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INHIBITION OF PHOTOPHOSPHORYLATION AND PHOTOSYNTHETIC CARBON CYCLE REACTIONS BY FATTY ACIDS AND ESTERS

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SUMMARY

Photosynthesis in Chlorella pyrenoidosa is quickly inhibited by the addition of 3×10^{-4} to 6×10^{-4} M lipoic acid, octanoic acid, or methyl octanoate. These inhibitions are wholly or partially reversible. Studies with ^{32}P-labeled phosphate and ^{14}C-labeled carbon dioxide of transient changes in the levels of photosynthetic intermediate compounds during the time following addition of inhibitor indicate blocking of (1) the carboxylation reaction of the photosynthetic carbon reduction cycle, (2) photophosphorylation (formation of ATP in the light), and (3) conversion of fructose-1,6-diphosphate to fructose-6-phosphate and of sedoheptulose-1,7-diphosphate to sedoheptulose-7-phosphate. Blocking of photophosphorylation results in decreased rates of conversion of ribulose-5-phosphate to ribulose-1,5-diphosphate, of glucose-6-phosphate to polysaccharides and sucrose, and other reactions. These metabolic effects, together with other published results, lead us to conclude that at least several key steps in the photosynthetic carbon reduction cycle may be mediated by enzymes in some organized system associated with the lamellae and photophosphorylation.

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*Royal Norwegian Council for Scientific and Industrial Research Fellow.

INTRODUCTION

Studies of the transient changes in the levels of intermediates of the carbon reduction cycle induced by the addition of certain chemicals have been undertaken in an effort to obtain information regarding the mechanisms whereby electrons and high energy chemical compounds from the light reactions of photosynthesis are utilized by the enzymes of the carbon reduction cycle of photosynthesis. A previous report[1] described results obtained by the addition of several compounds to photosynthesizing Chlorella pyrenoidosa. Some chemicals were found to produce effects similar to those obtained by turning off the light. Vitamin K_5 was found to induce cyclic photophosphorylation in vivo, as revealed by a stimulation of oligosaccharide formation and the oxidation of glucose-monophosphate via 6-phosphogluconic acid to ribose-5-phosphate (R5P).* Hexylresorcinol produced light-dark type transient effects, but at the same time also inhibited the transformation of fructose-1,6-diphosphate (FDP) and of sedoheptulose-1,7-diphosphate (SDP) to the respective monophosphates.

It was noted earlier that the addition of lipoic and methyl lipoic acids also resulted in the inhibition of the conversion of FDP and SDP to their respective monophosphates[2,3]. At the same time there was

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*Abbreviations: 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; PP_i, pyrophosphate; GMP, glucose-monophosphate (glucose-1-phosphate + glucose-6-phosphate).

observed an inhibition of the formation of ribulose-1,5-diphosphate (RuDP) from ribulose-5-phosphate (Ru5P) and of the carboxylation reaction which converts RuDP to 3-phosphoglyceric acid (PGA).

These effects have now been produced by the addition of several saturated fatty acids ranging in chain length from hexanoic acid to decanoic acid and by addition of methyl octanoate. Additional studies with both $^{14}CO_2$ and $H^{32}PO_4^{-2}$ have been carried out to relate changes in the concentrations of intermediates of the carbon reduction cycle to changes in the ratios of ADP to ATP. Some studies have been performed using air-adapted algae and 0.04% $^{14}CO_2$ in contrast to previous kinetic studies with higher CO_2 levels.

METHODS

For the experiments with 1-2% CO_2, unicellular algae, Chlorella pyrenoidosa, grown in continuous culture tubes[4], were harvested by centrifugation, washed once, and resuspended in 10^{-3} M KH_2PO_4.

The suspension of algae (for concentration see individual experiment) was placed in the steady-state photosynthesis apparatus which has been described previously[5]. In this apparatus, pH electrodes monitor the pH of the suspension. The signal from the pH meter passes to both the recorder and to a pH control relay which activates a solenoid and permits the admission of 0.1 N NH_4OH when the pH drops below the selected level. The pH of the suspension was initially brought below 5 (to about 4.8) by the addition of 0.1 N HCl. Then the control relay was turned on causing 0.1 N NH_4OH to flow into the suspension, bringing the pH to 5, at which level it was subsequently maintained by automatic addition of NH_4OH.

The steady-state photosynthesis apparatus has provision for monitoring the levels of CO_2, O_2, and $^{14}CO_2$ in the gas phase of the closed system in which the gas is made to recirculate through the suspension of algae. The signals from these instruments are continuously recorded, and from their rate of change with time and the known volumes of the system, rates of photosynthesis can be calculated.

For the experiments with air levels of CO_2 it was found desirable to increase the bubbling rate of gas through the algal suspension. This was achieved by adding an additional bubbler and recirculation pump to the steady-state apparatus. The 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 which was inserted into the suspension. 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 reentered the vessel through the bubbler. The rate of flow of this secondary gas recirculation was about one 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.

With the various fatty acids tested, preliminary experiments were carried out to determine the concentration required to obtain essentially complete inhibition. Subsequently, experiments with tracers were performed. Following a preliminary period of photosynthesis in 1-2% $^{12}CO_2$, $^{14}CO_2$ was introduced and its level and specific radioactivity maintained so that the photosynthetic steady state was not interrupted. Several 1 ml samples of algae suspension were taken into weighed test tubes

containing 4 ml of methanol for killing, in order to establish the steady-state level of intermediate compounds. After 10 min or more of photosynthesis with $^{14}CO_2$, some change was imposed on the system (such as addition of inhibitor, or turning out the light) and samples were taken periodically, as indicated in the results. Aliquot samples of the killed algal material were analyzed by two-dimensional paper chromatography and radioautography, as previously described[4,5]. The radioactivity of each compound was determined either by means of the automatic spot counter[6], or in the case of the ^{14}C and ^{32}P experiments, by elution of the radioactivity from the paper and counting in a liquid scintillation counter. 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[7]. The sample vials were counted automatically by the scintillation counter (Packard Instruments, Series 3000).

Experiments with labeled carbon and labeled phosphorus

In some experiments with inhibitors both ^{14}C-labeled carbon dioxide and ^{32}P-labeled inorganic phosphate were employed as tracers. In these experiments the algae were suspended in 10^{-4} M phosphate buffer, pH 5. After a period of pre-incubation, labeled phosphate (purchased from Oak Ridge National Laboratories) was added to the algae suspension in the steady-state apparatus. Since the solution was acidic it was necessary to adjust the pH back to pH 5 through the addition of 0.1 N ammonium hydroxide. After a further period of photosynthesis, $^{14}CO_2$ was admitted to the closed system and the experiment was continued as in the case with $^{14}CO_2$ only. The samples taken during the experiment were analyzed by two-dimensional paper chromatography in the same solvent system as described

previously. In order to achieve good separation of the phosphate compounds which travel only slowly in these solvents, the chromatograms were developed for 48 h in each solvent system, with Whatman No. 1 paper. It was anticipated that the high level of radiophosphorus in the inorganic phosphate spot might cause fogging of the medical X-ray film if the films were allowed to expose for the several days necessary to see the organic phosphate compounds. Therefore medical X-ray film was first placed in contact with the paper chromatograms for about one-half hour, after which it was developed and the film was used as a guide in cutting out the inorganic phosphate spot from the paper chromatograms. The paper chromatograms were then placed on fresh medical X-ray film and the film was allowed to expose for 2 days, after which it was developed. A typical radioautograph from such an experiment is shown in Figure 1.

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 phosphate spray[8]: ATP, ADP, UTP, UDPG, and pyrophosphate. The sugar monophosphates and diphosphates of the photosynthetic carbon reduction cycle whose positions are already well known were treated with a phosphatase and rechromatographed, after which the ^{14}C content of the free sugars was determined. The nucleotides already mentioned, as well as 3-phosphoglyceric acid (PGA), were eluted from the papers and their content of ^{32}P and ^{14}C determined by scintillation counting.

Experiments with air-adapted algae

Some experiments were performed with Chlorella pyrenoidosa which had been grown in shaker flasks with an unspecified, but limiting, level of carbon dioxide. These algae were harvested by centrifugation, washed once with water, and resuspended in 10^{-3} M KH_2PO_4. Their subsequent treatment was similar to that used in experiments with algae and 1-2% CO_2, described above, except that prior to the experiment they were illuminated and allowed to photosynthesize in the steady-state apparatus for at least one hour with air (.04% CO_2). In experiments with these algae, $^{14}CO_2$ was added from a 6-liter reservoir to the closed system to give an initial concentration of .06% CO_2 in air or less. The duration of the experiment and the amount of carbon dioxide used by the algae was such that the concentration of carbon dioxide did not fall below 0.02% CO_2 before the end of the experiment.

RESULTS

The levels of inhibitors used in preliminary experiments and the degree of inhibition and the degree of reversibility are indicated in Table I. It must be mentioned that the inhibition and its reversibility for a given level of inhibitor depends to some extent on the physiological state of the algae and has not proved to be precisely reproducible.

The effects of addition of 5×10^{-4} M lipoic acid on the levels of phosphoglyceric acid (PGA) and of ribulose-1,5-diphosphate (RuDP) are shown in Figure 2. The rapid drop in PGA level and the initial rise in RuDP level suggest a block of the carboxylation reaction. Figure 3 shows corresponding changes in the levels of sedoheptulose-1,7-diphosphate (SDP), fructose-1,6-diphosphate (FDP), fructose-6-phosphate (F6P),

TABLE I

Effects of Inhibitors on Rates of Photosynthesis in Chlorella

Inhibitor	Concentration	Photosynthesis Rate (μmole/min/cm³ algae) Initial O_2	Initial CO_2	After inhibitor O_2	After inhibitor CO_2	After washing O_2	After washing CO_2
butyric acid	3×10^{-4} M	16.7	15.9	15.0	13.0	-	-
hexanoic acid	3×10^{-4} M	17.1	16.8	4.4	4.5	10.6	9.3
heptanoic acid	3×10^{-4} M	16.7	15.5	5.9	4.9	15.9	14.5
octanoic acid	3×10^{-4} M	20.8	17.3	2.0	2.1	10.3	9.5
dodecanoic acid	3×10^{-4} M	14.5	11.9	1.3	0.6	9.8	7.9
lipoic acid	3×10^{-4} M	14.2	11.7	0	0	11.2	10.3
methyl octanoate	6×10^{-4} M	10.3	12.0	0	0	7.5	8.8

Rates of photosynthesis of Chlorella pyrenoidosa (2% v/v suspension in 10^{-3} M KH_2PO_4, pH 5) in 1-2% CO_2 in air, were calculated from the slopes of the recorded readings with time of the CO_2 and O_2 analyzers, together with the known volume of the gas-circulating system (small system: O_2, 315 cm^3; CO_2, 365 cm^3). Rates after inhibition were measured for the period 2 to 10 min after addition of inhibitor. Rates after washing were measured after the algae had been centrifuged from the suspension containing inhibitor and resuspended in fresh medium, pH 5, without inhibitor. During the entire experiment pH control was maintained by automatic addition of 0.1 N NH_4OH, as necessary.

and sedoheptulose-7-phosphate (S7P). The changes in levels of these sugars suggest a block of the conversion of FDP and SDP to their monophosphates.

