

tion showed that the theoretical RD curve is very sensitive to even small changes in the angular parameters. We therefore consider the preliminary parameters to be close to the final ones, though we intend to refine the structure further using least-squares refinement. The agreement between the experimental auto-correlation power spectrum (APS)<sup>5</sup> and the corresponding theoretical curve based on the bond distances determined from the RD function is quite good.

The angular parameters for the ring and methyl carbons in  $(F_3PNCH_3)_2$  are in good agreement with the corresponding parameters of  $(Cl_3PNCH_3)_2$ <sup>6</sup> and  $(PhF_2PNCH_3)_2$ <sup>7</sup> determined by Hoard and Jacobsen and Cox and Corey, respectively, using the X-ray crystallographic technique. The P—F bond lengths in  $(F_3PNCH_3)_2$  and  $(PhF_2PNCH_3)_2$  are not significantly different. While there is good agreement between the present values for the P—N bond distances with those found in the two crystal structures, we find a considerably longer C—N bond in  $(F_3PNCH_3)_2$ , 1.52 Å, compared to 1.475 Å in  $(Cl_3PNCH_3)_2$  and 1.44 Å in  $(PhF_2PNCH_3)_2$ . Theoretical RD curves calculated using the values determined for comparable bond distances in  $(Cl_3PNCH_3)_2$  and  $(PhF_2PNCH_3)_2$  are definitely not in agreement with our experimental RD curve.

*Acknowledgement.* We are indebted to Dr. R. Schmutzler who drew our attention to the problem and supplied us with a sample of the compound. One of us (B. A.) wants to express her gratitude to the *Norwegian Research Council for Science and Humanities* for financial support.

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Received June 25, 1969.

## Evidence Suggesting Independent Pathways for the Synthesis of Rat Liver Fatty Acids from Acetyl-CoA and Malonyl-CoA Respectively

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We have previously shown<sup>1</sup> that an addition of citrate to rat liver homogenates will result in a specific enhancement of the synthesis of myristic acid from (<sup>14</sup>C) acetate. Recent work by Smith and Dils<sup>2</sup> and Bartley and co-workers<sup>3</sup> has demonstrated a connection between the size of the available malonyl-CoA pool and the pattern of the fatty acids synthesized, small malonyl-CoA concentrations favouring the formation of shorter chained fatty acids such as myristic acid. In the paper by Bartley and co-workers<sup>3</sup> it is suggested that our findings may be due to the gradual synthesis and utilization of malonyl-CoA when acetate or acetyl-CoA are the precursors, the available malonyl-CoA pool thus being small at any given time.

In the present investigation we have studied the effect of adding malonyl-CoA to a rat liver homogenate which is synthesizing fatty acids from acetate or acetyl-CoA. The results show, that this does not lead to any relatively reduced synthesis of myristic acid. It seems that there are independent pathways for fatty acid synthesis from acetyl-CoA and malonyl-CoA, respectively.

*Experimental.* Liver tissue was taken from two mature albino rats. The homogenization medium was 0.05 M phosphate buffer pH=7.0 containing 0.01 M MgCl<sub>2</sub>, 0.02 M nicotinamide and 0.10 M sucrose. The ratio between liver (g) and medium (ml) was 1 to 2.5. Cell debris and nuclei were removed by centrifugation at 800 g for 10 min.

The incubation media contained further 0.001 M NADH and 0.001 M NADPH besides K-citrate and precursors as specified. No extra ATP was added, to avoid mitochondrial decarboxylation of (<sup>14</sup>C) malonyl-CoA as described by Nakada *et al.*<sup>4</sup> (1-<sup>14</sup>C) acetate and (1,3-<sup>14</sup>C<sub>2</sub>) malonyl-CoA were obtained from NEN Chem-

Table 1. Fatty acid synthesis from (1-<sup>14</sup>C) acetate and (1,3-<sup>14</sup>C<sub>2</sub>) malonyl-CoA by samples of the same rat liver homogenate.

Citrate conc. (mM)	Added substrate				Incorporated <sup>14</sup> C-activity (means ± S.E. of means)							
	Acetate expressed as:		Malonyl-CoA expressed as:		Total fatty acids expressed as:		Relative distribution among acids (%)					
	μCi × 10 <sup>3</sup>	mμ-moles	μCi × 10 <sup>3</sup>	mμ-moles	μCi × 10 <sup>3</sup>	mμ-moles precursor	Myristic	Palmitic	Palmitoleic	Stearic	Oleic	Rest
20	1000	225	0	0	337 ± 23	76	25 ± 4	56	2	9	3	5
0	0	0	30	30	8 ± 1	16	7 ± 2	41	26	13	8	5
20	0	0	30	30	9 ± 4	18	10 ± 2	42	26	11	8	3
0	0	225	30	30	10 ± 3	21	11 ± 1	38	27	15	5	4
20	0	225	30	30	8 ± 1	17	12 ± 1	43	27	10	4	4
20	1000	225	30	30	35 ± 10	—	31 ± 3	37	7	14	3	8

The incubation conditions and further analytical procedures are described in the text. The term "Rest" covers the fatty acids detected between C<sub>14-16</sub> and C<sub>16-18</sub> together with the unsaturated acids >C<sub>18</sub>. The average standard error in the last five columns was ± 1 %.

icals, Dreieichenhain/Frankfurt, West Germany. Total incubation volumes were between 2.1 and 2.3 ml. All incubations were run in duplicate for 2 h at 37.5° under aerobic conditions.

