phosphatidyl ethanolamine to phosphatidyl choline or phosphatidyl ethanolamine to phosphatidyl serine. Table II shows data of a time study of incorporation of $P_i^{32}$ into mitochondrial phospholipids of the liver of the hamster. It is apparent that there is very little difference in incorporation of $P_i^{32}$ into the various phospholipids of the liver of the hamster. Table III shows data of a time study of incorporation of $P_i^{32}$ into mitochondrial phospholipids of the kidney of the hamster. Phosphatidyl choline has the greatest incorporation of the isotope. The smallest amount of $P_i^{32}$ incorporated occurred in phosphatidyl ethanolamine. It is apparent from the data that the specific activity time curves give no evidence of any interconversion of the phospholipids in mitochondria.

*Summary.* *In vivo* incorporation of $P_i^{32}$ into the phospholipids of rat liver mitochondria, hamster liver and kidney mitochondria was investigated. The time course from 0.5 to 10 hours is given. The specific activity time curves give no evidence of any interconversion of the phospholipids in mitochondria.

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1. Dawson, R. M. C., Lindsay, D. B., Biochem. J., 1960, v77, 226.

2. Swanson, M. A., Artom, C., J. Biol. Chem., 1950, v187, 281.

3. Spiro, M. J., McKibbin, J. M., ibid., 1956, v219, 643.

4. Marinetti, G. V., Erbland, J., Stotz, E., Biochem. Biophys. Acta, 1958, v30, 642.

5. Youngs, J. N., Cornatzer, W. E., Comp. Biochem. Physiol., 1963, v9, 257.

6. Gurr, M. I., Finean, J. B., Hawthorne, J. N., Biochem. Biophys. Acta, 1963, v70, 406.

7. Youngs, J. N., Cornatzer, W. E., Proc. Soc. Exp. Biol. and Med., 1963, v112, 308.

8. Marinetti, G. V., Erbland, J., Albrecht, M., Stotz, E., Biochem. Biophys. Acta, 1958, v30, 543.

9. Dawson, R. M. C., Biochem. J., 1955, v61, 552.

10. Johnson, R. M., Dutch, P. H., Proc. Soc. Exp. Biol. and Med., 1951, v78, 662.

11. Fiske, C. H., Subbarow, Y., J. Biol. Chem., 1925, v66, 375.

12. Artom, C., ibid., 1941, v139, 953.

13. Schneider, W. C., Hogeboom, G. H., ibid., 1950, v183, 123.

14. Swanson, M. A., in S. P. Colowick and N. O. Kaplan, Methods in Enzymology II, New York, 1955, p541.

15. Cornatzer, W. E., Sandstrom, W. A., Reiter, J. H., Biochem. Biophys. Acta, 1962, v57, 568.

16. Marinetti, G. V., Erbland, J., Kochen, J., Fed. Proc., 1957, v16, 837.

17. Hevesy, G., Enzymologia, 1938, v5, 138.

18. Zilversmith, D. B., Entenman, C., Fishler, M. C., J. Gen. Physiol., 1943, v26, 325.

19. Artom, C., Biochem. Biophys. Res. Comm., 1964, v15, 201.

20. Borkenhagen, L. F., Kennedy, E. P., Fielding, L., J. Biol. Chem., 1961, v236, PC 28.

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## Fatty Acid Synthesis *in vitro* in a System from Rat Liver.* (30015)

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The experiments to be reported were carried out to investigate factors which may control the rate of fatty acid synthesis in a supernatant-microsomal system from rat liver. This synthesis comprises the following reactions: (a) activation of $CO_2$ with utilization of ATP in the presence of an enzyme which contains biotin, (b) carboxylation of acetyl-CoA with the activated $CO_2$ to form malonyl-CoA, (c) condensation of the malonyl-CoA with acetyl-CoA with the liberation, as $CO_2$ of the carbon previously fixed into acetyl-CoA, forming a $\beta$-ketoacyl derivative, and (d) subsequently the acyl derivatives are

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converted in several steps, some involving additional condensations, to give long-chained fatty acids. In this series of reactions the rate limiting step has been shown to be the condensation reaction (b) which leads to the formation of malonyl-CoA(1,2). This being the case, the control of fatty acid synthesis might occur either by modification of the enzyme level or activity, or because of changes in the availability of ATP, NADPH, (activated) $CO_2$ or of other intermediates in the reaction. In fasting and in diabetic rats the level of the condensing enzyme is actually reduced, but fatty acid synthesis suffers a disproportionately much greater reduction according to Korchak and Masoro(3). It has also been found that certain intermediates in the tricarboxylic acid cycle and other dicarboxylic acids affect the condensation reaction, *e.g.,* citrate, isocitrate, and malonate (see 1 for review).

