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Effect of Glycidate on Glycolate Formation and Photosynthesis in Isolated Spinach Chloroplasts[1]

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ABSTRACT

Glycidate (2,3-epoxypropionate) increased CO_2 photoassimilation in intact spinach (*Spinacia oleracea L.*) chloroplasts in the presence of various inhibitors of photosynthesis, including O_2, arsenite, azide, iodoacetamide, and carbonylcyanide 3-chlorophenylhydrazone. Although the mechanism by which glycidate enhances photosynthesis is obscure, the stimulatory effect cannot be ascribed to either an inhibition of glycolate formation, a specific interaction with the O_2 inhibition of photosynthesis, or a direct effect on the ribulose 1,5-bisphosphate carboxylase (EC 4.1.1.39) reaction. The lack of a differential effect of glycidate on photosynthesis and glycolate formation in the isolated chloroplast was confirmed in whole leaf studies by the CO_2 compensation concentration assay. These results are at variance with the report that glycidate stimulates net photosynthesis in tobacco leaf disks by irreversibly inhibiting glycolate formation and thus photorespiration (Zelitch, I., 1974, Arch. Biochem. Biophys. 163: 367-377).

Zelitch (21) has reported that potassium glycidate (2,3-epoxypropionate) irreversibly inhibits the synthesis of glycolate in illuminated tobacco (*Nicotiana tabacum* L.) leaf disks. Under conditions where glycolate formation and photorespiration (measured by the light-dark ^{14}C assay) were inhibited by 40 to 50%, net photosynthetic $^{14}CO_2$ uptake was similarly increased. It was concluded that glycidate is a specific and irreversible inhibitor of glycolate formation during photosynthesis in tobacco leaf disks. More recently, Zelitch (23) has extended these findings by determining the effect of this epoxide on the activity of Ru-P_2[2] carboxylase. These studies have shown that the *in vitro* activity of Ru-P_2 carboxylase extracted from glycidate-treated leaf tissue is not altered, nor is there any effect of the compound on the O_2 inhibition of carboxylase activity. Additionally, [1-^{14}C]glycidate was shown not to associate specifically with Ru-P_2 carboxylase *in vivo*. Based on these two lines of experimentation, Zelitch has reasoned that a large portion of the biosynthetic pathway for glycolate synthesis must occur by reactions other than the proposed Ru-P_2 oxygenase mechanism (23).

Although there is considerable controversy over the mechanism of glycolate formation during photosynthesis, there is little doubt that glycolate biosynthesis occurs in the chloroplast (5, 9, 19, 22). Since the plastid has a limited capacity for metabolizing glycolate (3, 13, 18), this metabolite may be regarded as an end product of chloroplast photosynthetic carbon metabolism. Therefore the isolated plastid provides a convenient and relatively simple system for studying glycolate formation during photosynthesis. This paper describes the effects of sodium glycidate on photosynthetic glycolate formation and $^{14}CO_2$ fixation in isolated spinach chloroplasts.

MATERIALS AND METHODS

Glycidate Synthesis. Sodium glycidate hemihydrate ($C_3H_3O_3NA \cdot 1/2H_2O$) was synthesized from redistilled glycidaldehyde (Aldrich Chemical Co.) and recrystallized following Procedure *A* of Payne and Van Ess (15). The nuclear magnetic resonance and IR absorption spectra of the recrystallized salt were consistent with the above structure. Stock solutions of glycidate were prepared in 33 mM HEPES (pH 7.8) just before use.

