Plant Physiol. (1981) 68, 28–32
0032-0889/81/68/0028/05/$00.50/0

Limitations on the Utilization of Glycolate by *Chlamydomonas reinhardtii*[1]

Received for publication August 28, 1980 and in revised form December 1, 1980

KENNETH G. SPENCER[2] AND ROBERT K. TOGASAKI
Department of Biology, Indiana University, Bloomington, Indiana 47401

ABSTRACT

Growth and shorter term incorporation measurements with both wild type *Chlamydomonas reinhardtii* and a mutant (F-60, lacking phosphoribulokinase activity) indicate that the rate of glycolate utilization is always relatively low. Growth support with external glycolate is restricted to cells with full photosynthetic capacity. A high concentration of glycolate is required for optimal growth support and incorporation of [^{14}C]glycolate. Glycolate incorporation is low at pH 3.8 even with the relatively free permeability. The F-60 mutant can take up only small quantities of glycolate in spite of photosynthetic electron transport and photophosphorylation competencies. This requirement for photosynthetic carbon metabolism indicates a significant difference in the glycolate pathway of this alga. No growth condition significantly increases glycolate incorporation rates. There is no evidence that one of the primary enzymes, glycolate dehydrogenase, is limiting utilization; measurements of glycolate uptake and excretion do not always correlate with its activity. Since the maximal utilization rate of glycolate is low, control of glycolate formation is important in preventing the loss of this fixed carbon from the algal cell.

Recent gas exchange measurements with *Chlamydomonas reinhardtii* and other green algae (3, 5, 13) have indicated very low levels of photorespiration, thereby changing the perception of the role of glycolate in these organisms. An inducible mechanism for concentrating CO_2 internally has been characterized (2). A high intracellular CO_2 concentration enables the algal cell to avoid glycolate formation and photorespiratory gas exchange. This mechanism provides a good explanation for many of the well known changes in measurements of whole cell glycolate release; prominent glycolate excretion is seen only in cells grown with elevated levels of CO_2 when there is little internal concentrating of CO_2. The likelihood of variable glycolate formation and the observations of glycolate excretion by the cell raise questions about the maximum capacity and the variability of glycolate utilization.

The very existence of glycolate excretion suggests that turnover is at times limited. However, radioisotopic and enzymic analyses have indicated that the potential for carbon flow through the glycolate pathway of higher plants exists in green algae (23). The capacity in several different algae for the assimilation of external glycolate has been considered important. Work with a bacterized diatom culture has shown that glycolate supplied in very low amounts can shorten the lag phase of the culture (19). Most work, including the present study, has noted the limitations of such utilization by green algae (23).

Before the awareness of active CO_2 concentration it was concluded that differences in metabolism were responsible for changes in the excretion of glycolate. A decrease in the activity of the enzyme responsible for its initial oxidation, glycolate dehydrogenase, that corresponded with the increased excretion following growth on high levels of CO_2 corroborated this notion (17). However, this correlation has been shown to break down in *Chlorella pyrenoidosa* (7) and *Euglena* (6) as well as in the following results with *Chlamydomonas*. In addition, the control of the glycolate balance by permeability and the availability of counterions was suggested in early work by Tolbert and Zill (24) and has been recently carefully examined in *Scenedesmus* by Findenegg (9). The latter study concluded that permeability can be the primary controlling factor in the uptake and release of glycolate.

In this report the potential for utilization of internal glycolate is estimated by several methods. At a low pH, the permeability barrier of glycolate into the cell is relieved. The effects of various growth conditions are determined. Incorporation studies with the F-60 mutant are particularly important in measuring true heterotrophic glycolate utilization. Because of its phosphoribulokinase lesion, this mutant can produce little glycolate. The effects of the metabolic inhibitor isonicotinyl hydrazide are also measured.

