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Biosynthesis of fatty acids: IV. Studies with inhibitors

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SUMMARY

Tetrolyl-CoA and propiolyl-CoA have been shown to be strong noncompetitive inhibitors of fatty acid synthesis. Acrylyl-CoA and isocrotonyl-CoA also inhibit, but much higher concentrations are required. It is suggested that this inhibition occurs through an interaction between the triple and double bonds of these compounds and enzyme sulfhydryl groups. Palmityl-CoA and free CoA have also been shown to inhibit fatty acid synthesis and to block the condensation of acetyl-CoA with malonyl-CoA, and the reduction of crotonyl-CoA to butyrate. The reduction of acetoacetyl-CoA to the β-hydroxy derivative was not inhibited by these materials, although the complete reduction to butyrate was prevented. The addition of flavin nucleotides also inhibited fatty acid synthesis catalyzed by brain enzyme preparations.

The impairment of fatty acid synthesis by several well-recognized inhibitors of sulfhydryl enzymes has been reported previously (1–3). In the course of studies of possible intermediates in fatty acid synthesis, it was discovered that alkyne acyl-CoA analogs are strongly inhibitory. Evidence for the inhibition of several steps in fatty acid biosynthesis is presented. Furthermore, free coenzyme A (CoA), a by-product of fatty acid synthesis, and palmityl-CoA inhibit similarly at concentrations equivalent to optimal substrate levels. The present communication describes experiments dealing with the inhibition of fatty acid synthesis by these and several flavin-containing compounds.

EXPERIMENTAL PROCEDURE

Preparation of Substrates. The anhydride of tetrolic acid was prepared by reacting sodium tetrolate with $POCl_3$ (4), or, alternatively, the mixed anhydride was formed with ethyl chloroformate (5). These compounds were converted to the CoA derivative (5), which was purified by paper chromatography (6). Propiolyl-CoA and acrylyl-CoA were prepared similarly. Palmityl-CoA was prepared from the mixed anhydride (7) or from palmityl chloride (8). Other CoA derivatives were prepared as previously described (9).

Tetrolic acid was obtained from Farchan Research Laboratory, Cleveland, Ohio. 1,1,3-Tricyano-2-amino-1-propene (U-9189) was a gift from Dr. P. W. O'Connell of the Upjohn Co.

Preparation of Enzymes. The preparation of fatty acid synthesizing enzymes from rat brain and liver has been described previously (3, 9). The enzyme preparation from rat adipose tissue was prepared according to Martin et al. (10).

Methods. The rate of fatty acid synthesis was determined with acetyl-1-C^{14}-CoA or malonyl-1,3-C^{14}-CoA, or spectrophotometrically by measuring the oxidation of triphosphopyridine nucleotide (TPNH) at 340 mμ. The stoichiometry of these reactions has been demonstrated in a previous communication from this laboratory (3).

RESULTS

Tetrolyl-CoA, the acetylenic analog of crotonyl-CoA, is not reduced by fatty acid synthesizing enzyme prep-

arations from brain, liver, or adipose tissue. The compound strongly inhibits the biosynthesis of long-chain fatty acids when assayed both by the oxidation of TPNH (Fig. 1-I) and by the incorporation of labeled precursors into fatty acids (Table 1). Similar concentrations of tetrolyl-CoA also inhibit the reduction of derivatives of α,β-unsaturated acids such as crotonyl-CoA (Fig. 1-II). Furthermore, the decarboxylation of the nonesterified carboxyl group of malonyl-CoA, which occurs upon the condensation of acetyl-CoA with malonyl-CoA (3), is also strongly inhibited (Table 2, Experiment 1). All of these reactions are approximately 50% inhibited with 1×10^{-5} M tetrolyl-CoA.

The rapid oxidation of one equivalent of TPNH observed spectrophotometrically (3, 11) in the presence of acetoacetyl-CoA, and indicating its reduction to the β-hydroxy derivative (12, 13), is virtually unimpaired by tetrolyl-CoA in concentrations up to 5×10^{-4} M. However, the complete reduction of acetoacetyl-1-C^{14}-CoA to butyrate-1-C^{14} (14) is 80% inhibited by this level of tetrolyl-CoA. Free potassium tetrolate has no effect on these reactions.