Preparation of Chloroplasts. Chloroplasts were isolated from leaves of spinach (*Spinacia oleracea* L., var. Virginia Blight Resistant) grown in a growth chamber at 21 C with a 10-hr day (2100 ft-c) and 16 C night. The light-dark cycle in the chamber was adjusted so that the plants received about 30 min of illumination before chloroplast isolation. Chloroplasts were prepared essentially as described by Jensen (12) with some modifications. The rinsed leaves, about 10 g after deribbing, were chopped gently at 4 C with razor blades in 45 ml of a grinding medium containing 330 mM sorbitol, 50 mM MES, 2 mM Na_2EDTA, 1 mM $MgCl_2$, 1 mM $MnCl_2$, 0.5 mM K_2HPO_4 (entire solution adjusted with NaOH to pH 6.5 at 4 C) plus 5 mM freshly prepared isoascorbate. The slurry was squeezed through two layers of Miracloth and centrifuged for 50 sec at 2000*g*. The chloroplast pellet was resuspended and stored in the dark at 4 C in the sorbitol-salt solution described above except that 50 mM HEPES (pH 6.7) replaced MES and the isoascorbate concentration was reduced to 2.5 mM. Chl was determined by the method of Arnon (1), and the chloroplast preparations contained 110 to 650 μg Chl/ml.

Photosynthetic $^{14}CO_2$ Assimilation. Photosynthetic CO_2 fixation by the isolated chloroplasts was determined by $H^{14}CO_3^-$ incorporation at 25 C and 3700 ft-c (74 nanoeinsteins/cm^2·sec, 400–700 nm) under various He/O_2 atmospheres. Unless noted otherwise, the reaction vessels contained chloroplasts (5–32 μg of Chl), resuspension medium adjusted to pH 7.8, 5 mM freshly prepared $Na_4P_2O_7$, ±3 mM glycidate, and 1 mM $NaH^{14}CO_3$ (2–4 Ci/mole) in a final volume of 1 ml. Vessels containing all the reaction medium components except chloroplasts and ^{14}C-bicarbonate were sealed and repeatedly evacuated and refilled with the appropriate He/O_2 mixture and chilled, after which the chloroplasts were introduced with a 20-gauge syringe needle. Following a 4-min preillumination without shaking in the photosynthetic Warburg bath, the reactions were initiated by injecting $NaH^{14}CO_3$ and terminated at varying times by injecting 0.1 ml of 6 N acetic acid. Contents of the flasks were thoroughly mixed, aliquots dried at 25 C under a stream of N_2, and dpm

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[2] Abbreviations: Ru-P_2: ribulose 1,5-bisphosphate; CCCP: carbonylcyanide 3-chlorophenylhydrazone.

determined by liquid scintillation spectroscopy. All experimental values were corrected for zero time controls. For the determination of ^{14}C-glycolate, the reactions were performed at 21% O_2 for 8 min; contents of the flasks were vigorously vortexed, centrifuged, and the acidified supernatant was bubbled with N_2 for 10 min. ^{14}C-glycolate was separated by one-dimensional descending paper chromatography in *n*-pentyl alcohol/5 N formic acid (1:1, upper), identified, eluted, and counted as previously described (4).

Glycolate Formation. Photosynthetic glycolate formation by the isolated chloroplasts at 21% O_2 was assayed colorimetrically as described below. The assay conditions were identical to those described above for $^{14}CO_2$ assimilation except that 1 mM $NaHCO_3$ replaced ^{14}C-bicarbonate and the reactions were terminated after 10 min. The contents of five replicate flasks were pooled, neutralized, vigorously vortexed, centrifuged for 15 min at 30,000*g*, and the pelleted chloroplast fragments washed twice by centrifugation with water. The combined supernatant was fractionated on a column (1 × 7.5 cm [about 6 ml]) of Dowex 1-acetate (X8, 200–400 mesh). The column was washed with 24 ml of H_2O and 24 ml of 1 N acetic acid, and the glycolate fraction eluted with 30 ml of 5 N acetic acid. The eluant was taken to dryness at 30 C under reduced pressure; residual acetic acid vapors were removed under a stream of N_2, and the residue was redissolved in 1 ml of 35 mM tris-HCl, pH 8. The glycolate content in 0.1- to 0.2-ml aliquots was determined by enzymic conversion to glyoxylate in the presence of glycolate oxidase (EC 1.1.3.1) and catalase (EC 1.11.1.6); then glyoxylate phenylhydrazone was formed and converted to the 1,5-diphenylformazan derivative as described by Vogels and Van Der Drift (20), and the absorbance at 535 nm was determined. Full details of this procedure are available elsewhere (14). Glycolate produced in the illuminated systems, ± glycidate, was always determined by comparing these samples against samples from identical systems maintained in the dark. The recovery of unlabeled glycolate and [1-^{14}C]glycolate standards was 79% ± 5%, and the per cent recovery was similar in both the presence and absence of glycidate. Glycidate did not interfere with the fractionation of glycolate or the enzymic-colorimetric assay for glycolate, nor were any nonphosphorylated organic acids, other than glycolate, detected as products of spinach chloroplast $^{14}CO_2$ assimilation at 21% O_2 and 1 mM $NaH^{14}CO_3$. In contrast to the modified Calkins assay employed by Robinson and Gibbs (16) in their study of glycolate formation in isolated chloroplasts, the method for glycolate quantitation described above did not require that isoascorbate and EDTA be omitted from the isolation and reaction media, thus enabling higher rates of CO_2 assimilation to be achieved.

