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Carbon Dioxide Fixation in *Trichomonas vaginalis*

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Reports of carbon dioxide fixation by non-chlorophyll-containing protozoa are rarely encountered in the literature. With the exception of a report by Kupferberg, Singher, Lampson, Levy & Romano (1953) on *Trichomonas vaginalis*, all other studies have been confined to *Tetrahymena* (van Niel, Thomas, Ruben & Kamen, 1942; Ryley, 1952; Lynch & Calvin, 1952) and *Trypanosoma* (Searle & Reiner, 1940; Moulder, 1948).

Kupferberg *et al.* (1953) demonstrated that *T. vaginalis* fixed carbon dioxide during growth and that the product was almost entirely outside the cell. This material isolated from the culture medium served as the starting material for this study. The ^{14}C -labelled product has been isolated and identified and the site of the radioactive carbon determined.

METHODS

Fixation material. The entire investigation was conducted with the $^{14}\text{CO}_2$ -fixation product isolated by Kupferberg *et al.* (1953). This material had been freeze-dried and stored in a closed container at 5° .

Chromatographic procedures. The freeze-dried material was reconstituted with water, passed through an Amberlite IR-120 cation-exchange column (1.1 cm. $\times$ 16 cm.) in the hydrogen form, and washed from the column with water at the rate of 14–16 drops/min. The material was prepared for chromatography by concentration of the eluate *in vacuo*. The dried material was resuspended in water (0.5–1.0 ml.) and was spotted or streaked onto Whatman no. 1 filter paper. The chromatograms were run ascending at 26° , either the ethyl ether–acetic acid–water (13:3:1, by vol.) system of Denison & Phares (1952) or a butanol–88% formic acid (95:5, v/v) system saturated with water, being used. The former system was used initially for a rapid differentiation of organic acids whereas the latter was employed when better definition of lactic acid and malic acid

was required. The chromatograms were air-dried and then steamed gently while being exposed to the heat of an infrared lamp, as described by Denison & Phares. The acids were identified on the paper chromatogram as yellow spots on a purple background after spraying with bromophenol blue (0.045 g./100 ml. of ethanol, pH 7.0). The location of the radioactivity on the paper chromatograms was determined either by placing the chromatogram in direct contact with X-ray film (Kodak Ltd. No-Screen) for 31 days, or by dividing it into small segments (5 mm. $\times$ 25 mm.) and making direct counts on each section with a windowless Geiger–Müller counter.

Preparation for infrared spectrophotometry. To accumulate sufficient material for the infrared studies, large sheets (50 cm. $\times$ 50 cm.) of Whatman no. 1 filter paper were streaked with the concentrated material along the length of the origin. The chromatograms were run ascending at 26° with the butanol–formic acid system. The location of the acids was established by cutting a thin vertical strip from each edge and a third from the centre and spraying them with the indicator. The acids were eluted from the unsprayed chromatogram with water, each zone being eluted several times with a total volume of 100 ml. Each eluate was filtered through Whatman no. 2 filter paper, concentrated *in vacuo* and the residue treated several times with 95% ethanol. The ethanol solutions were pooled and concentrated and the insoluble residue was discarded. The concentrated material was placed between KCl crystals and the infrared spectrum was determined in a Baird infrared spectrophotometer.

Degradations studies. Material to be degraded was obtained in the same manner as that for the infrared determinations. Lactic acid, with and without carrier, was degraded according to the procedure of Elsdon & Gibson (1954), and the aeration technique was used. The aldehyde was trapped as the bisulphite complex and the carbon dioxide was trapped in NaOH. The carbonate was precipitated as BaCO_3 by the addition of a saturated solution of BaCl_2 . Self-absorption corrections were made as described by Schweitzer & Stein (1950).

Enzymic studies. Cell-free extracts of *T. vaginalis* were prepared as described by Wellerson, Doscher & Kupferberg (1959), except that the one used for the carboxylase determination was neither washed nor frozen.

Carboxylase activity was tested for manometrically according to the procedure of Singer (1955). Glucose 6-phosphate-dehydrogenase activity was measured by following the reduction of the coenzyme at 340 m μ in a Beckman DU spectrophotometer. Phosphoriboisomerase activity was determined by the Dische & Borenfreund (1951) cysteine-carbazole test for ketopentose.