The effects of addition of octanoic acid to algae photosynthesizing in approximately 0.04% $^{14}CO_2$ in air are shown in Figures 4 through 9. Figures 4 and 6 through 8 are all from a single experiment in which both $^{14}CO_2$ and ^{32}P-labeled phosphate were used. Upon addition of the acid, complete inhibition of photosynthesis was achieved (according to $^{14}CO_2$ uptake). After 3-1/2 min, the pH of the suspending medium was raised to 7. The ^{14}C uptake was rapidly restored to about 40% of the initial rate. Figures 5 and 9 are from a parallel experiment except that no ^{32}P was used and the pH was not changed. Figure 4 shows (despite some scatter in the first 3 RuDP points) the high level of RuDP and low level of PGA seen under photosynthesis in air levels of CO_2. Octanoic acid addition blocks PGA formation somewhat more than its utilization, causing its level to dip. The block in the formation of RuDP is seen in its large decrease in level, despite the presumed inhibition of the carboxylation reaction leading to PGA. The recovery of the carboxylation reaction at pH 7 is seen in the rise in amount of PGA. Figure 5 shows the changes occurring in levels of pentose monophosphates upon addition of octanoic acid to the photosynthesizing algae. The increase in levels of these precursors to the formation of RuDP provides further evidence for the inhibition of RuDP formation.

Figure 6 shows the changes occurring in levels of ATP, ADP, UTP, and PP_1 accompanying the addition of octanoic acid, and later, pH change. The level of ATP drops rapidly and continues downward until the pH is

changed, at which point ATP level rises very rapidly. The smaller reciprocal change in ADP indicates that the first two phosphate groups are much less labeled during the 25 min allowed with ^{32}P-phosphate than the terminal phosphate of ATP. UTP level drops initially in similar fashion to ATP level, but appears (on the basis of one point) to recover before the pH change. This recovery is of possible significance in relation to the sucrose and glucose monophosphate curves (Figures 8 and 9). A sudden rise in level of pyrophosphate (PP_i) upon addition of octanoic acid is noted. Following the initial rise, the PP_i level falls to below its steady-state level and rises again, more slowly, when the pH is raised.

Figure 7 gives the changes in FDP and SDP induced with octanoic acid, and shows both the inhibition of the diphosphatase* and its very rapid release by pH change in the suspending medium. Figure 8 gives the corresponding changes in sugar monophosphates. The initial rapid drop in levels of F6P and S7P are indicative of rapid diphosphatase

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*Since it is not clear whether or not enzymes which have been isolated, characterized, and given systematic names are responsible, at least in their isolated form, for catalysis of the reactions occurring in vivo discussed in this paper, systematic names will be used only with reference to isolated enzymes. The reactions in vivo discussed in this paper include the removal of the phosphate groups from the number one carbon atom position of fructose-1,6-diphosphate and of sedoheptulose-1,7-diphosphate, referred to as the "diphosphatase reaction," and the carboxylation of ribulose-1,5-diphosphate, followed by hydrolytic splitting to give 3-phosphoglyceric acid, referred to as the "carboxylation reaction". The formation of ATP in the light during photosynthesis will be referred to as a "photophosphorylation".

inhibition. Later transient changes in these compounds are reflections of inhibitor-caused blocks at other points in the carbon pathway. The block in glucose monophosphate utilization responsible for the rapid initial rise in its level is released after about 2 min with the inhibitor, and the level then falls rapidly prior to the pH adjustment. Sucrose formation in a parallel experiment is shown in Figure 9. In that experiment, it is seen that sucrose formation was released after about 4 min, without any pH change. Thus, glucose monophosphate utilization, sucrose formation, and possibly UTP level all seem to have been restored several minutes after inhibitor addition and without other external cause. In contrast, restoration of ATP level, carboxylation reaction, and diphosphatase reaction were restored only after the pH of the suspending solution was raised to 7.

The effects of addition of methyl octanoate to photosynthesizing Chlorella are shown in Figures 10 through 13. From Figure 10, it is seen that changes in PGA and RuDP levels caused by methyl octanoate are similar to those seen when illumination is stopped suddenly. In both cases an interruption in the flow of ATP blocks the conversion of Ru5P to RuDP, and the conversion of PGA to phosphoryl-3-phosphoglyceric acid (prior to its reduction to triose phosphate). However, the rate of fall in the level of RuDP is not nearly as rapid when the inhibitor is added as it is when the light was turned off. Considering the rapid fall in ATP level in both cases (Figure 11), this difference suggests that the carboxylation reaction is inhibited by the methyl octanoate as it was by the free acid.

From Figure 11, it is clear that an important and rapid effect of methyl octanoate addition is the blocking of ATP formation. The drop in ATP is much more rapid than it was in the case of octanoic acid inhibition. The formation of ATP in the dark, presumably by oxidative phosphorylation, is clearly seen. If this oxidative formation of ATP is occurring in the light as well, it is inhibited by the addition of methyl octanoate, for there is no appreciable regeneration of ATP following the transient changes produced by adding the inhibitor as there was in the dark, following the light-dark transient. Also, the level of ATP falls much lower with the inhibitor in the light than it did in the dark.

Interesting transient changes in the level of pyrophosphate (PP_i) are noted. The largest change is the great increase in level when the light is turned on. This increase exceeds in magnitude, but not in rate, the light-induced increase in ATP level. Later, in the light, the PP_i level declines towards light steady-state value. It increases rapidly upon addition of methyl octanoate at the same moment that ATP level is declining, and then falls. Its behavior with methyl octanoate addition is essentially the same as with octanoic acid addition.

From Figure 12, it is seen that methyl octanoate blocks the conversion of FDP and SDP to their corresponding monophosphates. However, there is a drop in the level of these diphosphates during the first few seconds after addition of the fatty acid ester.

Figure 13 shows the changes in levels of glucose monophosphate and of sucrose formation in a parallel experiment with methyl octanoate. Since ^{32}P was not used in that experiment, there was no measurement of

UTP level. However, it appears that there was a partial recovery of sucrose synthesis, several minutes after addition of the inhibitor.

DISCUSSION

Addition of lipoic acid, octanoic acid, or methyl octanoate appears to cause inhibition of photosynthetic reactions in at least three primary sites (Figure 14): These are (1) formation of ATP by photophosphorylation (and possibly by oxidative phosphorylation as well), (2) carboxylation of RuDP to form PGA, and (3) conversion of FDP and SDP to F6P and S7P, respectively. The decrease in level of ATP leads to other blocks, including the conversion of Ru5P to RuDP, and the conversion of PGA to triose phosphate (and thence to FDP and SDP).

It will be noted throughout the discussion that for the levels of FDP and SDP to rise after the onset of inhibition requires not only blocking of the diphosphatase but also some continued slow formation of triose phosphates which can then react to make FDP and SDP. This triose phosphate might arise by reduction of PGA (but the low ATP level makes this seem unlikely), by oxidation of glucose-6-phosphate to R5P and CO_2 and transketolase reactions converting R5P to triose phosphate and S7P, or by direct reduction of RuDP carboxylation product to triose phosphate and PGA (as has sometimes been proposed[9,10]).

The decrease in level of ATP is accompanied by a decrease in level of UTP, resulting in blocking the conversion of glucose monophosphate to UDPG and then with fructose monophosphate to sucrose. In the case of the free acids, at least, it appears that this recovery may occur even in the absence of any recovery from the other effects just listed, including the depressed level of ATP.

In comparing the effectiveness of the fatty acids with that of the ester, it appears that the ester may produce a more rapid inhibition of the formation of ATP than does the free acid. The initial transient behavior of the PGA level depends on the relative rates of inhibition of the carboxylation reaction and the two reaction steps (phosphorylation and reduction) leading to the conversion of PGA to triose phosphate. Thus, the fatty acids, by inhibiting the carboxylation reaction more rapidly than photophosphorylation, cause the PGA level to drop initially, whereas the ester, which inhibits photophosphorylation more rapidly, causes PGA level to first rise and then drop. For similar reasons, RuDP (formed by a reaction requiring ATP and utilized by the carboxylation reaction) initially increases when lipoic acid is administered (to "high CO_2" algae) but decreases immediately when methyl octanoate is given. As already mentioned, the slower rate of fall of RuDP level with methyl octanoate addition as compared to its fall when the light is turned off demonstrates inhibition of the carboxylation reaction by the ester.

The acidity of the fatty acids may well cause some part of the inhibitory effects. Considering the effects found with the methyl ester of octanoic acid, it appears that the principal effects depend not on the acidity but rather on other physical properties of the molecules. The inhibition of the diphosphatase reaction was seen also in a previous study in which hexylresorcinol was added[1]. The ready reversibility of the inhibitions, seen either on resuspension of the algae in fresh solution not containing inhibitor, or (with acids) simply on raising the pH of the suspension so that the fatty acid

dissociates, shows that the photosynthetic apparatus is not completely and irreversibly damaged by the inhibitor, nor are any of the enzymes denatured. The fatty acids or esters are not likely to be competitive inhibitors of the steps seen to be blocked. The remaining possibility is that the acids and the ester reversibly cause some physical-chemical effect on the apparatus which interferes with its function. These substances are lipophyllic, and they may be acting upon some lipid membrane such as the lamellae of the chloroplasts.

Isolated chloroplasts have been found to undergo light-induced changes in structure as revealed by light-scattering changes[11], and with the Coulter Counter and microscopy[12]. Shrinkage of the chloroplasts was reported as occurring under conditions of electron transport, light-triggered ATPase, or both[13]. Dilley and Vernon[14], studying the light-induced scattering change in the presence of phosphorylation uncouplers, found evidence consistent with the hypothesis that the light scattering change is indicative of the generation of a precursor in the conversion of ADP to ATP. It has been found in this laboratory that when lipoic acid, octanoic acid, or methyl octanoate are added to Chlorella in the same concentrations used in the experiments reported in this paper, there is a large light-induced increase in light scattering[15]. This scattering could be abolished in part by washing the cells. Untreated cells in vivo do not seem to undergo appreciable light-induced scattering changes. These observations permit the tentative conclusion that the fatty acids and the ester used in these studies alter the properties of the lamellae in such a way that photophosphorylation is blocked and a light-induced conformational change occurs.