MATERIALS AND METHODS

Cell Culture. The MM[3] of Sueoka (21) was used with the following modifications: 15 mM potassium acetate was added to make MA; 15 mM potassium glycolate was added to make MG; 10 mM glycylglycine buffer (pH 3.8) was added to medium which contained only one-sixth of the phosphate buffer to make 3.8MM. Both of the strains of *C. reinhardtii* Dangeard used throughout this study were originally obtained from Dr. R. P. Levine. The wild type is from strain 137c(+). The F-60 mutant strain (16) showed no more than 5% of wild type photosynthesis. Stocks were maintained in the light on solid MA medium. Cells were routinely grown to mid log phase in 150 ml of medium in shake culture under constant illumination. Two liters of medium in a 3-liter stopcock flask were used for some growth measurements. Temperature for growth and all assays was 25 C unless otherwise indicated. Cultures in MM were bubbled with either air or a mixture of 25 ml/l CO_2 in air (the latter hereafter designated high CO_2-grown cells) at a rate of approximately 500 ml/min. The isolates are axenic and cultures were regularly examined microscopically for contamination. Synchronous cells were grown at 20 C according to the method of Surzycki (22). Cells were har-

[1] This research was supported in part by National Science Foundation Grant PCM 75-19643A2 (to R. K. T.).

[2] Present address: Botany Department, University of California, Berkeley, CA 94720.

[3] Abbreviations: MM, minimal medium; MA, acetate supplemented medium; MG, glycolate supplemented medium; 3.8MM, minimal medium at pH 3.8; DCPIP, dichlorophenolindophenol; INH, isonicotinyl hydrazide.

vested at or just after the stage of cell division, approximately 8 h into the dark phase of the 12:12 light:dark cycle.

Photosynthesis and Excretion Assays. Photosynthesis was measured as total $NaH^{14}CO_3$ incorporated into acid-stable products over a 20-min period. Cells were harvested by centrifugation at 400*g* for 5 min, washed once with MM, and resuspended to 50 μg total Chl/ml (1) in MM. A 4-ml aliquot of cell suspension was placed in a small test tube in a small constant temperature bath. Cells were kept in suspension with a small magnetic stir bar. Illumination for these assays and for glycolate incorporation measurements was from a 35-mm slide projector giving an irradiance of 4.0×10^4 ergs cm^{-2} h^{-1}. A 5-min preincubation period in the light was followed by the addition of 100 μl of $NaH^{14}CO_3$ (100 μmol and 25 μCi/ml) to give an initial HCO_3^- concentration of 2.5 mM. At 5-min intervals thereafter a 50-μl sample was added to 100 μl of glacial acetic acid. A 500-μl aliquot was filtered through a 1-ml syringe equipped with a Whatman GF/A glass fiber filter. Two hundred μl of the cell-free filtrate obtained were placed in a second vial containing 200 μl of acetic acid. Vials were subsequently heated to 50 C for 15 min to drive off all the unfixed CO_2. After the addition of 10 ml of ACS (Amersham) scintillation cocktail, samples were counted in a Beckman LS233 counter. A 10-mM concentration of isonicotinyl hydrazide (INH, K and K Laboratories) was added in some measurements during preincubation. Amounts of glycolate were tested with the Calkins colorimetric microdetermination (4).

Incorporation Studies. Cells for incorporation assays were washed once with MM (or 3.8MM if incorporation was to be done at pH 3.8) and resuspended to a density of 40 μg total Chl/ml in either MM (pH 6.8) with 10 mM glycolate or 3,8MM with 3.0 mM glycolate. A 2.5-ml portion of cell suspension was incubated in the light as with excretion assays with CO_2-free air bubbling before the addition of 50 μl of 2-[^{14}C]glycolate (2.5 μCi/ml, 55 μCi/μmol). The radioactive glycolate was purified by TLC before use. The 500-μl aliquots of these cells were removed at the indicated intervals and added to 10 ml of an ice cold 10 mM glycolic acid solution. Cells were then filtered onto a Whatman GF/A filter and washed once with 10 ml of the 10 mM glycolic acid solution. The filter was transferred to 10 ml of scintillation cocktail for counting. Any inhibitor used was added during preincubation. Acetate incorporation experiments were performed in the same manner using 20 μl of 2-[^{14}C]acetate (12.5 μCi and 20 μmol/ml) added to cells in 5 mM K-acetate. These and all other data are presented as the average of two trials unless otherwise indicated.

