Possible Reaction Pathway for Acylation of Coenzyme A

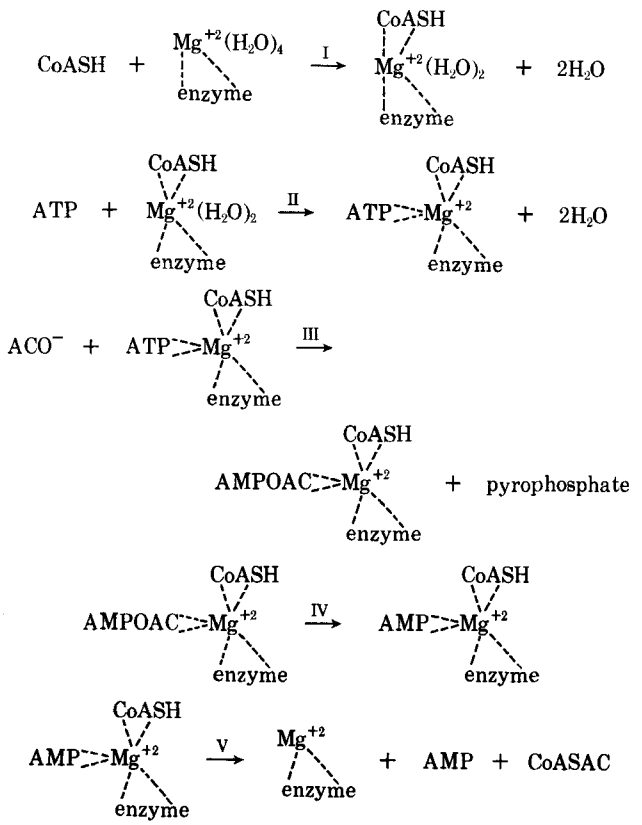
Keyphrases ☐ Coenzyme A, acylation—mechanism, anhydride intermediate ☐ Enzyme catalysis—formation of acyl coenzymes A ☐ Anhydride intermediates—coenzyme A acylation

Sir:

It is well known that acyl coenzymes A (CoA) such as acetyl, succinyl, and propionyl CoA act as catalysts in numerous enzymatic synthetic processes It is also known that the acylation process involves the sulfhydryl group of CoA.

Abundant evidence indicates that the overall reaction for the formation of an acyl CoA proceeds as follows: ATP + organic acid + CoA $\xrightarrow[\text{enzyme}]{Mg^{+2}}$ acyl CoA + AMP + pyrophosphate. However, the exact mechanism by which the acylation reaction takes place is still controversial.

Ingraham and Green (1) postulated a five-step mechanism for the formation of acetyl CoA from CoA ATP, acetate, and magnesium ions. They indicated, based on theoretical considerations, that the first step in the synthesis involves formation of a complex between CoA, Mg^{+2}, and the enzyme, and that this step is followed by reaction with ATP and acetate to form an acetyl–AMP–magnesium–enzyme complex. The final step involves the transfer of the acetyl group from the complex to the sulfhydryl group of CoA, as shown here:



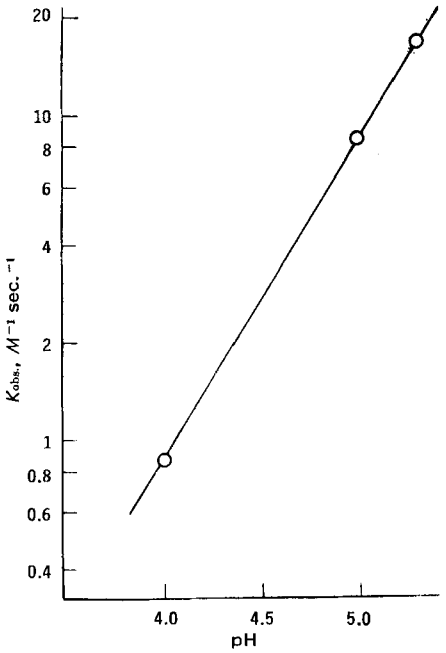


Figure 1—*The pH–rate profile of the reaction of succinic anhydride with cysteine at 25°.*

