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PHOTOSYNTHESIS OF CARBON COMPOUNDS

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INTRODUCTION

It is still possible to find in many textbooks of botany and plant physiology the unqualified formulation of photosynthesis as a simple chemical equation: $CO_2 + H_2O \xrightarrow{h\nu} (CH_2O) + O_2$. The clear implication, sometimes specifically stated, is that the sole products of photosynthesis are oxygen and carbohydrates. This concept of photosynthesis has been firmly entrenched until the past decade. If it were correct, it is possible that a discussion of the carbon metabolism of photosynthesis would hold little except academic interest to those engaged in agricultural research. One could attempt only an improvement in the overall rate of photosynthesis, and perhaps in the relative yields of usable carbohydrates such as sucrose and starch.

Fortunately, for the purposes of this symposium, as well as the future of agricultural research, this older formulation of photosynthesis is incorrect. It is true that the intermediate compounds of the primary photosynthetic carbon reduction cycle are a number of sugar phosphates, as well as 3-phosphoglyceric acid (PGA). However, any definition of photosynthetic products which includes free carbohydrates, such as sucrose and starch, should also include free amino acids and proteins, fatty acids and fats, and coenzymes, vitamins and pigments. There is

evidence that all these substances can be synthesized in the chloroplasts by reactions requiring cofactors produced by photo-electron transport and photophosphorylation. Mature leaves of certain species of plant may produce, under appropriate conditions, almost exclusively one product, such as sucrose. Rapidly growing and developing leaves of the same species make much higher levels of fats and proteins and other constituents. Unicellular algae, such as Chlorella pyrenoidosa, can be made to produce predominantly fat or predominantly protein, depending upon the choice of environmental conditions.

The reason for the variation in products of photosynthesis may sometimes be fairly obvious, as in the case of nitrogen deficiency or abundance. To determine the reasons for product variability in other less obvious cases, we must learn much more than we presently know about the control mechanisms within the chloroplasts and the relations between chloroplast metabolism and the metabolism of the nonphotosynthetic parts of the green cell.

We will need to know how control is effected of the flow of carbon into diverse biosynthetic pathways from key intermediates in the photosynthetic carbon metabolism such as PGA. We must discover how the enzymic machinery of the chloroplast is regulated for light and dark operation, and what determines the extent of export of specific photosynthetic intermediate compounds from the chloroplasts to the cytoplasm. We will be interested in how the regulatory mechanism adapts the metabolism to changes in environmental condition,

such as in the levels of carbon dioxide, phosphate, nitrate and other minerals, and light intensity and quality. A great many of these questions cannot be answered satisfactorily at the present time. However, some information is now available which may help point the way to future research and its application in this area.

THE PHOTOSYNTHETIC CARBON REDUCTION CYCLE

The photosynthetic carbon reduction cycle, as elucidated by Calvin and coworkers (1), is shown in Figure 1. The detailed reactions, and

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Insert Fig. 1 about here

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the arguments leading to their formulation, are discussed in other reviews (2) (3). Although this cycle has been widely accepted as essentially correct since its publication twelve years ago, some investigators closely concerned with the problem of carbon metabolism and photosynthesis maintain reservations about all, or part, of the cycle. Some criticisms of the postulated cycle have been reviewed elsewhere (4), as have our interpretations of the questions raised (5). It is appropriate to our present purposes to say that one of the principal types of objections raised to the cycle has been the inadequacy of enzymic activities isolated from green tissues to carry out key reactions of the proposed cycle. As we shall indicate, it appears that several steps in the proposed cycle may be reactions subject to strong metabolic regulation by changes brought about between light and dark, and perhaps by other environmental conditions. If this is the

case, it should not be surprising if enzymes isolated under inappropriate physiological conditions might be "switched off" and, therefore, show deficient biochemical activity. Factors controlling enzymic activity such as those involved in allosteric effects on enzymes can be very subtle. It seems possible that appropriate conditions for activating such regulated enzymes may have escaped even the most meticulous enzymologists.

Let us review the photosynthetic carbon reduction cycle briefly. It may be said to start with the priming of a pentose phosphate molecule by the phosphoribulokinase reaction with ATP. This step "energizes" the ribulose phosphate molecule by placing a second phosphate group in close proximity to the subsequent carboxylation site on the second carbon atom. According to the evidence and proposals of Rabin and Trown (6), an enzyme substrate complex is then formed through addition of enzyme sulfhydryl to the carbonyl group at carbon atom two.

The resulting thiohemiacetal could then lose water to give an enol form, sometimes postulated as necessary for addition of carbon dioxide across a double bond between carbon atoms two and three (7). The newly incorporated carbon bonds to carbon atom two. The bond between carbon three and two then breaks, releasing carbons three, four and five as PGA, and reducing carbon atom two which is still bound to the sulfur atom of the enzyme. Hydrolysis of the carbon two sulfur bond gives back the original enzyme and another molecule of PGA.

However, if the newly formed carboxyl group, bonded to carbon atom two, could be phosphorylated and reduced prior to liberation from

the enzyme, newly incorporated carbon could be converted directly into triose phosphate without first becoming part of a free molecule. The proposal of a reductive carboxylation reaction in photosynthesis (8), perhaps mediated by an organized enzyme system found only in the intact chloroplasts (5), has gained some support from kinetic tracer studies which indicate that the newly incorporated radiocarbon from photosynthesis with $^{14}CO_2$ finds its way too quickly into the sugar phosphates for it to have passed through the free pool of PGA (5). However, the postulated reductive carboxylation reaction remains only an interesting speculation for want of further and more convincing evidence. The kinetic data with tracers could equally well be accounted for by an enzyme-bound PGA whose equilibration with the free PGA pool is not as fast as the conversion of bound PGA to triose phosphate.

The proposed cycle specifies that PGA is phosphorylated with photochemically produced ATP to give phosphoryl 3-phosphoglyceric acid. This acyl phosphate is then reduced by NADPH in the presence of triose phosphate dehydrogenase. This reaction is the only reductive step in the photosynthetic carbon reduction cycle as it is usually written.

Part of the resulting glyceraldehyde-3-phosphate isomerizes to dihydroxyacetone phosphate. The two triose phosphates condense to give fructose 1,6-diphosphate (FDP), which then undergoes a phosphatase reaction to give fructose-6-phosphate (F6P). A reaction mediated by transketolase with thiamine pyrophosphate (TPP) as coenzyme converts fructose-6-phosphate to thiamine pyrophosphate glycolaldehyde addition compound (TPP-CHOH-CH_2OH) and erythrose-4-phosphate.

Aldolase condenses erythrose-4-phosphate and glyceraldehyde-3-phosphate to give sedoheptulose-1,7-diphosphate (SDP), which is converted by phosphatase to sedoheptulose-7-phosphate (S7P). A transketolase reaction on S7P produces another molecule of TPP-CHOH-CH_2OH and a molecule of ribose-5-phosphate (R5P).

Each of the two molecules of TPP-CHOH-CH_2OH undergoes a transketolase reaction with a molecule of glyceraldehyde-3-phosphate, giving two molecules of xylulose-5-phosphate (Xu5P). The Xu5P molecules are converted by an epimerase to ribulose-5-phosphate (Ru5P) while the R5P is converted by an isomerase to Ru5P, thus completing the cycle.

In each complete turn of the cycle three molecules of pentose diphosphate react with three molecules of carbon dioxide to give six molecules of PGA which can be reduced to yield, ultimately, three molecules of pentose phosphate plus one molecule of triose phosphate. The "extra" triose phosphate molecule thus represents the gain in end products from the reduction of three molecules of carbon dioxide. This reduction requires nine molecules of ATP, and six molecules of NADPH.

The reduced carbon thus generated by the basic photosynthetic carbon reduction cycle can be used as a starting point for a number of secondary biosynthetic pathways, only one of which is the formation of sucrose and polysaccharides.

PHOTOSYNTHETIC PATHWAYS TO END PRODUCTS

Sucrose and Polysaccharides.