Glycolate Dehydrogenase. In the crude preparation, membranes are disrupted by freezing a cell suspension containing 50 μg total Chl/ml in 1% Triton X-100 buffered with 13.5 mM phosphate (pH 6.8). The thawed sample was centrifuged at 28,000*g* for 20 min and the resulting supernatant was used. The assay was performed by a modification of the spectrophotometric method of Lord (14). A 500-μl portion of the crude enzyme was added to 1.2 ml of Tricine buffer (0.2 M, pH 8.0) along with 0.2 ml of DCPIP. The reaction was initiated by the addition of 10 μmol of neutralized glycolate. The *A* decrease at 600 nm was observed in a double beam spectrophotometer as DCPIP was reduced.

RESULTS

Figure 1 indicates the level to which growth can be supported by glycolate. Under the conditions used, high glycolate concentration, neutral pH, and relatively bright light, the effect is dramatic. Stimulation is initially comparable to that with acetate, but the cells reach stationary phase more quickly, *i.e.* at a significantly lower cell density. Glycolate utilization is distinguishable from acetate incorporation in several respects: (a) glycolate cannot support growth in the dark or in the presence of DCMU; (b) glycolate cannot substitute for acetate in sustaining the F-60 mutant; and (c) growth stimulation with glycolate is seen only after a lack of CO_2 has limited autotrophic growth.

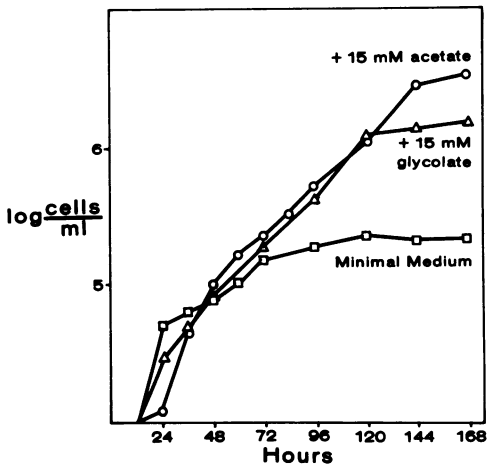

FIG. 1. Photoheterotrophic growth of *Chlamydomonas reinhardtii.* Cells were stirred in 2 liters of MM (21) with or without the addition of the potassium salt of acetate or glycolate. Incident light intensity was 500 ft-c. Replicate samples were removed and counted in a hemacytometer.

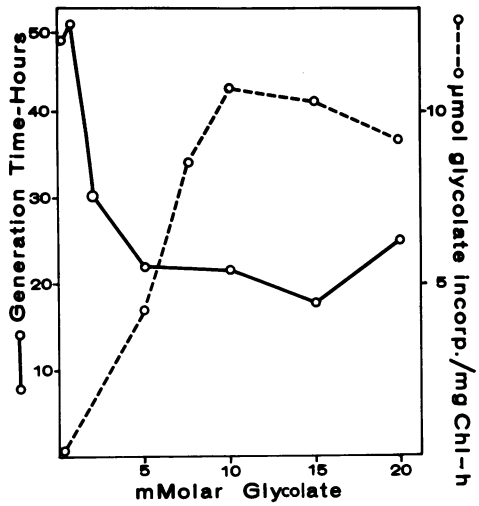

FIG. 2. Dependence of growth stimulation and incorporation upon glycolate concentration. Growth measurements were as in Figure 1 except that 100 ml of medium were used in shake culture. Generation time is for final 80 h of the 144-h experiment. Incorporation rates are for cells grown on medium supplemented with 15 mM K-glycolate. Average uptake of [2-^{14}C]glycolate (3 time points) over 6 h is shown.

The high levels of glycolate required for optimal incorporation and stimulation of growth are seen in Figure 2. Conditions for both were made analogous to those in Figure 1 except for the higher light intensity used for uptake measurements. The generation time is for the time during which CO_2 would limit autotrophic growth. The saturating concentration (about 10 mM) for the incorporation measurements is even slightly higher than that seen in the growth study. Determinations of the concentration of glycolate remaining after the growth study indicated that no more than half of the supplied glycolate was utilized.

