BBA 4047

THE INCORPORATION OF CITRATE CARBON INTO FATTY ACIDS

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(Received December 11th, 1962)

SUMMARY

Long-chain fatty acids are synthesized from citrate in an undialyzed soluble enzyme system from pigeon liver at a rate comparable to that observed when acetate is used as a precursor. The first enzymic step for citrate incorporation is probably its breakdown to acetyl-CoA and oxaloacetate catalyzed by the citrate-cleavage enzyme. Experiments with variously labeled citrate show good incorporation of the acetyl portion of citrate into fatty acids, and a poor incorporation of the oxaloacetyl portion into fatty acids. The incorporation of the oxaloacetyl portion of citrate as well as the acetyl portion has been shown to be avidin sensitive. The enzymic sequence from the oxaloacetyl portion remains uncertain.

INTRODUCTION

The biosynthesis of long-chain fatty acids from acetate is catalyzed by soluble enzymes of various tissues from several organisms[1-6]. We reported that citrate is as efficient a precursor of fatty acids as acetate in a pigeon-liver preparation[7]. Similar results have also been obtained by KALLEN AND LOWENSTEIN[8] using the lactating mammary gland of rats and by FORMICA[9]. We postulated that the initial step in this biosynthesis is the cleavage of citrate to acetyl-CoA and oxaloacetate by the citrate-cleavage enzyme[10] in the presence of ATP and CoA.

$$\text{Citrate} + \text{ATP} + \text{CoA} \rightarrow \text{acetyl-CoA} + \text{oxaloacetate} + \text{ADP} + P_i.$$

Acetyl-CoA is then incorporated into fatty acids through the malonyl-CoA pathway[11,12]. The fate of oxaloacetate formed form citrate is not yet clear. KALLEN AND LOWENSTEIN[8] suggested that oxaloacetate might be involved in fatty acid biosynthesis via an alternate route. HÜLSMAN[13] and ABRAHAM *et al.*[14] have suggested a role of oxaloacetate or citrate in transcarboxylation reactions leading to malonyl-CoA .The present communication presents further evidence that involves the citrate-cleavage enzyme in the conversion of citrate carbon into fatty acids. Data concerning the fate of the oxaloacetyl moiety of citrate in relation to fatty acid biosynthesis are also presented.

* United States Public Health Trainee (Grant No. 2G-187).

METHODS AND MATERIALS

ATP, CoA, NAD, NADH, NADP, NADPH, glucose 6-phosphate, hexokinase (EC 2.7.1.1), glucose-6-phosphate dehydrogenase (EC 1.1.1.49), lipoic acid, thiamine pyrophosphate and avidin were obtained from commercial sources.

Crystalline citrate-condensing enzyme (EC 4.1.3.7) was prepared by the method of SRERE AND KOSICKI[15]. Malate dehydrogenase (EC 1.1.1.37) was purchased from Boehringer and Sons. Crystalline fumarate hydratase (EC 4.2.1.2, formerly known as "fumarase") was a gift of Dr. T. P. SINGER. Citrate-condensing enzyme activity was measured by the method of OCHOA *et al.*[16]. Citrate-cleavage activity was measured according to the method of SRERE[10]. Hexokinase, glucose-6-phosphate dehydrogenase and pyruvate oxidase (EC 1.2.3.3) activities were assayed spectrophotometrically following the procedures in *Methods of Enzymology*, Vol. 1. Protein was estimated by the method of WARBURG AND CHRISTIAN[17]. Citric acid was estimated by the colorimetric method of HARTFORD[18].

Radioactive compounds

Sodium [2-^{14}C]acetate, [1-^{14}C]acetic anhydride and [1,5-$^{14}C_2$]citric acid were obtained from New England Nuclear Corporation. [1-^{14}C]Fumaric acid and [2-^{14}C]-fumaric acid were purchased from Nuclear Chicago Corporation. Sodium [1-^{14}C]-citrate, sodium [3,4-$^{14}C_2$]citrate and sodium [5,6-$^{14}C_2$]citrate were prepared as described below.