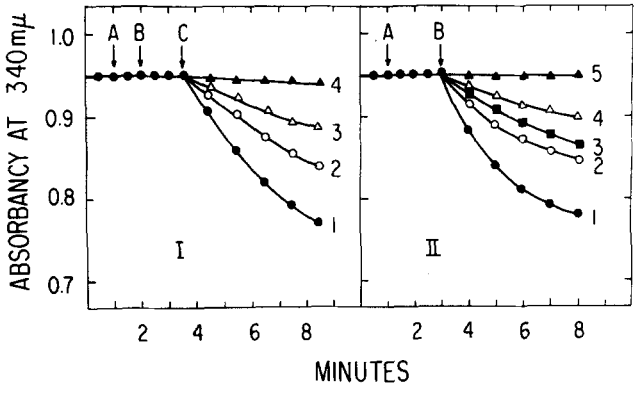


FIG. 1. Spectrophotometric demonstration of the inhibition of fatty acid synthesis and the reduction of crotonyl-CoA by tetrolyl-CoA. Each cuvette contained 10 μmoles of potassium phosphate buffer (pH 7.0), 25 mμmoles of TPNH, and brain fatty acid-synthesizing enzyme Fraction II (3) (0.8 mg. of protein), in a final volume of 0.2 ml. Graph I, arrow A: 0, 1.2, 6, and 12 mμmoles of tetroyl-CoA were added to cuvettes 1–4, respectively; arrow B: 60 mμmoles of malonyl-CoA were added; arrow C: 60 mμmoles of acetyl-CoA were added to each cuvette. Graph II, arrow A: 0, 0.3, 1.5, 9, and 60 mμmoles of tetroyl-CoA were added to cuvettes 1–5, respectively; arrow B: 60 mμmoles of crotonyl-CoA were added to each cuvette.

The inhibition by tetrolyl-CoA of the reduction of crotonyl-CoA is noncompetitive. This inhibition is of the classical type rather than the stoichiometric type (15), as indicated by the fact that the curve of enzyme concentration vs activity in the presence of inhibitor

TABLE 1. INHIBITION OF FATTY ACID SYNTHESIS BY TETROLYL-CoA*

Additions	Radioactivity in Fatty Acids
	cpm
None	1312
Tetrolyl-CoA, 6×10^{-6} M	1023
Tetrolyl-CoA, 1.2×10^{-5} M	751
Tetrolyl-CoA, 2.4×10^{-5} M	584
Tetrolyl-CoA, 3.6×10^{-5} M	497

* Each tube contained 10 μmoles of potassium phosphate buffer (pH 7.0), 1 μmole of TPNH, 70 mμmoles of malonyl-1,3-C^{14}-CoA (540 cpm/mμmole), 70 mμmoles of acetyl-CoA, and rat brain fatty acid-synthesizing enzyme Fraction II (0.8 mg of protein), in a final volume of 0.3 ml. Tubes were incubated for 5 min at 32°. The reaction was stopped by the addition of 0.2 ml of 1N H_2SO_4 and the fatty acids were recovered as described previously (9).

passes through the origin (Fig. 2). The inhibition of fatty acid biosynthesis by tetrolyl-CoA occurs at similar concentrations with enzyme preparations from liver and adipose tissue. In the preparations from adipose tissue, the reduction of crotonyl-CoA (11) is inhibited, while the first step of the reduction of acetoacetyl-CoA is not.

When tetrolyl-CoA was added to a solution of 2-mercaptoethanol at pH 7.8, a reaction occurred that was manifested by the appearance of a new peak in the