Our data do not permit a conclusion as to the origin of the pyrophosphate found on our chromatograms. It is clear that the level of PP_i, or of a labile compound which releases PP_i, is rapidly affected by light and by the addition of fatty acids and methyl octanoate. Perhaps the best tentative interpretation of the data is that PP_i, or the compound which releases it, is formed after ATP and is utilized in some way by a subsequent photosynthetic reaction which is also affected by the addition of the acid or ester inhibitors.

If the inhibition of photophosphorylation caused by the fatty acids and ester is a consequence of some alteration in the properties of the lamellae, can one assume a similar mechanism in the inhibitory effects on the diphosphatase and the carboxylation reactions? Neither of these reactions is known to be directly linked to the photophosphorylation reaction except through the reactions of the photosynthetic carbon reduction cycle, which have already been considered in some detail. It seems hardly to be expected that the methyl octanoate with its small water solubility would be capable of producing much effect on these reactions if they are mediated in vivo by enzymes in aqueous solution and unrelated to the lamellar structure of the chloroplasts. An earlier preliminary test of the lipoic acid dissolved in aqueous solution at pH 8 showed it to be without effect on the isolated 3-phospho-D-glycerate carboxylase (dimerizing), also known as carboxydismutase and as ribulose diphosphate carboxylase.

Peterkofsky and Racker[16] studied the activities of enzymes of the photosynthetic carbon reduction cycle found in cell-free extracts of spinach, Euglena gracilis, and two strains of Chlorella pyrenoidosa, and compared the activities of enzymes postulated for the cycle with

the rates required by photosynthesis in vivo in these organisms. They reported activities for three enzymes of the carbon reduction cycle which were inadequate to support the measured rate of CO_2 fixation in the two strains of Chlorella and in Euglena. (A fourth enzyme studied, transaldolase, No. 2.2.1.2., does not mediate a reaction of the cycle.) The three enzymes with deficient activity were (1) ribulose diphosphate carboxylase, 4.1.1.39, (2) D-fructose-1,6-diphosphate 1-phosphohydrolase, 3.1.3.10, and (3) sedoheptulose-1,7-diphosphate 1-phosphohydrolase. The reactions of the photosynthetic carbon reduction cycle which would be catalyzed by these enzymes are the same reactions which, along with photophosphorylation, have been found to be blocked by fatty acids and methyl octanoate in the present study. Peterkofsky and Racker recognized the possibility that the enzymes within the intact cell might operate in an organized way, and further arguments for this possibility have been advanced elsewhere[10,17]. It would now appear that if such an organization exists, it may include the lamellar surface and photophosphorylation plus the diphosphatase and carboxylation reactions.

Studies with isolated chloroplasts have revealed the existence of a light-activated and light-dependent ATPase, which requires the addition of phenazine methosulfate[18,19], a substance often used to promote cyclic photophosphorylation. If the sugar diphosphatase reaction is indeed dependent on the lamellar structure, as we have suggested, it is also conceivable that the diphosphatase might be light-dependent. Our recent studies[20] of the light-dark transient changes in the levels of intermediates of the photosynthetic carbon reduction cycle suggest that there is a light-dark switch in the diphosphatase reaction which results in blocking of the reaction after a minute or less of darkness.

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FIGURE LEGENDS

Fig. 1. Radioautograph of Chromatogram of ^{32}P and ^{14}C-Labeled Photosynthetic Products.

To Chlorella pyrenoidosa (80 ml 2% v/v suspension in 10^{-4} M phosphate buffer, pH 5) photosynthesizing with 1-2% CO_2 in air, ^{32}P-labeled phosphate (15 mc, carrier-free) was added for 43 min, and $^{14}CO_2$, 15 μC/μmole, maintained at about 1% total CO_2 in air for 17 min. The 1 ml sample was killed by addition of 4 ml methanol at room temperature. Of this 5 ml mixture, 1 ml was applied directly to origin of Whatman #1 filter paper, and developed 48 h in 88% liquefied phenol-water-acetic acid-1 M ethylene-diamine tetraacetic acid (840:160:10:1), dried, and developed 48 h in butanol-propionic acid-water (equal parts of butanol-water, 370:25 v/v and propionic acid-water, 18:22). A preliminary radioautograph was developed after 30 min exposure to locate the inorganic phosphate spot which was then cut from the paper to prevent fogging of the film. A second film was then exposed for 2 days. Spots A, B, C, D, F, G, and I are unidentified. Spot E is mostly dihydroxyacetone phosphate.

Fig. 2. Effects of Addition of Lipoic Acid on Levels of Photosynthetic Products. PGA and RuDP.

80 ml 2% suspension Chlorella, photosynthesizing with 1-2% CO_2 in air, 10^{-3} M phosphate buffer, pH 5, pH control by 0.1 N NH_4OH addition. After 10 min photosynthesis with $^{14}CO_2$, lipoic acid added in 200 μl ethanol to give final calculated concentration 6×10^{-4} M, if acid

FIGURE LEGENDS (Cont.)

were uniformly distributed in algal suspension. Level of ^{14}C in compounds expressed as μmoles of carbon corresponding to ^{14}C found in compound (per cm^3 algae, wet packed) divided by specific radioactivity of $^{14}CO_2$ which was 26 μC/μmole.

Fig. 3. Effects of Addition of Lipoic Acid on Levels of Photosynthetic Products. FDP, SDP, F6P, and S7P.

Conditions same as for Fig. 2.

Fig. 4. Effects of Addition of Octanoic Acid on Levels of Photosynthetic Products. PGA and RuDP.

80 ml 1% suspension Chlorella, photosynthesizing with approximately 0.04% CO_2 in air, 10^{-4} M phosphate buffer, pH 5, pH control by 0.01 N NH_4OH addition. After 26 min photosynthesis with added ^{32}P-labeled inorganic phosphate, carrier-free, 25 mC total, and 15 min with $^{14}CO_2$, 57 μC/μmole, octanoic acid in 200 μl ethanol was added to give a final calculated concentration of 3×10^{-4} M. After another 3-1/2 min, the pH of the suspending medium was raised to 7 by the addition of 0.1 N NaOH.

FIGURE LEGENDS (Cont.)

Fig. 5. Effects of Addition of Octanoic Acid on Levels of Photosynthetic Products. R5P and Ru5P.

Same general conditions as described for Fig. 4, but without ^{32}P-labeled phosphate or pH change. $^{14}CO_2$ specific radioactivity was 53 μC/μmole, which permitted accurate measurement of the small pools of pentose phosphates.

Fig. 6. Effects of Addition of Octanoic Acid on Levels of Photosynthetic Products. ^{32}P-Labeling of ATP, ADP, UTP, and Pyrophosphate.

Same conditions as described for Fig. 4.

Fig. 7. Effects of Addition of Octanoic Acid on Levels of Photosynthetic Products. FDP and SDP.

Same conditions as described for Fig. 4.

Fig. 8. Effects of Addition of Octanoic Acid on Levels of Photosynthetic Products. GMP, S7P, and F6P.

Same conditions as described for Fig. 4.

FIGURE LEGENDS (Cont.)

Fig. 9. Effects of Addition of Octanoic Acid on Levels of Photosynthetic Products. Sucrose.

Same conditions as described for Fig. 5.

Fig. 10. Effects of Addition of Methyl Octanoate on Levels of Photosynthetic Products. PGA and RuDP.

80 ml 2% suspension of Chlorella, photosynthesizing with 1-2% CO_2 in air, 10^{-4} M phosphate buffer, pH 5, pH control by 0.1 N NH_4OH addition. After 32 min photosynthesis with added ^{32}P-labeled inorganic phosphate, carrier-free, 15 mC total, and 6 min with $^{14}CO_2$, 15 μC/μmole, the light was turned off. After 5 min, the light was turned on and remained on for the duration of the experiment. After 5 more min, methyl octanoate in 200 μl methanol was added to give a final calculated concentration of 6×10^{-4} M, if the ester were uniformly distributed in the suspension.

Fig. 11. Effects of Addition of Methyl Octanoate on Levels of Photosynthetic Products. ^{32}P-Labeling of ATP, ADP, UTP, and PP_i.

Same conditions as described for Fig. 10.

FIGURE LEGENDS (Cont.)

Fig. 12. Effects of Addition of Methyl Octanoate on Levels of Photosynthetic Products. FDP, SDP, F6P, and S7P.

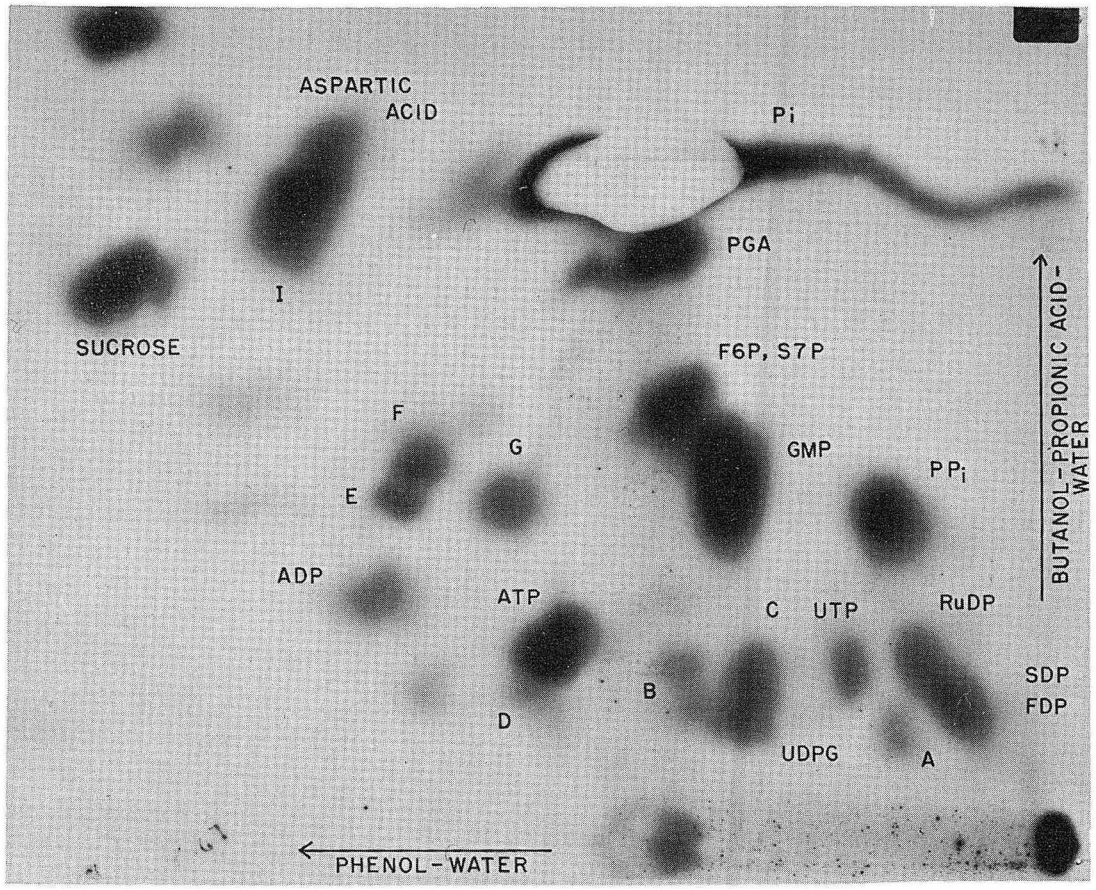
Same conditions as described for Fig. 10.

