ABSTRACT

A STUDY AND COMPARISON OF GLYCOLATE METABOLISM IN TOBACCO AND GREEN ALGAE

by John L. Hess

The Glycolate Pathway in Tobacco Leaves:

Kinetic studies on the rate of glycolate biosynthesis by tobacco leaves during $^{14}CO_2$ photosynthesis were run with α-hydroxypyridinemethanesulfonate, an inhibitor of glycolate oxidase. Although $^{14}CO_2$ fixation was reduced about 50 %, the inhibitor did not affect the initial rate of ^{14}C incorporation into phosphoglycerate or glycolate, nor the specific activity of these products. The rate of glycolate formation was slower than the rate of phosphoglycerate formation. At time periods of 4 and 11 seconds, phosphoglycerate was predominately carboxyl labeled and glycolate was uniformly labeled. The specific activity of the carboxyl carbon of phosphoglycerate was about 100 fold higher than the specific activity of either carbon of glycolate, but after 60 seconds, the specific activities of the carbon atoms of both compounds became nearly equal. The sulfonate blocked the glycolate pathway since the ^{14}C in glycolate rose from 3 % in the control to 50 % in the treated leaf, while the percent ^{14}C in the products of the glycolate pathway dropped from 32 % to 10 %. These data emphasize the magnitude of the glycolate pathway in higher plants, but do not indicate a second carboxylation reaction in photosynthesis for glycolate production.

The Glycolate Pathway in Algae:

Enzymes: No glycolate oxidase activity could be detected in cell extracts from four different algae by manometric, isotopic, and spectrophotometric assays. Isocitrate dehydrogenase, NADH:glyoxylate reductase, and phosphoglycolate phosphatase were present. The absence of glycolate oxidase is consistent with glycolate excretion by algae and represents a major metabolic difference between higher plants and algae.

Distribution of ^{14}C Within Products Formed During $^{14}CO_2$ Photosynthesis by Algae: For Chlorella and Chlamydomonas, after 12 seconds photosynthesis, the carboxyl carbon contained 70 % to 80 % of the ^{14}C in serine, but glycine was uniformly labeled. Comparison of these results with similar experiments with soybean leaves demonstrated that the complete glycolate pathway does not function in algae.

The Effect of α-hydroxymethanesulfonates on Photosynthesis by Algae: Addition of either 0.001 M α-hydroxypyridinemethanesulfonate or α-hydroxymethanesulfonate to Chlorella or Chlamydomonas produced four major alterations on algal photosynthesis in $^{14}CO_2$. At pH 8.3 the sulfonates restored CO_2 fixation rates to those at pH 6.5, a three-fold increase over the control. No change in rate occurred at

pH 6.5. The algae, in the presence of these inhibitors, did not accumulate glycolate at either pH 6.5 or 8.3 as did the tobacco. Both sulfonates caused a decrease in amino acid synthesis, and a corresponding increase in the ^{14}C accumulation in the keto acids. Sugar phosphate metabolism was also altered, since the α-hydroxymethanesulfonates caused an increase in these esters, particularly in ribulose-1,5-diphosphate. It was concluded that these sulfonate inhibitors are not specific for glycolate oxidase.

Growth and Photosynthesis of Algae in Red and Blue Light: Changes in absorption spectrum and $^{14}CO_2$ fixation patterns were observed with algae grown in red light (>600 mμ) or in blue light (400-500 mμ). When grown in blue light, Chlorella and Chlamydomonas showed a consistent decrease in their chlorophyll a/b ratio. Chlamydomonas adapted to blue light produced a larger amount of glycolate-^{14}C, while cells adapted to red light labeled mainly sugar phosphates and citric acid cycle products. At low light intensities (50-100 ft-c), cells adapted to blue light accumulated ^{14}C in sugar phosphates, while the cells grown in white formed mainly products of dark fixation.

Manganese Deficient Chlorella: These cultures were obtained after 20 days growth in a Mn^{+2} deficient medium. After

photosynthesis for 10 minutes the percent ^{14}C in glycolate formed by normal algae was 30 %; but that formed by the deficient algae was only 2 %. Simultaneously the percent ^{14}C in glycine and serine formed by the deficient algae increased. These data suggest that serine is not formed from glycolate in algae.

Glycolate Excretion and Uptake by Algae: Uptake was measured by the quantitative disappearance of glycolate in the medium or by the appearance of ^{14}C labeled glycolate or phosphoglycolate in the cells. No time dependent uptake of significant amounts of either glycolate or phosphoglycolate was observed. Only 2 % to 3 % of glycolate-2-^{14}C fed to Chlorella and Scenedesmus was metabolized after 10 minutes in the light. Glycolate excretion was maximal in 0.01 M bicarbonate and occurred in the light even in the presence of 10^{-5} M glycolate. Apparently algae were not capable of glycolate metabolism as was the higher plant.

Comparison of Glycolate Metabolism in Algae and Tobacco Leaves: It was concluded that there exists a basic difference between the metabolism of glycolate by algae and by the higher plant. Although in both plants, 3-phosphoglycerate was the initial product of photosynthesis with the subsequent formation of glycolate from the carbon reduction cycle, only in the higher plant has glycolate metabolism via glycolate oxidase been found. The algae, however, excrete their glycolate into the medium as an apparent end-product.

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Biochemistry

1966

ACKNOWLEDGEMENTS

I especially thank Dr. N. E. Tolbert whose creativity and requirements for excellence continue to affirm the current excitement of modern biochemical research. His encouragement, understanding and guidance throughout the development of my research program have been invaluable.

I am extremely thankful to Karen C. Swanson for her excellent help, particularly with the chromatography and radioautography so essential to the work of this thesis. I also thank Fang Hui Liao and P. L. Youngblood* for their work with environmental factors and glycolate excretion, and C.I. Byard** for help with the glycolate degradation technique.

I acknowledge the support and interest of the National Science Foundation in this research program.

*NSF Undergraduate Research Participant, Summer, 1964.

**NSF Undergraduate Research Participant, Summer 1965.

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B_6	Pyridoxal Phosphate
FMN	Flavinmononucleotide
ft-c	foot candles
α-KG	α-ketoglutarate
NAD	Nicotinamide-Adenine Dinucleotide
NADH	Nicotinamide-Adenine Dinucleotide (Reduced)
NADP	Nicotinamide-Adenine Dinucleotide Phosphate
NADPH	Nicotinamide-Adenine Dinucleotide Phosphate (Reduced)
α-NPO	α-naphthylphenyloxazole
OH-MSulfonate	α-hydroxymethanesulfonate
OH-PMSulfonate	α-hydroxypyridinemethanesulfonate or 2-pyridylhydroxymethanesulfonate
PGA	3-phosphoglycerate
P-Glycolate	Phosphoglycolate
POPOP	1,4-bis-2-(5-phenyloxazolyl)benzene
PPO	2,5-diphenyloxazole
PS	Photosynthesis
RuDP	Ribulose-1,5-diphosphate
S.A.	Specific Activity
Sugar-P	Sugar Phosphates
TCA	Trichloroacetic Acid
TCA cycle	Tricarboxylic Acid Cycle
UDPG	Uridine Diphosphate Glucose

INTRODUCTION

The "path of carbon in photosynthesis" describes how photosynthetic assimilatory power, ATP and NADPH produced in photosynthetic electron transport, is utilized for carbon dioxide fixation into the organic compounds of green plants, algae, and photosynthetic bacteria. Kinetic isotope tracer studies, initially developed for photosynthesis by Calvin's group, enabled a determination of the sequential formation of compounds during an isotope feeding. During early investigations carboxyl labeled 3-phosphoglycerate was identified as the initial product of photosynthetic $^{14}CO_2$ fixation. Subsequent metabolism of 3-phosphoglycerate successfully described the regeneration of the CO_2 acceptor, ribulose-1,5-diphosphate, through a sequence of reactions involving sugar mono- and diphosphate esters of both the Embden-Meyerhoff glycolytic pathway and the pentose phosphate pathway (9).

Many publications on the enzymes involved in this pathway and on the physiological significance of the carbon cycle have confirmed the initial proposals of Calvin's group. This study discusses some observations of CO_2 fixation which cannot be explained by that cycle, namely, the very rapid incorporation of $^{14}CO_2$ during photosynthesis into the components of the "glycolate pathway": phosphoglycolate,

glycolate, glyoxylate, glycine, serine, glycerate, and sucrose. The labeling of these products, which may vary from 10 % to 90 % of the total $^{14}CO_2$ fixed, depends upon many physiological conditions and the length of the experiment. These compounds of the glycolate pathway together with the compounds of the photosynthetic carbon cycle account for nearly all of the soluble ^{14}C products of short term $^{14}CO_2$ photosynthesis.

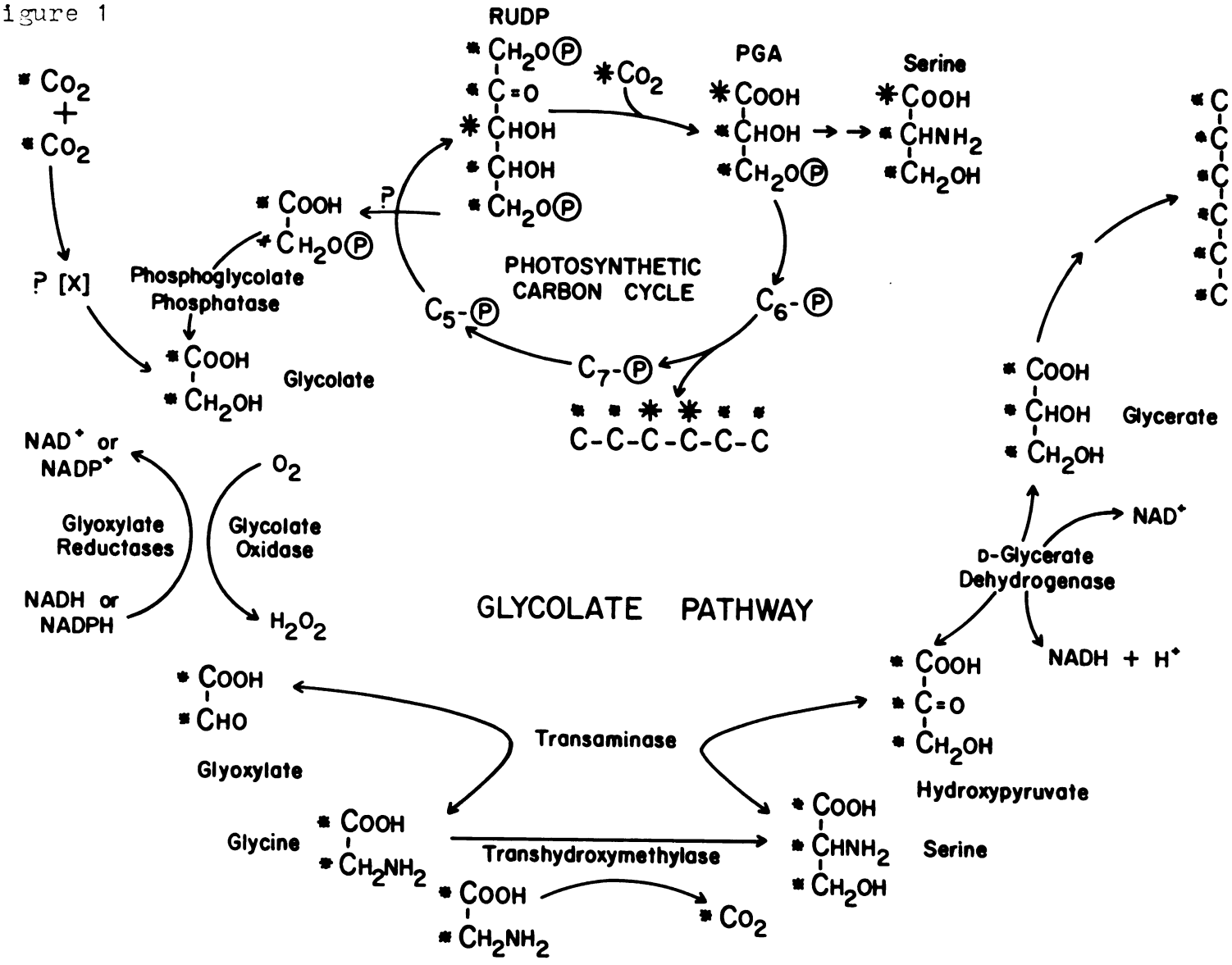
The importance of the two carbon acid, glycolic or α-hydroxyacetic acid, in photosynthesis remains obscure. Although rapidly labeled during $^{14}CO_2$ fixation, glycolate's synthesis has never been directly implicated in photosynthetic CO_2 fixation. Several laboratories, investigating the metabolic role of this acid in higher plants (47,90,120) and algae (88,89,125), have developed the glycolate pathway to describe the metabolism of this acid. A schematic representation of this pathway and its relationship to the carbon cycle is shown in Figure 1.

The photosynthetic research of O. Warburg has also suggested an important role for glycolate. Warburg has reported that up to 90 % of the CO_2 fixed during photosynthesis by his Chlorella is present as glycolate under certain conditions (121). From low quantum yields for photosynthesis in Chlorella Warburg has suggested that the additional energy

Figure 1: The Glycolate Pathway and Its Relationship with CO_2 Fixation.

It is apparent from this scheme (90) that hexoses, produced from the carbon reduction cycle, are initially 3,4 labeled. However, glycolate is initially uniformly labeled; consequently all of the products of the glycolate cycle, including hexose, are uniformly labeled.

Figure 1


RUDP
PGA
Serine
PHOTOSYNTHETIC CARBON CYCLE
Phosphoglycolate Phosphatase
Glycolate
Glycerate
NAD+ or NADP+
Glyoxylate Reductases
NADH or NADPH
Glycolate Oxidase
O2
H2O2
GLYCOLATE PATHWAY
D-Glycerate Dehydrogenase
NAD+
NADH + H+
Transaminase
Glyoxylate
Glycine
Hydroxypyruvate
Transhydroxymethylase
Serine

required for the reduction of CO_2 to carbohydrate is produced by a "Rückreaction" (122). We wonder whether this energy requirement might be obtained from the reoxidation of reduced carbohydrate components to glycolate or even the further oxidation of glycolate to glyoxylate.

This investigation evaluates the biosynthetic conditions and catabolic fates of glycolate in higher plants and algae. In both higher plants and algae, good comparative data has been lacking for the rate of ^{14}C labeling of each carbon atom of phosphoglycerate, glycolate, glycine, and serine. Data in previous publications (87,88,89,104,107, 125) have been interpreted assuming that the glycolate pathway does function in green algae, although no direct evidence supporting this assumption was presented. As reported in this thesis, the absence of glycolate oxidase and the glycolate pathway in algae requires a reevaluation of these previous experiments.

LITERATURE REVIEW

Glycolate Biosynthesis:

Benson and Calvin (13) and Wilson and Calvin (127) have reported the rapid labeling of glycolate by algae during photosynthesis; uniformly labeled glycolate was reported by Schou, et al. (95). The kinetics of ^{14}C accumulation in glycolate during photosynthesis (127) demonstrated that glycolate was likely a product of the carbon reduction cycle intermediates. A possible source of uniformly labeled glycolate is the sugar phosphates which when cleaved and oxidized would produce glycolate. Griffith and Byerrum (32) reported a conversion of ribose-1-^{14}C to glycolate in tobacco leaves although yields of glycolate-^{14}C were quite low. The formation of glycine from ribose-1-^{14}C has been demonstrated by Weissbach and Horecker (123) in which they implicated the intermediates glycolate and glyoxylate. Zelitch (138) has reported a 10 % conversion of uniformly labeled ribulose-^{14}C-phosphate to glycolate by spinach chloroplasts in the presence of an α-hydroxysulfonate.

Using the above arguments suggesting that glycolate could be formed from the sugar phosphates of the carbon reduction cycle, Bassham has created a hypothetical scheme for glycolate formation involving thiamine pyrophosphate (TPP) and other classical C_2-metabolites (8). He presented no new experimental evidence, however, to support this concept,

although it has been suggested by data from Holzer's group. Using TPP and pyruvate oxidase or transketolase they isolated a glycolaldehyde-TPP intermediate (28). Later, in agreement with reports from Racker's laboratory (15,31), Holzer found that a glycolaldehyde-TPP intermediate formed from a pentose or hexose phosphate could be oxidized to glycolyl-TPP in the presence of ferricyanide which then was hydrolyzed to release free glycolate (43). The *in vivo* significance of these reactions is unknown, but these experiments establish a background for the hypothesis that glycolate might be formed via a cleavage of a sugar phosphate to a TPP-C_2 complex which in turn is oxidized to free glycolate.

Richardson and Tolbert (92) have isolated and partially purified a specific phosphatase for phosphoglycolate. Yu, *et al.* (131) have also found this enzyme in spinach and wheat leaves. Recently Bassham and Kirk (11) have confirmed the rapid labeling of glycolate by Chlorella and also reported that O_2 stimulated the production of both glycolate and phosphoglycolate. Tolbert (107) has suggested that phosphoglycolate might be formed from the cleavage of one of the sugar diphosphates presumably through a TPP-C_2 intermediate. The oxidation of this C_2-phosphate fragment would produce the phosphoglycolate which phosphoglycolate phosphatase would hydrolyze to free glycolate. The inconsistent observation of

phosphoglycolate in photosynthesis experiments is probably a reflection of the resistance of the specific phosphatase to inactivation in methanol. Ullrich (116) has reported that terminating experiments in methanol does not prevent some hydrolysis of phosphate esters, and particularly the hydrolysis of phosphoglycolate.

In spite of the evidence against glycolate as an initial product of algal photosynthesis (81,127), Zelitch has recently published that glycolate cannot come from a phosphoglycerate metabolite (139). Stiller (101), in a recent review has criticized classical arguments for PGA as the first product of photosynthesis and discussed the possible de novo synthesis of glycolate. Using data from long time photosynthesis experiments, Zelitch (139) reported that the specific activity of glycolate is higher than the carboxyl carbon of phosphoglycerate; thus, he suggested that glycolate originates from a compound initially formed by the condensation of two CO_2 molecules. This route to glycolate is schematically represented in Figure 1 as a CO_2 + CO_2 condensation. In addition to the length of Zelitch's experiments (2 and 5 minutes), he used a killing time of about 30 seconds in $^{12}CO_2$ air. All of his reported experiments were inhibited with a "glycolic acid oxidase" inhibitor, α-hydroxypyridinemethanesulfonate (137). No short time kinetic experiments with tobacco leaves have been reported for the

initial formation of glycolate. In such experiments one must consider both the rate of ^{14}C incorporation into products as well as specific activity. A consideration of each fact, independent from the other, could lead to a misinterpretation of the results. The actual specific activity might be diluted if different resevoirs contributed to a common pool, e.g., extremely high specific activity of a compound within the chloroplast could be greatly reduced by a large cytoplasmic pool of the same compound. On the other hand, the rate at which the specific activity of a compound approaches the specific activity of the $^{14}CO_2$ may reveal the relative sizes of two different pools.

Work with mutants of Chlamydomonas reinhardtii, which lack the normal carboxydismutase of ribulose-1,5-diphosphate, did not have a light fixation pattern significantly different from that of dark fixation (62). The ^{14}C was distributed mainly in the acids of dark fixation: malate, aspartate, and glutamate. Since no glycolate formation was reported, no support was given to Zelitch's hypothesis. In the mutant algae, glycolate and the products of its metabolism should have been present, if a distinct, autonomous CO_2 fixation pathway existed which was different from the known carboxylation of ribulose diphosphate leading to the formation of phosphoglycerate.

The reduction of glyoxylate could also serve as a source of glycolate as shown in Figure 1. In 1955 the properties and action of a NADH specific glyoxylate reductase were described; this enzyme catalyzed the reduction of glyoxylate (135). In 1962 a second glyoxylate reductase was found associated with chloroplasts which preferentially reacted with NADPH (144). Since this reaction is reversible only with extremely high excesses of glycolate, the enzymatic reaction could function as a synthetic route from glyoxylate to glycolate. However, no direct evidence for the early formation of glyoxylate during photosynthesis has been reported.

Two major sources of glyoxylate might be the formation from isocitrate in the glyoxylate cycle, described by Kornberg (54) in Pseudomonas sp., or from the deamination of glycine. The glyoxylate cycle exists in many organisms, bacteria (50,54,83), algae (34), and higher plants (19,129). The glyoxylate cycle is most active in tissues which are required to metabolize much fat and therefore, not very active in the mature leaves of plants. Seeds usually have a very active enzyme complement for this cycle (16,76,98). In the algae and tetrahymena, these enzymes are induced only after growth in acetate (45,63,102). Apparently, the normal magnitude of this cycle must be small in actively photosynthesizing tissue. However, further information is needed in order to evaluate the low levels of isocitrate observed by Asada, et al. (4) in tobacco leaves inhibited with α-hydroxysulfonate. It

is unlikely that the turnover of this cycle can account for either the early labeling of glycolate in photosynthesis, or the distribution of ^{14}C in the organic acids when glycolate metabolism is inhibited. Therefore, this cycle should not be considered as important as other postulated sources of glycolate in higher plants.

The work of Homann (44) may also suggest another mechanism for glycolate biosynthesis. Using a model system with 2,3-diketogulonate, he proposed a possible oxidation mechanism with Mn^{+3} and the hydrated α,β-dicarbonyl moiety of the diketogulonate which resulted in the cleavage and oxidation of the 1,2 carbon fragment to oxalate with the concomitant reduction of Mn^{+3} to Mn^{+2}. Such a mechanism might also describe the formation of glycolate, since the requirement for Mn in glycolate formation has been reported (103).

Glycolate could also be formed from glycolaldehyde. Davies has reported that a NAD-dehydrogenase, specific for glycolaldehyde was present in the mitochondria from etiolated pea seedlings (24). However, a glycolaldehyde dehydrogenase has not been reported elsewhere, although Tolbert's laboratory has looked for it (personal communication).

Glycine Biosynthesis:

Vernon and Aronoff reported that glycine is not formed directly in photosynthesis (117). Tracer experiments with glyoxylate demonstrated its direct conversion to glycine (49,

60,97,99,120). The production of glycine from glyoxylate has been reported as a B_6 transamination reaction in carrot, pea leaves, and corn coleoptiles (22). In castor beans a transaminase specific for alanine has been reported by Kretovich (57), and Morgunova has also observed a glyoxylate transaminase in oil-bearing plants which preferentially utilizes γ-aminobutyric acid (73). The reductive amination of glyoxylate by a soy bean fraction is a reaction which may be important in nitrogen fixation (56), although this reaction has not been confirmed elsewhere.

The production of glycine from serine and the formation of "active-formaldehyde" using tetrahydrofolic acid by reversal of the serine hydroxymethylase could be another source of glycine (67,69,85). When serine-^{14}C was fed to leaves, however, little of the label appears in glycine but rather the ^{14}C moves into glycerate and sugars (90,119,14). Although the formation of glycine from serine has been reported in wheat (69,118) and Chlorella (14), this conversion is limited and does not likely function in C_1 metabolism as readily as in E. coli (85). The inhibition studies of Whittingham and Pritchard (125) presented evidence that the conversion of glycine to serine does occur to a significant extent in algae, but the general nature of isonicotinyl hydrazide inhibition suggests that glycine formation in algae proceeds via some pathway not described above.

Serine Biosynthesis:

The formation of serine in organisms has been well documented. Serine synthesis from glycine is an important reaction in plants as indicated by studies with formate and glycolate metabolism. The formyltetrahydrofolic synthetase has been isolated from higher plants as a cytoplasmic enzyme and can account for the activation of the C_1 unit (42). Isotope feeding studies with higher plants demonstrated that formate was incorporated into the β-carbon of serine and required tetrahydrofolic acid and B_6 (23,35,105). The tetrahydrofolic acid requirement has also been observed in mammalian tissue (52) and bacteria (93).

Evidence for serine formation from the transamination of hydroxypyruvate has been reported in pea seedlings (58), and an alanine:hydroxypyruvate transaminase has been described in higher plants (126). The formation of serine from PGA by algae or chloroplasts is supported by the labeling distribution in PGA and serine (20,90,133). Phosphatase can convert PGA to free glycerate (114) which can then be converted to hydroxypyruvate by D-glycerate dehydrogenase (100), and then the hydroxypyruvate transaminated to serine. Alternatively, the production of serine from PGA has also been postulated directly through phosphohydroxypyruvate to phosphoserine as shown in pea coleoptiles (33). Such a pathway has also been observed in human cells (84).

In summary, serine biosynthesis most likely occurs by one of the three pathways described above: the incorporation of a C_1 group activated by tetrahydrofolic acid into glycine in the serine hydroxymethylase reaction, the transamination of hydroxypyruvate formed from PGA and glycerate, or the transamination of phosphohydroxypyruvate from PGA and the hydrolysis of phosphoserine to serine.

The Glycolate Pathway in Higher Plants:

The initial isolation and characterization of glycolate oxidase by Tolbert, et al. (108) formed the basis for subsequent work on glycolate metabolism. Glycolate oxidase catalyzes the oxidation of glycolate to glyoxylate and H_2O_2 (Figure 1). This enzyme was further purified and characterized (142) and finally crystallized in the presence of FMN by Frigerio and Harbury (29). The oxidase has a molecular weight of 70,000 and a pH optimum of 8.3.

Glycolate oxidase is present in all higher green plant material capable of photosynthesis (59). Recently, however, Mitsui, et al. (71,72) have reported that glycolate oxidase is present in the root tips of low-land rice plants, and Tanner and Beevers (103) have reported glycolate oxidase activity in castor bean endosperm. Current investigations indicate that an alternate form of the enzyme may exist which may be associated with the electron carrier ferredoxin (6). Thus, the oxidase may interact in a coupled electron transport

system, although this has not yet been demonstrated. The oxidase activity increases as the plant becomes green, or the oxidase can also be activated in etiolated tissue by the addition of glycolate or flavin mononucleotide (59,109).

The importance of this oxidase in photosynthesis has been inferred from the dependence of the enzyme's synthesis on light, the early appearance of glycolate in photosynthesis, and the lack of serine formation in etiolated wheat tissue until the appearance of glycolate oxidase activity (111). An investigation of the possible role of the oxidase in oxidative phosphorylation was negative (141), so that further investigations concentrated on the role that the glycolate oxidase played in the metabolism of organic acids in higher plants.

The utilization of glycolate by plant materials has been extensively investigated and all of the data seem to be consistent with the pathway as shown in Figure 1. Tolbert and Cohen (110) in feeding higher plants labeled glycolate-2-^{14}C found that glycine and serine were the main metabolic products of glycolate and that α and β carbons of serine were uniformly labeled. These initial studies were later complemented with more data on the distribution of the labeled carbon in the compounds formed from glycolate. Rabson, Tolbert and Kearney (90) confirmed the above results and extended the sequence of reactions to glycerate, using additional data from serine-3-^{14}C

feeding and $^{14}CO_2$ photosynthesis. The data of Wang and Waygood (120) also indicate that a glyoxylate-serine pathway is present in higher plants. The ^{14}C distribution pattern in the sugars after the feeding of labeled serine proved that a sugar reservoir in wheat or soybean leaves could be formed from these precursors (47,90,120). A glycerate kinase as reported by Ozaki and Wetter (82) was found in rapeseed to function in the conversion of glycerate to phosphoglycerate. The hexoses of sucrose were labeled in the 3,4 positions after feeding serine-1-^{14}C, and in the 1,6 positions after feeding serine-3-^{14}C to the plants. The main pool of labeled glycerate, however, formed by the soybean (3) or wheat (90) is not uniformly labeled as expected if synthesized by the glycolate pathway.

To evaluate whether or not the glycolate pathway is associated with the cytoplasm or the chloroplast, the distribution of ^{14}C in serine formed by isolated chloroplasts was compared with that from the whole spinach leaves after photosynthesis in $^{14}CO_2$ (20). Carboxyl labeled serine was found in the chloroplast while that from the whole plant was uniformly labeled. These results were interpreted to indicate that two pathways exist for serine biosynthesis, but that the glycolate pathway was by far the most active since the serine pool from the whole plant was uniformly labeled after 20 seconds photosynthesis (90,95).

Other evidence which supports the metabolic function of the glycolate pathway comes from the use of inhibitors. Zelitch (134,137,143) and Asada, et al. (4) have used α-hydroxy-sulfonate inhibitors which Zelitch had described as specific for glycolic acid oxidase (134). These metabolic inhibitors have been used to evaluate the in vivo significance of the glycolate pathway, and have revealed that as much as 50 % or more of the fixed carbon accumulates in glycolate. Thus, much of the carbon dioxide fixed during photosynthesis must be channeled through this pathway.

The Glycolate Pathway in Algae:

Schou, et al. (95) fed glycolate-1-^{14}C and glycolate-2-^{14}C to Scenedesmus and reported that glycolate was metabolized into amino acids and sugar phosphates in the light with equal labeling in the α and β positions of glycerate. This distribution of ^{14}C in glycerate is consistent with its being formed via the glycolate pathway typical of higher plants. However, Smith, et al. (99) suggested that in algae both alanine and serine were formed from PGA. The presence of the glycolate pathway enzymes in algae has not been well documented. In a preliminary report of part of this work (40), we have shown the absence of glycolate oxidase in three genera of algae. A report by Kolesnikov (53) and another by Ohmann (78) have also suggested that glycolate oxidase, if present in algae, is probably different from the higher plant oxidase.

Also, in an initial report Tolbert, et al. (112) noted that in Chlorella and Chlamydomonas the α-hydroxysulfonate inhibitors described by Zelitch (134,137) did not cause an accumulation of glycolate during $^{14}CO_2$ photosynthesis as in the higher green plants.

Whittingham's laboratory has used another inhibitor, isonicotinyl hydrazide which caused the accumulation of glycolate and glycine during photosynthesis with Chlorella (87,88,89, 124,125). This inhibitor has been shown to inhibit pyridoxyl phosphate (B_6) enzymes (130) and therefore inhibits the possible conversion of glycine to serine in the glycolate pathway. Whittingham explains the accumulation of ^{14}C in glycolate and glycine, but never considers that inhibitors of B_6 enzymes should also decrease glycine accumulation since glycine too is likely formed via a pyridoxal phosphate mechanism (55).

In a Russian paper, Zak and Nichiporovich (133) reported the ^{14}C distribution in glycine, serine, and alanine after photosynthesis by Chlorella in $^{14}CO_2$. He found that alanine and serine were labeled similarly to PGA; and the labeling pattern of glycine excluded the possibility of its formation from serine. Vernon and Aronoff (117) reported that alanine and serine formed in soybean leaves, but only alanine was labeled similarly to PGA. This data and that outlined above suggest that the glycolate pathway may not exist in algae as it does in higher plants.

Glycolate Excretion and Uptake:

Tolbert and Zill (113) reported that free glycolate was excreted by Chlorella into the algal medium during photosynthesis. Later it was also shown that chloroplasts specifically excrete glycolate (49,106), since the photosynthetate found in the supernate was almost exclusively glycolate. These observations of excretion have been confirmed by Pritchard, et al. (87), Miller, et al. (70), Fogg, et al. (26,27), and Nalewajko, et al. (74), and recently such excretion has been reported for Chlamydomonas and Ankistrodesmus (40). The conditions for glycolate excretion are similar to those for glycolate production: high conditions of pH, light intensity and oxygen concentration; the presence of bicarbonate ion or low CO_2 concentration; and the presence of manganese.

As stated above, Schou, et al. (95) observed some glycolate-^{14}C could be converted by Scenedesmus at pH 2.8 in the light into amino acids and sugar phosphates, although no data was given for the amount of glycolate utilized. Fogg has developed a theory which considers the excreted glycolate as a reservoir of chemical energy, accumulated during photosynthesis, which can be later used by the phytoplankton. On the other hand Kearney and Tolbert (49) have reported that glyoxylate and not glycolate is taken up by

chloroplasts. The small quantities of glycolate uptake, under a variety of conditions, suggest limited metabolism of this acid (40), which is in contrast to acetate uptake and utilization by green algae (77).

Function of the Glycolate Pathway:

There have been several postulated functions for the glycolate pathway in higher plants (107). The excretion of glycolate by chloroplasts (106) and the cytoplasmic function of glycolate oxidase may operate together in transporting the fixed carbon from the chloroplast into the metabolic pools of the cytoplasm. Ongun and Stocking (79,80) have recently reported that the chloroplast membrane is freely permeable to serine and thus serine can function in a transport mechanism between the chloroplast and the cytoplasm. Their hypothesis, however, lacks any directional property which is required in a system expressing net transport. Their conclusions are also not supported by the recent evidence of Chang and Tolbert (20) that serine in the chloroplasts is labeled differently from that of the whole plant.

That algae also excrete glycolate (113) suggests that glycolate might be involved in an anion pumping mechanism e.g., for bicarbonate transport (106). The stimulation of glycolate production in low CO_2 concentration is consistent with the anion pumping mechanism, since at higher concentrations of CO_2 transport need not be dependent upon glycolate production.

Tolbert (107) has also suggested that the pathway might function in a permease system which is given direction by phosphoglycolate phosphatase. The phosphoglycolate may be hydrolyzed on the membrane and released as free glycolate which cannot reenter the chloroplast (49). Although many hypothetical reactions for the production of energy from glycolaldehyde etc. have been suggested, there has been no evidence for the coupling of these enzymes with electron transport. The production of glycolate might also be involved in the O_2 evolution steps of photosynthesis, since both processes are sensitive to CMU (107) and require Mn (103).

Since glycolate oxidase is cyanide insensitive (108) and very active in higher plants (21), the oxidation of glycolate may be involved in the respiration of leaves as a terminal oxidase (21). Based on both the amount of enzyme and the amount of glycolate in higher plants, the oxidation of glycolate could account for the total O_2 uptake of tobacco leaves (136,137,140).

The glycolate pathway might also be involved in the control of stomatal opening (143). Zelitch proposed that synthesis of sugars from glycolate via the glycolate pathway alters the osmotic potential of the guard cells. Glyoxylate reduction via glyoxylate reductase might function as a sink for excess reducing power produced as NADPH in photosynthesis, which if formed in large excess could inhibit photosynthetic

electron transport and phosphorylation. The oxidation of NADPH would favor the formation of ATP which may be required for stomatal opening. Both Heath, et al. (39) and Mansfield (65) have suggested that both of these hypotheses are incorrect.

A preliminary report suggested that glycolate oxidase might also function in glucose uptake (17). In an argument similar to that of Zelitch described above, the authors suggested that glyoxylate reduction could eliminate excess NADPH and therefore stimulate ATP production in photosynthesis. Since their only data was a correlation of glucose uptake and α-hydroxysulfonate inhibition of this uptake, the implication of glycolate metabolism in glucose transport is probably a misleading interpretation of results.

Environmental Factors Affecting Glycolate Formation:

The action spectrum for photosynthesis has long been recognized to be approximately that of chlorophyll a absorption, although the careful work of many investigators on responses of the initial photosynthetic events to different light quality has implicated two interacting pigment systems in photosynthesis. In green plants the two major pigment systems contain chlorophyll a and chlorophyll b. The dependence of the relative concentrations of related pigments on light quality has been reported. It has been difficult to evaluate whether the changes in pigment concentrations were

caused by the reduction of light intensity (91) or whether the quality of the light also induced these changes. The recent work of Fujita and Hattori with Tolypothrix indicated that changes in pigment ratios responded to a change in light quality rather than light intensity (30). Jones and Meyers have also completed similar work with Anacystis and conclude: "There is a preferential increase in the pigment of highest absorption for wavelengths of highest intensity" (48). These results clarify the role of light quality in pigment formation, while the light intensity apparently affects the rate of pigment conversions (48).

In 1955, Roux, et al. (94) and Tyszkiewicz (115) reported that plants, exposed to light with the emission spectrum of chlorophyll absorption, synthesized PGA and other sugar phosphates. However, if the incident light quality was limited to the region of the spectrum absorbed by carotenes, mainly amino acids were formed and no starch synthesis was observed. Three reports (Hauschild, et al. 36,37,38) implicated the interaction of red and blue light in CO_2 fixation by both green and blue-green algae. In their experiments blue light enhanced amino acid synthesis and decreased ^{14}C accumulation in sugar phosphates. These were long time (30 min) experiments, however, which had been preceded by a long period in the dark (up to 3 hr). Another recent report from Russia indicated that Chlorella in blue light fixed more ^{14}C

into the amino acids of the glycolate pathway and glycolate, especially in the presence of increased O_2 concentrations (132). This result is in agreement with a preliminary report of the work in this thesis (41). Likewise, Andreeva and Korzheva (1) have reported that less alanine, but more aspartate, serine, and glycine were formed in sunflower leaves if the plants were cultivated with blue light or if the photosynthesis experiment was performed in blue light.

Recently Horvath and Sazasz have shown that the light intensity can also affect the metabolism of the photosynthetic products (46). Their results confirm the vast amount of literature indicating that more amino acids are produced in low intensity white light and that in high intensities, starch is the main product. These results seem consistent with previous work and theories of photosynthetic production of ATP and NADPH.

That enzymes may respond to light has been shown in several cases. The accumulation of glycolate oxidase in the light has been discussed (59). The work of Margeulies on the formation of glyceraldehyde dehydrogenase in red light (66) may be relevant since this relates to photosynthetic requirements and metabolism in the cell.

METHODS

Plant Material:

Tobacco:

The tobacco plants used in these experiments, tobacco (var. Maryland Mammouth), were sown May 18, 1965 in the greenhouse and transplanted to an open field on June 11. Mature, but not fully expanded, leaves were harvested for the photosynthesis experiment July 21. Since the weather had been quite dry, the plants were only 45-55 cm tall.