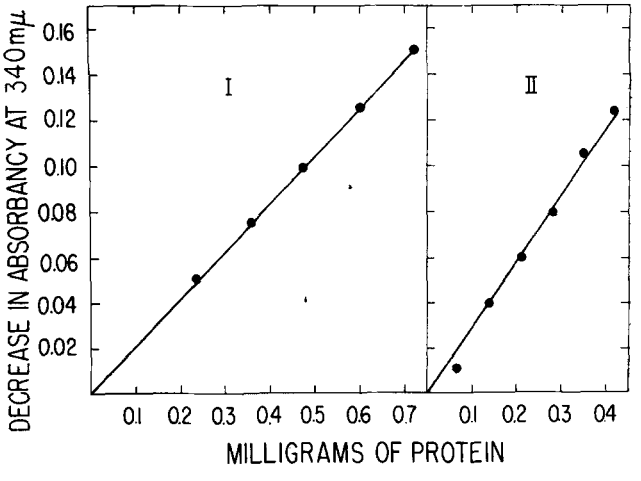


FIG. 2. A plot of the rates of fatty acid synthesis and of crotonyl-CoA reduction vs enzyme concentration in the presence of a constant concentration of tetrolyl-CoA. In both experiments, each cuvette contained 15 μmoles of potassium phosphate buffer (pH 7.0), 25 mμmoles of TPNH, and brain fatty acid-synthesizing enzyme Fraction II, in a final volume of 0.3 ml. Decrease in absorbancy at 340 mμ was measured after 5-min reaction time. Graph I: each cuvette contained 1.2 mμmoles of tetrolyl-CoA, 75 mμmoles of malonyl-CoA, and 75 mμmoles of acetyl-CoA. Graph II: each cuvette contained 0.4 mμmoles of tetrolyl-CoA and 50 mμmoles of crotonyl-CoA.

absorption spectrum at 308 mμ (Fig. 3). This nonenzymatic reaction did not occur at neutral pH. The addition of reduced glutathione also caused the appearance of a peak at 308 mμ, while oxidized glutathione did not. A reaction of this nature was not observed with potassium tetrolate or crotonyl-CoA. Using relatively large amounts of enzyme protein (5 mg), a similar change in the optical density occurred at 308 mμ on adding tetrolyl-CoA to a solution containing fatty acid synthesizing enzyme from brain.

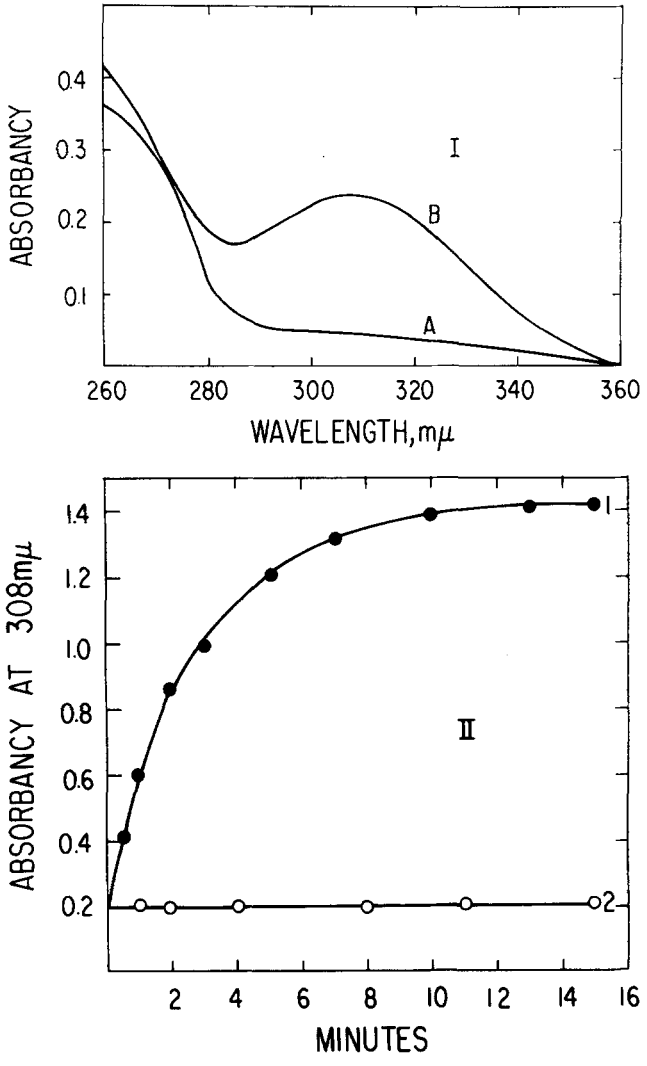


FIG. 3. Spectrophotometric demonstration of the nonenzymatic reaction between tetrolyl-CoA and 2-mercaptoethanol. Graph I: mixture A contained 50 μmoles of potassium phosphate buffer (pH 7.0) and 12 mμmoles of tetrolyl-CoA in a final volume of 1.0 ml; mixture B contained 50 μmoles of potassium phosphate buffer (pH 7.8), 5 μmoles of 2-mercaptoethanol, and 12 mμmoles of tetrolyl-CoA, in a final volume of 1.0 ml. The solutions were incubated for 20 min at room temperature before determining the absorption spectra. Graph II: cuvette 1 contained 50 μmoles of potassium phosphate buffer (pH 7.8), 1 μmole of 2-mercaptoethanol, and 35 mμmoles of tetrolyl-CoA, in a final volume of 0.2 ml; cuvette 2 contained the same reactants except that the pH of the buffer was 7.0.

