7. D. W. Schindler, *Science* **195**, 260 (1977).
8. The categorization is based on present understanding of phosphorus inputs to the Great Lakes. For example, industrial sources are not included since they generally contribute minor amounts of phosphorus, and measurements on the Great Lakes support this assumption. As an example, Appendix B of (*4*) reports that for 1975 direct industrial sources amounted to less than 5 percent of the direct point sources of total phosphorus to the Great Lakes.
9. Loss of phosphorus in streamflow is presently not well understood and is currently the subject of research in the Great Lakes Basin. It has been suggested [D. B. Baker and J. W. Kramer, *Proc. 16th Conf. Great Lakes Res.* (1973), p. 858] that a sizable fraction of upstream point sources could be lost during streamflow by incorporation into the sediments of the stream or its impoundments. Based on a preliminary analysis of runoff data, we assume that 75 percent of point source phosphorus originating in counties that are not contiguous with a Great Lake or their connecting channels is lost in streamflow. No losses are assumed for on-lake counties. The effect of this assumption on our predictions for western Lake Erie and lower Green Bay is minor, since most of the population lives in close proximity to these water bodies. The effect on Saginaw Bay is more substantial, because the cities of Flint and Saginaw lie in off-lake counties. It should be noted, however, that even if all point sources were assumed to reach Saginaw Bay, land runoff would be still greater for that basin.
10. Areal loading factors for the respective land uses are based on P. D. Uttormark, J. D. Chapin, and K. M. Green [*U.S. Environ. Prot. Agency Rep. EPA-660/3-74-020* (1974)].
11. In the original application (*5*), each of the lakes is treated as a completely mixed system with the exception of Lake Erie, which is divided into its three subbasins. The present model has been extended to treat Saginaw Bay and Green Bay explicitly. Since these bays have open boundaries, both advective and diffusive transport are modeled, with the latter being calibrated by using chloride and heat balances.
12. A general apparent settling velocity of 16 m/year is derived by S. C. Chapra [*Water Resour. Res.* **11**, 1033 (1975)]. This value works well for most of the Great Lakes with the exception of western and central Lake Erie and Green Bay, where the parameter is calibrated to existing data.
13. While total phosphorus concentration is a cause rather than an effect or symptom of eutrophication, it has been used as a measure of lake trophic state by a number of investigators. The present analysis uses values of 10 and 20 μg/liter as bounds for mesotrophy, as suggested by P. J. Dillon [*Limnol. Oceanogr.* **20**, 28 (1975)]. While Dillon's scheme is general, H. F. H. Dobson (personal communication) has used Great Lakes data to set the same bounds. It should be noted, however, that all such efforts are approximations to aid interpretation and that the boundaries are more realistically ranges rather than thresholds.
14. *Report to the International Joint Commission on the Pollution of Lake Erie, Lake Ontario, and the International Section of the St. Lawrence River* (International Joint Commission, Washington, D.C., and Ottawa, Ontario, 1969).
15. It should be noted that there are two shortcomings to the present approach. The first is that it does not consider the time that will be required for the predicted improvements to manifest themselves. A lake's response could be retarded by feedback from the sediments in cases where phosphorus levels have grown high owing to prolonged enrichment [see D. W. Schindler, in *Environmental Biogeochemistry*, J. Nriagu, Ed. (Ann Arbor Science, Ann Arbor, Mich., 1976), p. 647]. The effect is discussed in (*5*) with particular emphasis on Lake Erie. The second shortcoming, which is a problem of the phosphorus loading concept in general, is the assumption that phosphorus from all sources is equally available for algal growth. While it is unclear in terms of present understanding how this assumption affects our predictions, the question of phosphorus availability is presently the subject of much research and should be kept in mind when evaluating our results.
16. We thank the following for comments and discussions related to this study: R. Bowden, G. F. Lee, A. P. Pinsak, D. Rockwell, D. Scavia, S. Tarapchak, and N. Thomas. This report is GLERL Contribution No. 99.

10 March 1977

Increasing Photosynthesis by Inhibiting Photorespiration with Glyoxylate

Abstract. *Glyoxylate treatment doubles net photosynthetic carbon dioxide fixation by tobacco leaf disks because inhibition of glycolate synthesis by glyoxylate results in decreased photorespiration. These observations show that photorespiration can be metabolically regulated and suggest that genetic or chemical alteration of pool sizes of certain metabolites can produce plants with increased photosynthesis.*

In most crops at least half the CO_2 assimilated during photosynthesis is lost by photorespiration (*1*, *2*). This rapid loss of CO_2 diminishes net photosynthesis and yields (*3*). The synthesis and subsequent oxidation of glycolate (CH_2OHCOO^-) largely account for the production of photorespiratory CO_2 (*3*). Photorespiration is slow in the few photosynthetically efficient crops (*4*) such as maize, sorghum, sugarcane, and millet, presumably because the rate of glycolate synthesis is slow (*5*). Net photosynthesis is usually greater and crop yields are accordingly higher in these species (*6*).