Fig. 13. Effects of Addition of Methyl Octanoate on Levels of Photosynthetic Products. Sucrose and GMP.

Same general conditions as described for Fig. 10, but without ^{32}P-labeled phosphate and without any interruption of the light. Methyl octanoate added after 10 min photosynthesis with $^{14}CO_2$, 15 μC/μmole.

Fig. 14. The Photosynthetic Carbon Reduction Cycle.

Sites of inhibition by fatty acids and methyl octanoate are indicated by parallel thin lines (═).


ASPARTIC
ACID
Pi
PGA
I
SUCROSE
F6P, S7P
F
G
GMP
PPi
E
BUTANOL-PROPIONIC ACID-
WATER
ADP
ATP
C
UTP
RuDP
SDP
FDP
B
D
UDPG
A
PHENOL-WATER

ZN-5056

Fig. 1

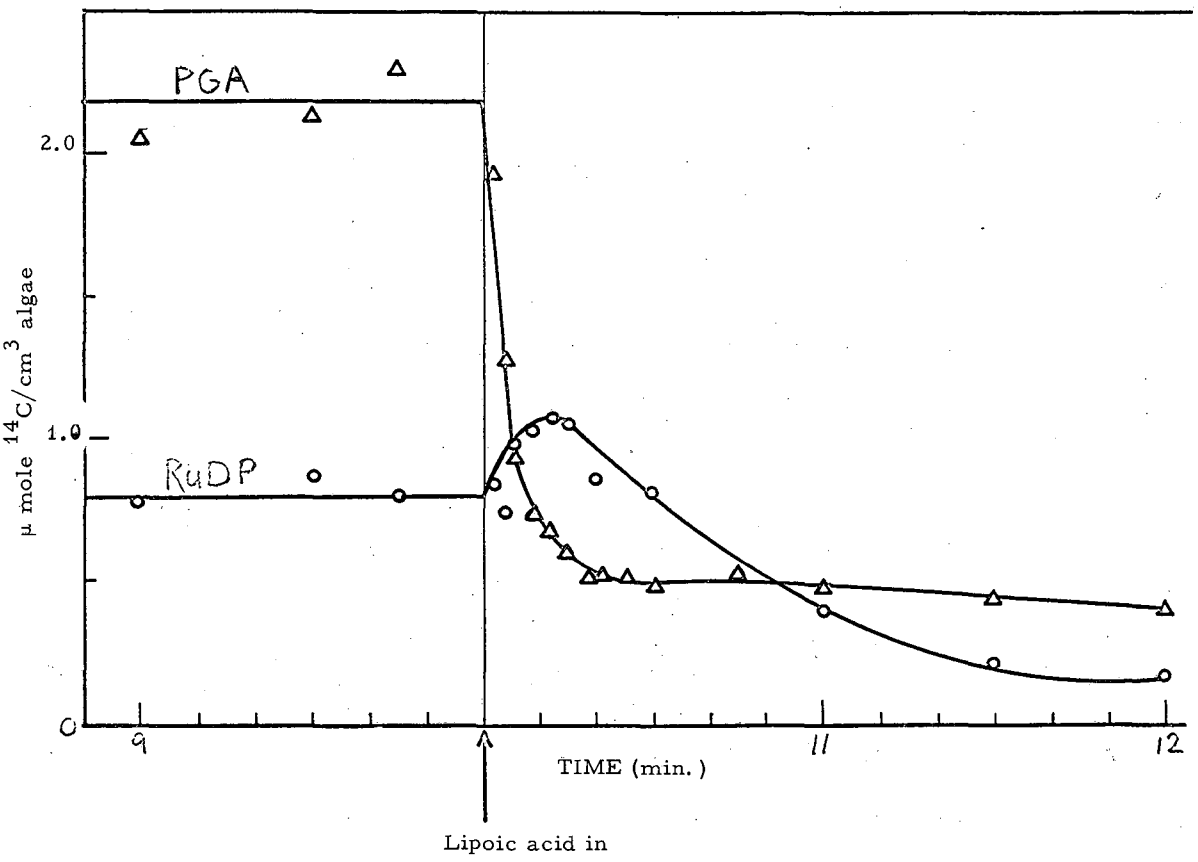
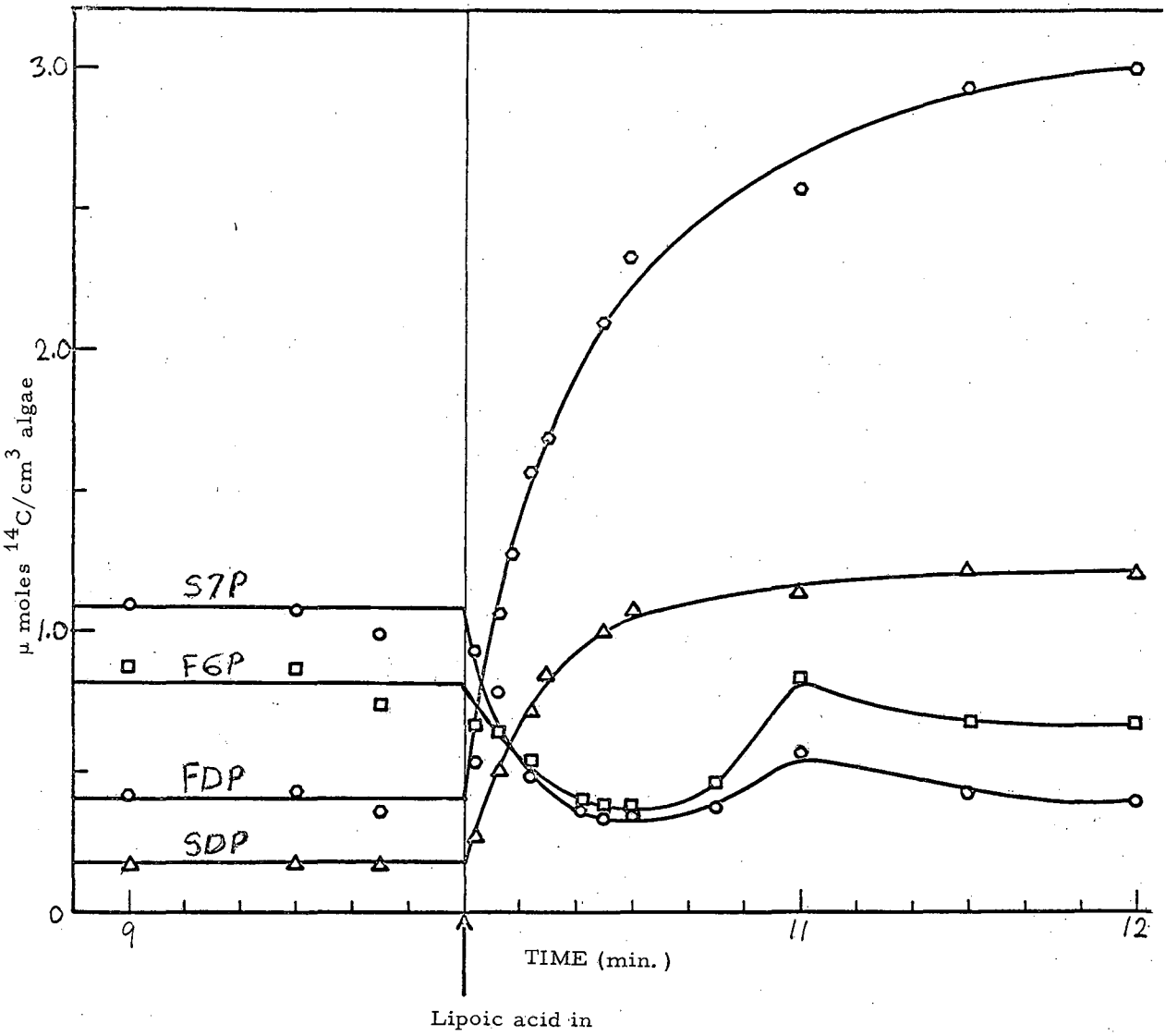

PGA
RuDP
2.0
1.0
0
μ mole $^{14}C/cm^3$ algae
9
11
12
TIME (min.)
Lipoic acid in
MUB-7150