Among the earliest labeled products of photosynthesis in *Chlorella*, as well as in spinach leaves and other plants, are uridine diphosphoglucose (UDPG), uridine diphosphogalactose (UDPGal) (9) and adenosine

diphosphoglucose (ADPG) (10). The kinetics of the labeling of UDPG led to the postulation that the pathway in photosynthesis leading to sucrose and polysaccharides involves the conversion of fructose-6-phosphate to glucose-monophosphate, which, in turn, reacts with uridine triphosphate (UTP) to give UDPG. It has been shown (11) that leaf homogenate and leaves convert radioactive glucose-1-phosphate, F6P and UTP to UDPG and, ultimately, to sucrose. Presumably other disaccharides and polysaccharides are synthesized by suitable condensations between UDPG or ADPG and the appropriate hexose phosphate, such as glucose phosphate or galactose phosphate. Although either ADPG or UDPG can function as glucosyl donor in starch synthesis (12), it appears that ADPG is specific for starch synthesis in leaf chloroplasts (13) (14) (15).

Insofar as the required UTP or ATP for UDPG and ADPG synthesis comes from photochemically generated ATP, these reactions may be considered as photosynthetic.

$$\text{Carbon reduction cycle} \rightarrow \text{F6P} \rightarrow \text{G6P} \rightarrow \text{G1P}$$

$$\text{ATP} + \text{G1P} \rightarrow \text{ADPG} + \text{PP}_i$$

$$\text{ADPG} \xrightarrow[\text{primer}]{\text{amylose}} \text{starch}$$

$$\text{ATP} + \text{UDP} \rightleftarrows \text{UTP} + \text{ADP}$$

$$\text{UTP} + \text{G1P} \rightarrow \text{UDPG} + \text{PP}_i$$

$$\text{UDPG} + \text{F6P} \rightarrow \text{sucrose phosphate} \rightarrow \text{sucrose} + \text{P}_i$$

Traces of ^{14}C-labeled sucrose phosphate are obtained from plants photosynthesizing with $^{14}CO_2$, while free ^{14}C-labeled fructose is absent.

Thus it seems that the sucrose phosphate route may be the photosynthetic one.

Amino Acids and Proteins

Among the first compounds found to be labeled by photosynthesis with $^{14}CO_2$ in algae were alanine, aspartic acid, serine, glycine, glutamic acid and other amino acids (16). Under appropriate physiological conditions, the labeling of some of these amino acids, notably alanine, proceeds more rapidly at short exposures to $^{14}CO_2$ than the labeling of sucrose. In fact, the labeling of alanine, which occurs within a very few seconds after the commencement of photosynthesis with $^{14}CO_2$, requires that this amino acid be derived quite directly from intermediates of the photosynthetic carbon reduction cycle (17).

It appears that the pathway of photosynthesis of alanine begins with phosphoglyceric acid from the photosynthetic cycle. The 3-phosphoglyceric acid formed by the carboxylation reaction is converted via 2-phosphoglyceric acid to phosphoenolpyruvic acid (PEPA) which then presumably is hydrolyzed to free pyruvic acid. Free pyruvic acid can then undergo transamination with glutamic acid to give free alanine.

The prominence of carbon labeling of alanine after short periods of photosynthesis for a time led us to suspect that alanine might be a site of primary nitrogen incorporation into amino groups (17). However, careful kinetic studies (18) using ^{14}C and the heavy isotope ^{15}N convinced us that the primary site of nitrogen incorporation into

amino groups is principally into glutamic acid, presumably via reductive amination of alpha-ketoglutaric acid. The relative lateness of ^{14}C labeling of glutamic acid is apparently a consequence of there being more pools of intermediate compounds lying between the carbon reduction cycle and glutamic acid.

It is supposed that carboxylation of phosphoenolpyruvic acid gives oxaloacetic acid which can condense with acetyl CoA to give citric acid. The citric acid presumably is converted by reactions of the Krebs cycle to alpha-ketoglutaric acid. The primary incorporation of nitrogen into amino groups of glutamic acid via reductive amination of alpha-ketoglutaric acid presumably utilizes NADH generated by photo-electron transport. Additional nitrogen incorporation may proceed via amidation, forming glutamine by a reaction utilizing ATP produced by photophosphorylation.

Carbon reduction cycle → 3-PGA → 2-PGA → PEPA

PEPA → pyruvic acid $\xrightarrow{\text{transamination}}$ alanine

2-PGA $\xrightarrow[\text{HOH}]{[O]}$ hydroxypyruvic acid $\xrightarrow{\text{transamination}}$ serine

PEPA + CO_2 → oxaloacetic acid

oxaloacetic acid $\xrightarrow{\text{transamination}}$ aspartic acid

oxaloacetic acid + acetyl CoA → citric acid

→ → → α-ketoglutaric acid $\xrightarrow[\text{NADH}\ NH_4^+]{}$ glutamic acid

PEPA + CO_2 + 4[H] → malic acid

As just mentioned, carboxylation of PEPA would give oxaloacetic acid. In addition to the reactions leading to glutamic acid, oxaloacetic acid could be transaminated to give aspartic acid, always seen as one of the early labeled products of photosynthesis in the presence of $^{14}CO_2$. Reduction of oxaloacetic acid, or reductive carboxylation of PEPA, would give malic acid, another of the earliest photosynthetic products outside the photosynthetic carbon reduction cycle. The reason for this rapid formation of labeled malic acid is not yet clearly understood, but it may well prove to have an as yet unsuspected biosynthetic role in photosynthesis.

Glycine synthesis requires the precursor glyoxylic acid. One possible source of this glyoxylic acid might be the splitting of isocitric acid (generated as described above) by isocitratase to give glyoxylate and succinate. Another source of glyoxylate could be the oxidation of glycolic acid, a compound frequently found to be labeled with ^{14}C following short periods of photosynthesis in the presence of $^{14}CO_2$. However, some plant cells, such as Chlorella pyrenoidosa, appear to be devoid of a glycolic acid oxidase (19).

Very important roles have sometimes been ascribed to glycolic acid as an intermediate in photosynthetic carbon reduction. It has been suggested (20) that glycolic acid itself, or an immediately related two-carbon compound, is synthesized de novo by photosynthetic carbon dioxide reduction and condensation of one-carbon units. This view has been supported by Zelitch (21), who found that after short periods of photosynthesis by tomato discs floating on bicarbonate

the specific activity of glycolic acid was higher than that of 3-PGA. The opposite result has been reported by Hess and Tolbert, who found the specific activity of PGA to be always higher than that of glycolate (22). Probably these seemingly contradictory results are explainable in terms of compartmentalization and separate pools of the same compound in different parts of the plant cell.

In our own laboratory, and in some others (23), kinetic data thus far obtained are consistent with the proposal that glycolic acid is derived by oxidation of sugar phosphate intermediates of the carbon reduction cycle. A recent result, obtained in kinetic studies of photosynthesis of $^{14}CO_2$ by isolated spinach chloroplasts, is shown in Figure 2.

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Insert Fig. 2 about here

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Possibly there is less ambiguity about compartmentalization with isolated chloroplasts than with whole cells. It is clear that labeling of PGA is much faster than that of glycolic acid. Moreover, the ^{14}C labeling of glycolic acid has a zero slope at the shorter times and only becomes labeled as sugar monophosphates are labeled. In fact, the rate of glycolic acid labeling is proportional to the degree of labeling of the sugar monophosphates. This suggests, but does not prove, a precursor-product relationship. It appears that the glycolic acid is formed from the carbon atoms one and two of the ketose phosphates. One possibility is that the glycoaldehyde thiamine pyrophosphate compound, formed in the course of the transketolase

reaction, undergoes oxidation to glycolic acid. Another proposal is that ribulose diphosphate can be oxidized, giving glycolic acid phosphate from carbon atoms one and two. We do know that glycolic acid formation is favored by high levels of oxygen (24) and low levels of carbon dioxide (25), and in unicellular algae, such as Chlorella pyrenoidosa, by high pH's (23). Since high levels of oxygen produce at the same time increased rates of formation of glycolic acid and diminished levels of intermediates of the carbon reduction cycle, particularly ribulose diphosphate (24), it has been suggested that the inhibition of photosynthesis by oxygen at low CO_2 pressures is a direct consequence of the draining off of carbon from the photosynthetic carbon reduction cycle, due to the oxidation of sugar phosphate intermediates. This would, in turn, decrease the supply of carboxylation substrate, ribulose diphosphate. In an elaboration of this idea, Coombs and Whittingham (26) suggest that the oxidation leading to the formation of glycolic acid from the sugar phosphates is accomplished by hydrogen peroxide or another peroxide formed by a "Mehler" reaction between oxygen and some primary reductant such as ferredoxin. In the presence of normal photosynthesis with carbon dioxide the level of reduced ferredoxin is presumed to be too low to react to produce the peroxide. It is postulated that in the absence of carbon dioxide, or at very low levels of carbon dioxide, photosynthesis would not utilize the reduced ferredoxin rapidly and its level would increase to a point where it could react with oxygen. This proposal was consistent with the findings that high light intensity in the absence of carbon dioxide and the presence of oxygen increased the rate of production of glycolic acid.