Sodium [1-^{14}C]citrate: [1-^{14}C]Acetyl-CoA was prepared from [1-^{14}C]acetic anhydride by the method of SIMON AND SHEMIN[19]. [1-^{14}C]Acetyl-CoA (1.5 μmoles) was added to a reaction mixture (3 ml) containing 200 μmoles of Tris–Cl buffer (pH 8.1), 20 μmoles of potassium malate, 4 μmoles of NAD, 0.8 unit of malate dehydrogenase and 1 unit of citrate-condensing enzyme. The reaction was followed spectrophotometrically at 340 mμ and at the end of a half-hour was 95 % complete. 40 μmoles each of sodium acetate, sodium malate and sodium citrate were added as carrier and the reaction was stopped and partially deproteinized by heating to 70° for 6 min. The organic acids were separated on a silicic acid column (21 × 1 cm) by the method of VARNER[20]. Effluent was collected in 10-ml fractions and titrated with 0.01 N NaOH with chlorophenol-red as indicator. Total radioactivity in each fraction was determined. The appearance of acid ([1-^{14}C]citric) corresponded with the appearance of radioactivity. The sodium [1-^{14}C]citrate was freed of the indicator by charcoal treatment at 60° for 5 min and the solution was concentrated on a flash evaporator. The [1-^{14}C]citric acid obtained was purified by rechromatography on silicic acid.

Sodium [3,4-$^{14}C_2$]citrate: For the enzymic synthesis of this compound 5 μmoles of potassium [2,3-$^{14}C_2$]fumarate ($7 \cdot 10^6$ counts/min) were added to 3 ml of a solution containing 500 μmoles potassium phosphate buffer (pH 7.4), 0.7 unit of malate dehydrogenase, 0.6 unit of fumarate hydratase, 15 units of citrate-condensing enzyme, 6 μmoles of acetyl-CoA and 9 μmoles of NAD. The rate and extent of formation of [3,4-$^{14}C_2$]citrate was followed by the increase in absorbancy due to NADH formation at 340 mμ. Within 30 min the reaction was virtually complete giving a conversion of around 60 % based on potassium fumarate added. 40 μmoles each of potassium fumarate, potassium malate and potassium citrate were now added. The sodium [3,4-$^{14}C_2$]citrate was purified using the same procedure as for sodium [1-^{14}C]citrate.

Sodium [5,6-$^{14}C_2$]*citrate*: For the enzymic preparation of this compound, the incubation mixture was the same as that used for the preparation of [3,4-$^{14}C_2$]citric acid. The reaction, however, was started with 7 μmoles of potassium [1-^{14}C]fumarate ($9 \cdot 10^6$ counts/min) instead of potassium [2,3-$^{14}C_2$]fumarate. The sodium [5,6-$^{14}C_2$]-citrate was purified as described above.

Preparation of the enzyme solution

Fresh livers obtained from decapitated pigeons were minced and suspended in 1.5 vol. of a buffer solution[21] containing 8.5 ml of 1 M K_2HPO_4, 0.9 ml of 1 M KH_2PO_4, 7 ml of 1 M $KHCO_3$ and 1 ml of 1 M $MgCl_2$ in a total volume of 100 ml. The tissue was homogenized by four strokes of the pestle in a motor-driven Potter–Elvejhem homogenizer. The homogenate was centrifuged at 20000 $\times$ g for 20 min. The supernatant solution was then recentrifuged at 100000 $\times$ g for 90 min in a Spinco Model-L preparative ultracentrifuge, and the resulting supernatant solution was used as the fatty acid-synthesizing enzyme system.