Fig. 2



MUB-7151

Fig. 3

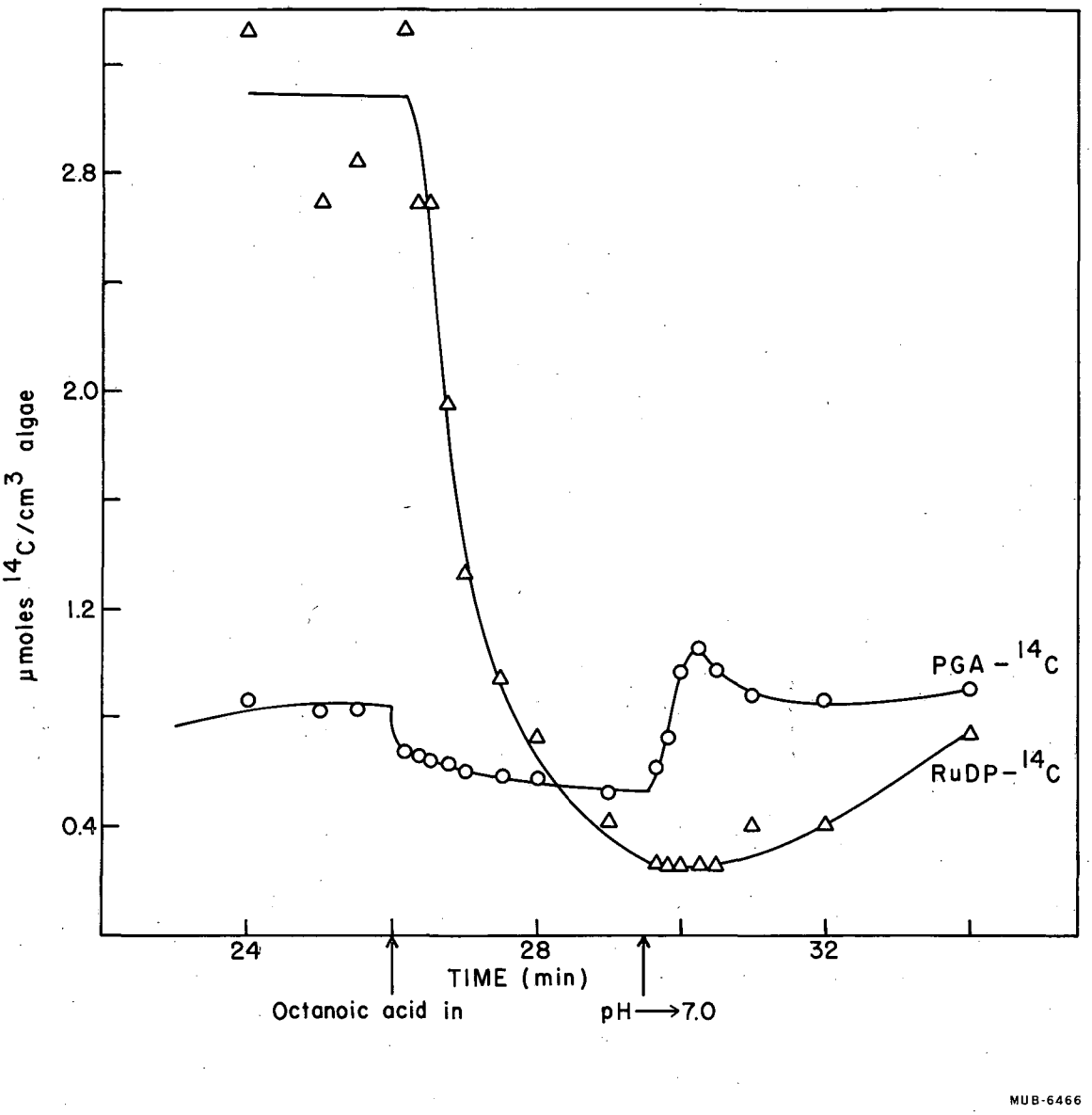
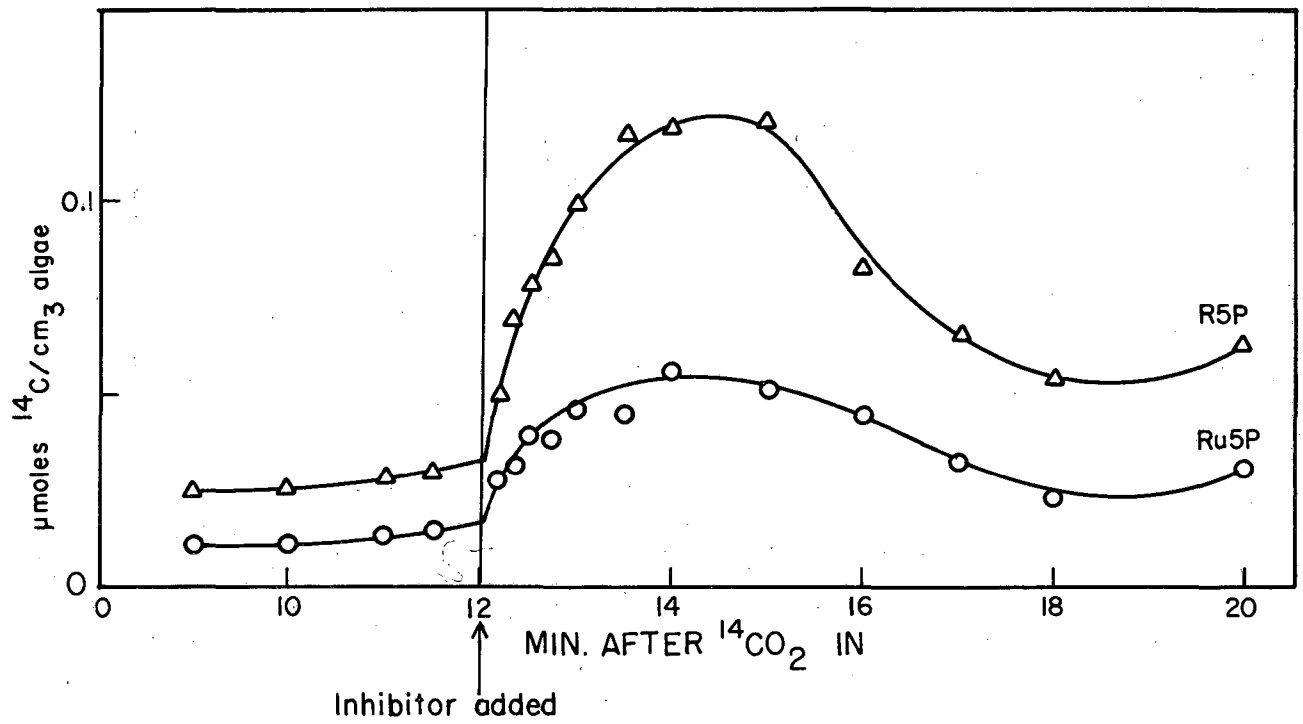

2.8
2.0
1.2
0.4
μmoles $^{14}C/cm^3$ algae
PGA - ^{14}C
RuDP - ^{14}C
24
28
32
TIME (min)
Octanoic acid in
pH → 7.0
MUB-6466

Fig. 4



MUB-5697

Fig. 5

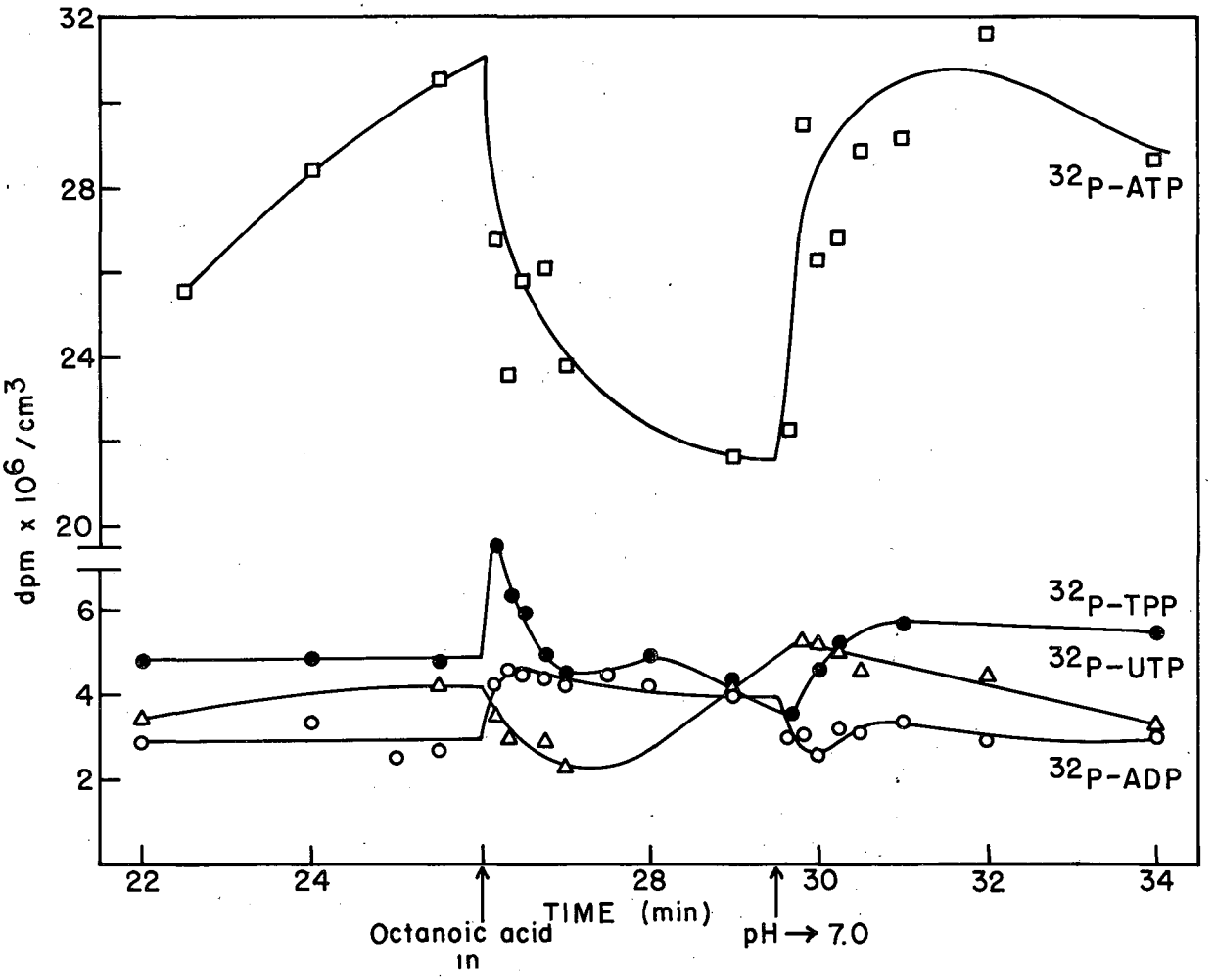

32
28
24
20
6
4
2
dpm x 10^6 / cm^3
32P-ATP
32P-TPP
32P-UTP
32P-ADP
22
24
28
30
32
34
TIME (min)
Octanoic acid in
pH → 7.0
MUB-6333-A