CO_2 Compensation Concentration Determinations. Mature cotton (*Gossypium hirsutum* L.) leaves were excised at the base of the petiole and individually placed in bags (20 × 45 cm) of Mylar (Vac Pac Inc.) containing 20 ml of a 0.2% (v/v) Tween 20 solution, ±sodium glycidate. The bags were inflated with air or 2% O_2 in 300 μl CO_2/l and sealed, and the excised leaf was thoroughly wetted with the solution. The inflated bags were placed in a growth room at 1400 ft-c and 25 C for 4 hr (positioned so that the leaf petioles were immersed in the Tween solution), after which the CO_2 concentration in the sealed bag was determined with a Beckman 215A CO_2 analyzer essentially as described by Goldsworthy and Day (10).

RESULTS AND DISCUSSION

The effect of glycidate on the time course of chloroplast CO_2 fixation is depicted in Figure 1. Each set of progress curves represents the results from experiments with different chloroplast preparations. In the absence of O_2, glycidate had little or no effect on either the kinetics or maximal rates of CO_2 assimilation. As the O_2 tension increased, glycidate caused a slight reduction of the lag phase of chloroplast photosynthesis. More noteworthy, with increasing O_2 concentration, glycidate increasingly stimulated the linear rate of CO_2 fixation in the isolated chloroplasts. Based on the results of numerous experiments, the epoxide increased the maximal rates of CO_2 uptake by 20 to 50% at 21% O_2 and 1.2- to 1.6-fold at 47% O_2 (Figs. 1, 2; Tables I, II). Glycidate stimulation of plastid photosynthesis in 21% O_2 was essentially saturated at 3 mM, with half-maximal stimulation occurring at less than 0.5 mM (Fig. 2). These results are consistent with the leaf disk studies of Zelitch (21) in which glycidate treatment stimulated net photosynthetic $^{14}CO_2$ uptake at 21% O_2.

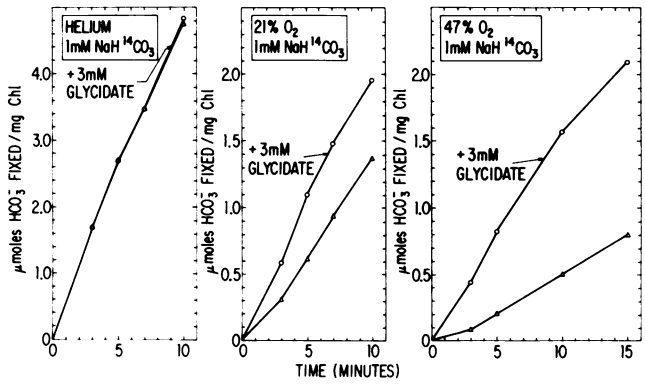

FIG. 1. Effect of glycidate on the time course of spinach chloroplast $^{14}CO_2$ photoassimilation. Following a 4-min preillumination, $^{14}CO_2$ fixation was assayed at 1 mM $NaHCO_3$, 3700 ft-c, and several O_2 tensions for varying times. Final reaction volumes were 1 ml and these contained 5–7 μg of Chl. Each of the three sets of progress curves represents the results from experiments with different chloroplast preparations. Maximum control (minus glycidate) rates of $^{14}CO_2$ uptake were (in μmoles/mg Chl·hr): 0% O_2(He), 32.5; 21% O_2, 9.0; 47% O_2, 3.6.

