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Metabolism of Glycollate by *Lemna minor* L. Grown on Nitrate or Ammonium as Nitrogen Source

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ABSTRACT

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Duckweed, *Lemna minor* L., grown on inorganic nutrient solutions containing either NH_4^+ or NO_3^- as nitrogen source was allowed to assimilate [1-^{14}C]- or [2-^{14}C]glycollate during a 20 min period in darkness or in light. The incorporation of radioactivity into water-soluble metabolites, the insoluble fraction, and into the CO_2 released was measured. In addition the extractable activity of phosphoenolpyruvate carboxylase was determined.

During the metabolism of [2-^{14}C]glycollate in darkness, as well as in the light, NH_4^+ grown plants evolved more $^{14}CO_2$ than NO_3^- grown plants. Formate was labelled only from [2-^{14}C]glycollate and in NH_4^+ grown plants it was significantly less labelled in light than in darkness. In NO_3^- grown plants formate showed similar radioactivity after dark and light labelling. The radioactivity in glycine was little influenced by the nitrogen source. Amounts of radioactivity in serine implied that the further metabolism of serine was reduced in darkness compared with its metabolism in the light under both nitrogen regimes. In illuminated NH_4^+ plants, serine was labelled through a pathway starting from phosphoglycerate. After [1-^{14}C]glycollate feeding NH_4^+ grown plants contained markedly more radioactive aspartate and malate than NO_3^- plants indicating a stimulated phosphoenolpyruvate carboxylation in plants grown on NH_4^+.

Key words—Photorespiration, glycollate, nitrogen, *Lemna*.

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INTRODUCTION

In chloroplasts in the presence of oxygen, ribulose-1,5-bisphosphate (RuBP) oxygenase produces P-glycerate and P-glycollate. P-glycerate is metabolized in the photosynthetic carbon reduction cycle while P-glycollate is a substrate for the photosynthetic carbon oxidation cycle (Tolbert, 1979). The latter pathway causes the loss of photorespiratory CO_2 and leads to a reduced efficiency of photosynthesis in C_3 plants (Zelitch, 1979). After dephosphorylation of P-glycollate in the chloroplasts the product, glycollate, is transferred to the peroxisomes. There it is oxidized to glyoxylate by glycollate oxidase, generating H_2O_2 by reduction of O_2. H_2O_2 (if it escapes catalase activity) can oxidize glyoxylate to CO_2 or to formate and CO_2 (Grodzinski, 1979; Halliwell and Butt, 1974; Zelitch, 1972). Alternatively glyoxylate can be either oxidized to oxalate, transaminated to glycine (Chang and Huang,

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Abbreviations: PEP: phosphoenolpyruvate; RuBP: ribulose 1,5-bisphosphate; DCM: dichloromethane.

1981; Tolbert, 1979) or converted to malate by reaction with acetyl-CoA (Miernyk and Trelease, 1981; Yamamoto and Beevers, 1960). The importance of metabolism of glyoxylate by each possible route *in vivo* is not yet clear. Apparently the availability of amino groups represents an important factor for the conversion of glyoxylate to glycine (Chang and Huang, 1981; Oliver, 1981; Somerville and Ogren, 1981; Yokota, Kawabata, and Kitaoka, 1983). Photorespiratory CO_2 has been proposed to be released from the carboxyl carbon of glycollate (Tolbert, 1979; Zelitch, 1972). The α-carbon forms a C-1 unit and/or formate, which can be oxidized to CO_2 by H_2O_2 or by a mitochondrial formate dehydrogenase (Grodzinski, 1979; Halliwell, 1974). It can also react with glycine to produce serine (Grodzinski, 1979). Serine synthesis, however, is assumed to occur mainly through the reaction of serine hydroxymethyltransferase (Tolbert, 1979). Two mols of glycine react to produce one mol each of serine, NH_3, and CO_2. Serine is converted to hydroxypyruvate, which may be decarboxylated by H_2O_2 (Walton and Butt, 1981) or metabolized to P-glycerate (Tolbert, 1979). The synthesis of serine can also proceed from P-glycerate through the glycerate pathway or through the phosphorylated pathway involving P-hydroxypyruvate and P-serine (Keys, 1980).

The pathways involved in photorespiration seem to be influenced by the type of supplied N-source (Emes and Erismann, 1982; Marques, Oberholzer, and Erismann, 1983; Vaklinova, Fedina, Vassileva, and Ananieva, 1981). This report describes effects of NH_4^+ and NO_3^- as sole N-sources on ^{14}C-incorporation from [1-^{14}C]- and [2-^{14}C]glycollate into metabolites of *Lemna minor* L. By feeding *Lemna* with [^{14}C]glycollate in darkness, fixation of released CO_2 by RuBP carboxylase could be excluded. Different effects of N-source on the metabolism of supplied [^{14}C]glycollate were to be expected in photosynthesizing *Lemna* plants, because in darkness the regeneration of glutamate in the photorespiratory nitrogen cycle is probably reduced (Keys, Bird, Cornelius, Lea, Wallsgrove, and Miflin, 1978) and the production of H_2O_2 in the chloroplasts is lacking (Yokota *et al.*, 1983).

The results obtained show that radioactivity in formate was strongly influenced by the N-source. In darkness the labelling of glycine and serine was not altered appreciably by the N-regime. Under photosynthetic conditions much of the label in serine in NH_4^+ grown plants apparently came through a pathway starting from P-glycerate. Furthermore, NH_4^+ nutrition compared to NO_3^- enhanced phosphoenolpyruvate (PEP) carboxylation.

MATERIALS AND METHODS

Plant material

Lemna minor L. (strain No. 6580-11'02) was grown in Fernbach flasks under sterile conditions for three weeks on an inorganic nutrient solution (pH 6·65) containing either NO_3^- or NH_4^+ as sole N-source (3·35 mol m^{-3}) (Kopp, Feller, and Erismann, 1974). The flasks contained 700 cm^3 nutrient solution and were aerated at a flow rate of 50 dm^3 h^{-1} at 25 °C under continuous light (13 000 lx, fluorescent tubes: Philips TL 40W/33).