Assay for fatty acid synthesis

Long-chain fatty acids were isolated from a copper-lime precipitate[22] according to the method of BRADY AND GURIN[23]. At the end of the incubation period the reaction was stopped by the addition of 0.5 ml of saturated KOH. 1 ml of absolute alcohol was added and the mixture was saponified at 95° for 45 min. The long-chain fatty acids were precipitated as their copper salts with 0.2 ml of 20% $CuSO_4$. 0.3 ml of 10% $Ca(OH)_2$ was added and the precipitate was washed thoroughly with water three times. The precipitate was suspended in 1 ml of water and the pH of this suspension was lowered to approx. 2 by addition of 2 N HCl. The free fatty acids thus formed were extracted into 5 ml of *n*-pentane by vigorous shaking for 2 min. An aliquot of the pentane solution was plated in aluminum cups and radioactivity determined in a gas-flow counter.

Gas chromatographic analysis

The fatty acids were first converted to their methyl esters with 2,2'-dimethoxypropane following the procedure of RADIN *et al.*[24]. Carrier methyl esters of myristate, palmitate and stearate were added and the methyl esters were separated quantitatively by gas chromatography. The radioactivity in each fraction was determined in a liquid scintillation counter (Nuclear Chicago Company).

RESULTS

Incorporation of [1,5-$^{14}C_2$]*citrate into fatty acids*

The results shown in Table I and Fig. 1 allow one to compare the efficiency of citrate and acetate as precursors for long-chain fatty acids. Since C-5 of citrate is not incorporated into fatty acids (Table VII below) the incorporation of C-1,5 labeled citrate is calculated on the basis of C-1 alone. Thus under similar circumstances 0.61 μmole of acetate or 0.58 μmole of citrate are used for fatty acid synthesis. The rate of incorporation of acetate and citrate into fatty acids was similar. With increasing amounts of unlabeled acetate a decrease in counts in fatty acids from sodium [1,5-$^{14}C_2$]-citrate was observed. Oxaloacetate lowers the incorporation of [1,5-$^{14}C_2$]citrate into

TABLE I

INCORPORATION OF [1,5-$^{14}C_2$]CITRATE AND [2-^{14}C]ACETATE INTO FATTY ACIDS

Each tube contained in a total volume of 0.78 ml, 0.6 ml of enzyme (75 mg protein/ml), 10 μmoles of magnesium fructose diphosphate, and the additions shown in the table. Tubes were flushed with N_2 and incubated for 60 min at 37°.

Precursor	*Additions (μmoles)*		*^{14}C-labeled compound incorporated in fatty acids (μmoles)*
		—	0.84
		0.5	0.70
		2.0	0.58
Potassium [1,5-$^{14}C_2$]citrate 10 μmoles	Sodium acetate	10.0	0.48
		25.0	0.45
		75.0	0.40
Sodium [2-^{14}C]acetate 2 μmoles	Potassium citrate	10.0	0.61

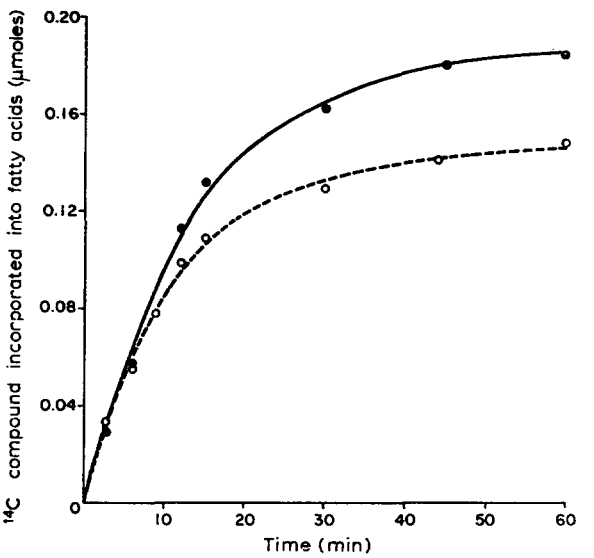

Fig. 1. Rate of [^{14}C]acetate and [^{14}C]citrate incorporation into fatty acids. Each tube contained in a total volume of 0.8 ml, 0.6 ml of enzyme (30 mg protein/ml) and 5 μmoles of magnesium fructose diphosphate. For the study of [2-^{14}C]acetate incorporation, those tubes contained 3 μmoles of [2-^{14}C]acetate (63 000 counts/min) and 10 μmoles of citrate (●—●). For the study of [1,5-$^{14}C_2$]-citrate incorporation, these tubes contained 10 μmoles of [1,5-$^{14}C_2$]citrate (300 000 counts/min) (○—○). Temperature of incubation was 37°.