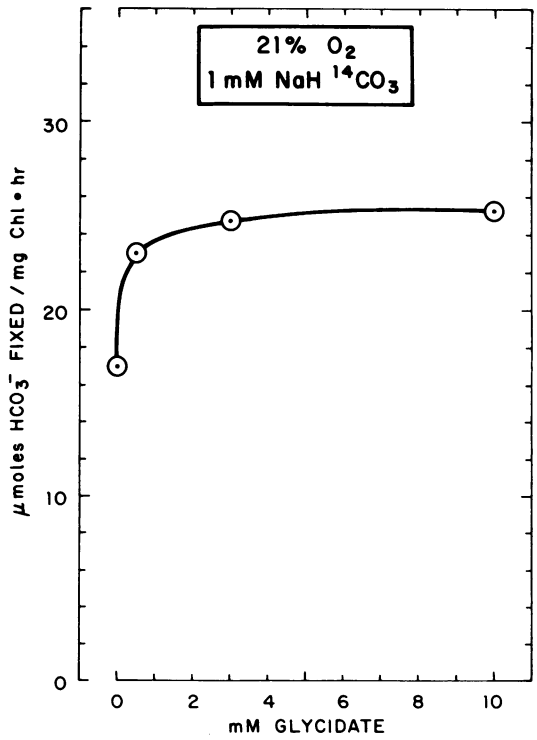

FIG. 2. Effect of glycidate concentration on spinach chloroplast $^{14}CO_2$ fixation. Following a 4-min preillumination, $^{14}CO_2$ photoassimilation was assayed at 1 mM $NaHCO_3$, 21% O_2, and 3700 ft-c for 8 min. Final reaction volumes were 1 ml and these contained 13.0 μg of Chl.

From the results presented in Figure 1, it appears that glycidate and O_2 interact to stimulate photosynthesis in the isolated chloroplast. The glycidate effect at 21% O_2 was essentially abolished in the absence of O_2 and greatly enhanced at elevated O_2. It is well documented that O_2 reversibly inhibits photosynthetic CO_2 assimilation and O_2 evolution in a variety of plant systems, including intact leaves, algae, leaf cells, and isolated chloroplasts (5, 9, 19). Although the mechanism of the inhibition of photosynthesis by O_2 is presently a controversial topic, there is little doubt that the site of O_2 interaction is the photosynthetic carbon reduction cycle (4, 7, 8). It may well be that glycidate stimulated the linear rates of chloroplast CO_2 fixation in the presence of O_2 by relieving the O_2 inhibition of photosynthesis. The results presented in Table I, obtained from experiments with the same plastid preparation, indicate that the epoxide partially relieves the Warburg effect in the isolated chloroplast. Since O_2 has been shown to inhibit reversibly Ru-P_2 carboxylase activity (5), a direct effect of glycidate on the carboxylase reaction was examined using the crystalline tobacco enzyme (6). Under the standard assay conditions (6), a 10-min preincubation of the enzyme with 3 mM glycidate and 1 mM $NaH^{14}CO_3$ had no effect on either the rate or the O_2 sensitivity of the carboxylase reaction (data not shown).