[^{14}C]*glycollate feeding*

Two hundred fronds of NO_3^- (331 ± 20·2 mg fr. wt., 0·298 ± 0·006 mg Chl, ± s.d., $n = 5$) or NH_4^+ (268·8 ± 12·3 mg fr. wt., 0·288 ± 0·008 mg Chl, ± s.d., $n = 5$) grown plants were placed in flasks (volume: 40 cm^3) on 15 cm^3 of the appropriate nutrient solution and aerated with filtered atmospheric air containing 370 mm^3 dm^{-3} CO_2 at a flow rate of 50 dm^3 h^{-1}. The temperature of the assimilation flasks was held constant at 25 °C. Fronds provided with [^{14}C]glycollate in darkness were first acclimated in darkness; those to be provided with [^{14}C]glycollate in the light were acclimated in the light. After 2 h of acclimation the nutrient solutions were replaced by 3·0 cm^3 of a nutrient solution containing 0·6 mol m^{-3} of either [1-^{14}C]- or [2-^{14}C]glycollate (INC, Calif.). For dark metabolism of [1-^{14}C]-

or [2-^{14}C]glycollate the specific activities were 1·7 and 2·65 Ci mol^{-1} respectively. Photosynthesizing fronds were kept at a light intensity of 13 000 lx (fluorescent tubes: Philips TL 40W/33), and provided with [1-^{14}C]- or [2-^{14}C]glycollate having specific activities of 2·0 and 5·6 Ci mol^{-1} respectively. The glycollate feeding was stopped after 0·5, 1·0, 5·0, 10 and 20 min by rapidly cooling the assimilation flask and quickly replacing the feeding solutions by 20 cm^3 ice-cold distilled H_2O. Then after draining the wash water, the plants were frozen in liquid N_2 and stored at −60 °C until analysis.

Estimation of $^{14}CO_2$ production

During metabolism of radioactive substrates, the air from each flask was bubbled through 10 cm^3 2-amino ethanol : 2-methoxyethanol (20 : 80, v/v) to absorb released CO_2. Ten cm^3 scintillation fluid (BBOT : naphthalene : toluene, 1 : 2 : 125, w/w/v) were added to the whole sample and radioactivity measured on an MR 300 automatic liquid scintillation system (Kontron). For blank values the *Lemna* fronds were replaced by parafin leaflets, which floated on the [^{14}C]glycollate feeding solution and which had approximately the same shape and the same area of surface as the plants.

Extraction and separation of ^{14}C-labelled products

The frozen plant material of one assimilation flask was homogenized in 3·0 cm^3 of absolute ethanol : dichloromethane (DCM) (E. Merck, Darmstadt) (2 : 1, v/v) using a glass homogenizer. The homogenate was centrifuged and the pellet washed with 1·0 cm absolute ethanol : DCM (2 : 1, v/v) and twice with 2·5 cm^3 distilled H_2O. To determine the radioactivity of the insoluble fraction 1·0 cm^3 distilled H_2O and 10 cm^3 scintillation fluid (2,5-diphenyloxazole (PPO) : 1,4-bis(5-phenyloxazol-2-yl)benzene (POPOP) : toluene : Triton-X 100, 3 : 0·09 : 500 : 250, w/w/v/v) were added to the pellet. Radioactivity in the suspension was measured. The combined supernatant fluid and washings were mixed thoroughly and centrifuged so that the two phases separated. The apolar phase (at the bottom) was washed with 2·0 cm^3 distilled H_2O and made up to 15 cm^3 with 96% (v/v) ethanol. The chlorophyll concentration of this solution was determined (Arnon, 1949). All operations were carried out at 0–4 °C.

The polar upper phase plus the 2·0 cm^3 of solution from washing the apolar phase were mixed and passed through a column of Dowex 50Wx8 (200/400 mesh, hydrogen form) packed in a Pasteur pipette. The neutral and anionic fraction was eluted with distilled H_2O and brought to a volume of 15 cm^3. Amino acids were eluted from the column with 6·0 cm^3 $1{\cdot}4 \times 10^2$ mol m^{-3} NH_4OH. The whole cationic fraction and 7·5 cm^3 of the neutral (and anionic) fraction were evaporated to dryness *in vacuo* at 40 °C. Compounds in these fractions were separated by thin layer chromatography and radioactivity determined as described previously (Marques *et al.*, 1983). The remaining 7·5 cm^3 of neutral fraction were used for separation and quantitative determination of glycollate, malate, glycerate and formate by HPLC. The acids were extracted from aqueous solution into DCM by ion-pair extraction using tetrapentylammonium ions (Greving, Jonkman, and de Zeeuw, 1978). Three cm^3 tetrapentylammonium solution and 0·56 cm^3 absolute ethanol (as solvating agent) were added to the fraction, so that the ethanol concentration was 20% (v/v). The fraction was then extracted 5 times with 5·0 cm^3 DCM. The fractions were pooled and DCM was removed at 25 °C by blowing N_2 upon the surface of the solution. The residue was taken up in 0·6 cm^3 distilled H_2O. This solution was analysed on an SP 8000 A liquid chromatograph (Spectra-Physics, Calif.) with two columns of AMINEX Ion Exclusion HP X 87 (300 mm × 7·8 mm) (Bio-Rad Labs, Calif.) arranged in series and eluted with a continuous flow (0·6 cm^3 min^{-1}) of 1·0 mol m^{-3} H_2SO_4 at 17 °C. The organic acids in the effluent were monitored at 200 nm with a Spectrophotometric Detector LC 75 (Perkin-Elmer) coupled with a recorder, and eluted into scintillation vials containing 10 cm^3 PPO/POPOP-scintillation fluid for determination of radioactivity.

Extraction and assay of PEP carboxylase

Using a Polytron homogenizer, plant material (1·25 g) was homogenized in 15 cm^3 of 10 mol m^{-3} imidazole-buffer, pH 7·1, containing 20% (v/v) ethylene glycol, 2·0 mol m^{-3} DTT and and 1% (w/v) insoluble PVP. The further procedure was as previously described (Marques *et al.*, 1983), except that the Sephadex G-25 was equilibrated with 50 mol m^{-3} Tris-HCl, pH 7·5. The enzyme activity was determined by the method of Lane, Maruyama, and Easterday (1969). The assay mixture contained 50 mol m^{-3} Tris-HCl, pH 7·5, 10 mol m^{-3} $MgCl_2$, 40 mol m^{-3} KCl, 1·0 mol m^{-3} PEP, 1·0 mol m^{-3} NADH, 6 units malate dehydrogenase and 32 mol m^{-3} $NaHCO_3$ in a total volume of 1·0 cm^3. Decrease in absorbance at 340 nm was measured at 25 °C with a Perkin-Elmer 55B spectrophotometer.