We have reinvestigated the effect of the partial pressure of $CO_2$, citrate, malonate and some other factors on the condensation reaction by measuring the incorporation of acetate-1-$^{14}$C, and malonate-2-$^{14}$C into fatty acids.

*Materials and methods.* Adenosine triphosphate (ATP), nicotinamide adenine dinucleotide phosphate (NADP), and Coenzyme A (CoA), Sigma Chemical Co.; sodium acetate-1-$^{14}$C, Research Specialties Co.; malonic acid-2-$^{14}$C, New England Nuclear Corp.; reagents for liquid scintillation counting, Packard Inst. Co.; p-dioxane, Eastman Organic Chemicals; toluene, Merck and Co.

*Homogenate.* Livers excised from male Long-Evans rats (195-205 g) were immediately chilled in ice-cold buffer, then minced in a blendor for 15 sec with 3 times their weight of potassium phosphate buffer (0.1 *M*, pH 7.5). The brei was homogenized in a Potter-Elvehjem homogenizer with a loose-fitting pestle as recommended by Abraham *et al*(4). A supernatant-microsome fraction (SMc) was prepared from the homogenate by spinning at an average force of 10,300 $\times$ *g*. Incubations were started by adding SMc to the incubation flask containing the medium and other components. The protein content of preparations was measured by the biuret method(5). In some experiments the SMc was first subjected to dialysis at 0° for 5 hr in 5 mm diameter Visking bags against several changes of $CO_2$-free phosphate buffer (pH 7.5) saturated with nitrogen. For some of the work the microsomes (Mc) were centrifuged out of the supernatant (S) at 105,000 $\times$ *g*.

*Incubation procedure.* Most incubations were carried out in a total volume of 4.5 ml, of which 2 ml consisted of SMc, 2 ml of medium, and 0.5 ml of water or other variable. Unless indicated otherwise, the final mixture invariably had the composition: 67.7 mM glycylglycine, 19.4 mM $MgCl_2$, 22.2 mM potassium citrate, 13.3 mM ATP, 0.53 mM NADP, 1.72 mM potassium acetate, 33 mM $K_2HPO_4$ at a final pH of 7.5. Radioactivity, added as a trace of sodium acetate-1-$^{14}$C, amounted to approximately $10^6$ dpm. Incubations were carried out at 30°, for 2 hours with gentle shaking.

*Extraction of fatty acid.* The reaction was stopped by addition of 2 ml of 17 *N* KOH to the contents of the reaction flasks(6). Hydrolysis was carried out for several hours on the steam bath. The reaction mixture, after being acidified with 10 *N* HCl was extracted 4 times with 10 ml heptane in an apparatus described elsewhere(6). The combined heptane extracts were washed once with 10 ml 5% acetic acid followed by 2 washes with 10 ml water. Subsequently they were dried with anhydrous sodium sulfate. The final heptane solution contains free fatty acids of medium to long chain length, non-saponifiable material, but no labeled acetic acid. Aliquots were counted in a scintillation counter in which efficiencies were checked routinely with an internal standard (toluene-$^{14}$C).

*Results. Incorporation with various cell fractions.* The behavior of the various cell fractions is recorded in Table I. When SMc is taken as standard, little incorporation occurs with the cell debris, addition of a 'natural' amount of mitochondria (Mt) has little effect but an excessive load of this fraction lowers the incorporation to 28% of the normal. The supernatant or microsomes alone are ineffective.