EXPERIMENTAL

Identification of radioactive product. The radioactive material was not effectively adsorbed by the cation-exchange column since 96–100% recovery was obtained when the column was washed with a small volume of water. Three acids were detected on paper chromatograms of the concentrated aqueous eluate. Lactic acid and malic acid were identified by comparing their R_f values with those of known standards with several solvent systems. The third acid had R_f 0.1–0.25 when run for 16 hr. in butanol-formic acid–water. The radioactivity was located entirely in the area occupied by the lactic acid. Final confirmation of the identity of the $^{14}\text{CO}_2$ -fixation product was obtained by comparison of the infrared spectrum of the radioactive material eluted from the chromatogram with that of lactic acid (Fig. 1).

The eluate of the radioactive area of a paper chromatogram was degraded to establish the location of the ^{14}C in the lactic acid molecule. In the procedure used (Elsden & Gibson, 1954) the carbon dioxide produced comes entirely from the carboxyl group. Table 1 shows that all of the tracer was located in the carboxyl carbon of the lactic acid.

Enzymic experiments. The absence of any label in the malic acid isolated eliminates the carboxylation

of pyruvate as the primary mechanism of fixation. A preliminary study of the role of carboxylase or ribulose diphosphate in the fixation of carbon dioxide by this protozoan was made by examining certain essential enzymic reactions. Carboxylase, an enzyme common to bacteria, fungi and plants, catalyses the following reaction:

By the manometric procedure of Singer (1955) the carboxylase activity of a freshly prepared cell-free extract was examined. There was no carbon dioxide liberated during the course of 120 min., in spite of the addition of both cocarboxylase and Mg^{2+} ions to the reaction mixture. A number of extracts of *T. vaginalis* were examined; all were negative for carboxylase.

Since ribose metabolism is associated with the fixation of carbon dioxide in certain bacteria and plants, certain of the steps involved in the synthesis of the key compound, ribulose diphosphate, were studied: glucose 6-phosphate dehydrogenase, because it catalyses the initial step in the conversion

Table 1. *Degradation of lactic acid*

Reaction flask contained 1 ml. of zinc lactate (55 μ moles) in $\text{N-H}_2\text{SO}_4$, 3 ml. of [^{14}C]lactic acid eluted from the chromatogram (6167 counts/min./ml.), 5 ml. of $\text{Ce}(\text{SO}_4)_2$ reagent. Bisulphite trap contained 5 ml. of NaHSO_3 and the alkali traps 25 ml. and 5 ml. of 0.1 M-NaOH.

Vessel	Total radioactivity* (counts/min.)
Reaction flask (start)	18 500
Reaction flask (finish)	0
Bisulphite trap	0
Alkali traps	16 700

* Corrected for self-absorption.

Fig. 1. A comparison of the infrared spectra of the fixation material and authentic lactic acid. Broken line, known lactic acid; continuous line, eluate from chromatogram (R_f 0.6).

of hexose into pentose; phosphoriboisomerase, for the isomerization of ribose 5-phosphate.

Glucose 6-phosphate dehydrogenase catalyses the reaction:

The reduction of the triphosphopyridine nucleotide (TPN) produces an absorption maximum at 340 m μ which allows the above reaction to be followed spectrophotometrically. The addition of 10 μ moles of glucose 6-phosphate to the cell-free extract produced a rapid reduction of TPN but did not reduce diphosphopyridine nucleotide (DPN) (Fig. 2). Inorganic phosphate, a known inhibitor of this enzyme, when present at a concentration of 0.06 M inhibited the reduction of TPN.

The extract was tested for phosphoriboisomerase activity with both ribose and ribose 5-phosphate as substrates. Portions were removed periodically and examined for the presence of ketopentose by the cysteine-carbazole reaction (Fig. 3). Only when ribose 5-phosphate was present was ketopentose produced. Both the ribose system and the endogenous control produced a slight decrease in E at 540 m μ . Because this particular extract did not possess an active phosphohexokinase, the decrease in E noted in the ribose 5-phosphate system after 60 min. indicates that the cysteine-carbazole colour resulted from ketopentose and not ketohexose.