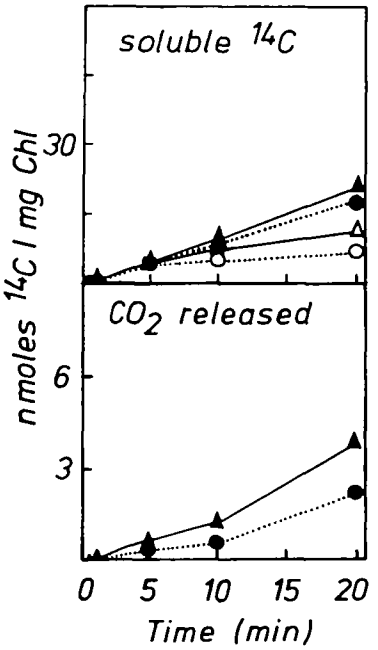

FIG. 1. Dark incorporation of ^{14}C from [1-^{14}C]glycollate (1·7 Ci mol^{-1}) (▵, ○) and [2-^{14}C]glycollate (2·65 Ci mol^{-1}) (▲, ●) into *Lemna minor* L. grown on nutrient solutions containing either NH_4^+ (▵, ▲) or NO_3^- (○, ●) as sole N-source (3·35 mol m^{-3} N). The concentration of glycollate in the nutrient solution was 0·6 mol m^{-3}. 'Soluble ^{14}C' was calculated for each sample as the sum of radioactivities in all measured water-soluble compounds except glycollate. Net $^{14}CO_2$ release in nmoles ^{14}C mg^{-1} chlorophyll during [2-^{14}C]glycollate feeding in darkness. Radioactivity was absorbed in 2-aminoethanol : 2-methoxyethanol (20 : 80, v/v). Similar results were obtained in additional experiments.

RESULTS

Metabolism of [^{14}C]glycollate in darkness

Total ^{14}C incorporated into the soluble fraction (Fig. 1) was calculated for each sample as the sum of the radioactivities incorporated in all measured, water-soluble compounds except glycollate. This 'soluble ^{14}C' was only little enhanced in NH_4^+ grown plants, but irrespective of the N-source all plants incorporated more ^{14}C from [2-^{14}C]- than from [1-^{14}C]glycollate. We attribute this difference to a loss of label from [1-^{14}C]glycollate through decarboxylation of glyoxylate and glycine (Grodzinski, 1979; Halliwell and Butt, 1974; Tolbert, 1979).

Ammonium grown plants evolved more $^{14}CO_2$ from [2-^{14}C]glycollate than NO_3^- plants (Fig. 1). Radioactive formate in the plants could only be detected after [2-^{14}C]glycollate feeding (Fig. 2) and was the first detectable radioactive product. The amount of labelled formate in NH_4^+ grown plants was about double that in NO_3^- grown plants. When [1-^{14}C]glycollate was supplied, the first labelled detectable metabolite was glycine. The labelling of glycine tended to level off during the feeding period, while that of serine continued to increase (Fig. 2). The rates of accumulation of ^{14}C in serine in the dark was slower initially than in the light (Fig. 5). The metabolically active pool of glycine was obviously much smaller than that of serine. Total radioactivity in these two amino acids was hardly influenced by the N-source. The lower percentage ^{14}C-incorporation into glycine and serine in NH_4^+ grown *Lemna* from [2-^{14}C]glycollate was due to higher ^{14}C in formate (Fig. 2) and from [1-^{14}C]glycollate was due to more ^{14}C in aspartate (Fig. 3) compared to NO_3^- grown plants. Radioactivity in serine was more than double after feeding

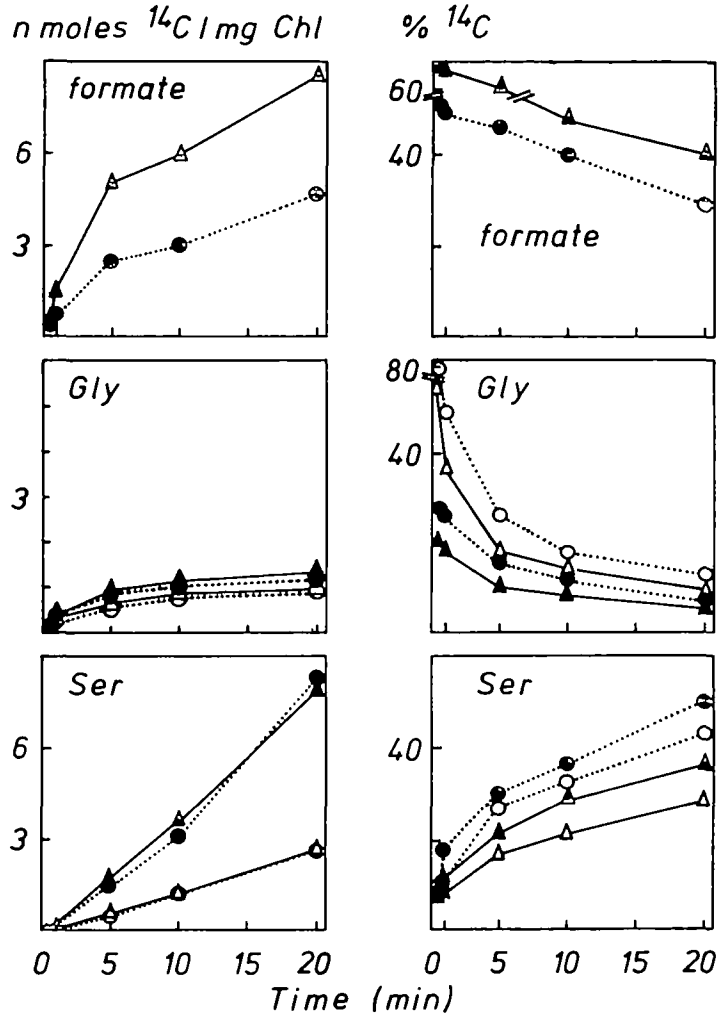

FIG. 2. Time course of ^{14}C incorporation into formate, glycine and serine in nmoles ^{14}C mg^{-1} chlorophyll, and the corresponding values as percent of soluble ^{14}C (see Fig. 1) after [^{14}C]glycollate feeding of *Lemna minor* L. in darkness. Effects of NH_4^+ (▲, △) or NO_3^- (○, ○) as sole N-source on the metabolism of [1-^{14}C]glycollate (▲, ○) and [2-^{14}C]glycollate (△, ○).

[2-^{14}C]glycollate compared with [1-^{14}C]glycollate; this is consistent with the conversion of two molecules of glycine to one of serine (Tolbert, 1979) in which both C-2 atoms are used but only one of the C-1 atoms and possibly also with the synthesis of serine from glycine and formate (Grodzinski, 1979).