*Incorporation into various lipid fractions.* By using a suitable modification of the meth-

TABLE I. Incorporation into Various Cellular Fractions.

| Cell fraction*(2) | Relative acetate incorporation | Cell fraction* (4) | Relative acetate incorporation |
|---|---|---|---|
| SMc | 100 | SMc | 100 |
| Cell debris | 22 | S | 3.6 |
| SMc, Mt (natural conc) | 116 | Mc | 1.8 |
| SMc, Mt (3 × natural conc) | 28 | | |

Values in the table are relative incorporations compared to those obtained for the SMc incubation mixtures which are set at 100.

*Conditions:* Incubations were anaerobic with substrates and cofactors (including bicarbonate, 10 mM) as indicated in text. There was 5% incorporation of the added acetate with SMc.

* S, supernatant alone; Mc, microsomes alone resuspended in buffer; SMc, supernatant + microsomes; Mt, mitochondria.

No. of rat livers used in parentheses.

od of extraction it was found that most of the activity was incorporated into the saponifiable fraction; *e.g.*, in one experiment there was 5.5% in the saponifiable fraction, and 0.04% in the nonsaponifiable fraction. Since similar ratios were found in other experiments, in those reported only total incorporation was measured. When changes were made in the extraction procedure the results shown in Table II were obtained. Most of the activity was found in fatty acids or their derivatives, judging by their solubilities.

*Effect of acetate and malonate concentrations.* When the concentration of acetate in the medium was varied by adding different levels of unlabeled acetate to the acetate-1-$^{14}$C the results shown in Table III were obtained. As acetate concentration goes up, the percent incorporation decreases rapidly but actual incorporation of acetate increases to a maximum at 1.5 mM. Hence a slightly higher concentration of acetate (1.72 mM) was used in subsequent experiments.

Incorporation of 10 mM malonate-2-$^{14}$C in the presence of 1.72 mM acetate was only 2 to 4% of the incorporation of 1.72 mM acetate-1-$^{14}$C when this was used alone (Table III, Exp. C). A further increase in the labeled malonate concentration did not result in a significant change in its incorporation. Little effect of bicarbonate on malonate incorporation could be seen.

TABLE II. Incorporation into Various Lipid Fractions.

| Fraction | Method of extraction | Avg results of 2 exp |
|---|---|---|
| | | (%) |
| Triglycerides and phospholipids | Incubation mixture at pH 8 extracted with chloroform | 34 |
| Free fatty acid | Incubation mixture acidified to pH 2 and extracted with heptane | 8 |
| Coenzyme A derivatives of fatty acids | Digested with KOH, acidified and reextracted with heptane | 58 |

*Conditions:* As in Table I and text except that they were carried out in air and there was a 10 min preincubation period with citrate (50 mM during preincubation, 22 mM during incubation).

*Effect of bicarbonate concentration.* When incubations were carried out under oxygen with various concentrations of $CO_2$ (1-5%) in the gaseous phase and $KHCO_3$ in the media adjusted by the method of Umbreit(7), results shown in Fig. 1 were obtained. Very little incorporation occurred in the absence of $CO_2$ and incorporation increased to a maximum at a terminal bicarbonate concentration of 30 mM as measured at the end of incubation by the Van Slyke manometric method.

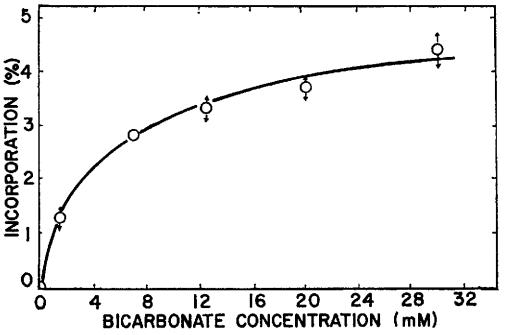



FIG. 1. Effect of bicarbonate concentration on acetate incorporation. *Conditions:* as in Table I. Lowest concentration of bicarbonate (1.5 mM) was attained in oxygen with 10 N KOH in center wells. Other bicarbonate levels were attained using oxygen mixtures containing 1, 2, 3, or 5% $CO_2$. In the presence of avidin (1 mg with 2500 units) only 0.1% acetate was incorporated. All incubations done in quadruplicate.

*Effect of citrate and malonate.* The effect of citrate concentration at various levels of bicarbonate is shown in Fig. 2. A sharp opti-

TABLE III. Effects of Acetate and Malonate on Incorporation of Same.

