PHOTOMETABOLISM OF GLYCOLLATE BY *EUGLENA GRACILIS*

By David R. Murray,*† J. Giovanelli,*‡ and Robert M. Smillie*

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Summary

The photometabolism of glycollate was investigated in *E. gracilis*, strain Z, an organism which can utilize glycollate as a single source of carbon in the light but not in the dark. The nature of the labelled products of the photometabolism of [1-^{14}C]glycollate, [2-^{14}C]glycollate, and [1-^{14}C]glycine and the inhibition of growth on glycollate by isonicotinyl hydrazide and by α-hydroxy-2-pyridine methane sulphonate were consistent with the operation of a glycollate pathway of the type found in the leaves of higher plants. In addition, several enzymes associated with glycollate metabolism in other photosynthetic organisms were demonstrated in cell-free extracts of *E. gracilis* grown with glycollate as the only carbon source. These included glycollate oxidase, NADPH: glyoxylate reductase, NADH : glyoxylate reductase (E.C. 1.1.1.26), glycine transaminase (E.C. 2.6.1.4), formyltetrahydrofolate synthetase (E.C. 6.3.4.3), and serine hydroxymethyltransferase (E.C. 2.1.2.1).

I. Introduction

Euglena gracilis can be grown autotrophically in the light, or heterotrophically in either light or darkness on a number of exogenous carbon substrates, including acetate, ethanol, and glucose. Recently, it was shown that *E. gracilis* can also utilize glycollate, glycine, or serine as sole carbon sources for growth (Murray, Giovanelli, and Smillie 1970). However, growth on these compounds was dependent on the presence of light. Since the photoassimilation of glycollate was inhibited by 3-(3,4 dichlorophenyl)-1,1-dimethylurea, a specific inhibitor of photosynthetic electron transfer, photosynthetic reactions in the chloroplast appeared to be involved in utilization of the glycollate.

Studies of *Chlorella* and several other algae have revealed that exogenous glycollate or glycine is poorly utilized by these cells (Droop and McGill 1966; Hess and Tolbert 1967). Instead, glycollate is often released to the external medium during photosynthesis (Pritchard, Griffin, and Whittingham 1962; Miller, Meyer, and Tanner 1963; Nalewajko, Chowdhuri, and Fogg 1963). These algae nevertheless appear to possess a pathway of glycollate metabolism leading to hexose phosphates via glyoxylate, glycine, serine, C_3 acids, and triose phosphates, and the enzyme glycollate oxidase has been detected in extracts of *Chlorella* and *Chlamydomonas* (Lord and Merrett 1968; Zelitch and Day 1968; Nelson and Tolbert 1969).

* Plant Physiology Unit, CSIRO, Division of Food Preservation, Ryde, and School of Biological Sciences, University of Sydney, Sydney, N.S.W. 2006.

† Present address: Botany Department, King's College, University of London, U.K.

‡ Present address: Laboratory of General and Comparative Biochemistry, National Institute of Mental Health, N.I.H., Bethesda, Maryland, U.S.A.

Since *E. gracilis* appears so far to be unique among unicellular photosynthetic organisms in utilizing substantial quantitities of glycollate for growth, the photometabolism of glycollate by this organism was investigated. [1-^{14}C]Glycollate, [2-^{14}C]glycollate, and [1-^{14}C]glycine were supplied to suspensions of *E. gracilis* and the products of metabolism were examined by paper chromatography and radioautography. In addition, cell-free extracts of cells grown on glycollate were examined for enzymes which are known to be associated with glycollate metabolism in other photosynthetic organisms.

II. Materials and Methods

(*a*) *Cell Cultures*

E. gracilis, strain Z, was cultured at 25°C in the basal medium of Hutner, Bach, and Ross (1956) supplemented with vitamin B_{12} (5 μg/l), except that all organic compounds other than vitamins and EDTA were omitted and glycollate (43·7 mM) was substituted. The cultures were grown in shaken flasks which were illuminated with 5000–7000 lux of white light. Air or N_2, both free of CO_2, was circulated through the flasks.