The contents of each incubation flask were saponified with alcoholic potassium hydroxide overnight at 50°. The saponified samples were extracted three times with pentane, acidified, and again extracted with pentane. The individual long-chain fatty acids were then assayed by paper chromatography as previously described.<sup>5,6</sup>

Results are shown in Table 1. The top row represents fatty acid synthesis from acetate alone. It is characterized by a relatively high incorporation into myristic acid. Further experiments with incubations based on (1-<sup>14</sup>C) acetyl-CoA have shown, in agreement with the findings of Lorch *et al.*,<sup>7</sup> that <sup>14</sup>C-labelled fatty acid patterns with substantial activities in myristic acid are common for both acetate and acetyl-CoA as precursors.

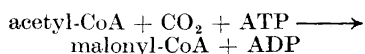
The next four rows represent fatty acid synthesis with <sup>14</sup>C-labelled malonyl-CoA as the precursor. They are primarily characterized by a relatively low incorporation into myristic

acid. The factors varied are the addition of citrate and non-labelled acetate, combined in such a way as to include the effect of acetate with and without activation of the carboxylase enzyme. It is seen that these factors have no influence on fatty acid synthesis from malonyl-CoA. Total incorporations have been calculated on the assumption that only half of the added (1,3-<sup>14</sup>C<sub>2</sub>) malonyl-CoA activity was available for condensation into fatty acids.

The results shown in the bottom row represent a combination of the two precursor systems. Above all one is struck by the fact that in spite of a marked metabolic interaction as far as total fatty acid synthesis is concerned, the fatty acid pattern resembles an incorporation from acetate alone. It seems that the same added malonyl-CoA that has been active enough to inhibit fatty acid synthesis from acetate by more than a factor of ten, is unable to penetrate the corresponding malonyl-CoA pool generated from acetate by the acetyl-CoA carboxylase enzyme. This appears most directly from a comparison between the results in row five and row six of the table. Here the chemical conditions are exactly the same: we have added 225 mμmoles acetate and 30

mμmoles malonyl-CoA. The only difference is, that the acetate added in row five is unlabelled, while that added in row six is  $^{14}\text{C}$ -labelled in the carboxyl group. The higher myristic acid value seen in row six (31 % of total activity compared to 12 %) can thus only be due to an incorporation of ( $^{14}\text{C}$ ) acetyl-CoA independent of added ( $^{14}\text{C}$ ) malonyl-CoA.

Our main conclusion from the results of the present investigation is that not all fatty acid synthesis from acetyl-CoA in rat liver homogenates is initiated by the reaction

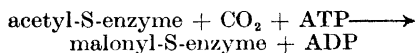


regarded as a chemical process in a homogeneous medium. The apparent lack of a common ( $^{14}\text{C}$ ) malonyl-CoA pool for fatty acid synthesis from ( $^{14}\text{C}$ ) acetate and ( $1,3\text{-}^{14}\text{C}_2$ ) malonyl-CoA stresses this point.

Since we have shown that myristic acid is a major product of fatty acid synthesis from acetate or acetyl-CoA, any influence from cell particle systems can be ruled out. *De novo* synthesis leads to stearic acid in both the mitochondria and the microsomes (Wakil *et al.*,<sup>8</sup> Lorch *et al.*<sup>7</sup>) and the addition of cell particles to the supernatant fraction will result in a lengthening of the synthesized fatty acid chains (Lorch *et al.*,<sup>7</sup> Gompertz and Greenbaum<sup>9</sup>).

It might be argued that the fact that we did not add extra ATP to our incubations hindered a direct carboxylation. However, we have shown in a separate experiment that the addition of 10 μmoles ATP as a supplement to the endogenous ATP produced in our system does not influence fatty acid patterns. Other preliminary results have shown that the two precursor systems run at constant rates relative to each other, at least during the first half hour of the incubation period.

Without further experimental evidence, we would suggest that there is a non-homogeneous carboxylation reaction



which under our conditions is functioning parallel to the normally seen homogeneous one involving CoA. Recent work by Lynen *et al.*<sup>10</sup> on yeast synthetase has shown that the acetyl group from acetyl-CoA can be transferred onto the "central" SH site of the multi-enzyme fatty acid synthetase complex. An additional carboxylation at this stage would make up an acetyl-activation pathway with obvious energy conservation advantages compared with the synthesis of malonyl-CoA. One could imagine the carboxylation coupled to the decarboxylation of the malonyl group that takes place in connection with the following condensation reaction with acyl-enzyme.

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Received May 28, 1969.