As discussed in the introductory remarks, Zelitch has attributed the glycidate stimulation of net photosynthesis in tobacco leaf disks to an irreversible inhibition of glycolate formation and thus decreased photorespiration (21, 23). Oxygen inhibition of photosynthesis consists of two components, (*a*) a direct inhibition of photosynthesis by O_2, and (*b*) and O_2-stimulated formation of glycolate and the subsequent photorespiratory oxidation of glycolate in part to CO_2 (5). Since, in the isolated chloroplast, glycidate reduced the total O_2 inhibition of photosynthesis (Table I), it was possible that the epoxide stimulated CO_2 fixation in the presence of O_2 by decreasing one or both components of the Warburg effect. Direct measurement of glycolate formation during photosynthesis in the isolated chloroplast indicated that glycidate actually stimulated the rate of glycolate biosynthesis (Table II). The increased rate of glycolate formation in the presence of the epoxide appeared to be a function of the enhanced overall photosynthetic activity of the chloroplasts in that the ratio of glycolate formation to $^{14}CO_2$ fixation remained constant, as did the percentage of total fixed carbon entering the glycolate pool (Table II). If, indeed, glycidate did stimulate photosynthesis by inhibiting glycolate formation in the chloroplast, one would expect a marked decline in the rate of glycolate synthesis, the percentage of total fixed carbon entering glycolate, and the ratio of glycolate produced to total CO_2 assimilated, as has been reported by Robinson and Gibbs (16) for high levels of CO_2. The lack of a differential effect of glycidate on photosynthesis and glycolate formation in the isolated chloroplast (Table II) was confirmed in whole leaf studies by the CO_2 compensation concentration assay. Treatment of mature cotton leaves with 5 and 10 mM glycidate caused no reduction of the CO_2 compensation concentration (56 μl/l), whereas a 20 mM solution increased the compensation point to 75 μl/l. In contrast to the glycidate treatments, low O_2 reduced the CO_2 compensation point to 5 μl/l due to increased photosynthesis and decreased photorespiration.

A general trend noted during this chloroplast study was that the magnitude of the glycidate stimulation of photosynthesis appeared to be correlated inversely with the photosynthetic activity of the plastid preparation. In our initial chloroplast experiments the linear rates of $^{14}CO_2$ photoassimilation were considerably lower than those reported in this paper and these relatively low rates were greatly enhanced by glycidate addition, irrespective of the O_2 tension. For example, during our preliminary studies the rate of $^{14}CO_2$ fixation at 0% O_2 was about 7 μmoles/mg Chl·hr and this activity was stimulated 70 to 80% in the presence of the epoxide (compare with data in Fig. 1 and Tables I and III). However, even in these initial experiments, glycidate had no effect on the percentage of ^{14}C incorporated into glycolate at 21% O_2. It was thus reasoned that the apparent interaction between O_2 and the glycidate stimulation of photosynthesis noted in Figure 1 and Table I was perhaps due to the reduced rates of CO_2 fixation caused by O_2 rather than to a specific effect of the epoxide on the O_2 inhibition of photosynthesis. This hypothesis was substantiated by the observation that glycidate enhanced the rate of chloroplast $^{14}CO_2$ uptake at 0% O_2 by up to 1.9-fold in the presence of certain inhibitors of photosynthesis (Table III), including uncouplers of photophosphorylation (CCCP; ref. 11) and inhibitors of the carbon reduction cycle (arsenite, azide, iodoacetamide; refs. 2, 17). An exception to the general case of inhibition relief by the epoxide was the inhibition of photosynthetic CO_2 fixation brought about by DCMU (Table III). These results are similar to those obtained with certain carbon cycle intermediates (ribose 5-P, fructose 1,6-bisphosphate) which also partially restore CO_2 photoassimilation inhibited by uncouplers (17), chemical inhibitors of the carbon reduction cycle (17), or O_2 (7, 16) but not by inhibitors of photosynthetic electron transport (17). Whereas at least part of the stimulatory effect of the photosynthetic carbon reduction cycle intermediates is due to the direct introduction of carbon into the cycle (7, 16, 17), it seems unlikely that the synthetic epoxide enhances photosynthesis by this mechanism as there was no concomitant decrease in the percentage of ^{14}C incorporated into glycolate (*i.e.*, no apparent isotopic dilution of glycolate; compare with Figs. 2 and 4 in ref. 16).

Table I. *Effect of Glycidate on O_2 Inhibition of Spinach Chloroplast Photosynthesis*

Following a 4-min preillumination, $^{14}CO_2$ fixation was assayed at 1 mM $NaHCO_3$, 3700 ft-c, and several O_2 tensions for varying times. The final reaction volumes were 1 ml and these contained 5.5 μg of Chl.