Glycolate is synthesized early in photosynthesis (*7*) and is oxidized to glyoxylate ($CHOCOO^-$) and further metabolized by reactions in the glycolate pathway of carbohydrate metabolism (*8*). Photorespiratory CO_2 is released during one or more steps of the pathway (*9*). Blocking glycolate oxidation with α-hydroxysulfonates (*10*), or glycolate synthesis with epoxides such as glycidate (*11*), inhibits photorespiration in disks cut from leaves of the inefficient species tobacco and increases net photosynthesis at least 50 percent.

We have sought ways of regulating the glycolate pathway that slow glycolate synthesis in order to lay a biochemical foundation for genetically altering photorespiration (*12*). Recently, we found that glycolate synthesis is inhibited in tobacco leaf disks when they are floated on solutions of L-glutamate, L-aspartate, phosphoenolpyruvate, or glyoxylate (*13*, *14*). We describe here the inhibition of photorespiration and the doubling of net photosynthesis when leaf tissue is treated with glyoxylate, an intermediate of the glycolate pathway.

Disks floated on 5 to 25 m*M* potassium glyoxylate (*p*H 4.6) showed a 1.2- to 2.3-fold increase in their net rate of photosynthetic $^{14}CO_2$ fixation compared with

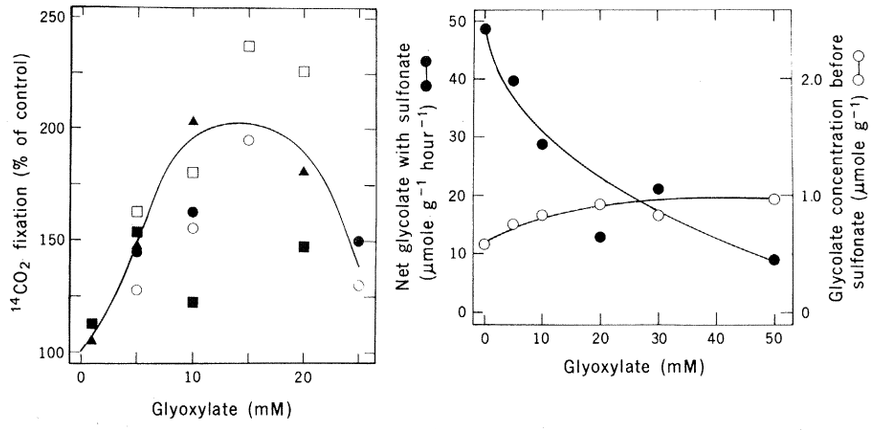

Fig. 1 (left). Effect of glyoxylate concentration on increase in net photosynthetic CO_2 fixation in tobacco disks. Control rates with water were arbitrarily taken to equal 100 percent. In each sample, six 1.6-cm-diameter disks from tobacco leaves (var. 'Havana Seed') whose leaf stomata opened well in the light (*21*) were strung together by a cotton thread and placed in a 75-ml Warburg flask containing 1.0 ml of the solution indicated. After 1 hour at 30°C with an irradiance of 325 μeinstein m^{-2} sec^{-1} (400 to 700 nm) a continuous stream of $^{14}CO_2$ (600 ppm) of known specific radioactivity was passed through the flask at 250 ml min^{-1} (*13*). After 5 minutes the disks were removed to boiling 20 percent ethanol and homogenized, and the amount of ^{14}C fixed was determined. The control rates [micromoles of $^{14}CO_2$ fixed per gram (fresh weight) per hour] were: (•) 60.3, (○) 52.4, (▲) 47.0, (■) 34.4, and (□) 38.0. Fig. 2 (right). Inhibition of glycolate synthesis in tobacco leaf disks floated on glyoxylate solution. In each sample, six 1.6-cm leaf disks were floated in 50-ml beakers on 1.5 ml of either water or the glyoxylate solutions for 1 hour with an irradiance of 390 μeinstein m^{-2} sec^{-1} at 30°C. The disks were then washed twice with 1.5 ml of water and plunged into boiling 20 percent ethanol either directly (○) or after the water had been replaced with 2.5 ml of 10 m*M* α-hydroxy-2-pyridinemethanesulfonic acid for 3 minutes (•). After the killed tissue was homogenized, the glycolate fraction was collected by ion exchange chromatography on Dowex-1 acetate (*5*) and assayed colorimetrically (*11*).