TABLE 2. INHIBITION OF THE ENZYMATIC DECARBOXYLATION OF MALONYL-CoA*

Experiment No.	Additions	Radioactivity in CO_2
		cpm
1	None	281
	Acetyl-CoA, 75 mμmoles	2008
	Acetyl-CoA, 75 mμmoles + tetrolyl-CoA, 4.5×10^{-6} M	1338
	Acetyl-CoA, 75 mμmoles + Tetrolyl, 9×10^{-6} + M	1054
	Acetyl-CoA, 75 mμmoles + tetrolyl-CoA, 1.3×10^{-5} M	987
	Acetyl-CoA, 75 mμmoles + tetrolyl-CoA, 1.8×10^{-5} M	843
2	None	157
	Acetyl-CoA, 75 mμmoles	1766
	Acetyl-CoA, 75 mμmoles + crotonyl-CoA, 1×10^{-4} M	1225
	Acetyl-CoA, 75 mμmoles + crotonyl-CoA, 2.5×10^{-4} M	903
3	None	661
	Acetyl-CoA, 75 mμmoles	2700
	Acetyl-CoA, 75 mμmoles + palmityl-CoA, 1.6×10^{-4} M	375
	Acetyl-CoA, 75 mμmoles + palmityl-CoA, 3.2×10^{-4} M	252
4	None	241
	Acetyl-CoA, 75 mμmoles	1171
	Acetyl-CoA, 75 mμmoles + CoA, 1.6×10^{-4} M	992
	Acetyl-CoA, 75 mμmoles + CoA, 3.2×10^{-4} M	386

* The incubation flasks (9) contained 30 μmoles of potassium phosphate buffer (pH 7.0), 1 μmole of $KHCO_3$, 75 mμmoles of malonyl-1,3-C^{14}-CoA (540 cpm/mμmole), and brain fatty acid-synthesizing enzyme Fraction II (1 mg of protein), in a final volume of 0.3 ml. The reaction was stopped after 10 min at 32° by the addition of 0.2 ml of 1 N H_2SO_4, and the $C^{14}O_2$ was recovered as described previously (3).

In order to determine if sulfhydryl compounds could overcome the inhibition by tetrolyl-CoA, a series of experiments was carried out in the presence of 2.4×10^{-5} M tetrolyl-CoA, a concentration that caused 87% inhibition of fatty acid synthesis. Pre-incubation of the enzyme and tetrolyl-CoA at this concentration, with 1×10^{-3} M cysteine, reduced glutathione, or reduced lipoic acid, for 10 min at room temperature before adding TPNH, malonyl, and acetyl-CoA, failed to overcome the inhibition by tetrolyl-CoA. When 1×10^{-3} M 2-mercaptoethanol was employed, the inhibition by tetrolyl-CoA decreased from 87 to 73%.

Propiolyl-CoA, the 3-carbon homolog of tetrolyl-CoA, is not reduced by the brain enzyme preparation and strongly inhibits fatty acid synthesis and reduction of crotonyl-CoA (Fig. 4). The concentration of propiolyl-CoA necessary to produce 50% inhibition is in the range of 1×10^{-5} M. The initial step in the reduction of acetoacetyl-CoA is not inhibited by propiolyl-CoA at this concentration.

Crotonyl-CoA is rapidly reduced to butyrate by the brain fatty acid-synthesizing enzyme in the presence of TPNH (3, 11) and is incorporated into long-chain fatty acids by this system in the presence of malonyl-CoA (14). Nevertheless, it appears to inhibit the condensation of acetyl-CoA with malonyl-CoA (Table 2, Experiment 2). Acrylyl-CoA, the 3-carbon homolog of crotonyl-CoA, is not reduced by this system and inhibits fatty acid synthesis. A much higher concentration of this compound is required than of the triple bond analogs; at 3×10^{-4} M, acrylyl-CoA caused only 65% inhibition. Isocrotonyl-CoA also is not reduced in these preparations nor does it condense with malonyl-