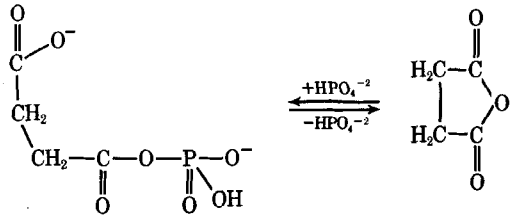
In their proposed theoretical mechanism, Ingraham and Green (1) hypothesized that the first two steps of the reaction, *i.e.*, the formation of the chelate complex between ATP, CoA, Mg^{+2}, enzyme, and ACO^- are the rate-limiting steps.

Berg (2), however, disagreed with Ingraham and Green's proposed mechanism. Supported by experimental evidence, Berg (2) indicated that the overall reaction between ATP, Mg^{+2}, acetate, and CoA occurs in at least two discrete steps:

$$\text{ATP} + \text{acetate} \underset{\text{Reaction I}}{\overset{Mg^{+2}\ \text{enzyme}}{\rightleftharpoons}} \text{acetyl AMP} + \text{pyrophosphate}$$

$$\text{acetyl AMP} + \text{CoA} \underset{\text{Reaction II}}{\rightleftharpoons} \text{acetyl CoA} + \text{AMP}$$

Reaction I, Berg (2) indicated, requires the presence of Mg^{+2} but not the presence of CoA. He also indicated that not only is CoA not required for Reaction I to take place, but if it is added, the rate of exchange is actually decreased. Reaction II, which is measured by the conversion of acetyl AMP to acetyl CoA, proceeds by the same rate in the absence of Mg^{+2}. However, a further observation made by Berg (2), which is inconsistent with this two-step reaction mechanism, is the fact that when acetyl AMP is added to the complete system (ATP, acetyl, CoA, Mg^{+2}, and enzyme), acetyl CoA synthesis continues but pyrophosphate is decreased to about one-tenth the rate found in the absence of acetyl AMP. For Berg's two-step reaction mechanism to be valid, the presence of acetyl AMP should not only affect the rate of formation of pyrophosphate, but it should also affect the rate of formation of acetyl CoA, since the addition of acetyl AMP should have an effect on both Reactions I and II.



Scheme I

A more attractive mechanism that is consistent with Berg's observations, I believe, involves another step in the reaction sequences. This step probably involves a rapid formation of acid anhydride intermediate from acyl AMP. The final step of the overall reaction then involves the reaction between the anhydrides and the sulfhydryl group of CoA to form the acyl CoA:

$$\text{ATP} + \text{organic acid} \xrightleftharpoons{Mg^{+2}\ \text{enzyme}} \text{acyl AMP} + \text{pyrophosphate}$$

$$\text{acyl AMP} \xrightarrow{\text{very fast}} \text{acid anhydride} + \text{AMP}$$

$$\text{acid anhydride} + \text{CoA} \longrightarrow \text{acyl CoA}$$

For this proposed mechanism to be valid, the following two requirements must be met.

1. *Anhydrides form as intermediates in the biosynthetic processes*—Anhydrides have not been isolated as products from biochemical reactions involving CoA. This may be due to their spontaneous hydrolysis in aqueous media which makes their isolation extremely difficult. However, anhydrides have been postulated as intermediates in both enzymatic and nonenzymatic reactions.

In enzymatic reactions (3), anhydrides were proposed as intermediates in the formation of succinyl CoA from succinate 18 and acetoacetyl CoA. In nonenzymatic reactions (4–6) involving the formation of anilidine in aqueous solution from aniline and succinate or tartrate buffers, the reaction was shown unequivocally to proceed *via* formation of acid anhydride intermediates:

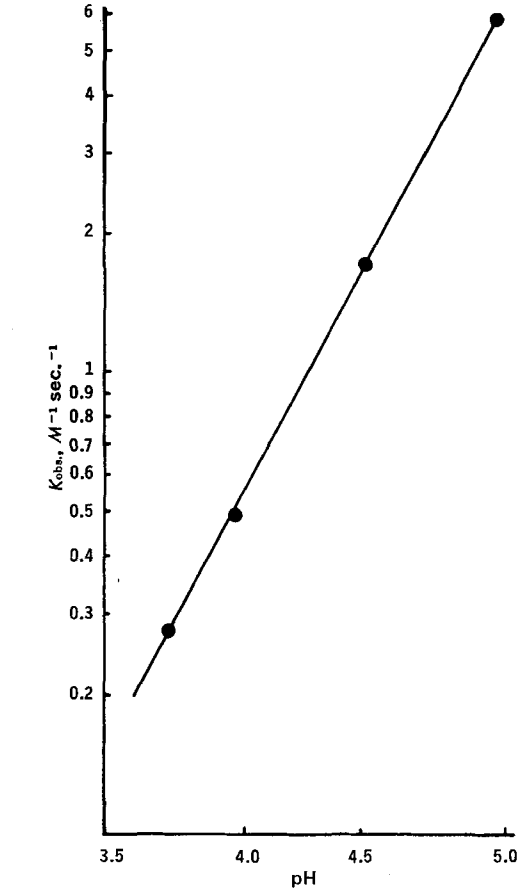
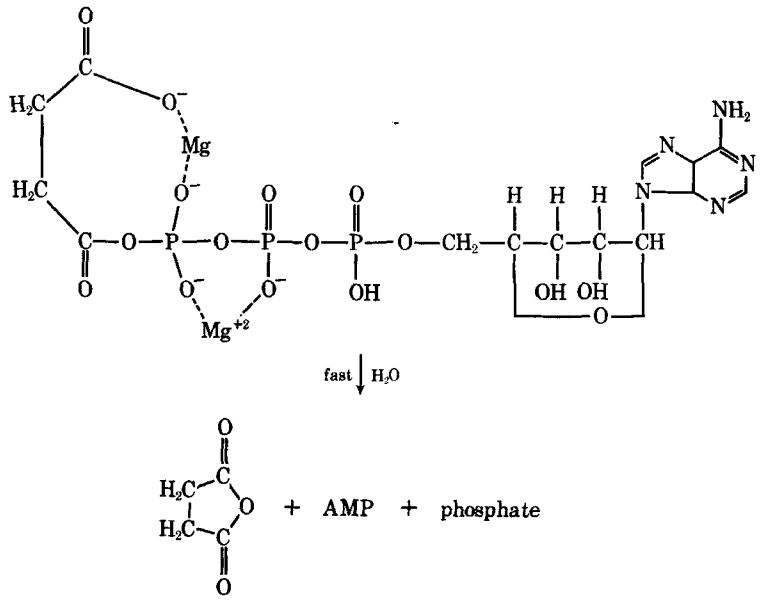


Figure 2—*The pH–rate profile of the reaction of propionic anhydride and 2-dimethylaminoethanethiol at 25°.*

$$\text{succinic acid} \rightleftharpoons \text{succinic anhydride} \xrightarrow{\text{aniline}} \text{anilide}$$

It was also shown (7) that anhydrides, such as succinic or phthalic anhydrides, could form with an ex-



Scheme II

tremely fast rate from the corresponding succinyl or phthalyl phosphate (Scheme I). Since ATP possesses phosphate groups in its molecule, it is conceivable that succinic anhydride is formed from succinyl ATP–magnesium-ion complex (Scheme II). A similar reaction pathway could be postulated for the formation of acetic anhydride.

2. *The rate of reaction between the sulfhydryl group of CoA and the anhydrides is much greater than the rate of hydrolysis of the anhydrides*—I have determined the rate of reaction between succinic anhydride and L-cysteine, a thiol-containing amino acid, and between propionic anhydride and 2-dimethylaminoethanethiol. The pH–rate profiles for these reactions are shown in Figs. 1 and 2.

The reaction rates were too rapid to be measured conveniently at pH's above 6. Thus, it would appear from these experiments that the reaction between a sulfhydryl group and anhydrides would proceed extremely fast under the conditions existing in the body, whereas the rates of hydrolysis of the anhydrides are relatively slow.