Assay Conditions	Reaction Time Interval	μmoles HCO_3^- fixed/mg Chl·hr	O_2 Inhibition
	min		%
0% O_2 (He)	0–5	27.0	
0% O_2 (He) + 3 mM glycidate	0–5	28.3	
21% O_2	5–10	9.8	64
21% O_2 + 3 mM glycidate	3–7	12.0	58
47% O_2	5–10	2.9	89
47% O_2 + 3 mM glycidate	3–7	7.5	73

Table II. *Effect of Glycidate on Glycolate Formation and Photosynthesis in Isolated Spinach Chloroplasts*

Following a 4-min preillumination, glycolate formation and $^{14}CO_2$ fixation were assayed at 1 mM $NaHCO_3$, 21% O_2, and 3700 ft-c for varying times. The final reaction volumes were 1 ml and these contained 23.5 μg of Chl.

Parameter	Reaction Time Interval	Control	+3 mM Glycidate
	min		
Glycolate formation (μmoles/mg Chl·hr)	0–10	3.97	4.86
$^{14}CO_2$ fixation (μmoles/mg Chl·hr)	3–7		14.4
	5–10	11.9	
^{14}C-glycolate (% of total ^{14}C fixed)	0–8	17.5	17.2
Rate of glycolate formation/rate of $^{14}CO_2$ fixation		0.33	0.34

Table III. *Effect of Glycidate on Inhibition of Spinach Chloroplast CO_2 Fixation by Various Photosynthesis Inhibitors*

Following a 4-min preillumination, $^{14}CO_2$ fixation was assayed at 1 mM $NaHCO_3$, 0% O_2 (He), and 3700 ft-c for 5 min. The final reaction volumes were 1 ml and these contained 27.9 μg of Chl. Rates of fixation in μmoles/mg Chl·hr were: control, 36.1; + glycidate, 36.7.

Changes in Reaction Medium	Rate of CO_2 Fixation	
	Control	+3 mM glycidate
	%	
None	100	100
+CCCP[1]		
0.5 μM	03	09
0.2 μM	25	40
+Iodoacetamide		
100 μM	06	16
+NaN_3		
10 μM	16	31
+$NaAsO_2$		
250 μM	16	39
150 μM	36	57
75 μM	54	73
+DCMU[1]		
0.25 μM	31	30

[1] Final ethyl alcohol concentration in reaction medium was 0.5% (v/v).

CONCLUSIONS

The mechanism by which glycidate stimulates photosynthesis in isolated chloroplasts is obscure. From the data presented in this paper, it appears that the glycidate stimulation of CO_2 photoassimilation is not site specific, but rather related to the ability of the plastid to carry out photosynthesis. The glycidate enhancement of photosynthesis in the presence of O_2 cannot be ascribed to an inhibition of glycolate formation, a specific interaction with the Warburg effect, or a direct effect on the Ru-P_2 carboxylase reaction. In marked contrast to Zelitch's leaf disk study (21), glycidate actually stimulated the rate of glycolate formation in the isolated chloroplast as a result of the enhanced overall photosynthetic activity. The reason for this discrepancy between the leaf disk and chloroplast data is not evident, although several possible explanations can be put forward. First, the two-stage assay for glycolate formation employed in the leaf disk study requires that the nonspecific inhibitor of glycolate oxidase, α-hydroxy-2-pyridinemethanesulfonate, be present to block the subsequent metabolism of glycolate by the photorespiratory pathway (21). Second, it is possible that glycidate is converted to an unknown compound outside the plastid and it is this compound which is the true inhibitor of glycolate formation in the chloroplast. Alternatively, the epoxide may inhibit an undefined extra-chloroplastic pathway of glycolate biosynthesis. However, concentrations of glycidate up to 20 mM failed to reduce the CO_2 compensation point of excised cotton leaves.

