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LIGHT-DARK TRANSIENTS IN LEVELS OF INTERMEDIATE COMPOUNDS DURING PHOTOSYNTHESIS IN AIR-ADAPTED *CHLORELLA*

By

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1. Introduction

Early studies of the kinetics of incorporation of radioactive carbon into the intermediate compounds of photosynthesis, such as those performed by Benson *et al.* (1952), Calvin and Massini (1952), and Wilson and Calvin (1955), employed unicellular algae, either *Chlorella* or *Scenedesmus*, which had been grown under high levels of carbon dioxide (typically 1-4% CO_2 in air). Difficulties in obtaining uniform samples of homogeneous biological material at very short intervals of time have generally precluded detailed kinetic studies of labeling rates and light-dark transient changes in leaves, which normally would have been grown in air. More recent kinetic studies of the incorporation of radiocarbon during photosynthesis, during which greater attention has been paid to maintenance of steady-state conditions, have been reported by Bassham and Kirk (1960), Smith *et al.* (1961) and Gould and Bassham (1965); but these studies have also employed *Chlorella pyrenoidosa*, grown in 2-4% CO_2 in air.

Whittingham (1952) has shown that *Chlorella pyrenoidosa* are capable of high rates of photosynthesis in air. However, *Chlorella* grown in 2-4% CO_2 in air, when placed in air, give very poor rates of photosynthesis for a long time. Thus it appears that there is some mechanism of adaption with algae grown in high levels of CO_2 which results in a temporary loss

in ability to photosynthesize efficiently in air. Because of the possible implications of this adaption to the mechanisms of the reactions responsible for carbon compound photosynthesis, it seemed desirable to conduct kinetic steady-state and light-dark transient studies with air-adapted algae photosynthesizing in air. The availability of very high specific activity ^{14}C, and improvement in our methods of conducting steady-state experiments, made such studies feasible. In addition, ^{32}P-labeled phosphate has been included as a second tracer in some of these studies in order to provide additional information about the levels of phosphorus-containing cofactors involved in the carbon reduction cycle of photosynthesis.

2. Material and Methods

In the first of the two experiments described, Chlorella pyrenoidosa were grown in continuous culture tube flasks, as described by Bassham and Calvin (1957). These algae were harvested, removed from old medium by centrifugation, and resuspended in fresh medium identical with that in which they were grown. The algal suspension, made up to 1% v/v, was placed in the steady-state apparatus which has been described in detail (Bassham and Kirk 1964). The photosynthesis chamber of this apparatus is a thin, transparent plastic vessel equipped with a water jacket, inlet and outlet valves for gas, a sampling valve, pH electrodes, a densitometer, and inlet valves for the automatic addition of nutrient solution and of a solution of 0.1 N ammonium hydroxide.

A mixture of carbon dioxide in air is circulated by means of a small diaphragm pump through the algae suspension and then through various instruments which monitor the levels of CO_2, O_2, and ^{14}C, all of which are automatically recorded against time on a Leeds & Northrup multipoint recorder. The gas handling system can be made to have large or small

volumes by means of gas reservoirs and stopcocks.

For the purposes of these experiments with air levels of CO_2, it was found desirable to introduce into the algal vessel a secondary bubbler in order to achieve better equilibration between gas and liquid phases. This bubbler consisted of a sintered glass cylinder about 1 cm long by 1/2 cm in diameter, hollowed out and joined to the end of a piece of glass tubing. The bubbler was inserted into the suspension of algae. Gas was taken from the outlet side of the vessel containing the suspension, passed through an auxiliary diaphragm pump similar to the one used in the main gas handling system of the apparatus, and then re-entered the vessel through the bubbler. The rate of flow of this secondary gas re-circulation was about 1 liter/min, and the rate of photosynthesis at air levels of CO_2 was significantly greater with this secondary bubbler in operation than without it.

After the suspension of algae had been photosynthesizing in air with density control and automatic addition of medium for 4 hours, the light was turned off for 8 hours, and then turned on again for another 4 hours of photosynthesis. The algae were then removed from the vessel, centrifuged, and resuspended in a medium consisting only of 10^{-3} M KH_2PO_4. The suspension was adjusted by the addition of a small quantity of 0.1 N HCl to a pH of about 4.8. The pH control mechanism was then turned on and 0.1 N NH_4OH flowed into the suspension, bringing the pH to 5, where it was automatically maintained throughout the duration of the experiment. The algae were permitted to photosynthesize in air in this medium for an additional 2 hours, at which time the tracer experiment was commenced.

At the beginning of the tracer experiment, the gas handling system was closed, and $^{14}CO_2$ (0.06%, 50 $\mu C/\mu mole$) was added to the system. This gas was contained in a 5-liter reservoir, so that the total amount of CO_2

at the start of the experiment was 120 μmoles. The rate of carbon dioxide uptake during the course of the experiment, which lasted for 13 min in the light, was subsequently found to be 3.16 μmoles/min/cm^3 algae.

After 13 min of photosynthesis with ^{14}C, the light was turned off for the remainder of the experiment. During the entire course of the experiment, samples of the algal suspension of approximately 1 ml volume were taken into weighed test tubes containing 4 ml of methanol for killing. These tubes were subsequently weighed to determine sample size. Samples were taken at 10-sec intervals just after the introduction of $^{14}CO_2$ and just after the light was turned off.

For analysis of the radioactive compounds formed by the algae, 1/5 aliquot samples of the killed algae-methanol mixture were dried on the origin of Whatman No. 1 chromatographic paper. These chromatograms were developed in two directions. The first solvent was made up of 840 ml "liquified" phenol (Malinckrodt, about 88% phenol, 12% H_2O), 160 ml water, 10 ml glacial acetic acid, and 1 ml 1.0 M ethylenediamine tetraacetic acid. The second solvent was made up of equal volumes of n-butanol-water (370:25 v/v) and of propionic acid-water (180:220 v/v), as described by Benson et al. (1950). In experiments containing only ^{14}C as a tracer, the chromatograms were developed for 24 hours in each direction. After radioautography to determine the position of the radioactive compounds on the paper, the carbon 14 content of each compound was measured by means of the "automatic spot-counter" developed by Moses et al. (1963).

Experiment with ^{14}C and ^{32}P as tracers

In the other experiment, Chlorella pyrenoidosa were grown in a low-form culture flask shaken on a water bath (Bassham and Calvin 1957) and harvested at approximately 2-day intervals. In this batch process, the rate of flow of the mixture of 4% CO_2 in air through the culture

flask was made so slow that carbon dioxide supply limited growth. The effect of this limitation was to reproduce roughly the condition of Chlorella grown in air. After harvesting and removal from the old medium by centrifugation, these Chlorella were resuspended (to 1% v/v) in 10^{-4} M KH_2PO_4 adjusted to pH of 4.8 with 0.1 N HCl. The pH control was set at 5 and enough 0.1 N NH_4OH was added to bring the pH to 5, after which it was maintained automatically. A solution of essentially carrier-free phosphoric acid, containing 10 mC of ^{32}P, was brought to pH 5 by titration with 0.1 N NaOH. This solution was added to the algae suspensions by injection through a rubber plug in the algae vessel. Steady-state photosynthesis in air was continued for 30 min prior to the addition of $^{14}CO_2$. At that time the gas system was closed and $^{14}CO_2$ (0.07%, 32 $\mu C/\frac{\mu moles}{}$) was added to the system from the large reservoir. No samples were taken until 10 min after addition of $^{14}CO_2$. Then samples were taken at intervals indicated in the figures for the duration of the experiment. After 13 min with $^{14}CO_2$, the light was turned off and the algae were kept in darkness until 30 min, at which point the light was turned on and remained on for the duration of the experiment.