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Received March 22, 1971.
Accepted for publication May 17, 1971.

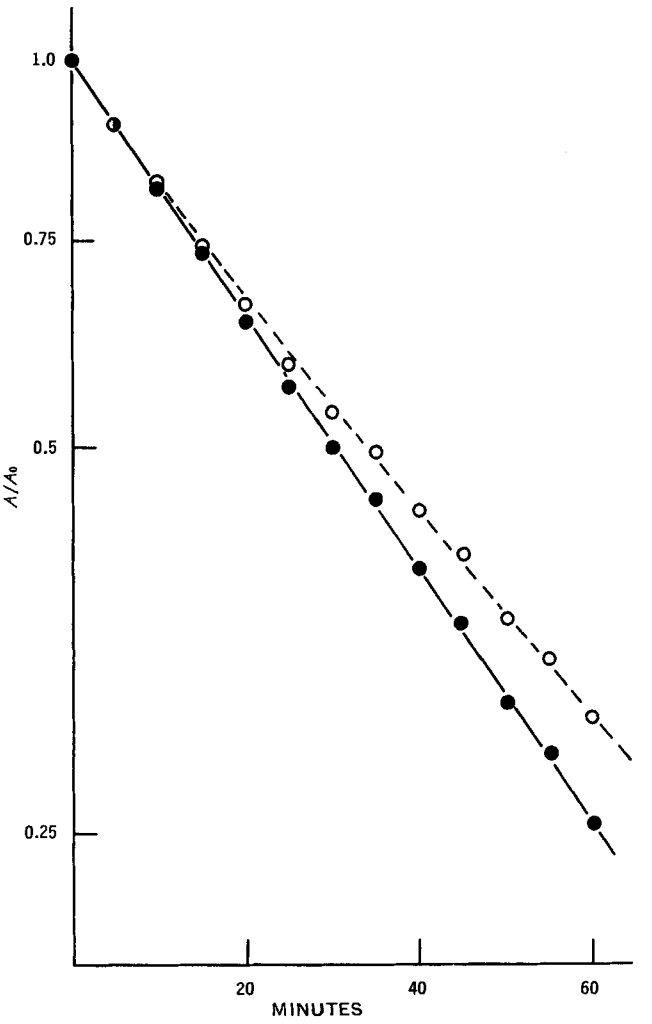

Figure 1—*Effect of dilution on diffusion of* p-*methoxyacetanilide from aqueous buffer to 1-octanol. Key:* ——, *in the absence of dilution;* - - -O- - -, *with dilution; and* —●—, *corrected for dilution.*

Correction for Effect of Dilution on Diffusion through a Membrane

Keyphrases ☐ Absorption kinetics, buccal—saliva dilution effect ☐ Dilution effect correction—first-order diffusion equation ☐ Diffusion equation, first order—dilution correction

Sir:

A number of steps in drug absorption and excretion involve simultaneous dilution of the fluid containing the drug. There does not, however, appear to be any published information concerning the effect of such dilution on diffusion rates. Beckett and Moffat (1) recognized that dilution by saliva affected buccal absorption, but they used an analog computer technique to allow for this condition. Our own work on buccal absorption, which showed that the rate of saliva production was constant during the period of a test (10 min.), led us to develop an equation to correct for the effect of such dilution on first-order diffusion.

The rate at which drug molecules enter the membrane is proportional to their concentration. Let A be the amount of drug in solution at time t, A_0 the initial amount of drug in solution, V_0 the initial volume of drug solution, and v the rate at which diluent is added.

For first-order diffusion in the absence of dilution, we thus have:

$$\frac{dA}{dt} = \frac{-kA}{V_0} \qquad \text{(Eq. 1)}$$

which on integration gives:

$$\ln \frac{A}{A_0} = \frac{-kt}{V_0} \qquad \text{(Eq. 2)}$$

When dilution occurs, we must write:

$$\frac{dA}{dt} = \frac{-kA}{V_0 + vt} \qquad \text{(Eq. 3)}$$