In previous studies (9, 18) division synchronized cells of green algae have shown more uptake of labeled glycolate immediately after cytokinesis. Using *Chlamydomonas* cells harvested at this stage results in a 13-fold increase in the uptake (from 0.084 to 11 nmol/mg Chl·h) when 1 μM glycolate was supplied. However, at the 10 mM concentration of glycolate, optimal for incorporation by asynchronous cells, there is only a very slight increase in the

uptake rate (from 10–12 μmol/mg Chl·h). To enable direct comparison with cells grown under conditions in which synchrony is not readily attained, only asynchronous cultures were used in all of the other measurements.

In addition, most measurements were at a neutral pH. The effect of enhancing permeability was tested by measurements at pH 3.8, the pK_a of glycolate. A 10 mM level of glycolate at pH 3.8 is quite toxic; the change in this toxicity corresponds directly to a change in pH. However, in relatively short-term measurements of incorporation at pH 3.8, a 3 mM level of glycolate is optimal for incorporation. At this low pH growth and overall metabolism are depressed. Maximal glycolate incorporation reached only 1.7 μmol/mg Chl·h (Table I). At pH values between 3.8 and 6.8, glycolate incorporation was never higher than at pH 6.8 (data not shown).

The requirement for photosynthesis is apparent in the incorporation measurements as it is in the growth studies (Tables I and II). Lowering the high light intensity used for these measurements results in proportionally lower uptake rates (data not shown). Increasing the concentration of DCMU has a similar effect. Although growth is not supported by glycolate in F-60, optimal incorporation rates at both pH values are about half of those of wild type (Table II). With regard to the role of photosynthesis it is also important to notice that the incorporation conditions with wild type are such that CO_2 is strictly limited. Bubbling the cell suspension with 1% CO_2 an air-reduced glycolate uptake by 25% in wild type but not in F-60. The inhibitor INH only partially blocks external glycolate uptake in wild type and seems to have little effect with F-60.

As has been reported previously (17), a decrease in glycolate dehydrogenase activity is observed following high CO_2 growth. Growth on acetate results in similarly low levels of glycolate dehydrogenase activity but does not cause low levels of incorporation or increased excretion (Table III). In the mutant F-60, a response to growth in the presence of elevated CO_2 is seen in the enzyme activity, but there was little effect upon glycolate incorporation. The amount of short-term photosynthate in this strain is small and there is no detectable excretion.

Excretion is measured as acid stable $H^{14}CO_3^-$ (incorporation) product found in the medium after cell removal. TLC identified at least 85% of this excretion as glycolate under all conditions. The specific radioactivity of excreted glycolate (determined from the Calkins assay) indicated that both glycolate carbons were derived from the supplied $H^{14}CO_3^-$. A marked change in the level of excreted glycolate is brought about by growth condition.

Table I. *Glycolate and Acetate Uptake*

Rates are presented as the average uptake of 2-[^{14}C]glycolate or 2-[^{14}C]acetate over 2 h. Acetate-grown cells were measured in 10 mM glycolate (pH 6.8), 3.0 mM glycolate (pH 3.8), or 5.0 mM acetate (pH 6.8). Unless otherwise indicated these and all other results are the average of two complete repetitions.

Strain and Conditions	Glycolate (pH 6.8)	Glycolate (pH 3.8)	Acetate (pH 6.8)
		μmol/mg Chl·h	
Wild type			
Light	8.8	1.5	17
Dark	1.3	1.7	9.3
F-60			
Light	4.2	1.0	51[a]
Dark	2.2	0.6	31[a]

[a] Represents a single experiment.

Table II. *The Effect of Inhibitors on Glycolate Growth Stimulation and Glycolate Incorporation*

Growth stimulation is the ability for growth in medium with 15 mM glycolate compared with that in MM. A "no" indicates growth equal to or less than that in MM. Incorporation measurements were performed at pH 6.8 as in Table I.