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LITERATURE CITED

1. Arnon, D. I. 1949. Copper enzymes in chloroplasts. Polyphenoloxidases in *Beta vulgaris*. Plant Physiol. 24: 1–15.
2. Avron, M. and M. Gibbs. 1974. Carbon dioxide fixation in the light and in the dark by isolated spinach chloroplasts. Plant Physiol. 53: 140–143.
3. Chan, H. W.-S. and J. A. Bassham. 1967. Metabolism of ^{14}C-labeled glycolic acid by isolated spinach chloroplasts. Biochim. Biophys. Acta 141: 426–429.
4. Chollet, R. 1974. $^{14}CO_2$ fixation and glycolate metabolism in the dark in isolated maize (*Zea mays* L.) bundle sheath strands. Arch. Biochem. Biophys. 163: 521–529.
5. Chollet, R. and W. L. Ogren. 1975. Regulation of photorespiration in C_3 and C_4 species. Bot. Rev. 41: 137–179.
6. Chollet, R., L. L. Anderson, and L. C. Hovsepian. 1975. The absence of tightly bound copper, iron, and flavin nucleotide in crystalline ribulose 1,5-bisphosphate carboxylase-oxygenase from tobacco. Biochem. Biophys. Res. Commun. 64: 97–107.
7. Ellyard, P. W. and M. Gibbs. 1969. Inhibition of photosynthesis by oxygen in isolated spinach chloroplasts. Plant Physiol. 44: 1115–1121.
8. Ellyard, P. W. and A. San Pietro. 1969. The Warburg effect in a chloroplast-free preparation from *Euglena gracilis*. Plant Physiol. 44: 1679–1683.
9. Gibbs, M. 1971. Carbohydrate metabolism by chloroplasts. *In:* M. Gibbs, ed., Structure and Function of Chloroplasts. Springer-Verlag, New York. pp. 169–214.
10. Goldsworthy, A. and P. R. Day. 1970. A simple technique for the rapid determination of plant CO_2 compensation points. Plant Physiol. 46: 850–851.
11. Izawa, S. and N. E. Good. 1972. Inhibition of photosynthetic electron transport and photophosphorylation. Methods Enzymol. 24B: 355–377.
12. Jensen, R. G. 1971. Activation of CO_2 fixation in isolated spinach chloroplasts. Biochim. Biophys. Acta 234: 360–370.
13. Kisaki, T. and N. E. Tolbert. 1969. Glycolate and glyoxylate metabolism by isolated peroxisomes or chloroplasts. Plant Physiol. 44: 242–250.
14. Laing, W. A. 1974. Physiological and biochemical properties of soybean ribulose diphosphate carboxylase. Ph.D. thesis. University of Illinois, Urbana.
15. Payne, G. B. and P. R. Van Ess. 1961. Oxidation of glycidaldehyde by alkaline hydrogen peroxide. J. Org. Chem. 26: 2984–2985.
16. Robinson, J. M. and M. Gibbs. 1974. Photosynthetic intermediates, the Warburg effect, and glycolate synthesis in isolated spinach chloroplasts. Plant Physiol. 53: 790–797.
17. Schacter, B., J. H. Eley, and M. Gibbs. 1971. Involvement of photosynthetic carbon reduction cycle intermediates in CO_2 fixation and O_2 evolution by isolated chloroplasts. Plant Physiol. 48: 707–711.
18. Thompson, C. M. and C. P. Whittingham. 1968. Glycolate metabolism in photosynthesizing tissue. Biochim. Biophys. Acta 153: 260–269.
19. Tolbert, N. E. 1973. Glycolate biosynthesis. *In:* B. L. Horecker and E. R. Stadtman, eds., Current Topics in Cellular Regulation, Vol. 7. Academic Press, New York. pp. 21–50.
20. Vogels, G. D. and C. Van Der Drift. 1970. Differential analyses of glyoxylate derivatives. Anal. Biochem. 33: 143–157.
21. Zelitch, I. 1974. The effect of glycidate, an inhibitor of glycolate synthesis, on photorespiration and net photosynthesis. Arch. Biochem. Biophys. 163: 367–377.
22. Zelitch, I. 1975. Pathways of carbon fixation in green plants. Annu. Rev. Biochem. 44: 123–145.
23. Zelitch, I. 1976. Biochemical and genetic control of photorespiration. *In:* C. C. Black and R. H. Burris, eds., CO_2 Metabolism and Productivity of Plants. University Park Press, Baltimore. In press.