and enhanced levels of mercaptoethanol sensitive antibody, with an increase in the level of low molecular weight antibody appearing later. Similarly, C58 mice receiving endotoxin and BGG formed antibody earlier and to greater levels than did those receiving BGG alone. The increased antibody production in this strain also was due to both mercaptoethanol sensitive and insensitive antibody.

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Coenzyme A-Induced Inhibition of Conversion of Palmitic Acid to Ketone Bodies in Rat Liver Homogenates.* (29136)

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The oxidation of fatty acids to ketone bodies by the liver has long been recognized and the early literature on this phase of fat metabolism has been reviewed(1). A study of factors which affect the rate of ketone body formation from fatty acids in rat liver preparations has been undertaken in this laboratory. During this study the effect of added cofactors on the oxidation of short-chain and long-chain fatty acids to ketone bodies by a liver homogenate system was measured. It was observed that the addition of ATP, cytochrome C and coenzyme A together decreased the conversion of added long-chain fatty acids to ketone bodies. The individual cofactor responsible for this reduction in ketogenesis was then determined and the specificity of this inhibition was further studied. These observations are presented here.

Materials and methods. Male, Wistar rats, 3-5 months old, fed Purina Laboratory Chow *ad libitum* were decapitated after which the livers were rapidly removed and placed in ice-cold 0.9% NaCl. Liver homogenates, 12.5%, in Krebs-Ringer phosphate medium without calcium were prepared with a Potter-Elvehjem, all-glass homogenizer. Tissue homogenates were kept at ice bath temperature throughout their preparation until incubation in beakers in a Dubnoff shaker with a gas phase of air. Substrates and cofactors were added just prior to incubation. Caprylate solutions were adjusted to pH 7.4 with KOH before addition. Palmitate solutions were prepared by an alternate procedure(2) due to the more limited solubility of long-chain fatty acids. Coenzyme A was obtained from Pabst Laboratories. Similar results were obtained with coenzyme A from Sigma Chemical Co. At the end of incubation, proteins were precipitated by treating the homogenates with $CuSO_4$ and $Ca(OH)_2$(3) and filtered. Total ketone bodies in the filtrates were then determined(4). Endogenous ketogenesis, both

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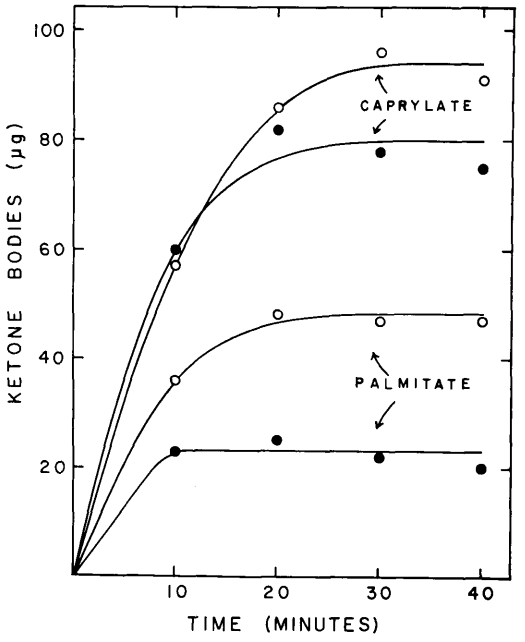

FIG. 1. Effect of added cofactors on conversion of palmitate and caprylate to ketone bodies by rat liver homogenates. Each flask contained 1.6 ml of 12.5% liver homogenate plus 0.2 ml of 5 mM palmitate or 10 mM caprylate and 0.2 ml of water (○—○) or ATP, cytochrome C and CoA in water (●—●). Final concentrations of added cofactors were 1.5 mM ATP, 0.015 mM cytochrome C and 0.5 mM coenzyme A. Total ketone bodies determined at the indicated times are expressed as μg of acetone/100 mg of tissue. Temperature was 37°. Similar results were obtained by repetition of this experiment.

in the presence and absence of the various cofactors and sulfhydryl compounds tested, was determined and subtracted from ketogenesis observed in the respective homogenates containing added fatty acids.

Results and discussion. In the presence of added cytochrome C, ATP, and coenzyme A the conversion of palmitic acid to ketone bodies was decreased 50% in the liver homogenate system (Fig. 1). Ketogenesis from caprylate was not significantly affected by addition of these cofactors. The accumulation of ketone bodies formed from added substrates was complete in approximately 20 minutes of incubation. The amounts of added caprylate and palmitate were equivalent in terms of potential ketone body formation. The short-chain fatty acid was more ketogenic (Fig. 1) in agreement with the earlier observations of Kennedy and Lehninger(5).