(*b*) *Radiochemicals*

[1-^{14}C]Glycollate (calcium salt, 2·16 mCi/mmole) was obtained from Volk Radiochemicals, U.S.A. [2-^{14}C]Glycollate (calcium salt, 2·43 mCi/mmole) was obtained from Calbiochem, U.S.A. These were converted to the potassium salts by dissolving with a slurry of Zeokarb 225 (H^+ form) and neutralizing the filtrate with KOH. [2-^{14}C]Glyoxylate (sodium salt, 16·0 mCi/mmole), [1-^{14}C]glycine (7·90 mCi/mmole), [^{14}C]paraformaldehyde (0·1 mCi, 1·58 mg), and n-[U-^{14}C]hexadecane (0·781 μCi/g) were products of Amersham Radiochemical Centre, England. The [^{14}C]-paraformaldehyde was converted to [^{14}C]formaldehyde by hydrolysis in 0·3 ml water at 110°C for 24 hr and diluted with phosphate buffer (0·01M, pH 7·5) and unlabelled formaldehyde to yield a solution containing 2 μCi (5·8 μmoles) of [^{14}C]formaldehyde per millilitre. Radiochemical purity of ^{14}C-labelled glycollate, glyoxylate, and glycine was checked by paper chromatography and radioautography.

(*c*) *Provision of ^{14}C-labelled Compounds to Suspensions of* E. gracilis

Cells in the exponential phase of growth were centrifuged from the growth medium at 2000 *g* and washed twice by resuspension in "inorganic medium" (growth medium from which glycollate had been omitted). The final suspension was introduced to the outer compartment of a feeding vessel [a 150-ml conical flask fitted with a centre well containing 0·2 ml of 20% (w/v) KOH solution] and illuminated for 20–30 min before the addition of the labelled carbon source. To determine $^{14}CO_2$ released by the cells, the contents of the centre well were removed quantitatively and the absorbed $^{14}CO_2$ counted.

(*d*) *Extraction Procedures*

Ethanol (95% v/v) was added to the cell suspension to give a final ethanol concentration of 80% (v/v). After standing at room temperature for 2 hr in darkness, ethanol-insoluble compounds were collected by centrifugation. These were extracted at 60°C for 10 min with 20% ethanol (v/v), and then for 10 min with water (Benson 1955). All the soluble fractions were combined (40–50 ml) and pigments and lipids removed by three successive extractions with 40–80 ml of cyclohexane. After sampling for measurement of radioactivity, the aqueous-ethanol fraction was taken to dryness under a flow of air and dissolved in a small quantity of water for paper chromatography.

(*e*) *Chromatography and Radioautography*

Compounds in the fraction soluble in aqueous ethanol were separated by two-dimensional descending paper chromatography at room temperature with Whatman No. 1 and No. 4 papers. Aqueous phenol (80 g/100 ml)–18·5N ammonia (200 : 1 v/v), prepared according to Smith (1961),

or the isobutyrate solvent of Crowley, Moses, and Ullrich (1963) was used in the first (machine) direction, and n-butanol–propionic acid–water (6 : 3 : 4 v/v) in the second direction (Benson *et al.* 1950).

Other solvents used for particular separations in ascending and descending directions respectively were: triethylamine–acetone–water (5 : 80 : 15 v/v) (Wilkinson and Davies 1958), to separate glycine and serine; and t-amyl alcohol–chloroform–formic acid (98 g/100 ml)–water (80 : 80 : 27 : 83 v/v) (Ranson 1955), to separate organic acids. Radioautographs were prepared by stapling air-dried chromatograms to sheets of Ilford X-ray film. Areas visible on radioautographs were first assigned an identity according to relative position. Preliminary confirmation of identity was obtained with detection methods commonly employed for phosphate esters (Rosenberg 1959), organic acids, and amino acids (Smith 1961). Final confirmation of identity by elution and co-chromatography with authentic compounds was carried out for glycine, serine, aspartate, glutamate, lysine, glycollate, malate, and citrate. Phosphopyruvate was confirmed by its reaction with alkaline iodine solution (Lohman and Meyerhof 1934) and phosphoglycerate was confirmed by its reaction with chromotropic acid in concentrated sulphuric acid (Bartlett 1959).

(*f*) *Measurement of Radioactivity*

Liquid samples of up to 0·20 ml were counted in 10 ml of Diotol scintillator (Herberg 1960). Ethanol-insoluble material was counted by first degrading to $^{14}CO_2$ with persulphate (Katz, Abraham, and Baker 1954). The $^{14}CO_2$ was absorbed in 20% (w/v) KOH (0·2 ml) which was diluted with water to 10 ml before sampling for counting with Diotol. Areas cut from chromatograms were counted in 8–15 ml of the scintillator of Wang and Jones (1959). Counting was done using a Tricarb liquid scintillation spectrometer (series 3000, Packard Instrument Co., Illinois, U.S.A.) with n-[U-^{14}C]hexadecane as an internal standard.