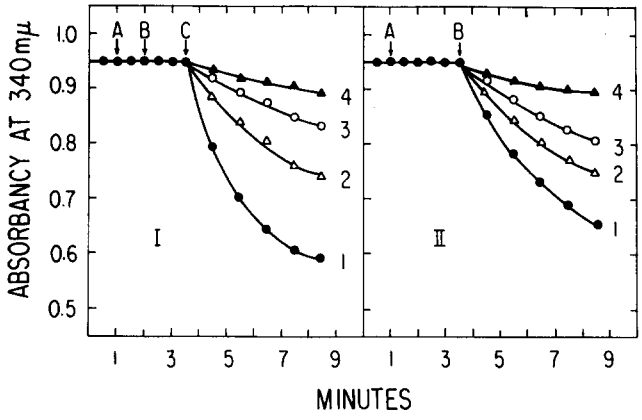


FIG. 4. Spectrophotometric demonstration of the inhibition by propiolyl-CoA of fatty acid synthesis and the reduction of crotonyl-CoA. Each cuvette contained 10 μmoles of potassium phosphate buffer (pH 7.0), 25 mμmoles of TPNH, and brain fatty acid-synthesizing enzyme Fraction II (0.7 mg of protein), in a final volume of 0.2 ml. Graph I, arrow A: 0, 1.8, 3.6, and 7.2 mμmoles of propiolyl-CoA were added to cuvettes 1–4, respectively; arrow B: 70 mμmoles of malonyl-CoA were added; arrow C: 70 mμmoles of acetyl-CoA were added to each cuvette. Graph II, arrow A: 0, 1.8, 3.6, and 7.2 mμmoles of propiolyl-CoA were added to cuvettes 1–4, respectively; arrow B: 70 mμmoles of crotonyl-CoA were added to each cuvette.

CoA. It is a somewhat less effective inhibitor than acrylyl-CoA; a concentration of 3×10^{-4} M caused only a 40% inhibition of fatty acid synthesis.

Palmityl-CoA inhibits both fatty acid synthesis and crotonyl-CoA reduction (Fig. 5), 50% inhibition occurring at a concentration of 4×10^{-4} M. It does not

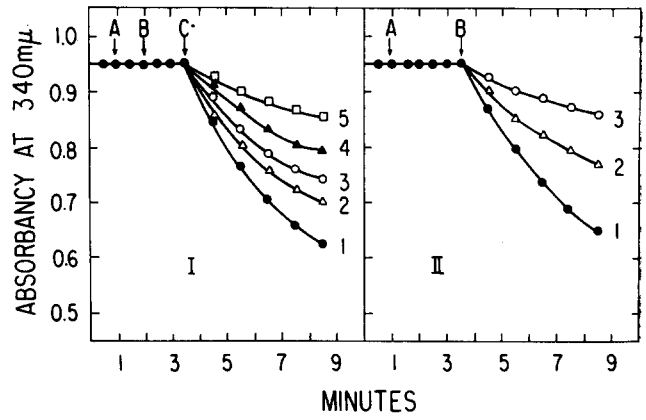


FIG. 5. Spectrophotometric demonstration of the inhibition by palmityl-CoA of fatty acid synthesis and the reduction of crotonyl-CoA. Each cuvette contained 10 μmoles of potassium phosphate buffer (pH 7.0), 25 mμmoles of TPNH, and brain fatty acid-synthesizing enzyme Fraction II (1 mg of protein), in a final volume of 0.2 ml. Graph I, arrow A: 0, 25, 50, 75, and 100 mμmoles of palmityl-CoA zere added to cuvettes 1–5, respectively; arrow B: 70 mμmoles of malonyl-CoA were added; arrow C: 70 mμmoles of acetyl-CoA were added to each cuvette. Graph II, arrow A: 0, 50, and 100 mμmoles of palmityl-CoA were added to cuvettes 1–3, respectively; arrow B: 70 mμmoles of crotonyl-CoA were added to each cuvette.

affect the reduction of acetoacetyl-CoA to β-hydroxybutyrate. The inhibition by palmityl-CoA of the condensation of acetyl-CoA with malonyl-CoA is shown in Table 2, Experiment 3. Neither free palmitic acid nor linoleic acid inhibit fatty acid biosynthesis at concentrations up to 3×10^{-4} M.