Algae:

The strains of algae used in these experiments were obtained from the "Culture Collection of Algae" at Indiana University, Bloomington, Ind. Ankistrodesmus braunii (Naeg) Collins (#245); Chlorella pyrenoidosa (#395) Chick, Emerson; and Scenedesmus obliquus (#393) Krüger, Gaffron D-3, were cultured on an inorganic salt medium with Hoagland's micronutrients (75). Chlamydomonas reinhardtii (#90) Dangeard (-) strain grew well on the high phosphate medium described by Orth et al. (81). The cells were cultured in 2.5 liter "low form" Fernbach flasks fitted with air inlets; these were placed on a reciprocating shaker of about 60 excursions per min., thus providing a gentle but thorough agitation of the 1500 ml of medium. For culturing of algae in white light the shaker was held in a controlled environment chamber (Sherer-Gillet) at 15°C which maintained a temperature in the

culture medium of 20°. The chambers provided 1200 ft-c of continuous light from Westinghouse cool white super high fluorescent bulbs (F96T12/CW/5HO). For aeration and gassing of the medium, air from an oil-free compressor was passed through a cotton filter and distilled water and then mixed with CO_2 (also bubbling through distilled water) so that a final concentration of 0.2% CO_2 v/v in air was obtained.

For the culture of cells in portions of the spectrum, either blue and red filter systems were used as shown in Figure 2. The red filter was "Fire Red" #110 gelatin (Grand Stage and Lighting Co., Chicago, Ill.), and light was obtained from 15 watt red fluorescent tubes (General Electric - F15T12-R). This combination effectively eliminated all radiation below 600 mμ. Blue light was obtained from 15 watt blue fluorescent lights (General Electric F15T12-B or Sylvania F15T8-B) which was filtered through a blue celluloid (supplied by Kliegel Bros. as medium blue #32 Cinemoid filter) and 5 cm of a $CuSO_4$ solution of 30 g per 1. The combination of blue celluloid and $CuSO_4$ solution produced a rather narrow band of emission between 400 mμ and 520 mμ. All of the culture work and photosynthesis experiments in blue or red light were performed in dark rooms. Intensities of 350-400 ft-c in the blue light and 200 ft-c in red light were used. These cultures were grown under continuous light and aeration with 0.2% CO_2 in air, at 20-21° as maintained by a water bath.

Figure 2

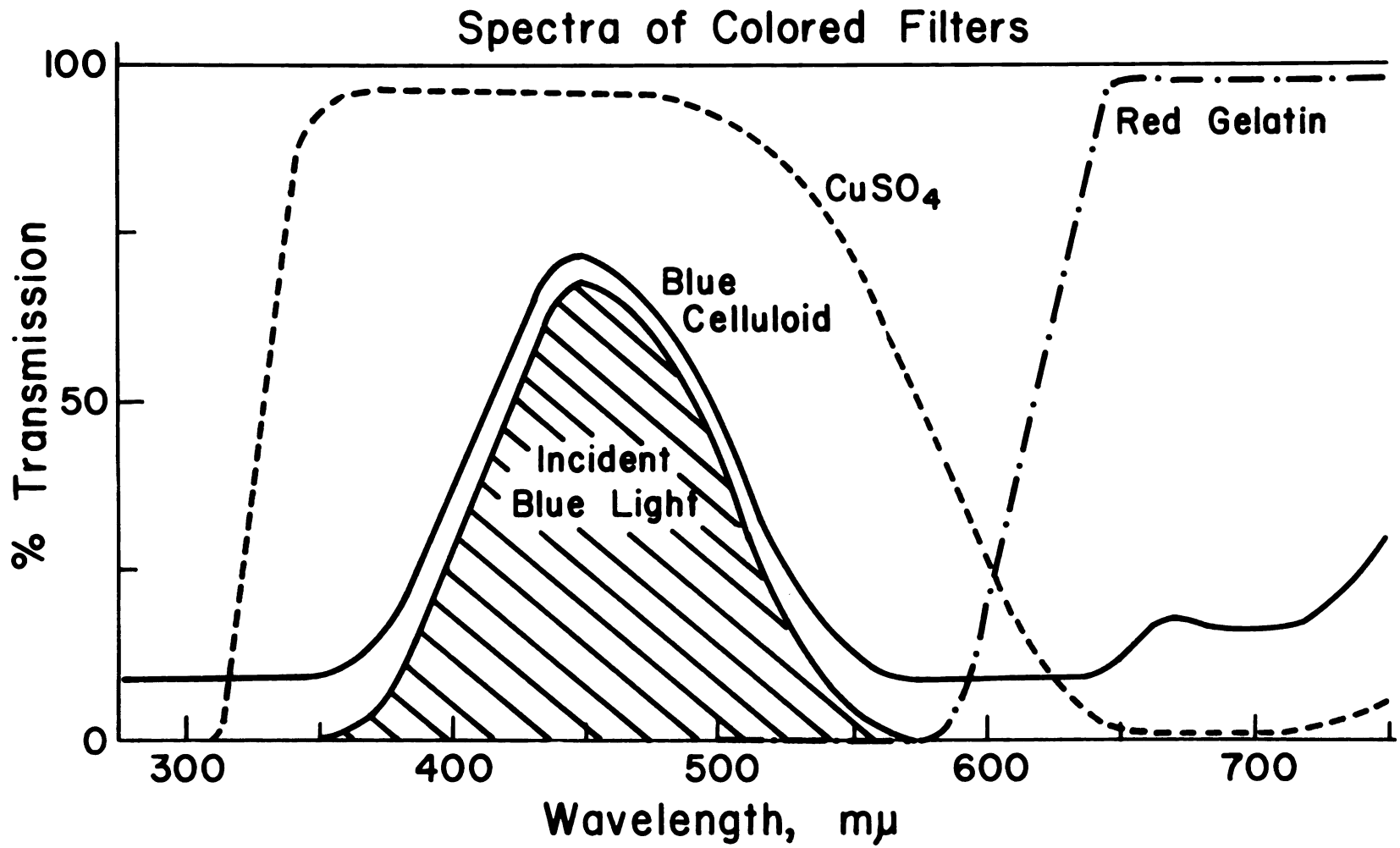
Absorption Spectra of Filter Systems Used for Algal Culture and Photosynthesis Experiments

Spectra were measured with a Cary 15 recording spectrophotometer.

The red gelatin filter (_._._._) transmitted only those wavelengths above 600 mμ.

The blue celluloid filter (———) blue the $CuSO_4$ solution filter (-----) transmitted only those wavelengths included in the cross-hatched area.

Figure 2


Spectra of Colored Filters
100
50
0
% Transmission
300
400
500
600
700
Wavelength, mμ
Red Gelatin
CuSO4
Blue
Celluloid
Incident
Blue Light

Chemicals:

All of the chemicals used in this work were standard reagent grade. The glyoxylate and hydroxypyruvate were obtained from Nutritional Biochemicals (Cleveland, Ohio), and phosphoglycolate as the cyclohexylammonium salt from General Biochemical Co. (Chagrin Falls, Ohio). Chromatographically pure sodium glycolate was produced by Matheson, Coleman and Bell Co. FMN and the pyridine nucleotides, NAD, NADH, and NADPH, were products of Sigma Chemical Co. (St. Louis, Mo.); NADP and isocitrate came from Calbiochem Co. (Los Angeles, Calif.). Eastman Kodak (Rochester, N.Y.) supplied 2,7-dihydroxynaphthalene (#4408) and chromotropic acid (#P230). The inhibitor, 2-pyridylhydroxymethanesulfonate (OH-PMSulfonate), was obtained from Aldrich Chemical Co. (Milwaukee, Wis.). N. E. Tolbert prepared the hydroxymethanesulfonate.

The counting systems utilized the following reagents: p-dioxane (spectroquality) Matheson, Coleman and Bell; phenyl-ethylamine, BP 75-76°/6mm, (#2642) Eastman Kodak (Rochester, N.Y.); PPO (2,5-diphenyloxazole), α-NPO (α-naphthylphenyl-oxazole), and POPOP (1,4-bis-2-(5-phenyloxazolyl)benzene) all scintillation grade, Packard Instruments (Downers Grove, Ill.).

The $Ba^{14}CO_3$ received from Oak Ridge National Lab. was used routinely in our photosynthesis experiments. The source of uniformly labeled 3-phosphoglycerate (1.35 mc/mmole) was Calbiochem Co., while uniformly labeled glycolate was prepared

and isolated from long time photosynthesis experiments in $^{14}CO_2$ with plant tissue. The following radioactive compounds were purchased from Nuclear Research Chemicals, Inc. (Orlando, Fla.): glycine-1-^{14}C (3.4 mc/mmole), glycine-2-^{14}C (2.0 mc/mmole), DL-serine-1-^{14}C (4 mc/mmole), DL-serine-3-^{14}C (6 mc/mmole), glycolate-2-^{14}C (4 mc/mmole), glycolate-1-^{14}C (0.5 mc/mmole), and phosphoglycolate-2-^{14}C (2.5 mc/mmole).

Photosynthesis Experiment with Tobacco Leaves:

Young leaves from field grown tobacco (var. Maryland Mammouth) were cut from plants in the morning and immediately placed in a beaker containing either water or 0.01 M 2-pyridylhydroxymethanesulfonate (OH-PMSulfonate) at pH 3.5. These leaves were removed at 5 minute intervals so that each leaf remained in solution exactly 1 hour before the photosynthesis experiment. Partial shade and good ventilation insured high transpiration rates so that the leaves took up enough solution to become turgid in 10 minutes.

After 1 hour, the leaf was removed from the solution and the blade tip cut so that the remaining leaf was about 15 cm long and 10 cm across at the widest point. The tissue was immediately put in the apparatus which had been designed for rapid equilibration of the atmosphere with added CO_2 and for a rapid killing procedure. The reaction chamber (leak-proof) was a plexiglass box with internal dimensions of 11 cm x 15 cm

x 1.7 cm. The removable cover had two holes, 1.5 cm in diameter; one held a stopper with a stopcock attached to a vacuum pump, the other held a stopper with a 60 ml separatory funnel. This separatory funnel contained a known amount of $Ba^{14}CO_3$, and 2 ml of lactic acid was introduced through a syringe cap on the top of the funnel before the experiment was begun. A partial vacuum of 75-90 mm Hg was pulled on the chamber and the vacuum line closed. Then, at zero time, the stopcock of the funnel containing the liberated $^{14}CO_2$ was opened and nearly simultaneously the syringe cap removed by a second operator so that the $^{14}CO_2$ was swept into the chamber. The leaf was held perpendicular to the sunlight of an intensity of 7,000 ft-c as measured with a Weston photometer. At the end of the experimental period the lid of the chamber was slid off while a second operator poured hot absolute methanol into the chamber. Less than 2 seconds were required to remove the lid and add the methanol. The methanol and killed tissue were rapidly transferred to a beaker and boiled for 5 minutes. After decanting the MeOH, the leaves were boiled in water 2 minutes. The extracts were combined and aliquots counted in the scintillation counter. Two-dimensional chromatography (12) of these extracts permitted evaluation of the product distribution of the fixed ^{14}C, while column chromatography permitted isolation of larger quantities of several compounds (139). The data in Table 1 represent the approximate amounts of ^{14}C used in the experiment and the dry weight of the

leaf tissue (air dried for 1 week at 28°). Using $Ba^{14}CO_3$ with a specific activity (S.A.) of 52.6 μc/mg and assuming the leaf tissue volume negligible in a total chamber volume of 375 ml, the final CO_2 concentration of the samples was between 0.2% and 0.3%.

Table 1

Photosynthesis Experiment with Tobacco Leaves

Sample No.	Time of PS	Pretreatment	Amt. of ^{14}C μcurie	Dry Wt. mg.
1	4"	1 hr, water	328	384
2	4"	1 hr, OH-PMSulfonate	197	391
3	11"	1 hr, water	180	413
4	11"	1 hr, OH-PMSulfonate	160	471
5	30"	1 hr, water	234	431
6	30"	1 hr, OH-PMSulfonate	178	364
7	60"	1 hr, water	470	378
8	60"	1 hr, OH-PMSulfonate	282	388

Photosynthesis Experiments with Algae:

The normal photosynthetic experiments with algae were similar to those of Bassham and Calvin (9). The cells, in a log phase of growth, were centrifuged from the medium at 1000 x g for 10 minutes. The pellet was resuspended in a minimum of distilled water and transferred to a pointed centrifuge tube and again centrifuged 10 min at 1000 x g. The packed cell volume was recorded and then the cells were resuspended in the reaction medium (usually 0.001 M phosphate at pH 6.5) so that a final suspension of 1% algae cells (v/v) was obtained.

The time-rate experiments were performed in a small "lollipop" containing 15-20 ml of suspended algal cells. White light of 3000 ft-c was obtained from two 300 watt Kenrad reflector floodlamps, positioned perpendicular to the plane of the lollipop, one on each side. The lollipop was suspended in a water bath maintained at 20°. Five-liter diphtheria toxin culture bottles, filled with distilled water, were placed between the lamps and the lollipop in order to absorb the heat from the lamps. Air from the compressed air line, passing through a cotton filter, gently aerated the cultures during the five minute preincubation period. At zero time 100 µl of $H^{14}CO_3^-$ in 0.1 N KOH (0.5 µc/ml) was introduced into the suspension and the lollipop briskly shaken in the light path.

At various time intervals, approximately 2-4 ml aliquots were drained into hot absolute methanol so that the final concentration was about 70-80% methanol. After gentle boiling, acidification with acetic acid, and aeration with non-radioactive CO_2, a 0.1 or 0.2 ml aliquot of the extracts was counted in 15 ml of scintillation fluid suitable for aqueous solutions (51). Total fixation was calculated as counts per minute per ml of algal suspension.

When photosynthesis experiments in colored light were performed, several modifications of the above procedure produced excellent results. The algae suspension in the lollipop was 0.5% rather than 1.0% since the light intensities of the

filtered light sources were quite low. Filter systems and light sources identical to those described for the culture conditions provided the incident radiation for these experiments (Figure 2). The distance between the light source and the face of the lollipop determined the light intensity which was approximately measured with a Weston photometer.

The lollipop was illuminated from only one side and the experiments were performed in total darkness except for the filtered light used in the experiment. A gentle stream of cold air maintained the temperature of the lollipop at approximately 20° during long experiments (10 min). All other procedures for handling the algae, isotopes, and killed samples, were followed as described above.

Separation and Identification of Photosynthetic Products:

The two-dimensional paper chromatographic techniques of Benson et al. (12) routinely were used for the photosynthetic fingerprint of the percent distribution of ^{14}C among the products. A suitable aliquot of the methanol-water extract was evaporated to a very small volume (under reduced pressure at 30-35°). This was spotted on Whatman #1 paper for chromatography and the chromatogram developed in the first direction with water-saturated phenol and in the second direction with butanol-propionic acid-water. After drying the approximate glycolate area on the chromatograms was sprayed with 0.1 M $NaHCO_3$ to prevent glycolate sublimation. Exposure of the dried

chromatogram to Kodak No-Screen X-ray film for $2\frac{1}{2}$-3 weeks revealed the location of the radioactive spots which were then counted with a thin window (DuPont Mylar film) gas flow counter using a Nuclear Chicago scaler (Model 161A). Helium, passing through ethanol at 0° provided the gas for the counting chamber. Since only the relative distribution of the radioactivity on a chromatogram was needed, the absolute efficiency of the system was never accurately determined; however, an approximate value of 10% efficiency was used for gross approximations. Cochromatography of radioactive spots with authentic samples and the use of spray reagents permitted positive identification of unknown compounds.

When larger samples of a particular compound were needed for degradation procedures, ion-exchange resins provided the easiest initial purification. The technique of Plaisted (86), using Dowex-50-H^{+}, effectively separated the amino acids from the other plant material. Further purification was achieved using two-dimensional chromatography on Whatman #3 with the solvents described above. Water eluted the purified spots from the chromatogram into test tubes.

Zelitch's procedure (139) with Dowex-1-acetate columns was employed to separate glycolate and phosphoglycerate. Of the fractions collected, one contained glycolate and the other phosphoglycerate (PGA). These fractions were further purified by paper chromatography in a basic solvent system (butanol: 95%

ethanol:water:diethylamine, 80:10:20:1). The chromatography removed from the radioactive acids small quantities of unlabeled plant substances also present in these fractions.

Colorimetric Assays:

The colorimetric assay of Calkins (18) with 2,7-dihydroxy-naphthalene produced a chromophore with glycolic acid which absorbs at 540 mμ. In the assay 0.2 ml of sample was boiled for 20 min with 2 ml of a 0.01% solution of the naphthalenediol in concentrated sulfuric acid. The color response was linear from 0 to 0.1 μmole glycolate. The sample was diluted with 4 ml of 2 N sulfuric acid and the absorbance of the solution measured with the Coleman Jr. Spectrophotometer.

Hydrolysis of 3-phosphoglycerate was necessary so that the free glycerate could be degraded and assayed colorimetrically. Fractions containing PGA, or the spots eluted from chromatograms were combined and taken to dryness. The residue was dissolved in a minimum volume of water and titrated to the blue-green endpoint of bromthymol blue indicator (approximately pH 7.5-8.0). A 0.1 ml aliquot of neutralized alkaline phosphatase solution (2-3 mg per 10 ml) completely hydrolyzed the PGA in 2 hr at 30°. The free glycerate was then purified by paper chromatography in the basic solvent system described above.

The procedure, outlined by Bartlett (7), for a quantitative glycerate determination with chromotropic acid (4,5-dihydroxy-2,7-naphthalenedisulfonic acid) produced a good reproducable

color with a maximum absorbance at 690 mμ. A 0.2 ml sample was heated in a boiling water bath for 30 min with 5.8 ml of the chromotropic acid solution (0.01 % chromotropic acid in concentrated sulfuric acid). After cooling the colored solution gave a linear absorbancy response with the Coleman Jr. Spectrophotometer from 0.01-0.15 μmoles of free glycerate.

Degradation Procedures:

The modified degradation technique of Sakami, described by Chang and Tolbert (20) was equally effective for degrading serine and free glycerate. The degradation vessel was a 50 ml, 3 necked, pear-shaped flask fitted with a separatory funnel for adding reagents, a condenser and attached CO_2 trap, and an inlet tube for aeration. The degradation followed three separate steps outlined in the following procedure.

Step one: Cleavage of serine into one mole each of CO_2, formate, and formaldehyde.

To the flask was added radioactive glycerate or serine, 0.2 mmole cold carrier (21 mg serine), and 2 ml 0.5 M phosphate at pH 5.8. Then, to the closed system, 107 mg $NaIO_4$ in 3 ml water was introduced and the system was aerated 1 hr at room temperature. Most of the excess periodate was consumed by reaction with an additional 0.2 mmole of carrier during another hour of aeration.

Step two: Oxidation of formate to CO_2 (carbon 2 of the acid).

To the flask was added 1.5 ml 3.0 M phosphate at pH 2.5. Then one gram of $HgCl_2$, dissolved in 5 ml hot water, was introduced into the closed system through the funnel. The system was aerated for 1 hr while boiling gently.

Step three: Oxidation of formaldehyde to CO_2 (carbon 3 of the acid).

To the flask was added 1 ml 5% $AgNO_3$; the system was closed, and 1.5 g $K_2S_2O_8$ in 5 ml water was added through the funnel. First the solution was aerated for 45 min with gentle heating, then aeration continued 30 min longer while boiling.

After each step the 5 ml of the CO_2-trapping solution (128) (27 ml redistilled phenylethylamine, 27 ml absolute methanol, 500 mg POP, and 10 mg POPOP taken to a final volume of 100 ml with toluene) was quantitatively transferred to a polyethylene counting vial. The trap was washed with scintillation fluid (5 g POP and 100 mg POPOP per liter of toluene) three times so that the final volume in the counting vial approximated 15 ml.

The degradation of glycine followed the exact procedure of Chang and Tolbert (20). Ninhydrin released the carboxyl carbon of glycine at room temperature, and boiling with potassium persulfate in the presence of $AgNO_3$ oxidized the second carbon to CO_2. The same degradation apparatus and CO_2 trapping system were used as described for the serine degradation.

The degradation of glycolate proceeded according to the technique outlined by Zelitch (139) with slight modifications. The degradation was performed in the degradation vessel previously described rather than a Warburg flask. The trap contained 5 ml of the amine CO_2-trapping solution rather than KOH as used by Zelitch. This degradation also proceeded in three steps:

Step one: Oxidation of glycolate to glyoxylate.

To the flask were added 1.0 ml 0.1 M phosphate at pH 8.0, 1.0 ml 6×10^{-4} M FMN, and approximately 2,000-5,000 cpm of the radioactive glycolate. Gentle aeration with O_2 was started and 0.2 ml of a glycolate oxidase preparation was added through the funnel with a total volume of 2 ml water. After 4.0 ml sodium glycolate carrier solution (500 µg/ml) were added, aeration was continued for 1.5 hr. The oxidase prepared from tobacco sap, was the ammonium sulfate fraction precipitated between 15-30 g $(NH_4)_2SO_4$ per 100 ml solution.

Step two: Cleavage of glyoxylate into one mole each of CO_2 and formate.

Through the funnel was added 0.6 ml of 0.2 N $(NH_4)_4Ce(SO_4)_4$ in 2 N sulfuric acid, and the solution boiled gently 1 hr without heating. Thus, the possible distillation of formic acid at this pH was minimized. The flask was cooled to room temperature and aerated gently with air for 30 min to quantitatively remove the liberated CO_2. Care must be taken to avoid excessive

bubbling, since the protein solution may foam through the condenser and ruin the CO_2-trap.

Step three: Oxidation of the formate to CO_2 (carbon 2 of glycine)

After addition of a second CO_2-trap, 2 g $HgCl_2$ in 10 ml hot water was added through the funnel. This mixture was boiled gently for 1 hr with continuous aeration.

For each degradation the counting efficiency with the amine CO_2-trapping solution approximated 55-60% as compared with 20% with the Cab-O-Sil gel counting of KOH solutions. The distinct advantage of being able to count the entire amount of CO_2 trapped, rather than a small aliquot of KOH solution allowed the use of fewer counts per degradation.

The calculation of percent yield was obtained by counting an aliquot of the non-degraded material and comparing it with the total recovered. The percent distribution of ^{14}C within the compound was directly calculated from the amounts of ^{14}C recovered at each degradation step. Results of standard degradations with acids of known radioactive distribution (Tables 2 and 3) indicated the reliability of the glycerate, glycine, and serine degradations. In all cases at least two degradations were run on each standard sample, but more were run on glycolate samples to confirm the low recovery of the second carbon atom.

Table 2

Percent Distribution of ^{14}C in Standard C_3 Compounds

Compound		Percent Recovery	Percent ^{14}C C_1	C_2	C_3
3-PGA-U-^{14}C	1	95	33.9	33.3	32.7
	2	96	34.7	33.1	31.4
Serine-1-^{14}C	1	97	92.6	1.6	5.8
	2	--	95.3	1.0	3.3
Serine-3-^{14}C	1	96	0.4	6.8	92.8
	2	--	0.9	1.7	97.4

The total yield of ^{14}C from glycolate-2-^{14}C was consistently low and varied between 90-97 %. The recovery of the first carbon of glycolate was excellent (Table 3). It was concluded that the most valid calculation for the ^{14}C distribution in glycolate would be to determine the percent of ^{14}C recovered in the first carbon with respect to the total activity added to the reaction rather than with respect to the total ^{14}C recovered. Since the total recovered was always less than 100%, due to poor yields of the second carbon, calculations on the amount of recovered radioactivity would increase the percent of ^{14}C in the first carbon of glycolate. The effect of this argument is presented in Table 3. Uniformly labeled glycolate yielded a 55/45 (C_1/C_2) ratio if the distribution was based on total ^{14}C recovered in the degradation. This ratio became 51/49 if the

distribution in C_1 was based on the total ^{14}C added to the reaction and the C_2 was calculated by difference.

Table 3

Percent Distribution of ^{14}C in Standard C_2 Compounds

Compound		Percent Recovery	Percent of ^{14}C Recovered		Percent of ^{14}C Added	
			C_1	C_2	C_1	C_2
Glycine-1-^{14}C	1	100	87.4	12.4		
	2	100	84.8	15.1		
Glycine-2-^{14}C	1	96	1.3	98.7		
Glycolate-1-^{14}C	1	100	99.5	0.5	99.5	0.5
	2	94.4	99.0	1.0	93.5	6.5
Glycolate-2-^{14}C	1	90.9	13.8	86.2	12.6	87.4
	2	97.3	14.4	85.6	14.0	86.0
Glycolate-1,2-^{14}C		94.6	54.5	45.5	51.4	48.6

Calculation of Specific Activities for Glycerate and Glycolate:

From the concentration of the acid solutions, determined colorimetrically, as μmoles/ml, and the activity of the solution evaluated as counts per minute per ml (cpm/ml) of the same solution, the specific activity as cpm/μmole acid was determined by division. The specific activity per μmole of carbon (cpm/μmole C) resulted as the product of the total specific activity and the percent of the ^{14}C in a particular carbon. A convenient check on this calculation was the sum of all the specific

activities of the carbons in a compound, which must equal the specific activity of the whole compound.

Sonication of Algae:

The algae were harvested as previously described for the photosynthesis experiments. The packed cell volume was diluted to a 30% (v/v) suspension in a designated medium. The sonic converter (Branson Sonifier, Heat Systems (Melville, L.I.)) was a probe instrument and rupture of the cells was accomplished in "rosette cells" supplied by Heat Systems Co. Routinely 15 ml of algal suspension at pH 7.5-8.5 in the 25 ml rosette cell was cooled in an ethanol-water-ice bath at -5°. The regular tip of the sonicator was positioned as close to the bottom of the cell as possible, and the instrument operated at the maximum output level so that the ammeter indicated 0.55-0.65 amps. The Sonifier ruptured 90% of the Chlamydomonas cells after five minutes, but only 60-70 % of the Chlorella or Ankistrodesmus cells after 10-15 min. The apparent turbidity of the suspension decreased as the cells were broken and particle size decreased. The pH values of the broken suspensions varied as indicated by the data of Table 4.

Table 4

pH of Algal Suspensions Before and After Sonication

Suspending Medium	pH Before Sonication	pH After Sonication
Water	6.5	5.7-5.8
0.06 M phosphate buffer	8.3	7.5
0.02 M cacodylate buffer	6.3	6.3

It was observed that at pH values less than pH 5.5, the suspension would sometimes coagulate and greatly reduce the efficiency of the sonication.

Centrifugation at 3.5×10^4 x g for 10 min removed the unbroken cells and cellular debris. The clear dark green supernate was then used in experiments as a cell extract or fractionated for a specific enzymatic activity.

Enzyme Assay Procedures:

Glycolate Oxidase:

The manometric technique (21) employed 0.5 ml of 0.1 M phosphate buffer at pH 8.3, 0.5 ml 6×10^{-4} M FMN, 0.5 ml water, and 1 ml cell extract (pH 7.5-8.0) in the main portion of the Warburg vessel. The side arm contained 0.5 ml 2×10^{-2} M Na-glycolate and was added with the main chamber at zero time. The oxidase activity was evaluated from the volume of O_2 uptake per unit time.

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In the very sensitive isotopic technique a known amount of glycolate-2-^{14}C was added to the crude cell sonicate (not centrifuged) at zero time. The complete mixture was incubated in the light with gentle shaking and aliquots killed by mixing them with hot methanol. Glycolate oxidase activity was measured by the identification of glycine and serine on two dimensional chromatography of this reaction mixture. The relative amounts of glycine and serine were compared with the amount of glycolate added as substrate.

Phosphoglycolate phosphatase:

The phosphoglycolate phosphatase assay was that described by Yu et al. (131), except that cacodylate rather than Tris-acetate buffer was used. The mixture contained 0.1 ml buffer (0.1 M cacodylate plus 0.02 M $MgSO_4$ at pH 6.3), 1.0 ml 0.01 M phosphoglycolate and 1.0 ml enzyme. The mixture was incubated at 30° for 30 min after the addition of the enzyme, and then was killed with 1.0 ml of TCA (10%). After centrifugation, 1 ml of the supernate was assayed for orthophosphate (25).

Spectrophotometric Assays:

The Gilford automatic cuvette changer and absorbance converter, Beckman DU spectrophotometer, and Sargent recorder were used to measure the rates of the following enzymatic reactions.

Glycolate Oxidase:

A spectrophotometric assay observed the increased absorbancy at 324 mμ due to the formation of glyoxylate phenylhydrazone. This reaction mixture contained: 2 ml 0.1 M phosphate pH 8.3; 0.1 ml 0.1 M phenylhydrazine hydrochloride adjusted to pH 6.8; 0.1 ml 0.1 M cysteine; 0.5 ml enzyme (cell extract); 0.3 ml 0.1 M glycolate (total volume of assay = 3 ml). The increased absorbancy formed during a given time interval was defined as glycolate oxidase activity.

Glyoxylate Reductase:

The assay procedure of Zelitch (135) followed the oxidation of reduced pyridine nucleotide, measured as an absorbancy reduction at 340 mμ after the addition of glyoxylate. The assay mixture contained: 1 ml 0.1 M phosphate pH 6.5, 0.5 ml 0.1 M glyoxylate, 0.05 ml 0.02 M NADH, 0.1 ml cell extract, and water to 3 ml.

Isocitrate Dehydrogenase:

Isocitrate dehydrogenase activity was detected using the assay procedure described by Syrett, et al. (102). The assay followed the rate of NADP reduction measured as increasing absorbancy at 340 mμ. The assay mixture contained: 1 ml 0.1 M phosphate pH 7.5; 1 ml 0.05 M $MgCl_2$, 0.1 ml 0.06 M cysteine,

0.05 ml 0.02 M NADP, 0.2 ml enzyme preparation (cell extract), and 0.1 ml 0.1 M isocitrate used to start the reaction.

Extraction and Determination of Chlorophyll:

The 80% acetone chlorophyll extraction which has been used for extracting chlorophyll from chloroplast preparations (2) did not extract much of the chlorophyll from algal extracts. The extinction coefficients, which Mackinney (64) has reported for absolute methanol solutions of chlorophyll, made it possible to determine the chlorophyll concentration directly on a methanol extract from algae. Ten ml of a 0.5 % algal suspension provided sufficient chlorophyll for the spectrophotometric determination. The algae were centrifuged in an International Clinical centrifuge at maximum speed for 3 min. Immediately the supernate was discarded and the tube inverted on an absorbant surface so that the excess water would drain from the sides of the tube. After several minutes, the cells were re-suspended in 3 ml of absolute methanol (using a Vortex mixer), and were placed in stoppered centrifuge tubes in the dark for at least 2 hr to insure complete extraction of the pigment. After centrifugation, absorbancy measurements on the green supernate were made with the Beckman DU spectrophotometer. From Mackinney's extinction coefficients at 665 mμ and 650 mμ the following equations for chlorophyll concentration were calculated:

mg Chlorophyll *a* per liter = 16.2 Abs_{665} - 7.53 Abs_{650}

mg Chlorophyll *b* per liter = 30.6 Abs_{650} - 11.3 Abs_{665}

The absorbancy measurements at these wavelengths were converted to mg chlorophyll/liter, and a final value as mg chlorophyll/ml of packed cells was obtained by multiplying by 0.06 to account for the 3 ml of methanol used and the volume of packed cells (0.05 ml).

Measurement of in vivo Absorption Spectra:

Light scattering presents the main problem in the accurate measurement of the spectral absorption of *in vivo* algal suspensions. The technique of Shibata, *et al.* (96) employed a neutral density filter made of filter paper saturated with mineral oil which was placed between the light source and the cuvette. It was found that a double thickness of waxed weighing paper (Schleicher and Schuell Co. No. B-2) placed next to the cuvette on the face toward the light source most effectively elucidated the fine structure of the *in vivo* chlorophyll spectrum. The ratio of chlorophyll a/b absorption could be visibly evaluated from spectra measured on the Cary 15 spectrophotometer. Good resolution was achieved with a slit width of approximately 0.7 mm. Under these conditions the Absorbance of a 1 % algal suspension was approximately 0.5 Absorbancy units at 750 mμ.

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Growth rates of algal suspensions were obtained by measuring aliquots of algal cultures at various time intervals after innoculation. The log of the Absorbancy of the suspension measured at 680 mμ with the Coleman Jr. Spectrophotometer, was plotted against time. Typical data revealed whether a culture was in a log phase or a lag phase of growth.

RESULTS AND DISCUSSION

The Rate of Glycolate Formation in Tobacco:

In the photosynthetic experiments with tobacco leaves, as described in the methods section, the linear rate of $^{14}CO_2$ fixation observed during the first 60 sec in the control experiment (Figure 3) indicates that the tissues did not run out of $^{14}CO_2$, i.e. $^{14}CO_2$ did not become rate-limiting. It is important for the arguments about the movement of ^{14}C into the metabolic pools, as represented by glycolate, that these leaves were at all times saturated with $^{14}CO_2$. The abnormally high rate of fixation by the inhibited tissue for the 30 second experiment might have been due to a difference in the biological activity of the tissue rather than its size, since as seen in Table 1, the dry weight of the tissue was not larger than the other samples. That the inhibitor was taken up by this particular sample was evident in the product distribution.

The percent distribution of ^{14}C fixed into the various products of photosynthesis from 4 to 60 seconds are shown in Figure 4. The percent of ^{14}C fixed into glycolate and glycerate increased with time, but that fixed into PGA decreased. These data agree with the classical results obtained by Bassham and Calvin (9), indicating that initially PGA contains most of the newly fixed ^{14}C and that other compounds are formed subsequently.

Figure 3

Total Fixation of $^{14}CO_2$ by Tobacco Leaves

Leaves photosynthesized $^{14}CO_2$ in full sunlight.

Closed symbols: Control (1 hr pretreatment with water)

Open symbols: Inhibitor (1 hr pretreatment with 0.01 M OH-PMSulfonate

Figure 3

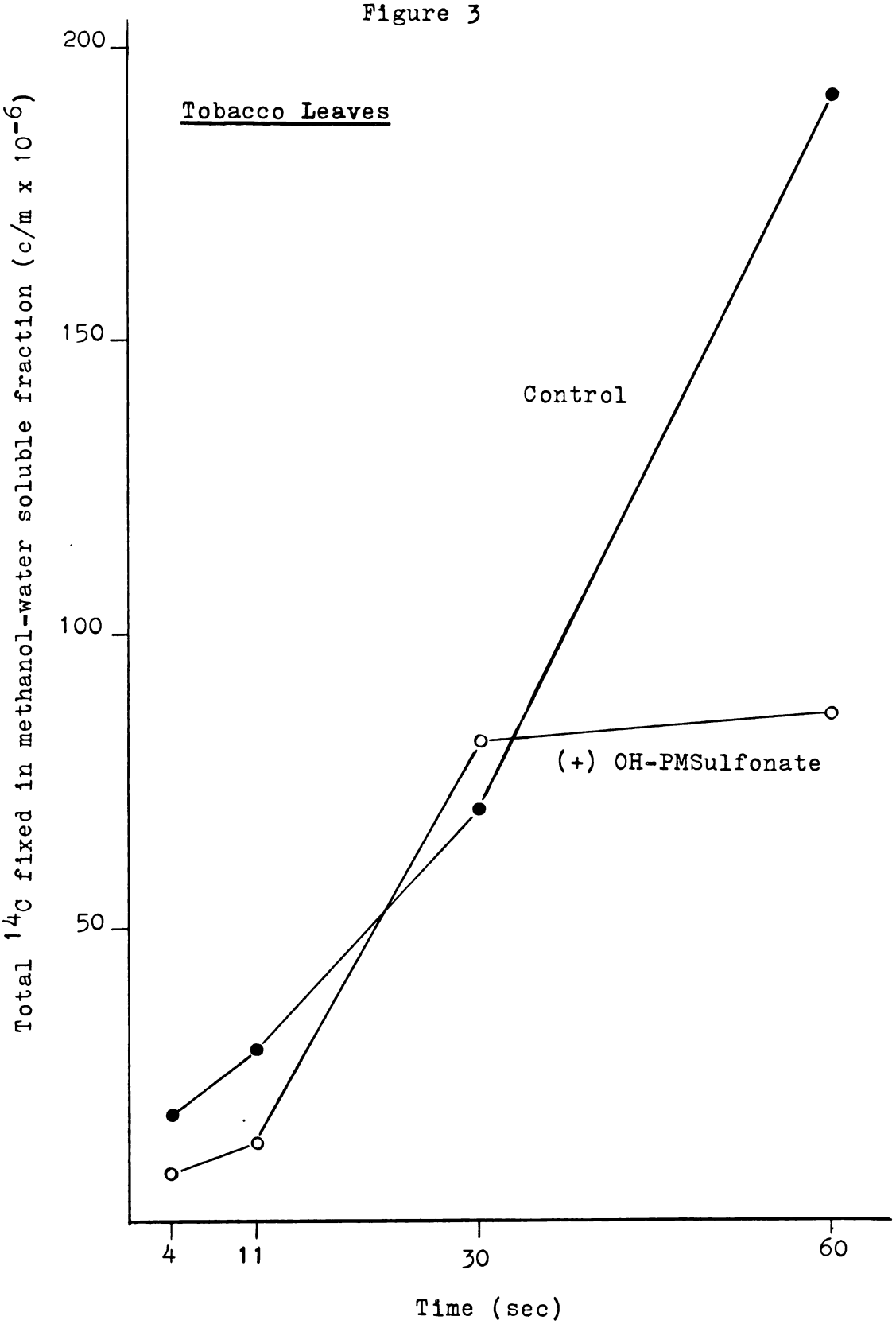

Tobacco Leaves
Total ^{14}C fixed in methanol-water soluble fraction (c/m x 10^{-6})
200
150
100
50
Control
(+) OH-PMSulfonate
4
11
30
60
Time (sec)

Figure 4

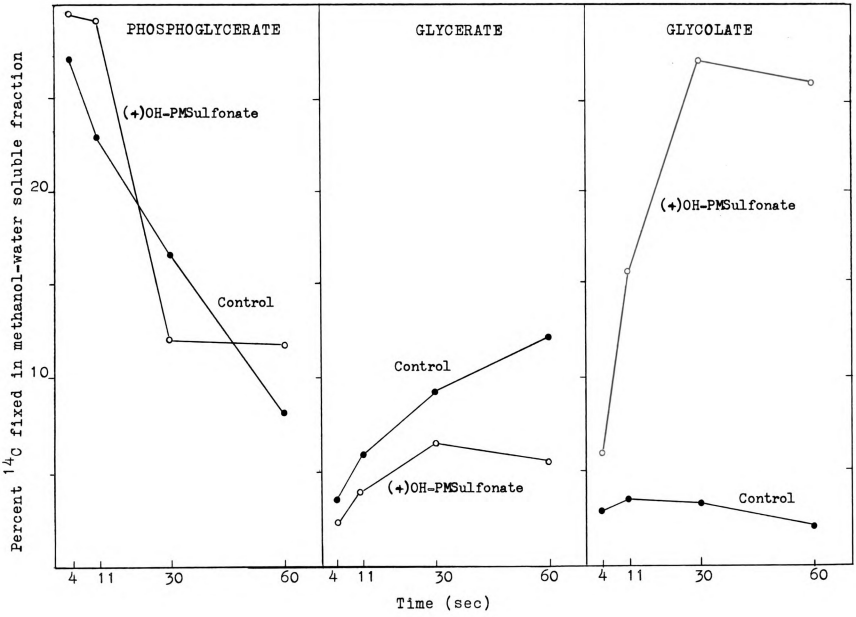
Percent Distribution of ^{14}C in PGA, Glycerate, and Glycolate Formed by Tobacco Leaves

Leaves photosynthesized $^{14}CO_2$ in full sunlight.