Aspartate was only labelled when [1-^{14}C]glycollate was supplied (Fig. 3). The increase of radioactivity in aspartate was faster than that in serine. Aspartate received about three times more ^{14}C-activity in NH_4^+- than in NO_3^- grown plants, whereas the percentage of soluble ^{14}C in asparagine was not influenced by the N-source (data not shown). Higher radioactivity in NH_4^+ grown plants was detected in malate (Fig. 3), although these plants had smaller total malate pools ($1{\cdot}6 \pm 0{\cdot}2$ μmol mg^{-1} Chl, $\pm$s.d., $n = 5$) than those fed with NO_3^- ($7{\cdot}5 \pm 0{\cdot}07$ μmol mg^{-1} Chl). Thus the specific activity of malate in NH_4^+ plants was strikingly higher than in NO_3^- plants. However, the metabolically active malate pool receiving the

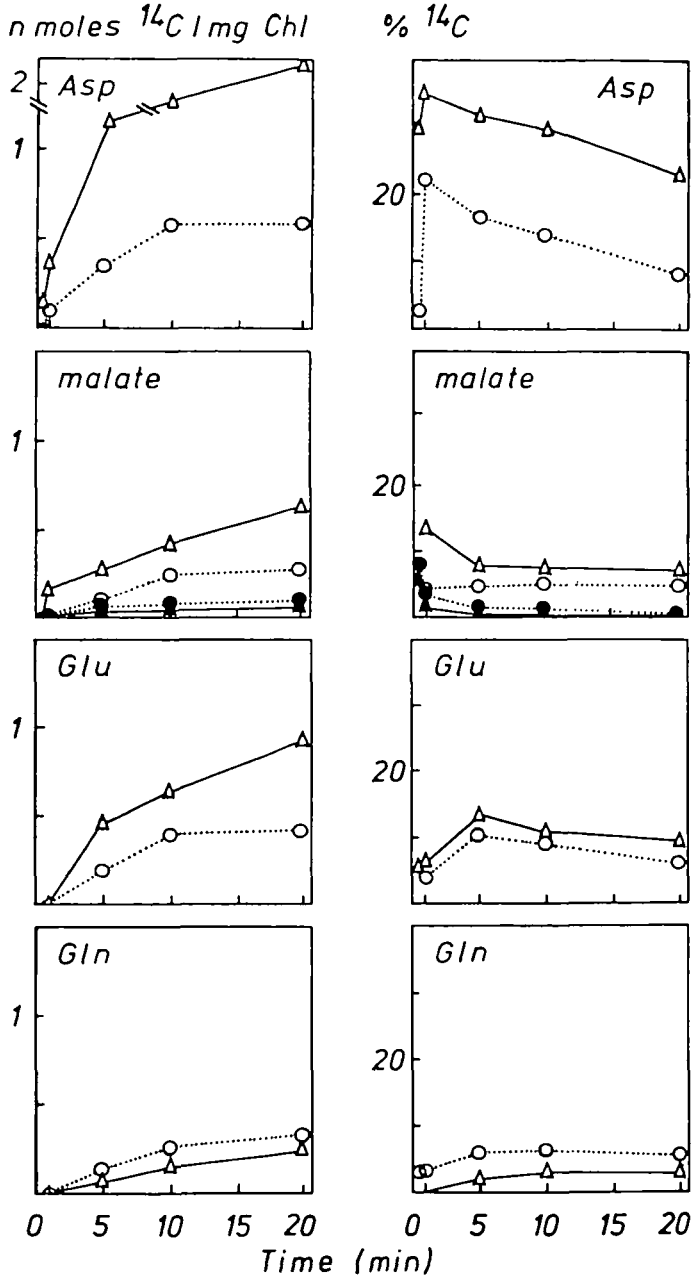

FIG. 3. Time course of ^{14}C incorporation into aspartate, malate, glutamate and glutamine in nmoles ^{14}C mg^{-1} chlorophyll, and corresponding activities expressed as percent of soluble ^{14}C (see Fig. 1) after [^{14}C]glycollate feeding of *Lemna minor* L. in darkness. Effects of NH_4^+ (▲, △) or NH_3 (○, ○) as sole N-source on the metabolism of [1-^{14}C]glycollate (△, ○) and [2-^{14}C]glycollate (▲, ○).

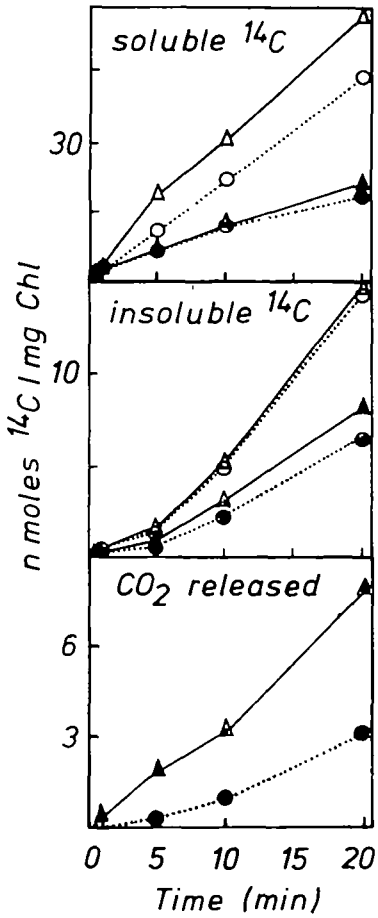

FIG. 4. Incorporation of ^{14}C from [1-^{14}C]glycollate (2·0 Ci mol^{-1}) (▲, ○) and [2-^{14}C]glycollate (5·6 Ci mol^{-1})(▲, ●) into photosynthesizing *Lemna minor* L. grown on nutrient solutions containing either NH_4^+ (▲, △) or NO_3^- (○, ●) as sole N-source (3·35 mol m^{-3} N). The concentration of glycollate in the nutrient solution was 0·6 mol m^{-3}. Other details as for Fig. 1.

^{14}C-label from [1-^{14}C]glycollate contributed only very little to the total amount of malate in the plants. When [2-^{14}C]glycollate was supplied, little radioactivity was found in malate (Fig. 3).

Glutamate and glutamine, like aspartate were labelled only after feeding [1-^{14}C]glycollate (Fig. 3). More labelled glutamate was found in NH_4^+ grown plants, while glutamine labelling was greater in NO_3^- grown plants. Labelling of the insoluble fractions increased linearly over 20 min and was not influenced by the N-source or the position of labelled carbon within glycollate (data not shown). In the dark there was apparently no detectable flux of ^{14}C from glycollate into sugar phosphates and free sugars.