Fig. 6

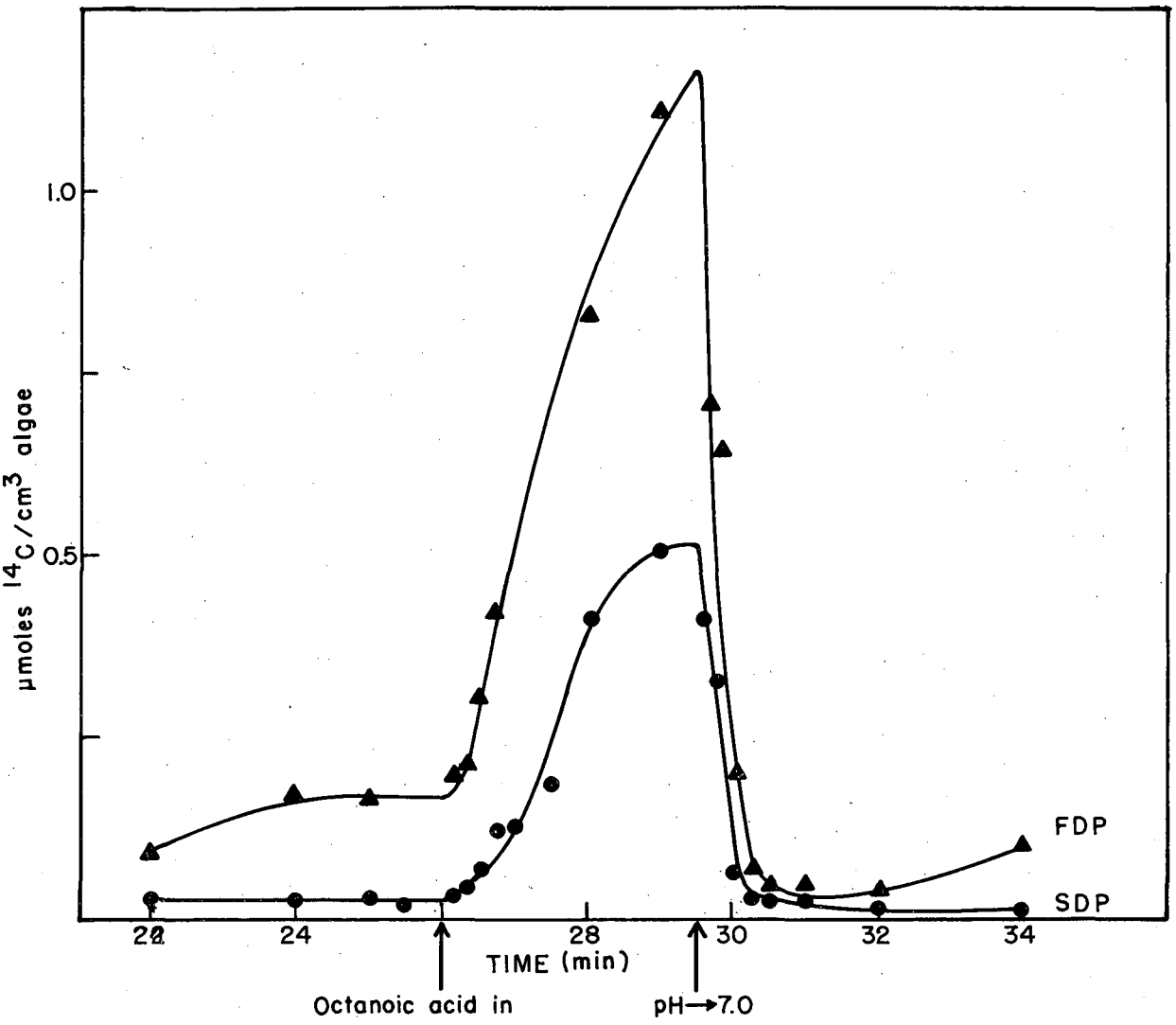

1.0
0.5
µmoles $^{14}C/cm^3$ algae
22
24
28
30
32
34
TIME (min)
Octanoic acid in
pH→7.0
FDP
SDP
MUB-6329

Fig. 7

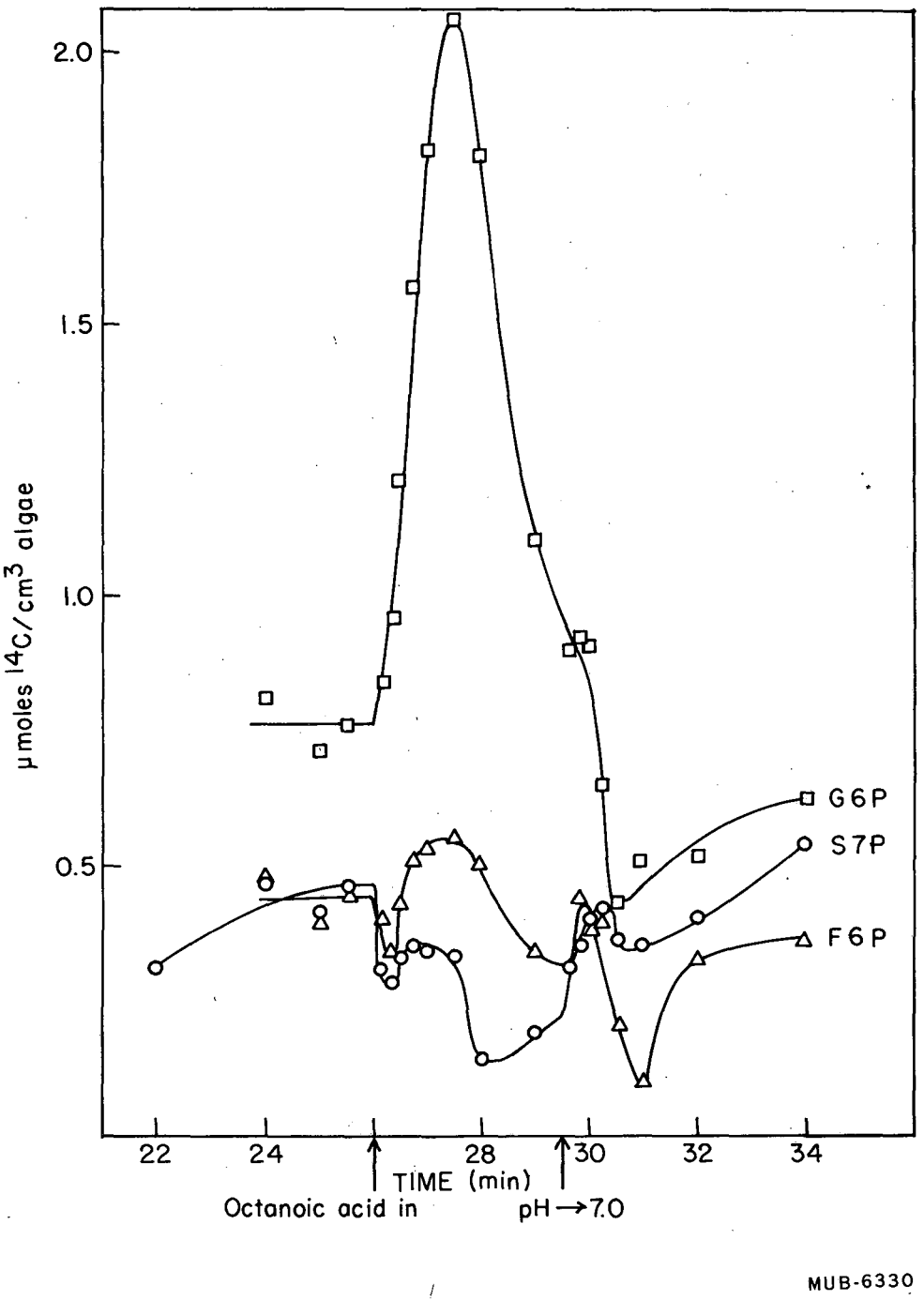

2.0
1.5
1.0
0.5
μmoles $^{14}C/cm^3$ algae
G6P
S7P
F6P
22
24
28
30
32
34
TIME (min)
Octanoic acid in
pH→7.0
MUB-6330

Fig. 8

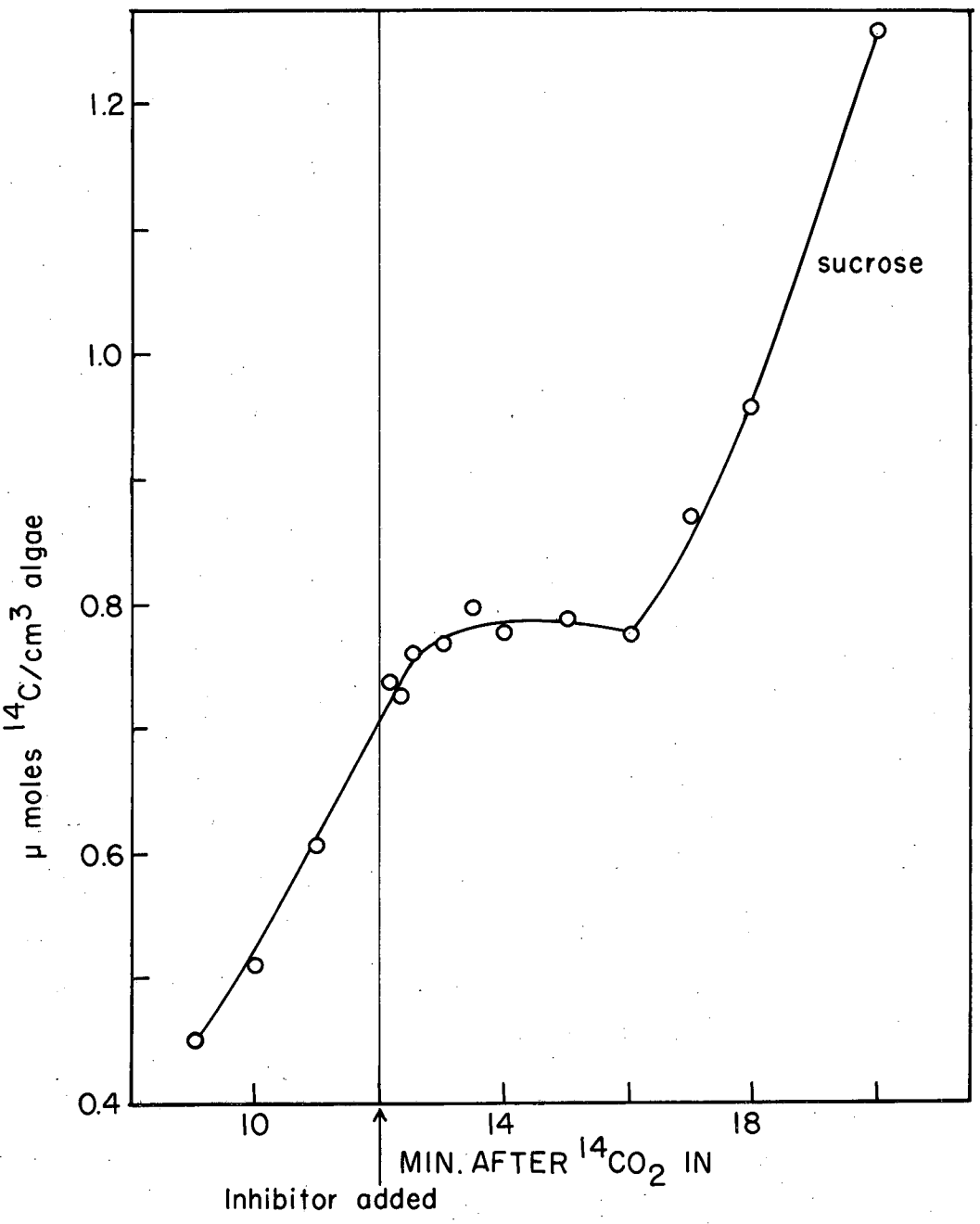

1.2
1.0
0.8
0.6
0.4
μ moles $^{14}C/cm^3$ algae
sucrose
10
14
18
MIN. AFTER $^{14}CO_2$ IN
Inhibitor added
MUB-6268

