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.

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

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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).

reaction mixture on a Dowex 1-Cl column with a 0.015 *M* HCl–0.2 *M* KCl mixture revealed several radioactive peaks. The major peak, which contained no inorganic phosphorus or adenine derivatives, gave positive reactions for keto-pentose and organic phosphorus. These fractions were treated with barium and ethanol and the precipitated barium salt washed with ethanol and dried. The pentose was characterized as ribulose by color tests and paper chromatography. After dephosphorylation with a potato phosphatase the product gave an absorption spectrum in the orcinol test identical to that given by ribulose, with peaks at 540 and 670 mμ.[2] In the cysteine–carbazole[3] test the maximum color intensity developed in 15–20 minutes with a peak at 540 mμ. This behavior is characteristic of ribulose.[4] Paper chromatography of the dephosphorylated sugar revealed only a single component. This had the same R_f as ribulose and gave the characteristic ribulose color when sprayed with an orcinol reagent[5] or the aniline phthalate reagent[6] (Table I).

TABLE I

CHROMATOGRAPHY OF THE DEPHOSPHORYLATED REACTION PRODUCT

	Rf		Color of Spot	
	Acetone–H_2O[a]	Phenol[b]	Orcinol	Aniline-phthalate
Ribose	0.60	0.56	None	Pink
Sedoheptulose	.52	...	Blue	...
Xylulose	.67	.56	Purple	...
Ribulose	.63	.63	Brown	Brown
Reaction product	.63	.62	Brown	Brown

[a] 10 parts acetone, 3 parts water. [b] Water-saturated phenol.

The composition of the reaction product isolated from the ion-exchange chromatogram is shown in Table II. It contained two equivalents of organic phosphate per mole of ribulose. For the quantitative ribulose assays the dephosphorylated sugar was used, since the behavior of the phosphate ester in these reactions has not been determined.

TABLE II

ANALYSIS OF RIBULOSE DIPHOSPHATE[a]

	μ*M*/ml.
Total P	10.5
Inorganic P[b]	9.7
Reducing sugar[b]	5.1
Ribulose (orcinol)[b]	5.5
Ribulose (cysteine carbazole)[b]	4.7

[a] To 25 mg. of the dried barium salt in 1.8 ml. H_2O were added 0.2 ml. of 0.21 *N* H_2SO_4 and 0.04 ml. of 0.57 *M* K_2SO_4. After removal of $BaSO_4$, the solution (*p*H 5) was incubated for 60 minutes at 34° with 0.03 ml. of potato phosphatase solution. [b] After phosphatase treatment.

The rate of hydrolysis of RuDP in normal acid at 100° was found to follow first order kinetics, indicating that both acid groups were nearly equally acid labile. The half time of hydrolysis was about thirty minutes, which is similar to that of ribulose 5-phosphate. The enzyme which catalyzes the esterification reaction appears to be a phosphopentokinase; it remains to be determined whether isomerization of ribose 5-phosphate to ribulose 5-phosphate preceeds the reaction with ATP.

Ribulose diphosphate was first described by Benson as one of the early products of photosynthesis.[7] Quayle, *et al.*, have reported the formation of phosphoglycerate from ribulose diphosphate with algal extracts.[8] The results reported here are consistent with the view that RuDP is an intermediate in the carbon dioxide fixation system in which ribose 5-phosphate is the substrate.[1] The availability of substrate amounts of RuDP will facilitate further studies on the carboxylation reaction.

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(9) Aided by a grant from the National Foundation for Infantile Paralysis.

THE SITE OF ATTACK BY THROMBIN ON FIBRINOGEN[1]

Sir:

Although two principal fragments are released from fibrinogen when it is activated by thrombin,[2] it has been deduced from the kinetics of clotting that only one collision is involved,[3] and it may be inferred that the fragments come from a relatively small area on the fibrinogen surface. The resultant change in electrostatic charge configuration probably sets the pattern for the subsequent polymerization. Hypothetical illustrations have been given[4] with the interaction site at one end of the rod-shaped fibrinogen molecule or on one side midway between the ends; either arrangement could explain the subsequent lateral dimerization with partial overlapping which has been postulated as the primary polymerization process.[5] We now present evidence that the site is actually midway between the ends.

Electrical birefringence measurements have been made on bovine fibrinogen in 3 *M* urea in 64% aqueous glycerol, before and after activation by throm-

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