Closed symbols: Control (1 hr pretreatment with water)

Open symbols: Inhibitor (1 hr pretreatment with 0.01 M OH-PMSulfonate)

Figure 4


PHOSPHOGLYCERATE
GLYCERATE
GLYCOLATE
(+)OH-PMSulfonate
Control
(+)OH-PMSulfonate
Control
(+)OH-PMSulfonate
Control
Percent ^{14}C fixed in methanol-water soluble fraction
20
10
4
11
30
60
Time (sec)

These data also demonstrate that the inhibitor, OH-PMSulfonate, effectively blocked glycolate oxidase as evidenced by the gigantic accumulation of ^{14}C in this acid. The demonstration that movement of ^{14}C into the rest of the glycolate pathway was also blocked by OH-PMSulfonate is shown in Figure 5. A reciprocal relationship existed between the accumulation of glycolate-^{14}C in the presence of the inhibitor and the decrease in the ^{14}C within the products of the glycolate pathway, glycine, serine, and sucrose. I conclude, therefore, from ^{14}C pool sizes that the inhibitor, OH-PMSulfonate, as described by Zelitch (134), blocks glycolate oxidase with the resultant accumulation of ^{14}C in glycolate. Further, I confirm that PGA and not glycolate is the initial product of photosynthetic CO_2 fixation, even in the presence of an inhibitor which favors glycolate accumulation.

In order to more completely defend this latter conclusion, it was necessary also to evaluate the relative specific activities of PGA, glycolate, and glycerate. The relative total amounts of radioactivity isolated in these compounds (Figure 6) suggest that not only did the inhibitor cause the accumulation of glycolate but it also reduced the amount of free glycerate. The latter conclusion is to be expected if glycerate were a product of the glycolate pathway, although labeling data to be presented does not support this argument.

Figure 5

Percent Distribution of ^{14}C in Glycolate, Glycine, Serine, and Sucrose Formed by Tobacco Leaves

Leaves photosynthesized $^{14}CO_2$ in full sunlight.

Closed symbols: Control (1 hr pretreatment with water)

Open symbols: Inhibitor (1 hr pretreatment with 0.01 M OH-PMSulfonate)

Figure 5

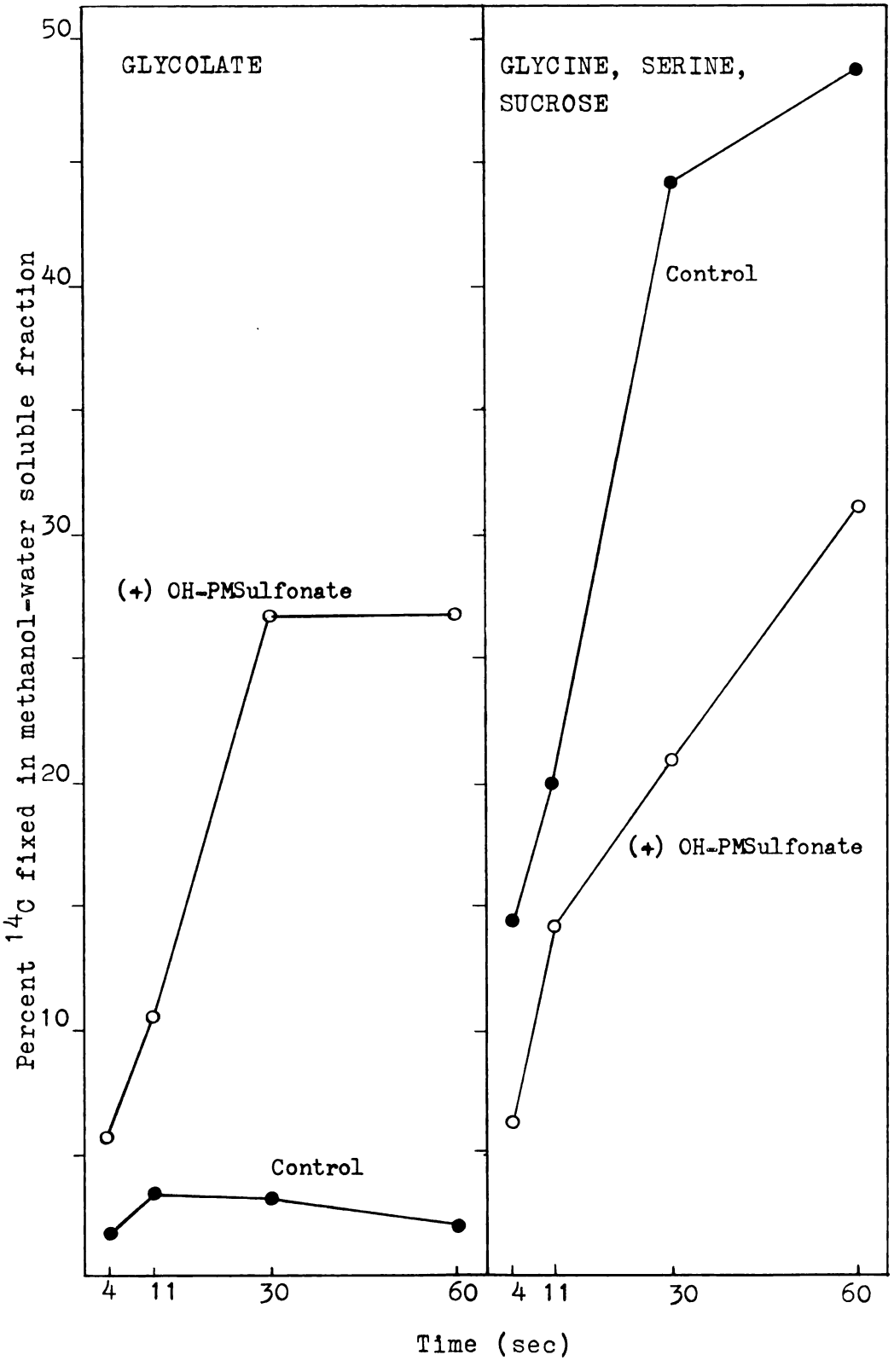

GLYCOLATE
GLYCINE, SERINE, SUCROSE
Control
(+) OH-PMSulfonate
(+) OH-PMSulfonate
Control
Percent ^{14}C fixed in methanol-water soluble fraction
50
40
30
20
10
4
11
30
60
4
11
30
60
Time (sec)

Figure 6

Total ^{14}C in PGA, Glycerate, and Glycolate Formed by Tobacco Leaves

Leaves photosynthesized in full sunlight.

Control: 1 hr pretreatment with water.

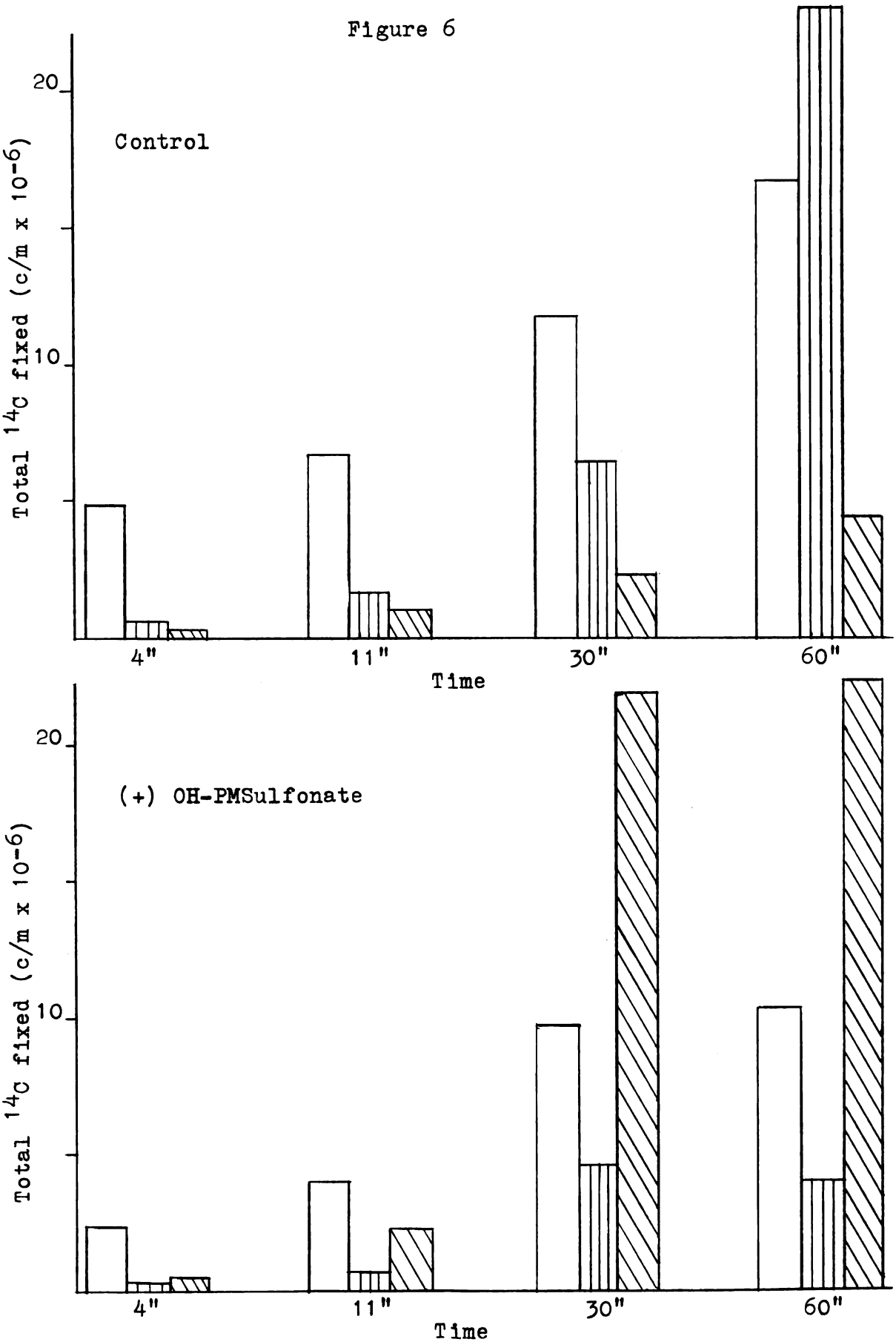
Inhibitor: 1 hr pretreatment with 0.01 M OH-Sulfonate.


^{14}C in phosphoglycerate (PGA)


^{14}C in glycerate


^{14}C in glycolate

Figure 6


Control
20
10
Total ^{14}C fixed (c/m x 10^{-6})
4"
11"
30"
60"
Time
(+) OH-PMSulfonate
20
10
Total ^{14}C fixed (c/m x 10^{-6})
4"
11"
30"
60"
Time

That most of the free glycerate did not arise from the hydrolysis of PGA during the killing procedure is indicated by the changes in the ratios of PGA/glycerate (Figure 6). The ratio of PGA/glycerate was not approximately constant as would be expected from phosphatase hydrolysis of PGA during killing.

The specific activities of the chromatographically pure samples of PGA, glycerate, and glycolate, are presented in Table 5. These values can be corrected to approximate disintegrations per minute by multiplying by 2, since we observed 50-60% counting efficiency in our scintillation counting procedure. These data, as plotted in Figure 7, demonstrate that the S.A. of PGA immediately reached a plateau (within 4 seconds) and that by 30 or 60 seconds even the S.A. of glycerate and glycolate were approximately that of PGA. These data do not confirm Zelitch's claim (139) that the S.A. of glycolate is greater than the S.A. of PGA. Zelitch used experimental times of 2 and 5 minutes and killing times of 30 seconds in $^{12}CO_2$ air.

Since the specific activity of the carboxyl group of PGA and the carbon atoms of glycolate must be compared, both compounds and also glycerate were degraded as described in the methods section. The results (Tables 6 and 7) indicate that PGA and glycerate were predominantly carboxyl labeled at short times, but that they became almost uniformly labeled by 60 seconds. The glycolate (Table 8), however, showed at all times

Table 5

Specific Activities of Compounds Isolated from Tobacco Leaves After Photosynthesis in $^{14}CO_2$

		Specific Activities (cpm/μmole acid)		
Time of PS	Pretreatment	Phosphoglycerate	Glycolate	Glycerate
4"	Water	6.8×10^7	1.4×10^6	7.3×10^5
4"	OH-PMSulfonate	3.0×10^7	7.7×10^5	3.0×10^5
11"	Water	1.9×10^7	2.7×10^6	1.1×10^6
11"	OH-PMSulfonate	2.0×10^7	2.4×10^6	2.5×10^5
30"	Water	1.5×10^7	1.3×10^7	9.8×10^6
30"	OH-PMSulfonate	1.1×10^7	1.6×10^7	3.8×10^6
60"	Water	8.1×10^7	1.6×10^7	2.2×10^7
60"	OH-PMSulfonate	8.4×10^7	3.3×10^7	6.3×10^6

Figure 7

Specific Activities of PGA, Glycerate, and Glycolate Formed by Tobacco Leaves

Leaves photosynthesized $^{14}CO_2$ in full sunlight.

Closed symbols: Control (1 hr pretreatment with water)

Open symbols: Inhibitor (1 hr pretreatment with 0.01 M OH-PMSulfonate)

Figure 7

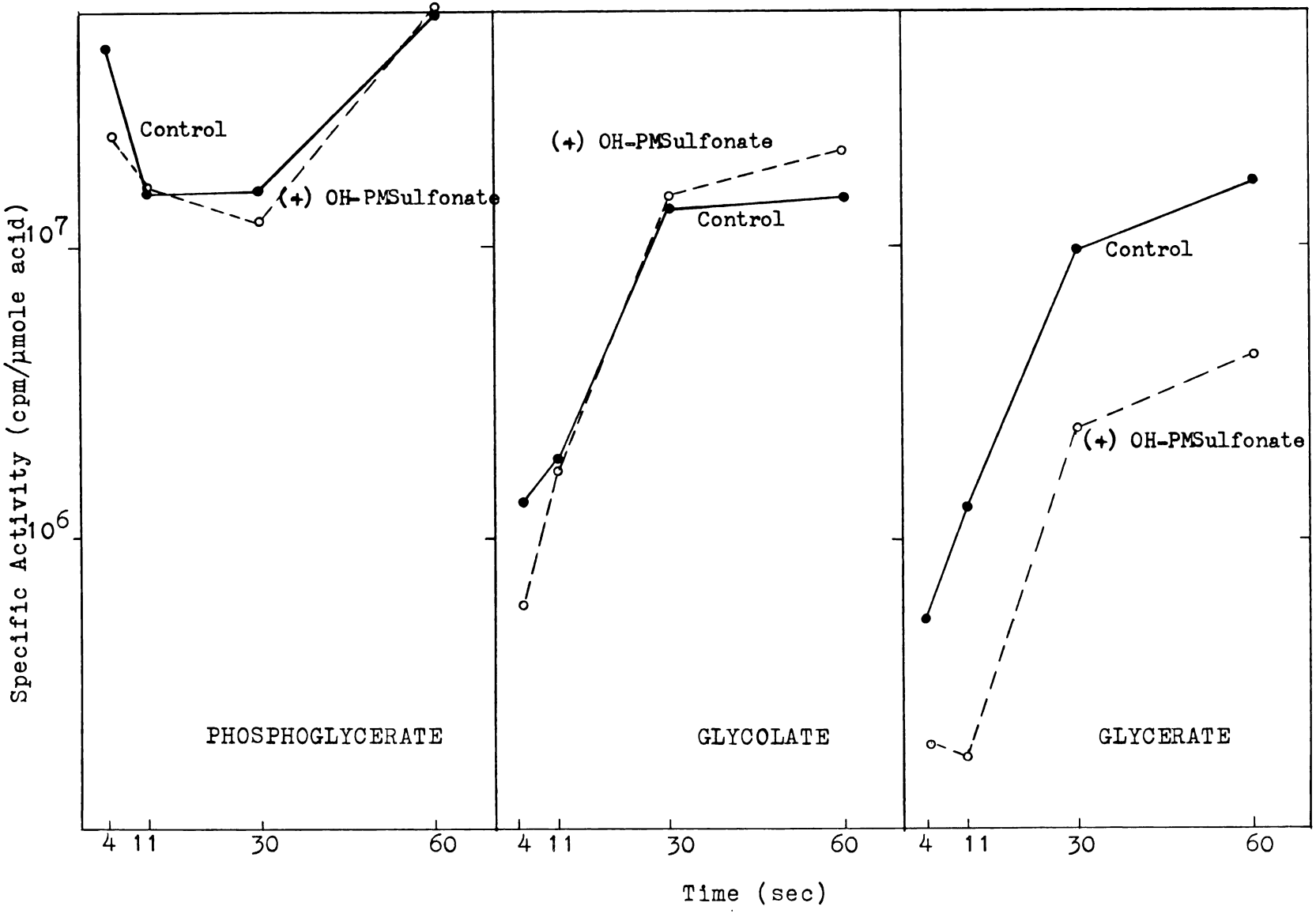

Specific Activity (cpm/μmole acid)
10^7
10^6
Control
(+) OH-PMSulfonate
PHOSPHOGLYCERATE
(+) OH-PMSulfonate
Control
GLYCOLATE
Control
(+) OH-PMSulfonate
GLYCERATE
4
11
30
60
Time (sec)

Table 6

Percent Distribution of ^{14}C in 3-Phosphoglycerate Isolated From Tobacco Leaves After Photosynthesis in $^{14}CO_2$

		% Distribution of ^{14}C		
Time of PS	Pretreatment	C_1	C_2	C_3
4"	Water	83.2	8.9	7.9
4"	OH-PMSulfonate	87.4	7.1	3.5
11"	Water	64.9	16.1	19.0
11"	OH-PMSulfonate	64.0	16.5	19.5
30"	Water	51.1	23.0	25.9
30"	OH-PMSulfonate	49.6	28.1	22.3
60"	Water	36.7	33.3	30.1
60"	OH-PMSulfonate	43.1	28.8	28.1

Table 7

Percent Distribution of ^{14}C in Glycerate Isolated From Tobacco Leaves After Photosynthesis in $^{14}CO_2$

Time of PS	Pretreatment	% Distribution of ^{14}C C_1	C_2	C_3
4"	Water	83.9	9.2	6.9
4"	OH-PMSulfonate	89.1	4.9	6.0
11"	Water	73.5	12.5*	13.0*
11"	OH-PMSulfonate	72.6	15.0	12.4
30"	Water	54.1	27.4	18.5
30"	OH-PMSulfonate	42.9	29.9	26.2
60"	Water	34.4	35.9	29.7
60"	OH-PMSulfonate	40.5	34.0	25.6

% error for these averages was generally 5% although those marked * were 10%; the values were averaged from two separate degradations.

Table 8

Percent Distribution of ^{14}C in Glycolate Isolated from Tobacco Leaves after Photosynthesis in $^{14}CO_2$

Time of PS	Pretreatment	Percent Distribution of ^{14}C Recovered		Percent Distribution of ^{14}C Added	
		C_1	C_2	C_1	C_2
4"	Water	50.2	49.8	42.6	57.4
4"	OH-PMSulfonate	59.3	40.7	52.2	47.8
11"	Water	59.5	40.6	52.2	47.8
11"	OH-PMSulfonate	56.6	42.4	51.0	49.0
30"	Water	57.6	46.4	51.6	48.4
30"	OH-PMSulfonate	53.8	46.2	51.4	48.6
60"	Water	53.9	46.1	52.4	47.6
60"	OH-PMSulfonate	56.8	43.2	49.9	51.1

equal distribution of the label in both carbon atoms. Using the S.A. data in Table 5 and the percent distribution data in Tables 6, 7, and 8, the S.A. of the individual carbon atoms of these compounds have been calculated (Table 9). Thus, the S.A., 5.7×10^7 cpm/μmole C, for the carboxyl carbon of PGA is 100 times greater than the S.A., 6.0×10^5 cpm/μmole C, of either carbon of glycolate at 4 seconds. The rapidity of photosynthesis in this tissue is emphasized by observing that the S.A. of these same carbons was almost equal after 60 seconds. The very high initial S.A. of PGA confirms that carboxy labeled PGA was formed initially by the carboxylation reaction of the photosynthetic carbon cycle. Labeling of glycolate occurred subsequently. That Zelitch observed a S.A. of glycolate higher than PGA must reflect a rapid incorporation of non-radioactive CO_2 into his samples during his long killing procedure, thus reducing first the specific activity of PGA.

The effect of the hydroxysulfonate on glycerate formation invites speculation. As already mentioned the inhibition of glycerate formation by OH-PMSulfonate (Figures 4 and 6) might be explained as a result of blocking the glycolate pathway. However, if the glycolate pathway were contributing to the pool of free glycerate, the distribution of label in glycerate should be either uniform or more quickly randomized in the control leaves than when the inhibitor was present. This was not observed (Tables 6 and 7). The lower S.A. of glycerate in the presence

Table 9

Specific Activities of Carbon Atoms in PGA, Glycerate, and Glycolate Which Were Isolated From Tobacco Leaves After Photosynthesis in $^{14}CO_2$

Time of PS	Pretreatment	Specific Activity (cpm/μmole C)		
		3-phosphoglycerate		
		C_1	C_2	C_3
4"	Water	5.7×10^7	6.1×10^6	5.4×10^6
4"	OH-PMSulfonate	2.6×10^7	2.1×10^6	1.1×10^6
11"	Water	1.2×10^7	3.1×10^6	3.6×10^6
11"	OH-PMSulfonate	1.3×10^7	3.3×10^6	3.9×10^6
30"	Water	7.7×10^6	3.5×10^6	3.9×10^6
30"	OH-PMSulfonate	5.5×10^6	3.1×10^6	2.5×10^6
60"	Water	2.9×10^7	2.7×10^7	2.5×10^7
60"	OH-PMSulfonate	3.6×10^7	2.4×10^7	2.4×10^7
		Glycerate		
		C_1	C_2	C_3
4"	Water	6.1×10^5	6.7×10^4	5.0×10^4
4"	OH-PMSulfonate	2.7×10^5	1.5×10^4	1.8×10^4
11"	Water	8.1×10^5	1.4×10^5	1.5×10^5
11"	OH-PMSulfonate	1.8×10^5	3.8×10^4	3.1×10^4
30"	Water	5.3×10^6	2.7×10^6	1.8×10^6
30"	OH-PMSulfonate	1.7×10^6	1.1×10^6	1.0×10^6
60"	Water	7.6×10^6	7.9×10^6	6.6×10^6
60"	OH-PMSulfonate	2.5×10^6	2.2×10^6	1.6×10^6
		Glycolate		
		C_1	C_2	
4"	Water	6.0×10^5	7.9×10^5	
4"	OH-PMSulfonate	4.0×10^5	3.7×10^5	
11"	Water	1.4×10^6	1.3×10^6	
11"	OH-PMSulfonate	1.2×10^6	1.2×10^6	
30"	Water	6.7×10^6	6.3×10^6	
30"	OH-PMSulfonate	8.3×10^6	7.7×10^6	
60"	Water	8.4×10^6	7.7×10^6	
60"	OH-PMSulfonate	1.6×10^7	1.7×10^7	

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of OH-PMSulfonate (Table 5) suggests that the inhibitor decreased the amount of $^{14}CO_2$ incorporation into glycerate. This block in glycerate formation may not have been solely in the glycolate pathway, since the ^{14}C-distribution in the remaining glycerate does not correspond to that expected from glycolate. If the radioactivity, passing through the glycolate pathway, did not contribute significantly to the pool of glycerate until after 60 seconds, then one may not expect to see more rapid randomization of glycerate in the control system than in the inhibited one. A possible solution to this problem might be found in the degradation of serine from these experiments. Rabson, Tolbert, and Kearny (90) have shown uniform labeling of serine after 20 sec by the intact leaf. One should be able to differentiate between serine from the glycolate pathway in the control experiments and serine formed from glycerate in the inhibited system. Similarly Chang and Tolbert (20) were able to differentiate between two reservoirs of serine, one in the chloroplasts and one in the cytoplasm.

Enzymes in Algae:

Glycolate Oxidase:

For many reasons, investigators have tacitly assumed that the glycolate pathway operated in green algae. The ubiquitous distribution of glycolate oxidase in higher plants has been well demonstrated. Algae also rapidly label glycolate, glycine, and serine during photosynthesis as do higher plants. However,

there have been no literature citations to this enzyme from algae except for three preliminary reports (40,53,78) which suggest that if the oxidase is present it is likely different from that of higher plants. Therefore experiments were run to attempt to isolate and characterize glycolate oxidase from sonicated preparations of Chlorella, Chlamydomonas, and Ankistrodesmus.

Extracts from cells of 2 or 3 day old cultures were prepared as described in the methods section. Before sonication the cells were suspended in either, 0.1 M phosphate buffer at pH 8.3, 0.1 M phosphate at pH 8.3 containing 2×10^{-2} Na-glycolate, or 0.1 M phosphate at pH 8.3 containing 2×10^{-2} cysteine. After sonication, these suspensions were centrifuged so that the original crude extract, the supernatant fluid and the residue could be assayed manometrically. No glycolate oxidase activity was observed in any of these preparations from any of the algal strains under any conditions. One-half ml of an active glycolate oxidase preparation from tobacco when added to flasks which contained samples of the above extracts demonstrated no inhibition of activity. Addition of glycolate oxidase from tobacco to the cell suspension before sonication indicated a 10% reduction of the initial activity (detected manometrically) after the cells were sonicated. No oxidase activity was observed as glycolate dependent O_2 uptake. Flushing the flasks with pure O_2 gas for 5 minutes before the experiment also did not promote any activity.

When glycolate-2-^{14}C was added to these algal extracts, little or none of the isotope was converted to glycine or serine or any other compound as measured by paper chromatography. These results are in agreement with a recent report of Whittingham (125). To test the possibility that the transaminase, converting glyoxylate to glycine, might have been inactivated, and that glyoxylate cannot be easily detected by paper chromatography, I used an assay for glycolate oxidase which follows the rate of glyoxylate formation as the accumulation of the glyoxylate-phenylhydrazone. This assay also did not indicate any glycolate metabolism by any of the algal extracts. These experiments were also done with Chlamydomonas cultures at various stages of growth, lag phase, log phase, and senescent algae, but no oxidase could be detected in any of the preparations.

In the above experiments numerous controls were run and they are summarized as follows: (a) No inhibitor for the normal glycolate oxidase from tobacco leaves was present in the algae cell extracts. (b) No enzyme activity was detected when the algal extracts were prepared in the presence of 2×10^{-2} M glycolate which Baker and Tolbert (6) had used to isolate a form of glycolate oxidase from etiolated wheat tissue. (c) Oxidase from tobacco leaves was not significantly destroyed by sonication. (d) Increasing the partial pressure of oxygen had no effect on the endogenous respiration. Glycolate oxidase from green leaves has a low affinity for oxygen and is dependent upon ample oxygen in the atmosphere (108).

Thus, the assumption that the typical glycolate pathway is operating in algae must be modified. Certainly the conclusions from these studies on the oxidase eliminated the possibility that glycolate metabolism, if it occurs at all in algae, proceeds via a typical glycolate oxidase. Recently other reports have suggested that glycolate oxidase in algae may be different from that in the higher plant (53,78). The theory that glycolate is excreted by algae, as if it were an end metabolic product (125), is confirmed by these enzymatic studies reported here.

Isocitrate Dehydrogenase:

To evaluate whether or not the lack of glycolate oxidase activity reflects a general inactivation of algal enzymes during preparation of extracts, isocitrate dehydrogenase was assayed as described in the methods. The algal extracts demonstrated a rate of NADP reduction (30 μmoles NADPH formed per 10 min per 0.1 ml extract) comparable to that reported by Syrett, et al. for Chlorella vulgaris (102). This activity was dependent only on isocitrate and specific for NADP, since NAD was completely inactive. That isocitrate dehydrogenase and other enzymes to be mentioned below could be detected, under conditions identical with those used for preparation of glycolate oxidase, demonstrated that active enzymes were present in the sonicated cell-free extracts.

Glyoxylate Reductase:

An assay for glyoxylate reductase also showed the presence of an active enzyme which was dependent upon NADH, but was inactive with NADPH. A reaction of 30 μmoles NADH oxidized per 5 min per 0.05 ml extract was observed with glyoxylate substrate. The enzyme preparation was also capable of reducing hydroxypyruvate with NADH. Laudahn (61) has reported that both the glyoxylate reductase from spinach leaves and D-glycerate dehydrogenase from tobacco leaves have approximately the same K_m values of 1.2×10^{-4} for hydroxypyruvate and 1.2×10^{-2} for glyoxylate (cf. 9.1×10^{-3} for glyoxylate reported by Zelitch, 135). Thus, the observation of glyoxylate and hydroxypyruvate reduction by NADH in algal extracts could indicate activity for both glyoxylate reductase and D-glycerate dehydrogenase, or that one enzyme was acting on both substrates. Further isolation and characterization of these reductive enzymes from the algal extracts are needed before any conclusions can be made about their function in algal cells.

Phosphoglycolate Phosphatase:

Extracts from Chlorella, Chlamydomonas and Ankistrodesmus all contained active phosphoglycolate phosphatase. The phosphatase, detected in extracts prepared in 0.02 M cacodylate at pH 6.3, gave a ratio of 20:1 with respect to hydrolysis of phosphoglycolate and phosphoglycerate. This ratio compared favorably

Table 10

A Summary: Preparation of Cell-Free Extracts From Algae And Detection of Enzymes in These Extracts.

Procedure:

1. Cells were grown in 1200 ft-c white light with 0.2 % CO_2 in air.

2. Algae were harvested and resuspended (30 % v/v) in water, 0.1 M phosphate buffer, or buffer plus 0.01 M glycolate, each at pH 7.5.

3. Cells were sonicated with maximum output at 5°, 15 min for Chlorella or Ankistrodesmus and 5 min for Chlamydomonas.

4. Extracts were assayed by Warburg respirometer, isotopic products and spectrophotometric techniques.

Results :

GLYCOLATE OXIDASE _ _ _ _ _ _ _ _ _ _ ABSENT

Phosphoglycolate Phosphatase_ _ _ _ _ Present

NADH:Glyoxylate Reductase _ _ _ _ _ _ Present

Isocitrate Dehydrogenase_ _ _ _ _ _ _ Present

with the partially purified phosphoglycolate phosphatase isolated from tobacco leaves which gave a phosphoglycolate/phosphoglycerate ratio of 3:1 after an acetone fractionation (131).

As summarized in Table 10, the active enzymes, NADP: glyoxylate reductase, phosphoglycolate phosphatase, and isocitrate dehydrogenase, have been demonstrated in the sonicates of three strains of algae. In contrast, no glycolate oxidase was observed with these same algal preparations. Similar negative results for glycolate oxidase have also been obtained from sonicated preparations of Scenedesmus obliquus (Gaffron D-3) and Chlorella pyrenoidosa (Warburg).

Distribution of ^{14}C in Products of the Glycolate Pathway Formed by Algae During Photosynthesis in $^{14}CO_2$:

During initial $^{14}CO_2$ fixation by algae, glycolate is uniformly labeled (107) and PGA is carboxyl labeled (9) as is also observed in higher plants. However, the absence of glycolate oxidase (as reported in the previous section) raised speculation about ^{14}C distribution in glycine and serine. Therefore, the ^{14}C distribution in the carbons of phosphoglycerate, glycine and serine, formed by Chlamydomonas reinhardtii and Chlorella pyrenoidosa (Chick) during short time photosynthesis was determined.

The isolation and degradation procedures for PGA, glycine, and serine were described in the methods section. Both PGA and serine were identically carboxyl labeled (Table 11). The formation

Table 11

Percent Distribution of ^{14}C in the Carbons of Phosphoglycerate, Glycine and Serine Formed by Algae During Photosynthesis.

A 2 % suspension of algae in 0.001 M phosphate buffer at pH 6.5 photosynthesized $^{14}CO_2$ in white light (3000 ft-c).

		% Distribution of ^{14}C		
		C_1	C_2	C_3
Chlorella pyrenoidosa				
(7 sec PS)	P-glycerate	84	4	12
	Serine	78	6	16.
(12 sec PS)	P-glycerate	82	3	15.
	Serine	80	4	16
	Glycine	50	50	
Chlamydomonas reinhardtii				
(12 sec PS)	P-glycerate	79	4	17
	Serine	69	7	24
	Glycine	49	51	
Soybean*				
(20 sec PS)	P-glycerate	74	11	16
	Serine	28	38	34

*From Rabson et al. (90)

of carboxyl labeled serine by algae differs markedly from the formation of uniformly labeled serine by soybean. In both plant tissues, the PGA was still carboxyl labeled. (See data and reference cited in Table 11). In these experiments the labeling of serine in algae did not become more uniform than that of PGA.

These results further support the concept that the glycolate pathway does not function in algae. As is seen in Figure 1, an alternate pathway for serine formation via PGA, glycerate, and hydroxypyruvate, could explain the formation of carboxyl labeled serine. My observation of an active D-glycerate dehydrogenase or glyoxylate reductase in the algal extracts would also be consistent with this hypothesis. Chang and Tolbert (20) have reported that serine formed by isolated chloroplasts is also carboxyl labeled; Zak reported that alanine and serine formed by Chlorella were carboxyl labeled (133).

The glycine formed by the algae was uniformly labeled and this finding must be compared with uniformly labeled glycolate and carboxyl labeled serine. Chang and Tolbert also observed that glycine formed by chloroplasts was uniformly labeled (20). In agreement with my results, Zak (133) reported that glycine formed by Chlorella after 13 sec photosynthesis had 49 % of its ^{14}C in the C_1, while for the same length of time serine contained 82 %, 0 %, and 17 % respectively for C_1, C_2, C_3. This labeling pattern eliminates the possibility that the reversal of serine

hydroxymethylase can account for the glycine production, since the glycine thus formed would necessarily be carboxyl labeled as is the serine. Uniformly labeled glycine might come from the uniformly labeled glycolate, but the absence of glycolate oxidase and the excretion of glycolate make this possibility unlikely. As with the work of Whittingham (89,125), the source of glycine in algae remains unexplained. Perhaps a third, as yet unrecognized, pathway exists for the production of uniformly labeled glycine.

Effect of α-Hydroxymethanesulfonates On $^{14}CO_2$ Photosynthesis by Algae:

α-Hydroxypyridinemethanesulfonate has been used to inhibit glycolate oxidase and to evaluate the function of the glycolate pathway in plants (4,134,137,139,143). Algal experiments were initiated for a similar purpose. The only report of this inhibitor's interaction with algae has been from Asada, et al. (4) who observed an inhibition of CO_2 fixation.

The normal photosynthesis experiments with $NaH^{14}CO_3$ were run with 1.0 % suspensions of Chlamydomonas reinhardtii and Chlorella pyrenoidosa (Chick) with and without the OH-PMSulfonate. Although data with the Chlorella were qualitatively similar, only data with the Chlamydomonas experiments have been presented (Tables 3-7 of the Appendix). A comparison of typical radioautographs from two dimensional chromatography of the methanol-water

soluble fraction from tobacco (Figure 8) and Chlamydomonas (Figure 9) reveals the striking difference between the ^{14}C distribution in these two plants when treated with OH-PMSulfonate. The remainder of this discussion is concerned with a presentation and interpretation of data obtained from such chromatograms.

The data in Figure 10 indicates a linear $^{14}CO_2$ fixation rate by the algae for the first 4 minutes of fixation, and that this rate was greatly stimulated in the presence of 0.001 M OH-PMSulfonate at pH 7.6. The distribution data of ^{14}C in products after 2 and 4 min photosynthesis experiments, described in Figure 10, are presented in the histogram (Figure 11), and may be directly compared with similar data on the percent distribution of ^{14}C in products formed by tobacco leaves with and without OH-PMSulfonate after nearly similar periods of $^{14}CO_2$ fixation (Figure 12). Several differences are apparent. The sugar phosphates (including PGA) contained more ^{14}C when the algae were in OH-PMSulfonate, while in the tobacco leaves with the sulfonate, the sugar phosphates were lowered; similar changes were noted in ribulose-1,5-diphosphate.

Most dramatic were the differences in the amount of glycolate-^{14}C formation: in the higher plant a tremendous accumulation of the acid was observed as a result of OH-PMSulfonate addition, but even at these relatively long times, no similar effect was shown with the algae. This difference is visually apparent in the radioautographs from tissue treated with

Figure 8

Radioautographs of Methanol-Water Extract from Tobacco Leaves After 30 and 300 Second Photosynthesis in $^{14}CO_2$ in Full Sunlight

A portion of the percent distribution data in Tables 1 and 2 of the Appendix were obtained from these chromatograms.

Control (Pretreatment 1 hr with water).

Inhibitor (Pretreatment 1 hr with 0.01 M OH-PMSulfonate).