(*g*) *Enzyme Assays*

Cell-free extracts of *E. gracilis* were prepared as follows. Cells (100–300 ml of culture at 2–5 $\times 10^6$ cells/ml) were collected by centrifuging for 10 min at 2000 *g* at 2°C. All subsequent operations were carried out at 0–2°C using precooled materials. The cells were washed free of growth medium by resuspension in 0·01M phosphate buffer, pH 7·5, and centrifugation. After final resuspension in 20–30 ml of the same buffer, the cells were placed in a French pressure cell (Milner, Lawrence, and French 1950) and broken at 3000–5000 lb/in^2. After centrifugation for 15 min at 12000 *g*, the supernatant solution was sampled for enzyme assays. The protein content of extracts was determined by the method of Lowry *et al.* (1951) using bovine serum albumin as a reference standard.

Spectrophotometric assays were carried out in cuvettes (3 ml capacity, 1 cm light path) at 25°C and the rate of change in absorbancy was recorded.

Glycollate oxidase was assayed by the reduction of 2,6-dichlorophenolindophenol, which serves as an electron acceptor for this enzyme (Zelitch and Ochoa 1953; Frigerio and Harbury 1958).

Glyoxylate reductase (glycollate : NAD oxidoreductase, E.C. 1.1.1.26) was assayed by the method of Zelitch and Gotto (1962).

Glycine transaminase (glycine : 2-oxoglutarate aminotransferase, E.C. 2.6.1.4) was measured by the incorporation of label from [^{14}C]glyoxylate into glycine in the presence of an amino-group donor (Cossins and Sinha 1965).

Formyltetrahydrofolate synthetase (formate : tetrahydrofolate ligase (ADP), E.C. 6.3.4.3) was assayed by the method of Hiatt (1965).

Serine hydroxymethyltransferase (L-serine : tetrahydrofolate 5,10-hydroxymethyltransferase, E.C. 2.1.2.1) was assayed according to Cossins and Sinha (1967), except that pyridoxal phosphate (0·2 μmole) was included. [^{14}C]Formaldehyde and tetrahydrofolate were first incubated to produce [^{14}C]methanyltetrahydrofolate, which then yields [3-^{14}C]serine by transfer of the ^{14}C to unlabelled glycine. Labelled serine was isolated by paper chromatography and counted by scintillation spectrometry. Tetrahydrofolate used in these assays was prepared by catalytic hydrogenation of folic acid (Rabinowitz and Pricer 1957) and was lyophilized and stored in evacuated Thunberg tubes at −15°C.

III. Results

(*a*) *Photometabolism of [1-^{14}C]- and [2-^{14}C]Glycollate and of [1-^{14}C]Glycine*

The distribution of radioactivity in the products of the photometabolism of [1-^{14}C]- and [2-^{14}C]glycollate and [1-^{14}C]glycine is shown in Table 1. A high proportion of the label in products remained in ethanol-soluble compounds, although a

Table 1

DISTRIBUTION OF RADIOACTIVITY IN PRODUCTS OF THE PHOTOMETABOLISM OF [1-^{14}C]- AND [2-^{14}C]GLYCOLLATE AND [1-^{14}C]GLYCINE

[1-^{14}C]- or [2-^{14}C]Glycollate was fed to cells which had been grown at 7000 lux under nitrogen with glycollate (43·7 mM) as sole carbon source. The cells were separated from the growth medium by centrifuging at low speed and resuspended in growth medium minus glycollate. Following pre-illumination for 30 min, each treatment (2×10^7 cells in 5 ml) was incubated with 1·90 μmoles (4·1 μCi) of [1-^{14}C]- or [2-^{14}C]glycollate at 7000 lux and 25°C. Total activities in products at 5, 10, and 30 min were 0·7, 1·6, and $4·0 \times 10^6$ disintegrations per minute respectively, representing uptakes of 10, 22, and 54% of both the [^{14}C]glycollate solutions supplied. [1-^{14}C]Glycine was fed to cells grown and treated as described above except that growth was in an atmosphere of CO_2-free air. Each treatment ($9·5 \times 10^6$ cells in 5 ml) was incubated with 0·12 μmole (0·92 μCi) of [1-^{14}C]glycine. Uptake of [1-^{14}C]glycine was 1·8, 3·5, and $5·7 \times 10^5$ disintegrations per minute after 6·5, 13, and 65 min respectively