It is perhaps worth noting that in the study by Coombs and Whittingham, as well as in the earlier studies by Bassham and Kirk (24), an increased production in glycolic acid was accompanied by an increased formation of labeled glycine, despite the fact that both studies were done with Chlorella pyrenoidosa. Thus, there may be some mechanism for the conversion of glycolic acid to glycine in this organism, despite the reported absence of a glycolic acid oxidase (19).

The synthesis of protein in the chloroplasts and higher plants is greatly accelerated during photosynthesis (27) (28). This protein synthesis appears to utilize intermediates of the photosynthetic carbon reduction cycle, since the proteins are labeled when $^{14}CO_2$ is administered but not when ^{14}C-labeled carbohydrates were supplied (28). Illuminated, isolated chloroplasts synthesize protein from non-protein nitrogen (29). Moreover, spinach chloroplasts, isolated after a period of photosynthesis with $^{14}CO_2$, are found to have incorporated ^{14}C into the soluble protein of the chloroplasts much more rapidly than it was into the soluble cytoplasmic protein.

With the development of techniques for achieving steady-state photosynthesis in the presence of labeled carbon dioxide over long periods of time (30), it became possible to study the product-precursor relationships for the route from CO_2 to protein. Such studies indicated that there are in Chlorella pyrenoidosa at least two pools of amino acids, only one of which is rapidly labeled with ^{14}C during photosynthesis with $^{14}CO_2$. In the case of some amino acids, such as

alanine, the maximum rate of labeling was achieved as soon (5 minutes) as the immediate precursors, intermediates of the carbon reduction cycle, were saturated with $^{14}CO_2$ (17). No other labeled compounds in the cell, except such intermediates and phosphoenolpyruvic acid, were saturated with $^{14}CO_2$ by this time, indicating precursor-product relationship between cycle intermediates and amino acids.

The next step was to isolate, during a period of photosynthesis lasting several hours, both the free amino acids and the labeled proteins which were hydrolyzed to give their amino acid substituents. When this was done (31), it was found that the labeling of the bound amino acids in every case reached a maximum rate only after the pool of actively turning over free amino acids had become saturated with ^{14}C.

The ratio between the size of the actively turning over amino acid pool and the size of the total pool of the same amino acid varies over a wide range for different amino acids. Thus, under the conditions chosen, the specific radioactivity of the free pool of alanine at the time of saturation of the actively turning over pool was about 0.45, indicating that the actively turning over pool of alanine was about 45% of the total alanine pool. The relationship between the labeling of this free pool and the bound amino acid moieties is shown in Figure 3.

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Insert Fig. 3 about here

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In sharp contrast, the actively turning over pool of glycine represents only about 1.5% of the total free pool of glycine under the conditions chosen. The relationship between the labeling of this pool

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Insert Fig. 4 about here

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and the bound moieties of glycine is shown in Figure 4. These data explain why it was thought earlier (2) that the bound glycine moieties of protein might arise from some precursor other than free glycine. We suspect that the boundary between the actively turning over pool of amino acids and the other pools of amino acids is provided by the chloroplast membrane. This cannot be proved by kinetic studies alone and must await confirmation by studies with isolated chloroplast systems which are capable of total biosynthetic activity. In any event, protein synthesis in the chloroplast from the pools of actively turning over amino acids may be considered as photosynthetic. Such synthesis would probably utilize ATP from photophosphorylation for the activation of the amino acid moieties preparatory to the formation of peptide bonds and proteins.

There seems now to be abundant evidence, which I shall not attempt to review, that chloroplasts contain the necessary genetic information in the form of DNA and protein synthesizing machinery in the form of ribosomes and soluble RNA to permit the chloroplasts to synthesize their own components of protein. Perhaps at some future date it will become possible to prepare from suitable plants, for example spinach, isolated chloroplasts of sufficient biochemical integrity to permit their injection into large single cells of other plants, such as Nitella, without loss of biochemical activity.

Fats and Pigments

During photosynthesis by unicellular algae, it is not uncommon for as much as 30% of the carbon dioxide taken up to be incorporated

into fats. Probably a high percentage of this incorporated carbon is bound into the lipids of the lamella in the chloroplast. These lipids and phospholipids are thought to play important roles in the physical-chemical processes associated with photo-electron transport and photophosphorylation in photosynthesis.

Glycerol phosphate required for lipid synthesis is probably derived by the reduction with NADPH of dihydroxyacetone phosphate (DHAP), an intermediate in the carbon reduction cycle. Galactose-6-phosphate, required for the synthesis of various galactolipids may be formed from UDP-galactose, derived from UDPG.

It is presumed that fatty acid biosynthesis follows routes similar to those reported for nonphotosynthetic systems. Such systems begin with acetyl CoA. In the presence of biotin and ATP, acetyl CoA is carboxylated to give malonyl CoA. According to Lynen (32) the bond to CoA is replaced by a bond to the sulfhydryl group of an enzyme, and the resulting malonyl-S-enzyme decarboxylates to give acetoacetyl enzyme plus CO_2. This compound then undergoes reduction of the carbonyl, dehydration and further reduction to give butyryl-S-enzyme, which is then converted to butyryl CoA. This series of reactions is then repeated with another molecule of malonyl CoA, the end result being the lengthening of the fatty acid chain by two carbons, with each cycle of reactions. Long-chain fatty acids then presumably esterify with glycerol phosphate or galactose phosphate to make the lipids and phospholipids found in the lamellae of the chloroplast (33).

Perhaps the most important unanswered question regarding photosynthesis of fats is the question of the origin of the acetyl CoA,

or acetate moiety for the starting point of fatty acid synthesis. Several possibilities may be suggested, but there is little evidence to favor any one of these in photosynthesis.

1. Pyruvic acid derived from 3-PGA could undergo oxidative decarboxylation yielding CO_2 and acetyl Coenzyme A. Such a process, involving both loss of CO_2 and oxidation, runs counter to the general direction of photosynthesis. Since in bright light it is presumed that the NADP is mostly in the reduced form, oxidative reactions would require a separate pool of oxidized cofactor, perhaps NAD. It would seem that the oxidative decarboxylation of pyruvic acid, useful in supplying both reducing power and acetyl CoA in nonphotosynthetic reactions, would not be such a useful reaction in photosynthesis.

2. Acetate might arise by some cleavage of malic acid to acetate and glyoxylate. However, in view of the high requirement for acetate moieties for fatty acid synthesis, such a reaction would tend to produce too much glyoxylate.

3. Perhaps the most efficient production of acetyl Coenzyme A in photosynthesis would be by a phosphoketolase reaction which would convert some of the TPP-CHOH-CH_2OH directly to acetyl phosphate. The acetyl phosphate could then be converted without energy loss to acetyl Coenzyme A. Part of the attractiveness of this proposal lies in the fact that TPP-CHOH-CH_2OH must be formed anyway in the course of the transketolase reactions which are two of the key steps in the photosynthetic carbon reduction cycle.

It has already been suggested that glycolic acid might be formed by an oxidation of TPP-CHOH-CH_2OH. If these proposals are correct,

TPP-CHOH-CH_2OH is at an extremely important branch point for the diversion of carbon from the basic carbon reduction cycle and the beginning of biosynthetic pathways. Indeed, this could be a primary regulatory point for the distribution of carbon to the three major classes of end products—fats, proteins and carbohydrates. Clearly the control of the supply of acetyl Coenzyme A would regulate fat synthesis. Glycolic acid formation and oxidation to glyoxylic acid, followed by transamination to give glycine, could serve to regulate the rate of protein synthesis. The small pool size of the actively turning over glycine pool may provide a limitation to total protein synthesis. Carbohydrate synthesis could then depend upon the net accumulation of carbon in the cycle in excess of that drained off for the synthesis of proteins and fats.

Obviously this is a most tentative speculation. It is intended merely to illustrate the kind of branch points and control mechanisms that we must look for if we are to make a serious effort in the direction of regulating the quality, as well as the quantity of products of photosynthesis.

Aside from the synthesis of lipids and phospholipids, a considerable portion of the carbon in a developing chloroplast must go into the biosynthesis of key pigments, such as chlorophyll and carotene. The biosynthetic pathways to these substances are well known in the literature, and there is no reason not to suppose that the synthetic pathways occurring in the chloroplast follow similar routes and utilize appropriate starting compounds derived from the photosynthetic carbon reduction cycle.