Fig. 9

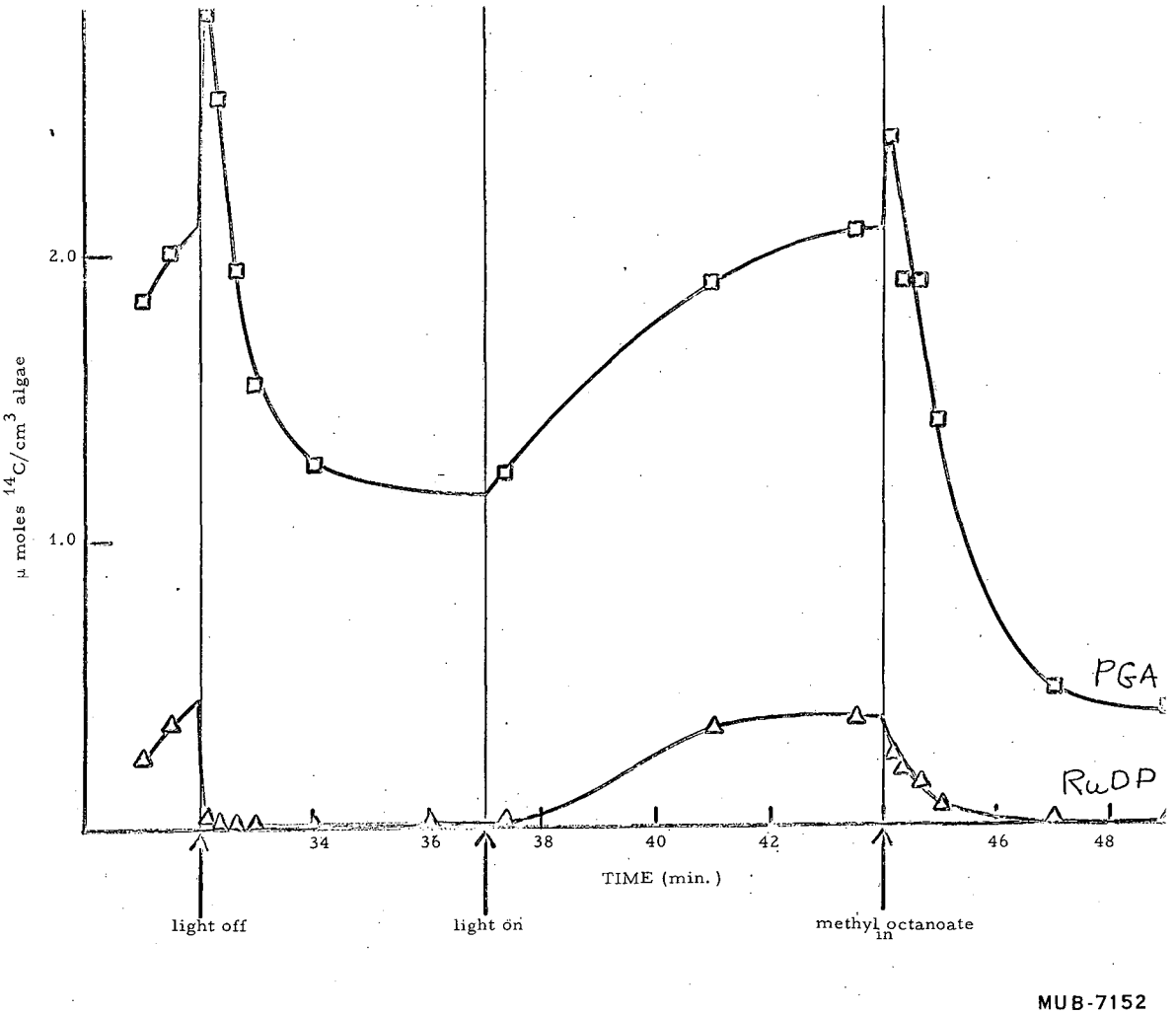

2.0
1.0
μ moles $^{14}C/cm^3$ algae
PGA
RuDP
34
36
38
40
42
46
48
TIME (min.)
light off
light on
methyl octanoate in
MUB-7152

Fig. 10

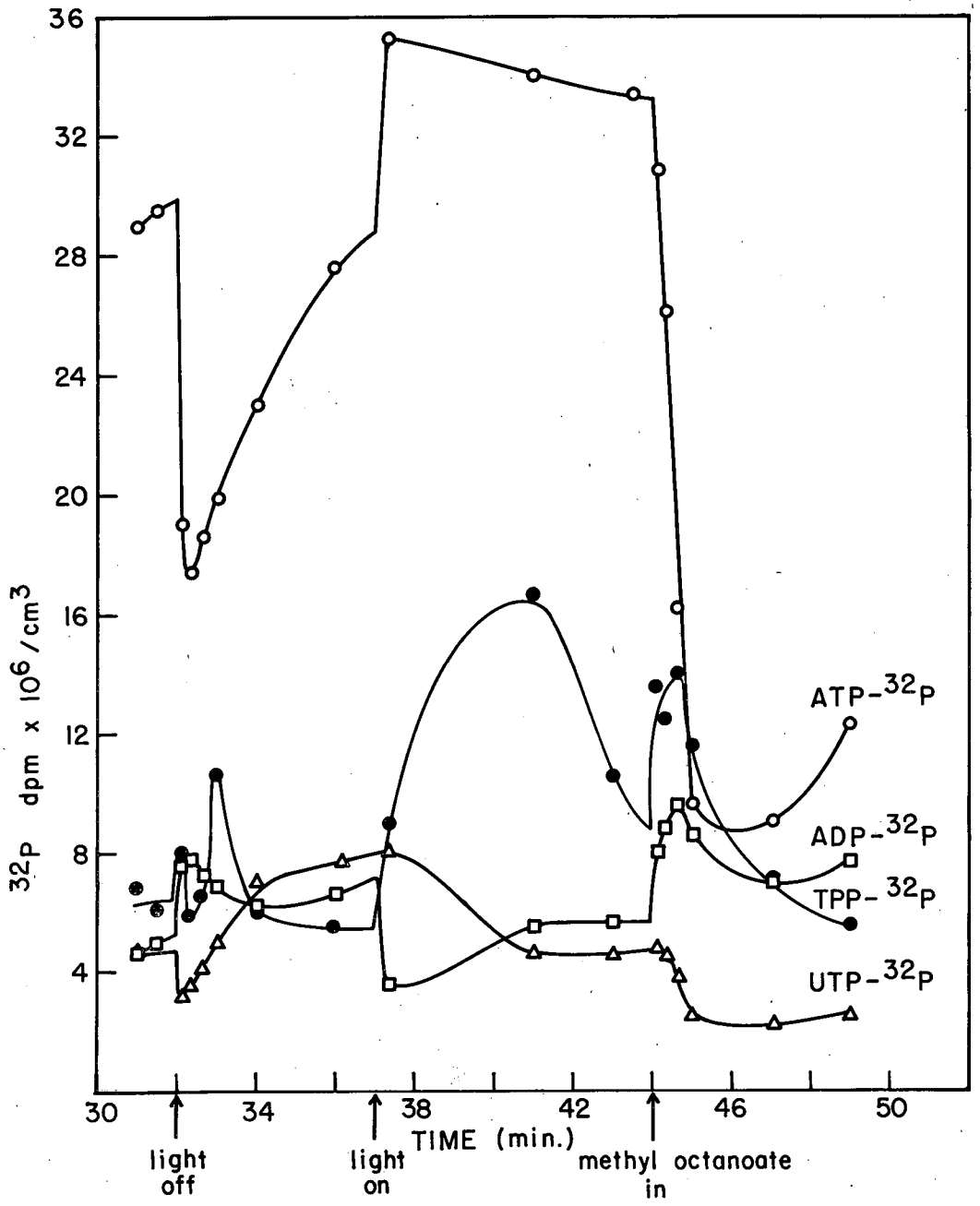

36
32
28
24
20
16
12
8
4
32P dpm x 10^6/cm^3
ATP-32P
ADP-32P
TPP-32P
UTP-32P
30
34
38
42
46
50
TIME (min.)
light off
light on
methyl octanoate in
MUB-6270-A