disks on water (Fig. 1). The stimulation was dependent on glyoxylate concentration, and at 15 m*M* glyoxylate was about 2.0-fold. For photosynthetic measurements a CO_2 concentration of 600 parts per million (ppm) was used because half-maximal rates of CO_2 fixation occurred at this concentration with leaf disks (*13*). Glyoxylate treatment could not have stimulated CO_2 fixation by supplying a substrate for some rate-limiting step, because the increase in net photosynthesis approached 50 μmole of CO_2 per gram (fresh weight) per hour (Fig. 1), while the rate of metabolism of added [^{14}C]glyoxylate under similar conditions was only 1.8 to 1.9 μmole g^{-1} $hour^{-1}$.

The increase in net photosynthesis caused by glyoxylate results from an inhibition of photorespiratory CO_2 release. Photorespiration was measured as the ratio of previously fixed $^{14}CO_2$ evolved into a stream of CO_2-free air in the light over that in the dark (*10*). The ratio was decreased from 4.4 for disks floated on water to 1.9 for disks on glyoxylate (10 m*M*, *p*H 4.6, for 60 minutes). Although the rate of $^{14}CO_2$ release in the dark remained unchanged throughout the treatment, the rate in the light was decreased by 57 percent. Thus the glyoxylate treatment inhibited only the light-dependent component of respiration (that is, photorespiration).

The inhibition of photorespiration by glyoxylate is associated with a decrease in glycolate synthesis. The synthesis was determined by measuring glycolate accumulation during a 3-minute exposure to α-hydroxy-2-pyridinemethanesulfonic acid, a potent inhibitor of glycolate oxidase in leaf disks (*2*). Floating disks in the light on glyoxylate solution for 1 hour without sulfonate causes a 40 to 60 percent increase in the low steady-state concentration of glycolate normally in the tissue (Fig. 2). This small increase in the glycolate pool, which may result from the rapid activities of reduced nicotinamide-adenine dinucleotide (or reduced nicotinamide-adenine dinucleotide phosphate) glyoxylate reductases (*5, 15*), was subtracted from the glycolate accumulation in the presence of sulfonate. The rate of glycolate accumulation in the presence of the sulfonate was inhibited 20 to 80 percent, depending on glyoxylate concentration (Fig. 2). At 15 m*M* glyoxylate, glycolate synthesis was inhibited about 50 percent [equivalent to at least 50 μmole of CO_2 fixed per gram (fresh weight) per hour].

The inhibition of glycolate synthesis in disks caused by 20 m*M* potassium glyoxylate (*p*H 4.6) can be reversed by replacing the glyoxylate solution with water. Inhibition is partially reversed after 1 hour and fully reversed after 2 hours. Floating disks for 1 hour on 20 m*M* glyoxylate increased the glyoxylate concentration in the tissue from 0.61 to 0.87 μmole g^{-1} (*16*). After the glyoxylate had been replaced by water for 1 hour the concentration fell to 0.66 μmole g^{-1}, and after 2 hours it was back to 0.59 μmole g^{-1}. The glyoxylate concentration in control disks floated on water throughout the experiment remained constant. Whereas these values are based on the glyoxylate contained in the whole tissue, glycolate synthesis and elevated glyoxylate concentration may be compartmentalized.

The reestablishment of rapid glycolate synthesis after the glyoxylate solution is replaced with water differs from the prolonged inhibition caused by glutamate, which is not reversed even 4 hours after glutamate is replaced with water (*13*). The differences in the reversibility of their inhibitory effects and in the magnitude of their increase in net photosynthesis suggest that glutamate and glyoxylate inhibit glycolate synthesis by different mechanisms. Because glyoxylate is the first product of glycolate metabolism, it may inhibit glycolate synthesis in vivo by a feedback mechanism early in the pathway to block photorespiration. Alternatively, a metabolic product of glyoxylate may be regulating glycolate synthesis.