To determine which cofactor(s) was responsible for the inhibition of ketone body production from palmitate, the added cofactors were tested individually and in various combinations (Table I). It was established that the inhibitory agent was coenzyme A. Addition of coenzyme A did not alter the pH of the homogenate system.

The effect of a number of other sulfhydryl-containing compounds on the oxidation of palmitic acid to ketone bodies was also measured to study the specificity of the coenzyme A-induced inhibition of ketogenesis in this system (Table II). Cysteine, β-mercaptoethylamine, ergothioneine and reduced glutathione each had no effect on the production of ketone bodies under these experimental conditions.

The inhibition of ketogenesis from palmitate by coenzyme A in liver homogenates is not in conflict with the requirement of co-

TABLE I. Effect of Added Cofactors on Ketogenesis from Palmitate by Rat Liver Homogenate. The test system contained 1.6 ml of 12.5% liver homogenate and 0.2 ml of 5 mM palmitate. Cofactors were added in final concentrations of 1.5 mM ATP, 0.5 mM coenzyme A and 0.015 mM cytochrome C where indicated. Final volume of all homogenates was 2.0 ml. Incubation time was 20 min. Temperature was 37°. Total ketone bodies are expressed as acetone determined/100 mg fresh liver.

Additions	Ketone bodies, μg
None	46
ATP + coenzyme A + cytochrome C	20
ATP + coenzyme A	19
ATP + cytochrome C	43
Coenzyme A + cytochrome C	12
ATP	45
Coenzyme A	16
Cytochrome C	40

TABLE II. Effect of Sulfhydryl Compounds on Ketogenesis from Palmitate by Rat Liver Homogenate. Conditions as in Table I. Sulfhydryl compounds were each added in a final concentration of 0.5 mM where indicated.

Additions	Ketone bodies, μg
None	54
Glutathione	53
β-Mercaptoethylamine	51
Cysteine	59
Ergothioneine	51
Coenzyme A	12

enzyme A in the β-oxidation of fatty acids by mitochondria. That fatty acyl CoA derivatives are intermediates in β-oxidation of fatty acids to acetyl CoA was demonstrated with soluble enzyme systems isolated from mitochondria(6). Several types of inhibition are dependent on the level of organization of the biological system studied(7). Also, the conversion of fatty acids to ketone bodies is a combination of the β-oxidation of fatty acids to acetyl CoA and the formation of ketone bodies therefrom.

In the liver acetyl CoA produced from fatty acids may undergo condensation to form acetoacetyl CoA plus coenzyme A(8). Mechanisms for subsequent formation of acetoacetate from acetoacetyl CoA also liberate coenzyme A(9,10). It is doubtful, however, that the added coenzyme A in the system reported herein is reversing acetoacetyl CoA formation from acetyl CoA or acetoacetate formation from acetoacetyl CoA since ketone body formation from caprylic acid was unaffected. A number of other differences in the metabolism of short-chain and long-chain fatty acids have been summarized(11). It is also unlikely that the added coenzyme A stimulated incorporation of added palmitate into phospholipids and glycerides sufficient to account for the reduction in ketogenesis since an acyl coenzyme A acceptor was not added. The actual mechanism by which coenzyme A decreased the conversion of palmitic acid to ketone bodies under these experimental conditions is undergoing further study.

Summary. A cofactor mixture containing ATP, cytochrome C and coenzyme A decreased ketone body formation from added palmitic acid in a rat liver homogenate system. The conversion of caprylic acid to ketone bodies, however, was not appreciably altered under these conditions. It was found that the decreased ketogenesis from palmitate was caused by coenzyme A. Other sulfhydryl-containing compounds tested did not affect ketone body production from palmitate in this system.

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Thin Layer Chromatography of Blood Lipids.* (29137)

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Thin layer chromatography (TLC) has proved to be a rapid, simple and versatile procedure for the separation of complex substances into their constituents. Although the technique was described as early as 1938(1), it has only recently been applied to the separation of lipids(2). The use of thin layer chromatography in the analysis of blood lipids has not been explored fully as yet. This report presents a simple application of a method originally described by Weicker(3)

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