Strain and Inhibitor	Growth Stimulation by Glycolate	Normal Wild Type Glycolate Uptake
		%
Wild type		
None	Yes	100
DCMU, 1 μM	No	11
INH, 10 mM	No	49
F-60		
None	No	41
INH, 10 mM	No	47[a]

[a] Single experiment.

DISCUSSION

The results in Figure 1 and Table I demonstrate that while external glycolate can support the growth of *C. reinhardtii*, there are strict limitations to this utilization. Complete photosynthetic competence is required, growth could not be stimulated in darkness or in the presence of DCMU. The requirement for the Calvin-Benson cycle is indicated by the results with the mutant lacking phosphoribulokinase. Yet, glycolate growth stimulation could not be detected when high levels of CO_2 were available for fixation. This peculiar form of photoheterotrophy is like that found with glycolate in other green algae (23).

The level of glycolate required for optimal uptake in *C. reinhardtii* (Fig. 2) is approximately one-third of the 20 mM level observed by Lord and Merrett with *Chlorella* (15). Algal cells have been observed to take up more glycolate in synchronous culture just after cell division (9, 18). The glycolate affinity of *C. reinhardtii* at this stage of the life cycle is observed here to be higher without a significant increase in the rate of uptake. The highest concentration of glycolate observed in natural waters is approximately 1 μM (10). Therefore, affinity of these cells for glycolate is still at least several orders of magnitude too low to support growth in natural environments. Although subtle effects of low levels of glycolate, such as seen by Pant and Fogg (19) in the diatom *Skeletonema*, have not been eliminated in the present study, it is apparent that *Chlamydomonas* has not adapted for natural use of external glycolate. The strain of *C. reinhardtii* used here has been

Table III. *The Effects of Changes in Glycolate Dehydrogenase Activity with Growth Conditions*

Glycolate dehydrogenase is assayed in a 1% Triton X-100 crude cell extract as the reduction of DCPIP. Glycolate incorporation is determined over 2 to 6 h at pH 6.8 as in Table I. Glycolate excretion is release of acid stable $^{14}CO_2$ products, assumed to be fully labeled glycolate for calculation of rates.

Strain and Conditions	Glycolate Dehydrogenase	Glycolate Uptake	Glycolate Excretion
		μmol/mg Chl·h	
Wild type			
MM (air)	1.6	8.5	0.82
MM (2.5% CO_2)	0.32	3.5	8.7
MG	1.7	11.	1.1
MA	0.36	8.0	0.55
MA (2.5% CO_2)	0.18	ND[a]	3.4
F-60			
MA	1.3	3.3	
MA (2.5% CO_2)	0.14	2.8	

[a] Not determined.

in laboratory culture for more than 30 years. The effect of such isolation on response to an environmental parameter remains a question. Table III indicates that growth dependence upon glycolate does not enhance glycolate uptake substantially. A 50-fold higher affinity for acetate demonstrates the potential for efficient organic acid incorporation by these cells.

The remaining experiments were designed to determine whether or not *C. reinhardtii* is adapted for utilizing large amounts of internally produced glycolate. Characterization of excretion or external glycolate incorporation under different conditions gives an indication of whether glycolate metabolism is actually quite limited or if other factors are just limiting external usage in these whole cell experiments. The data here deal with three such limitations of external usage: (a) lack of ATP for active transport when photosynthesis is limited; (b) insufficient levels of glycolate dehydrogenase for metabolism; (c) exclusion of external glycolate by a high concentration of internally produced substrate. These last two would be especially likely limitations in cells adapted to grow on high (2.5%) CO_2.

Photosynthesis is likely to affect glycolate transport as well as its formation. Findenegg's study (9) of glycolate permeability indicates that an energy source for transport is in fact required at high pH in *Scenedesmus*. It is probably needed when a neutral pH is used here. At pH 3.8, the pK_a of glycolate, this acid is toxic in both the light and dark at a concentration of 10 mM (20). The toxicity of acetate and other organic acids at low pH values has frequently been observed in the algae and has been attributed to the acidification of the cytoplasm resulting from free permeability of the undissociated acid (8). Table I shows that such free permeability is not sufficient to permit rapid utilization of glycolate by *Chlamydomonas* at pH 3.8.