fatty acids but only at much higher concentrations (Table II). This dilution must be of C-1 because C-5 of citrate is not converted to fatty acid carbon (Table VII).

Effect of citrate-condensing enzyme

Citrate-cleavage activity can be measured by trapping the acetyl-CoA formed with hydroxylamine and subsequently determining the amount of acetyl hydroxamate

TABLE II

EFFECT OF INCREASING CONCENTRATION OF ACETATE AND OXALOACETATE ON [1,5-$^{14}C_2$]CITRATE INCORPORATION

In a total volume of 0.85 ml, each tube contains 0.55 ml of enzyme (47 mg protein/ml), 5 μmoles of magnesium fructose diphosphate, 10 μmoles of potassium [1,5-$^{14}C_2$]citrate (260000 counts/min). Tubes were flushed with N_2 for 30 sec before incubation for 60 min at 37°.

Expt.	*Sodium acetate (μmoles)*	*Potassium oxaloacetate (μmoles)*	*[^{14}C]Citrate incorporated into fatty acids (μmoles)*
1	—	—	0.33
	0.5	—	0.30
	2.0	—	0.17
	10.0	—	0.13
	50.0	—	0.12
	100.0	—	0.10
	100.0	0.5	0.11
	100.0	2.0	0.12
	100.0	10.0	0.13
	100.0	50.0	0.10
	100.0	100.0	0.04
2	—	0.5	0.32
	—	2.0	0.34
	—	10.0	0.30
	—	50.0	0.21
	—	100.0	0.08
	—	160.0	0.06

formed. If condensing enzyme is added to a citrate-cleavage system, hydroxamate formation is drastically reduced because the acetyl-CoA and oxaloacetate are converted to citrate faster than acetyl-CoA can react with hydroxylamine. In a system where acetyl-CoA production is used to assay the cleavage activity the addition of condensing enzyme would seem to inhibit the reaction. Increasing concentration of condensing enzyme should therefore decrease the incorporation of ^{14}C from [^{14}C]-citrate into fatty acids. Addition of crystalline citrate-condensing enzyme (pig heart) to the fatty acid-synthesizing system results in an 80% reduction of [^{14}C]citrate incorporation (Table III).

Effect of ATP and CoA

A number of laboratories[4,6,25,26] have observed that low concentrations of citrate, ATP and CoA increase the incorporation of labeled acetate into fatty acids, but high concentrations result in a decreased incorporation. We feel the action of the citrate-cleavage enzyme explains these results. Initially citrate is needed for NADPH generation (see below) while ATP and CoA are necessary for acetate activation. When the concentration of citrate, ATP or CoA is increased, then the amount of citrate cleaved is increased which lowers the quantity of citrate available for NADPH production and increases non-radioactive acetyl-CoA in the medium. Both these effects will reduce the radioactive acetate incorporated into fatty acids.

5 μmoles of ATP and CoA also reduce the incorporation of labeled citrate into fatty acids. This is presumably caused by removing a source of NADPH by increasing

TABLE III

EFFECT OF CITRATE-CONDENSING ENZYME ON INCORPORATION OF [1,5-$^{14}C_2$]CITRATE INTO FATTY ACIDS

Each tube contained in a total volume of 0.65 ml, 0.5 ml of enzyme extract (50 mg protein/ml), 5 μmoles of magnesium fructose diphosphate and 10 μmoles of sodium [1,5-$^{14}C_2$]citrate (260 000 counts/min) in addition to varying amounts of condensing enzyme. Incubation was for 60 min at 37°. Each tube was flushed with nitrogen for 30 sec before starting the incubation.