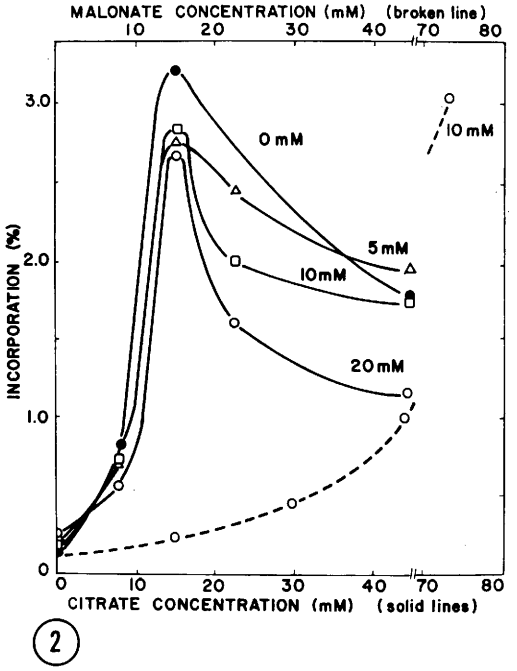
| Acetate conc (mM)*† | Malonate conc (mM) | Bicarbonate conc (mM)‡ | Relative incorporation† |
|---|---|---|---|
| Exp A | | | |
| .46 (3) | 0 | 10 | 56 |
| 1.72 (2) | 0 | 10 | ≡100 |
| 3.41 (3) | 0 | 10 | 97 |
| 5.11 (2) | 0 | 10 | 101 |
| Exp B | | | |
| 1.72 (2) | 0 | 10 | ≡100 (A) |
| 1.72 (2) | 15 | 10 | 180 (A) |
| 1.72 (2) | 30 | 10 | 460 (A) |
| 1.72 (2) | 45 | 10 | 1040 (A) |
| 1.72 (2) | 75 | 10 | 3170 (A) |
| Exp C | | | |
| 1.72 (2) | 0 | 10 | ≡100 (A) |
| 1.72 (1) | 10 | 0 | 2 (M) |
| 1.72 (2,A) (1,M) | 10 | 10 | 128 (A), 4 (M) |
| 1.72 (2,A) (2,M) | 25 | 10 | 129 (A), 5 (M) |

*Conditions:* As in Table I except that in the incubation mixture for the flasks in Exp B, only 6.7 mM ATP (½ usual conc) was used, citrate was absent, and ionic strength was kept constant by adding KCl to give a total concentration of 0.150 mE. In Exp C, glutathione was present (10 mM).

* No. of samples incubated shown in parentheses.

† Acetate-1-$^{14}$C, A; Malonate-2-$^{14}$C, M. Incorporation relative to acetate at 1.72 mM conc.

‡ Bicarbonate added. Actual conc after incubation not measured.

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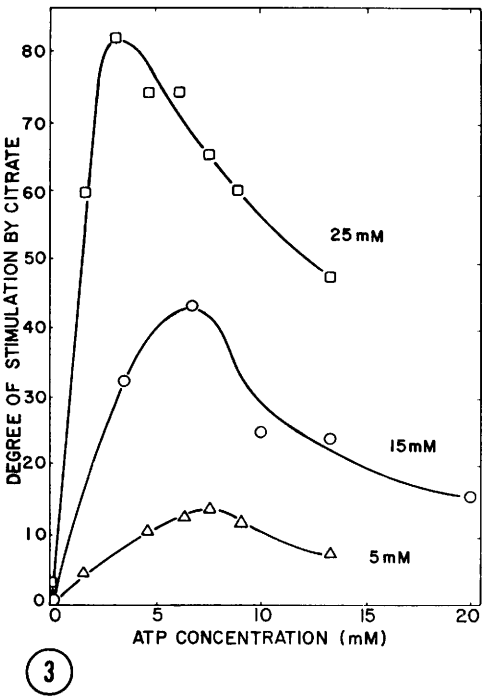


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FIG. 2. Effect of citrate and malonate on acetate incorporation with various levels of bicarbonate. *Conditions:* incubations done in air, otherwise as in Table I except in malonate experiments the ATP concentration was only half that normally used (6.7 mM) and total ionic strength of malonate plus chloride was 150 mE.