The F-60 mutant is severely restricted in its ability to form glycolate so the level of external incorporation should be a good estimate of heterotrophic glycolate utilization if permeability is not limiting. Corresponding to the absence of growth stimulation by glycolate in F-60, the incorporation rates are low in this mutant (Table I). Phosphorylating capacity for glycolate transport is not lacking. Grossman (11) has demonstrated the dependence of acetate incorporation upon the phosphorylating capacity of the *Chlamydomonas* cell, and F-60 has enough phosphorylating capacity to support high rates of acetate uptake (Table I). Inasmuch as there is no other apparent barrier to glycolate utilization in F-60, it seems likely that the actual glycolate turnover rate is limited. Because the glycolate pathway of higher plants does not require another photosynthetic product this result indicates a significant difference of the glycolate pathway in *Chlamydomonas*.

The partial blockage of incorporation by the inhibitor INH may indicate at least the partial presence of the glycolate pathway. Most results with INH have been interpreted as resulting from an inhibition of the aminotransferase mediating the glycine to serine conversion (23). One study (25) suggested that this reagent was promoting glycolate formation directly in *Chlorella*. It is possible that such increased formation rather than decreased metabolism is causing the results observed in Table II. More extensive experimentation with F-60 which should always lack glycolate formation could indicate the primary effect of this inhibitor.

One explanation of the requirement for full photosynthetic competence for glycolate assimilation would be that much glycolate carbon is released as CO_2 and then refixed. Product analysis experiments have failed to indicate any recent fixation products from glycolate, but the low affinity for glycolate makes good chromatographic resolution of products difficult (20). The direct effects of CO_2 on glycolate utilization are complex. In addition to the possible role of refixation, CO_2 must be limiting for optimal formation of glycolate and for optimal permeability as described for *Scenedesmus* (9).

The enzyme believed to be limiting glycolate turnover was glycolate dehydrogenase. As noted by Nelson and Tolbert (17) in *Chlamydomonas*, the lowering of the activity of this enzyme as measured by dye reduction is quite marked following growth on high CO_2 (Table III). In MM the low enzyme activity of high CO_2-grown cells is accompanied by low incorporation rates and high excretion levels. These same correlations cannot be seen when low glycolate dehydrogenase activity is found following mixotrophic growth in wild type or high CO_2 growth in F-60. In other studies excretion and glycolate dehydrogenase have also been shown not to correlate in *Euglena* (6) and *Chlorella* (7). In the present study the fact that measured glycolate dehydrogenase rates frequently are so low compared with incorporation rates indicates that the assay for this enzyme needs to be examined.

There is no direct evidence that glycolate metabolism is limited in CO_2-grown cells. The limitation of the utilization of external glycolate (Table III) could be a result of exclusion by increased glycolate formation. A similar inhibition with CO_2-grown *Scenedesmus* cells has been observed (at pH 5.7) in the dark or in the presence of high (5% CO_2) (9). These latter results suggested that the permeability of glycolate was altered by such growth conditions.

The data from all attempts to optimize external glycolate uptake suggest that the total capacity for glycolate metabolism is low, not more than 10% of the maximum photosynthetic rate. Higher plants have been observed to process as much as 50% of their photosynthate through the glycolate pathway (12). Measurements of excretion (Table III) indicate that when a large amount of glycolate is formed by *C. reinhardtii*, much of it is lost to the cell. To prevent loss of fixed carbon, the CO_2 concentrating mechanism and the resulting limitation of glycolate formation must be of primary importance to this species. Elucidating the mechanism of the CO_2-concentrating mechanism and establishing the *in situ* importance of the inducible nature of this response would greatly add to our understanding of this versatile green unicell.

Acknowledgments—The authors wish to thank Ms. Maureen Fisher for expert technical assistance and Dr. Stephen Lien for constructive discussion.