Figure 9

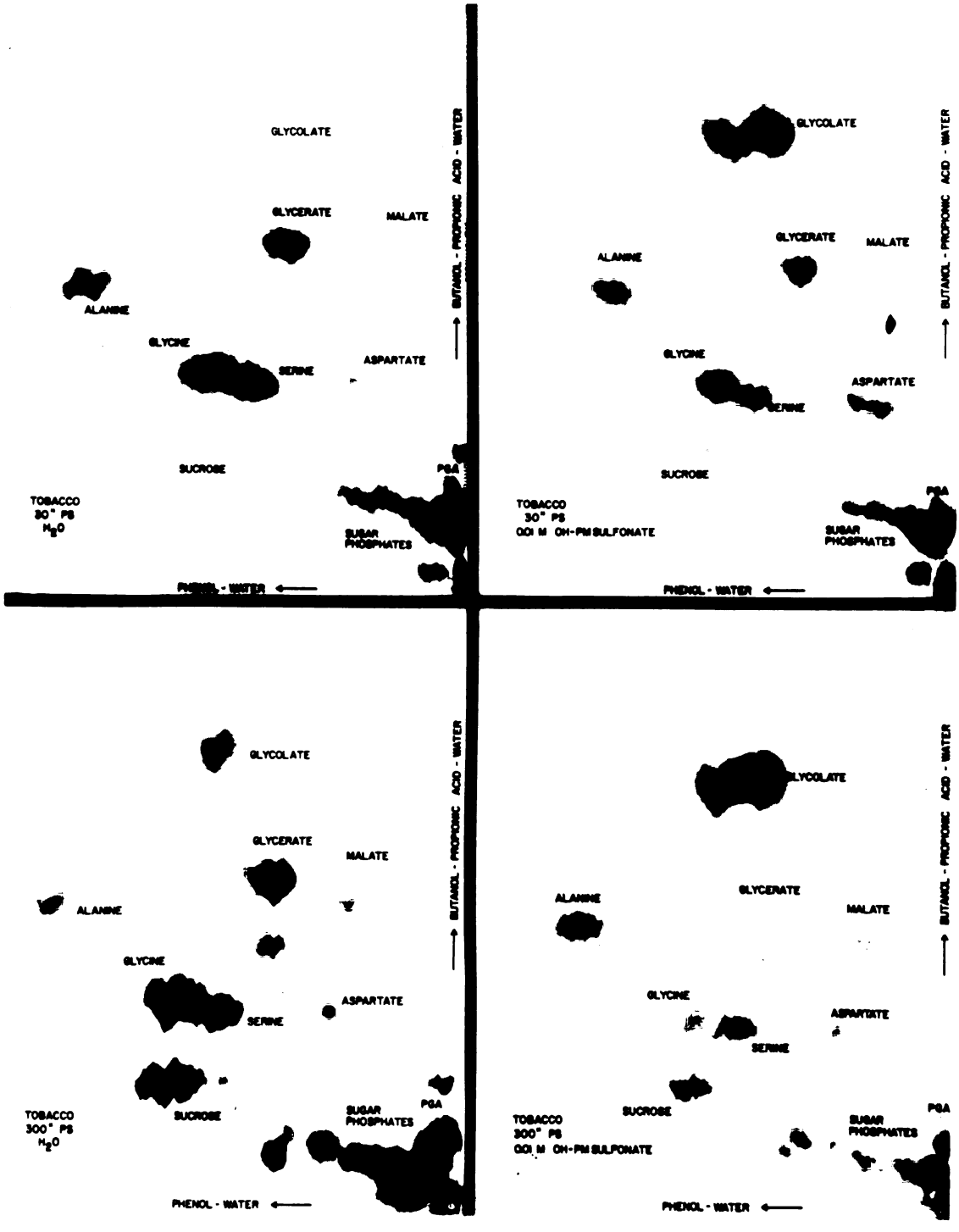

GLYCOLATE
GLYCERATE
MALATE
ALANINE
GLYCINE
SERINE
ASPARTATE
SUCROSE
PGA
SUGAR PHOSPHATES
TOBACCO 30° PS H2O
TOBACCO 30° PS 001 M OH-PM SULFONATE
TOBACCO 300° PS H2O
TOBACCO 300° PS 001 M OH-PM SULFONATE
BUTANOL - PROPIONIC ACID - WATER
PHENOL - WATER

Figure 9

Radioautographs of Methanol-Water Extract from Chlamydomonas After 60 Seconds Photosynthesis in $^{14}CO_2$ and 3000 ft-c White Light

The control was suspended in 0.001 M phosphate. The 0.01 M OH-PMSulfonate designated in the figure represents the initial concentration of inhibitor added to the 1 % algal suspension. The final concentration was 0.001 M and conditions were those of normal photosynthesis.

A portion of the percent distribution data in Table 4 of the Appendix was obtained from these chromatograms.

Figure 8

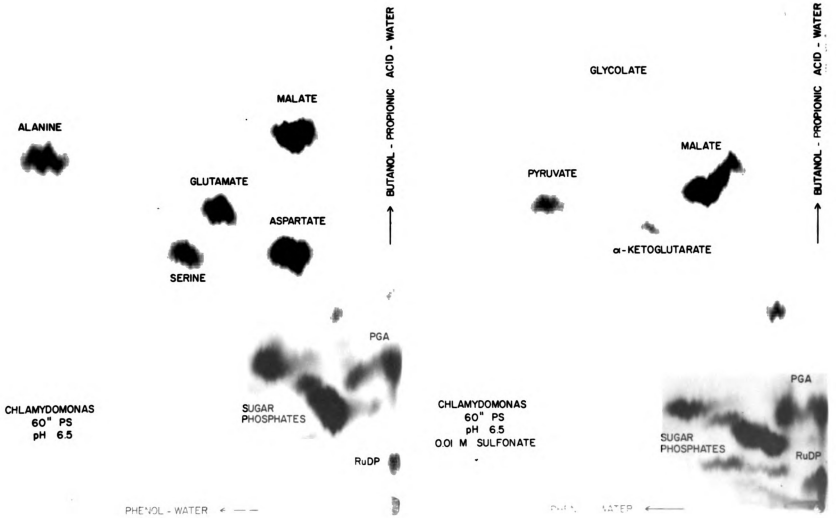

ALANINE
MALATE
GLUTAMATE
ASPARTATE
SERINE
BUTANOL - PROPIONIC ACID - WATER
PGA
SUGAR PHOSPHATES
RuDP
CHLAMYDOMONAS
60" PS
pH 6.5
PHENOL - WATER
GLYCOLATE
PYRUVATE
MALATE
α-KETOGLUTARATE
BUTANOL - PROPIONIC ACID - WATER
PGA
SUGAR PHOSPHATES
RuDP
CHLAMYDOMONAS
60" PS
pH 6.5
0.01 M SULFONATE

Figure 10

Total Fixation of $^{14}CO_2$ by *Chlamydomonas reinhardtii* at pH 7.6

A 1 % algal suspension at pH 7.6 in 0.001 M phosphate buffer. Preincubation time was 5 min in the light with and without 0.001 M OH-PMSulfonate.

Closed symbols - Control (0.001 M phosphate)

Open symbols - Inhibitor (0.001 M OH-PMSulfonate)

Figure 10

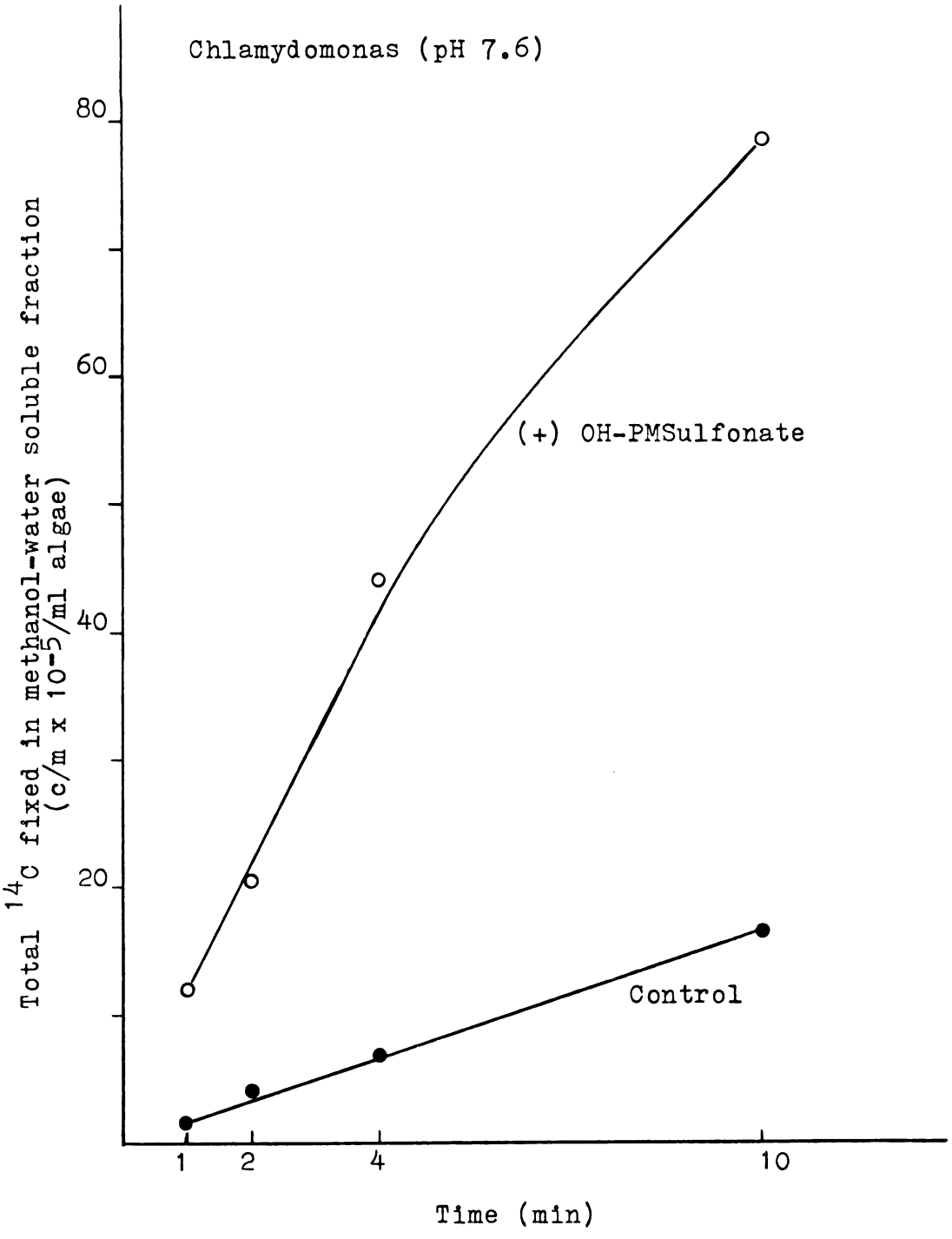

Chlamydomonas (pH 7.6)
80
60
40
20
Total ^{14}C fixed in methanol-water soluble fraction (c/m x 10^{-5}/ml algae)
(+) OH-PMSulfonate
Control
1
2
4
10
Time (min)

Figure 11

Percent Distribution of ^{14}C in Some Products in the Methanol-Water Soluble Fraction Formed by Chlamydomonas During Photosynthesis in $^{14}CO_2$

Figure 11

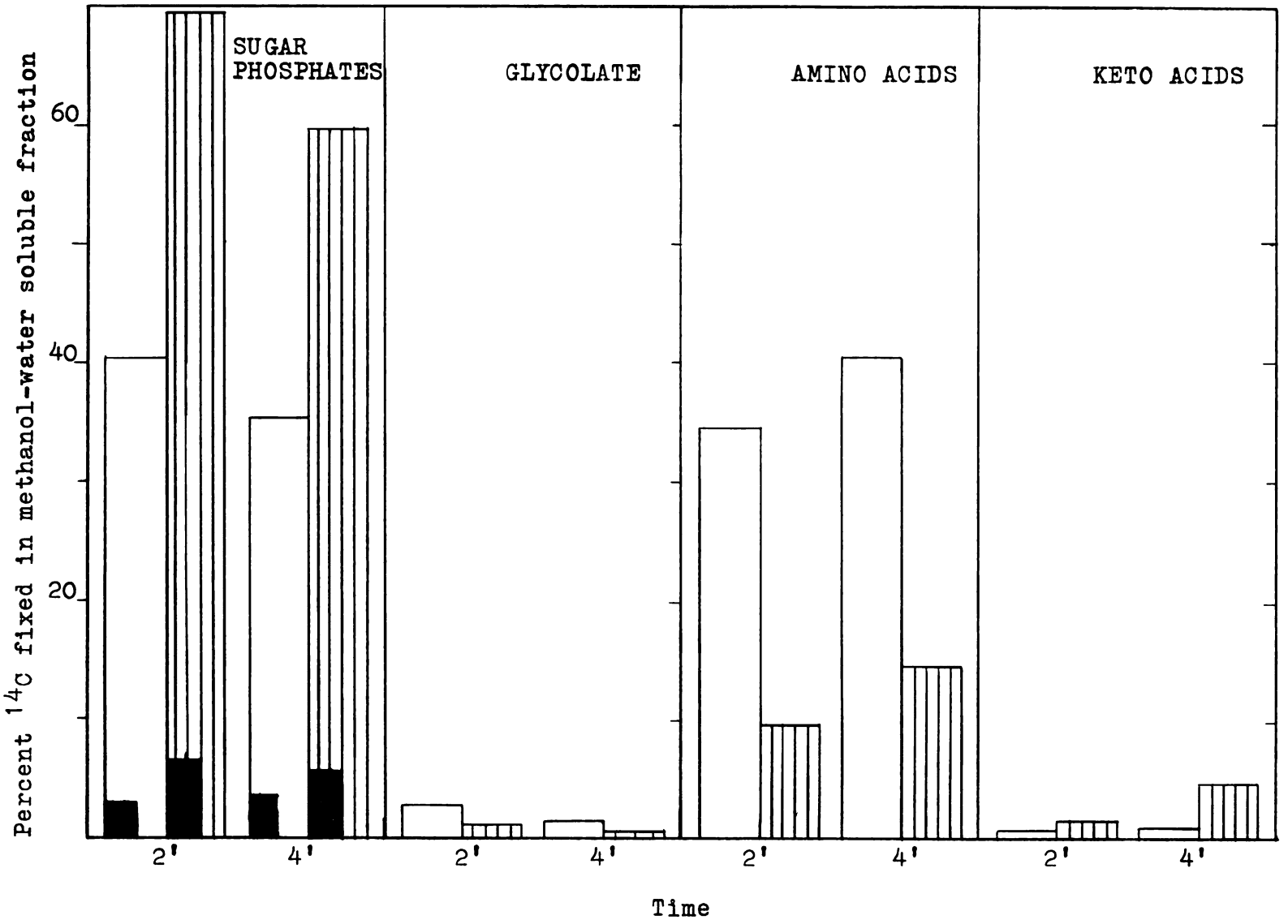

SUGAR PHOSPHATES
GLYCOLATE
AMINO ACIDS
KETO ACIDS
Percent ^{14}C fixed in methanol-water soluble fraction
60
40
20
2'
4'
2'
4'
2'
4'
2'
4'
Time

Figure 12

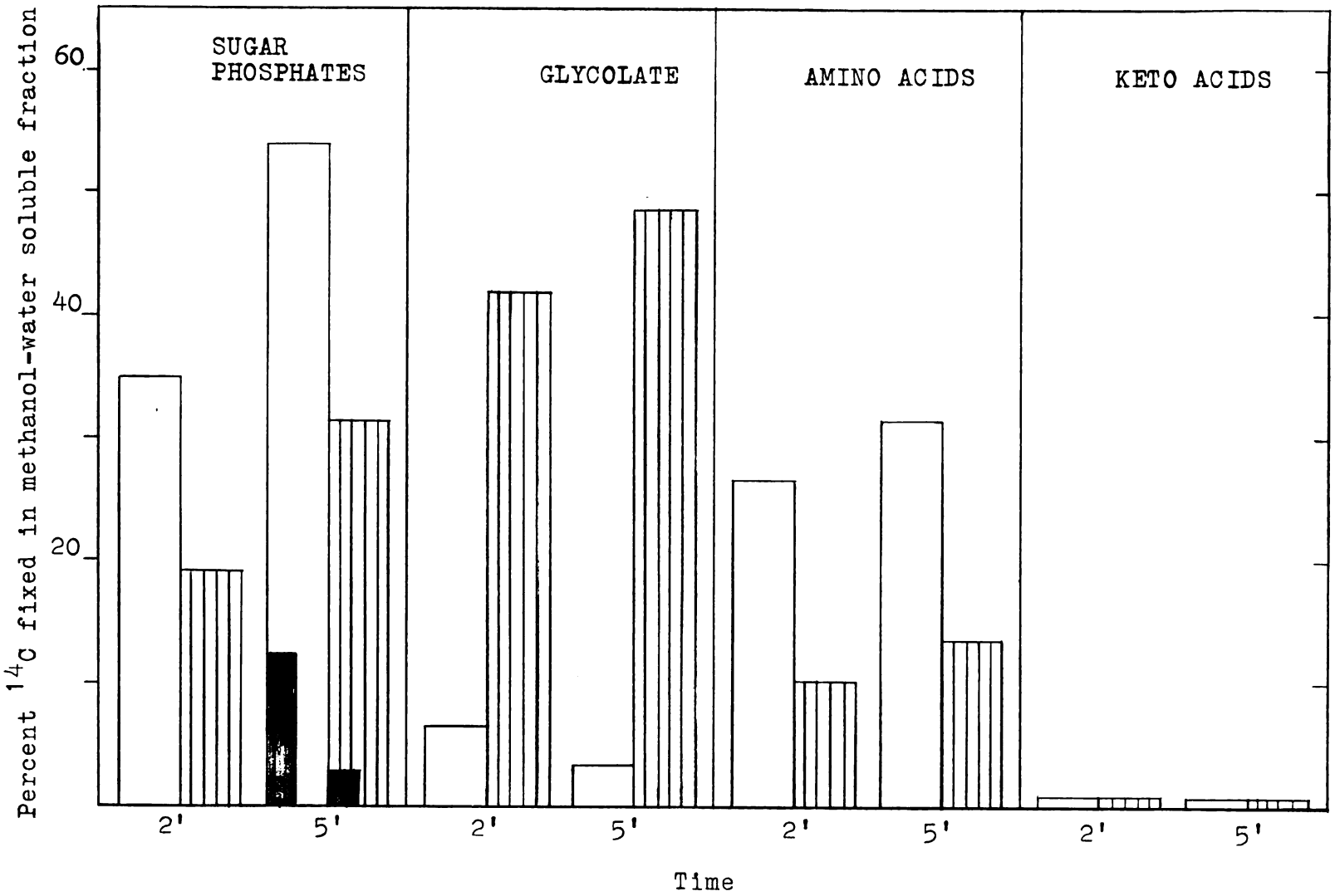
Percent Distribution of ^{14}C in Some Products in the Methanol-Water Soluble Fraction Formed by Tobacco Leaves During Photosynthesis in $^{14}CO_2$

Control - Pretreatment 1 hr with water.

Inhibitor - Pretreatment 1 hr with 0.01 M OH-PMSulfonate at pH 3.5.

^{14}C Fixed in Sugar Diphosphates.

Figure 12


SUGAR PHOSPHATES
GLYCOLATE
AMINO ACIDS
KETO ACIDS
Percent ^{14}C fixed in methanol-water soluble fraction
60
40
20
2'
5'
2'
5'
2'
5'
2'
5'
Time

OH-PMSulfonate, which indicate the large accumulation of glycolate-^{14}C after 30 seconds, as well 300 seconds (Figure 8), while no increase in glycolate-^{14}C was observed in Chlamydomonas even after 60 seconds photosynthesis (Figure 9).

A significant inhibition of labeled amino acid formation probably resulted from a block in the glycolate pathway in the tobacco experiments; in algae, the sulfonate apparently blocked the sites of transamination or perhaps glutamate dehydrogenase. This conclusion is supported by the large decrease of mainly glycine-^{14}C and serine-^{14}C formed by tobacco leaves with OH-PMSulfonate, and the accumulation of glycolate-^{14}C. In the algae, however, in the presence of the sulfonate the percent ^{14}C in alanine, aspartate, and glutamate was more dramatically reduced than that in serine and glycine. The OH-PMSulfonate in algae also caused an increased accumulation of ^{14}C in the keto acids, pyruvate and α-ketoglutarate; this effect was not observed in tobacco.

It can be concluded from the above data that the "specific" glycolate oxidase inhibitor described by Zelitch (134,137) functions at sites other than the oxidase. These data from Chlamydomonas, showing no glycolate accumulation in the presence of OH-PMSulfonate, can be interpreted to imply that glycolate oxidase and the glycolate pathway are absent in these algae.

A more thorough investigation of the effects of OH-PMSulfonate on algae during shorter periods of photosynthesis in bicarbonate

was undertaken. The results of these experiments are summarized under four general headings: (a) the effect of sulfonates on total fixation; (b) the effect of sulfonates on glycolate formation; (c) the effect of sulfonates on amino acid formation; (d) the effect of sulfonates on the sugar diphosphates.

(a) In the presence of the OH-PMSulfonate, $^{14}CO_2$ fixation during photosynthesis is rapid and slightly inhibited at pH 6.5 (Figure 13). On the other hand, the rate of CO_2 fixation is normally reduced at pH 8.3 (81), but the addition of OH-PMSulfonate to the Chlamydomonas stimulated the rate of CO_2 fixation to that which existed at pH 6.5 even though the pH was maintained at 8.3 (Figure 14). The reason for stimulation is not known.

(b) There was no accumulation of glycolate-^{14}C in the presence of the OH-PMSulfonate (Figures 15 and 16) at pH 6.5, or even at the alkaline pH of 8.3 which favors glycolate formation in algae (81). These results are to be compared with the previous data from tobacco leaves where the addition of the sulfonate resulted in the accumulation of large amounts of glycolate.

(c) ^{14}C incorporation into the amino acids was severely inhibited by OH-PMSulfonate at either pH 6.5 or 8.3. Correspondingly ^{14}C labeled keto acids appeared on the chromatograms, although these keto acids normally do not accumulate in sufficient amounts to be readily detectable. Thus, in the presence of the

Figure 13

Total Fixation of $^{14}CO_2$ by Chlamydomonas reinhardtii at pH 6.5

Standard photosynthesis experiment with 1 % algae suspended in 0.001 M phosphate at pH 6.5. Preincubation time was 5 min in the light with and without 0.001 M OH-PMSulfonate.

Closed symbols - Control (0.001 M phosphate)

Open symbols - Inhibitor (0.001 M OH-PMSulfonate)

Figure 13

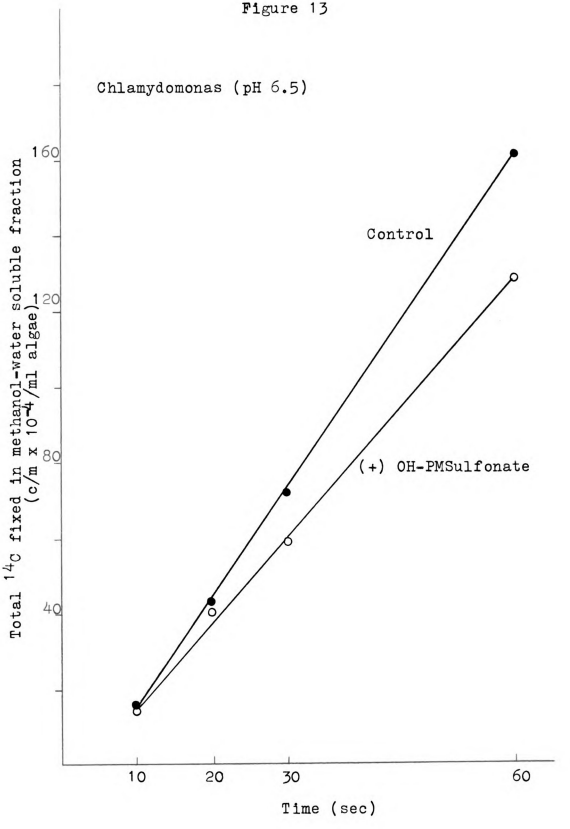

Chlamydomonas (pH 6.5)
Total ^{14}C fixed in methanol-water soluble fraction (c/m x 10^{-4}/ml algae)
160
120
80
40
Control
(+) OH-PMSulfonate
10
20
30
60
Time (sec)

Figure 14

Total Fixation of $^{14}CO_2$ by Chlamydomonas reinhardtii at pH 8.3

Standard photosynthesis epxeriment with 1 % algae suspended in 0.001 M phosphate at pH 8.3. Preincubation time was 5 min in the light with and without 0.001 M OH-PMSulfonate.

Closed symbols - Control (0.001 M phosphate)

Open symbols - Inhibitor (0.001 M OH-PMSulfonate)

Figure 14

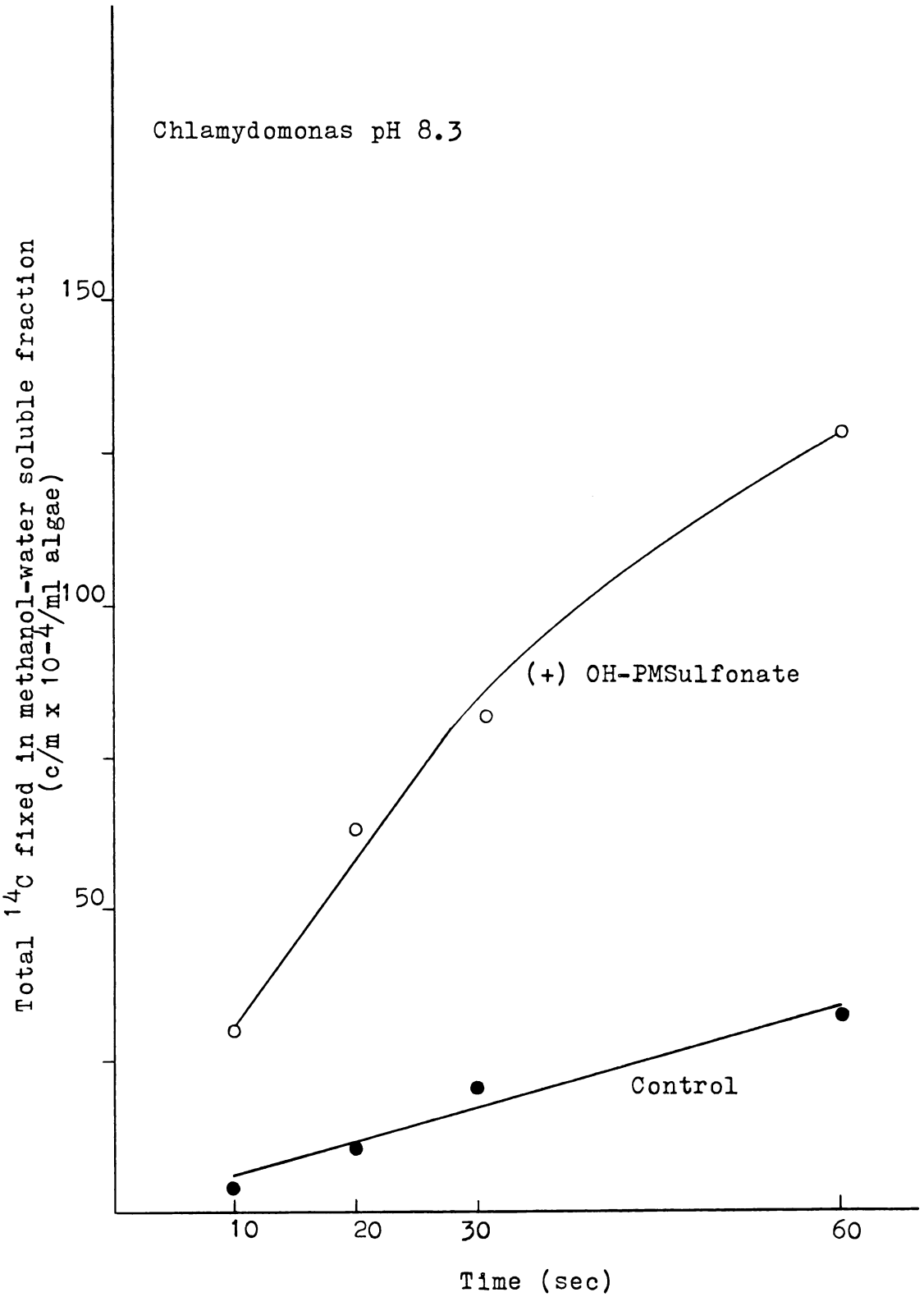

Chlamydomonas pH 8.3
Total ^{14}C fixed in methanol-water soluble fraction (c/m x 10-4/ml algae)
150
100
50
(+) OH-PMSulfonate
Control
10
20
30
60
Time (sec)

Figure 15

Percent Distribution of ^{14}C in PGA, Glycolate, and Amino Acids Formed by Chlamydomonas at pH 6.5

Standard photosynthesis experiment at pH 6.5 in $^{14}CO_2$.

Closed symbols - Control (0.001 M phosphate).

Open symbols - Inhibitor (0.001 M OH-PMSulfonate).

Figure 15

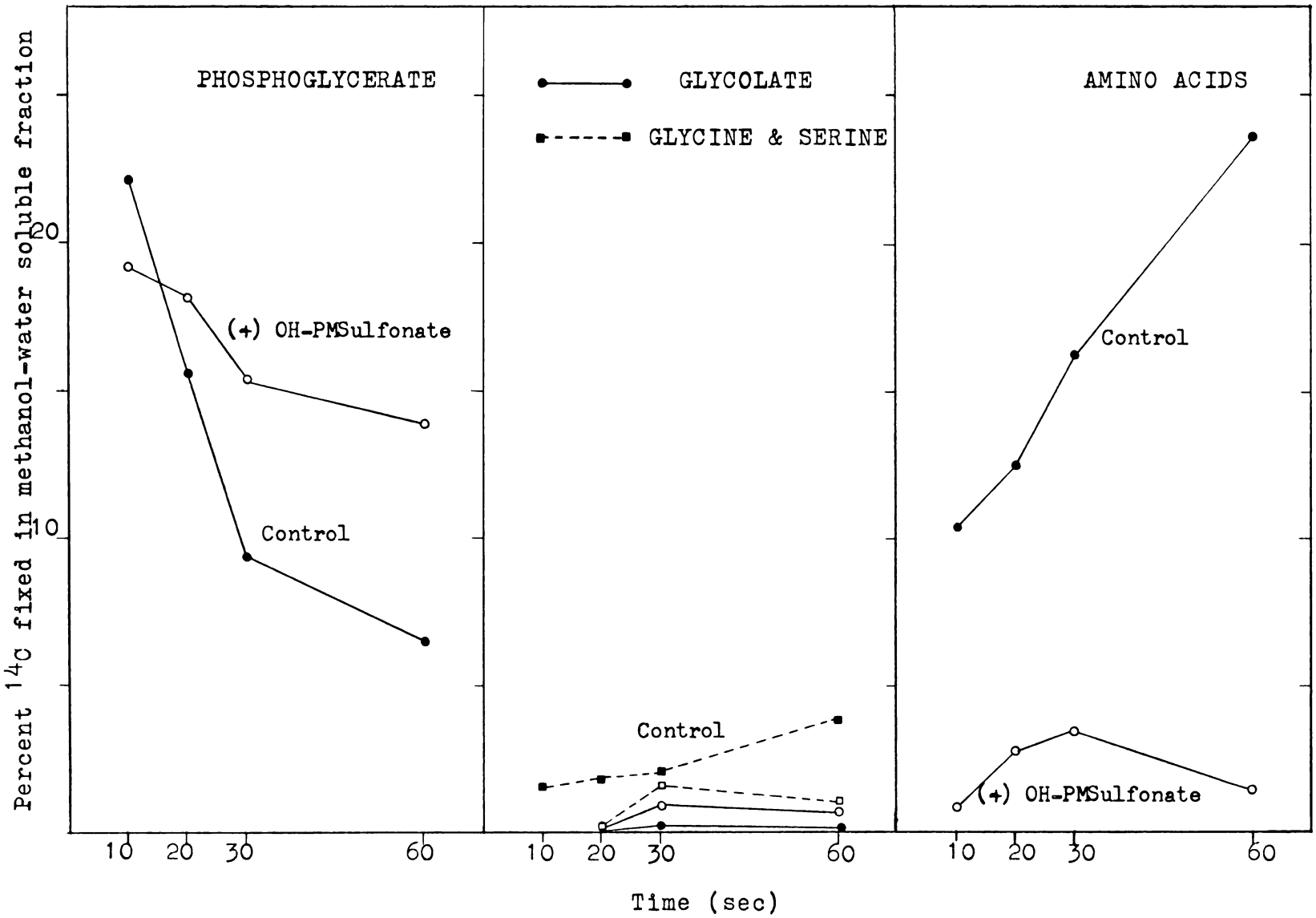

PHOSPHOGLYCERATE
GLYCOLATE
GLYCINE & SERINE
AMINO ACIDS
Percent ^{14}C fixed in methanol-water soluble fraction
20
10
(+) OH-PMSulfonate
Control
Control
Control
(+) OH-PMSulfonate
10 20 30 60
10 20 30 60
10 20 30 60
Time (sec)

Figure 16

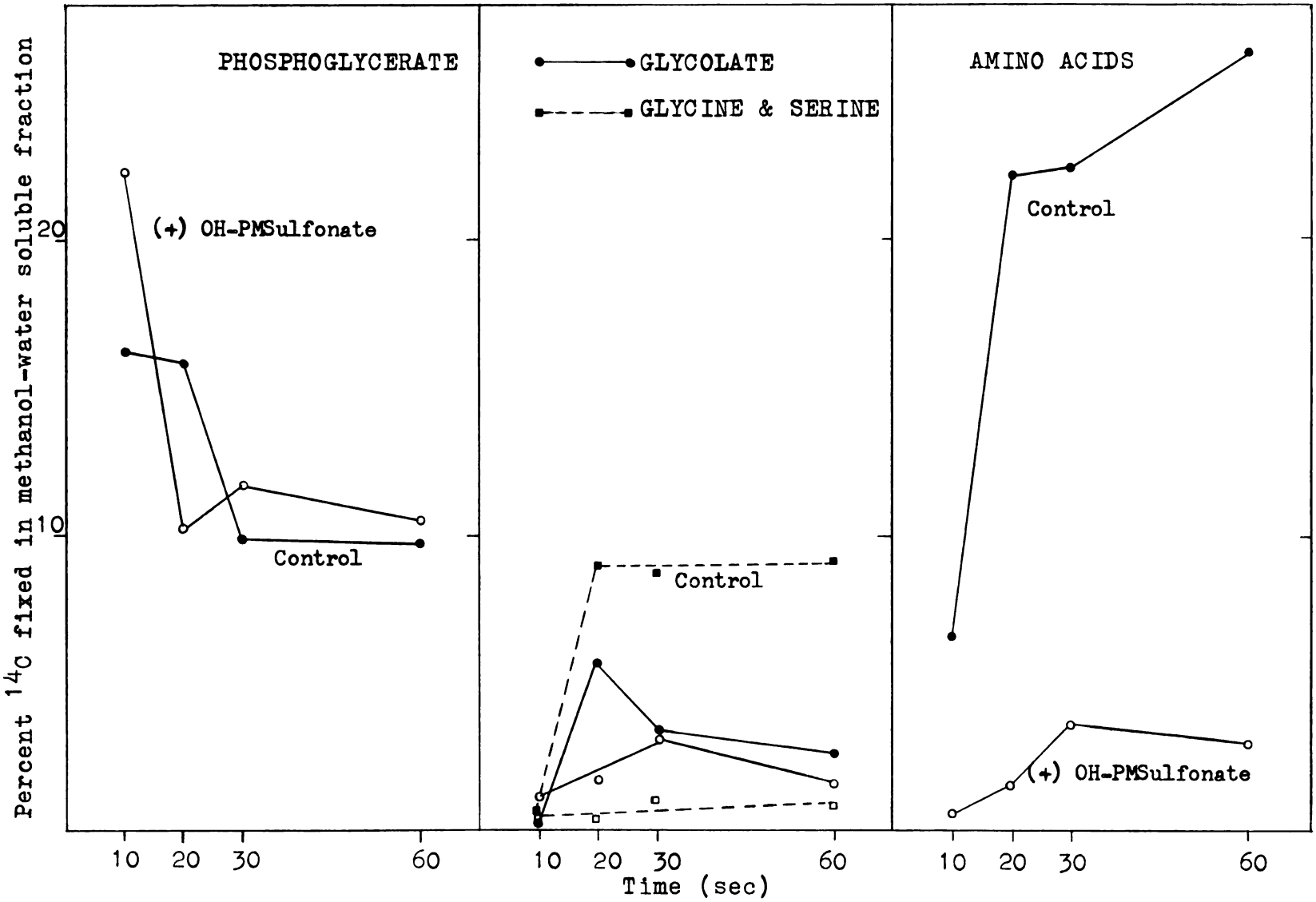
Percent Distribution of ^{14}C in PGA, Glycolate, and Amino Acids Formed by Chlamydomonas at pH 8.3

Standard photosynthesis experiment at pH 8.3 in $^{14}CO_2$.

Closed symbols - Control (0.001 M phosphate)

Open symbols - Inhibitor (0.001 M OH-PMSulfonate)

Figure 16


PHOSPHOGLYCERATE
GLYCOLATE
GLYCINE & SERINE
AMINO ACIDS
(+) OH-PMSulfonate
Control
Control
Control
(+) OH-PMSulfonate
Percent ^{14}C fixed in methanol-water soluble fraction
20
10
10 20 30 60
10 20 30 60
10 20 30 60
Time (sec)

sulfonate inhibitor, ^{14}C in alanine was much less, while pyruvate was strongly labeled; similarly, glutamate-^{14}C was labeled less and α-ketoglutarate-^{14}C more.