Killing of the algae samples and analysis of the radioactive compounds by two-dimensional paper chromatography was performed as described for the first experiment, except that two-dimensional paper chromatography was carried out with one set of chromatograms for 48 hours in each dimension in order to obtain better separation of the phosphate-labeled compounds. In order to preclude fogging of the radioautograph by the high levels of ^{32}P-labeled inorganic phosphate, a preliminary radioautography for 1/2 hour was performed. This radioautograph was used as a guide for cutting out the central part of the inorganic phosphate spot. The paper chromato-

grams were then placed back on fresh X-ray film and a new radioautograph was prepared by exposure of the film to the paper for 1-1/2 days. With the radioautograph as a guide, areas of the paper containing the labeled compounds were cut out and their radioactivity determined. The sugar phosphates and diphosphates were dephosphorylated by treatment with a phosphatase. After rechromatography of the free sugars, the individual sugars were eluted from the paper chromatogram into sample vials for counting. In this case 0.3 ml or less of aqueous eluate from the paper was placed in 18 ml of water-miscible scintillation counting liquid, as described by Kinard (1957). The sample vials were counted automatically by the scintillation counter (Packard Instruments, Series 3000). Other phosphate-labeled compounds were eluted from the original chromatogram into vials, and their content of both ^{14}C and ^{32}P was determined by counting in the automatic liquid scintillation counter.

The identities of the following compounds were verified by elution of the suspected radioactive spot from the paper chromatogram and co-chromatography with authentic samples, followed by spraying with the Hanes-Isherwood (1949) phosphate spray: ATP, ADP, UTP, UDPG,* and PP_1.* Other compounds of the carbon reduction cycle and related compounds were identified by their positions or co-chromatography either before or after treatment with phosphatase.

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*Abbreviations: UDPG, uridine diphosphoglucose; PP_1, pyrophosphate; PGA, 3-phosphoglyceric acid; RuDP, ribulose-1,5-diphosphate; Ru5P, ribulose-5-phosphate; R5P, ribose-5-phosphate; FDP, fructose-1,6-diphosphate; F6P, fructose-6-phosphate; SDP, sedoheptulose-1,7-diphosphate; S7P, sedoheptulose-7-phosphate; DHAP, dihydroxyacetonephosphate; PEPA, phosphoenolpyruvic acid; GMP, glucose monophosphate.

3. Results and Discussion

The levels of ^{14}C found in various intermediate compounds of the carbon reduction cycle as a result of the first experiment can be obtained from the saturation levels seen in Figures 1 through 4. The total radioactivity per cm^3 of algae found in each compound has been divided by the specific radioactivity of the $^{14}CO_2$, which is measured continuously, to obtain the concentration of labeled carbon found in the intermediate compounds. When the level of ^{14}C in a given compound no longer increases with time, it is assumed that as much ^{14}C is entering the metabolic pool of molecules of the intermediate compound as is leaving. After this time (8 min in this experiment), the ^{14}C in each compound is a measure of the size of the actively-turning-over metabolic pool (Bassham and Kirk 1960), provided biosynthesis of molecules in the pool utilizes only fully-labeled precursors. During active photosynthesis, the rate of photosynthetic formation of intermediate compounds of the carbon reduction cycle from $^{14}CO_2$ is so much greater than the rate of synthesis of these compounds from other (unlabeled) precursors, that this condition is met. However, when the light is turned off, syntheses of photosynthetic carbon reduction cycle intermediate compounds from precursors which are not fully labeled may alter both pool sizes and specific radioactivities. An example of this condition will be seen in Figure 9.

The pool sizes obtained from this experiment are compared in Table I with previously published pool sizes for algae photosynthesizing under steady-state conditions with 1-2% CO_2. The pool size of the carbon dioxide acceptor, ribulose-1,5-diphosphate (RuDP), is greatly increased, whereas the pool sizes of other intermediates in the cycle are much smaller in the air-adapted algae as compared with the algae grown and photosynthesizing with high levels of CO_2.

This greatly increased RuDP pool size with the air-adapted algae might suggest that the mechanism of adaptation is simply an increase in the concentration of the carboxylation substrate. That this is not the case was seen in preliminary experiments in which Chlorella grown and photosynthesizing in 1-2% CO_2 in air were suddenly given only air (.04% CO_2). Although the level of RuDP rose as high as 2 μmoles of carbon per cm^3 of algae, the photosynthesis rate was less than 0.8 μmoles CO_2 per min per cm^3 of algae.

The initial labeling rates, saturation, and light-dark transient changes in the levels of intermediates of the photosynthetic carbon reduction cycle in the first experiment are shown in Figures 1 through 5. Although the levels of RuDP and of 3-phosphoglyceric acid (PGA), (Figure 1), are quite different than in experiments previously reported with algae in 1% CO_2, the general shape of the transient changes is the same. The level of PGA rises very rapidly when the light is turned off and then declines more slowly. The level of RuDP drops very rapidly. However, in this experiment, in contrast to all previous light-dark transient studies with higher levels of carbon dioxide, the level of RuDP does not fall to zero but instead remains from 4 to 8 min after the light at a nearly constant and easily measurable level. That the RuDP under these conditions of .04% CO_2 does not decline to zero in the dark may be of considerable significance with respect to the possible mechanism of the carboxylation reaction in vivo at low CO_2 levels. It suggests that, contrary to previous indications, the carboxylation reaction in vivo with low levels of CO_2 may depend upon some form of light activation. Otherwise the RuDP would be used up beyond the limits of detection. The free energy change of the carboxylation reaction in photosynthesis as calculated by Bassham (1963) is so negative that no

measurable amount of RuDP could be expected to be seen when the reaction has gone to equilibrium.

In Figure 2 it can be seen that the levels of sugar diphosphate fall rapidly towards zero when the light is turned off. In the dark, reduced NADP is no longer available for the reduction of PGA to triose phosphate with subsequent formation of hexose and heptose diphosphates. Following the initial drop to zero there is a very pronounced rise in the levels of fructose-1,6-diphosphate (FDP) and sedoheptulose-1,7-diphosphate (SDP) which pass through a maximum about 3 min after the light is turned off. This transient rise in the levels of these diphosphates indicates a device of the regulatory mechanism in adjustment of the metabolism to the dark condition. It appears that there may be a dark inhibition of the diphosphatase reaction which converts FDP and SDP to their corresponding monophosphates. Without such a diphosphatase inhibition these diphosphates should be broken down as rapidly as they are formed, for one must presume that their formation cannot be as fast in the dark as during photosynthesis.