REGULATION OF PHOTOSYNTHETIC METABOLISM

Let us now turn to the principal subject of this discussion—that is, the mechanisms of metabolic regulation in the photosynthetic apparatus. I shall then attempt some suggestions about possible ways in which we might hope to utilize these regulatory mechanisms in influencing by external means the course of metabolism during photosynthesis.

During the past several years we have conducted a number of investigations on the kinetics of photosynthetic metabolism in unicellular algae, during conditions of both steady-state photosynthesis and sudden environmental change. These changes have included variation in physical factors, such as light and carbon dioxide pressure, and also the application of certain chemical substances which penetrate the cell and interrupt, or alter, the normal course of metabolism.

The basic technique for carrying out these studies is to establish a condition of steady-state photosynthesis (30), usually with a species of unicellular algae such as Chlorella pyrenoidosa. The radioactive tracer, ^{14}C in $^{14}CO_2$, ^{32}P in phosphate, or both, is then added in such a way that the chemical level and specific radioactivity of the isotope is maintained throughout the course of the experiment. Small samples of the algae are taken from time to time and are quickly killed in 80% methanol.

The environmental change, whether physical or chemical, is then imposed and samples are rapidly taken during and after the period of transition from the condition of steady-state photosynthesis to the new

physiological state. Subsequently, all of the samples of killed plant material are subjected to two-dimensional paper chromatography and radioautography. The amount of each isotope in each individual intermediate compound from each sample is then determined by appropriate counting techniques. Finally, the level of tracer in each sample as a function of time is plotted.

An analysis of the steady-state levels of intermediate compounds and of the changes in these levels upon the imposition of the environmental change permits us to draw conclusions about the dynamic behavior of the system. This behavior provides clues about the interaction between the various subcellular compartments in the cell, the chloroplasts and the cytoplasm. Also we can learn something about metabolic regulation, as, for example, between light and dark. Information can be obtained about alterations in the flow of carbon along various biosynthetic pathways that have been induced by these physical changes or by added chemicals.

Some of the earlier studies were performed with various well known inhibitors of photo-electron transport, and photosynthetic phosphorylation (34). In general, the results of these studies were predictable: inhibitors of photo-electron transport resulted in changes in the carbon reduction cycle very similar to those induced by turning off the light. The interruption of the supply of electrons and of ATP stopped the formation of RuDP from Ru5P, and the reduction of PGA to triose phosphate. The transient changes produced were as would be predicted.

An interesting result was found upon the addition of the compound

Vitamin K_5. This compound had been reported to be a stimulator of cyclic photophosphorylation (35). Cyclic photophosphorylation is, in a sense, accomplished at the expense of net photo-electron transport, which results in the reduction of NADP. Electrons, raised by light reaction 1 to a high reducing potential, are cycled back through a phosphorylation step instead of being used for NADP reduction. The addition of Vitamin K_5 should therefore produce an excess of ATP and a deficiency of NADPH. Thus, it might be possible to simulate in the extreme an effect of an inbalance between the supply of electrons and high energy phosphate. It seems possible that the balance between the supply of these two cofactors may constitute one facet of metabolic regulation of end products in photosynthesis.

Addition of Vitamin K_5 in sufficient concentration to inhibit photosynthesis caused a greatly accelerated formation of oligosaccharides and polysaccharides. Such an acceleration would indeed be consistent with the concept that an exaggerated supply of ATP might favor those reactions of macromolecular synthesis which require only ADP and not NADPH.

Soon after Vitamin K_5 addition, 6-phosphogluconic acid was seen to accumulate in the light for the first time (Figure 5). Later the

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Insert Fig. 5 about here

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amount of ribose-5-phosphate also increased, clearly indicating the operation of the oxidative pentose phosphate cycle in the light in contrast to the usual finding that it was seen only in the dark. It

therefore appeared that the interruption of photo-electron transport caused NADP to be converted entirely to its oxidized form, thereby stimulating sugar phosphate oxidation via the oxidative pentose phosphate pathway.

Another interesting class of inhibitors proved to be the fatty acids of chain length six to eight carbons, and the fatty acid ester, methyl octanoate (36). The effect of the addition of these inhibitors at the level of around 5×10^{-4} M is to inhibit reversibly photosynthesis in whole algae cells. The fatty acids operate in their undissociated form, since, to be effective, they must be added at pH 5. If the pH is raised to 7, the effect can be reversed, and photosynthesis is restored, indicating that the fatty acids have dissociated and diffused out of the algae into the medium.

This behavior suggests that these inhibitors act at some lipid-containing surface, or lamellae. Our current hypothesis is that the introduction of the fatty acid, or its ester, somehow reversibly alters the structure of the lipid layer. This structural change would cause a loss in capacity for photophosphorylation.

The metabolic effects of the addition of these inhibitors are a sudden cessation of photophosphorylation, inactivation of the fructose diphosphatase and sedoheptulose diphosphatase reaction, and inhibition of the carboxylation reaction. The sites of these inhibitions of the carbon cycle are shown in Figure 6.

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Insert Fig. 6 about here

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It is of particular interest that these three seemingly unrelated reactions of photosynthesis should all be affected be the same inhibitor. Whatever the mechanism of the relationship between photophosphorylation and the diphosphatase and carboxylation reactions, the fact that they are related strongly suggests some type of close metabolic regulation which will permit the plant to switch its metabolism. As we shall see, the fatty acids and their esters seem to be mimicking light-dark regulation in some respects.

Light-Dark Transient Studies

Using the techniques just described, and employing both ^{32}P and ^{14}C as tracers, we have studied the metabolic behavior of unicellular algae during light-dark and dark-light transitions (37). These studies gave some interesting indications about the relation between photosynthetic and glycolytic reactions in the green cell, and the mechanism of enzymic regulation of these reactions between light and dark.

Insert Fig. 7 about here

The ^{14}C labeling of PGA (in this experiment saturated in the light) increases rapidly when the light is turned off, due to the sudden stopping of the supply of ATP and electrons from photochemical reactions(Fig. 7). Shortly thereafter, the level of PGA rapidly falls and continues to decline in the dark, due to the conversion of PGA to various biosynthetic secondary products of photosynthesis. Prominent amongst such products are amino acids, for example, alanine. When the light is turned on again, the level of ^{14}C labeling of PGA drops slightly, due to the sudden supply of electrons and ATP for its reduction, and then rises gradually toward steady-state light level.

The ^{32}P labeling of PGA parallels the ^{14}C labeling in the light, as would be expected by the operation of the photosynthetic carbon reduction cycle, which continuously introduces completely labeled $^{14}CO_2$ and ^{32}P-labeled PGA. When the light is turned off, the ^{32}P labeling of PGA rises and begins to drop, still following the ^{14}C labeling.

Then, during the continued dark period, the ^{32}P labeling of PGA levels off and does not drop in parallel with the ^{14}C labeling. This clearly shows that new PGA is being made by the plant cell from carbon sources which have not become appreciably labeled with ^{14}C during the previous period of photosynthesis. Of course, either photosynthetic or glycolytic formation of PGA introduces completely ^{32}P-labeled phosphate. So far, the results could be explained in terms of separate sites for PGA formation in photosynthesis and in glycolysis. The presumption would be that the photosynthetic pool of PGA had declined to a low level while the pool of glycolytic PGA had risen.

However, when the light is turned on again, the ^{32}P-labeled pool of PGA, which now must be presumed to be primarily glycolytic in origin, drops very rapidly. This indicates that reduction of PGA is immediately caused by electrons and ATP from the light reactions. It therefore appears that there is a rapid interaction between photosynthesis and glycolysis.

The carbon and phosphorus labeling of other sugar phosphate intermediates in the carbon reduction cycle followed similar patterns. In the light, and in the first few seconds of darkness, the labeling

with carbon and phosphorus was parallel. In the subsequent dark period and in the first few seconds of light there was a large differential between phosphorus and carbon labeling.

From these tracer studies it may be concluded that intermediate compounds of photosynthesis and glycolysis are readily interchangeable. There are two ways in which intermediate compounds could be acted upon by enzymes of both photosynthesis and glycolysis: (1) Intermediates of the two processes could diffuse freely between chloroplasts and cytoplasm, while the enzymes responsible for the two processes would be segregated, as between chloroplast and cytoplasm. (2) Enzymes in the chloroplasts might be capable of carrying out reactions of photosynthesis in the light, and then through some switching mechanism perform a type of glycolysis in the dark.