(d) A large accumulation of sugar diphosphates occurred in the presence of OH-PMSulfonate (Figure 17). This unusually large amount of diphosphate accumulated at both pH 6.5 and pH 8.3. Phosphatase hydrolysis of these sugar diphosphate areas from the chromatograms and rechromatography indicated that the radioactive material was predominantly ribulose-1,5-diphosphate.

Since the results with Chlamydomonas and Chlorella (data for Chlorella not presented) in the presence of OH-PMSulfonate were both unexpected and striking, they were further extended by repeating similar experiments with hydroxymethanesulfonate (OH-MSulfonate) another of the sulfonate derivatives which inhibits glycolate oxidase (134). The OH-MSulfonate derivative was chosen because it was not a pyridine analog and its use might have differentiated between effects caused by the pyridine moiety and those caused by the α-hydroxysulfonate moiety.

Results with OH-MSulfonate are presented in Figures 18 through 22 and are essentially similar to the four main effects on algal metabolism obtained with OH-PMSulfonate. (a) This OH-MSulfonate apparently had no effect at pH 6.5 (Figure 18), but stimulated CO_2 fixation at pH 8.1 (Figure 19). This stimulation restored the rate of fixation at pH 8.1 to that which normally occurs at pH 6.5. (b) There was no accumulation of glycolate-^{14}C

Figure 17

Total ^{14}C in Ribulose-1,5-diphosphate Formed by Chlamydomonas

Standard photosynthesis experiments at pH 6.8 or 8.3 in $^{14}CO_2$.

Closed symbols - Control (0.001 M phosphate)

Open symbols - Inhibitor (0.001 M OH-PMSulfonate)

Figure 17

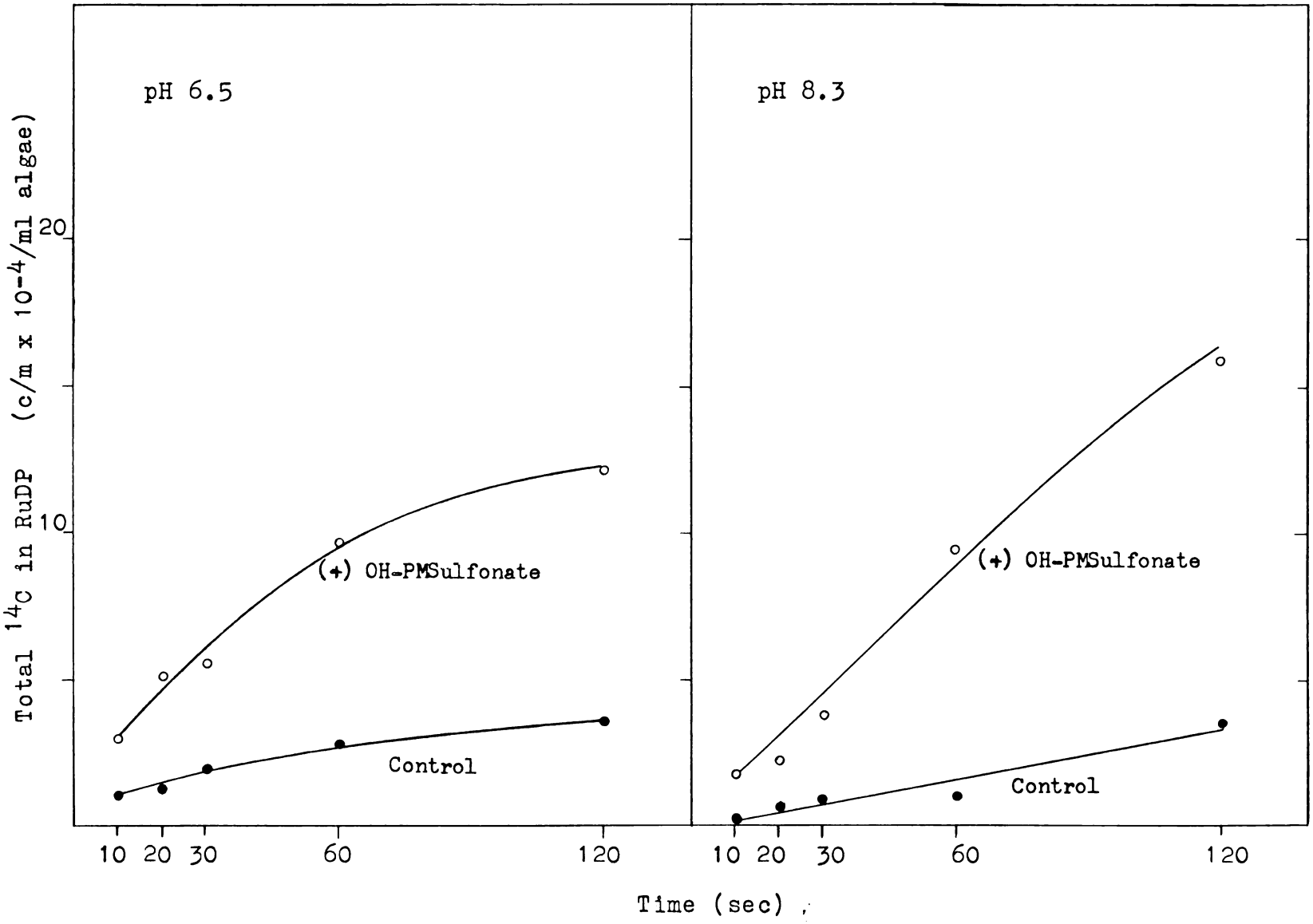

pH 6.5
pH 8.3
Total ^{14}C in RuDP (c/m x 10^{-4}/ml algae)
20
10
(+) OH-PMSulfonate
Control
(+) OH-PMSulfonate
Control
10 20 30 60 120
10 20 30 60 120
Time (sec)

Figure 18

Total Fixation of $^{14}CO_2$ by Chlamydomonas reinhardtii at pH 6.5

Standard photosynthesis experiment with 1 % algae suspended in 0.001 M phosphate at pH 6.5. Preincubation time was 5 min in the light with and without 0.001 M OH-methanesulfonate.

Closed symbols - Control (0.001 M phosphate)

Open symbols - Inhibitor (0.001 M OH-MSulfonate)

Figure 18

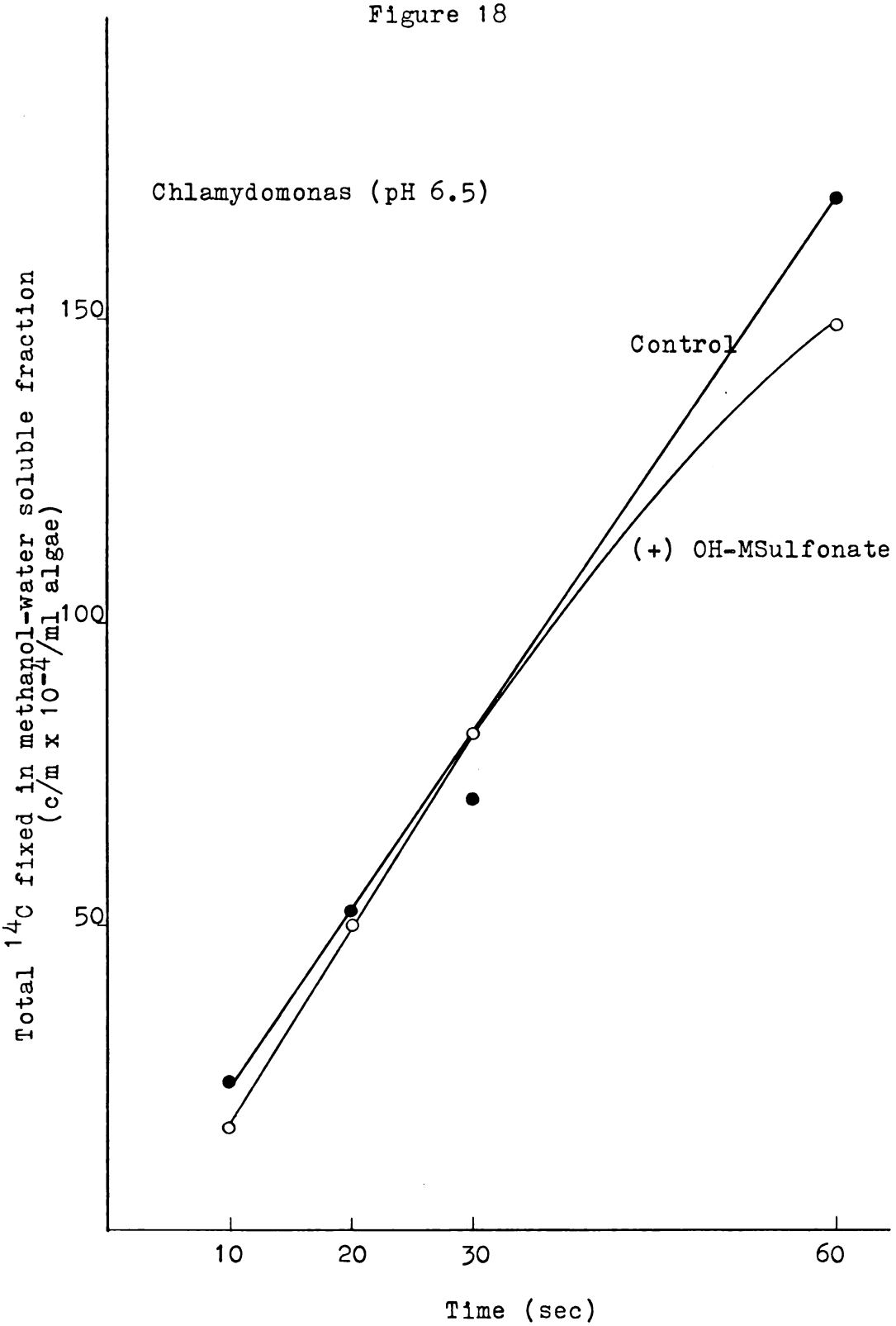

Chlamydomonas (pH 6.5)
Control
(+) OH-MSulfonate
Total ^{14}C fixed in methanol-water soluble fraction (c/m x 10^{-4}/ml algae)
150
100
50
10
20
30
60
Time (sec)

Figure 19

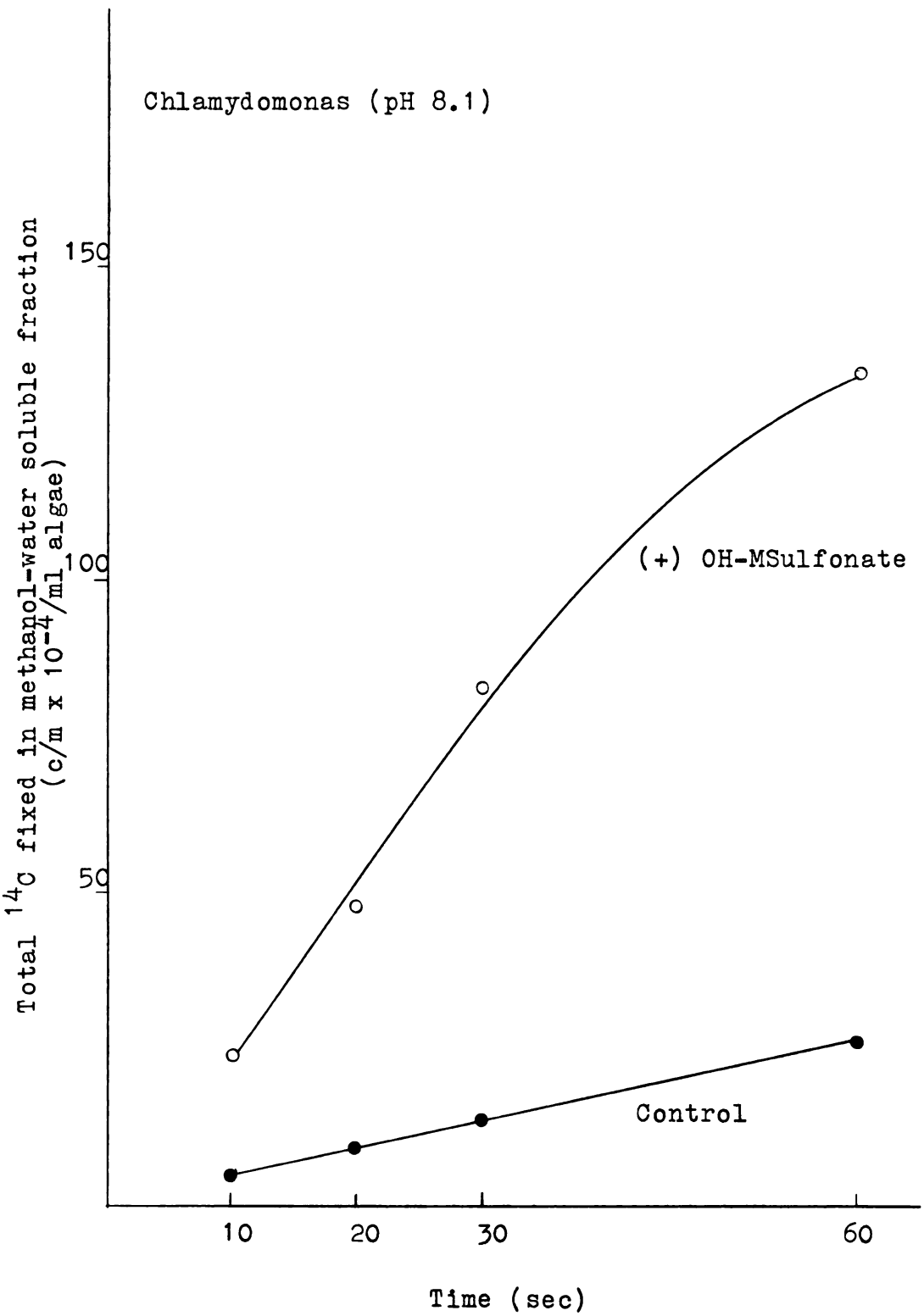
Total Fixation of $^{14}CO_2$ by Chlamydomonas reinhardtii at pH 8.1

Standard photosynthesis experiment with 1 % algae suspended in 0.001 M phosphate at pH 8.1. Preincubation time was 5 min in the light with and without 0.001 M OH-methanesulfonate.

Closed symbols - Control (0.001 M Phosphate)

Open symbols - Inhibitor (0.001 M OH-MSulfonate)

Figure 19


Chlamydomonas (pH 8.1)
Total ^{14}C fixed in methanol-water soluble fraction (c/m x 10^{-4}/ml algae)
150
100
50
(+) OH-MSulfonate
Control
10
20
30
60
Time (sec)

at either pH (Figures 20 and 21). Again this observation stands in direct opposition to the data expected if glycolate oxidase were inhibited. (c) The inhibition of amino acid formation was more pronounced at pH 8.1 than at pH 6.5 (Figures 20 and 21), and this inhibition suggested that the pyridine moiety of OH-PMSulfonate was not primarily responsible for this effect. (d) A linear accumulation of ^{14}C in ribulose-1,5-diphosphate occurred in the presence of the OH-MSulfonate (Figure 22); thus, reinforcing the similar observation of OH-PMSulfonate's effect on algae.

Additional data demonstrating the unusual accumulation of ribulose-1,5-diphosphate-^{14}C is presented in Figure 23. The linear accumulation is shown to be a function of the high constant percent ^{14}C in the labeled diphosphate and the increasing total fixation of $^{14}CO_2$ by Chlamydomonas. This abnormally high percent of ^{14}C in ribulose-1,5-diphosphate and the sugar phosphates in general (Figure 11), and the decreased rate at which the percent of ^{14}C of 3-phosphoglycerate-^{14}C decreases at pH 6.5 (Figures 15 and 21) suggest that a site of α-hydroxysulfonate activity might exist within the reactions of the sugar phosphates. Much additional information and data, however, are required before confirming any relationships or interactions of these sulfonates and sugar phosphate metabolism.

Perhaps ribulose-1,5-diphosphate is involved in some reaction other than CO_2 fixation, for example, bicarbonate or CO_2 membrane

Figure 20

Percent Distribution of ^{14}C in PGA, Glycolate, and Amino Acids Formed by Chlamydomonas at pH 6.5

Standard photosynthesis experiment at pH 6.5 in $^{14}CO_2$.

Closed symbols - Control (0.001 M phosphate)

Open symbols - Inhibitor (0.001 M OH-MSulfonate)

Figure 20

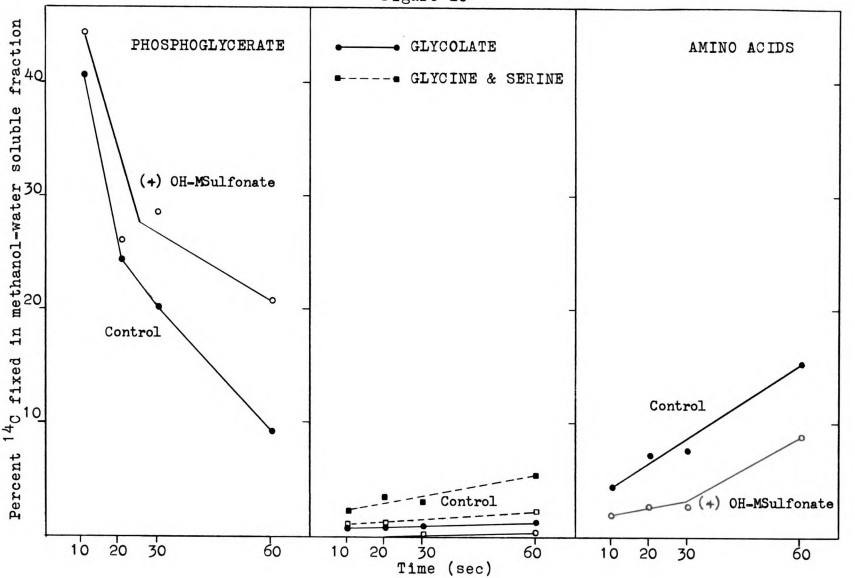

PHOSPHOGLYCERATE
GLYCOLATE
GLYCINE & SERINE
AMINO ACIDS
(+) OH-MSulfonate
Control
Control
Control
(+) OH-MSulfonate
Percent ^{14}C fixed in methanol-water soluble fraction
10
20
30
40
10 20 30 60
10 20 30 60
10 20 30 60
Time (sec)

Figure 21

Percent Distribution of ^{14}C in PGA, Glycolate, and Amino Acids Formed by Chlamydomonas at pH 8.1

Standard photosynthesis experiment at pH 8.1 in $^{14}CO_2$.

Closed symbols - Control (0.001 M phosphate)

Open symbols - Inhibitor (0.001 M OH-MSulfonate)

Figure 21

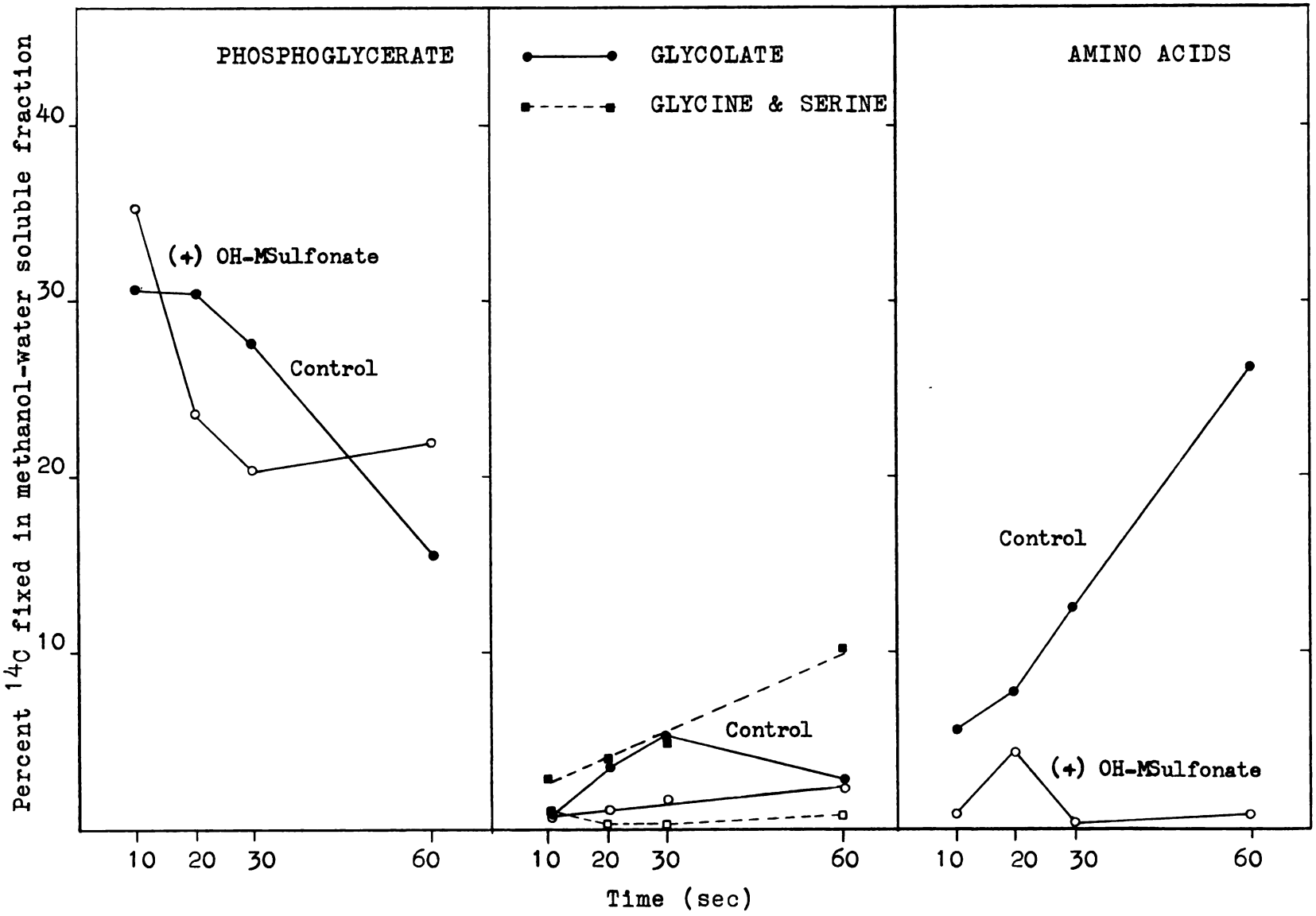

PHOSPHOGLYCERATE
GLYCOLATE
GLYCINE & SERINE
AMINO ACIDS
(+) OH-MSulfonate
Control
Control
Control
(+) OH-MSulfonate
Percent ^{14}C fixed in methanol-water soluble fraction
10
20
30
40
10
20
30
60
Time (sec)

Figure 22

Total ^{14}C in Ribulose-1,5-diphosphate Formed by Chlamydomonas

Standard photosynthesis experiments at pH 6.5 or 8.3 in $^{14}CO_2$.

Closed symbols - Control (0.001 M phosphate)

Open symbols - Inhibitor (0.001 M OH-MSulfonate)

Figure 22

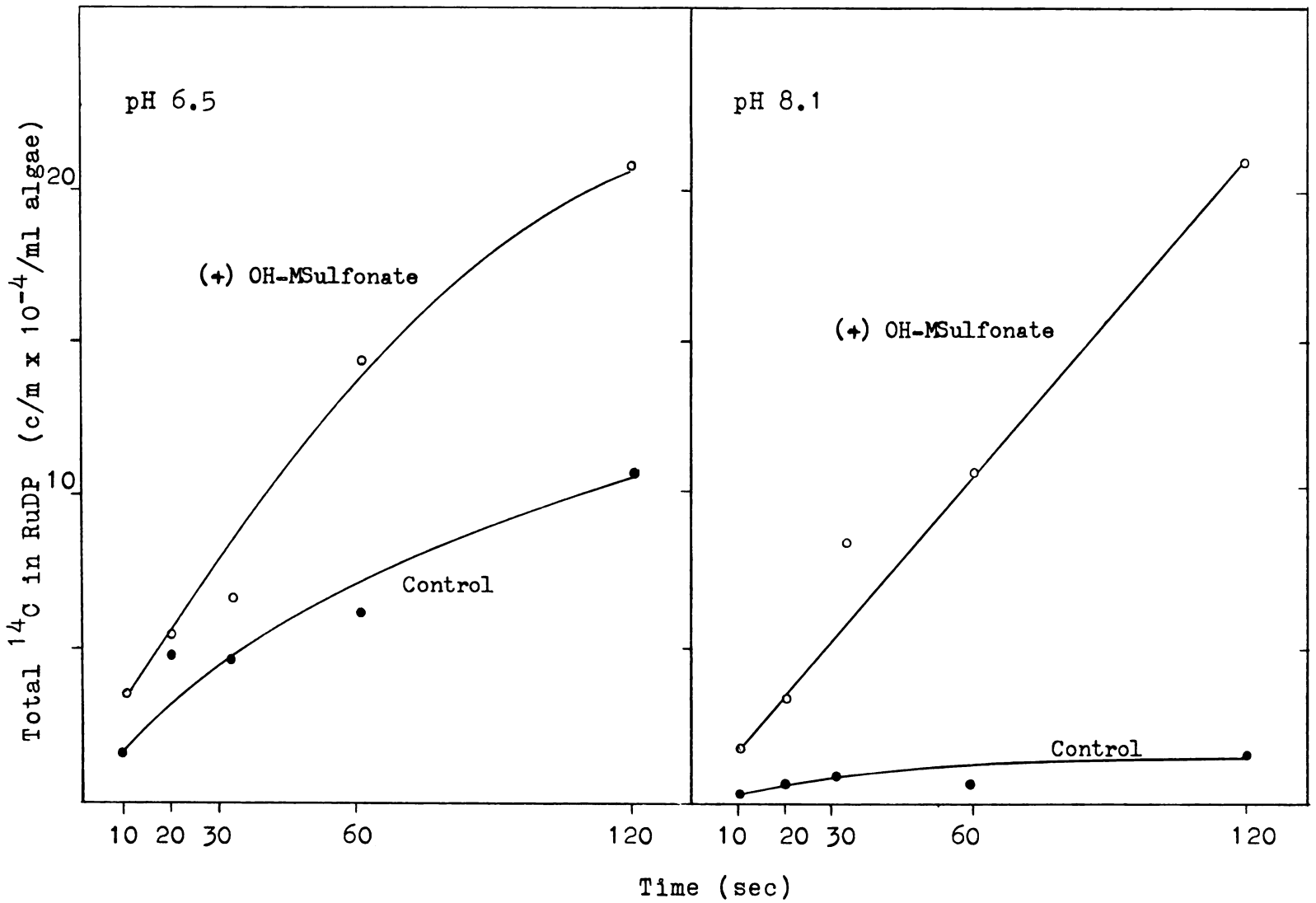

pH 6.5
pH 8.1
(+) OH-MSulfonate
(+) OH-MSulfonate
Control
Control
Total ^{14}C in RuDP (c/m x 10^{-4}/ml algae)
20
10
10 20 30 60 120
10 20 30 60 120
Time (sec)

Figure 23

Percent and Total ^{14}C in Ribulose-1,5-diphosphate formed by Chlamydomonas

Standard photosynthesis experiments at pH 8 in $^{14}CO_2$.

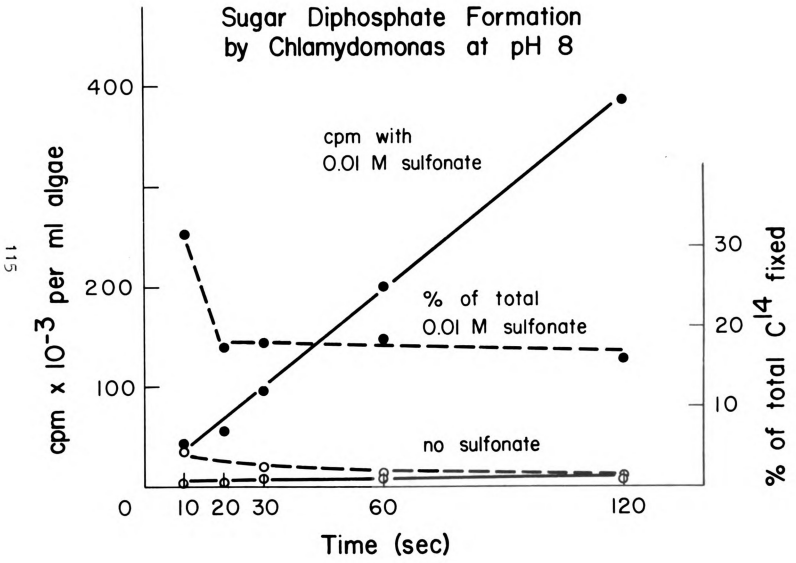
Open symbols - Control (0.001 M phosphate).

Closed symbols - Inhibitor (0.001 M OH-PMSulfonate).

----- Percent of the ^{14}C of the methanol-water fraction in RuDP.

_____ Total ^{14}C of the methanol-water fraction in RuDP.

Figure 23


Sugar Diphosphate Formation
by Chlamydomonas at pH 8
cpm with
0.01 M sulfonate
% of total
0.01 M sulfonate
no sulfonate
cpm x 10-3 per ml algae
% of total C14 fixed
Time (sec)
0
100
200
400
10
20
30
10 20 30
60
120

transport. The stimulated accumulation of ribulose-1,5-diphosphate-^{14}C at pH 8.3 was greater than the stimulation at pH 6.5 (Figures 17 and 22); thus, it is suggested that the excess sugar diphosphate might account for the increased total fixation observed at pH 8 (Figures 14 and 19). Further investigations, perhaps at various CO_2 concentrations are needed to completely evaluate this hypothesis.

Asada, et al. have reported that another of the hydroxysulfonates, Na-glyoxal-bisulfite, reduced the total CO_2 fixation by Chlorella ellipsoidea by 50 % (4). Besides the use of a different hydroxysulfonate, they also used a much higher concentration of bicarbonate (100 µM/3 ml) than was used in my experiments (less than 10 µM/15 ml). However, the differences in results and conditions merit further investigations.

The reasons for and the significance of, the four major observations on the effect of OH-PMSulfonate and OH-MSulfonate upon algal photosynthesis are not obvious. The inhibitor was used in all the algae experiments above pH 6, but the tobacco leaves transpired while in the 0.01 M inhibitor solution at pH 3.5. Since Zelitch has shown up to 60 % inhibition of glycolate oxidase at pH 8.3 (134), and that most likely the sulfonate within the plant cell was no longer at pH 3.5, the block in the glycolate pathway, caused by α-hydroxysulfonates will be observed at physiological pH values (4,137,139). Therefore the differences

between the effects of these inhibitors on tobacco and algae cannot be explained in terms of the pH difference of the α-hydroxysulfonate solutions.

The observed decrease in the amount of glycolate-^{14}C most likely reflects a block in its synthesis rather than an enhancement of its metabolism. This block in glycolate synthesis might be related to the accumulation of ribulose-1,5-diphosphate, since cleavage and oxidation of this diphosphate between carbons 2 and 3 could lead to phosphoglycolate formation. No phosphoglycolate was observed in any of our experiments with tobacco or algae, but it has been detected in both plants (11,137). Inefficient inactivation of the large excess of phosphoglycolate phosphatase (92,131) by methanol solutions has been reported by Ullrich (116). That little or no phosphoglycolate was observed in our experiments probably reflected the poor inactivation of this phosphatase.

The inhibition of amino acid formation during photosynthesis by the sulfonate derivatives should be further investigated. The inhibitor of B_6 (pyridoxal phosphate) transaminases by OH-PMSulfonate might be expected since both contain the pyridine moiety. However, the main reason for the inhibition of amino acid synthesis cannot be explained this way, since the OH-MSulfonate, containing no pyridine ring, was a very effective inhibitor of amino acid formation.

All the experiments, with both enzymes and inhibitors, provided no evidence that a glycolate oxidase, having any of the properties of the oxidase of higher plants, was present in algae. Tanner and Beevers (104) have just published the assumption that glycolate oxidase "present in algae" was responsible for the increased respiration rates in Chlorella which they observed in the presence of OH-PMSulfonate. Their assumption is clearly in error according to the data in this thesis, as well as the data showing this compound to be an inhibitor of glycolate dependent O_2 uptake (137). The observation, however, of increased respiration in algae merits further investigation before we can understand the mechanism of OH-PMSulfonate action.

The data presented here might also be compared with the work of Whittingham's group with Chlorella. In their work, isonicotinyl hydrazide caused glycolate accumulation in algae (84,85). They interpreted these results as a block in the glycolate pathway which prevents the conversion of glycine to serine. Yet they simultaneously observed increased sucrose production. These results can be better interpreted to suggest that the glycolate pathway in algae does not contribute to the synthesis of sucrose as it does in the higher plant. If this pathway were functioning, then the block in the pathway would cause an increase in glycolate (as was found with α-hydroxysulfonate inhibition in tobacco), but then the end-product, hexoses, should decrease (Figure 1). The simultaneous accumulation of ^{14}C in sucrose and

glycolate by Chlorella in the presence of the inhibitor supports an argument for a second autonomous pathway for sucrose synthesis unrelated to glycolate, and that both compounds are likely end-products of metabolism (125).

Asada, et al.(5) reported that inhibition of photosynthesis in spinach leaves by isonicotinylhydrazide caused the expected accumulation of ^{14}C in glycine, but none in glycolate. These data are consistent with the difference between algae and higher plant metabolism that I have found. In the higher plant glycolate does not accumulate, although the hydrazide might enhance its synthesis; because glycolate oxidase, not inhibited by isonicotinylhydrazide, causes the conversion of glycolate to glyoxylate to glycine. Alternatively, because algae lack a glycolate oxidase these latter reactions are impossible with the end result that glycolate accumulates.

There are two basic conclusions from these experiments. First, the α-hydroxysulfonate inhibitors are not specific for glycolate oxidase as stated previously. Their apparent specificity, however, in the higher plant might reflect the large amount of the oxidase present. Limited absorption and transport within higher plant tissue and the possible priority given to the use of the α-hydroxysulfonates for oxidase inhibition may prevent achieving concentrations sufficient for effecting amino acid labeling or for entering the chloroplast to alter sugar phosphate metabolism.

Second, and most important, a comparison of algal and higher plant metabolism as developed from these enzymatic, product degradation, and in vivo inhibition studies, reveals that algae do not have an active glycolate pathway which is typical of the higher plant. The algae, however, rapidly form and excrete glycolate during photosynthesis.

Thus, many unsolved problems remain in our understanding of the metabolic role that glycolate plays in algae and higher plant metabolism. If the function of glycolate in the higher higher plant is simply to move carbon from the chloroplast to the cytoplasm (106,107), then the reason for the excretion of glycolate to the medium by algae remains obscure. Glycolate excretion probably represents a more basic function necessary for maintaining the integrity of the algal cell or the chloroplast, for example, ion transport (107).

The difference in glycolate metabolism between algae and higher plants is also emphasized by the source of glycine in these organisms. The glycolate pathway in higher plants adequately accounts for sufficient quantities of uniformly labeled glycine (107). In algae, however, neither serine nor glycolate are likely candidates for glycine precursors, since serine does not account for the labeling pattern in glycine, and glycolate is not likely oxidized to glyoxylate by glycolate oxidase. Although Whittingham argues that the effect of isonicotinylhydrazide

inhibits the glycine-serine reaction as a B_6 inhibitor (89, 124,125), he never considers that other B_6 requiring enzymes like transaminases for glycine (22) should also be inhibited. Perhaps glycine formation in algae proceeds via some yet unknown mechanism.

Environmental Factors Affecting Glycolate Production and Utilization by Algae:

The following series of experiments demonstrated that pigment changes and metabolic changes did occur when algae were cultured in limited regions of the spectrum, i.e., different light qualities. The results which support this hypothesis were obtained from measurements of growth rates, pigment absorption changes, and the percent distribution of ^{14}C in products formed during short time photosynthesis in various light qualities at various intensities.

Algae cultures, when removed from white light and put in predominately red or blue light (as described in the methods section) grew more slowly for 4 or 5 days before attaining a relatively constant, rapid growth rate which approximated that of the cultures in white light. Thus, Chlamydomonas and Chlorella in blue light grew slowly at first and then more rapidly, as measured by the culture's Absorbance at 680 mμ (Figures 24 and 25). All measurements were made on cultures which initially had similar cell populations as judged from

Figure 24

Growth Rates of a *Chlamydomonas reinhardtii* Culture in Blue Light (400-500 mμ)

Curve 1: First day of growth in blue light.

Curve 2: Second day of growth in blue light.

Curve 3: Fifth day of growth in blue light.

Curve 4: Average rate of growth after 10 days culture in blue light.