FIG. 3. Effect of ATP and citrate on incorporation. *Conditions:* as in Table I except acetate concentration 2.0 mM, citrate as indicated on individual curves, and bicarbonate 10 mM. For definition of degree of stimulation see Table IV.

TABLE IV. Bicarbonate and Stimulation by Citrate or by Malonate.

| Bicarbonate conc (mM) | Degree of stimulation* | | | | | |
|---|---|---|---|---|---|---|
| | Citrate conc (mM) | | | Malonate conc (mM) | | |
| | 0 | 14.8 | 44.4 | 1 | 10 | 50 |
| 0 | 1 | 18 | 10 | 1 | 1.8 | 4.6 |
| 5 | 1 | 14 | 10 | – | – | 6.5 |
| 10 | 1 | 14 | 9 | 1 | 2.5 | 6.6 |
| 20 | 1 | 10 | 5 | – | – | 5.6 |

*Conditions:* As in Table I except as indicated in Table. Incorporation of acetate at 0 citrate or malonate was approximately 0.2% (degree of stimulation, 1).

* Degree of stimulation is the quotient of incorporation observed and incorporation at 0 conc of di-(or tri-)carboxylic acid.

mum occurs at a citrate concentration of about 15 mM. With malonate there is no such optimum concentration but the effect of malonate becomes more and more pronounced up to the highest concentration tested (75 mM). Increase in bicarbonate in the presence of citrate does not enhance the citrate effect whereas the effect of malonate is enhanced (Table IV). It is of interest that malonate stimulates in the presence of optimum concentrations of citrate and vice versa, and above all further stimulation occurs when the supernatant is preincubated with either citrate or malonate (Table V).

*Effect of ATP.* The incorporation of acetate at various levels of citrate varies with the concentration of ATP as shown in Fig. 3. Incorporation rises to a maximum at 3 to 7 mM ATP, depending on citrate concentration. At high citrate concentrations less ATP seems to be necessary.

*Effect of miscellaneous additions.* The effect of various other factors in the incorporation is shown in Table VI. It is evident that the supernatant is somewhat deficient in CoA, particularly in male animals but that addition of NADPH has little effect. ADP in high concentrations is inhibitory. Fluoroacetate has little effect and oxaloacetate is like-

TABLE VI. Effect of Miscellaneous Additions.*

| Addition | Concentration (mM) | Relative incorporation† |
|---|---|---|
| None | — | 100 (♂ or ♀) |
| Coenzyme A | .06 | 191 (♂), 139 (♀) |
| NADPH‡ | .56 | 97 (♀) |
| ADP‡ | 14.0 | 64 (♂) |
| ADP | 40.0 | 29 (♂) |
| Fluoroacetate§ | 1.7 | 105 (♂) |
| Fluoroacetate | 17.0 | 60 (♂) |
| Oxaloacetate | 4.4 | 110 (♂) |
| (Boiled enzyme mixture) | — | 1 (♂ or ♀) |

*Conditions:* As in Table I except that incubations were carried out in air, ATP 6.7 mM rather than 13.4 mM in exp on first 5 lines.

* For effect of methionine, ethionine and their S-adenosyl derivatives see accompanying paper (Doering, C. H., and Natori, Y.).

† Sex of animals indicated in parentheses. For definition of relative incorporation see Table IV.

‡ NADPH, reduced nicotinamide-adenine dinucleotide phosphate; ADP, adenosine diphosphate.

§ Gift of Dr. I. Edelman.

TABLE V. Effect of Preincubation with Citrate and Malonate.

| Concentration during preincubation (mM) | Concentration during incubation (mM) | Incorporation (%) | Degree of stimulation* |
|---|---|---|---|
| 36 KCl | 40 KCl | 0.3 | 1 |
| 36 KCl | 20 KCl<br>25 malonate | 1.0 ± .1 | 3 |
| 36 KCl | 15 citrate<br>25 malonate | 4.9 ± .1 | 16 |
| 27 citrate | 15 citrate<br>25 malonate | 7.0 ± .3 | 23 |
| 45 malonate | 15 citrate<br>25 malonate | 7.2 ± .2 | 24 |