Experiments with isolated chloroplasts can help us to decide between these possibilities. During the past year it became possible, as a result of improved techniques of chloroplast isolation and incubation in our laboratory, to achieve high rates of photosynthesis of carbon compounds from carbon dioxide with isolated chloroplasts (38). These rates of complete photosynthesis with isolated chloroplasts approach 65% of the in vivo rates for 10 to 15 minutes. These periods of time are sufficient for us to study the kinetics of intermediate formation and transport from the chloroplasts. Also we can investigate light-dark transients and the possibility of conversion from photosynthetic to glycolytic metabolism.

When kinetic studies were performed on the labeling of photosynthetic intermediate compounds by chloroplasts in the presence of ^{14}C

and ^{32}P, it was found that a disproportionate amount of labeled carbon and phosphorus appear in dihydroxyacetone phosphate (DHAP), and, to a lesser extent, other compounds. A typical radioautograph of the products of photosynthesis with isolated chloroplasts and labeled carbon and phosphorus is shown in Figure 8.

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Insert Fig. 8 about here

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The reason for this accentuation of certain photosynthetic intermediates was found when we carried out experiments in which the chloroplasts were quickly centrifuged from the suspending medium prior to killing with methanol. Analysis of the separated pellet material and supernatant material showed that there had been a very large export of photosynthetic intermediate compounds into the supernate.

Of equal interest was the fact that the export of compounds from the chloroplast did not appear to be an indiscriminate leakage. If the transfer of compounds from the chloroplast to the suspending medium were the result merely of holes in the chloroplast membrane, one would expect an approximately equal proportion of all of the intermediate compounds to come out of the chloroplasts. But this was not the case, as shown by the data in Table I which gives the rates of

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Insert Table I about here

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appearance of radiocarbon in intermediate compounds and other products of photosynthesis in the pellet and in the supernatant solution at

3 minutes. By far, the greater part of the compounds found to be heavily labeled in isolated chloroplasts are quickly transported into the supernate, while other intermediates of the photosynthetic carbon reduction cycle, such as F6P, S7P and RuDP, are rather well-retained in the pellet.

Of course, the pellet must retain a certain minimal amount of all of the intermediates of the carbon cycle, if the carbon cycle is to continue to run. It might be supposed that the fall-off in the rate of photosynthesis of carbon compounds, after 15 or 20 minutes in isolated chloroplasts, is due to the loss in the ability of the chloroplast to retain this minimal quantity of photosynthetic intermediates. However, the reason for the rate decline is probably more subtle. We shall see in a moment that in transient studies, such as between light and dark, the chloroplasts have the ability to reabsorb intermediates from the suspending medium. In any event, if intermediate compounds diffuse freely between chloroplasts and cytoplasm in vivo, as they do between isolated chloroplasts and the suspending medium, the transient changes between light and dark resulting in utilization of photosynthetic intermediates by glycolysis can be understood.

Nevertheless, interaction between the two processes at the level of metabolites poses other questions. If the metabolic intermediates are in contact with enzymes of both photosynthesis and glycolysis, then certain switching mechanisms for some enzymic activities would seem to be required.

Consider the enzymes fructose-1,6-diphosphatase (catalyzing a reaction of photosynthetic carbon reduction) and phosphofructokinase (a reaction of glycolysis). The diphosphatase would convert FDP to F6P and inorganic phosphate as a step in the photosynthetic carbon reduction cycle. Then F6P would diffuse to the site of the phosphofructokinase, where it would be phosphorylated with ATP to give FDP. The FDP could then recycle through the photosynthetic step to give F6P and inorganic phosphate. The net effect would be equivalent to an ATPase activity resulting in the rapid hydrolysis of photosynthetically-produced ATP in the light, and of ATP produced by oxidative phosphorylation in the dark.

Perhaps this undesirable effect is partly prevented by the apparent retention of F6P by the chloroplast. However, light-dark kinetic studies with algae cells indicate that there is an additional switching mechanism (37). In Figure 9 is shown the ^{14}C labeling of FDP and SDP during

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Insert Fig. 9 about here

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photosynthesis in the light and during the transition to darkness. The level of FDP fell rapidly when the light was turned off, as would be expected from the fact that PGA is no longer being reduced to triose phosphate, which condenses to make FDP. However, after about one and a half minutes the levels of FDP and SDP rise markedly, passing through maximums and then declining to steady-state levels which are characteristic of the dark.

It appears that FDP is being formed by a phosphofructokinase reaction utilizing ATP, as glycolysis begins in the dark. At present, no good reason can be suggested for the formation of SDP. Perhaps it is an accidental result of the release of triose phosphate by aldolase following the formation of FDP. This triose phosphate can then react with some erythrose-4-phosphate, which presumably can still come from F6P by transketolase-mediated reactions. In any event, it is clear that if FDP and SDP can diffuse freely back and forth between the sites of photosynthesis and glycolysis, as the experiments with isolated chloroplasts indicate, the diphosphatase which was active while the light was on must have been switched off in the dark, beginning about one to two minutes after the onset of darkness.

That there is sufficient ATP to permit phosphofructokinase reactions in the dark is clear from the data shown in Figure 10. In

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Insert Fig. 10 about here

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this and other experiments we have frequently seen that the ATP level, which declines momentarily when the light is turned off rapidly, comes back to an equal, or higher, level than that observed in the light.

The transient changes in pyrophosphate (PP_i) seen in the same figure should be interpreted with caution. It must be noted that the light-dark transients seen in this experiment with pyrophosphate have not always proved to be reproducible in other experiments, and the reason for this variation is not yet clearly understood.

It has been suggested (39) that pyrophosphate may arise, at least in bacterial photosynthesis, as a side reaction from a precursor of ATP. In our experiments with algae photosynthesizing in the presence of ^{32}P-labeled phosphate, addition of methyl octanoate caused a very rapid decline in labeling of ATP and, at the same time, a momentary increase in the level of pyrophosphate. This finding could also be interpreted as indicating PP_i derivation from a precursor to ATP in photophosphorylation.

However, it must be remembered that pyrophosphate can be produced by a variety of metabolic reactions, some of which are of great importance in photosynthesis. As noted earlier, pyrophosphate is produced by the reaction of UTP with glucose-1-phosphate to give UDPG. The formation of ADPG from ATP and glucose-1-phosphate also produces pyrophosphate. Presumably these reactions occur at a much greater rate in the light than in the dark, and the increased level of pyrophosphate might result from a shift in the steady-state concentration due to its increased formation by such reactions. An additional possibility is that pyrophosphatase is itself subject to light-dark control, perhaps as a part of some regulatory mechanism.

This brief discussion of PP_i changes serves to point up the complexity of the interactions of metabolites from diverse biosynthetic pathways, and the need for experiments designed to observe simultaneously the levels of transient changes in as many as possible of the metabolic compounds involved in these networks of reactions. The simultaneous employment of ^{14}C and ^{32}P in careful kinetic studies during steady-state photosynthesis with homogeneous populations of

cells or intact chloroplasts seems to offer considerable hope for future research in this area.

Another facet of light-dark control in photosynthetic cells seems to be indicated by the data shown in Figure 11, which was obtained

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Insert Fig. 11 about here

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from the experiment with unicellular algae grown under levels of CO_2 approximating those of air (37). Under these conditions the level of ribulose diphosphate is much larger than that of any other intermediate of the carbon reduction cycle. Consequently, ribulose diphosphate is found for a significant time after the light is turned off. However, if the enzymic activity remains constant for the carboxylation reaction in light and dark, one would expect the rate of disappearance of RuDP to be always proportional to the concentration of RuDP, unless the level of RuDP were above saturation.

What we see is that as the level of ribulose diphosphate decreases, its rate of disappearance is not in a constant proportion to the level of the RuDP. The result is that after several minutes the rate of disappearance of RuDP is practically zero, and the RuDP persists for many minutes of darkness. This clearly indicates that the activity of the carboxylation reaction is greatly diminished in the dark.