Figure 24

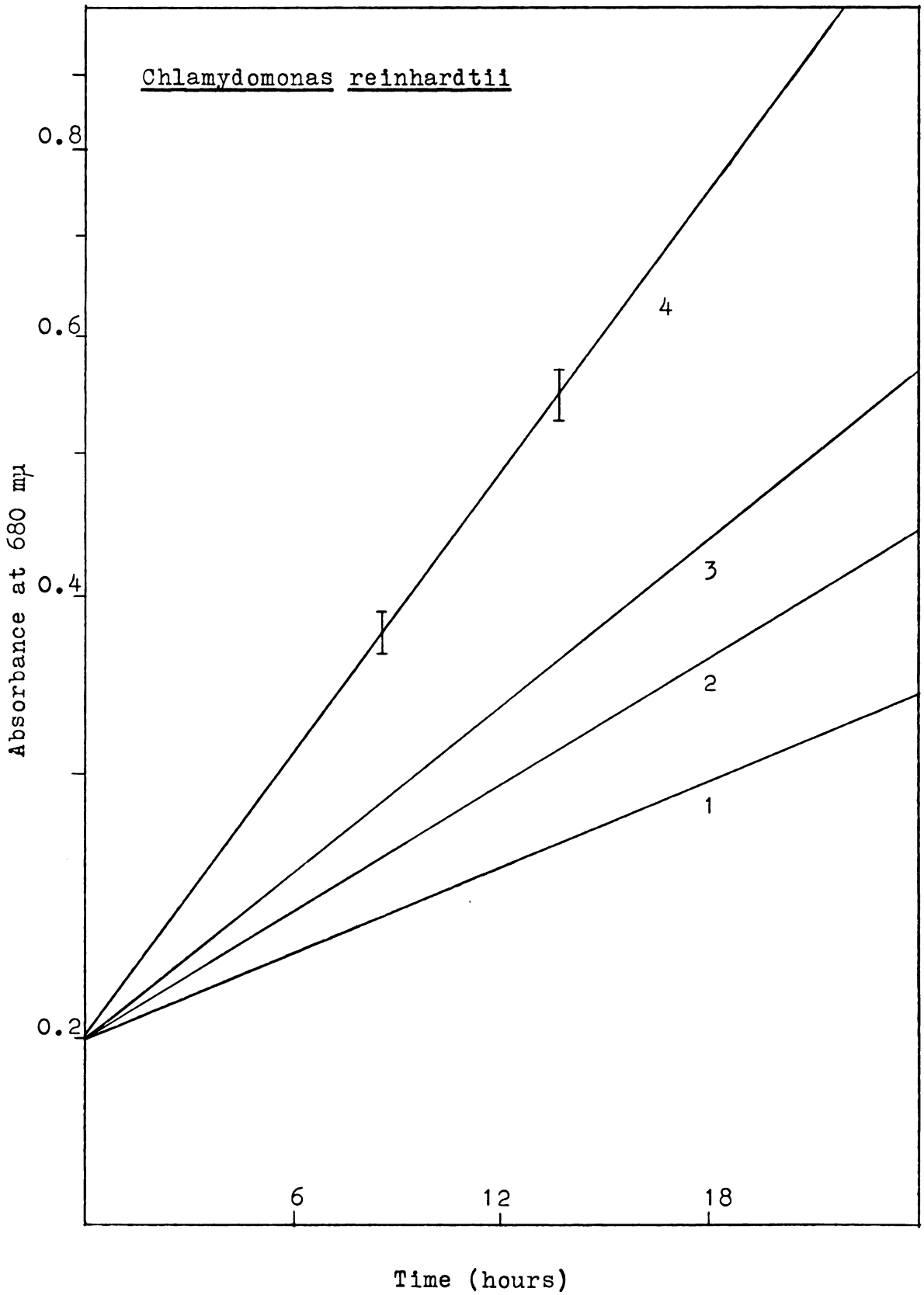

Chlamydomonas reinhardtii
0.8
0.6
0.4
0.2
Absorbance at 680 mμ
4
3
2
1
6
12
18
Time (hours)

Figure 25

Growth Rates of a Chlorella pyrenoidosa Culture in Blue Light (400-500 mμ)

Curve 1: First day of growth in blue light.

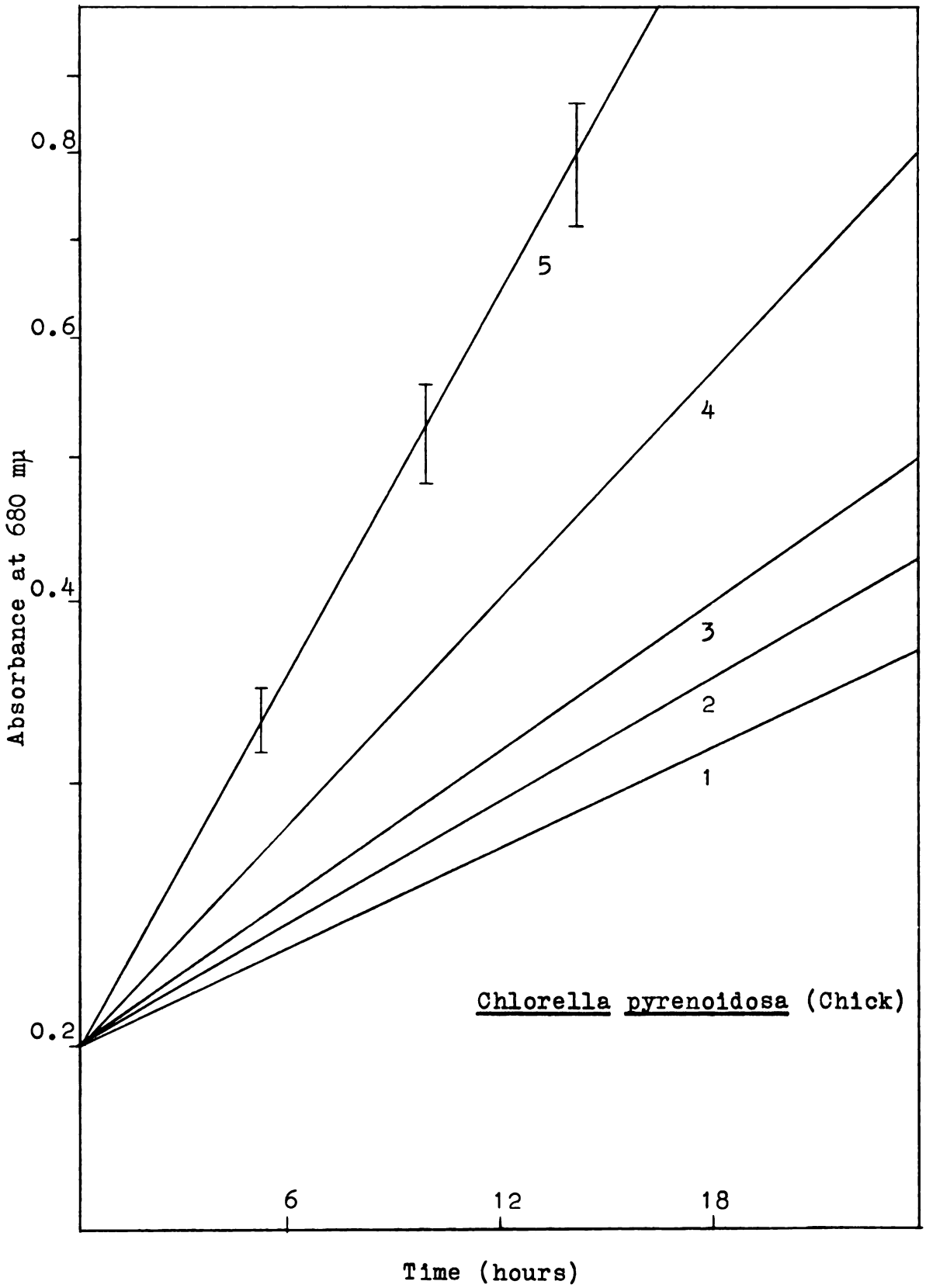
Curve 2: Second day of growth in blue light.

Curve 3: Third day of growth in blue light.

Curve 4: Fourth day of growth in blue light.

Curve 5: Average rate of growth after 10 days culture in blue light.

Figure 25


0.8
0.6
0.4
0.2
Absorbance at 680 mμ
5
4
3
2
1
Chlorella pyrenoidosa (Chick)
6
12
18
Time (hours)

approximately similar Absorbance values. Chlamydomonas grown in red light also grew more slowly at first, but after several days in the red light they also grew more rapidly.

During the first six days of culture in blue light there appeared a significant decrease in the chlorophyll a/b ratio when compared to the constant chlorophyll a/b ratio from cultures grown in white light. The values reported in Tables 12 and 13 are averages of two methanol extractions from the algae. Variation in total chlorophyll may be unexpectedly large because of the difficulty in accurately estimating the packed cell volume while using a constant for this volume in the calculations. The data in Table 12 were obtained from a different culture than those of Table 13, but the causes of the large difference in the absolute values of chlorophyll a/b ratios and total chlorophyll remain obscure. A consistent trend in these data, however, was the decrease in the chlorophyll a/b ratio during culture of the algae in blue light. The data from Chlamydomonas extracts were more consistent than data from Chlorella, perhaps, because of the greater difficulty of quantitatively extracting pigments from Chlorella. To emphasize the general reduction of the chlorophyll a/b ratio, these data are plotted in Figure 26.

This decrease in the chlorophyll a/b ratio could also be demonstrated by measurements on whole algae suspensions

Table 12: Chlorophyll Content of Cultures Grown in Blue Light

Amount of chlorophyll per ml packed cells after growth in blue light (400-500 mμ).

	Chlamydomonas		Chlorella	
Days of Culture in Blue Light	Chlorophyll mg/ml	Ratio a/b*	Chlorophyll mg/ml	Ratio a/b*
0	1.09	0.63	0.70	0.77
1	2.03	0.45	1.39	0.60
2	2.15	0.45	1.50	0.36
3	2.63	0.46	1.03	0.77
4	2.02	0.58	1.15	0.71
5	2.56	0.44	2.13	0.51

*Ratio of chlorophyll a/chlorophyll b

Table 13: Chlorophyll Content of Cultures Grown in Blue Light

Amount of chlorophyll per ml packed cells after growth in blue light (400-500 mμ).

	Chlamydomonas		Chlorella	
Days of Culture in Blue Light	Chloro-phyll mg/ml	Ratio a/b*	Chloro-phyll mg/ml	Ratio a/b*
6	1.55	2.09	1.32	1.88
7	1.83	1.87	1.64	2.01
8	1.54	2.15	1.83	2.38
9	1.69	1.87	1.94	2.11
10	--	--	1.35	1.49
11	1.64	1.65	--	--
12	1.53	1.61	1.15	1.83
13	1.82	1.58	1.20	1.86
Cells Grown in White Light	1.05	2.93	1.14	2.11

*Ratio of chlorophyll a/chlorophyll b

Figure 26

Chlorophyll a/b Ratio in Green Algae During Growth in Blue Light (400-500 mμ)

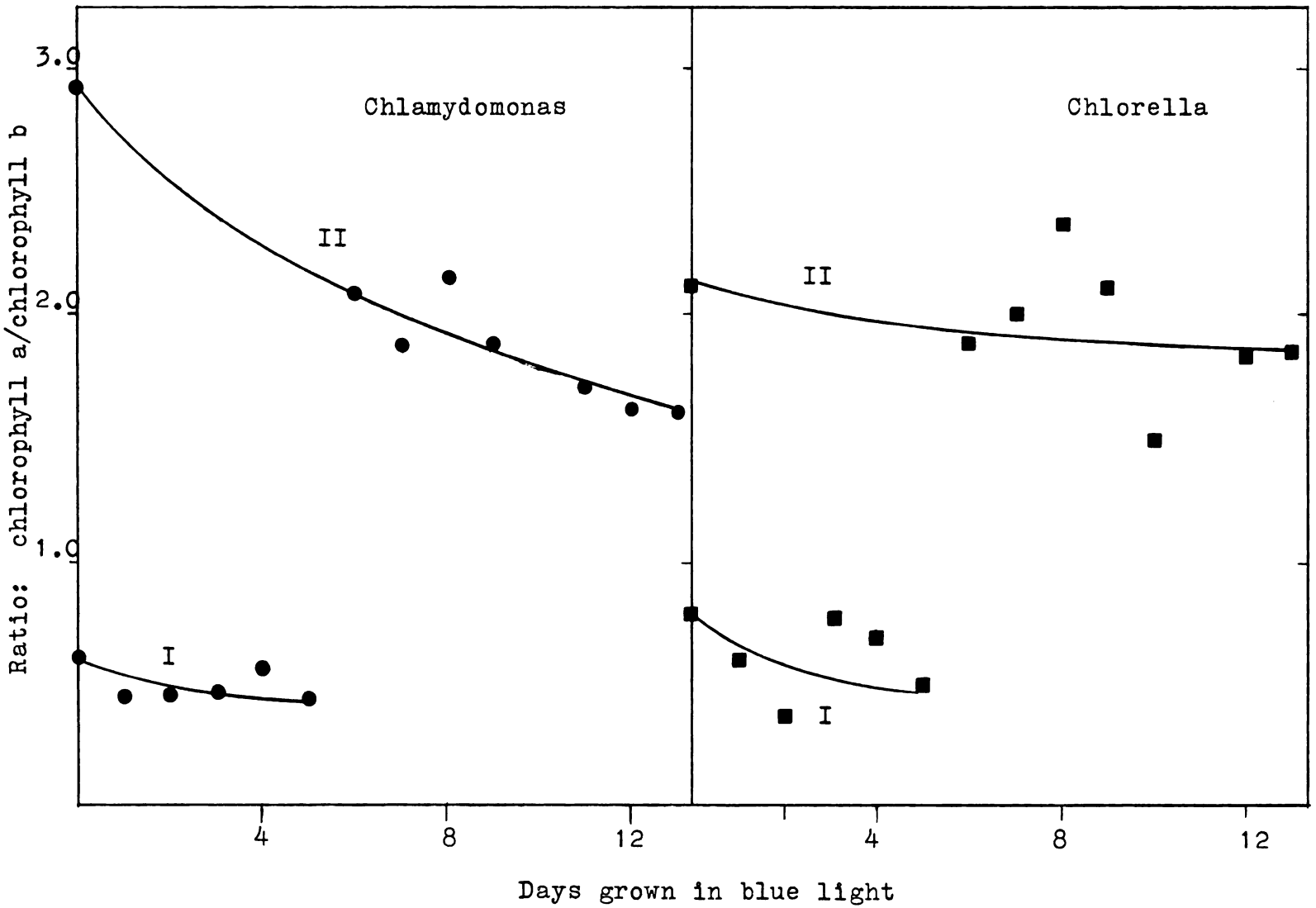
The data from Tables12 and 13 have been plotted to show that there was a consistent decrease in the a/b ratio as the cells continued to grow in the blue light (400-500 mμ).

●——● Chlamydomonas reinhardtii

■——■ Chlorella pyrenoidosa

Curve I: Data from Table 12. Curve II: Data from Table 13.

Figure 26


Chlamydomonas
Chlorella
II
II
I
I
3.0
2.0
1.0
4
8
12
4
8
12
Ratio: chlorophyll a/chlorophyll b
Days grown in blue light

(Figure 27). Using a Cary 15 recording spectrophotometer, absorption spectra were obtained according to Shibata's technique (96) modified as described in the methods section. The spectra in Figure 27 do have equal Absorbance values at 550 mμ, but, for clarity of presentation, the curves have been separated in the figure. The absorbance change of the 680 mμ/655 mμ ratio (*in vivo* maxima for chlorophylls *a* and *b* respectively) shows a significant *in vivo* decrease in the chlorophyll a/b ratio with length of culture in blue light. No significant changes in these regions of the spectrum were observed for algae grown in red light. Other regions of the spectrum did reflect absorption changes, but these were not consistent and too complicated for a careful evaluation by my techniques.

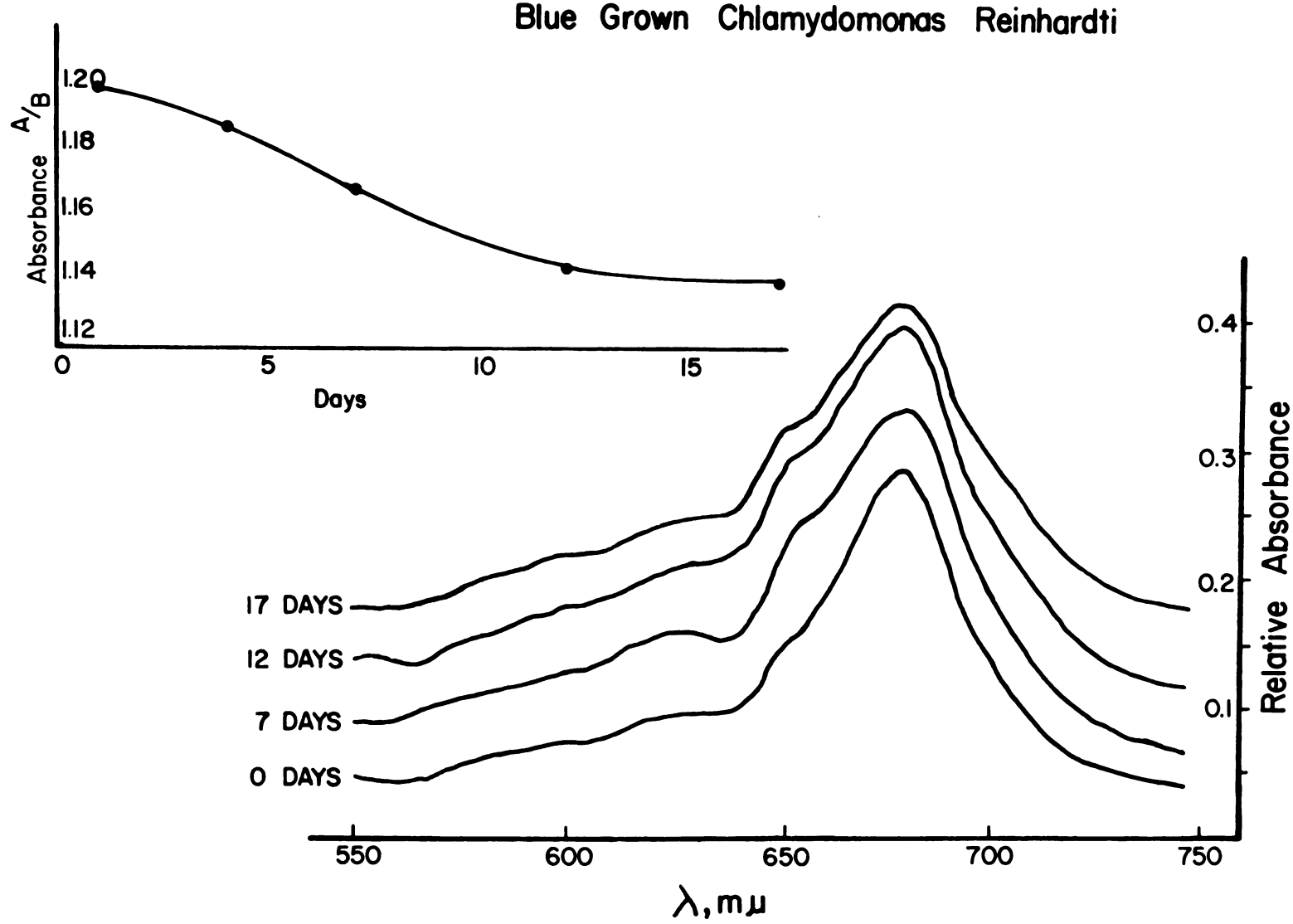
As indicated by data in Tables 8 and 9 of the Appendix, a change in the distribution of the ^{14}C labeled products of $^{14}CO_2$ fixation was apparent in algae after growth in blue light. In Chlamydomonas (Table 8, Appendix), the most significant change was related to a large increase in the percent of ^{14}C accumulated in glycolate, but this change was not observed until six days after growth in blue light. In Chlorella, accumulation of excess ^{14}C in serine occurred almost immediately after growth in the blue light was begun (Table 9, Appendix). At all times a consistently high percent of the

Figure 27

In Vivo Spectra of *Chlamydomonas reinhardtii* Grown in Blue Light (400-500 mμ)

The spectra were measured on approximately 1% algal suspensions and recorded with a Cary 15 Spectrophotometer. All absorbance values at 550 mμ were approximately equal. The Absorbance ratio a/b equals the Absorbance ratio 680 mμ/ 655 mμ which was considered a measure of the chlorophyll a/ chlorophyll b ratio.

Figure 27


Blue Grown Chlamydomonas Reinhardti
Absorbance A/B
1.20
1.18
1.16
1.14
1.12
0
5
10
15
Days
17 DAYS
12 DAYS
7 DAYS
0 DAYS
0.4
0.3
0.2
0.1
Relative Absorbance
550
600
650
700
750
λ, mμ

^{14}C was incorporated into serine as compared with the percent found in the control, and a gradual increase in the amount of ^{14}C-glycolate was observed, but this accumulation was not as large as that observed in Chlamydomonas.

In conclusion, both the pigment data and the fixation data (particularly that for Chlamydomonas) support an adaptation phenomenon of growth and photosynthesis to blue light. The most significant changes which I have observed were a decrease in the chlorophyll a/b ratio and an increase in the accumulation of ^{14}C in glycolate or serine during short periods of $^{14}CO_2$ fixation.

Using fully adapted Chlamydomonas (after at least 10 days growth in either red or blue light), kinetic photosynthesis experiments were performed in varying intensities of red light, blue light, or white light. All of the experiments demonstrated linear fixation rates (Tables 10, 11, and 12, Appendix). A significant difference was observed with algae grown in blue light. These algae rapidly accumulated a large percent of the newly fixed ^{14}C into glycolate during a 10 minute period in blue light (Figure 28). Concurrently there was a decrease in the percent of the ^{14}C in TCA cycle acids and the associated amino acids (mainly malate, aspartate and glutamate) with respect to algae grown in red light. Apparently the amount of ^{14}C in the sugar phosphate reservoirs in Chlamydomonas adapted to blue light was much smaller than in algae adapted to red

Figure 28

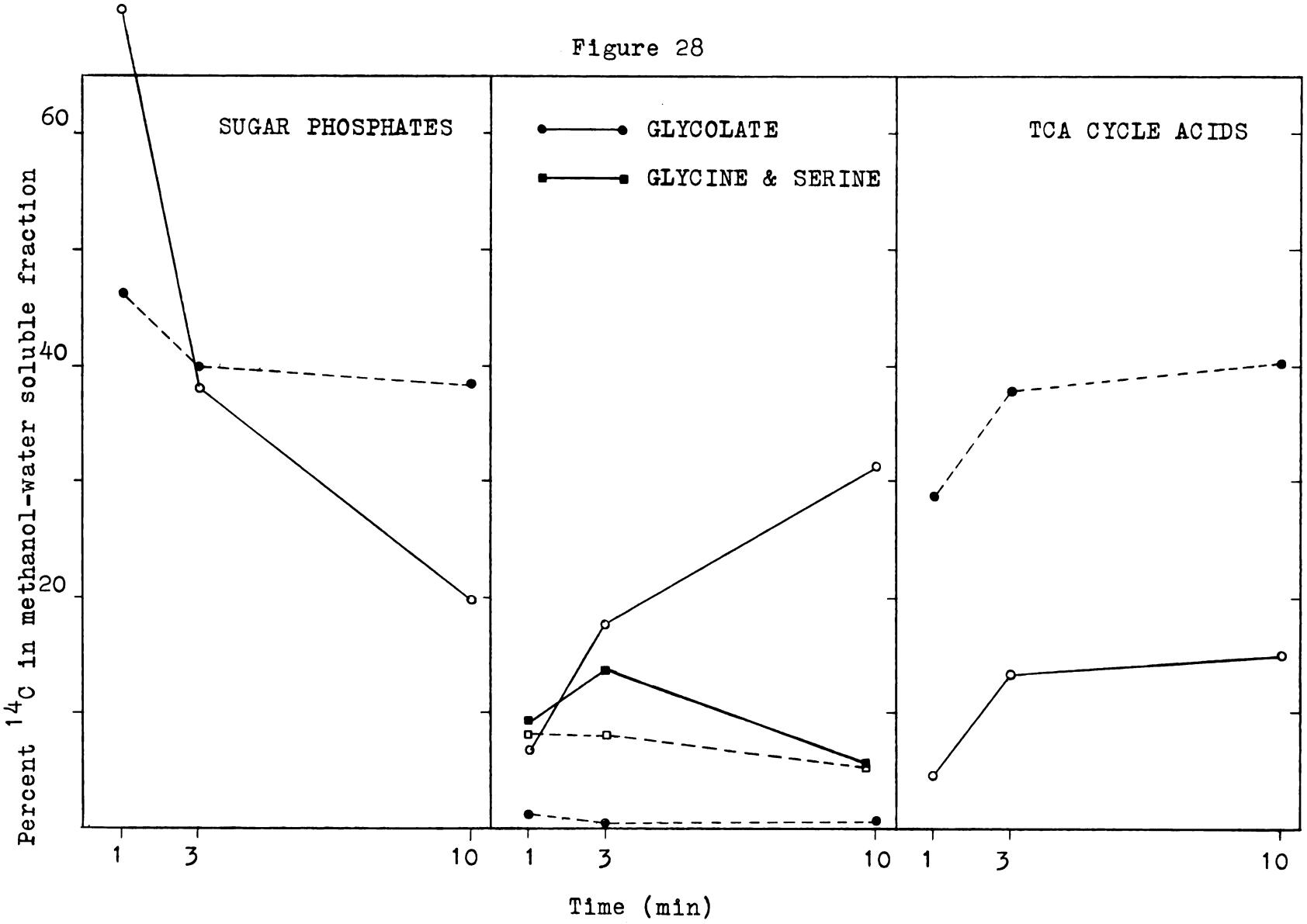
Percent Distribution of ^{14}C in Products Formed by *Chlamydomonas reinhardtii* after Adaptation to Colored Light During Photosynthesis in $^{14}CO_2$

Cells were adapted to red light (>600 mμ) or blue light (400-500 mμ) for at least ten days.

_____ Cells grown in blue light, $^{14}CO_2$ photosynthetic fixation in blue light at 110 ft-c.

----- Cells grown in red light, $^{14}CO_2$ photosynthetic fixation in red light at 200 ft-c.

Figure 28


SUGAR PHOSPHATES
GLYCOLATE
GLYCINE & SERINE
TCA CYCLE ACIDS
Percent ^{14}C in methanol-water soluble fraction
60
40
20
1
3
10
Time (min)

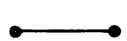
light, since the percent ^{14}C in these reservoirs decreased more rapidly with time. The percent ^{14}C in glycine and serine did not parallel that in glycolate, but instead remained rather constant and equal for algae adapted to either red or blue light; thus, the metabolic pools of glycolate and serine probably are not directly related.

The data in Figures 29 and 30 should be compared directly to emphasize the effect that growth of Chlamydomonas in blue light has on glycolate production. The total ^{14}C in glycolate accumulated during photosynthesis in 110 ft-c blue light almost equaled that fixed at 1200 ft-c white light by cells adapted to red light. This labeling of glycolate at low blue light intensity is in contrast to the high light intensity normally required for glycolate production. It is also apparent in Figure 30, that glycolate accumulation increased much more rapidly than glycine and serine, which is again consistent with the hypothesis that serine synthesis is independent of glycolate formation and excretion.

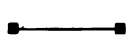
The accumulation of labeled carbon in the TCA cycle acids by red light adapted Chlamydomonas during photosynthesis in 200 ft-c of red light was slightly greater than that accumulated by the blue adapted cells utilizing 100 ft-c white light (Figure 31). As indicated by Figure 32, the total ^{14}C accumulated in the sugar phosphates was lower in the blue-adapted

Figure 29

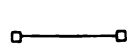
Total ^{14}C in Glycolate, Glycine, and Serine by Chlamydomonas reinhardtii Adapted to Red Light (>600 mμ) During Photosynthesis in $^{14}CO_2$



Photosynthesis in red light (200 ft-c, >600 mμ)



Photosynthesis in white light (1200 ft-c)



Photosynthesis in white light (100 ft-c)

Figure 29

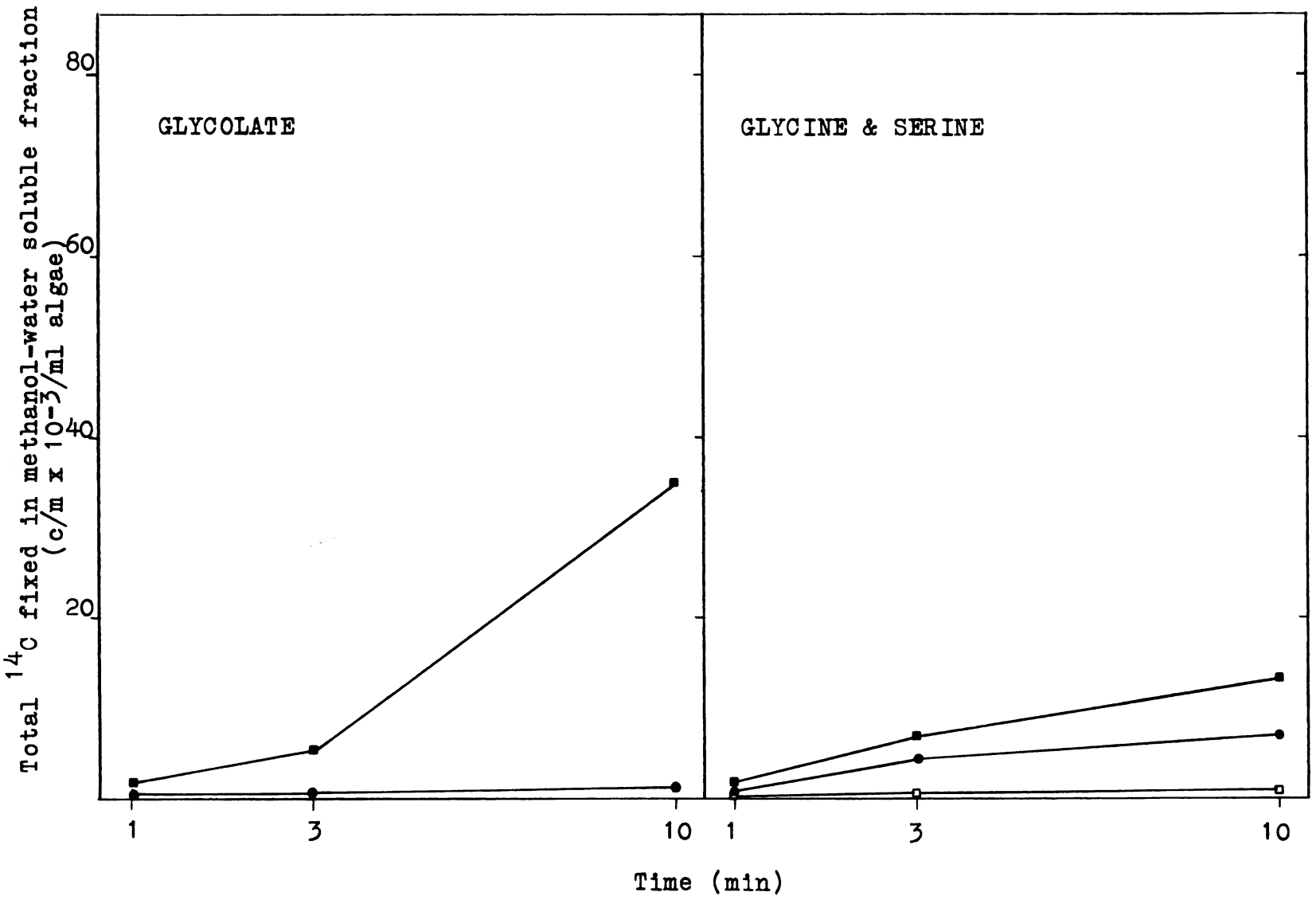

GLYCOLATE
GLYCINE & SERINE
Total ^{14}C fixed in methanol-water soluble fraction (c/m x 10^{-3}/ml algae)
80
60
40
20
1
3
10
1
3
10
Time (min)

Figure 30

Total ^{14}C in Glycolate, Glycine, and Serine by Chlamydomonas reinhardtii Adapted to Blue Light (400-500 mμ) During Photosynthesis in $^{14}CO_2$

Photosynthesis in blue light (110 ft-c, 400-500 mμ).

Photosynthesis in white light (1200 ft-c).

Photosynthesis in white light (100 ft-c).

Figure 30

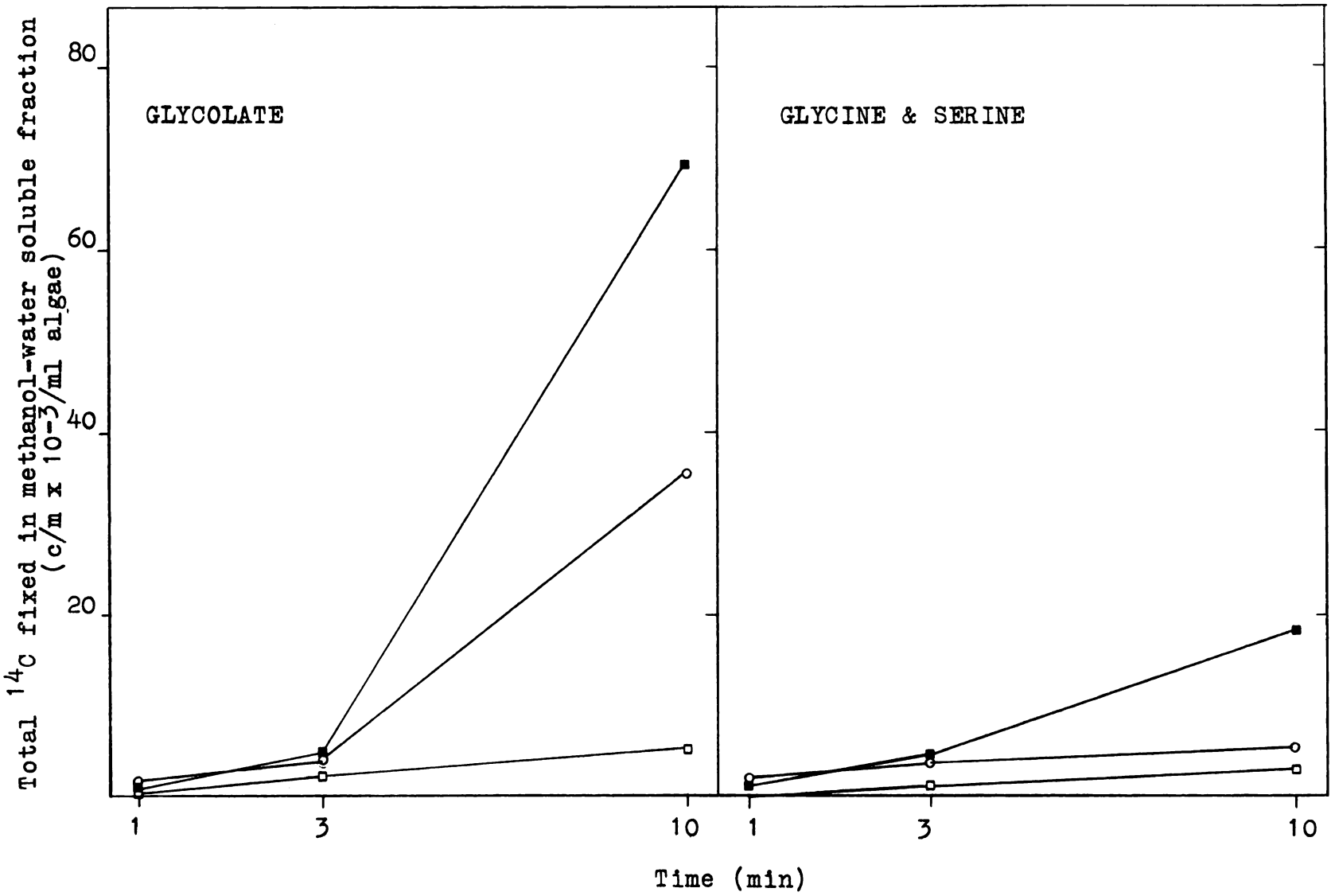
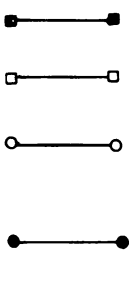

GLYCOLATE
GLYCINE & SERINE
Total ^{14}C fixed in methanol-water soluble fraction (c/m x 10-3/ml algae)
80
60
40
20
1
3
10
1
3
10
Time (min)

Figure 31

Total ^{14}C in TCA Cycle Acids Formed by Chlamydomonas reinhardtii Adapted to Red or Blue Light During Photosynthesis in $^{14}CO_2$



Photosynthesis in 1200 ft-c white light.

Photosynthesis in 100 ft-c white light.

Photosynthesis in 200 ft-c red light (>600 mμ) by algae adapted to red light.

Photosynthesis in 110 ft-c blue light (400-500 mμ) by algae adapted to blue light.