Secondly, the transient peak in levels of these diphosphates implies a dark-activated mechanism or the release from a light-inhibited step leading to the formation of these compounds. Such a reaction might be the phosphofructokinase reaction. From the results of the second experiment reported later, it is clear that after one min of darkness the level of ATP which fell initially due to the cessation of photophosphorylation and the temporary continued demand for ATP by the photosynthetic carbon reduction cycle, rises again due to respiratory reactions.

During photosynthesis, FDP and SDP are continuously and rapidly converted to their respective monophosphates. A high level of diphosphatase activity is required. At the same time there must not be any kinase activity

which would convert hexose and heptose monophosphates to diphosphates. The only kinase permissible during photosynthesis is the kinase which forms the carboxylation precursor, RuDP. A hexokinase would waste ATP by utilizing it for the conversion of F6P to FDP, which would be immediately broken down again by the phosphatase.

If both photosynthesis and glycolysis proceed at the same site in the light and in the dark respectively, it is essential that there be a light-dark switching mechanism which (when the light is turned off) inactivates the diphosphatase and activates the kinase. Figure 3 shows the level of ^{14}C in various sugar monophosphates, and it is of interest to note that following the initial transients, the levels of ^{14}C in these sugars continually decline in the dark despite the fact that no further carboxylation of RuDP is occurring. This may be a further indication of glycolysis.

Figure 4 shows that the level of dihydroxyacetone phosphate (DHAP) parallels that of FDP, while the level of phosphoenolpyruvic acid (PEPA) parallels that of PGA. Figure 5, the levels of pentose monophosphate, shows that these levels do not change much when the light is turned off, an indication that both their formation and utilization are effectively blocked in the dark.

There is a relatively rapid drop in the labeled glycolic acid level when the light is turned off. Possibly this drop is related to a cessation of O_2 production in the chloroplast, since the formation of glycolic acid has been found to be very dependent upon O_2 tension (Bassham et al 19). Many explanations for the dark decline and subsequent rise in malic acid label are possible. For example, reduced cofactors required for reductive carboxylation of PEPA may be quickly exhausted when the light is turned off and then may be regenerated when glycolysis rate increases after 2-4

minutes of darkness.

Figure 7 shows the transient changes in levels of several amino acids. The most pronounced light-dark effects are the rapid synthesis of alanine and of serine immediately following turning off the light and the rapid synthesis of aspartic acid and glutamic acid after about a minute of darkness. The syntheses of these acids no doubt accounts for the major part of the drop in level of PGA following its initial rise in the darkness. The delayed dark-enhanced syntheses of aspartic acid and glutamic acid are probably a reflection of the time taken for the reestablishment of ATP level by respiratory reactions as seen in later results.

Figure 8 shows the levels of ATP, ADP, UTP and PP_i observed in the second described experiment. When the light is first turned off, the level of ATP declines rapidly due to cessation of photophosphorylation and continued ATP requirement for the reduction of PGA to sugar phosphate. After one minute of darkness the ATP begins to rise again rapidly and is soon reestablished to the previous level. Further transitory fluctuations follow and then the ATP level rises steadily in the dark. When the light is again turned on the ATP level rises sharply due to photophosphorylation and then falls perhaps as a result of increased demand by the operation of the photosynthetic carbon reduction cycle.

In general, the behavior of the ADP level is inverse to that of the ATP, while the level of UTP parallels the changes in the levels of ATP.

The changes in the level of PP_i are striking. The PP_i spot which we observe on our chromatograms may well reflect a decomposition product of some more labile high energy phosphate present in the living cell. The level of PP_i has been observed to undergo rapid changes upon the introduction of inhibitors which also affect photophosphorylation (Pedersen *et al.*, In the press). The results of those studies led to the conclusion

that the PP_i, or its biochemical precursor, probably was formed from or parallel to ATP and was utilized in some way by the carbon reduction cycle.

Santarius and Heber (1965) have reported that the level of AMP in chloroplasts of some leaves undergoes substantial light-dark changes. They suggest the possibility that there might be a reaction activating CO_2 *in vivo* which could generate ATP. From our results which indicate a light activation of the carboxylation reaction, and from the rapid light-dark transient changes in the level of PP_i, it is tempting to speculate that PP_i may be the decomposition product of some cofactor involved in the activation of a CO_2 for *in vivo* photosynthesis. Thus far, however, we have not succeeded in sufficiently labeling AMP to permit us to observe possible changes in its level.

The levels of ^{14}C and ^{32}P in PGA during the second experiment are shown in Figure 9. The change in ^{32}P and ^{14}C during the first part of the light-dark transients are the same—that is, both increase rapidly and then begin to decline. From that point on, however, there is an interesting and significant difference between the ^{14}C and ^{32}P level of the PGA pool. As observed previously, the ^{14}C level of PGA declines rapidly and steadily in the dark, reaching a very low level after some minutes of darkness. However, the level of ^{32}P after an initial small decline stays virtually unchanged for the duration of the period of darkness. These results indicate metabolism of ^{14}C and ^{32}P-labeled PGA and synthesis of PGA from unlabeled carbon and labeled phosphate. This new synthesis of PGA in the dark from an unlabeled carbon source may be expected if sugar reserves which have not become appreciably labeled during the few minutes of photosynthesis with $^{14}CO_2$ are broken down by glycolysis to make PGA as rapidly as the PGA is utilized for subsequent

biosynthetic reactions such as those leading to the synthesis of amino acids and respiratory combustion to carbon dioxide.

The pool of PGA formed in this way could be separate from the photosynthetic pool of PGA. However, when the light is turned on again the level of ^{32}P-labeled PGA drops very rapidly without a concommitant drop in the level of ^{14}C-labeling. Such behavior suggests that the PGA labeled with ^{32}P only is being rapidly reduced by cofactors formed from the light reaction of photosynthesis. Thus it appears that most of the changes in levels observed during this experiment may be accounted for in terms of a single pool which is synthesized from $^{14}CO_2$ and ^{32}P-labeled phosphate in the light but is formed from unlabeled carbon precursors and labeled phosphate in the dark. This observation lends strong support to the idea that there is a pool of PGA common to the photosynthetic carbon reduction cycle and to a significant part of dark glycolysis. If triose and hexose phosphate pools are also common to glycolysis and photosynthesis, the triose phosphate dehydrogenase reaction may be reversed in the transition from light to dark through the change in the supply of reduced NADP. However, the light-dark switch in the activities of hexose and heptose diphosphatase and phosphofructokinase would still be required since neither photosynthesis nor respiration can operate efficiently in the simultaneous presence of both enzymic activities.

A further point of interest is the apparent absence of a kinase reaction forming RuDP in the dark. In Figure 5, the level of Ru5P is about the same in the dark as in the light. The overall level of ATP is higher after some minutes in the dark than it is in the light. Thus, there might be some light-dark control mechanism regulating the conversion of Ru5P to RuDP as well as the subsequent carboxylation reaction leading

to PGA. Alternitavely, it may be that Ru5P kinase does not have access to the ATP which is produced in the dark but must use only ATP formed during photophosphorylation. If there is compartmentalization of this type for Ru5P, can there be pools of PGA and perhaps hexose phosphates that are common to both respiration and photosynthesis?

From their studies of intracellular translocation of photosynthetic products in leaves, Heber and Willenbrink (1964) and Santarius et al. (1964) concluded that there is a considerable translocation of many sugar phosphates and of ATP from chloroplast to cytoplasm. Significantly, they found that RuDP was not translocated from the chloroplast.