It was noted some years ago (40) (41), and was recently confirmed (42), that the level of bicarbonate required for half-saturation of the carboxylation reaction with RuDP in the presence of the isolated

carboxylation enzyme is of the order of 2×10^{-2} M. However, in whole cells, as well as in isolated chloroplasts (38) the level of bicarbonate required to achieve half the saturating rate of carboxylation is approximately 6×10^{-4} M. Thus, it seems that there may be some light activation for the carboxylation reaction (not necessarily the enzyme activity itself) which requires a degree of organization not found with the isolated enzymes. The light-dark switching of the activity of the carboxylation reaction indicated by the kinetic data with the whole cells may be a light-dark switching of this activation of the carbon dioxide, or bicarbonate.

We have been able recently to carry out light-dark transient studies with spinach chloroplasts. In these experiments both ^{32}P-labeled phosphate and $^{14}CO_2$ were employed as tracers. The levels of labeling in 3-PGA during photosynthesis and then in the dark are shown in Figure 12. In contrast to the results with whole algae cells, there

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Insert Fig. 12 about here

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is no evidence for glycolysis in this case. The curves for ^{14}C and ^{32}P labeling remain exactly parallel throughout the course of the experiment.

Also, it is clear that there is little dark conversion of PGA to secondary products, indicating either that the isolated chloroplasts have lost the capability for such conversion or that most of such conversion occurs outside the chloroplast. It should be remembered from

Table I that most of the PGA is exported from the isolated chloroplasts to the medium, and the remaining level of PGA may well be too low to permit such biosynthetic pathways to be followed.

Figure 13 shows the behavior of RuDP labeling. Labeling with

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Insert Fig. 13 about here

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^{32}P and ^{14}C is quite parallel throughout the experiment. There is a clear indication of light-dark switching of the carboxylation enzyme in this figure. The level of RuDP falls during the first minute of darkness. Then the level of RuDP remains quite constant for the remainder of the dark period, suggesting the inactivation of the carboxylation reaction. When the light is turned on, we see a very large rise in the level of RuDP, suggesting that the light activation of the carboxylation reaction is not complete during the first minute of light. Then the level of RuDP falls back towards some steady-state level. Presumably the carboxylation reaction is now functioning at the steady-state light rate.

The level of ^{32}P and ^{14}C in dihydroxyacetone phosphate (DHAP) is shown in Figure 14. The labeling of DHAP falls about 40% during the

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Insert Fig. 14 about here

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period of darkness, despite the fact that other experiments indicate that 80% or more of the DHAP is in the supernatant at the time the light is turned off. Thus, some DHAP diffuses back into the chloroplast from the suspending medium. The transitory peak in DHAP labeling immediately

after the light is turned on has been observed in other experiments as well as this one. It may be the result of the dark inactivation postulated for the diphosphatase reaction. Thus, an increase in the levels of FDP and SDP before the activation of the diphosphatase could be reflected in an increase in the level of DHAP.

Figure 15 shows that the level of ATP declines greatly in the

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Insert Fig. 15 about here

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dark and rises rapidly in the light. More surprising is the fact that the level of ADP also declined in the dark and rose again in the light. This fall and rise in ADP in this particular experiment is well outside the limits of experimental error, but with other isolated chloroplast experiments this effect has so far not proved reproducible. Nonetheless, it suggests that with this particular chloroplast preparation, there must have been an active myokinase, or adenylate kinase, activity which brought about the following reaction: $2\ ADP \rightleftarrows ATP + AMP$. Thus, when the light is turned off and photophosphorylation stops, the level of ATP falls due to the continued requirement of the operation of the carbon reduction cycle. Upon the depletion of ATP, the equilibrium would cause ADP to be converted to ATP and AMP, with the ultimate result being the conversion of both ADP and ATP to AMP.

The consequences of this myokinase activity could well be the regulation of key enzymic steps by the level of AMP. Another possibility is that the level of inorganic phosphate rises in the chloroplasts during the dark and itself causes an inhibition of certain key

reactions. Thus, the inhibition of ADPG pyrophosphorylase by inorganic phosphate, according to Priess, et al. (43), may be a means of light-dark regulation of starch synthesis in leaf chloroplasts. During photosynthesis the level of inorganic phosphate would be kept low and the activity of the ADPG pyrophosphorylase, stimulated by PGA, would be high, thereby permitting active starch synthesis. In the dark the level of inorganic phosphate might rise and thereby quench the activity of this biosynthetic pathway.

SUMMARY AND CONCLUSIONS

Photosynthesis produces as end products not only sugars and carbohydrates, but proteins, fatty acids, fats, and a great variety of other compounds required for the regeneration of chloroplasts. When green cells are rapidly growing and dividing, so also are the chloroplasts growing and dividing, and a large proportion of photosynthetically incorporated carbon dioxide is utilized for the production of these diverse end products.

I have attempted to outline, insofar as they are known to us, the pathways leading from carbon dioxide to these end products. It seems most likely that the green plant cell employs a variety of regulatory mechanisms to control the flow of carbon to various end products, depending upon the requirements imposed by environment and upon the physiological state of the cell. I have given some experimental evidence indicating that light-dark regulation of metabolism in chloroplasts is not accomplished solely by the supply, or lack of it, of electrons and high energy phosphate from the photochemical reaction.

Rather, there are additional control points, particularly at the level of the diphosphatase reaction and the carboxylation reaction. It may be that the administering of fatty acid esters, such as methyl octanoate, can mimic the effect of darkness, since the same reactions are affected.

The need for these regulatory mechanisms becomes more apparent when one views the evidence for interaction between reactions of photosynthesis and glycolysis and the diffusion of intermediate compounds between the sites of these two processes. Many interesting questions remain to be answered regarding the apparent differential export of photosynthetic intermediates from the chloroplasts. Of great importance are questions about branch points at which carbon is drained from the primary photosynthetic process and utilized in the synthesis of various end products. How is the flow of photosynthetically reduced carbon into end products distributed at these branch points?

A better understanding of these regulatory mechanisms should be of value to future agricultural research. Perhaps it will be possible to manipulate the regulatory mechanisms to produce more or better end products in photosynthesis in green leaves.

Experiments with various chemical inhibitors already suggest that we can alter the ratio of ATP to electrons supplied for the photosynthetic reaction of carbon reduction to end products, and that we can mimic certain phases of light-dark regulation. Thus far, experiments have been limited to levels of inhibitors which cause complete inhibition of photosynthesis. Experiments with carefully controlled

lower levels of such inhibitors might well bring about interesting changes in the quality of photosynthetic products over longer periods of time. Obviously, such applications and extensions of our knowledge to agricultural research will be of primary interest to those concerned with the quality of green leaves as agricultural products.

The importance of such control of products of green leaves must not be underrated. Leaves which can be consumed directly as food by people may well become much more important in agriculture in the future. If leaves used for fodder are enriched in fats and protein, greater productivity of animal protein can be achieved. Utilization of such potentially vast crops as jungle foliage might become an economic reality if a light chemical spraying by airplane could result, a few days or weeks later, in leaves greatly enriched in fats and protein.

Such hopeful predictions are mere speculations today. Nonetheless, today's and tomorrow's discoveries of the nature of the distribution of photosynthetically reduced carbon, and the mechanisms of regulation of this distribution, provide a basis for a new era of agricultural experimentation.

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Table I

Rates of Formation of ^{14}C-Labeled Compounds in Isolated Spinach Chloroplasts Photosynthesizing with $^{14}CO_2$.

Compound		Rates at 3 min µm ^{14}C/mg Chl/hr	
		Pellet	Supernatant solution
3-Phosphoglyceric acid		2.90	26.70
Dihydroxyacetone phosphate		0.19	16.20
Phosphoglyceraldehyde		0.07	1.58
Sugar diphosphates		0.15	2.70
Hexose and Heptose monophosphates		3.15	0.93
Pentose monophosphates		0.27	1.20
Sucrose		0.17	-
Unidentified Spot "M"		0.12	0.99
Aspartic acid		0.035	0.15
Malic acid		0.055	-
Serine		0.036	0.036
Alanine		0.044	-
Glycine		Negligible	0.10
Glycolic acid		0.07	4.50
Total		7.26	55.11
Grand Total	62.37		
Measured externally	65.0		

(legends)

Fig. 1. The Carbon Reduction Cycle of Photosynthesis.

Asterisks denote the approximate order of ^{14}C labeling of specific carbon atoms of intermediate compounds of the cycle in experiments in which plants were allowed to photosynthesize for a short time with $^{14}CO_2$. Such experiments and their interpretation have been reviewed elsewhere (2) (3) (5).