Condensing enzyme (μmoles of citrate formed/h)	*[^{14}C]Citrate incorporated into fatty acids (μmoles)*
—	0.53
2.4	0.42
12.0	0.31
60.0	0.22
120.0	0.16
300.0	0.14
600.0	0.11

TABLE IV

EFFECT OF ATP AND CoA ON INCORPORATION OF RADIOACTIVITY INTO FATTY ACIDS FROM [2-^{14}C]ACETATE AND [1,5-$^{14}C_2$]CITRATE

In addition to the components shown in the table, each tube contained 0.6 ml of enzyme extract (47 mg protein/ml) and 5 μmoles of magnesium fructose diphosphate in a total volume of 0.85 ml. The glucose 6-phosphate regeneration system for NADPH consisted of the following: glucose 6-phosphate, 10 μmoles; NADP, 0.2 μmoles; K_2 ATP, 2 μmoles; glucose-6-phosphate dehydrogenase (0.6 μmole of NAD reduction/min) and crystalline hexokinase, 0.2 mg.

Substrate	*ATP (μmoles)*	*CoA (μmoles)*	*NADPH-regenerating system*	*^{14}C-labeled compound incorporated into fatty acids (μmoles)*
[2-^{14}C]acetate, 2 μmoles	—	—	Sodium citrate, 10 μmoles	0.64
	2	2		0.07
	5	5		0.01
	20	10		0.002
[1,5-$^{14}C_2$]citrate, 10 μmoles	—	—		0.81
	2	2		0.37
	5	5		0.070
	5	5	Glucose 6-phosphate systems	0.70

citrate cleavage, for when another NADPH-generating system is added then the incorporation is increased to normal levels (Table IV).

Fatty acid analysis

A gas chromatographic analysis of the fatty acids synthesized from [1,5-$^{14}C_2$]-citrate was undertaken in order to ascertain whether the composition of the fatty acids resembles the composition of fatty acids obtained from [2-^{14}C]acetate. The result of such an analysis is shown in Table V. The major fatty acids synthesized from labeled citrate are palmitate (66 %) and myristate (26 %). Incorporation into other fatty acids is small. A similar distribution of radioactivity into fatty acids synthesized from [2-^{14}C]acetate has been reported by PORTER AND TIETZ[27].

TABLE V

GAS CHROMATOGRAPHIC ANALYSIS OF FATTY ACIDS SYNTHESIZED FROM [^{14}C]CITRATE

This table shows a complete analysis for the fatty acids in the *n*-pentane extract from an incubation mixture containing [1,5-$^{14}C_2$]citric acid.

Fraction	*Counts/min*
n-Pentane extract	29 900
Recovered as methyl esters of fatty acids	24 750
$< C_{14}$	480
C_{14} methyl myristate	6 380
C_{15} methyl pentadecanoate	140
C_{16} methyl palmitate	16 400
C_{17} methyl heptadecanoate	270
C_{18} methyl stearate	670
$> C_{18}$	210

TABLE VI

INHIBITION OF FATTY ACID SYNTHESIS BY AVIDIN

Each tube contained in a total volume of 1.25 ml, 0.8 ml of enzyme, 12.5 μmoles of potassium citrate, 7.5 μmoles of magnesium fructose diphosphate. A 0.1% solution of avidin in 0.02 M phosphate buffer (pH 7.4) was used. The other components are shown below. Incubation was for 60 min at 37° after N_2 flush.