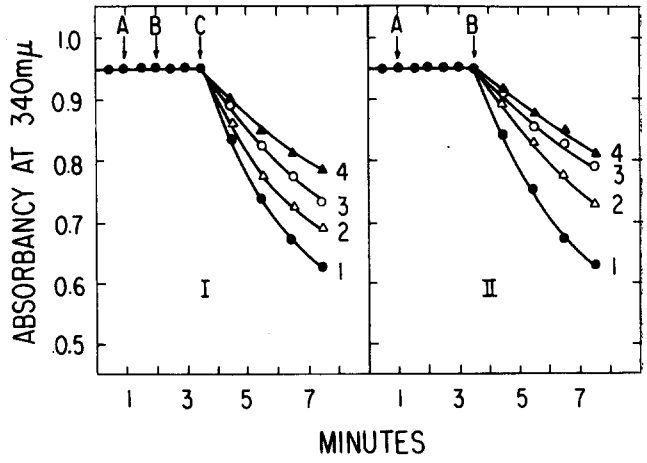


FIG. 6. Spectrophotometric demonstration of the inhibition by CoA of fatty acid synthesis and the reduction of crotonyl-CoA. Each cuvette contained 10 μmoles of potassium phosphate buffer (pH 7.0), 25 mμmoles of TPNH, and brain fatty acid-synthesizing enzyme Fraction II (0.9 mg of protein), in a final volume of 0.2 ml. Graph I, arrow A: 0, 50, 100, and 200 mμmoles of CoA were added to cuvettes 1–4, respectively; arrow B: 50 mμmoles of malonyl-CoA were added; arrow C: 40 mμmoles of acetyl-CoA were added to each cuvette. Graph II, arow A: 0, 50, 100, and 200 mμmoles of CoA were added to cuvettes 1–4, respectively; arrow B: 70 mμmoles of crotonyl-CoA were added to each cuvette.

Free CoA inhibits both fatty acid synthesis and crotonyl-CoA reduction 50% at a concentration of 4×10^{-4} M (Fig. 6). The condensation of acetyl-CoA with malonyl-CoA is also inhibited (Table 2, Experiment 4). As with all the other inhibitors in this series, the first step of the reduction of acetoacetyl-CoA is unaffected. The enzyme system obtained from brain tissue catalyzes the deacylation of CoA derivatives. The deacylation of butyryl-CoA (Table 3) and palmityl-CoA (Fig. 7) is also inhibited by free CoA.

It has been shown that 1,1,3-tricyano-2-amino-1-propene completely inhibited the reduction of crotonyl-CoA by rabbit liver microsomes at a concentration of 4×10^{-4} M (17). This compound did not inhibit either crotonyl-CoA reduction or fatty acid synthesis in the brain enzyme preparation at a concentration of 1×10^{-3} M.

TABLE 3. INHIBITION BY FREE CoA OF THE DEACYLATION OF BUTYRYL-CoA*

Additions	Decrease in Butyryl-CoA	
	After 15 min	After 30 min
	mμmoles	*mμmoles*
None	17	21
Fatty acid-synthesizing enzyme Fraction II (0.5 mg of protein)	67	91
+ CoA, 2.5×10^{-3} M	22	34

* Each tube contained 20 μmoles of potassium phosphate buffer (pH 7.0) and 380 mμmoles of butyryl-CoA in a final volume of 0.19 ml. The tubes were incubated for 0, 15, and 30 min at 37°. The reaction was stopped by adding 0.1 ml of neutralized 2 N hydroxylamine, and this mixture was then incubated for 10 min at room temperature. Residual butyryl-CoA was determined by the method of Lipmann and Tuttle (16).

Attempts were made to demonstrate a requirement for flavin nucleotides in fatty acid biosynthesis in this system. Treatment of the brain fatty acid synthesizing enzyme with acid-ammonium sulfate (12, 18) decreased the synthetic activity of the preparation, and its activity was largely abolished when the pH was lowered to 3.9. But the addition of flavin nucleotides failed to reverse this inhibition. On the contrary, inhibition by flavin adenine mononucleotide (FMN) and flavin adenine dinucleotide (FAD) was observed with enzyme preparations not treated with acid-ammonium sulfate (Table 4). Attempts to demonstrate the presence of TPNH-FMN or FAD diaphorase activity in such a preparation showed that only the oxidation of less than 1 mμmole of TPNH during a 30-min incubation could be attributed to the presence of a diaphorase in enzyme Fraction III. The presence of amytal had no effect on fatty acid synthesis. Acriflavin, at a concentration of 1×10^{-4} M strongly inhibited fatty acid synthesis. This inhibition was not reversed by the addition of flavin nucleotides (19).

