synthesis by ADP.[8] It may therefore be proposed that in the synthesis of GSH from glutamylcysteine and glycine, phosphorylation of the enzyme by ATP is the initial step.

Acknowledgment.—The author wishes to express his appreciation for the facilities provided by Dr. Konrad Bloch and for his interest in the above work, which was supported by a grant in aid from the U.S. Public Health Service.

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RECEIVED AUGUST 27, 1953

δ-AMINOLEVULINIC ACID, ITS ROLE IN THE BIOSYNTHESIS OF PORPHYRINS AND PURINES[1]

Sirs:

We wish to report our finding that δ-aminolevulinic acid (II) can replace the two substrates, "active" succinate[2,3,4] and glycine[5,6] for porphyrin synthesis. It would appear, therefore, that II is the source of all the atoms of protoporphyrin as outlined in Fig. 1.

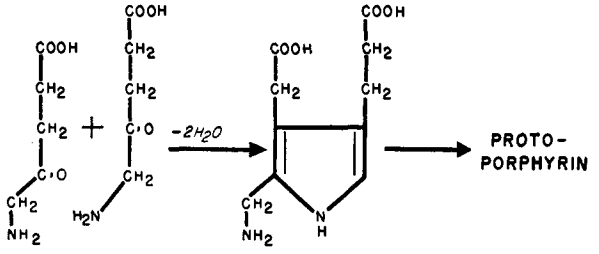

Fig. 1.—The formation of the precursor pyrrole for porphyrins from two moles of α-aminolevulinic acid.

The radioactivity of hemin obtained from either C^{14} glycine plus unlabeled succinate or C^{14} succinate plus unlabeled glycine, in duck blood, is reduced by 80 to 90% by the addition of an equimolar amount of unlabeled II. Also, II labeled with N^{15} or with C^{14} in the δ-carbon atom formed heme containing the isotope in many times the concentration of that formed from labeled glycine; hemin, synthesized from 0.05 mM. of C^{14}-labeled II and from 0.05 mM. of C^{14}-labeled glycine of equal activity had an activity of 22,000 c.p.m. and 500 c.p.m., respectively. II is the decarboxylated product of α-amino-β-ketoadipic acid (I), which can be formed from a condensation of "active" succinate and glycine. The above finding is evidence that I is an obligatory intermediate in the process (Figs. 1 and 2). The formula of the precursor pyrrole (Fig. 1), suggested on theoretical grounds, is the same as that proposed[7,8,9] for porphobilinogen.

II, which has not been previously described, was synthesized by three different procedures: (1) by exhaustive benzoylation of imidazole propionic acid[10] followed by hydrolysis, (2) by nitrosation of β-ketoadipic acid followed by reduction and (3) by a phthalimide synthesis on δ-chlorolevulinic acid.

Other metabolic pathways for II seem probable; for instance, a route by which the α-carbon atom of glycine may be utilized for the ureido carbon atoms of the purines,[11] for the β-carbon atom of serine,[12,13] etc., since II may be considered to be a derivative of the α-carbon atom of glycine. Testing the implications of Fig. 2 we have found that the δ-carbon atom of II is incorporated into the ureido groups of purines in much higher concentration than is the α-carbon atom of glycine in comparable experiments. This accords with the key steps in the postulated cycle (Fig. 2).

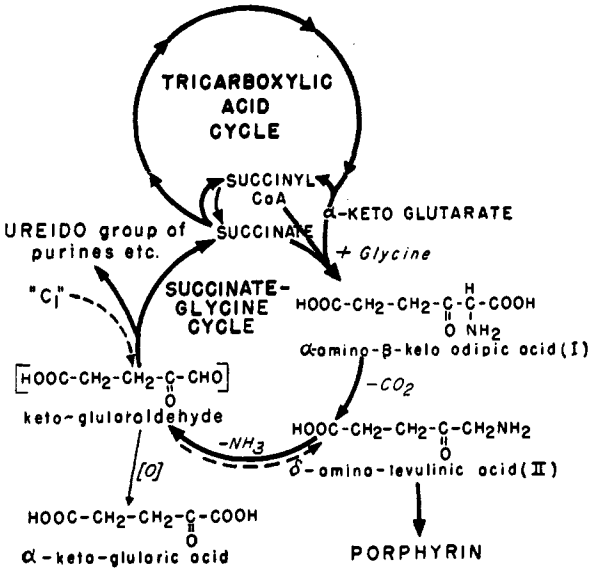

Fig. 2.—Succinate–glycine cycle: a metabolic pathway for the oxidation of glycine.

Further, this succinate–glycine cycle geared with the Kreb's citric acid cycle may account for the serine–glycine reaction,[12,13,14] may explain the metabolic reactions of the "one-carbon atom" compounds and provide via the oxidation of the proposed ketoglutaraldehyde to ketoglutaric acid, a pathway by which succinate can be converted to ketoglutarate. Consistent with the possible reversibility of these reactions outlined in Fig. 2 is our earlier finding that isotopic formate is utilized,

(1) This work was supported by grants from the National Institutes of Health, United States Public Health Service, from the American Cancer Society on the recommendation of the Committee on Growth of the National Research Council, and from the Rockefeller Foundation.

(2) D. Shemin and J. Wittenberg, *J. Biol. Chem.*, **192**, 315 (1951).

(3) D. Shemin and S. Kumin, *ibid.*, **198**, 827 (1952).

(4) D. Shemin, Abstracts, Amer. Chem. Soc. Meeting, Atlantic City, N. J., 1952, p. 35c.

(5) D. Shemin and D. Rittenberg, *J. Biol. Chem.*, **166**, 621, 627 (1946).

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(7) R. G. Westall, *Nature*, **170**, 614 (1953).

(8) G. H. Cookson and C. Rimington, *ibid.*, **171**, 875 (1953).

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(10) We wish to thank Dr. H. Tabor for a generous sample of urocanic acid.

(11) J. L. Karlsson and H. A. Barker, *J. Biol. Chem.*, **177**, 597 (1949).

(12) T. Winnick, I. Moring-Claesson and D. M. Greenberg, *ibid.* **175**, 127 (1948).

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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.

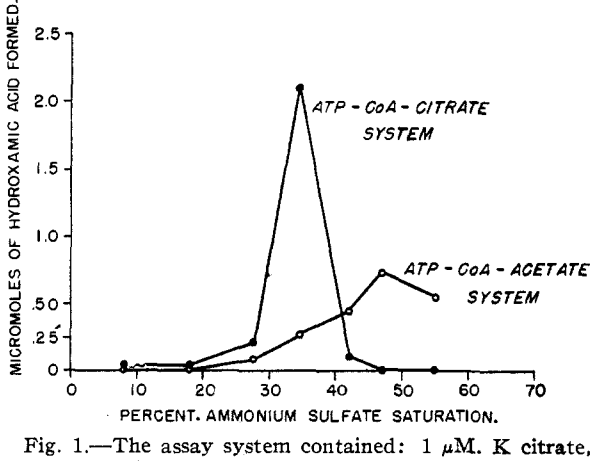

Fig. 1.—The assay system contained: 1 μM. K citrate, 10 μM. $MgCl_2$, 10 μM. glutathione, 5 μM. ATP, 32 units CoA, 200 μM. NH_2OH, enzyme, *p*H 7.4 in a total volume of 1 ml. Controls were run without citrate. Hydroxamic acid was determined according to Lipmann and Tuttle.[2] The ATP-CoA-acetate reaction 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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(6) T. E. Friedeman and G. E. Haugen, *J. Biol. Chem.*, **147**, 415 (1943).