Fraction of Products	Percentage of Total Activity in Products: Cells Fed [1-^{14}C]Glycollate for Following Periods (min)			Cells Fed [2-^{14}C]Glycollate for Following Periods (min)			Cells Fed [1-^{14}C]Glycine for Following Periods (min)		
	5	10	30	5	10	30	6·5	13	65
CO_2 released	2·4	2·3	4·4	0·3	0·3	0·6	2·4	5·3	9·0
Ethanol-insoluble	10·3	16·2	10·8	3·4	8·4	4·5	3·6	10·3	31·8
Ethanol-soluble:									
Cyclohexane-soluble	22·4	15·0	41·4	20·5	25·2	54·6	4·4	3·6	37·1
Cyclohexane-insoluble	64·9	66·5	43·3	75·9	66·1	40·3	89·6	80·8	22·1

substantial proportion of this had transferred to compounds in the cyclohexane-soluble fraction (pigments, lipids) at the longest times. The proportion of label released as $^{14}CO_2$ was low at all times, less being released from [2-^{14}C]glycollate than from [1-^{14}C]glycollate or [1-^{14}C]glycine.

Table 2 shows the labelling of ethanol-soluble (cyclohexane-insoluble) products at some of the shortest time intervals studied. Glycine was labelled equally from either position of ^{14}C in glycollate, and contained the greatest individual proportion of label in the products. Serine derived almost twice as much label from the 2 position (or α-carbon) of glycollate as from the 1 position, and also contained the greatest individual proportion of label in the products from [1-^{14}C]glycine. Collectively, sugar phosphates accounted for a major proportion of label from all substrates. As the time of feeding was increased this proportion rose, accompanied by declines in the proportion of label in both glycine and serine (from [2-^{14}C]glycollate) or in glycine only (from [1-^{14}C]glycollate).

(*b*) *Metabolism of [2-^{14}C]Glycollate in the Absence of Light*

The uptake of glycollate by *E. gracilis* is known to continue for a short time in darkness following a period of illumination, although the darkened cells are unable

to utilize the glycollate for growth (Murray, Giovanelli, and Smillie 1970). It was therefore of interest to determine whether any appreciable changes in the metabolic fate of glycollate occurred after transfer from light to darkness. Table 3 shows the distribution of label in products of [2-^{14}C]glycollate metabolism after 90 min in either light or dark.

TABLE 2

DISTRIBUTION OF RADIOACTIVITY IN ETHANOL-SOLUBLE, CYCLOHEXANE-INSOLUBLE PRODUCTS OF THE PHOTOMETABOLISM OF [1-^{14}C]- AND [2-^{14}C]GLYCOLLATE AND OF [1-^{14}C]GLYCINE

Experimental details are given with Table 1. Of the sugar phosphates not listed separately, glucose monophosphate and ribulose diphosphate were also labelled but were only partially separated by the solvents employed

Product	Percentage of Total Activity in Products		
	Cells Fed [1-^{14}C]Glycollate (5 min)	Cells Fed [2-^{14}C]Glycollate (5 min)	Cells Fed [1-^{14}C]Glycine (13 min)
Glycollate	—	—	3·1
Glycine	16·8	16·5	—
Serine	4·8	9·2	7·3
Glutamine	0·5	0·6	0·5
Glutamate	0·7	1·6	3·5
Aspartate	1·5	1·1	4·0
Alanine	0·2	0·2	0·3
Threonine	0·2	0·3	0·1
Lysine	2·4	2·2	1·3
Malate	1·8	2·0	1·5
Succinate	0·7	0·4	0·2
Citrate	1·2	2·1	1·5
Glycerate	0·8	1·0	0·7
Phosphoglycerate	4·5	4·4	4·5
Phosphopyruvate	0·7	1·2	2·3
Ribose monophosphate	0·4	0·3	1·5
Fructose monophosphate	5·6	8·3	13·4
Fructose diphosphate	3·3	2·7	4·8
Total sugar phosphates	19·4	24·5	35·7
Total other compounds	9·3	8·3	14·3

In the dark the proportion of label released as $^{14}CO_2$ increased while the proportions of label entering ethanol-insoluble compounds (proteins, paramylon) or cyclohexane-soluble compounds (pigments, lipids) were much reduced. Nevertheless, incorporation of label from glycollate into sugar phosphates still occurred in the dark, and the distribution of label among products within the ethanol-soluble, cyclohexane-insoluble fraction was similar for cells incubated in either light or dark (Table 4).