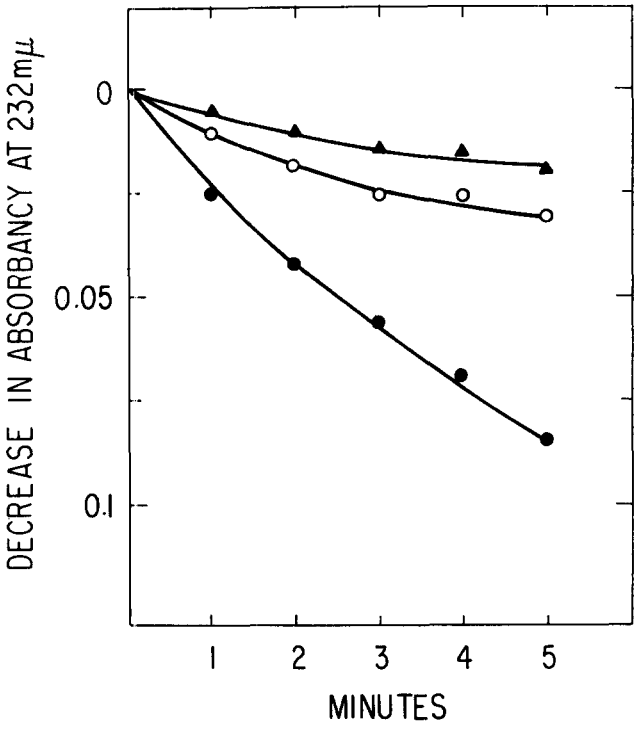


FIG. 7. Spectrophotometric demonstration of the inhibition by CoA of the deacylation of palmityl-CoA. Each cuvette contained 10 μmoles of potassium phosphate buffer (pH 7.0) and 6 mμmoles of palmityl-CoA in a final volume of 0.2 ml. One cuvette (closed triangles) contained no enzyme. To the other cuvettes were added brain fatty acid-synthesizing enzyme Fraction III (60 μg of protein) (closed circles), or brain fatty acid-synthesizing enzyme Fraction III(60 μg of protein) plus 150 mμmoles of CoA (open circles).

TABLE 4. THE EFFECT OF FLAVIN NUCLEOTIDES ON FATTY ACID SYNTHESIS*

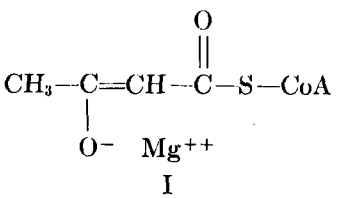
Experiment No.	Additions	Radioactivity in Fatty Acids
		cpm
1	None	1,888
	FMN, 7×10^{-6} M	562
	FMN, 7×10^{-5} M	84
	FMN, 7×10^{-4} M	39
2	None	1,952
	FMN, 2×10^{-4} M	281
	FAD, 2×10^{-4} M	511

* Each tube contained 30 μmoles of potassium phosphate buffer (pH 7.0), 500 mμmoles of TPNH, 100 mμmoles of malonyl-2-C^{14}-CoA (1,400 cpm/mμmole), 75 mμmoles of acetyl-CoA, and fatty acid-synthesizing enzyme Fraction III (0.12 mg of protein), in a final volume of 0.25 ml. The incubation time was 30 min at 37°. The reaction was stopped by the addition of 0.2 ml of 2 N H_2SO_4.

DISCUSSION

Free CoA and the acyl-CoA compounds examined in this study inhibited long-chain fatty acid synthesis, the condensation of acetyl-CoA with malonyl-CoA, and the reduction of crotonyl-CoA, but had no effect on the reduction of acetoacetyl-CoA to the β-hydroxy derivative. These findings suggest either that the initial reduction of β-keto acyl-CoA compounds proceeds at another, and structurally different, site, or that distinct enzymes are involved in the reduction of acetoacetyl-CoA and in fatty acid synthesis (13). The concept of a multi-enzyme aggregate being involved in fatty acid synthesis (12) is compatible with the observation that various steps in the biosynthetic process have differing sensitivities to various inhibitors. It could be argued that the reduction of β-keto derivatives in fatty acid synthesis proceeds only with the enzyme-bound β-keto compound (12, 20), and that the reduction of acetoacetyl-CoA is a separate, although analogous, reaction. However, a partially purified enzyme system from brain can reduce acetoacetyl-CoA to butyrate and also incorporate it after reduction as an intact 4-carbon unit into palmitate (14).