Fig. 2. Glucose 6-phosphate-dehydrogenase activity of *T. vaginalis*. Each cuvette contained: cell-free extract (equivalent of 5.2×10^7 cells); 2-amino-2-hydroxymethylpropane-1:3-diol buffer, pH 7.7, 600 μ moles; MgSO_4 , 1 μ mole. Glucose 6-phosphate (G 6-P; 10 μ moles), TPN or DPN (0.77 μ mole) and Na_2HPO_4 (200 μ moles) were added as shown. The total volume was 3 ml. and incubation was at 26°.

DISCUSSION

Succinic acid has been reported as playing a major role in the fixation of carbon dioxide by both *Tetrahymena* (Lynch & Calvin, 1952) and *Trypanosoma* (Searle & Reiner, 1940). Sixty-three per cent of the labelled carbon dioxide was fixed in the form of succinate by *Tetrahymena*. The fact that no labelled succinate was isolated from *Trichomonas vaginalis* and that lactic acid accounts for nearly all the carbon dioxide fixed suggests that the mechanisms involved are different. The identification of a carboxyl-labelled lactic acid as the end product of carbon dioxide fixation eliminates the malic acid dehydrogenase or 'malic' enzyme system as possible routes of fixation. Failure to

Fig. 3. Phosphoriboisomerase activity of *T. vaginalis*. The reaction mixtures contained: cell-free extract (equivalent of 1.25×10^8 cells), M/15 phosphate buffer, pH 7.0, and ribose 5-phosphate (7.2 μ moles) or ribose (6.6 μ moles). The control was without substrate. Volume of the reactants, 15 ml. Incubation temperature, 37°. Ketopentose was determined by the cysteine-carbazole method of Dische & Borenfreund (1951). ○, Ribose 5-phosphate; ●, ribose; ---, control.

demonstrate carboxylase activity also excludes the carboxylation of acetaldehyde as a means of fixation. One route to which the data do seem to apply is that of a pentose diphosphate carboxylation. This mechanism has generally been associated only with photosynthetic cells. Santer & Vishniac (1955). Trudinger (1956), Aubert, Milhaud & Millet (1956) and Suzuki & Werkman (1958) described this type of fixation as occurring in chemoautotrophic bacteria of the genus *Thiobacillus* and found that both whole cells and cell extracts formed phosphoglyceric acid when supplied with carbon dioxide and ribulose diphosphate. According to the enediol mechanism suggested by Calvin (1954) 3-phosphoglyceric acid resulting from a carboxylation with $^{14}\text{CO}_2$ would contain ^{14}C only in the carboxyl carbon atom. A carboxyl-labelled 3-phosphoglyceric acid therefore, if metabolized via the Embden-Meyerhof route, should produce carboxyl-labelled lactic acid.

The presence of glucose 6-phosphate dehydrogenase and phosphoriboisomerase activity as well as the evidence for an Embden-Meyerhof system in this organism (Wirschafter & Jahn, 1956) lend support to the suggestion of the existence of such a system.

SUMMARY

1. The end product of the fixation of $^{14}\text{CO}_2$ by *Trichomonas vaginalis* was characterized by chromatography and by its infrared spectrum as lactic acid.
2. The ^{14}C occurred only in the carboxyl group of lactic acid.
3. Extracts of the organism contained glucose

6-phosphate dehydrogenase and phosphoriboisomerase, but no carboxylase.

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Phospholipid Metabolism in Nervous Tissue

2. METABOLIC STABILITY*

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Davison & Dobbing (1959) put forward a provisional hypothesis that the brain constituents, in common with the constituents of other tissues, may be divided into at least two main groups according to their biological half-lives. Evidence was presented for the possible existence of a phospholipid

compartment in the brain whose metabolic stability was much greater than could be accounted for by the known turnover rates of even its most slowly metabolized components. A possible explanation was that phospholipids of certain anatomical structures, for example the myelin sheaths, were metabolically inert, whereas in other parts of the brain these same phospholipids were metabolic-

* Part 1: Davison & Dobbing (1959).