(*c*) *Effects of Enzyme Inhibitors on Growth in the Presence of Glycollate*

Isonicotinyl hydrazide and α-hydroxy-2-pyridine methane sulphonate, compounds used *in vivo* as inhibitors of serine hydroxymethyltransferase (Dac and Wriston 1958; Pritchard, Griffin, and Whittingham 1962) and glycollate oxidase

(Zelitch 1957, 1959) respectively, inhibited the growth of *E. gracilis* when glycollate was the sole carbon source. Very slight growth occurred in the presence of 1 mM isonicotinyl hydrazide, while at 0·1 mM recovery to normal growth rate followed an

TABLE 3

EFFECTS OF LIGHT OR DARK ON THE DISTRIBUTION OF LABEL IN PRODUCTS OF [2-^{14}C]GLYCOLLATE METABOLISM

Cells were grown in light and resuspended in inorganic medium as described in Table 1. This procedure took 30 min. Each treatment (29×10^6 cells in 5 ml) was immediately incubated with [2-^{14}C]glycollate (2·06 μmoles, 4·45 μCi) for 90 min at 25°C in light (7000 lux) or in darkness. The activities in products were equivalent to uptake of 65% (light) and 49% (dark) of the [2-^{14}C]glycollate provided

Fraction	Light		Dark	
	$10^{-3} \times$ No. of Disintegrations/min	% of Total Radioactivity	$10^{-3} \times$ No. of Disintegrations/min	% of Total Radioactivity
CO_2 released	125	3·1	552	18·0
Ethanol-insoluble	1958	48·8	440	14·4
Ethanol-soluble:				
Cyclohexane-soluble	257	6·4	35	1·1
Cyclohexane-insoluble	1670	41·7	2040	66·5

extended lag phase. At 1 mM, α-hydroxy-2-pyridine methane sulphonate inhibited growth completely, but cells remained viable and eventually growth recommenced. Either inhibitor at 1 mM inhibited growth when CO_2 was the sole carbon source.

TABLE 4

EFFECTS OF LIGHT OR DARK ON THE DISTRIBUTION OF LABEL IN ETHANOL-SOLUBLE, CYCLOHEXANE-INSOLUBLE PRODUCTS OF [2-^{14}C]GLYCOLLATE METABOLISM

The experiment is described in Table 3

Product	% of Total Activity*		Product	% of Total Activity*	
	Light	Dark		Light	Dark
Glycine+serine +glutamine	9·8	12·8	Citrate	2·0	2·7
			Glycerate	2·2	2·4
Glutamate	3·3	2·8	Phosphoglycerate	8·8	4·2
Aspartate	5·5	4·4	Phosphopyruvate	4·0	3·5
Alanine	1·5	1·4	Ribose monophosphate	2·0	2·5
Threonine	1·2	1·3	Fructose monophosphate	14·4	12·0
Lysine	3·8	9·0	Total sugar phosphates	33·2	29·0
Malate	1·1	1·8	Total other compounds	22·6	22·3
Succinate	1·0	1·4			

* Values are percentages of total activity in the ethanol-soluble, cyclohexane-insoluble fraction.

(*d*) *Enzymes Associated with Glycollate Metabolism*

The presence of glycollate oxidase in cell-free extracts of *E. gracilis* was demonstrated by reduction of 2,6-dichlorophenolindophenol and also by measuring the production of glyoxylate as the phenylhydrazone derivative (Dixon and Kornberg 1959). It was not determined whether the oxidase links with oxygen like the enzyme glycollate oxidase (glycollate: oxygen oxidoreductase, E.C. 1.1.3.1) from higher

plants or whether it is the type found in *Chlamydomonas* by Nelson and Tolbert (1969) and named by them glycollate: 2,6-dichlorophenolindophenol oxidoreductase. Table 5 compares the activities of glycollate oxidase and glyoxylate reductase for