Tetrolyl-CoA inhibits fatty acid synthesis noncompetitively. The observation that the CoA derivative is required for inhibition may reflect activation of the triple bond through the thiolester. The known nonenzymatic addition of sulfhydryl compounds to double (21) and triple (22) bonds, together with the demonstration of a reaction between tetrolyl-CoA and mercaptans, suggests that a similar reaction has occurred between tetrolyl-CoA and the closely juxtaposed sulfhydryl groups of the enzyme (2). There is a structural analogy between the enolate anion of acetoacetyl-CoA (I), which has an absorption maximum at 303 mμ (23), and the hypothetical addition product of mercaptoethanol and tetrolyl-CoA (II), which also has an absorption peak in this region.



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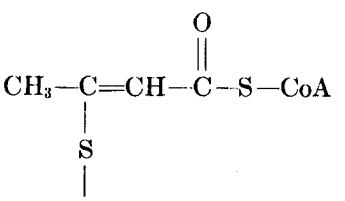

II

Attempts to demonstrate spectrophotometrically such an interaction between tetrolyl-CoA and the brain fatty acid enzyme were successful. It is conceivable that the rest of the enzyme protein facilitates the reaction at pH 7.0 between tetrolyl-CoA and enzyme sulfhydryl groups. If tetrolyl-CoA reacted with the enzyme to form a discreet complex, one might expect to find a stoichiometric type of inhibition (15); this, however, does not seem to be the case.

The double bond analogs, isocrotonyl-CoA and acrylyl-CoA, are not reduced in the presence of TPNH and were found to inhibit fatty acid synthesis. Although crotonyl-CoA does not condense with malonyl-CoA, it does block the condensation of acetyl-CoA with malonyl-CoA. Crotonyl-CoA has been shown to be readily converted to butyrate (11) and, after reduction, can serve as a primer for long-chain fatty acid synthesis in the presence of malonyl-CoA (14). The *trans*-configuration is necessary for substrate activity. The mechanism of the inhibition by these compounds is probably similar to that of the triple-bond compounds. The requirement for higher concentrations in order to exert inhibitory activity most likely reflects the lesser affinity for nucleophilic addition to alkene compounds compared to alkyne compounds (24).

The lack of inhibition of either the reduction of crotonyl-CoA or fatty acid synthesis by 1,1,3-tricyano-2-amino-1-propene, which strongly inhibits crotonyl-CoA reduction in liver microsome preparations (17), is consistent with the contention that the reduction of α,β-unsaturated compounds observed in the soluble fatty acid-synthesizing enzyme preparations is an integral part of the fatty acid-synthesizing system.

Palmityl-CoA has previously been shown to inhibit fatty acid synthesis in a pigeon liver enzyme preparation (25). Since free palmitic acid is not inhibitory in the brain enzyme preparation, the most likely explanation for the inhibition by palmityl-CoA is interaction with the enzyme site. Palmitic acid appears to be the product of fatty acid synthesis in this enzyme preparation (3), and it is conceivable that the inhibitory nature of palmityl-CoA could be due to an impairment of the release of the newly synthesized product from the enzyme. It is also possible that palmityl-CoA causes an inhibitory effect on fatty acid synthesis because of the surface activity of this material. The inhibition of fatty acid synthesis by free CoA has also been shown in a pigeon liver preparation (1). The marked inhibition of the deacylation of acyl-CoA compounds by CoA suggests that inhibition of the release of the product from the enzyme may be involved.

Attempts to demonstrate the presence of flavin nucleotides in the brain enzyme preparation (11), or the

requirement for these nucleotides in presumably flavin nucleotide-depleted preparations, were unsuccessful. The most likely explanation for the inhibitory action of these compounds would be the presence of a diaphorase that catalyzed the oxidation of TPNH; however, very little diaphorase activity could be demonstrated. Accordingly, we feel that the participation of flavin nucleotides in fatty acid synthesis in mammalian enzyme preparations remains to be established.

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