Unlike the effect on intact leaf tissue, glyoxylate is a potent inhibitor of photosynthetic $^{14}CO_2$ fixation by spinach chloroplasts isolated and assayed as in (*17*); half-maximal inhibition occurred with 25 μM glyoxylate. The inhibition may result from the ability of glyoxylate to remove photosynthetically produced NADPH by the glyoxylate reductase reaction (*15, 18*). Glyoxylate (10 m*M*) had no effect, however, on the rate of $^{14}CO_2$ fixation by purified spinach ribulose diphosphate carboxylase, assayed as described in (*19*), and did not alter the inhibition of this activity by 100 percent O_2 (Warburg effect). Glyoxylate, therefore, is not inhibiting glycolate synthesis by acting directly on the oxygenase activity of ribulose diphosphate carboxylase, a possible source of glycolate (*20*). Other reactions may be responsible for glycolate synthesis (*1, 6*).

Since glyoxylate treatment under these conditions can stimulate net photosynthesis by a factor of 2.0, CO_2 assimilation is not limited by the activity of ribulose diphosphate carboxylase or photosynthetic electron transport and adenosine triphosphate production, but rather by the partitioning of photosynthate between the carbon reduction (Calvin) cycle and the glycolate pathway. By specifically inhibiting glycolate production with an intermediate of the glycolate pathway, albeit one that is readily metabolized to other compounds, one can double the photosynthetic rate. This demonstrates that at least 50 percent of the carbon fixed is lost through photorespiration.

Our knowledge that altering the pool size of common metabolites can increase photosynthesis by inhibiting glycolate synthesis and photorespiration suggests that chemical or genetic regulation of such compounds can produce plants with higher rates of photosynthesis. Mutant plant cells may be selected for slower glycolate synthesis and photorespiration (*6, 12*), perhaps by changing their concentrations of glyoxylate or glutamate. Plants derived from such cultures may convert solar to chemical energy more efficiently with a consequent increase in yield.

DAVID J. OLIVER
ISRAEL ZELITCH

Department of Biochemistry, Connecticut Agricultural Experiment Station, New Haven 06504

References and Notes

1. G. J. Kelly, E. Latzko, M. Gibbs, *Annu. Rev. Plant Physiol.* **27**, 181 (1976).
2. I. Zelitch, *Annu. Rev. Biochem.* **44**, 123 (1975).
3. ———, *Photosynthesis, Photorespiration, and Plant Productivity* (Academic Press, New York, 1971), p. 347.
4. C. C. Black, *Annu. Rev. Plant Physiol.* **24**, 253 (1973).
5. I. Zelitch, *Plant Physiol.* **51**, 299 (1973).
6. ———, *Science* **188**, 626 (1975).
7. A. A. Benson and M. Calvin, *J. Exp. Bot.* **1**, 63 (1950).
8. N. E. Tolbert, *Annu. Rev. Plant Physiol.* **22**, 45 (1971).
9. ——— and R. K. Yamazaki, *Ann. N.Y. Acad. Sci.* **168**, 325 (1969).
10. I. Zelitch, *Plant Physiol.* **43**, 1829 (1968).
11. ———, *Arch. Biochem. Biophys.* **163**, 367 (1974).
12. M. B. Berlyn and I. Zelitch, *Plant Physiol.* **56**, 752 (1975).
13. D. J. Oliver and I. Zelitch, *ibid.* **59**, 688 (1977).
14. I. Zelitch, D. J. Oliver, M. B. Berlyn, in *Biological Solar Energy Conversion*, A. Mitsui, S. Miyachi, A. San Pietro, S. Tamura, Eds. (Academic Press, New York, 1977).
15. I. Zelitch and A. M. Gotto, *Biochem. J.* **84**, 541 (1962).
16. I. Zelitch, *Arch. Biochem. Biophys.* **150**, 698 (1972); E. Robins, N. R. Roberts, K. M. Eydt, O. H. Lowry, D. E. Smith, *J. Biol. Chem.* **218**, 897 (1956). Glyoxylate was isolated from leaf extracts by ion exchange chromatography on columns of Dowex-1 acetate, and its concentration determined spectrophotometrically.
17. R. G. Jensen and J. A. Bassham, *Proc. Natl. Acad. Sci. U.S.A.* **56**, 1095 (1966).
18. E. B. Nelson, N. E. Tolbert, J. L. Hess, *Plant Physiol.* **44**, 55 (1969).
19. J. M. Paulsen and M. D. Lane, *Biochemistry* **5**, 2350 (1966).
20. G. Bowes, W. L. Ogren, R. H. Hageman, *Biochem. Biophys. Res. Commun.* **45**, 716 (1971).
21. N. C. Turner and J. Y. Parlange, *Plant Physiol.* **46**, 175 (1970).
22. D.J.O. was supported in part by an NSF energy-related postdoctoral fellowship.

31 January 1977; revised 4 March 1977