I. ribulose-5-phosphate (Ru5P); II. ribulose-1,5-diphosphate (RuDP); III. 3-phosphoglyceric acid (PGA); IV. phosphoryl-3-phosphoglyceric acid; V. 3-phosphoglyceraldehyde (GA3P); VI. fructose-6-phosphate (F6P); VII. dihydroxyacetone phosphate (DHAP); VIII. fructose-1,6-diphosphate (FDP); IX. sedoheptulose-7-phosphate (S7P); X. sedoheptulose-1,7-diphosphate (SDP); XI. ribose-5-phosphate (R5P); XII. thiamine pyrophosphate glycolaldehyde addition compound (TPP-CHOH-CH_2OH), phosphoenolpyruvic acid (PEPA), thiamine pyrophosphate (TPP); XIII. xylulose—5-phosphate (Xu5P), glucose-6-phosphate (G6P).

Enzymes:

7. ribulose-5-phosphate kinase; 8. ribulose diphosphate carboxylase (carboxydismutase); 9. phosphoglyceryl kinase; 10. triose phosphate dehydrogenase; 13. triose phosphate isomerase; 14 and 17. aldolase; 15 and 18. diphosphatase; 16, 19 and 21. transketolase; 20. phosphoribose isomerase; 22. ribulose phosphate-xylulose phosphate isomerase; 23. hypothetical reduction of PGA moiety.

Fig. 2. Comparison of ^{14}C labeling of PGA, F6P and S7P, and glycolic acid during photosynthesis in isolated spinach chloroplasts.

Fig. 3. Photosynthetic ^{14}C labeling of free and bound alanine.

Fig. 4. Photosynthetic ^{14}C labeling of free and bound glycine.

Fig. 5. Changes in levels of F6P, R5P and 6-phosphogluconic acid induced by addition of Vitamin K_5 to photosynthesizing Chlorella pyrenoidosa.

Fig. 6. Sites of methyl octanoate inhibition of the photosynthetic carbon reduction cycle.

Fig. 7. Labeling of PGA with ^{32}P and ^{14}C during photosynthesis and respiration of Chlorella pyrenoidosa.

Fig. 8. Radioautograph of ^{32}P and ^{14}C-labeled compounds from chloroplast photosynthesis.

Fig. 9. Labeling of FDP and SDP with ^{14}C during photosynthesis and respiration of Chlorella pyrenoidosa.

Fig. 10. Levels of ^{32}P-labeled ATP, ADP, UTP and PP_i during light and dark with Chlorella pyrenoidosa.

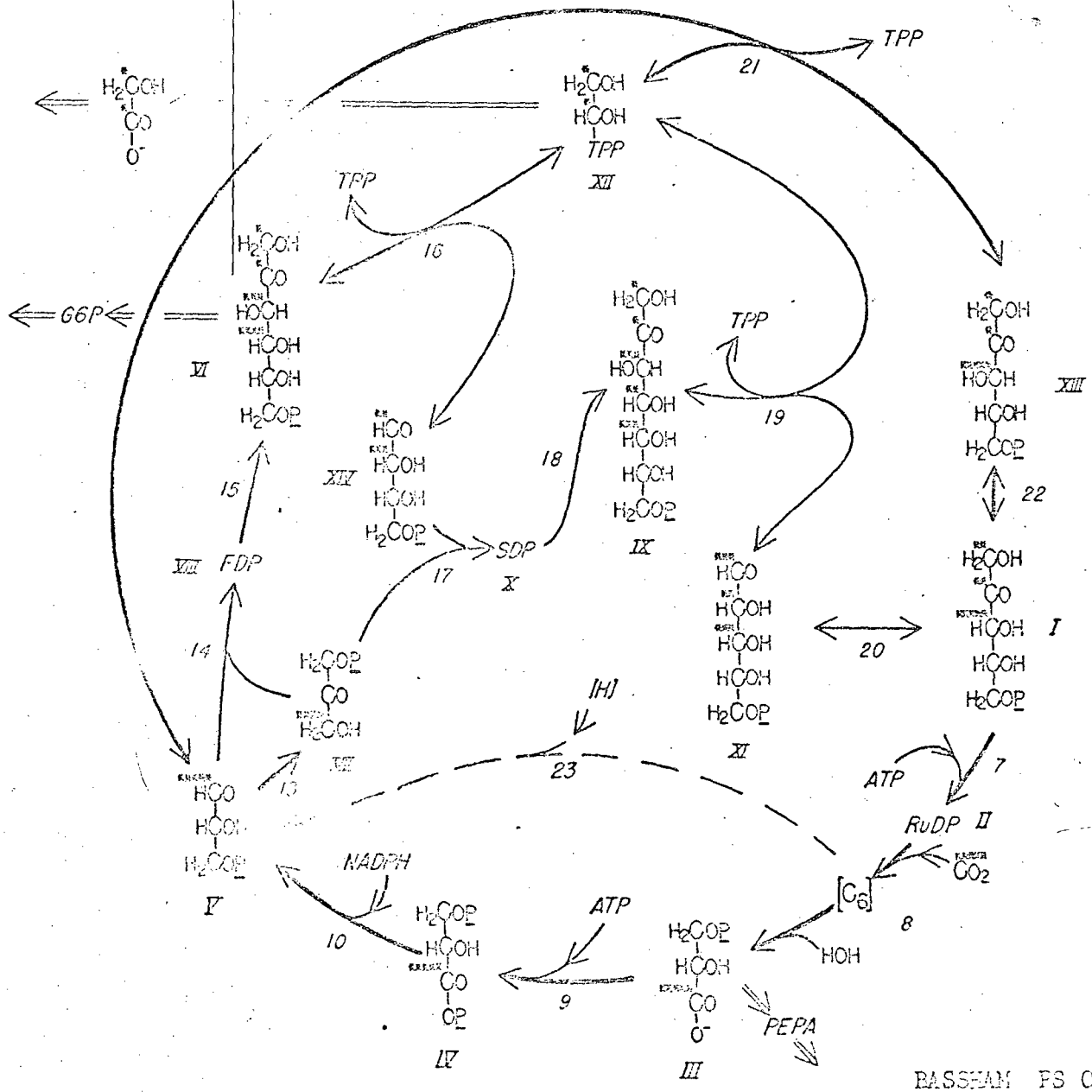
Fig. 11. Labeling of RuDP with ^{14}C during photosynthesis and respiration of Chlorella pyrenoidosa.

Fig. 12. Labeling of PGA with ^{32}P and ^{14}C during photosynthesis and dark in isolated spinach chloroplasts.

Fig. 13. Labeling of RuDP with ^{32}P and ^{14}C during photosynthesis and dark in isolated spinach chloroplasts.

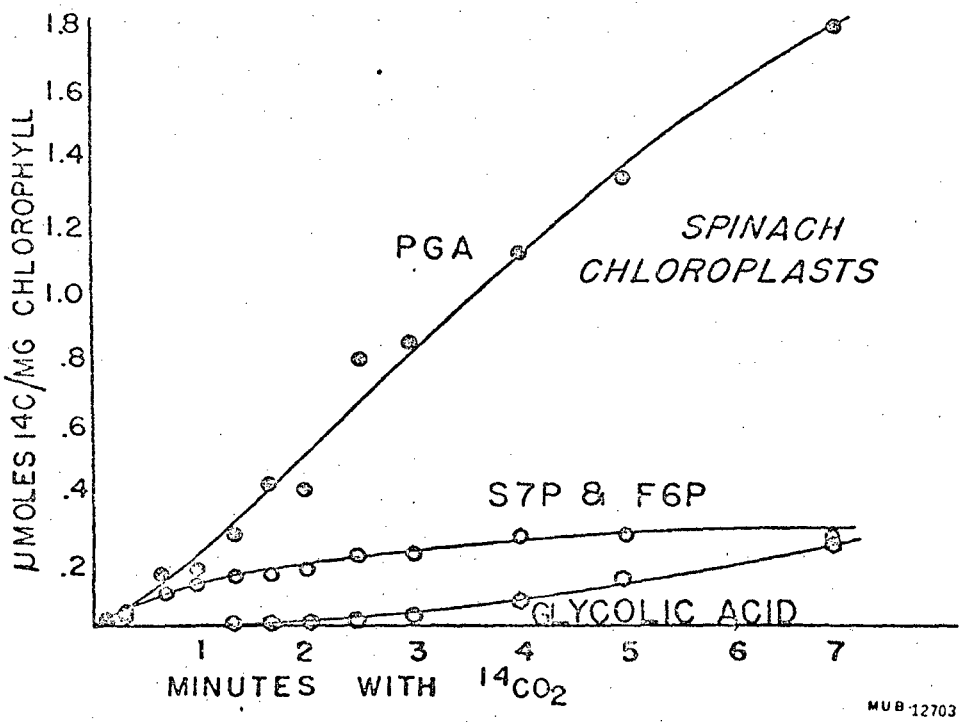
Fig. 14. Labeling of DHAP with ^{32}P and ^{14}C during photosynthesis and dark in isolated spinach chloroplasts.