Metabolism of [^{14}C]*glycollate in the light*

Nitrate- as well as NH_4^+-grown plants incorporated a similar amount of ^{14}C into total soluble ^{14}C from [2-^{14}C]glycollate in the light (Fig. 4) and in the dark (Fig. 1). After [1-^{14}C]glycollate feeding, however, photosynthesizing plants had metabolized more to soluble ^{14}C products than plants in the dark given either [1-^{14}C]- or [2-^{14}C]glycollate

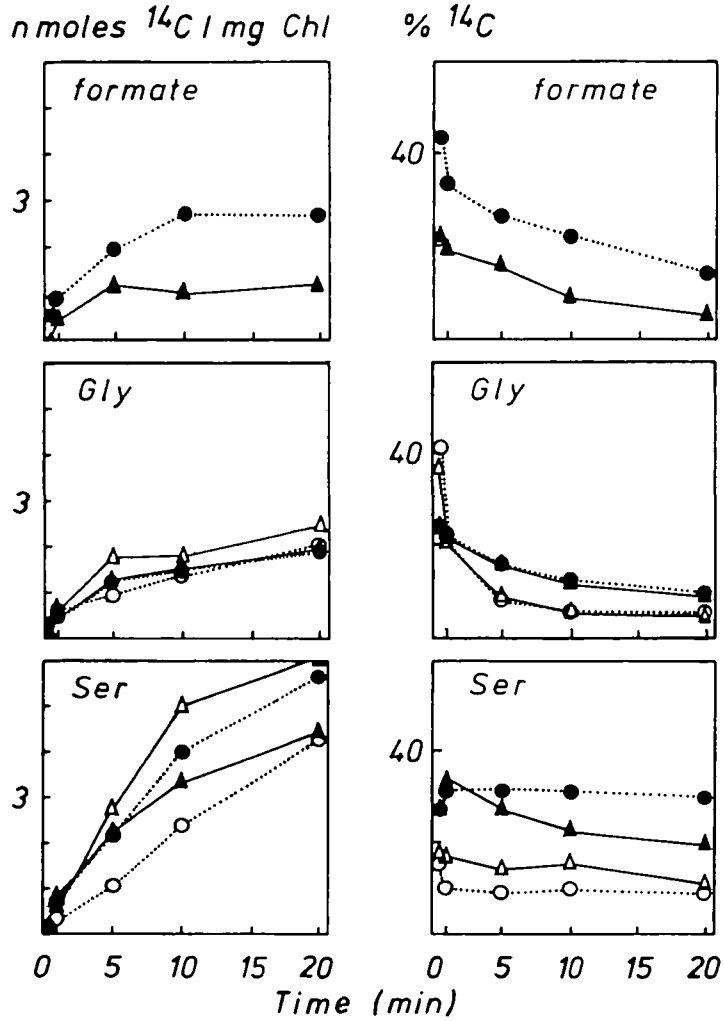

FIG. 5. Time course of ^{14}C incorporation into formate, glycine and serine in nmoles ^{14}C mg^{-1} chlorophyll, and corresponding activities expressed as percent of soluble ^{14}C (see Fig. 4) after [^{14}C]glycollate feeding of *Lemna minor* L. in the light. Effects of NH_4^+ (△, ▲) or NO_3^- (○, ●) as sole N-source on the metabolism of [1-^{14}C]glycollate (△, ○) and [2-^{14}C]glycollate (▲, ●).

(Figs 1, 4). Labelling of insoluble fractions (Fig. 4) was markedly higher after [1-^{14}C]- than after [2-^{14}C]glycollate feeding and was little influenced by the N-source. The difference of the $^{14}CO_2$ release between NH_4^+ and NO_3^- grown plants during the metabolism of [2-^{14}C]glycollate in the light (Fig. 4) was strikingly greater than during the dark metabolism (Fig. 1).

Formate and glycine were the earliest-labelled compounds measured (Fig. 5). Surprisingly, after [2-^{14}C]glycollate feeding in the light NO_3^- grown plants contained more labelled formate than NH_4^+ plants. This is in contrast to plants labelled in the dark (Fig. 2). The type of N-source had little influence on the labelling of glycine (Fig. 5). Radioactivity in serine (Fig. 5) showed distinct differences between NH_4^+ and NO_3^- grown plants. In NO_3^- grown plants the metabolism of [2-^{14}C]glycollate resulted, at least at the beginning of the labelling period, in double the amount of ^{14}C-activity in serine compared to [1-^{14}C]glycollate feeding,