Perhaps the simplest explanation of our results with Chlorella would be that there is considerable movement of PGA and sugar phosphates between chloroplast and cytoplasm. To explain the kinetics of changes in PGA labeling, one must then suppose that the level of PGA in the cytoplasm is quickly affected by the existence or absence of photosynthesis in the chloroplast. When the light is turned on and PGA inside the chloroplast is reduced, PGA from the cytoplasm might flow back into the chloroplast to maintain its concentration during the induction period when the level of RuDP had not yet built up sufficiently to permit a rapid carboxylation. On the basis of kinetic studies, in vivo, it is difficult to distinguish between the possibility of a single locus for photosynthesis and glycolysis, and the alternative possibility of two loci, with rapid diffusion of some intermediate compounds between the loci. In either case, a light-dark change in enzymic activities at key steps in the metabolic pathways would be required, and is indicated by the results of this study.

Summary

The labeling of intermediate compounds and photosynthetic cofactors during photosynthesis and periods of darkness by Chlorella pyrenoidosa in the presence of ^{32}P-labeled phosphate and $^{14}CO_2$ have been investigated. Algae adapted to photosynthesis in air were used, and the level of carbon dioxide was maintained at approximately 0.04% and at constant specific radioactivity during the course of the experiments.

The transient changes which occur in the levels of labeled fructose-1,6-diphosphate and in sedoheptulose-1,7-diphosphate, and in the corresponding monophosphates when the light is turned off suggest a light activation of the diphosphatase enzymes which decays after about 2 minutes of darkness. It is suggested that a light-dark switch in enzymic activities permits photosynthesis and glycolysis to occur in light and dark respectively with the same enzymic apparatus.

The greatly diminished rate of disappearance of the carboxylation substrate, ribulose-1,5-diphosphate, after about 2 min suggests that there is also a light activation of the carboxylation reaction in vivo. Large transient changes in the level of pyrophosphate between light and dark indicate that there may be an unstable cofactor which decomposes to give pyrophosphate during or after killing of the algal cells. The possibility that this cofactor is involved in an activation of carbon dioxide for the carboxylation reaction in vivo is suggested.

Light-dark transient changes in labeling of other compounds of the photosynthetic carbon reduction cycle and related compounds were determined, and possible significance of these changes is discussed.

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References

Bassham, J. A. & Calvin, M.: The Path of Carbon in Photosynthesis.— Prentice-Hall, Englewood Cliffs, New Jersey. 1957.

Bassham, J. A. & Kirk, M.: Dynamics of the Photosynthesis of Carbon Compounds. I. Carboxylation Reactions.— Biochim. Biophys. Acta 43: 447-464. 1960.

Bassham, J. A.: Photosynthesis: Energetics and Related Topics.— In: Advances in Enzymology, pp. 40-117, Interscience Publishers, New York. 1963.

Bassham, J. A. & Kirk, M.: Photosynthesis of Amino Acids.— Biochim. Biophys. Acta 90:553-562. 1964.

Benson, A. A., Bassham, J. A., Calvin, M., Goodale, T. C., Haas, V. A. & Stepka, W.: The Path of Carbon in Photosynthesis. V. Paper Chromatography and Radioautography of the Products.— J. Am. Chem. Soc. 72: 1710-1718. 1950.

Benson, A. A., Kawauchi, S., Hayes, P. & Calvin, M.: The Path of Carbon in Photosynthesis. XVI. Kinetic Relationships of the Intermediates in Steady-State Photosynthesis.— J. Am. Chem. Soc. 74:4477-4482. 1952.

Calvin, M. & Massini, P.: The Path of Carbon in Photosynthesis. XX. The Steady-State.— Experientia VIII/12:445-457. 1952.

Gould, E. S. & Bassham, J. A.: Inhibitor Studies on the Photosynthetic Carbon Reduction Cycle in Chlorella pyrenoidosa.— Biochim. Biophys. Acta 102:9-19. 1965.

Hanes, C. S. & Isherwood, F. A.: Separation of the Phosphoric Esters on the Filter Paper Chromatogram.— Nature 164:1107-1112. 1949.

Heber, U. & Willenbrink, J.: Sites of Synthesis and Transport of Photosynthetic Products Within the Leaf Cell.— Biochim. Biophys. Acta 82:313-324. 1964.

Kinard, F. E.: Liquid Scintillator for the Analysis of Tritium in Water.— Rev. Sci. Inst. 28:293-295. 1957.

Moses, V. & Lonberg-Holm, K. K.: A Semi-automatic Device for Measuring Radioactivity on Two-dimensional Paper Chromatograms.— Anal. Biochem. 5:11-27. 1963.

Pedersen, T. A., Kirk, M. & Bassham, J. A.: Inhibition of Photophosphorylation and Photosynthetic Carbon Cycle.— Biochim. Biophys. Acta. In the press.

Santarius, K. A., Heber, U., Ullrich, W. & Urbach, W.: Intracellular Translocation of ATP, ADP and Inorganic Phosphate in Leaf Cells of Elodea densa in Relation to Photosynthesis.— Biochem. Biophys. Res. Commun. 15:139-146. 1964.

Santarius, K. A. & Heber, U.: Changes in the Intracellular Levels of ATP, ADP, AMP and PP_i and Regulatory Function of the Adenylate System in Leaf Cells during Photosynthesis.— Biochim. Biophys. Acta 102:39-54. 1965.

Smith, D. C., Bassham, J. A. & Kirk, M.: Dynamics of the Photosynthesis of Carbon Compounds. II. Amino Acid Synthesis.— Biochim. Biophys. Acta 48:299-313. 1961.

Whittingham, C. P.: Rate of Photosynthesis and Concentration of Carbon Dioxide in Chlorella.— Nature 170:1017-1018. 1952.

Wilson, A. T. & Calvin, M.: The Photosynthetic Cycle, CO_2 Dependent Transients.— J. Am. Chem. Soc. 77:5948-5957. 1955.

Table I. Calculated concentrations of photosynthetic intermediate compounds. Concentrations are calculated as described in text by dividing radioactivity at "saturation" by specific radioactivity.

Compound	Concentration per cm^3 algae, μgram atoms of carbon	
	0.04% CO_2	1% CO_2*
3-phosphoglyceric acid	0.40	3.0
dihydroxyacetone phosphate	0.096	
fructose-1,6-diphosphate	0.086	
fructose-6-phosphate	0.27	
sedoheptulose-1,7-diphosphate	0.01	
sedoheptulose-7-phosphate	0.30	1.8
ribose-5-phosphate	0.02	
ribulose-1,5-diphosphate	1.89	0.36

* From Bassham and Kirk (1960)

Figure Legends

Figure 1. Levels of photosynthetic intermediate compounds in light and dark: phosphoglyceric acid and ribulose diphosphate.

Figure 2. Levels of photosynthetic intermediate compounds in light and dark: hexose and heptose diphosphates.

Figure 3. Levels of photosynthetic intermediate compounds in light and dark: hexose and heptose monophosphates.

Figure 4. Levels of photosynthetic intermediate compounds in light and dark: dihydroxyacetone phosphate and phosphoenolpyruvate.