Figure 31: ^{14}C in TCA Cycle Acids

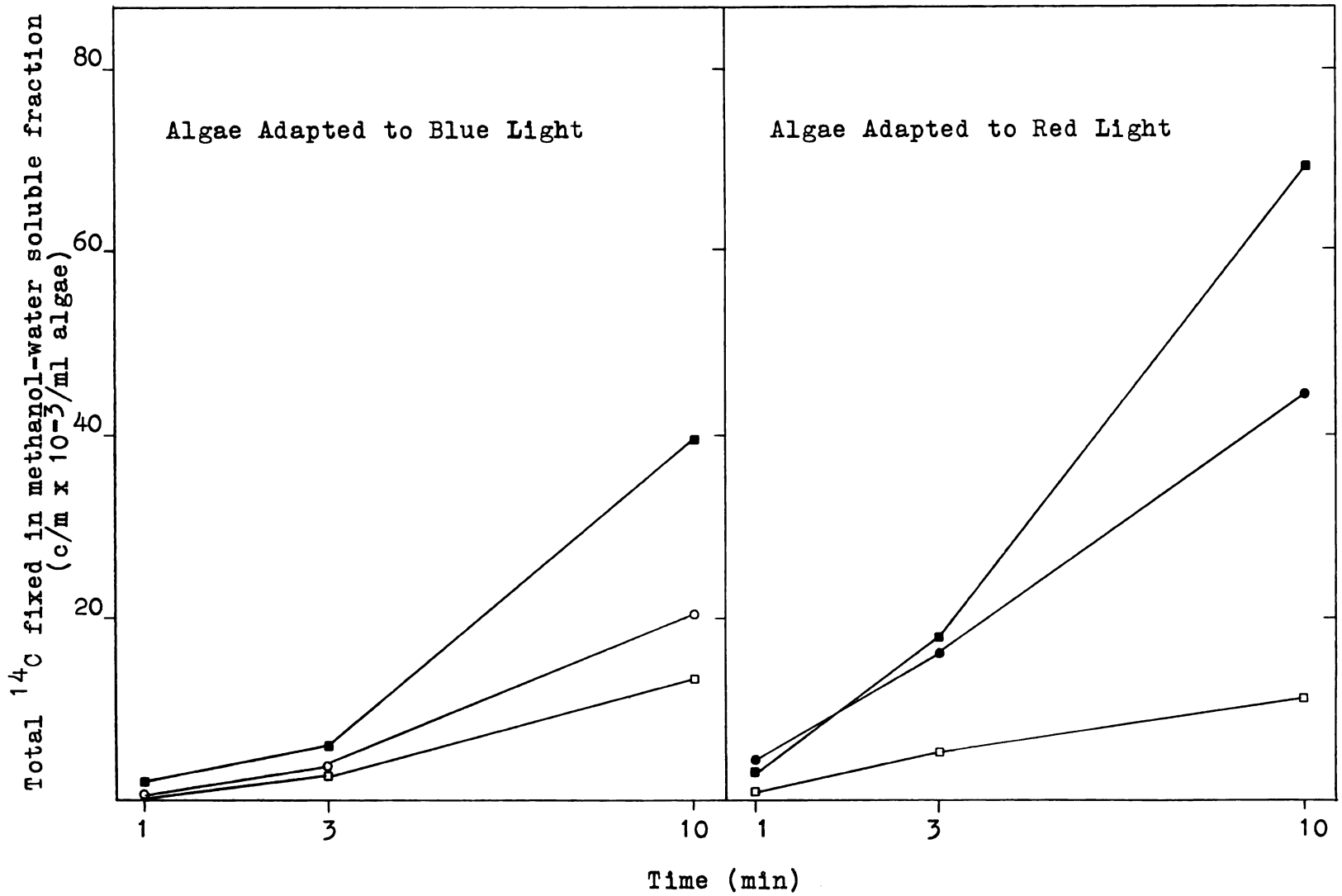


Figure 32

Total ^{14}C in Sugar Phosphates by Chlamydomonas reinhardtii Adapted to Either Red or Blue Light During Photosynthesis in $^{14}CO_2$

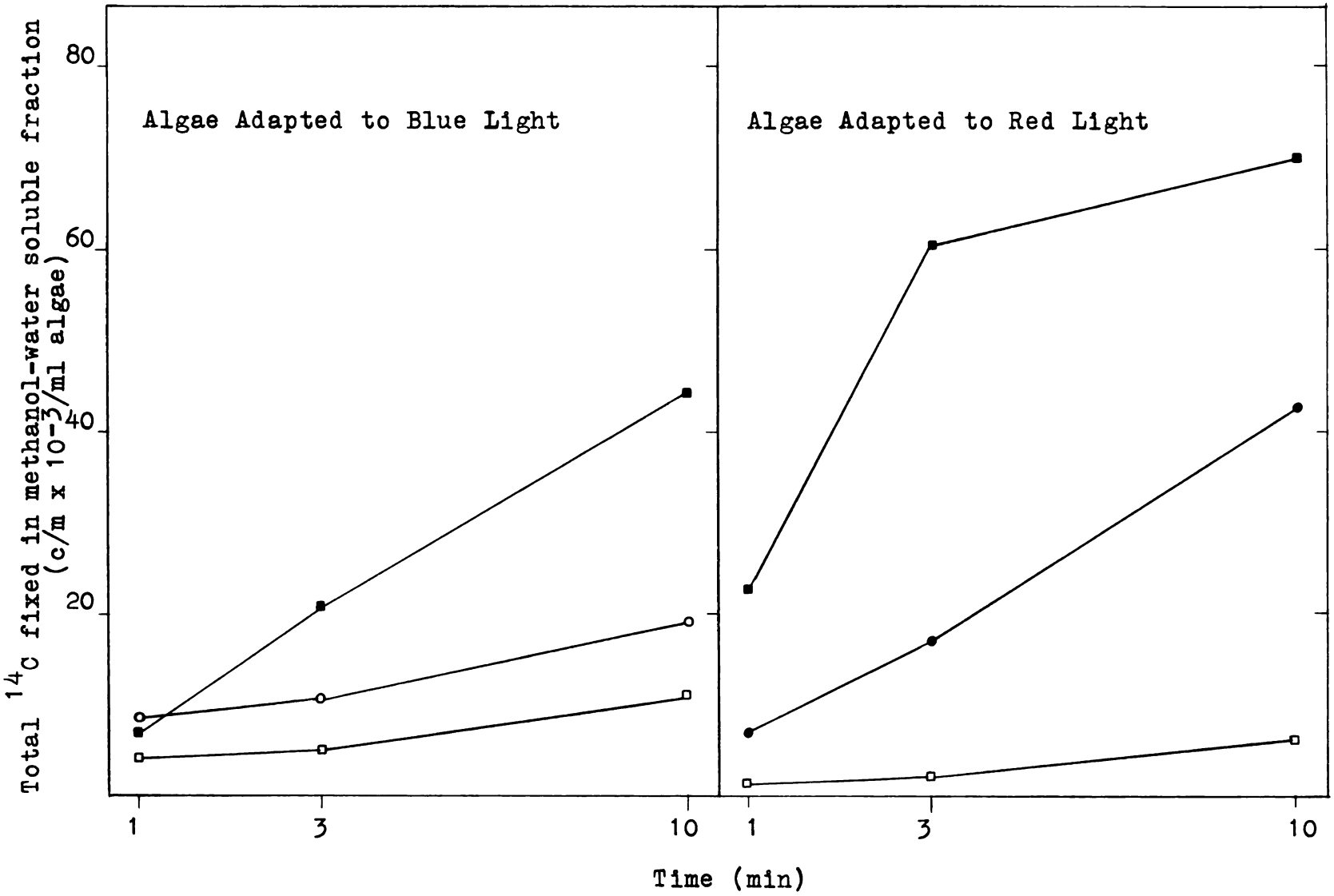
■——■ Photosynthesis in 1200 ft-c white light.

□——□ Photosynthesis in 100 ft-c white light.

○——○ Photosynthesis in 110 ft-c blue light (400-500 mμ) by algae adapted to blue light.

●——● Photosynthesis in 200 ft-c red light (>600 mμ) by algae adapted to red light.

Figure 32: ^{14}C in Sugar Phosphates



algae than in the red-adapted cells, and this data is consistent with other reports (36,37). Thus, in both algae adapted to blue or red light, the sugar phosphates were labeled; however, the pool size in the blue-adapted Chlamydomonas appeared smaller as shown by the large reduction of the percent ^{14}C in these esters (Figure 28). Perhaps these esters were rapidly metabolized to glycolate which was continually increasing. On the other hand, the large amounts of phosphate esters accumulated by the red-adapted cells were probably more slowly converted in the red light to TCA cycle acids.

All of the experiments with white light (Tables 10, 11, 12, Appendix) demonstrated that a full spectrum of light had little effect on the percent distribution of ^{14}C in the products formed by the adapted algae. Thus, algae adapted to blue light produced about the same percent of ^{14}C in products formed during 1 to 10 minutes of $^{14}CO_2$ fixation in white light as in blue light. These facts supported the hypothesis that the cultures grown in blue light actually experienced a period of adaptation which altered the cellular constituents. Reports, on the red light dependent formation of glyceraldehyde phosphate dehydrogenase (66), and on the formation of glycolate oxidase in light (59,109,111), suggest that light dependent changes in enzymatic composition of a cell can occur.

A series of experiments, with algae adapted to blue light were performed at various intensities of blue light, to evaluate the extent of light saturation of $^{14}CO_2$ fixation by the available light. Chlorella grown in white light (Figure 33A) and Chlamydomonas adapted to blue light (Figure 33C) fixed $^{14}CO_2$ with normal light saturation. However, the fixation rate with blue-adapted Chlorella has an unusual curve since very high levels of fixation occurred at very low light intensities (Figure 33B), and further evaluation of this phenomenon seemed necessary. The percent distribution of ^{14}C after 3 and 10 minute exposures of algae (blue-adapted) to $^{14}CO_2$ in blue light is presented in Tables 14-17.

Table 14

Percent Distribution of ^{14}C in Products Formed by Chlorella (Adapted to Blue Light) After 3 and 10 Minutes in the Dark in $^{14}CO_2$

Time of PS	3 min	10 min
Total Fixed c/m x 10^{-3}/ml algae	3.4	4.9
% Fixed in:		
Aspartate	52.7	44.4
Glutamate	7.5	9.5
Glutamine	32.3	41.3
Misc.	7.5	4.8

Figure 33

Total ^{14}C Fixed by Green Algae During Photosynthesis in Various Intensities of Blue Light (400-500 mμ) in $^{14}CO_2$

Open symbols = 3 min photosynthesis experiments
Closed symbols = 10 min photosynthesis experiments

Graph A: Chlorella pyrenoidosa grown in white light.

Graph B: Chlorella pyrenoidosa adapted to blue light.

Graph C: Chlamydomonas reinhardtii adapted to blue light.

Figure 33

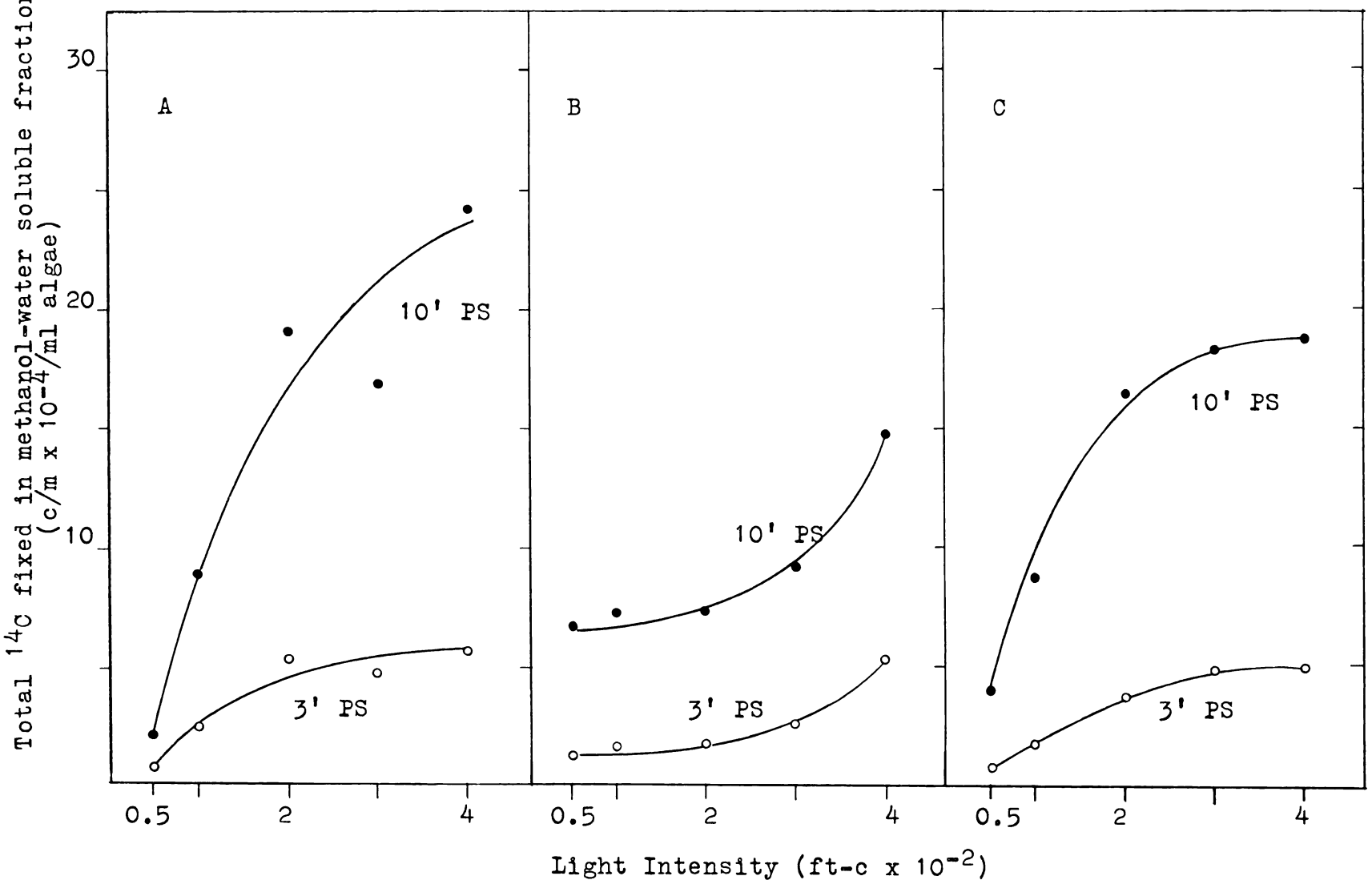

Total ^{14}C fixed in methanol-water soluble fraction (c/m x 10^{-4}/ml algae)
30
20
10
A
B
C
10' PS
3' PS
10' PS
3' PS
10' PS
3' PS
0.5
2
4
0.5
2
4
0.5
2
4
Light Intensity (ft-c x 10^{-2})

Table 15: Percent Distribution of ^{14}C Fixed by a 0.5 % Suspension of Chlorella (Grown in White Light) During Photosynthesis in Blue Light (400-500 mμ)

Time of PS	3 min.		10 min.	
Light Intensity	50 ft-c	400 ft-c	50 ft-c	400 ft-c
Total Fixed ($c/m \times 10^{-3}$/ml algae)	8.1	57.1	22.5	242.7
% Fixed in:				
PGA	6.8	6.0	10.1	8.9
RuDP	--	10.3	16.9	15.5
Sugar-P	21.1	26.1	18.8	33.8
UDPG	--	--	2.9	2.2
Sucrose	--	1.1	--	2.2
Glycolate	--	4.3	--	3.4
Glycine	--	4.9	--	2.1
Serine	2.0	10.9	4.8	10.0
Glycerate	--	--	--	--
Alanine	4.1	2.2	5.8	2.8
Pyruvate	--	--	--	--
Aspartate	40.1	4.3	27.1	6.6
Malate	13.6	8.8	7.2	5.3
Glutamate	4.8	7.1	5.8	1.9
Glutamine	2.7	--	1.4	--
α-KG	--	--	--	--
Fumarate	--	2.7	--	--
Succinate	--	--	--	--
Citrate	--	--	--	--
Lipids	--	--	--	--
Misc.	4.8	11.4	--	5.3

Table 16: Percent Distribution of ^{14}C Fixed by a 0.5 % Suspension of Chlorella (Adapted to Blue Light) During Photosynthesis in Blue Light (400-500 mμ)

Time of PS	3 min.		10 min.	
Light Intensity	50 ft-c	400 ft-c	50 ft-c	400 ft-c
Total Fixed (c/m x 10^{-3}/ml algae)	13.9	26.1	68.4	148.5
% Fixed in:				
PGA	24.2	14.4	18.4	7.3
RuDP	6.7	22.6	7.3	19.6
Sugar-P	26.1	20.5	27.7	20.9
UDPG	1.7	0.2	0.9	1.3
Sucrose	0.4	0.6	1.9	1.1
Glycolate	1.5	5.5	1.1	4.8
Glycine	2.6	4.9	3.4	6.7
Serine	7.6	11.5	14.4	18.6
Glycerate	--	--	--	--
Alanine	1.9	1.4	2.9	2.8
Pyruvate	--	--	--	--
Aspartate	18.8	7.8	6.6	4.4
Malate	3.9	4.9	3.9	3.1
Glutamate	0.6	--	1.2	0.7
Glutamine	1.5	1.6	0.8	0.6
α-KG	--	--	--	--
Fumarate	--	0.6	0.3	0.5
Succinate	--	--	--	0.6
Citrate	--	--	--	--
Lipids	--	--	--	--
Misc.	2.4	3.3	11.0	7.0

Table 17: Percent Distribution of ^{14}C Fixed by a 0.5 % Suspension of Chlamydomonas (Adapted to Blue Light) During Photosynthesis in Blue Light (400-500 mμ)

Time of PS	3 min.		10 min.	
Light Intensity	50 ft-c	100 ft-c	50 ft-c	100 ft-c
Total Fixed (c/m x 10^{-3}/ml algae)	8.8	18.0	41.7	186.2
% Fixed in:				
PGA	9.8	9.6	4.5	5.8
RuDP	--	10.4	4.2	5.2
Sugar-P	11.3	18.1	24.2	17.7
UDPG	--	1.9	3.3	0.9
Sucrose	2.8	0.4	1.4	1.7
Glycolate	11.2	13.1	19.7	17.5
Glycine	--	4.6	2.9	1.9
Serine	7.1	6.2	3.9	4.7
Glycerate	--	1.2	--	0.2
Alanine	7.1	1.5	1.2	1.9
Pyruvate	--	--	--	--
Aspartate	14.1	5.8	5.3	3.9
Malate	15.5	18.1	11.3	16.4
Glutamate	11.3	2.3	4.3	4.5
Glutamine	--	1.9	0.8	4.3
α-KG	--	--	--	--
Fumarate	2.8	1.5	0.6	0.9
Succinate	--	--	0.4	1.5
Citrate	--	--	--	--
Lipids	--	--	--	--
Misc.	7.1	3.5	11.1	11.0

In the dark, normal Chlorella primarily incorporated ^{14}C into aspartate and glutamine as did Chlorella adapted to blue light (Table 14), as would be expected from CO_2 exchange reactions. The percent radioactivity in glutamine decreased at low light intensity, and the percent ^{14}C in aspartate decreased at higher light intensity (Table 15). Blue-adapted Chlorella accumulated a much smaller percentage of ^{14}C in aspartate even at 50 ft-c, and much more ^{14}C in PGA and RuDP (Table 16). The very high fixation of $^{14}CO_2$ by the blue-adapted Chlorella (Figure 33B) at low light intensities was not an expression of increased aspartate or glutamate metabolism, but rather of the increased ^{14}C accumulation in components of the photosynthetic carbon cycle.

The blue-adapted Chlamydomonas accumulated an abnormally high percentage of ^{14}C in glycolate, even at low light intensity and a relatively low percentage in aspartate and glutamate (Table 17). The large amount of ^{14}C in glycolate could be interpreted as a rapid conversion of the sugar phosphate pools into glycolate by blue-adapted Chlamydomonas, and thus both Chlorella and Chlamydomonas respond somewhat similarly to growth in blue light. These changes in ^{14}C product distribution from $^{14}CO_2$ fixation suggested that photosynthesis and metabolism in algae adapted to blue light were altered. Perhaps

a more efficient utilization of the incident light energy could account for the increased photosynthetic CO_2 fixation at low light intensities.

Such data, as presented in this section, invite speculation that perhaps the two major areas of photosynthesis, electron transport and carbon dioxide fixation, are so closely related that complementary changes in both systems compensate for environmental alterations. These changes would tend to maximize the efficiency with which an organism would photosynthesize and metabolize CO_2. Results from many further experiments, however, are needed before any final conclusions can be made with regard to the physiological significance of these changes. Complete interpretation of the results with light quality must await further basic information on the reasons for and mechanism of glycolate biosynthesis during photosynthesis.

Manganese Deficient Culture of Chlorella:*

Cultures of Chlorella pyrenoidosa (Chick) were maintained on the inorganic salt medium of Norris et al. (75), with and without $MnCl_2$ added to the micro-nutrients and with continuous

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*Work done in conjunction with P. L. Youngblood, NSF Undergraduate Research Participant in 1964.

0.2 % CO_2 aeration. The chemicals used were analyzed for Mn^{++} and recrystallized in doubly-distilled deionized water. The Mn-deficient medium was prepared with redistilled deionized water. Only after 20 days, with nutrient changes every third day, was a reduction of growth rate observed. The slower growth rate observed with this culture could be restored to the normal growth rate by adding an appropriate amount of $MnCl_2$ to the deficient medium (Figure 34). The "Mn-restored" culture achieved normal growth rates after 24 hours. Since the rate curves were measured on cultures with similar initial cell populations, the Mn-deficient rate actually indicated a slower log phase of growth (Figure 34).

The fixation data in Table 15 were obtained from standard photosynthetic experiments in $^{14}CO_2$ (see methods). The nearly linear rate of total fixation over the 10 minute period indicated that the $^{14}CO_2$ concentration never became limiting. Glycolate production in the manganese deficient culture was inhibited but glycine and serine synthesis increased. The increased accumulation of ^{14}C in glycine and serine in a system not producing glycolate, supports the concept that glycolate oxidase is absent and glycolate conversion to serine does not occur. The unknown precursors for glycolate formation might, however, be diverted toward increased glycine and serine production in the manganese

Figure 34

Growth Rates for Cultures of Chlorella pyrenoidosa (Chick) During Growth in White Light.

Curve 1: Normal Chlorella culture.

Curve 2: Manganese deficient culture.

Curve 3: Manganese deficient culture with $MnCl_2$ added.

Figure 34

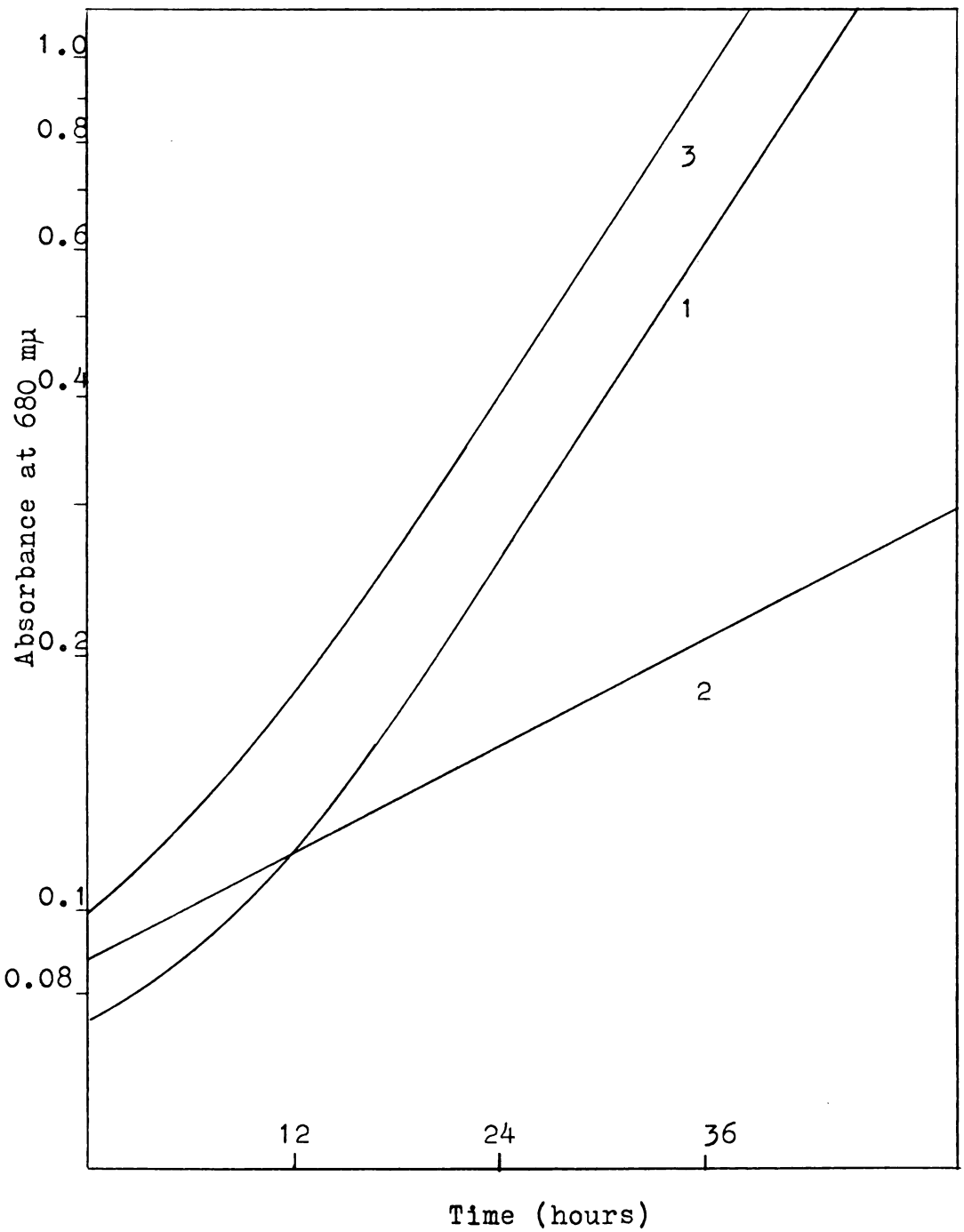
1.0
0.8
0.6
0.4
0.2
0.1
0.08
Absorbance at 680 mμ
3
1
2
12
24
36
Time (hours)

Table 18

Percent Distribution of ^{14}C Fixed by *Chlorella pyrenoidosa* (Chick)

Condition of PS	Normal Control			Mn-Deficient		
Time of PS	1'	3'	10'	1'	3'	10'
Total Fixation (c/m x 10^{-3}/ml algae)	21.2	79.0	211.6	44.8	178.9	461.5
% Fixed in:						
Sugar-P	76.5	63.1	37.3	61.9	59.0	42.8
Glycolate	7.0	14.5	30.9	4.0	3.3	2.4
Glycine	2.6	5.2	5.2	7.1	6.9	5.7
Serine	4.3	9.5	10.1	13.5	15.8	20.4
Other	9.6	7.7	16.5	13.6	14.8	28.6

deficient algae. These results confirm an earlier report on the requirement for Mn^{++} in glycolate formation during photosynthesis by Chlorella (103). Tanner, et al. (103) also reported the increased amount of ^{14}C labeling of glycine and serine by manganese deficient Chlorella.

Glycolate Excretion and Uptake:**

The excretion of glycolate by algae has been well documented (49,70,87,113) and there is one report of glycolate excretion by chloroplasts (106). No metabolic function has yet been elucidated for glycolate excretion. Tolbert (106), Whittingham and Pritchard (125), Warburg and Krippahl (121), and Orth, et al. (81) have reported maximum glycolate excretion at low CO_2 concentrations (0.1 to 0.3 %), high light intensity, high O_2 and high pH. The excretion of glycolate was measured in the algal supernatant medium as ^{14}C-glycolate or by the colorimetric procedure of Calkins for glycolate (18). During the course of my research additional conditions for glycolate excretion and metabolism were considered in our research group, and the results are reported in this section.

**Uptake and excretion work done in conjunction with Fang Hui Liao, Graduate Research Assistant, during 1965.

The amount of glycolate excreted by the algae was found to be greatest in the presence of 0.01 M bicarbonate buffer, but much less in 0.1 M $NaHCO_3$ (Table 19).

Table 19

Glycolate Excretion by Chlorella pyrenoidosa (Chick)

Glycolate excreted by 2% Chlorella suspension in water during photosynthesis in 3000 ft-c.

Final HCO_3^- Conc.	Glycolate in Supernate
moles/l	µg/hr/ml
None	9
0.0001	9
0.001	25
0.01	78
0.1	10

In 0.01 M $NaHCO_3$ glycolate excretion was linear during the first hour. These results seem inconsistent with prior observations on the requirement for low levels of CO_2 for maximum glycolate formation. However, all measurements with CO_2 were done at pH ranges of 4.5 to 6.5, while the pH of a bicarbonate solution is about 8.3.

The uptake of glycolate by algae has been generally measured by the disappearance of glycolate determined by the

colorimetric assay (26,27,70). Nalewajko, et al. (74) recently reported, however, a small uptake of ^{14}C labeled glycolate by a strain of Chlorella isolated from the Arctic Ocean. However, we have observed, in many attempts with Chlorella and Chlamydomonas, no significant uptake of ^{14}C labeled glycolate or phosphoglycolate during time periods of 10 to 120 minutes. Typical results are presented in Table 20. The small amount

Table 20

Uptake of Glycolate-2-^{14}C or Phosphoglycolate-2-^{14}C by Chlamydomonas reinhardtii in White Light.

A 3 % Chlamydomonas suspension in 0.04 M phosphate (initial pH 4.0) in 3000 ft-c White Light.

Time (min)	Percent ^{14}C in Algae	
	Glycolate-2-^{14}C	P-glycolate-2-^{14}C
1	1.1	4.1
3	1.3	1.9
10	2.6	2.0
30	0.5	1.0

of activity remaining with the cells did not increase with time, and thus, could have been caused by surface adsorption of the labeled acid by the algae or filter. The quantitative colorimetric measurement of glycolate added to algal cultures

also indicated no uptake with respect to time (Table 21, part 1). In fact, when bicarbonate buffer was used in the presence of 10^{-5} M glycolate, a time-dependent excretion of glycolate with a linear rate occurred for at least one hour. Thus, the presence of glycolate in the supernatant fluid did not inhibit further glycolate excretion (Table 21, part 2).

Table 21

Excretion and Uptake of Glycolate by *Chlorella pyrenoidosa* (Chick)

Part 1: A 2% Chlorella (grown in 5% CO_2) suspension was prepared in water and 10^{-5} M Na-glycolate.

Time (min)	Total Glycolate µg/ml	Net Glycolate Change µg/ml
0	24.5	0
5	26.7	+2
15	22.8	-2
30	19.3	-5
60	25.9	+2

Part 2: A 2% Chlorella (grown in 5% CO_2) suspension was prepared in 0.01 M $NaHCO_3$ and 10^{-5} M glycolate.

Time (min)	Total Glycolate µg/ml	Net Glycolate Change µg/ml
0	21.7	0
5	25.2	+4
15	30.6	+9
30	38.0	+16
60	60.5	+39

A significant earlier report in 1950 on glycolate metabolism in algae by Schou, et al. (95) claimed that at pH 2.8 in the light, Scenedesmus metabolized glycolate in a period of 10 minutes to amino acids and sugar phosphates. On the basis of this report and the fact that glycolate is formed rapidly by algae during photosynthesis, the assumption was established that the glycolate pathway was present in algae. These experiments of Schou, et al. were repeated by feeding glycolate-2-^{14}C to three strains of algae: Chlorella pyrenoidosa (Chick), Chlorella pyrenoidosa (Warburg), and Scenedesmus obliquus (Gaffron D-3) (the same Scenedesmus used by Schou). Typical results with Chlorella are shown in Table 22 and similar results were obtained with Scenedesmus. The cells were not filtered from the medium as Schou, et al. described, so that the percent of glycolate conversion could be estimated. Very little glycolate metabolism was observed during the 10 minute photosynthesis experiment with glycolate-2-^{14}C in the light. These data also demonstrate that metabolism at pH 6.0 was equal to or greater than that at pH 2.3 (also found with Scenedesmus).

Table 22

Percent Distribution of ^{14}C After Feeding Glycolate-2-^{14}C

Approximately 1 μc glycolate-2-^{14}C was added to 2% Chlorella pyrenoidosa suspensions at pH 2.8 and pH 6.0 in 0.001 M phosphate. After 10 min photosynthesis in 300 ft-c white light the suspension was killed with MeOH and the percent distribution determined.

	Chick strain		Warburg strain	
Compound	pH 2.8	pH 6.0	pH 2.8	pH 6.0
Glycolate	97.7	98.7	97.7	94.3
Glycine	0.4	0.1	0.3	0.1
Serine	0.6	0.3	0.7	1.0
Misc.	1.3	1.2	1.4	4.6

No estimation of the percent of glycolate metabolized by the Scenedesmus was given by Schou, et al., but only the percent ^{14}C distributed in glycine, serine, and other labeled products. On the basis of the amount of glycolate-^{14}C used by Schou, et al. and the intensity of their radioautographs, it is likely that we have both observed similar limited metabolism of glycolate-^{14}C by impure (not bacteria-free) algal suspensions. Several possible reasons might account for the limited (2 to 3 %) conversion of glycolate-2-^{14}C to other products. An impurity in the glycolate-^{14}C, such as glyoxylate, could have been present which could not be detected by the

chromatographic procedures employed, but which would be readily used by plant cells (55). As mentioned above the algal cultures were not free of bacteria which could possibly account for some glycolate metabolism. There is also the possibility that the algae have a very limited ability to metabolize glycolate to glycine and serine, particularly since most of the serine in algae is formed directly from PGA. The colorimetric determination of glycolate disappearance might also be criticized, since such a disappearance need not reflect an uptake of the acid by algae. Another product, e.g. peroxide, also formed by the cells could cause the breakdown of glycolate; and such interactions could account for the inconsistent results that have been observed for glycolate uptake and metabolism.

However, from all of the results presented here, I must conclude that glycolate metabolism in algae is minimal; none of these data indicate sufficient glycolate uptake or metabolism to contribute significantly to the metabolism of the cell or the utilization of the glycolate formed and excreted during photosynthesis. Speculation, about the role of glycolate as an end-product of algal metabolism emphasizes the need for determining the biochemical reason for glycolate's formation. The lack of the typical glycolate pathway in algae

dictates that we find an alternate metabolic route for glycolate utilization, or else discover the biochemical systems of the cell, dependent upon glycolate formation; so that the hypothesis of its excretion as an end-product of algal photosynthesis becomes rational.

SUMMARY

The Glycolate Pathway in Tobacco Leaves:

Kinetic studies on the rate of glycolate biosynthesis by tobacco leaves during $^{14}CO_2$ photosynthesis were run either in the presence or in the absence of α-hydroxypyridinemethanesulfonate, an inhibitor of glycolate oxidase. Although $^{14}CO_2$ fixation was reduced about 50 %, the inhibitor did not affect the initial rate of ^{14}C incorporation into phosphoglycerate or glycolate, nor the specific activity of these products. The rate of glycolate formation was slower than the rate of phosphoglycerate formation. At time periods of 4 and 11 seconds, phosphoglycerate was predominately carboxyl labeled and glycolate was uniformly labeled. The specific activity of the carboxyl carbon of phosphoglycerate was about 100 fold higher than the specific activity of either carbon of glycolate. Between 30 to 60 seconds, phosphoglycerate became uniformly labeled and the specific activities of the carbon atoms of phosphoglycerate and glycolate became nearly equal.

The inhibitor blocked the glycolate pathway *in vivo*. As a result in a 5 minute experiment the percent of the total ^{14}C in glycolate rose from 3 % in the control to 50 % in the inhibited leaf. At the same time the percent ^{14}C in the products of the glycolate pathway, which were formed from glycolate (glycine, serine, and sucrose), dropped from 32 % to 10 % of the total. These data emphasize the magnitude of the

glycolate pathway in higher plants. The results extend the concept that glycolate is formed after PGA and from a component of the photosynthetic carbon cycle. The results do not support the hypothesis of a second autonomous carboxylation reaction in photosynthesis for the production of glycolate.

The Glycolate Pathway in Algae:

Enzymes: No glycolate oxidase activity could be detected in cell extracts from four strains of algae. Manometric, isotopic, and spectrophotometric techniques were used to detect the oxidase activity. Repeated attempts to rupture cells in buffers either with or without glycolate were unsuccessful in demonstrating the glycolate dependence of either oxygen uptake or glyoxylate production. Active isocitrate dehydrogenase, NADH:glyoxylate reductase, and phosphoglycolate phosphatase were present in the cell extracts. The lack of a typical glycolate oxidase, suggests that the glycolate pathway in algae does not exist as it does in the higher plant. However, these observations do not exclude an alternative pathway for glycolate metabolism.

Distribution of ^{14}C in Carbons of Glycolate Pathway Products Formed During $^{14}CO_2$ Photosynthesis in Algae: The serine formed by Chlorella after 12 seconds contained 80 % of the total ^{14}C in the carboxyl carbon and that formed by Chlamydomonas contained 70 % of the ^{14}C in the carboxyl carbon.

Thus, serine is most probably formed from PGA and not from the glycolate pathway. Glycine, however, from the same experiments was uniformly labeled. These results were compared with the formation of uniformly labeled serine after 4 to 20 seconds photosynthesis by soybean leaves. The labeling pattern in serine formed by algae during photosynthesis in $^{14}CO_2$ demonstrated that the complete glycolate pathway does not function in algae.

The Effect of α-hydroxysulfonates on $^{14}CO_2$ Photosynthesis by Algae: Preincubating 1 % suspensions of Chlamydomonas or Chlorella in 0.001 M phosphate with either 0.001 M α-hydroxypyridinemethanesulfonate or α-hydroxymethanesulfonate produced four main effects on algal photosynthesis in $^{14}CO_2$. Little or no change in total $^{14}CO_2$ fixation occurred at pH 6.5, but at pH 8.3 the presence of the sulfonate restored the fixation rate to that at pH 6.5. This rate represented a 3-fold increase over the fixation rate in the control. In contrast to the gigantic accumulation of glycolate observed in tobacco in the presence of these inhibitors, no glycolate accumulated in algae at either pH 6.5 or 8.3. Amino acid synthesis was greatly inhibited in the presence of either α-hydroxysulfonate as expressed by a decreased accumulation in alanine, glutamate and aspartate. Correspondingly there was an increased accumulation of ^{14}C in the keto acids, pyruvate and α-ketoglutarate. These inhibitors at either pH 6.5 or 8.3 also altered sugar phosphate metabolism.

A larger percentage of the total ^{14}C accumulated in the sugar phosphates, particularly in ribulose-1,5-diphosphate. From these results, it was concluded that the α-hydroxysulfonates used in this study are not specific glycolate oxidase inhibitors. These compounds affected both amino acid and sugar phosphate metabolism in algae, as well as glycolate oxidase in tobacco. The lack of a typical glycolate oxidase and the glycolate pathway in algae was supported by the inability of these inhibitors to cause an increase in glycolate accumulation.

Growth and Photosynthesis of Algae in Red and Blue Light: Changes in absorption spectra and $^{14}CO_2$ fixation patterns were observed with algae grown in red light (>600 mμ) or in blue light (400-500 mμ). When grown in blue light Chlorella and Chlamydomonas showed a consistent decrease in their chlorophyll a/b ratio. The spectra of both methanol extracts and *in vivo* algal suspensions showed this change. The algae adapted to blue light accumulated 35 % of the ^{14}C in glycolate; but a normal accumulation was observed in glycine and serine (7 %). This enhanced accumulation in glycolate occurred at 110 ft-c of blue light in contrast to the normal requirement of high light intensity for glycolate formation. On the other hand, Chlamydomonas adapted to red light formed TCA cycle acids and associated amino acids which contained 40 % of the fixed ^{14}C, and accumulated little or no ^{14}C in

glycolate (5 %) during photosynthesis in 200 ft-c of red light. The percent of sugar phosphates formed by cells adapted to red light was greater after 10 minutes (40 %) than the percent of ^{14}C in these compounds formed by algae adapted to blue light (20 %). That glycolate accumulation increased without much change in the percent ^{14}C in glycine and serine is consistent with the hypothesis that serine synthesis is independent of glycolate formation, i.e., these algae did not contain a complete glycolate pathway.

