acid, ammonia and carbon dioxide provided that glutamate or carbamylglutamate was present.

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THE DIRECT FORMATION OF ACETYL-COENZYME A FROM SUCCINATE[1]

Sir:

Although extracts of the ciliated protozoan, *Tetrahymena*, reduce cytochrome c in the presence of succinate,[2] the low cytochrome oxidase activity of the extracts[3] indicates that the main route of succinate utilization is not through the usual succinic dehydrogenase–cytochrome system path. Recent experiments with ciliate extracts have revealed that in the presence of succinate, ATP[4] and Co A, there occurs a rapid formation of acetyl-Co A, as measured by the hydroxamate method.[5]

Washed suspensions[6] of *Tetrahymena pyriformis*, strain S, were homogenized by five passages through a Logeman hand mill. After centrifugation for 20 min. at 1350 × *g* the precipitate was suspended in 1 *M* tris buffer *p*H 8.4. Most of the succinic dehydrogenase activity, as measured by the reduction of cytochrome c[2], is in the supernatant. A slight but measureable activity is retained in the precipitate. In the presence of ATP, Co A, Mg^{++} and succinate, the precipitate forms a hydroxamic acid. Table I shows that there is no significant amount of hydroxamate formation in the absence of these components.

On first examination the reaction seems to be similar to the decarboxylation of succinate where succinyl Co A and propionyl Co A are intermediates.[7] However, the results obtained upon extraction of the formed hydroxamate and chromatography on paper with water saturated butanol as solvent[8] excludes this possibility. The developed chromatograms show only a single spot at R_F 0.51 which is identical to that of acetyl hydroxamate prepared from acetyl phosphate. Mixtures of the extracted hydroxamate and acetyl phosphate also yield a single spot. In addition, no significant amounts of carbon dioxide are formed when the enzyme is incubated under nitrogen with the components listed in Table I. No hydroxamate is recovered when hydroxylamine is added to the mixture (containing 100 μM. of potassium phosphate buffer in place of hydroxylamine) at the end of the incubation period; this appears to preclude the formation of an intermediate acyl phosphate.

Since the extract contains very low succinic dehydrogenase activity, and, as would thus be anticipated, malonate does not inhibit the reaction (Table I), acetyl formation is not achieved through Krebs cycle oxidation of the succinate. Formation of the acetyl group by reversal of the cycle, through α-ketoglutarate, also seems unlikely, since no trace of a succinyl hydroxamate or phosphate is obtained on the paper chromatograms.

TABLE I

Components	μM. hydroxamate formed
Complete	2.23
No succinate	0.64
No ATP	0.30
No Co A	0.06
No Mg^{++}	0.87
Complete + 100 μM. malonate	2.73

The complete mixture contained in 1.0 ml.: 200 μM. Na succinate, 25 units Co A, 10 μM. K-ATP, 50 μM. NaF, 15 μM. $MgCl_2$, 400 μM. hydroxylamine, 20 μM. glutathione, 100 μM. tris buffer *p*H 8.4, and extract containing 22 mg. of protein.

In the absence of Co A, succinate and the protozoan enzyme do not replace acetate in the formation of acetyl phosphate by extracts of *Streptococcus faecalis* which contain acetokinase activity.[9] Acetyl formation is thus not the combination of a C_2–C_2 split to free acetate and acetate activation to form acetyl-Co A.

Equimolar amounts of inorganic phosphate and acetyl groups are formed by the reaction. The ratio is not affected by fluoride.

Succinic acid is the only carboxylic acid revealed when ether extracts of the reaction products are chromatographed on paper with *n*-butanol–formic acid–water (5:1:4) as the solvent system.[10]

These observations indicate the direct formation of acetyl-Co A from succinate by extracts of *Tetrahymena*.

(1) Aided by grants E-159 and G-3364 from the National Institutes of Health, United States Public Health Service.

(2) G. R. Seaman, *Arch. Biochem. Biophys.*, **35**, 132 (1952).

(3) G. R. Seaman, *ibid.*, **48**, 424 (1954).

(4) The following abbreviations are used: ATP, adenosine triphosphate; ADP, adenosine diphosphate; Co A, coenzyme A; tris, tris-(hydroxymethyl)-aminomethane; Pi, inorganic phosphate.

(5) F. Lipmann and L. C. Tuttle, *J. Biol. Chem.*, **161**, 415 (1945).

(6) G. R. Seaman, *J. Gen. Microbiol.*, **11**, 300 (1954).

(7) H. R. Whiteley, *Proc. Natl. Acad. Sci. U.S.*, **39**, 779 (1953).

(8) E. R. Stadtman, and H. A. Barker, *J. Biol. Chem.*, **184**, 769 (1950).

(9) I. Rose, *et al.*, *Fed. Proc.*, **13**, 283 (1954).

(10) W. H. Lugg and B. T. Overell, *Aus. J. Sci. Res.*, **1**, 98 (1946).

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THE ENZYMATIC FORMATION OF RIBULOSE DIPHOSPHATE

Sir:

We have recently reported that a soluble extract from spinach leaves is capable of fixing carbon dioxide in the carbonyl group of phosphoglyceric acid in the absence of light.[1] Ribose 5-phosphate, TPN, ATP, and Mg^{++} are required in this reaction. Fractionation of this crude extract has now yielded a preparation which, in the absence of TPN, catalyzes the formation of ribulose diphosphate (RuDP) from ATP and ribose 5-phosphate. This reaction requires Mg^{++}. The activity has been purified about ten-fold by ammonium sulfate precipitation and adsorption and elution on calcium phosphate gel.

To isolate the product formed in the reaction, ATP labeled with P^{32} in the two terminal phosphate groups was incubated with ribose 5-phosphate and Mg^{++} in the presence of the partially purified enzyme. Ion exchange chromatography of the

(1) A. Weissbach, P. Z. Smyrniotis and B. L. Horecker, THIS JOURNAL, **76**, 3611 (1954).