Figure 5. Levels of photosynthetic intermediate compounds in light and dark: pentose monophosphates.

Figure 6. Levels of photosynthetic intermediate compounds in light and dark: malic and glycolic acids.

Figure 7. Levels of photosynthetic intermediate compounds in light and dark: amino acids.

Figure 8. Levels of photosynthetic cofactors in light and dark: ATP, ADP, UTP, and PP_i.

Figure 9. Levels of ^{14}C and ^{32}P labeling in PGA during photosynthesis and in the dark.

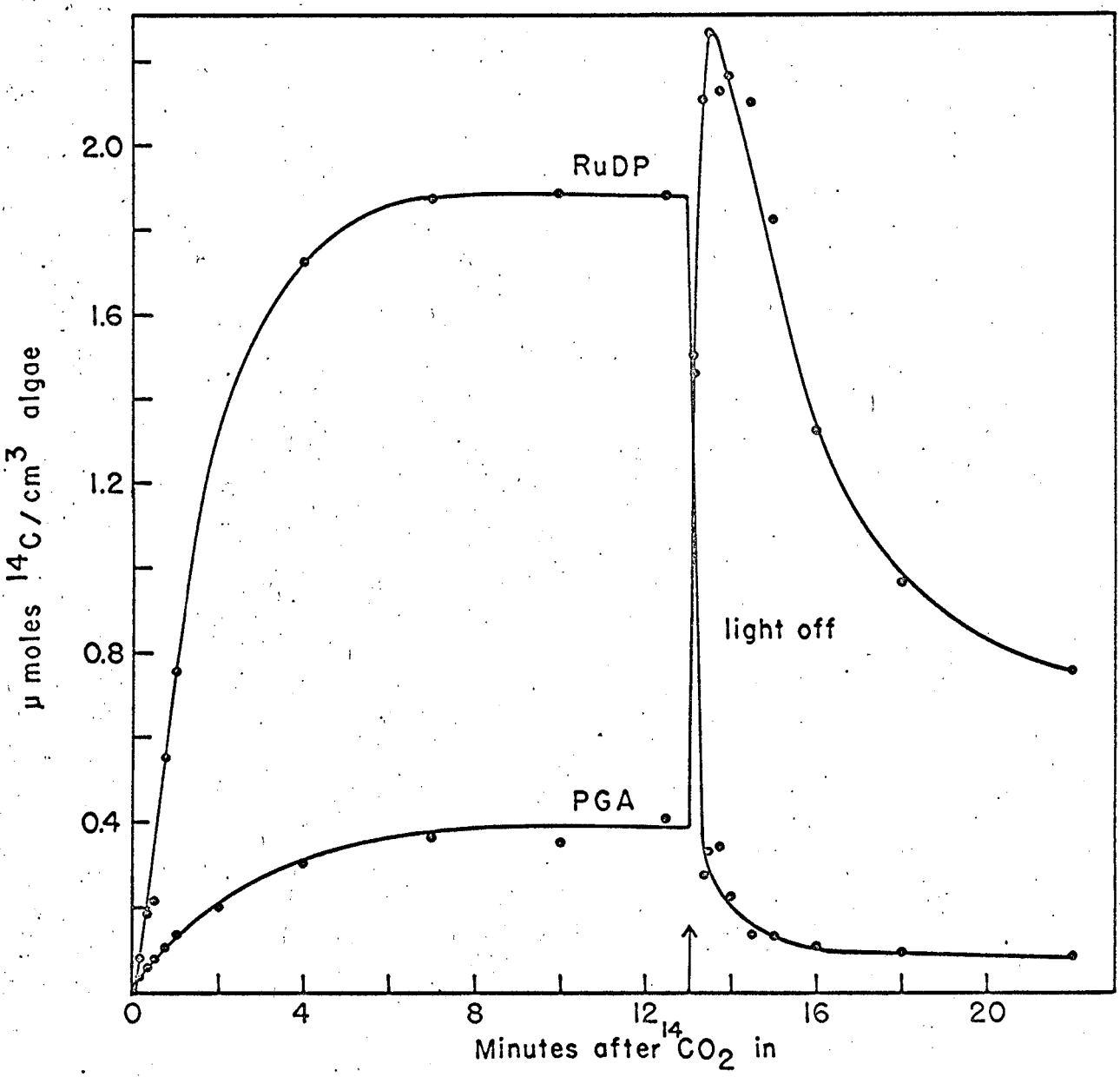

2.0
1.6
1.2
0.8
0.4
μ moles ^{14}C / cm^3 algae
RuDP
PGA
light off
0
4
8
12
16
20
Minutes after $^{14}CO_2$ in
MUB-4694

Figure 1

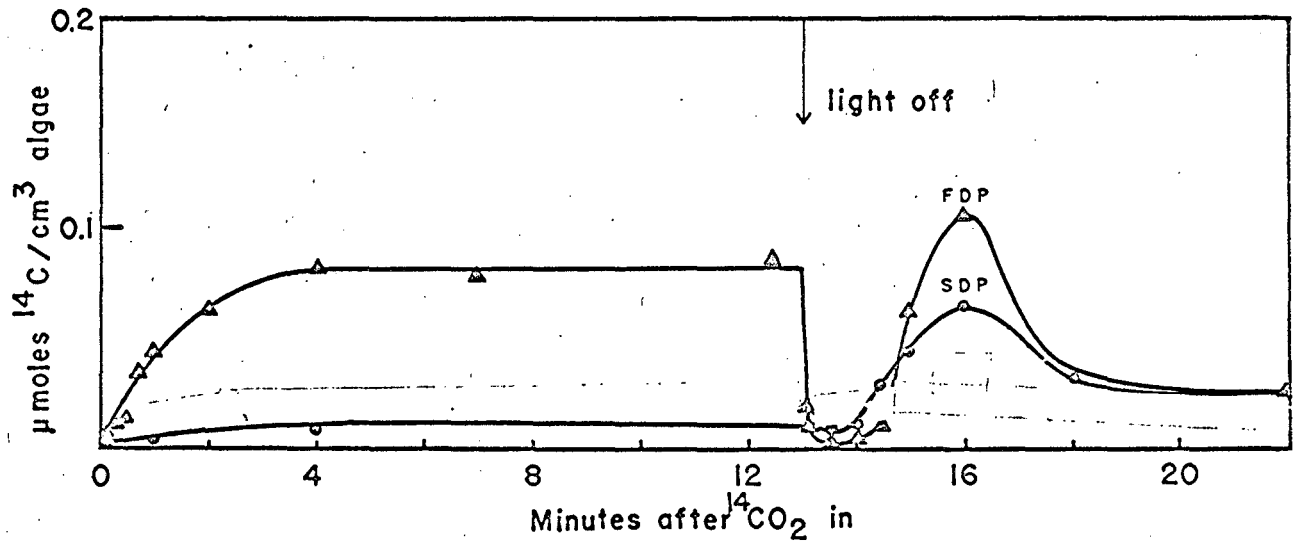

0.2
0.1
light off
FDP
SDP
μmoles $^{14}C/cm^3$ algae
0
4
8
12
16
20
Minutes after $^{14}CO_2$ in
MUB-4691