Substrate	*Avidin (μg)*	*^{14}C-labeled compound incorporated into fatty acids (μmoles)*
Sodium [2-^{14}C]acetate	—	0.41
2.5 μmoles,	20	0.29
95 000 counts/min	60	0.12
	150	0.06
	300	0.02
Potassium [1,5-$^{14}C_2$]citrate	—	0.51
10 μmoles,	20	0.34
155 000 counts/min	60	0.13
	150	0.05
	300	0.023

Inhibition by avidin

Table VI shows that avidin affects the incorporation of radioactivity from acetate and citrate into fatty acids to the same extent, which would imply that only the malonyl-CoA pathway was operative for citrate utilization. However, the inability of acetate to dilute out completely the acetyl-CoA pool from citrate (Table I) and the lowering of incorporation of radioactivity from labeled citrate by high concentrations of oxaloacetate (Table II) suggested that the oxaloacetyl moiety from citrate might give rise to malonyl-CoA by a route other than acetyl-CoA carboxylation or to fatty acids by a different mechanism. This was tested by using citrate labeled in the oxaloacetyl portion of the molecule.

Incorporation of variously labeled citrates

[1-^{14}C]Citrate, [5,6-$^{14}C_2$]citrate, [3,4-$^{14}C_2$]citrate were compared as precursors for fatty acid synthesis (Table VII). The cleavage enzyme has the same stereospecificity as the condensing enzyme, *i.e.*, it breaks the citrate exactly at the same carbon to

TABLE VII

INCORPORATION OF RADIOACTIVITY FROM VARIOUSLY LABELED CITRATES INTO FATTY ACIDS

Each tube contains in a total volume of 0.8 ml, 0.6 ml of enzyme (45 mg/ml of protein), 5 μmoles of magnesium fructose diphosphate and 10 μmoles of variously labeled potassium citrates as shown below. The tubes were flushed with N_2 for 30 sec and incubated for 60 min at 37°.

Radioactive citrate used	*Counts/min used*	*Counts/min in fatty acids*
[1,5-$^{14}C_2$]Citrate	12 360	470 ± 20
[1-^{14}C]Citrate	12 400	920 ± 40
[3,4-$^{14}C_2$]Citrate	12 540	50 ± 5
[5,6-$^{14}C_2$]Citrate	12 500	0

TABLE VIII

INCORPORATION OF [2-^{14}C]PYRUVATE INTO FATTY ACIDS

Each tube contains in a total volume of 0.8 ml, 0.6 ml of enzyme (52 mg protein/ml), 10 μmoles of potassium citrate, 5 μmoles of magnesium fructose diphosphate and 310 000 counts/min of [2-^{14}C]pyruvate.

Sodium pyruvate (μmoles)	*Sodium acetate (μmoles)*	*[^{14}C]Pyruvate incorporated into fatty acids (μmoles)*
0.3	—	0.0003
5	—	0.004
50	—	0.038
0.3	2	0.0003
5	2	0.004
50	2	0.033

TABLE IX

AVIDIN INHIBITION OF FATTY ACID SYNTHESIS FROM LABELED CITRATES

Each tube contains in a total volume of 1.25 ml, 0.8 ml of enzyme (50 mg protein/ml), 7.5 μmoles of magnesium fructose diphosphate and 12.5 μmoles of potassium citrate.

Citrate used	*Counts/min/tube*	*Avidin (μg)*	*^{14}C-labeled compound incorporated into fatty acids* (μmoles)*
[1,5-$^{14}C_2$]Citrate	150 000	—	0.90
[1,5-$^{14}C_2$]Citrate	150 000	300	0.025
[3,4-$^{14}C_2$]Citrate	49 000	—	0.03
[3,4-$^{14}C_2$]Citrate	49 000	300	0.0013

* In the case of [1,5-$^{14}C_2$]citrate this refers to C-1 only. However, in case of [3,4-$^{14}C_2$]citrate both C-3 and C-4 are taken into account (see text).

carbon linkage where it is formed (Bandurski and Topper, Bhaduri and Srere). These data show no incorporation of C-5 or C-6; very slight incorporation of C-3 and C-4 and good incorporation of C-1. When potassium [2-^{14}C]pyruvate was tested as a precursor in comparable systems only 1/3 the activity found for C-3 and C-4 was found to be incorporated (Table VIII). No pyruvate oxidase was found in the enzyme system.

