albeit poorly, for the methene bridge atoms of protoporphyrin.

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AN ENZYMATIC REACTION BETWEEN CITRATE, ADENOSINE TRIPHOSPHATE AND COENZYME A[1]

Sirs:

During studies on synthetic functions of pigeon liver preparations, it was observed that acetone powder extracts and the supernatant fraction of pigeon liver homogenate catalyze a reaction between ATP, CoA, citrate and hydroxylamine which leads to the accumulation of large amounts of hydroxamic acid. Acetone powder extracts of other livers showed slight activity with ATP, CoA and citrate, while yeast extracts appeared to be inactive. The enzyme system was partially purified by ammonium sulfate fractionation and in this manner can be separated from the ATP-CoA-acetate system. As shown in Fig. 1, between 18 and 34% ammonium sulfate saturation, most of the activity in the ATP-CoA-citrate reaction precipitates, while the ATP-CoA-acetate system remains in solution. In a similar manner, fractions were obtained which contained the ATP-CoA-citrate system, but did not show a citrate formation on incubation with CoA and oxalacetate. This observation appears to differentiate our system from Ochoa's condensing enzyme. The purified system did not react appreciably with succinate, malate, aconitate or isocitrate, instead of citrate. The reaction is dependent on the presence of magnesium ions.

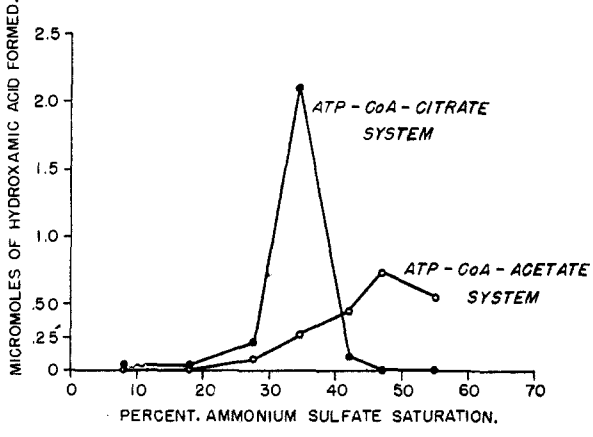

Fig. 1.—The assay system contained: 1 μM. K citrate, 10 μM. $MgCl_2$, 10 μM. glutathione, 5 μM. ATP, 32 units CoA, 200 μM. NH_2OH, enzyme, *p*H 7.4 in a total volume of 1 ml. Controls were run without citrate. Hydroxamic acid was determined according to Lipmann and Tuttle.[2] The ATP-CoA-acetate reaction was measured by substituting acetate for citrate.

(1) This investigation was supported by a research grant from the National Institutes of Health, Public Health Service. The following abbreviations are used: adenosine triphosphate, ATP; adenosine diphosphate, ADP; inorganic phosphate, P_i; and coenzyme A, CoA or HS·CoA.

(2) F. Lipmann and L. C. Tuttle, *J. Biol. Chem.*, **159**, 21 (1945).

The hydroxamic acid formed behaved chromatographically like acethydroxamic, not excluding definitely small amounts of other hydroxamic acids. In order to identify the other reaction products, stoichiometric amounts of CoA were incubated with citrate and ATP. The characteristic thioester absorption at 232 μM[3] appeared immediately and, as shown in Table I, equivalent amounts of acetyl CoA, keto acid and inorganic phosphate were formed. No pyrophosphate was detected as a product of this reaction which differentiates it further from the ATP-CoA-acetate reaction.[4] For further identification of the acyl CoA, the ATP-CoA-citrate enzyme was combined with sulfonamide-acetokinase[5]; on incubation of this system

TABLE I

The system contained: 40 μM. citrate, 20 μM. of $MgCl_2$, 20 μM. glutathione, 10 μM. ATP, 2.6 μM. CoA, twice fractionated enzyme and water in a total volume of 2 ml., *p*H 7.4. Incubated 40 minutes at 37°. Keto acid was measured according to the method of Friedeman and Haugen.[6] For estimation of acetyl CoA, after incubation, an equal volume of 2 *M* NH_2OH was added to an aliquot and hydroxamic acid determined as usual.[2]

System	Acetyl CoA, μM.	P_i, μM.	Keto acid, μM.
Complete	3.0	2.9	2.7
No citrate	0	0	0
No CoA	0.17	0	0

with citrate, ATP and CoA, acetyl sulfonamide and keto acid were formed in equivalent amounts. The keto acid was identified as oxalacetic acid by measuring the decarboxylation with aniline hydrochloride at 15°. Under these conditions, the product of our enzymatic reaction is decarboxylated like oxalacetate within 5–10 minutes, while acetoacetate reacts only rather slowly.

It is concluded from these observations that we are dealing here with an interaction between ATP, CoA and citrate, which yields acetyl CoA, oxalacetate, ADP and inorganic phosphate:

$$\text{ATP} + \text{Citrate} + \text{HS·CoA} \rightleftharpoons \text{acetyl·CoA} + \text{Oxalacetate} + \text{ADP} + P_i \quad (1)$$

The reversibility of the reaction is indicated by incorporation of inorganic phosphate into ATP when ATP, citrate and CoA were incubated with P_i^{32}. Exchange was found only with the complete system but not by incubation of ATP and P_i^{32} alone. Preliminary experiments indicate a net synthesis of ATP by reversal of reaction (1) if the glucose-hexokinase system is added as phosphate acceptor.

The reaction described here appears to represent a new variant of citrate degradation and synthesis. Various attempts to show a primary formation of citryl-CoA which is a plausible intermediary, have so far not given promising results.

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