondria to change the distribution of radioactivity in fatty acids in a citrate-stimulated system confirms this conclusion. The mitochondrial enzymes primarily involved are probably those involved in chain elongation and desaturation as indicated by the recent observations of Harlan and Wakil (15). If the distribution of radioactivity in the various fatty acids is assumed to mirror the composition of fatty acid synthesized, then the composition of the fatty acids synthesized in the reconstructed system more closely resembles the composition of the fatty acids in the whole cell (28, 29).

The mitochondria in the reconstructed system showed their full stimulatory capacity only when oxidative phosphorylation was taking place and the mitochondrial structure remained intact. In some experiments, the mitochondrial preparations which had released considerable amounts of ATPase, as detected by enzymatic test, still retained the full stimulatory capacity. The reason for the discrepancy between this observation and the sensitivity of the mitochondrial stimulation to dinitrophenol is not clear to us at the moment. In the reconstructed system, the bulk of the synthesized fatty acids are incorporated into mitochondrial lipids. Removal of synthesized fatty acids from the site of synthesis would not be expected to increase the rate of fatty acid synthesis here. The report that palmityl-CoA is an inhibitor for several enzymes involved in fatty acid synthesis[1] (30, 31), however, offers a possible explanation of increased synthesis.

The possibility that mitochondria can directly activate the carboxylase enzyme needs to be investigated. Den and Klein (32) presented evidence to show that small particles, needed in fatty acid synthesis, catalyzed the carboxylation of acetyl-CoA to malonyl-CoA in the presence of ATP and Mn^{++}. The avidin sensitivity of the system studied here makes it seem unlikely that an unknown pathway is involved.

Abraham, Matthes, and Chaikoff (33, 34) reported that the addition of microsomes to the supernatant fraction caused a 5- to 8-fold stimulation of lipogenesis from acetate whereas earlier workers could not find such stimulation (2, 5, 35). On the other hand, Masoro, Korchak, and Porter (36) showed that liver microsomes of unfed rats exert a strong inhibitory effect on fatty acid synthesis by soluble enzyme systems. They correlated this microsomal effect with the ATPase content of microsomes. Recent work by Abraham *et al.* (37) has shown that the effect of microsomes depends upon their concentration and the concentration of ATP. While they report large stimulation of fatty acid synthesis by microsomes, the effect is still dependent upon the presence of 20 mM citrate. Whatever the situation may be, the possible participation of microsomes in fatty acid synthesis cannot be excluded.

The current idea that cellular fatty acid synthesis occurs exclusively in the cytoplasmic portion of cells should be modified. In a pigeon liver system, at least, we have shown that the mitochondria have a decisive effect on the nature and rate of fatty acid synthesis. It seems likely that the microsomes may have to be taken into account for a proper understanding of the fatty acid biosynthetic process.

[1] S. Abraham, personal communication.

SUMMARY

In a soluble enzyme system from pigeon liver, the stimulatory effect of high concentrations of citrate (10 mM) on the biosynthesis of fatty acids from acetate could be replaced with pigeon liver mitochondria. If the mitochondria were subjected to heat or sonic oscillations, or if they were incubated anaerobically, the stimulatory effect was abolished.

If the enzyme system was prepared so that adenosine triphosphate, coenzyme A, nicotinamide adenine dinucleotide phosphate, and HCO_3^- were required for fatty acid biosynthesis, then mitochondria could replace singly each of these requirements. Rat liver mitochondria could replace pigeon liver mitochondria, but rat heart and pigeon heart sarcosomes were unable to stimulate fatty acid synthesis in the system.

The fatty acids synthesized in the reconstructed system (soluble enzyme and mitochondria) were located primarily in the mitochondria and contained a higher percentage of stearic acid than the fatty acids synthesized in a citrate-stimulated system.

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