Avidin inhibited the incorporation of the radioactivity both from [1,5-$^{14}C_2$]- and [3,4-$^{14}C_2$]citrates (Table IX).

DISCUSSION

The results using the variously labeled citrates for fatty acid biosynthesis indicate that only the acetyl-CoA moiety from citrate is incorporated into fatty acids to a significant extent. Nonetheless a fraction of the oxaloacetyl portion of the molecule is also incorporated into fatty acids. The lack of incorporation of C-5 or C-6 of citrate into fatty acids eliminates a pathway in which oxaloacetate is oxidatively decarboxylated to malonate with a subsequent incorporation into fatty acids.

The fact that pyruvate was a poorer source of fatty acid synthesis than oxaloacetate would seem to eliminate a pathway where oxaloacetate gave rise to pyruvate (by transcarboxylation[28] or decarboxylation) which was then converted to acetyl-CoA and thence to fatty acids. It is possible, however, that pyruvate generated continuously during the incubation would be a better precursor than added pyruvate. There may exist a pathway in which oxaloacetate is converted to malonyl-CoA and thence to fatty acids. Such an oxidative decarboxylation would be similar to those of pyruvate to acetyl-CoA or α-ketoglutarate to succinyl-CoA and one would predict the involvement of lipoate thiamine pyrophosphate, NAD and CoA, but not of biotin. Since the conversion is avidin sensitive this mechanism seems an unlikely one. Whatever the pathway, it does not seem to be quantitatively significant in the pigeon-liver system.

MARTIN AND VAGELOS[29] have shown that citrate stimulates the acetyl-CoA carboxylase (EC 6.4.1.2) reaction in rat adipose tissue preparations. This reaction is considered to be the rate-limiting step in fatty acid biosynthesis[30,31]. It would seem likely that part of the stimulatory effect of citrate observed with soluble enzyme systems is due to the activating effect of citrate on the carboxylating enzyme. Citrate is mostly localized inside the mitochondria[32] and the concentration of the extramitochondrial citrate is considerably lower than what these authors use for the activation of the carboxylase enzyme. Since citrate can act as a carbon source, a source of hydrogen, and as an activator for carboxylase, the concentration of citrate outside the mitochondria would be an important factor for regulating the fatty acid biosynthesis in the whole cell. The problem of citrate transport across the mitochondrial membrane under various conditions needs to be thoroughly explored.

Although for a number of years it has seemed unequivocal that fatty acid synthesis takes place chiefly in the cytoplasm a number of recent experiments have altered these views. HÜLSMAN[33] and WAKIL *et al.*[34] have shown that mitochondrial synthesis of fatty acids occurs and ABRAHAM *et al.*[14] have implicated microsomal enzymes in fatty acid synthesis. These experiments have been done with isolated cell fractions and to date there is no information on the site of synthesis in intact cells. One must also consider the availability of substrates to the various parts of the cell. It is likely that most of the acetyl-CoA formation of a cell takes place in the mito-

chondria therefore it must also be the precursor of cytoplasmic acetyl-CoA. This may occur in at least three ways: (a) by diffusion of acetyl-CoA, (b) by activation of acetate which has been formed in the mitochondria by deacylation of acetyl-CoA or (c) by cleavage of citrate which has been formed in the mitochondria and diffused out. Because of the distribution of citrate-utilizing and citrate-forming enzymes we feel the last possibility is an important one. Citrate may indeed be the precursor of acetyl-CoA for fatty acid biosynthesis in more physiological systems. Thus GORDON[35] has isolated labeled lipids from rats injected with [^{14}C]citrate and D'ADAMO AND HAFT[36] have shown that the isotope distribution in fatty acids of livers perfused with [^{14}C]glutamate indicates the operation of the citrate cleavage enzyme in their system.

ACKNOWLEDGEMENT

This work was supported in part by a grant from the National Institutes of Health (Grant No. A-2252).

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