*Conditions:* As in Table I except that SMc was subjected to dialysis against $O_2$-, $CO_2$-free phosphate buffer (pH 7.5), saturated with nitrogen. *Preincubations* were carried out for 30 min at 30° in a total volume of 2.5 ml (2.0 ml SMc, 0.5 ml additions). At this stage the total conc of malonate, citrate and chloride was 40 mM. *Incubations* were started by addition of glycyl-glycine, Mg ion, bicarbonate (final conc 10 mM), ATP, NADP, and acetate-1-$^{14}$C in a total vol of 1.5 ml. All incubations were done in duplicate.

* For degree of stimulation see Table IV.

wise without effect. Boiled supernatant is ineffective as might be anticipated.

*Discussion.* The properties of the experimental system used in these studies are shown by (1) incorporation of $^{14}C$ as acetate into fatty acids and more complex fatty acid derivatives, (2) a requirement for both microsomes and supernatant, neither fraction being active alone, (3) negligible incorporation of malonate, (4) sensitivity of the system to changes in concentration of bicarbonate, citrate, malonate, ATP, and CoA, (5) enhancement of the activity of the system by preincubation with citrate or malonate, (6) inhibition by ADP but not by fluoroacetate, (7) inhibition by avidin. These observations largely serve to confirm those made by other workers who have used systems prepared somewhat differently either from liver or from other tissues and organisms. We shall confine our discussion to the effects of bicarbonate, citrate, and malonate.

The stimulating effect of (mono-), di- and polycarboxylic acids on the synthesis of fatty acids have been dealt with by many workers, *e.g.*,(8,9,1,2) who have attempted to explain the results in different ways: (1) Participation of citrate by providing a source of (a) NADPH, (b) bicarbonate, (c) carboxyl groups (transcarboxylation), (d) complex formation with metallic inhibitors or (2) specific effect of citrate and other acids on the acetyl-CoA carboxylase enzyme(1) as seen in the experiments of Vagelos *et al*(10). No doubt other mechanisms might be brought forward to explain the enhancement of acetate incorporation by citrate etc., and other mechanisms exist for control of fatty acid synthesis as for example, through inhibition of synthesis by CoA derivatives(2), or through the promotion by citrate of acetyl-CoA transfer between mitochondria and cytoplasm(11,12), but our present concern is with the citrate-malonate effect.

To deal with the above points in order, it is clear (1a) that in the *in vitro* system there is no lack of NADPH. Additional reduced coenzyme has no effect on the system. This does not mean that *in vivo* there might not be control *via* NADPH/NADP adjustments (9) as postulated by Wieland(13).

The results shown in Fig. 2 and Tables III and IV show that the bicarbonate concentration has an effect on acetate incorporation and that this effect is related to that of the stimulating acids (1b). The effect of bicarbonate concentration had been noted previously by Gibson *et al*(14), but in view of the high concentrations required to elicit significant effects it does not appear as a likely part of a control mechanism. Moreover, low concentrations of bicarbonate suffice for the attainment of nearly maximum rates of incorporation. On the other hand transcarboxylation (1c) from citrate or other similar acids has been considered to be important by Abraham *et al*(15,16) and by Hülsmann(17). However, a number of objections to this concept have been considered in detail by Lynen *et al*(2) and, since nearly all the evidence is against this theory, we will not repeat the arguments here. It is of interest to draw attention to the inhibitory effect of citrate (but not of malonate) at high concentration as seen in Fig. 2. It may be explained on the basis of a cleavage of the molecule which would provide unlabeled acetyl-CoA to dilute the labeled material being incorporated(11,12,18). Malonate, not being cleaved, is not inhibitory at high concentrations.

Lynen and coworkers(2) have noted that the action of the various acids such as citrate is not related to their ability to complex metallic ions. Hence mechanism (1d) may be eliminated. It remains, therefore, to consider the direct action of the acids on the carboxylation reaction. The present results (particularly the preincubation experiments, Table V) strongly support the conclusion that the carboxylase enzyme itself is affected by the presence of the malonate or citrate, and confirm the conclusions of Vagelos *et al*(10) and Lynen *et al*(2). Activation of the incorporation of acetate requires time, as does a configurational change for a protein-enzyme. It must be emphasized that malonate, although it is as effective or more effective than citrate in stimulating incorporation (Fig. 2, and Table IV) is not incorporated to any extent into lipids in the system we used in these experiments (Table III). Presumably it is not made into a CoA-derivative in

the present system and hence does not enter the synthetic pathway.