TABLE 5

GLYCOLLATE OXIDASE AND GLYOXYLATE REDUCTASE ACTIVITIES IN *E. GRACILIS* CULTURED UNDER THREE CONDITIONS

Activities are μmoles of substrate converted/min/g protein. The cell-free extracts were prepared from cells cultured at 6000–7000 lux and 25°C with glycollate (43·7 mM) as carbon source in CO_2-free air, or in air, or with glucose (18·3 mM) as carbon source in air

Carbon Source for Growth	Glycollate Oxidase	NADPH: Glyoxylate Reductase	NADH: Glyoxylate Reductase
Glycollate	6·7	13·9	9·2
CO_2 (from air)	7·8	11·0	10·3
Glucose + CO_2 (from air)	9·5	13·0	19·0

cells grown in the light with glycollate, CO_2 (0·03%), or glucose (plus CO_2) as carbon sources. The activities of these enzymes were similar for cells cultured with glycollate or CO_2, while cells which had utilized glucose showed a higher level of glyoxylate reductase.

TABLE 6

GLYCINE TRANSAMINASE IN A CELL-FREE EXTRACT OF *E. GRACILIS*

The cell-free extract was prepared from cells cultured on glycollate (43·7 mM) under CO_2-free air at 7000 lux. Each complete reaction mixture (in 2·0 ml) contained: potassium phosphate buffer (pH 7·5), 100 μmoles; L-amino acid, 2 μmoles; pyridoxal phosphate, 0·2 μmole; sodium [2-^{14}C]glyoxylate, 0·2 μmole (3·2 μCi); and cell extract (0·4 mg protein)

Amino Acid Provided	Extract Present	Incubation Period (hr)	Radioactivity into Glycine* (10^{-3} × No. of counts/min)
None	+	2	10
Glutamate	+	0·5	1970
Glutamate	−	0·5	720
Serine	+	2	39
Serine	−	2	62
Alanine	+	2	54
Alanine	−	2	111
Aspartate	+	2	62
Aspartate	−	2	308

* Average values per 1 hr.

Glutamate served as an amino-group donor for enzyme-catalysed transamination of glyoxylate to glycine (Table 6). However, alanine, aspartate, and serine either did not, or did so at a lower rate.

Formyltetrahydrofolate synthetase (Table 7) and serine hydroxymethyltransferase (Table 8), enzymes catalysing C_1-unit activation and transfer, were also present in extracts of cells cultured with glycollate as sole carbon source.

TABLE 7

FORMYLTETRAHYDROFOLATE SYNTHETASE IN AN EXTRACT OF *E. GRACILIS*

The extract was prepared from cells cultured as described in Table 6

Treatment			Increase in Absorbance at 355 nm in 10 min*
Tetrahydrofolate (μmoles)	ATP (μmoles)	Extract (mg protein)	
2	2	0·22	0·030
2	2	0·44	0·056
2	4	0·44	0·068
4	2	0·44	0·110

* Corrected for absorbance change in control treatments with ATP omitted.

TABLE 8

SERINE HYDROXYMETHYLTRANSFERASE IN AN EXTRACT OF *E. GRACILIS*

The extract was prepared from cells cultured as described in Table 6

Treatment	Time (min)	Radioactivity in [^{14}C]Serine	
		$10^{-3} \times$ No. of Counts/min	%
Complete	15	105	100
Minus extract	15	5	5
Complete	30	198	100
Minus pyridoxal phosphate	30	140	70·4
Minus glycine	30	21·5	11
Minus tetrahydrofolate	30	2	1
Complete	60	375	100
Minus extract	60	9	2

IV. DISCUSSION

In the leaves of plants, glycollate is converted to carbohydrates via glyoxylate, glycine, serine, hydroxypyruvate, glycerate, and 3-phosphoglycerate to triose phosphates and hexose phosphates (Tolbert 1963; Cheung, Rosenblum, and Sallach 1968). The data presented here indicate that a similar pathway for glycollate metabolism operates in *E. gracilis*. At the shortest period of photometabolism studied (5 min), glycine was labelled equally from either carbon atom of glycollate and also contained