Fig. 11

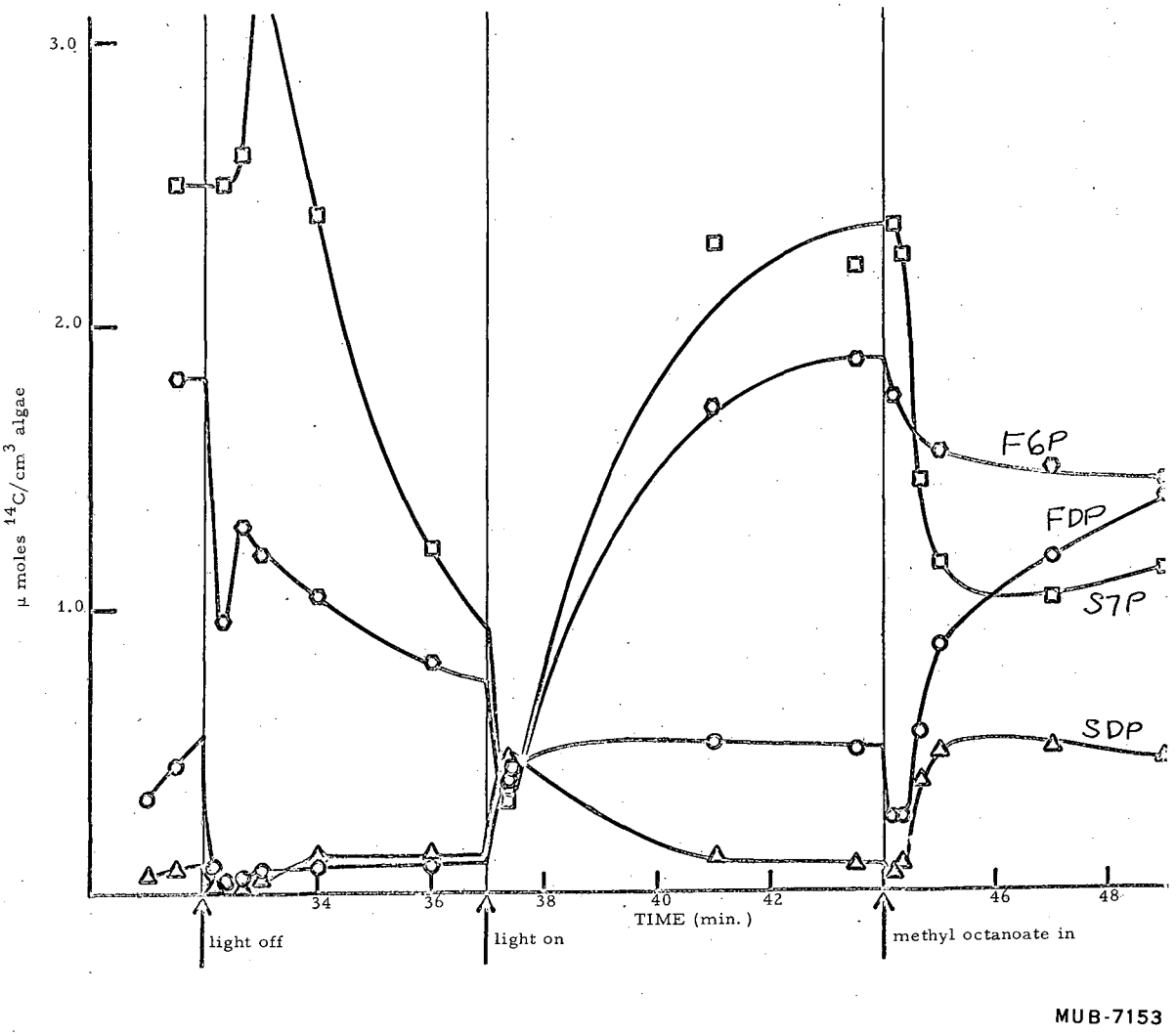

3.0
2.0
1.0
μ moles $^{14}C/cm^3$ algae
F6P
FDP
S7P
SDP
34
36
38
40
42
46
48
TIME (min.)
light off
light on
methyl octanoate in
MUB-7153

Fig. 12

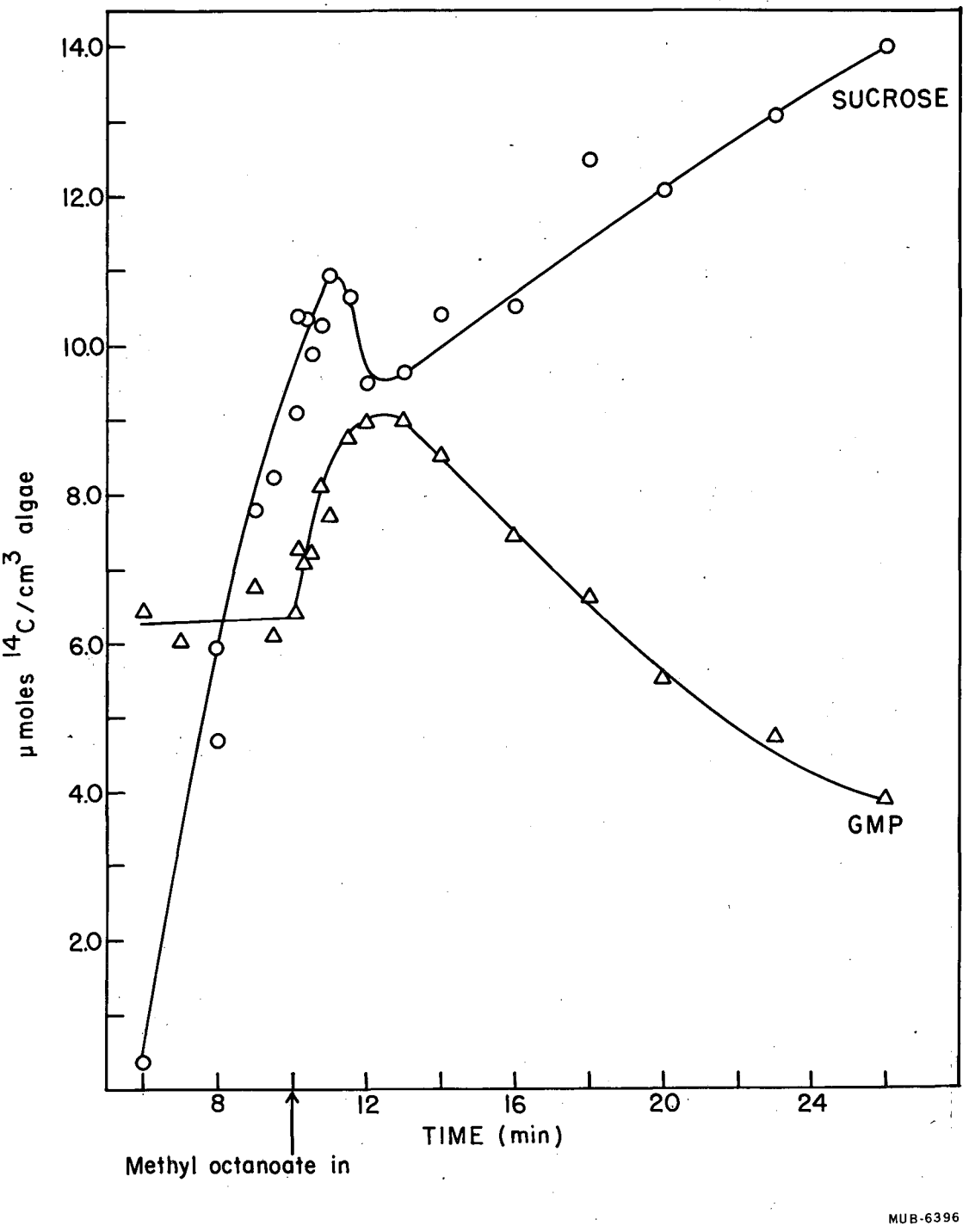

14.0
12.0
10.0
8.0
6.0
4.0
2.0
μ moles $^{14}C/cm^3$ algae
SUCROSE
GMP
8
12
16
20
24
TIME (min)
Methyl octanoate in
MUB-6396

Fig. 13

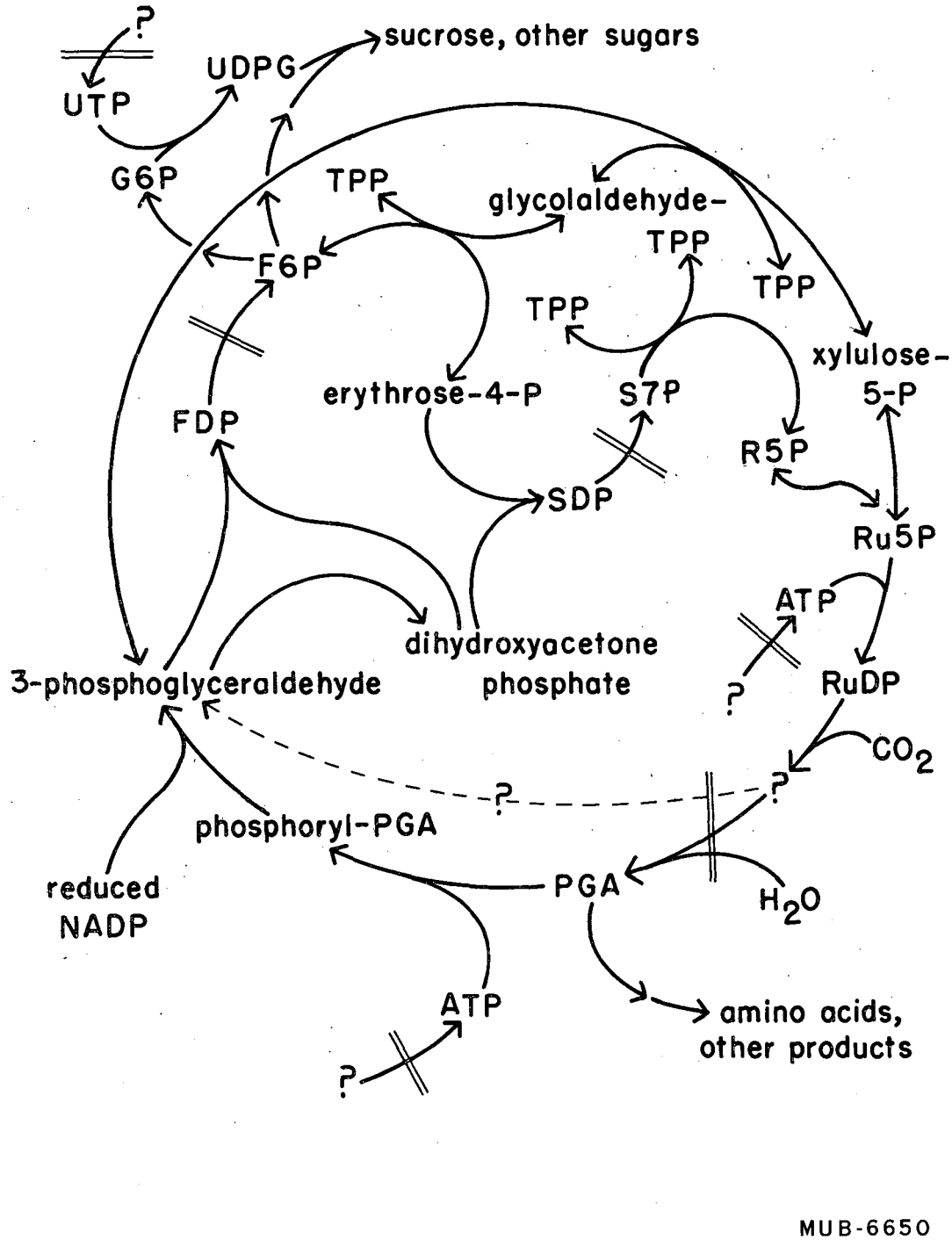

?
UTP
UDPG
sucrose, other sugars
G6P
TPP
glycolaldehyde-
TPP
F6P
TPP
TPP
xylulose-
5-P
erythrose-4-P
S7P
FDP
R5P
SDP
Ru5P
ATP
dihydroxyacetone
phosphate
3-phosphoglyceraldehyde
?
RuDP
CO_2
?
?
phosphoryl-PGA
reduced
NADP
PGA
H_2O
ATP
?
amino acids,
other products
MUB-6650

Fig. 14