The data in Table IV and Fig. 2 show that at high concentrations of citrate, there is an inhibition of incorporation by high bicarbonate concentrations. This is in agreement with the recent findings of Majerus *et al*(19) who have shown, in agreement with Lynen(20), that the activated intermediates in fatty acid synthesis are protein bound. Moreover, the acyl groups which are bound to acyl carrier protein (ACP) are in reversible equilibrium with their products(19): Acetyl-S-ACP + malonyl-S-ACP enzyme $\rightleftarrows$ acetoacetyl-S-ACP + ACP-SH + $CO_2$. This clearly explains the $CO_2$ effect.

*Summary.* A preparation from rat liver containing both microsomes and soluble enzymes when supplemented with the necessary cofactors incorporates acetate-1-$^{14}$C preponderantly into the saponifiable fraction of the lipids. Bicarbonate has no significant stimulating effect on the synthesis whereas citrate and malonate have pronounced and similar stimulatory effects, particularly when they are preincubated with the synthesizing system. However, at high citrate concentrations bicarbonate becomes inhibitory. Malonate stimulates fatty acid synthesis without participating as a metabolite. The same is concluded with citrate, at least with concentration up to 15 mM. An interrelationship between citrate and ATP concentrations and the stimulation of fatty acid synthesis is indicated.

1. Numa, S., Matsuhashi, M., Lynen, F., Biochem. Z., 1961, v334, 203.

2. Lynen, F., Matsuhashi, M., Numa, S., Schweizer, E., in The control of lipid metabolism, J. K. Grant, ed., Academic Press, London & New York, 1963.

3. Korchak, H. M., Masoro, E. J., Biochim. Biophys. Acta, 1962, v58, 354.

4. Abraham, S., Matthes, K. J., Chaikoff, I. L., J. Biol. Chem., 1960, v235, 2551.

5. Gornall, A. G., Bardawill, C. J., David, M. M., ibid., 1949, v177, 751.

6. Doering, C. H., Tarver, H., Anal. Biochem., 1964, v9, 498.

7. Umbreit, W. W., Burris, R. H., Stauffer, J. F., eds., Manometric Techniques, Burgess, Minneapolis, 1957.

8. Brady, R. O., Gurin, S., J. Biol. Chem., 1950, v186, 461.

9. Popjak, G., Tietz, A., Biochem. J., 1955, v60, 147.

10. Vagelos, P. R., Alberts, A. W., Martin, D. B., J. Biol. Chem., 1963, v238, 533.

11. Spencer, A. F., Lowenstein, J. M., ibid., 1962, v237, 3640.

12. Srere, P. A., Bhaduri, A., Biochim. Biophys. Acta, 1962, v59, 487.

13. Wieland, O., 4th Congres de la Fed. Int. du Diabetes, Geneva, 1961, p10.

14. Gibson, D. M., Titchener, E. B., Wakil, S. J., Biochim. Biophys. Acta, 1958, v30, 376.

15. Abraham, S., Matthes, K. J., Chaikoff, I. L., Biochem. Biophys. Res. Comm., 1960, v3, 646.

16. ———, Biochim. Biophys. Acta, 1961, v46, 197.

17. Hülsmann, W. C., ibid., 1960, v45, 623; 1962, v62, 620.

18. Formica, J. V., ibid., 1962, v59, 739.

19. Majerus, P. W., Alberts, A. W., Vagelos, P. R., Proc. Nat. Acad. Sci., 1964, v51, 1231.

20. Lynen, F., Fed. Proc., 1961, v20, 941.

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## Studies on Ethionine. VII. Fatty Acid Synthesis in Livers of Ethionine-Treated Rats.* (30016)

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Administration of ethionine, the S-ethyl analog of methionine, to female rats causes a marked and rapid accumulation of lipid in the liver(1,2,3). This ethionine-induced fatty liver is accompanied by a fall in triglyceride

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