the greatest individual proportion of label in the products (Table 2). Thus the assimilation of glycollate could not have been solely by decarboxylation followed by refixation of the released CO_2 photosynthetically, since the 2 position of glycollate would not be expected to contribute label to glycine as efficiently as the carboxyl position of glycollate. Nevertheless, the relatively low proportions of ^{14}C appearing in CO_2 released in the light from glycollate and glycine labelled in the carboxyl position (Table 1) suggest that some refixation of CO_2 produced endogenously is occurring. Apart from decarboxylations concomitant with the operation of the tricarboxylic acid cycle, one CO_2 molecule would be produced for each C_3-unit produced from two C_2-units by the glycollate pathway. Refixation of endogenous CO_2 was also indicated by the labelling of ribose phosphate and ribulose 1,5-diphosphate among the sugar phosphates and by the similar amount of label found in 3-phosphoglycerate after 5 min of feeding with either [1-^{14}C]- or [2-^{14}C]glycollate. Without refixation of released CO_2 into 3-phosphoglycerate, more label in 3-phosphoglycerate might have been expected from [2-^{14}C]glycollate than from [1-^{14}C]glycollate. Feeding for very short times would be necessary to establish this point with certainty, but the low specific activity of the labelled glycollate available precluded such experiments. It may also be noted that the proportion of ^{14}C released as CO_2 increased substantially during metabolism of [2-^{14}C]glycollate by cells transferred from light to dark conditions (Table 3).

The greater contribution of the α-carbon atom of glycollate to serine (Table 2) confirms the expected formation of serine by condensation of glycine with a C_1-unit derived largely from the 2 position of glycollate. In recent experiments with *Chlorella pyrenoidosa*, Lord and Merrett (1970) showed that most of the label from [1-^{14}C]-glycollate is incorporated into glycine and serine in short-term feeding experiments (10–30 sec). Interconversion of [^{14}C]glycine and [^{14}C]serine prior to their incorporation into protein in *E. gracilis* cells growing on glutamate has previously been noted (Kempner and Miller 1965). Although enzymes catalysing the addition of a C_1-unit to glycine have now been detected in extracts of *E. gracilis* cells (Tables 7, 8), it is not known whether glyoxylate or glycine is the C_2-unit from which the transferred C_1-unit is derived. This point should prove interesting phylogenetically, since there is evidence for glycine cleavage with α-carbon activation in some higher plants (Wang and Waygood 1962; Miflin, Marker, and Whittingham 1966) as well as in certain bacteria (Klein and Sagers 1967*a*, 1967*b*).

The low activity of glycine transaminase for serine as amino-group donor is surprising, as serine serves as a donor for this enzyme in several plant tissues (Cossins and Sinha 1965; King and Waygood 1968). With serine and the other amino acids tested except glutamate, the rate of non-enzymic conversion to glycine in the absence of cell extract was always greater than that in the presence of cell extract. The reason for the lower activity in glycine in the presence of cell extract is not apparent. However, serine is not a good donor for the spinach leaf enzyme, which shows best activity with glutamate or alanine (Kisaki and Tolbert 1969). The specificity of the *E. gracilis* enzyme appears to resemble the glutamate-specific glycine transaminase from mammalian liver (Thompson and Richardson 1966), an enzyme which is distinct from the alanine-specific transaminase from the same source (Thompson and Richardson 1967).

In higher plants several enzymes metabolizing C_2 compounds are localized in organelles, notably glyoxysomes (Breidenbach and Beevers 1967; Breidenbach, Kahn, and Beevers 1968) and peroxisomes (Tolbert *et al.* 1968, 1969; Kisaki and Tolbert 1969). Most of the glycollate oxidase and NADH : glyoxylate reductase activities of the leaves of some plants are localized in peroxisomes. While peroxisomes have not yet been isolated from algae or from *Euglena*, the existence of similar organelles in these organisms is not unlikely. If photoassimilation of glycollate in *E. gracilis* does involve peroxisome-like microbodies, then it will be interesting to determine whether enzymes contained in these organelles, or even the organelles themselves, are inducible. From comparisons made between cells of *E. gracilis* grown with different carbon sources in the light (Table 5), glycollate oxidase and the glyoxylate reductases did not appear to be induced by exogenous glycollate. However, since glycollate utilization is linked to chloroplast metabolism, light or CO_2 concentration (Nelson and Tolbert 1969) may be more important in regulating synthesis of enzymes of the glycollate pathway than glycollate itself.

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VI. References

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