Figure 2

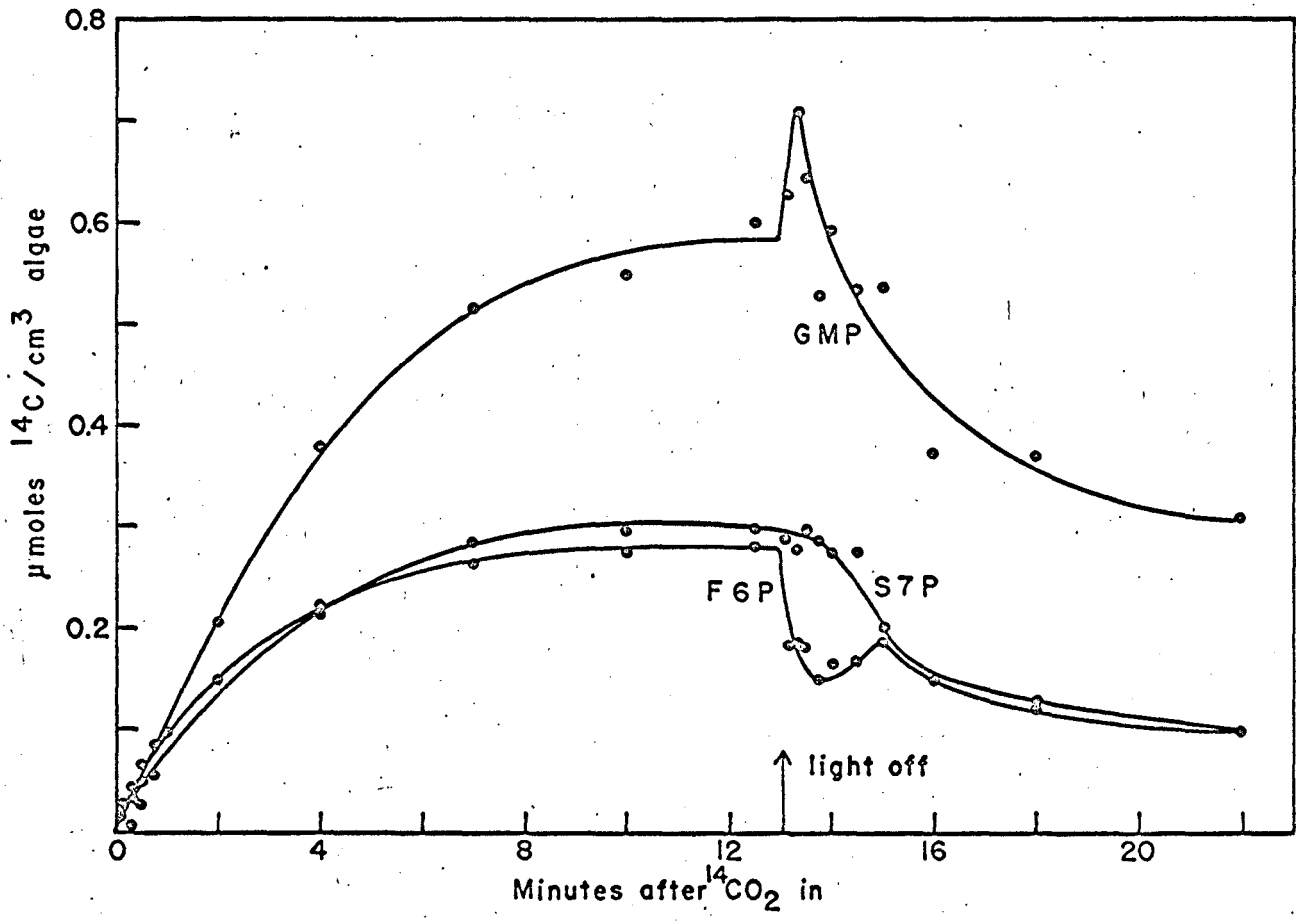

0.8
0.6
0.4
0.2
μmoles $^{14}C/cm^3$ algae
GMP
F6P
S7P
light off
0
4
8
12
16
20
Minutes after $^{14}CO_2$ in
MUB-4693

Figure 3

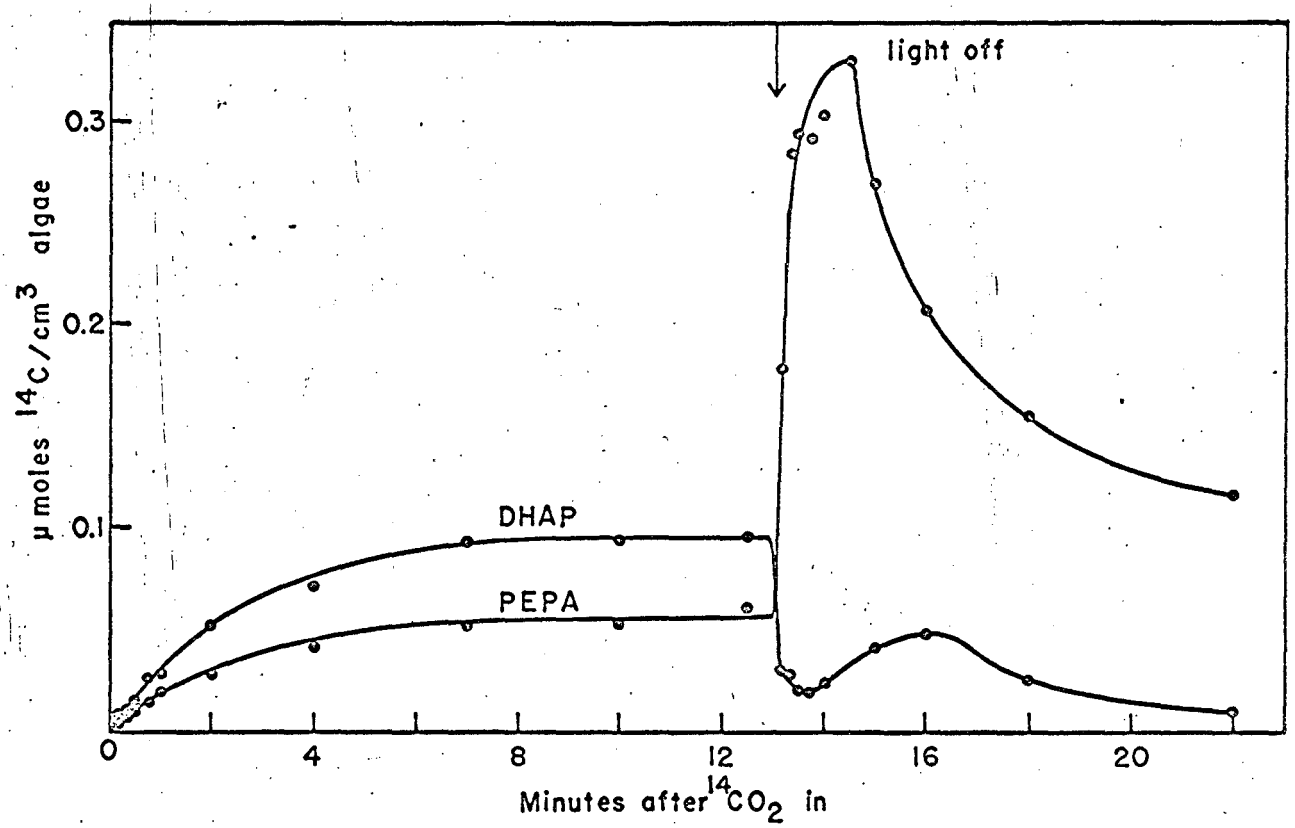

light off
0.3
0.2
0.1
μ moles $^{14}C/cm^3$ algae
DHAP
PEPA
0
4
8
12
16
20
Minutes after $^{14}CO_2$ in
MUB-4692