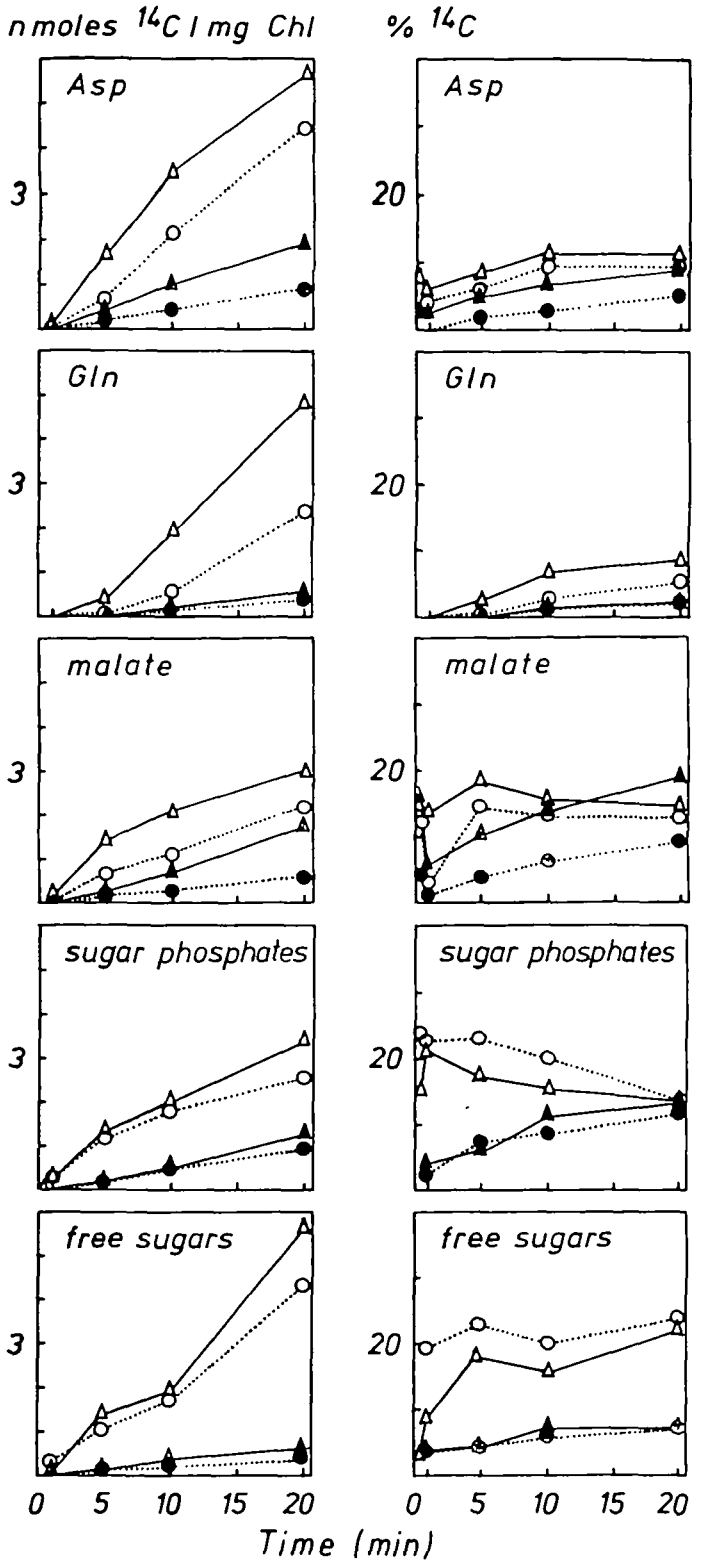

FIG. 6. Time course of ^{14}C incorporation into aspartate, glutamine, malate, sugar phosphates and free sugars in nmoles $^{14}C\ mg^{-1}$ chlorophyll, and corresponding activities expressed as percent of soluble ^{14}C (see Fig. 4) after [^{14}C]glycollate feeding of *Lemna minor* L. in the light. Effects of NH_4^+ (▲, △) or NO_3^- (●, ○) as sole N-source on the metabolism of [1-^{14}C]glycollate (△, ○) and [2-^{14}C]glycollate (▲, ●).

as would be expected (Tolbert, 1979). In NH_4^+ grown plants, however, more label was incorporated into serine from [1-^{14}C]- than from [2-^{14}C]glycollate.

Aspartate (Fig. 6), asparagine, glutamate (data not shown) and glutamine (Fig. 6) were labelled much more from [1-^{14}C]- than from [2-^{14}C]glycollate. After supplying both forms of [^{14}C]glycollate more radioactivity was present in aspartate in NH_4^+ than in NO_3^- grown plants, while labelling of asparagine and glutamate was not changed by the N-source. After [1-^{14}C]glycollate feeding, glutamine in NH_4^+ grown plants contained about double the radioactivity of that in NO_3^- grown plants. Ammonium grown plants incorporated markedly more labelled carbon into malate than NO_3^- grown plants (Fig. 6) with either [1-^{14}C]- or [2-^{14}C]glycollate, and the same pattern was observed with alanine (data not shown). *Lemna* plants, which metabolized [^{14}C]glycollate in the dark, contained no detectable radioactive alanine. Sugar phosphates and free sugars (Fig. 6) were much more labelled when the plants had been supplied with [1-^{14}C]- instead of [2-^{14}C]glycollate.

PEP carboxylase

The extractable activity of PEP carboxylase in NH_4^+ grown *Lemna* ($0{\cdot}82 \pm 0{\cdot}07$ μmol mg^{-1} Chl min^{-1}, ±s.d., $n = 4$) was apparently higher than in NO_3^- grown plants ($0{\cdot}69 \pm 0{\cdot}06$ μmol mg^{-1} Chl min^{-1}, ±s.d., $n = 4$).

DISCUSSION

Metabolism of [^{14}C]glycollate in darkness

The substrate immediately involved in photorespiratory CO_2 release has been proposed to be glycine and glyoxylate (Grodzinski, 1979; Halliwell and Butt, 1974; Tolbert, 1979). Another possible source of $^{14}CO_2$ from [1-^{14}C]glycollate is the decarboxylation of hydroxypyruvate by H_2O_2 (Walton and Butt, 1981). Radioactive CO_2 released during metabolism of [2-^{14}C]glycollate may be generated by oxidation of formate (Grodzinski, 1979; Halliwell, 1974), which is produced by oxidation of glyoxylate (Grodzinski, 1979; Zelitch, 1972). Further metabolism of recycled glycollate, produced by the mentioned decarboxylation of hydroxypyruvate, would lead to a $^{14}CO_2$ release from supplied [2-^{14}C]glycollate. The higher $^{14}CO_2$ release from [2-^{14}C]glycollate of NH_4^+ grown *Lemna* plants (Fig. 1) could originate from more available radioactive formate (Fig. 2) for oxidation. The high amount of [^{14}C]formate found only after [2-^{14}C]glycollate feeding was probably generated through the decarboxylation of glyoxylate (Grodzinski, 1979; Zelitch, 1972). In the presence of enough amino donors, glyoxylate is transaminated to glycine and not oxidized to CO_2 (Chang and Huang, 1981; Oliver, 1981; Somerville and Ogren, 1981). When feeding *Lemna* plants with glycollate the availability of amino groups for the amination of glyoxylate possibly could have been rate-limiting, even in the presence of an inorganic N-source, so that an excess of glyoxylate produced by oxidation of the supplied glycollate was decarboxylated.

Total ^{14}C incorporated into the soluble fraction (Fig. 1) and the labelling of glycine and formate (Fig. 2) suggest, that [^{14}C]glycollate was metabolized more rapidly in NH_4^+ grown plants. This conclusion is supported by the higher extractable activity of glycollate oxidase in NH_4^+ grown *Lemna* (Emes and Erismann, 1982).