LITERATURE CITED

1. ARNON DI 1949 Copper enzymes in isolated chloroplasts. Plant Physiol 24: 1–15
2. BADGER MR, A KAPLAN, JA BERRY 1980 Internal inorganic carbon pool of *Chlamydomonas reinhardtii*. Plant Physiol 66: 407–413
3. BIDWELL RGS 1977 Photosynthesis and light and dark respiration in freshwater algae. Can J Bot 55: 809–818
4. CALKINS VP 1943 Microdetermination of glycolic and lactic acids. Ind Eng Chem Anal Ed 15: 762–763
5. CHENG KH, B COLMAN 1974 Measurements of photorespiration in some microscopic algae. Planta 115: 207–212
6. CODD GA, MJ MERRETT 1971 The regulation of glycolate metabolism in division synchronized cultures of *Euglena*. Plant Physiol 47: 640–643
7. COLMAN B, AG MILLER, B GRODZINSKI 1974 A study of the control of glycolate excretion in *Chlorella*. Plant Physiol 53: 395–397
8. DANFORTH WF 1962 Substrate assimilation and heterotrophy. *In* RA Lewin, ed, Physiology and Biochemistry of Algae. Academic Press, New York, pp 99–123
9. FINEDNEGG GR 1977 Interaction of glycolate-, HCO_3^--, Cl^--, and H^+-balance of *Scenedesmus obliquus*. Planta 135: 33–38
10. FOGG GE, DJ EAGLE, ME KINSON 1969 The occurrence of glycolic acid in natural waters. Verh Int Verin Limnol 17: 480–484
11. GROSSMAN AR 1978 Characterization of photosynthetic mutants and the ATPase activity in *Chlamydomonas reinhardi*. PhD thesis. Indiana University, Bloomington
12. JACKSON WA, RJ VOLK 1970 Photorespiration. Annu Rev Plant Physiol 21: 385–431
13. LLOYD NDH, DT CANVIN, DA CULVER 1977 Photosynthesis and photorespiration in algae. Plant Physiol 59: 936–940
14. LORD JM 1972 Glycolate oxidoreductase in *Escherichia coli*. Biochim Biophys Acta 267: 227–237
15. LORD JM, MJ MERRETT 1971 The growth of *Chlorella pyrenoidosa* on glycollate. J Exp Bot 22: 60–69
16. MOLL B, RP LEVINE 1970 Characterization of a photosynthetic mutant strain of *Chlamydomonas reinhardi* deficient in phosphoribulokinase activity. Plant Physiol 46: 576–580
17. NELSON EB, NE TOLBERT 1969 The regulation of glycolate metabolism in *Chlamydomonas reinhardtii*. Biochim Biophys Acta 184: 263–270
18. NELSON EB, NE TOLBERT, JL HESS 1969 Glycolate stimulation of oxygen

evolution during photosynthesis. Plant Physiol 44: 55–59
19. PANT A, GE FOGG 1976 Uptake of glycollate by *Skeletonema costatum* (Greve.) Cleve in bacterized culture. J Exp Mar Biol 22: 227–234
20. SPENCER KG 1978 Control of glycolate metabolism and photorespiration as a growth response in *Chlamydomonas reinhardtii*. PhD thesis. Indiana University, Bloomington
21. SUEOKA N 1960 Mitotic replication of deoxyribonucleic acid in *Chlamydomonas*. Proc Natl Acad Sci USA 46: 83–87
22. SURZYCKI S 1972 Synchronously grown cultures of *Chlamydomonas reinhardi*. Methods Enzymol 24: 67–73
23. TOLBERT NE 1974 Photorespiration. *In* WDP Stewart, ed, Algal Physiology and Biochemistry. University of California Press, Berkeley, pp 474–504
24. TOLBERT NE, LP ZILL 1956 Excretion of glycolic acid by algae during photosynthesis. J Biol Chem 222: 895–906
25. VENNESLAND B, JETSCHMANN 1976 The effect of cyanide and some other carbonyl binding reagents on glycolate excretion by *Chlorella vulgaris*. Planta 128: 81–84.