Figure 4

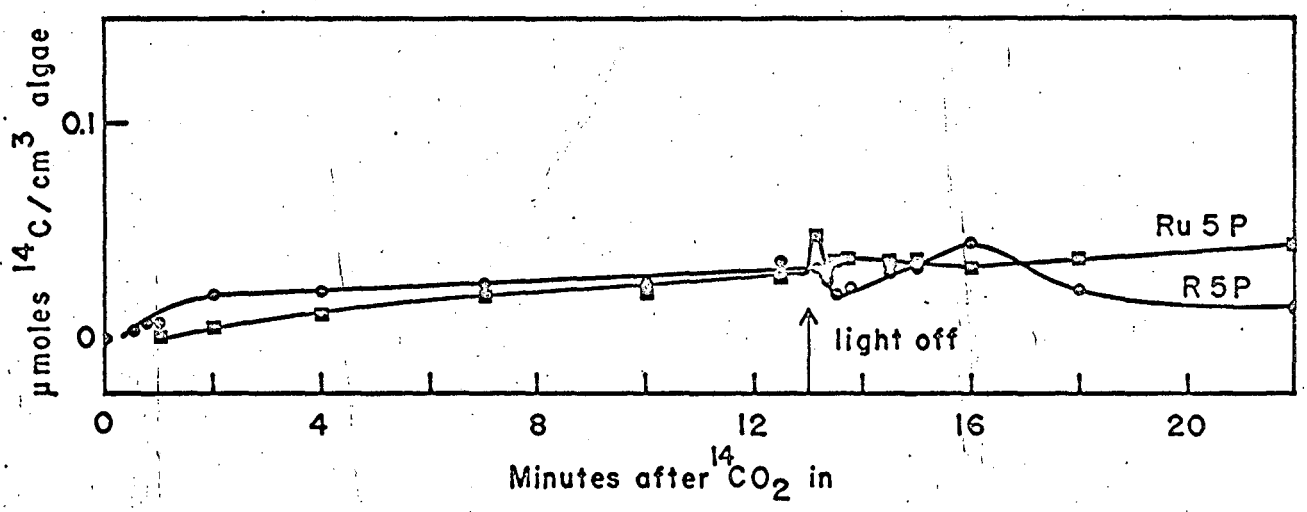

μmoles $^{14}C/cm^3$ algae
0.1
0
Ru 5 P
R 5P
light off
0
4
8
12
16
20
Minutes after $^{14}CO_2$ in
MUB-4690

Figure 5

Figure 6

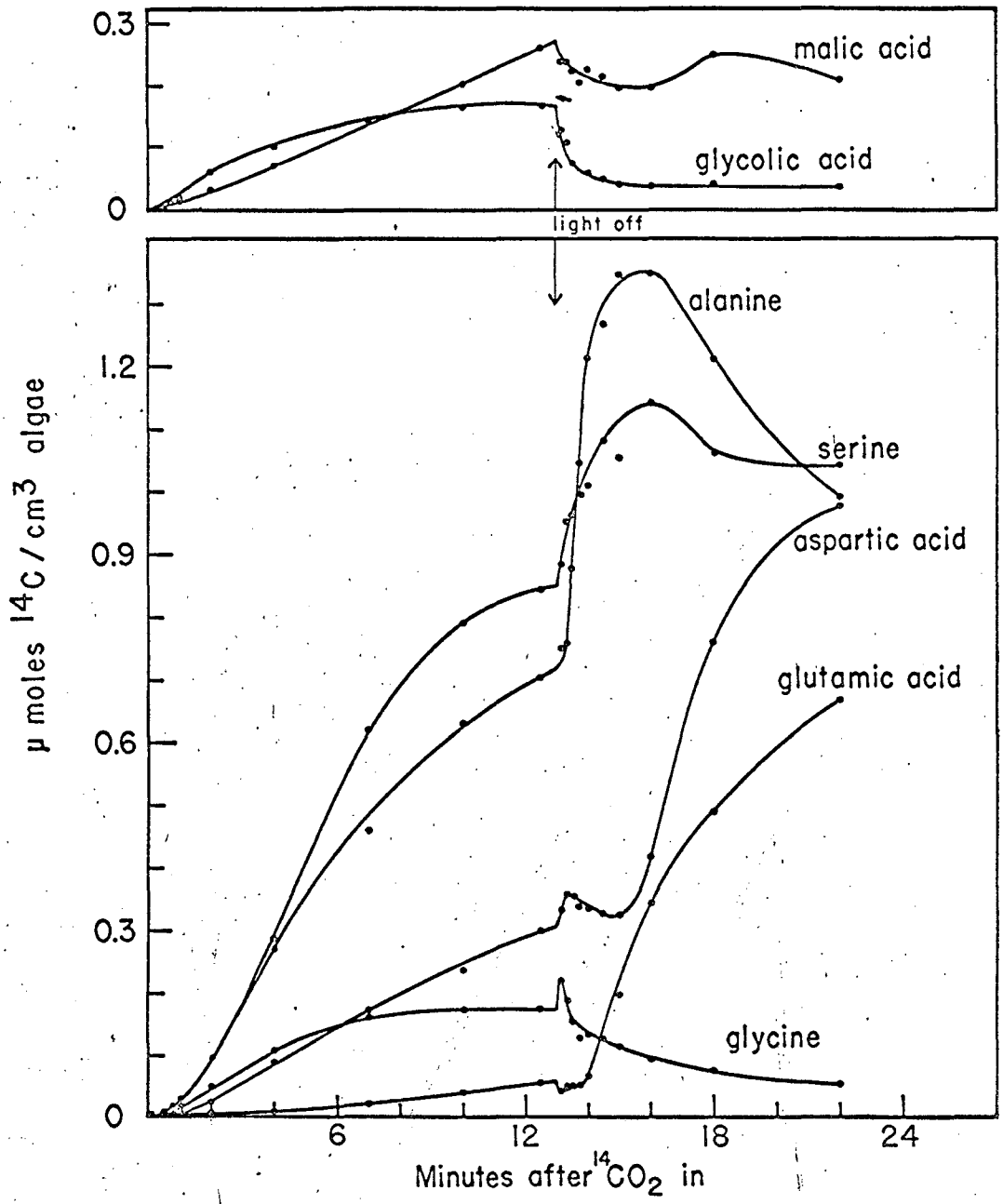
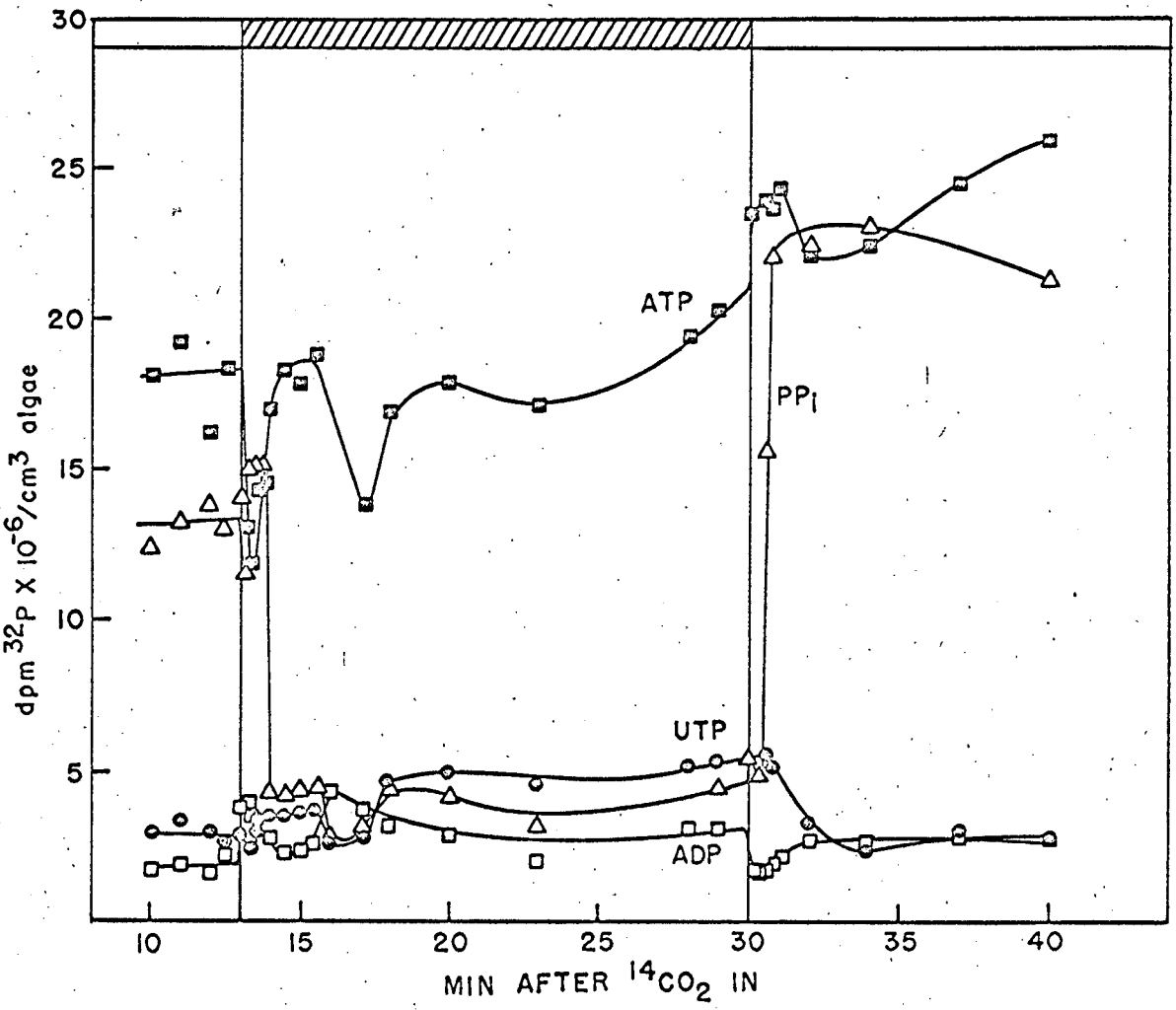