In photosynthesizing plants fed with [^{14}C]glycollate the radioactivity in serine tended to reach a constant level within 20 min (Fig. 5), whereas in darkness the increase of radioactivity in serine was linear during the same time and was slower at the beginning of the labelling period (Fig. 2). These results point to a reduced metabolism of the serine synthesized through the glycollate pathway in plants kept in the dark. This conclusion and the fact that we could

not find any labelled sugar phosphates in darkness (see results) imply that the supply of carbon to the Calvin cycle through the glycollate pathway by further metabolism of serine is, at least indirectly, light dependent. A corresponding observation has previously been made with pea leaves (Miflin, Marker, and Whittingham, 1966). Aspartate and malate were significantly labelled only when [1-^{14}C]glycollate had been fed and they contained more ^{14}C in NH_4^+ grown plants (Fig. 3). Glycollate is not a substrate for malate synthesis (Yokota *et al.*, 1983), and the incorporation of [1-^{14}C]glycollate into malate is not thought to be via glycine and serine (Asada, Saito, Kitoh, and Kasai, 1965; Miflin *et al.*, 1966). If malate synthase (Miernyk and Trelease, 1981; Yamamoto and Beevers, 1960) were active, or if hydroxyaspartate was produced by condensation of glycine and glyoxylate (Kornberg and Morris, 1963), aspartate and malate should have similar radioactivities after [1-^{14}C]glycollate and [2-^{14}C]glycollate feeding. The extractable activity of PEP carboxylase in NH_4^+ grown *Lemna* was enhanced compared to plants with NO_3^- nutrition (see results). In other plants, PEP carboxylation was also found to be stimulated by NH_4^+ (Paul, Cornwell, and Bassham, 1978). All these observations suggest that aspartate and malate were labelled via oxaloacetate after dark fixation of $^{14}CO_2$. An enhanced PEP carboxylation in NH_4^+ grown plants could possibly be a consequence of more available $^{14}CO_2$ in NH_4^+ plants. A higher level of PEP carboxylation would deliver more carbon skeletons, which are required for the assimilation of NH_4^+ to synthesize more amino acids (Erismann and Kirk, 1969) and proteins (Dicht, Kopp, Feller, and Erismann, 1976). In contrast, primary leaves of *Phaseolus vulgaris* L. grown on NH_4^+ as sole N-source showed a reduced PEP carboxylation rate (Marques *et al.*, 1983) compared to NO_3^- nutrition. Here, as in the case of other terrestrial plants, NH_4^+ is mainly assimilated in the roots. In *Lemna* and other aquatic plants, NH_4^+ is taken up directly into the photosynthetic cells.

It is likely that ^{14}C-label in glutamate and glutamine (Fig. 3) also arose through fixation of $^{14}CO_2$ by further conversions of malate and/or oxaloacetate in the Krebs cycle. The labelling pattern of glutamate compared to that of glutamine points to a reduced rate of glutamate amidation in NH_4^+ grown plants in darkness, a view supported by measurements of glutamine synthetase activities in *Lemna minor* L. (Rhodes, Sims, and Stewart, 1979).

Metabolism of [^{14}*C*]*glycollate under photosynthetic conditions*

In the light the *Lemna* plants assimilated [^{14}C]glycollate as well as $^{14}CO_2$ originating mainly from photorespiration and apparently from a glycollate degradation in the feeding solution. Despite sterile conditions, control flasks with a [^{14}C]glycollate feeding solution but without plants evolved $^{14}CO_2$. The experimental production of $^{14}CO_2$ was several times more from [1-^{14}C]- than from [2-^{14}C]glycollate. This explains why sugar phosphates, free sugars (Fig. 6) and thus total soluble ^{14}C were more rapidly and more highly labelled from [1-^{14}C]- than from [2-^{14}C]glycollate in photosynthesizing plants. In the case of [1-^{14}C]glycollate feeding, high blank values prevented an accurate determination of $^{14}CO_2$ released from *Lemna* plants.

Ammonium grown plants accumulated more total soluble ^{14}C during [1-^{14}C]glycollate feeding than NO_3^- grown plants (Fig. 4). Labelling of sugar phosphates, free sugars (Fig. 6) and the insoluble fractions as well as soluble ^{14}C in the case of [2-^{14}C]glycollate feeding were little influenced by the N-source (Fig. 4). This may indicate that NH_4^+ grown plants assimilated $^{14}CO_2$ not only through RuBP carboxylation but also through another carboxylation reaction. The higher radioactivity of aspartate and malate (Fig. 6) in NH_4^+ compared to NO_3^- grown plants points to a stimulated PEP carboxylation.

The rate of glutamine labelling (Fig. 6) was also increased in NH_4^+ grown plants, this is in contrast to the labelling in darkness (Fig. 3). This means that the higher concentration of

glutamine measured in NH_4^+ grown *Lemna* (Erismann and Kirk, 1969) could likely be realized only in plants receiving light.

The fact that formate was only labelled in plants which were supplied with [2-^{14}C]glycollate, implies the labelling of formate by decarboxylation of [^{14}C]glyoxylate (Grodzinski, 1979; Zelitch, 1972) and certainly argues against a direct formation of formate from CO_2 (Kent, 1972). In NH_4^+ grown plants under photosynthetic conditions formate was markedly less labelled and since its radioactivity levelled off after 5 min (Fig. 5) the metabolically active formate pool was smaller than in NO_3^- grown plants. After [2-^{14}C]glycollate metabolism in the light (Fig. 5) as well as in darkness (Fig. 2) NO_3^- grown plants contained similar amounts of labelled formate, whereas NH_4^+ grown plants showed several times more radioactive formate in the dark than in the light. The increased $^{14}CO_2$ release from [2-^{14}C]glycollate of photosynthesizing NH_4^+ plants (Fig. 4) compared to plants in darkness (Fig. 1) was possibly generated by a stimulated rate of formate oxidation, so that the metabolically active formate pool of NH_4^+ grown plants became smaller (Fig. 5) and the flow of carbon through it faster in the light than in the dark. A possible explanation is that formate was oxidized by H_2O_2 (Grodzinski, 1979; Halliwell, 1974; Yokota *et al.*, 1983) produced in the chloroplasts by an excess of reduction equivalents due to NH_4^+ nutrition (Weissmann, 1972).

The time course of labelling of serine after [^{14}C]glycollate metabolism was completely different in the light (Fig. 5) and in darkness (Fig. 2), while radioactivity in glycine (Figs 2, 5) was not markedly influenced either by light or by the N-source. Considering the proposed pathways for the synthesis of serine in higher plants (Keys, 1980), we suppose that in NH_4^+ grown *Lemna* plants serine was mainly labelled from P-glycerate, which incorporated ^{14}C through RuBP carboxylation. The labelling pattern of serine in the light could not have been expressed in the dark, because here the sugar phosphates were not labelled (see results). The rate of serine synthesis through the glycollate pathway (Tolbert, 1979) is possibly limiting, so that an increased requirement of serine for stimulated protein synthesis evident in NH_4^+ grown plants (Dicht *et al.*, 1976) is covered through a pathway starting from P-glycerate (Keys, 1980).

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LITERATURE CITED

Arnon, D. I., 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiology*, **24**, 1-15.

Asada, K., Saito, K., Kitoh, S., and Kasai, Z., 1965. Photosynthesis of glycine and serine in green plants. *Plant and Cell Physiology*, **6**, 47-59.