Further experiments with algae adapted to blue light revealed that even at low blue light intensities (50-100 ft-c) the cells fixed a higher percent of ^{14}C in the sugar phosphates than did a control culture grown in white light. The white grown cells formed mainly aspartate and glutamine from $^{14}CO_2$ at this low intensity of blue light. Such experimental results could suggest that the systems for CO_2 fixation and electron transport are related in their response to environmental conditions.

Manganese Deficient Culture of Chlorella: Manganese deficient cultures were obtained after 20 days growth in a Mn^{++} deficient medium. The decreased growth rate of this culture could be restored to a normal rate by the addition of $MnCl_2$ to the culture. After photosynthesis for 10 minutes the percent ^{14}C in glycolate formed by normal algae was 30 % while that formed by the deficient algae was only 2 %. Concurrently there

was an increase in the percent of glycine and serine formed by the Mn-deficient Chlorella. These data also suggest that glycolate metabolism to form serine does not occur as it does in the higher plant.

Glycolate Excretion and Uptake by Algae: The amount of glycolate uptake by algae was measured either by disappearance of the glycolate in the medium or by appearance of labeled glycolate or phosphoglycolate in the cells. No time dependent uptake of significant amounts of either glycolate or phosphoglycolate was observed. Feeding glycolate-2-^{14}C to Chlorella and Scenedesmus revealed that only 2-3 % of the radioactive acid was metabolized by these cultures after 10 minutes photosynthesis. Several reasons, including bacterial contamination or impure substrates, could explain this small percent conversion. Again the conclusion must be that the algae were not capable of glycolate metabolism as is the higher plant. Glycolate excretion was maximal in 0.01 M bicarbonate and occurred in the light even in the presence of 10^{-5} M glycolate.

Comparison of Glycolate Metabolism in Algae and Tobacco Leaves: From the results in this thesis it is concluded that there exists a basic difference between the metabolism of glycolate by algae and that of the higher plant. In both plant tissues, 3-phosphoglycerate is the initial

product of photosynthesis with the subsequent formation of glycolate from the carbon reduction cycle. However, only in the higher plant is there glycolate oxidase and the subsequent reactions of the glycolate pathway. Algae contain no typical glycolate oxidase and therefore do not produce glycine, serine or sugars from glycolate as does the higher plant. Instead the algae excrete the glycolate into the medium as an apparent end product of metabolism.

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APPENDIX

Table 1: Percent Distribution of ^{14}C Fixed by Tobacco Leaves

Control (Con) was pretreatment with water; inhibitor (Inh) was pretreatment with 0.01 M OH-PMSulfonate.

Time of PS	4"		11"		30"		60"	
Conditions	Con	Inh	Con	Inh	Con	Inh	Con	Inh
Total Fixation (c/m x 10^{-5})	185.7	836.0	294.0	137.7	714.0	821.1	1,922.9	871.0
% Fixed in:								
PGA	26.9	29.2	22.9	28.9	16.6	12.0	8.2	11.8
RuDP	3.8	2.0	4.6	8.2	1.3	1.7	1.3	4.8
Sugar-P	18.9	28.8	29.5	26.0	20.5	17.3	8.1	11.6
UDPG	--	--	1.1	--	1.8	1.8	2.1	--
Sucrose	--	--	1.3	--	1.3	1.9	3.7	3.4
Glycolate	1.9	2.2	3.5	12.7	1.9	16.6	1.7	20.4
Glycine	6.7	3.1	7.4	6.4	14.8	7.8	15.5	13.2
Serine	3.6	0.9	4.1	2.7	15.5	4.5	10.6	7.9
Glycerate	3.6	2.4	5.9	4.0	9.2	5.5	12.1	4.5
Alanine	12.2	12.2	5.7	6.9	4.9	5.5	10.9	4.2
Pyruvate	--	--	--	--	1.5	1.1	2.4	0.8
Aspartate	1.9	--	3.7	0.3	2.0	4.1	1.5	1.2
Malate	15.4	9.5	8.3	--	2.2	6.8	4.0	4.0
Glutamate	--	--	--	--	0.7	0.7	1.4	1.1
Glutamine	--	--	--	--	--	--	--	--
α-KG	--	--	--	--	--	--	--	--
Fumarate	--	--	--	--	--	--	0.8	--
Succinate	--	--	--	--	--	--	--	--
Citrate	--	--	--	--	--	--	--	--
Lipids	--	--	--	--	--	--	--	--
Misc.	5.0	9.7	2.0	4.0	5.9	12.4	15.6	10.7

Table 2: Percent Distribution of ^{14}C Fixed by Tobacco Leaves

Control (Con) was pretreatment with water; Inh-1, pretreatment with 0.01 M OH-PMSulfonate; Inh-2, pretreatment with 0.01 M OH-MSulfonate.

Time of PS	2'			5'	
Conditions	Con	Inh-1	Inh-2	Con	Inh-1	Inh-2
Total Fixation (c/m x 10^{-4})	506.4	98.1	133.4	3,233.6	986.0	1,979.9
% Fixed in:						
PGA	10.8	3.6	2.2	9.2	3.1	1.1
RuDP	--	--	--	12.6	3.1	--
Sugar-P	13.3	11.3	17.7	8.7	7.1	10.3
UDPG	--	--	--	1.7	2.0	--
Sucrose	6.5	4.6	9.0	14.9	4.2	7.5
Glycolate	6.4	42.1	45.3	2.9	48.6	57.5
Glycine	12.6	7.7	1.9	9.4	3.0	2.9
Serine	8.8	2.6	2.4	7.2	3.1	4.7
Glycerate	10.1	2.1	--	6.2	1.0	0.4
Alanine	2.0	--	1.7	1.7	6.6	2.4
Pyruvate	--	--	--	--	--	--
Aspartate	0.5	--	--	0.9	1.3	--
Malate	1.1	2.6	--	4.1	4.9	--
Glutamate	2.7	--	--	1.9	1.3	0.7
Glutamine	--	--	--	--	--	--
α-KG	--	--	--	--	--	--
Fumarate	--	--	--	--	--	--
Succinate	--	--	--	--	--	--
Citrate	--	--	--	--	--	--
Lipids	--	--	--	--	--	--
Misc.	25.7	23.6	19.9	18.6	10.7	12.7

Table 3: Percent Distribution of ^{14}C Fixed by Chlamydomonas at pH 7.6

Control (Con) was 1 % algal suspension in 0.001 M phosphate pH 7.6; inhibitor (Inh) was 0.001 M OH-PMSulfonate added to the control.

Time of PS	1'		2'		4'		10'	
Conditions	Con	Inh	Con	Inh	Con	Inh	Con	Inh
Total fixation (c/m x 10^{-3}/ml algae)	16.2	121.6	40.7	206.1	68.6	441.4	163.9	786.6
% Fixed in:								
PGA	22.2	22.8	13.9	16.4	16.1	11.5	4.7	8.7
RuDP	7.9	5.8	2.9	6.9	3.1	6.0	6.5	4.0
Sugar-P	30.2	44.9	21.0	38.8	13.7	34.3	21.8	20.0
UDPG	--	4.9	1.7	6.3	2.5	8.1	2.7	8.4
Sucrose	--	--	0.4	1.2	3.7	1.8	3.1	2.2
Glycolate	4.0	0.7	2.6	0.7	1.2	0.4	5.8	0.7
Glycine	3.2	0.1	1.7	0.1	1.3	0.2	1.3	0.8
Serine	4.8	1.8	6.2	3.2	7.9	3.5	7.4	2.9
Glycerate	--	0.8	0.6	1.5	0.2	--	0.4	--
Alanine	5.6	0.2	7.7	1.2	5.3	3.6	3.8	3.5
Pyruvate	--	0.8	--	0.8	--	3.4	--	2.0
Aspartate	4.8	2.6	11.7	3.9	18.8	5.5	14.9	3.9
Malate	13.5	11.7	14.5	13.0	11.8	11.5	13.9	19.2
Glutamate	4.0	0.2	7.3	1.2	6.9	1.6	7.8	3.7
Glutamine	--	--	--	--	1.2	--	0.9	0.4
α-KG	--	0.1	--	0.1	--	2.6	--	5.5
Fumarate	--	0.4	0.4	1.1	0.6	0.6	0.5	1.0
Succinate	--	--	--	--	--	--	--	--
Citrate	--	0.5	1.5	0.7	0.6	1.0	--	0.6
Lipids	--	--	--	--	--	3.4	--	7.3
Misc.	--	2.1	5.0	1.7	5.0	1.5	4.5	5.1

Table 4: Percent Distribution of ^{14}C Fixed by Chlamydomonas at pH 6.5

Control (Con) was 1 % algal suspension in 0.001 M phosphate, pH 6.5; inhibitor (Inh) 0.001 M OH-PMSulfonate added to the control system.

Time of PS	10"		20"		30"		60"		120"	
Conditions	Con	Inh	Con	Inh	Con	Inh	Con	Inh	Con	Inh
Total fixation ($c/mx10^{-4}$/ml algae)	16.6	15.3	44.1	40.6	72.7	59.8	162.4	128.6	220.0	236.9
% Fixed in:										
PGA	22.1	19.2	15.6	18.2	9.4	20.4	6.5	13.9	5.9	6.4
RuDP	6.6	20.1	2.8	12.7	2.7	9.0	1.7	7.5	1.6	3.5
Sugar-P	39.8	38.4	52.8	50.9	49.9	43.3	40.4	41.5	26.3	34.1
UDPG	--	0.3	1.9	2.8	1.2	3.8	1.1	3.5	0.6	1.9
Sucrose	--	--	--	--	0.1	--	0.3	--	0.6	0.6
Glycolate	--	--	--	--	0.3	0.9	--	0.7	0.6	1.4
Glycine	--	--	0.3	--	0.4	0.6	0.5	1.0	1.3	0.3
Serine	1.6	--	1.6	0.4	1.7	1.0	3.4	--	4.6	1.8
Glycerate	--	--	--	--	--	--	0.3	--	0.3	0.2
Alanine	1.6	--	3.0	0.3	4.8	0.5	5.0	--	4.4	0.5
Pyruvate	--	1.0	--	1.3	0.7	2.1	0.4	3.4	0.3	4.7
Aspartate	6.6	0.9	6.9	1.7	8.1	1.2	9.5	0.4	10.9	1.5
Malate	16.3	8.9	11.3	8.4	13.9	13.1	16.9	16.0	18.8	18.5
Glutamate	--	--	0.7	0.2	1.2	0.2	4.6	--	12.3	0.4
Glutamine	--	--	--	--	--	--	0.6	--	1.9	--
α-KG	--	--	--	--	--	0.3	--	1.0	0.3	2.9
Fumarate	--	--	--	--	0.6	--	--	0.8	0.5	0.5
Succinate	--	--	--	--	--	--	--	--	0.8	0.6
Citrate	--	--	--	--	0.2	--	0.5	0.5	0.5	0.6
Lipids	--	--	--	--	0.7	--	1.3	--	1.6	7.6
Misc.	6.0	11.0	3.1	3.1	4.1	3.8	6.9	9.8	5.9	12.2

Table 5: Percent Distribution of ^{14}C Fixed by Chlamydomonas at pH 8.3

Control (Con) was 1 % algal suspension in 0.001 M phosphate pH 8.3; inhibitor (Inh) was 0.001 M OH-PMSulfonate added to the control.

Time of PS	10"		20"		30"		60"		120"	
Conditions	Con	Inh	Con	Inh	Con	Inh	Con	Inh	Con	Inh
Total fixation (c/mx10^{-4}/ml algae)	4.3	29.9	10.4	63.1	20.4	81.2	32.2	128.8	69.7	209.3
% Fixed in:										
PGA	16.1	22.1	15.8	10.2	9.8	11.6	9.7	10.5	8.1	6.0
RuDP	3.8	6.1	7.0	3.5	4.5	4.7	3.2	7.4	5.2	2.8
Sugar-P	56.1	53.6	26.8	62.7	39.1	50.8	29.7	45.0	20.2	26.5
UDPG	--	3.0	--	3.9	--	3.0	--	4.0	1.0	2.0
Sucrose	--	--	--	--	--	--	0.2	--	0.7	0.6
Glycolate	0.3	1.2	4.8	1.8	3.4	3.4	2.6	1.7	1.9	3.7
Glycine	0.3	--	4.5	--	4.8	--	2.7	--	1.6	0.8
Serine	0.3	0.7	4.5	0.4	3.9	1.3	6.5	0.9	7.3	5.0
Glycerate	--	--	1.1	0.3	1.4	0.6	0.6	0.8	0.4	3.6
Alanine	2.2	--	3.9	0.5	6.7	1.5	5.4	1.3	3.3	1.0
Pyruvate	1.3	0.5	1.1	1.4	--	1.4	0.2	1.5	--	4.2
Aspartate	2.8	--	6.2	0.3	4.2	0.9	8.2	0.7	10.8	1.5
Malate	13.6	6.1	--	8.5	18.8	11.3	22.9	18.7	16.5	24.1
Glutamate	0.9	--	1.1	0.4	0.6	--	3.3	--	8.7	0.7
Glutamine	--	--	--	--	--	--	0.3	--	1.2	--
α-KG	--	--	19.2	--	--	--	--	--	0.2	0.4
Fumarate	--	--	1.1	--	0.6	--	0.3	--	0.9	0.9
Succinate	--	--	--	--	0.6	--	1.0	--	1.2	--
Citrate	--	--	--	--	--	--	--	0.5	0.9	0.9
Lipids	--	--	--	0.7	--	1.4	0.3	1.9	3.7	--
Misc.	2.2	6.6	2.8	5.5	1.7	8.1	3.0	5.2	5.3	16.2

Table 6: Percent Distribution of ^{14}C Fixed by Chlamydomonas at pH 6.5

Control (Con) was 1 % algal suspension in 0.001 M phosphate pH 6.5; inhibitor (Inh) was 0.001 M OH-MSulfonate added to the control.

Time of PS	10"		20"		30"		60"		120"	
Conditions	Con	Inh	Con	Inh	Con	Inh	Con	Inh	Con	Inh
Total fixation ($c/mx10^{-4}/ml$ algae)	24.2	16.9	52.0	50.7	70.4	82.7	169.0	147.9	220.7	273.6
% Fixed in:										
PGA	40.6	44.4	24.4	25.6	20.1	28.1	9.2	20.7	7.3	16.5
RuDP	7.4	21.4	9.4	10.8	6.6	8.0	3.6	9.8	4.9	7.6
Sugar-P	29.3	16.8	42.6	40.9	47.7	41.0	37.3	30.5	27.6	22.8
UDPG	1.2	--	1.1	0.6	1.6	1.2	1.3	1.4	0.5	1.4
Sucrose	--	--	--	--	--	--	--	0.7	0.7	0.7
Glycolate	0.6	--	0.6	--	0.8	0.5	1.2	0.5	1.8	0.9
Glycine	0.6	--	2.2	--	1.1	0.3	1.7	0.9	2.1	0.1
Serine	1.6	0.9	1.3	0.7	2.0	0.4	3.6	1.5	5.6	2.5
Glycerate	1.0	--	0.4	--	0.2	0.2	0.4	--	0.4	0.3
Alanine	0.8	--	1.9	0.6	2.6	1.3	3.6	1.9	3.3	1.6
Pyruvate	--	--	--	0.6	--	0.4	--	0.2	--	0.4
Aspartate	1.4	0.9	2.3	2.1	2.7	2.1	6.7	4.6	8.6	5.8
Malate	8.0	11.1	7.4	7.6	7.5	9.3	18.6	16.8	17.7	18.0
Glutamate	--	0.3	--	0.7	0.6	0.4	4.0	2.3	8.6	9.2
Glutamine	0.5	--	--	--	--	--	0.6	--	1.7	0.7
α-KG	--	--	--	--	--	--	0.2	0.4	0.6	0.5
Fumarate	--	--	--	--	0.4	0.2	0.4	0.5	0.6	0.6
Succinate	--	--	--	--	--	--	--	--	--	--
Citrate	--	--	--	--	--	0.3	0.5	1.3	0.4	1.5
Lipids	--	--	--	--	--	--	--	--	--	--
Misc.	7.0	4.3	6.4	9.6	6.6	5.7	7.1	6.0	7.9	8.9

Table 7: Percent Distribution of ^{14}C Fixed by Chlamydomonas at pH 8.1

Control (Con) was 1 % algal suspension in 0.001 M phosphate pH 8.1; inhibitor (Inh) was 0.001 M OH-MSulfonate added to the control.

Time of PS	10"		20"		30"		60"		120"	
Conditions	Con	Inh	Con	Inh	Con	Inh	Con	Inh	Con	Inh
Total fixation ($c/mx10^{-4}$/ml algae)	2.6	12.1	4.8	24.2	7.1	41.6	13.4	66.7	26.1	136.9
% Fixed in:										
PGA	30.6	35.2	29.7	23.6	27.7	20.5	15.8	22.4	11.7	26.1
RuDP	11.1	15.2	12.2	14.2	13.9	20.7	4.5	16.3	6.1	15.3
Sugar-P	38.9	34.1	25.8	32.3	23.5	30.4	23.3	31.6	17.2	31.2
UDPG	--	--	--	--	--	--	--	--	--	--
Sucrose	--	--	--	--	--	--	--	--	--	--
Glycolate	--	0.7	3.9	0.9	5.4	1.5	3.0	2.1	2.0	0.9
Glycine	--	--	1.7	--	1.8	--	3.0	--	1.8	--
Serine	2.8	0.7	2.6	--	3.0	--	7.4	0.7	6.1	0.6
Glycerate	--	--	0.4	--	--	--	--	--	--	--
Alanine	--	--	0.9	1.7	2.4	--	6.9	--	4.5	--
Pyruvate	--	--	--	--	--	1.6	--	--	--	--
Aspartate	2.8	--	4.8	2.4	5.4	--	8.9	--	13.9	--
Malate	13.9	10.0	14.8	12.7	15.7	14.1	22.2	9.2	25.0	7.4
Glutamate	--	--	--	--	--	--	4.5	--	6.4	--
Glutamine	--	--	--	--	--	--	--	--	--	--
∝-KG	--	--	--	--	--	--	--	--	--	--
Fumarate	--	--	--	--	--	--	--	--	--	--
Succinate	--	--	--	--	--	--	--	--	--	--
Citrate	--	--	--	--	--	--	--	--	--	--
Lipids	--	--	--	--	--	--	--	--	--	--
Misc.	--	4.1	3.1	12.2	1.2	11.0	0.5	17.7	5.1	18.4

Table 8: Percent Distribution of ^{14}C Fixed by Chlamydomonas During Initial Days of Growth in Blue Light (400-500 mμ)

Photosynthesis occurred for 10 min. in blue light (300-400 ft-c) with a 0.5 % algal suspension (water).

Days grown in Blue Light	1	2	3	6	8	11	12	White Grown Control
Total fixation ($c/mx10^{-3}$/ml algae)	447.7	709.5	541.3	151.8	531.0	439.9	128.9	515.9
% Fixed in:								
PGA	1.8	5.6	4.9	2.0	3.9	2.8	0.7	7.9
RuDP	1.4	1.9	1.4	3.4	3.6	5.0	0.6	0.4
Sugar-P	18.0	25.3	19.3	21.6	19.6	27.6	18.9	33.0
UDPG	2.4	--	1.5	--	1.8	1.4	0.8	--
Sucrose	4.2	2.5	2.8	0.3	2.6	2.3	2.8	--
Glycolate	9.3	1.6	5.5	26.7	4.4	3.3	35.0	3.1
Glycine	2.2	1.0	1.8	2.1	2.9	1.6	2.1	2.7
Serine	4.9	6.1	9.0	5.8	4.7	5.7	5.8	3.6
Glycerate	4.2	--	--	--	1.2	1.1	2.5	1.7
Alanine	2.1	1.3	2.6	1.4	2.4	1.1	1.4	1.6
Pyruvate	--	--	--	--	--	--	--	--
Aspartate	7.7	6.0	8.6	7.2	7.8	4.7	3.4	5.1
Malate	17.1	15.4	12.0	18.2	14.1	16.8	9.4	8.0
Glutamate	10.8	16.8	16.2	3.8	12.5	10.7	3.3	18.6
Glutamine	2.7	2.2	3.5	4.5	3.0	3.3	3.2	1.0
α-KG	--	--	--	--	--	--	--	--
Fumarate	1.3	0.6	0.6	1.0	1.2	1.1	0.8	--
Succinate	2.7	1.2	2.4	--	2.2	1.5	1.6	4.0
Citrate	--	--	--	--	--	--	--	--
Lipids	--	--	--	--	--	--	--	--
Misc.	7.3	12.6	7.8	2.1	12.2	10.0	7.5	9.2

Table 9: Percent Distribution of ^{14}C Fixed by Chlorella During Initial Days of Growth in Blue Light (400-500 mμ)

Photosynthesis for 10 min. occurred in blue light (400-500 mμ) with a 0.5 % algal suspension (water).

Days grown in Blue Light	1	2	3	6	7	8	11	12	White Grown Control
Total fixation (c/mx10^{-3}/ml algae)	243.9	492.4	264.6	132.4	189.1	43.0	27.7	48.2	68.4
% Fixed in:									
PGA	5.8	2.1	5.1	8.8	--	4.4	0.4	4.8	6.9
RuDP	5.7	2.9	8.1	4.8	10.8	9.8	5.9	10.0	16.1
Sugar-P	28.5	24.8	25.1	31.1	21.6	24.5	28.8	23.6	31.5
UDPG	1.8	1.1	--	--	3.3	1.1	0.7	2.1	--
Sucrose	2.3	1.3	--	--	18.6	2.2	--	1.1	2.6
Glycolate	2.1	8.0	9.9	1.8	2.3	13.6	15.3	6.0	0.9
Glycine	3.9	6.2	7.4	11.5	7.5	12.2	11.5	9.8	10.7
Serine	13.8	17.4	17.5	22.4	17.1	16.6	22.6	20.3	--
Glycerate	--	--	--	--	--	--	--	--	--
Alanine	6.7	6.5	5.0	4.8	8.5	4.1	2.4	3.8	3.2
Pyruvate	--	--	--	--	--	--	--	--	--
Aspartate	6.2	12.3	7.4	8.2	2.5	3.8	3.1	4.9	9.6
Malate	4.8	5.7	2.3	3.9	3.8	1.9	1.4	2.7	10.5
Glutamate	--	2.3	2.5	--	0.8	0.8	2.8	1.1	2.1
Glutamine	--	--	5.1	--	--	--	--	--	--
α-KG	--	--	--	--	--	--	--	--	--
Fumarate	3.4	--	1.2	--	1.3	--	--	0.6	--
Succinate	--	--	--	--	--	--	--	--	--
Citrate	--	--	--	--	--	--	--	--	--
Lipids	--	--	--	--	--	--	--	--	--
Misc.	15.0	9.4	3.3	2.7	2.0	5.2	5.2	9.3	6.0

Table 10: Percent Distribution of ^{14}C Fixed by Chlamydomonas Adapted to Blue Light (400-500 mμ)

Photosynthesis with a 0.5 % algal suspension (water) occurred under various conditions of blue and red light.

Light Quality	Blue (400-500 mμ) Light			Red (>600 mμ) Light		
Intensity	110 ft-c			200 ft-c		
Time of PS	1'	3'	10'	1'	3'	10'
Total fixation (c/m x 10^{-3}/ml algae)	9.8	28.5	98.4	9.6	24.3	99.8
% Fixed in:						
PGA	21.7	8.0	7.8	27.1	10.1	5.5
RuDP	19.4	12.0	5.3	7.9	4.7	5.7
Sugar-P	24.8	15.4	4.4	21.0	23.7	20.2
UDPG	4.7	2.9	2.4	2.6	2.7	1.7
Sucrose	--	--	--	--	--	--
Glycolate	7.0	17.7	36.4	9.7	19.6	17.8
Glycine	7.0	8.6	2.4	3.5	4.7	2.1
Serine	2.3	5.1	2.9	--	4.1	5.2
Glycerate	--	--	1.0	--	--	--
Alanine	--	2.9	--	--	--	1.9
Pyruvate	--	--	--	--	--	--
Aspartate	--	2.9	7.3	2.6	3.4	6.7
Malate	3.1	8.0	3.9	7.0	14.2	9.5
Glutamate	--	2.9	3.9	--	4.1	10.0
Glutamine	--	--	2.4	--	--	1.4
α-KG	--	--	--	--	--	--
Fumarate	--	--	--	--	--	--
Succinate	0.8	--	--	0.9	--	--
Citrate	--	--	--	--	--	--
Lipids	--	--	--	--	--	--
Misc.	9.3	13.7	19.8	17.5	8.8	12.4

Table 11: Percent Distribution of ^{14}C Fixed by Chlamydomonas Adapted to Blue Light (400-500 mμ)

Photosynthesis with a 0.5 % algal suspension (water) occurred under two intensities of white light.

Light Intensity	100 ft-c			1200 ft-c		
Time of PS	1'	3'	10'	1'	3'	10'
Total fixation (c/m x 10^{-3}/ml algae)	4.7	14.1	41.5	11.2	45.6	191.0
% Fixed in:						
PGA	27.9	13.1	5.4	17.0	9.0	3.3
RuDP	17.7	2.2	4.4	16.9	12.8	3.3
Sugar-P	19.1	19.7	18.6	28.8	20.5	16.7
UDPG	7.4	5.1	--	1.7	3.9	--
Sucrose	--	--	1.0	--	--	--
Glycolate	13.2	18.3	12.3	3.4	12.8	36.2
Glycine	--	4.4	1.5	5.1	7.7	5.7
Serine	--	4.4	2.5	--	2.6	3.8
Glycerate	--	--	--	--	--	--
Alanine	--	--	2.0	--	--	--
Pyruvate	--	--	--	--	--	--
Aspartate	--	11.0	9.3	5.1	2.3	4.3
Malate	5.9	11.0	11.3	6.8	10.3	13.8
Glutamate	--	0.7	12.3	1.7	--	2.9
Glutamine	--	--	2.5	--	--	--
α-KG	--	--	--	--	--	--
Fumarate	--	--	--	--	--	--
Succinate	--	--	--	--	--	--
Citrate	--	--	--	--	--	--
Lipids	--	--	--	--	--	--
Misc.	8.2	10.2	9.8	13.6	17.9	10.0

Table 12: Percent Distribution of ^{14}C Fixed by Chlamydomonas Adapted to Red Light (>600 mμ)

Photosynthesis with a 0.5 % algal suspension (water) occurred under various conditions of red and white light.

Light Quality	Red (>600 mμ)			White			White		
Intensity	200 ft-c			100 ft-c			1200 ft-c		
Time of PS	1'	3'	10'	1'	3'	10'	1'	3'	10'
Total fixation (c/m x 10^{-3}/ml algae)	16.5	42.6	111.1	3.9	10.2	23.2	34.2	109.9	280.7
% Fixed in:									
PGA	8.9	12.1	10.1	12.7	15.0	7.6	27.5	8.7	2.5
RuDP	8.0	1.6	3.5	4.5	1.7	1.7	3.8	2.5	2.5
Sugar-P	22.1	24.8	21.7	18.7	13.3	17.4	34.3	38.1	20.1
UDPG	7.1	1.3	1.7	--	--	0.6	2.3	5.9	--
Sucrose	--	0.5	2.3	--	--	1.3	2.3	5.6	16.8
Glycolate	1.3	0.3	0.9	--	--	--	3.1	5.3	13.0
Glycine	1.3	2.6	1.5	--	--	0.2	1.5	2.5	2.5
Serine	7.1	5.5	4.1	3.0	4.3	3.4	4.6	4.2	2.3
Glycerate	2.2	1.1	1.5	--	0.4	1.5	--	1.1	--
Alanine	8.4	5.5	2.9	4.5	7.3	5.3	3.8	2.8	4.8
Pyruvate	4.9	--	1.7	--	0.4	1.0	--	--	--
Aspartate	7.1	8.7	8.1	17.2	15.4	11.7	5.3	4.5	6.1
Malate	7.5	12.4	12.7	20.2	20.5	13.6	5.3	8.4	7.8
Glutamate	8.0	11.9	13.3	6.7	12.4	19.5	1.5	1.4	6.7
Glutamine	--	1.6	3.8	--	0.9	1.5	--	--	2.5
α-KG	--	--	--	--	--	--	--	--	--
Fumarate	0.9	2.1	1.7	1.5	0.9	1.9	--	1.4	1.8
Succinate	0.4	2.6	2.3	--	1.3	0.4	--	--	1.8
Citrate	--	--	--	--	--	--	--	--	--
Lipids	--	--	--	--	--	--	--	--	--
Misc.	4.9	5.3	6.4	11.2	6.4	11.5	4.6	7.6	8.7

Table 13: Percent Distribution of ^{14}C Fixed by a 0.5 % Suspension of Chlorella (Grown in White Light) During Photosynthesis in Blue Light (400-500 mμ).

Time of PS			3 min.		
Lt. Intensity (ft-c)	50	100	200	300	400
Total Fixed (c/m x 10^{-3}/ml algae)	8.1	25.3	53.7	40.1	57.1
% Fixed in:					
PGA	6.8	24.1	19.9	13.3	6.0
RuDP	--	7.7	10.2	14.8	10.3
Sugar-P	21.1	33.5	36.7	32.5	26.1
UDPG	--	--	--	--	--
Sucrose	--	--	--	--	1.1
Glycolate	--	--	1.2	1.6	4.3
Glycine	--	--	1.2	0.5	4.9
Serine	2.0	3.0	6.3	7.4	10.9
Glycerate	--	--	--	--	--
Alanine	4.1	3.8	2.7	2.7	2.2
Pyruvate	--	--	--	--	--
Aspartate	40.1	12.4	6.6	6.4	4.3
Malate	13.6	10.9	9.9	12.2	8.8
Glutamate	4.8	1.1	1.5	3.2	7.1
Glutamine	2.7	--	--	--	--
α-KG	--	--	--	--	--
Fumarate	--	1.3	2.1	2.7	2.7
Succinate	--	--	--	--	--
Citrate	--	--	--	--	--
Lipids	--	--	--	--	--
Misc.	4.8	2.3	1.5	2.7	11.4

Table 14: Percent Distribution of ^{14}C Fixed by a 0.5 % Suspension of Chlorella (Grown in White Light) During Photosynthesis in Blue Light (400-500 mμ).

Time of PS			10 min.		
Lt. Intensity (ft-c)	50	100	200	300	400
Total Fixed (c/m x 10^{-3}/ml algae)	22.5	88.7	191.1	169.6	242.7
% Fixed in:					
PGA	10.1	12.7	11.2	4.5	8.9
RuDP	16.9	8.8	7.8	2.9	15.5
Sugar-P	18.8	26.5	42.2	41.5	33.8
UDPG	2.9	9.8	1.5	1.9	2.2
Sucrose	--	2.0	--	2.9	2.2
Glycolate	--	--	--	2.9	3.4
Glycine	--	--	--	3.1	2.1
Serine	4.8	6.5	14.9	16.2	10.0
Glycerate	--	--	--	--	--
Alanine	5.8	6.2	3.0	3.3	2.8
Pyruvate	--	--	--	--	--
Aspartate	27.1	13.1	5.2	7.8	6.6
Malate	7.2	6.9	6.0	8.0	5.3
Glutamate	5.8	3.0	0.7	2.3	1.9
Glutamine	1.4	--	--	--	--
α-KG	--	--	--	--	--
Fumarate	--	1.6	--	--	--
Succinate	--	--	--	--	--
Citrate	--	--	--	--	--
Lipids	--	--	--	--	--
Misc.	--	3.0	7.5	2.9	5.3

Table 15: Percent Distribution of ^{14}C Fixed by a 0.5 % Suspension of Chlorella (Adapted to Blue Light) During Photosynthesis in Blue Light (400-500 mμ).

Time of PS	3 min.				
Lt. Intensity (ft-c)	50	100	200	300	400
Total Fixed ($c/m \times 10^{-3}$/ml algae)	13.9	14.3	17.7	52.9	26.1
% Fixed in:					
PGA	24.2	29.8	19.6	10.4	14.4
RuDP	6.7	11.1	25.0	26.7	22.6
Sugar-P	26.1	17.1	18.9	22.7	20.5
UDPG	1.7	1.4	--	3.4	0.2
Sucrose	0.4	1.1	--	--	0.6
Glycolate	1.5	2.9	7.5	6.3	5.5
Glycine	2.6	3.9	5.7	8.5	4.9
Serine	7.6	7.5	8.2	8.7	11.5
Glycerate	--	--	--	--	--
Alanine	1.9	2.5	1.8	2.2	1.4
Pyruvate	--	--	--	--	--
Aspartate	18.8	10.0	8.6	5.1	7.8
Malate	3.9	3.2	1.7	2.7	4.9
Glutamate	0.6	--	--	--	--
Glutamine	1.5	3.9	2.5	--	1.6
α-KG	--	--	--	--	--
Fumarate	--	--	--	--	0.6
Succinate	--	--	--	--	--
Citrate	--	--	--	--	--
Lipids	--	--	--	--	--
Misc.	2.4	5.4	1.8	3.4	3.3

Table 16: Percent Distribution of ^{14}C Fixed by a 0.5 % Suspension of Chlorella (Adapted to Blue Light) During Photosynthesis in Blue Light (400-500 mμ).

Time of PS	10 min.				
Lt. Intensity (ft-c)	50	100	200	300	400
Total Fixed ($c/m \times 10^{-3}$/ml algae)	68.4	73.0	73.3	92.1	148.5
% Fixed in:					
PGA	18.4	14.2	8.9	5.1	7.3
RuDP	7.3	11.9	23.6	14.6	19.6
Sugar-P	27.7	24.5	18.8	20.3	20.9
UDPG	0.9	1.5	1.1	1.2	1.3
Sucrose	1.9	1.9	1.7	1.8	1.1
Glycolate	1.1	2.3	3.7	11.7	4.8
Glycine	3.4	4.5	6.9	8.1	6.7
Serine	14.4	16.2	16.4	14.1	18.6
Glycerate	--	--	--	--	--
Alanine	2.9	2.1	2.8	1.3	2.8
Pyruvate	--	--	--	--	--
Aspartate	6.6	7.4	5.0	5.5	4.4
Malate	3.9	1.9	2.6	2.6	3.1
Glutamate	1.2	1.4	0.6	1.1	0.7
Glutamine	0.8	1.1	0.7	1.2	0.6
α-KG	--	--	--	--	--
Fumarate	0.3	--	--	0.7	0.5
Succinate	--	0.6	--	0.7	0.6
Citrate	--	--	--	--	--
Lipids	--	--	--	--	--
Misc.	11.0	9.2	7.1	10.0	7.0

Table 17: Percent Distribution of ^{14}C Fixed by a 0.5 % Suspension of Chlamydomonas (Adapted to Blue Light) During Photosynthesis in Blue Light (400-500 mμ).

Time of PS			3 min.		
Lt. Intensity (ft-c)	50	100	200	300	400
Total Fixed ($c/m \times 10^{-3}$/ml algae)	8.8	18.0	38.9	49.1	50.0
% Fixed in:					
PGA	9.8	15.2	8.2	7.8	9.6
RuDP	--	8.8	9.2	5.0	10.4
Sugar-P	11.3	13.1	16.5	14.6	18.1
UDPG	--	--	--	0.6	1.9
Sucrose	2.8	--	1.3	1.4	0.4
Glycolate	11.2	4.7	11.8	32.4	13.1
Glycine	--	3.7	3.0	3.4	4.6
Serine	7.1	4.1	6.3	7.0	6.2
Glycerate	--	1.0	1.6	1.4	1.2
Alanine	7.1	2.0	3.6	1.2	1.5
Pyruvate	--	--	--	--	--
Aspartate	14.1	7.4	5.3	3.4	5.8
Malate	15.5	26.6	20.4	11.4	18.1
Glutamate	11.3	4.0	3.0	1.2	2.3
Glutamine	--	2.0	1.0	0.6	1.9
α-KG	--	--	--	--	--
Fumarate	2.8	1.7	0.7	1.6	1.5
Succinate	--	--	--	--	--
Citrate	--	--	--	--	--
Lipids	--	--	--	--	--
Misc.	7.1	5.7	8.2	7.2	3.5

Table 18: Percent Distribution of ^{14}C Fixed by a 0.5 % Suspension of Chlamydomonas (Adapted to Blue Light) During Photosynthesis in Blue Light (400-500 mμ).

Time of PS	10 min.				
Lt. Intensity (ft-c)	50	100	200	300	400
Total Fixed (c/m x 10^{-3}/ml algae)	41.7	87.9	165.0	183.1	186.1
% Fixed in:					
PGA	4.5	12.6	5.9	5.4	5.8
RuDP	4.2	3.7	5.3	4.3	5.2
Sugar-P	24.2	28.1	24.8	17.8	17.7
UDPG	3.3	0.7	1.9	1.1	0.9
Sucrose	1.4	1.0	2.5	1.8	1.7
Glycolate	19.7	9.4	11.2	28.0	17.5
Glycine	2.9	1.0	1.6	1.4	1.9
Serine	3.9	4.4	5.3	3.7	4.7
Glycerate	--	--	1.2	0.8	0.2
Alanine	1.2	3.4	1.6	3.5	1.9
Pyruvate	--	--	--	--	--
Aspartate	5.3	5.9	5.9	3.9	3.9
Malate	11.3	17.2	16.5	10.8	16.4
Glutamate	4.3	6.7	5.9	4.2	4.5
Glutamine	0.8	1.5	4.3	3.0	4.3
α-KG	--	--	--	--	--
Fumarate	0.6	--	0.9	0.5	0.9
Succinate	0.4	0.5	0.6	0.8	1.5
Citrate	--	--	--	--	--
Lipids	--	--	--	--	--
Misc.	11.1	4.0	4.7	9.1	11.0