Figure 7



MUB-7268

Figure 8

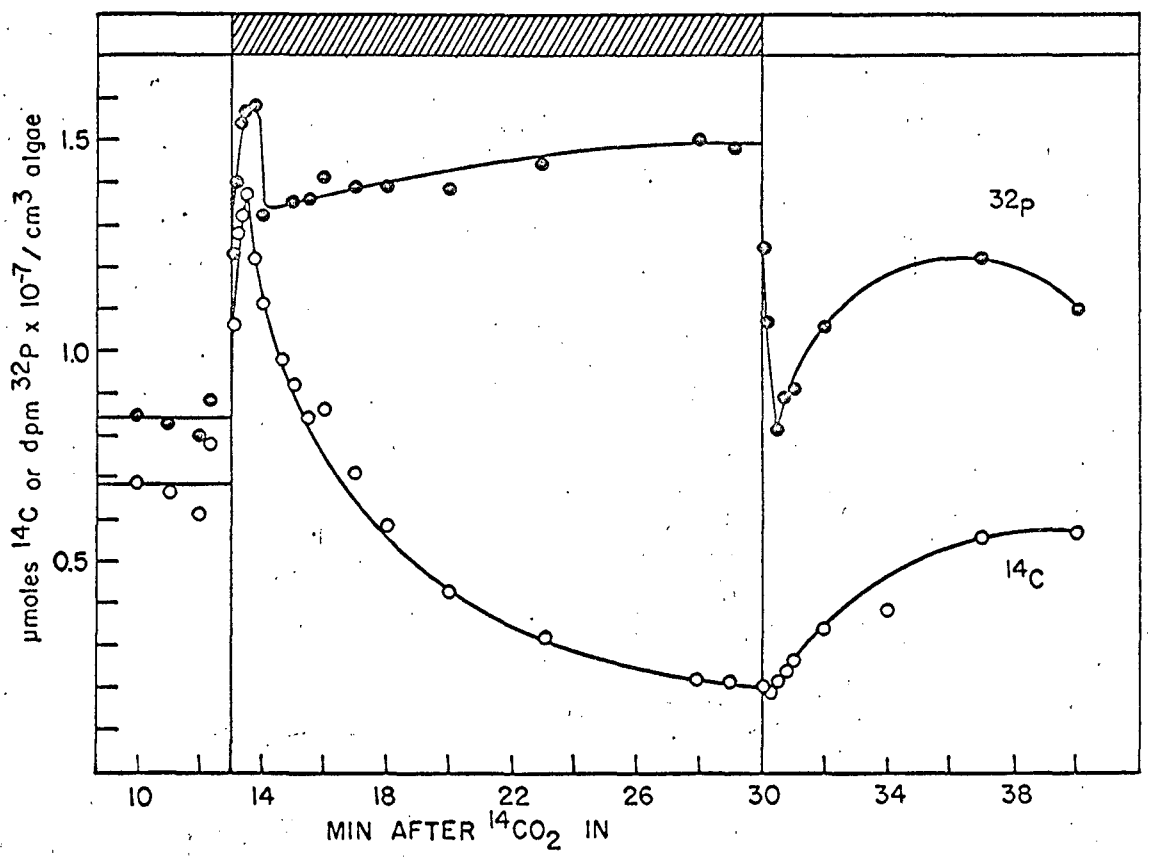

μmoles ^{14}C or dpm ^{32}P x 10^{-7} / cm^3 algae
1.5
1.0
0.5
10
14
18
22
26
30
34
38
MIN AFTER $^{14}CO_2$ IN
^{32}P
^{14}C
MUB-7269

Figure 9