Chang, C. C., and Huang, A. H. C., 1981. Metabolism of glycollate in isolated spinach leaf peroxisomes. Kinetics of glyoxylate, oxalate, carbon dioxide, and glycine formation. *Plant Physiology*, **67**, 103-6.

Dicht, M., Kopp, A., Feller, U., and Erismann, K. H., 1976. Influence of ammonium and nitrate on protein content of *Lemna minor* L. during photosynthesis. *Biochemie und Physiologie der Pflanzen*, **170**, 531-4.

Emes, M. J., and Erismann, K. H., 1982. The influence of nitrogen supply on the structure and activity of glycollate oxidase in *Lemna minor* L. *Plant Science Letters*, **27**, 103-9.

Erismann, K. H., and Kirk, M. R., 1969. The influence of nitrogen source on metabolic intermediates in steady-state photosynthesis by *Lemna minor* L. In *Progress in photosynthesis research, Volume 3*. Ed. H. Metzner. Tübingen. Pp. 1538-45.

Greving, J. E., Jonkman, J. H. G., and de Zeeuw, R. A., 1978. Determination of carboxylic acids in

the picomole range after derivatization with pentafluorbenzyl bromide and electron capture gas chromatography. *Journal of Chromatography*, **148**, 389–95.

GRODZINSKI, B., 1979. A study of formate production and oxidation in leaf peroxisomes during photorespiration. *Plant Physiology*, **63**, 289–93.

HALLIWELL, B., 1974. Oxidation of formate by peroxisomes and mitochondria from spinach leaves. *Biochemical Journal*, **138**, 77–85.

——and BUTT, V. S., 1974. Oxidative decarboxylation of glycollate and glyoxylate by leaf peroxisomes. Ibid. **138**, 217–24.

KENT, S. S., 1972. Photosynthesis in the higher plant *Vicia faba*. II. The non-Calvin cycle origin of acetate and its metabolic relationship to the photosynthetic origin of formate. *Journal of Biological Chemistry*, **247**, 7293–7302.

KEYS, A. J., 1980. Synthesis and interconversion of glycine and serine. In *The biochemistry of plants. A comprehensive treatise. Volume 5*. Eds P. K. Stumpf and E. E. Conn. Academic Press, New York. Pp. 359–74.

——BIRD, I. F., CORNELIUS, M. J., LEA, P. J., WALLSGROVE, R. M., and MIFLIN, B. J., 1978. Photorespiratory nitrogen cycle. *Nature*, **275**, 741–3.

KOPP, A., FELLER, U., and ERISMANN, K. H., 1974. Investigations on the regulation of nitrogen assimilation of *Lemna minor* during the transition from ammonium to nitrate nutrition and from nitrate to ammonium nutrition during photosynthesis. *Zeitschrift für Pflanzenphysiologie*, **73**, 456–60.

KORNBERG, H. L., and MORRIS, J. G., 1963. β-Hydroxyaspartate pathway: a new route for biosynthesis from glyoxylate. *Nature*, **197**, 456–7.

LANE, M. D., MARUYAMA, H., and EASTERDAY, R. L., 1969. Phosphoenolpyruvate carboxylase from peanut cotyledons. *Methods in Enzymology*, **13**, 277–83.

MARQUES, I. A., OBERHOLZER, M. J., and ERISMANN, K. H., 1983. Effects of different inorganic nitrogen sources on photosynthetic carbon metabolism in primary leaves of non-nodulated *Phaseolus vulgaris* L. *Plant Physiology*, **71**, 555–61.

MIERNYK, J. A., and TRELEASE, R. N., 1981. Role of malate synthase in citric acid synthesis by maturing cotton embryos: a proposal. Ibid. **67**, 875–81.

MIFLIN, B. J., MARKER, A. F. H., and WHITTINGHAM, C. P., 1966. The metabolism of glycine and glycollate by pea leaves in relation to photosynthesis. *Biochimica et biophysica acta*, **120**, 266–73.

OLIVER, D. J., 1981. Role of glycine and glyoxylate decarboxylation in photorespiratory CO_2 release. *Plant Physiology*, **68**, 1031–4.

PAUL, J. S., KORNWELL, K. L., and BASSHAM, J. A., 1978. Effects of ammonia on carbon metabolism in photosynthesizing isolated mesophyll cells from *Papaver somniferum* L. *Planta*, **142**, 49–54.

RHODES, D., SIMS, A. P., and STEWART, G. R., 1979. Glutamine synthetase and the control of nitrogen assimilation in *Lemna minor* L. In *Nitrogen assimilation of plants*. Eds E. J. Hewitt and C. V. Cutting. Academic Press, London. Pp. 501–20.

SOMERVILLE, C. R., and OGREN, W. L., 1981. Photorespiration-deficient mutants of *Arabidopsis thaliana* lacking mitochondrial serine transhydroxymethylase activity. *Plant Physiology*, **67**, 666–71.

TOLBERT, N. E., 1979. Glycollate metabolism by higher plants and algae. In *Encyclopedia of plant physiology, New series, Volume 6*. Eds M. Gibbs and E. Latzko. Springer-Verlag, Berlin. Pp. 338–52.

VAKLINOVA, S., FEDINA, I., VASSILEVA, V., and ANANIEVA, L., 1981. Effect of nitrate and ammonium nitrogen on the intensity of photosynthesis, photorespiration and on the ribulose-1,5-diphosphate carboxylase/oxygenase in C_3 and C_4 types of plants. In *Photosynthesis VI, photosynthesis and productivity, photosynthesis and environment*. Ed. G. Akoyunoglou. Philadelphia. Pp. 55–62.

WALTON, N. J., and BUTT, V. S., 1981. Metabolism and decarboxylation of glycollate and serine in leaf peroxisomes. *Planta*, **153**, 225–31.

WEISSMANN, G. S., 1972. Influence of ammonium and nitrate nutrition on the pyridine and adenine nucleotides of soybean and sunflower. *Plant Physiology*, **49**, 142–5.

YAMAMOTO, Y., and BEEVERS, H., 1960. Malate synthetase in higher plants. Ibid. **35**, 102–8.

YOKOTA, A., KAWABATA, A., and KITAOKA, S., 1983. Mechanism of glyoxylate decarboxylation in the glycollate pathway in *Euglena gracilis* Z. Ibid. **71**, 772–6.

ZELITCH, I., 1972. Comparison of the effectiveness of glycolic acid and glycine as substrates for photorespiration. Ibid. **50**, 109–13.

——1979. Photorespiration: studies with whole tissues. In *Encyclopedia of plant physiology, New series, Volume 6*. Eds M. Gibbs and E. Latzko. Springer-Verlag, Berlin. Pp. 353–67.