Fig. 15. Labeling of ATP, ADP and PP_i with ^{32}P during photosynthesis and dark in isolated spinach chloroplasts.



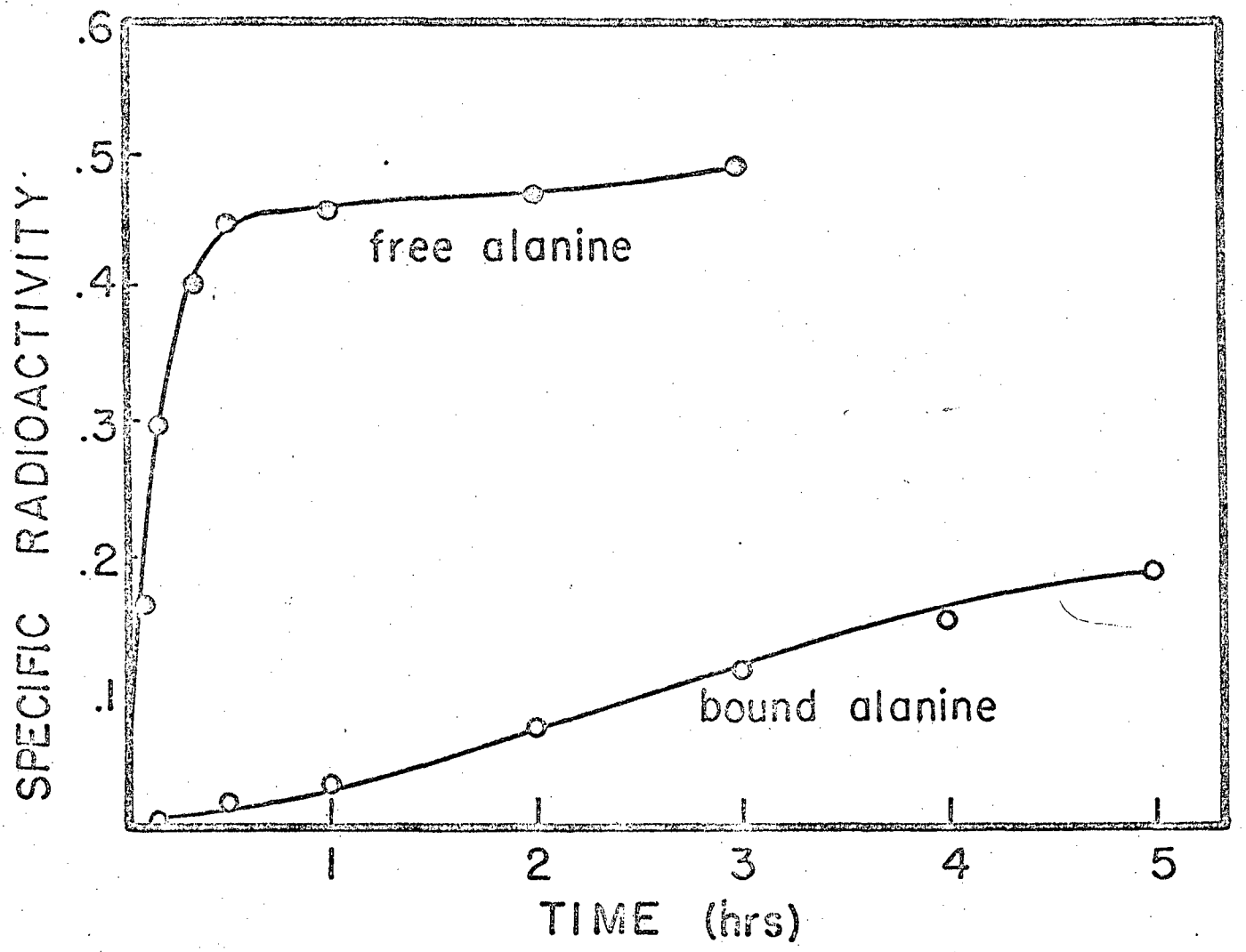
BASSHAM PS OF C COMPOUNDS

FIGURE 1



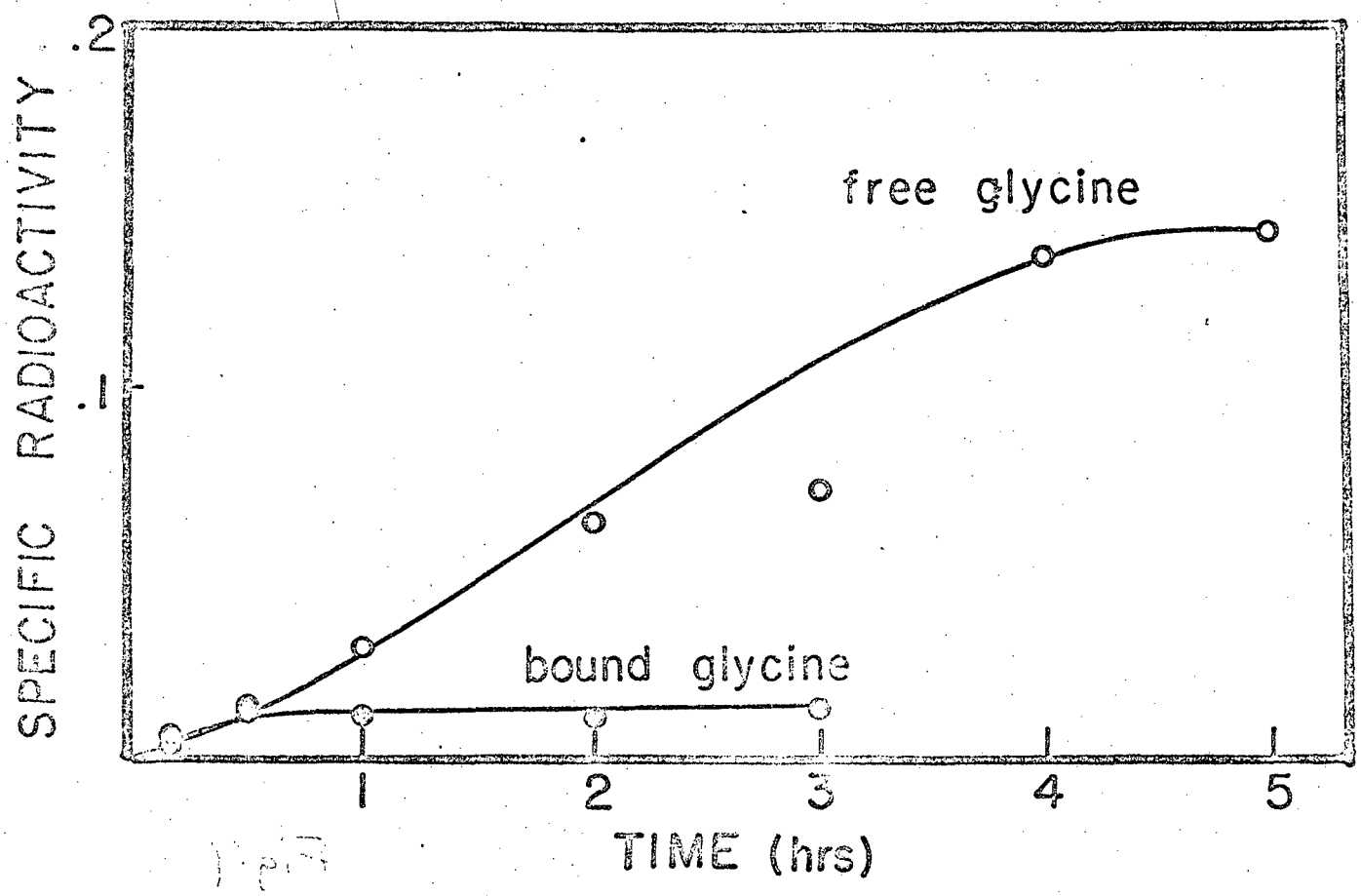
BASSHAM PS OF C COMPOUNDS

FIGURE 2



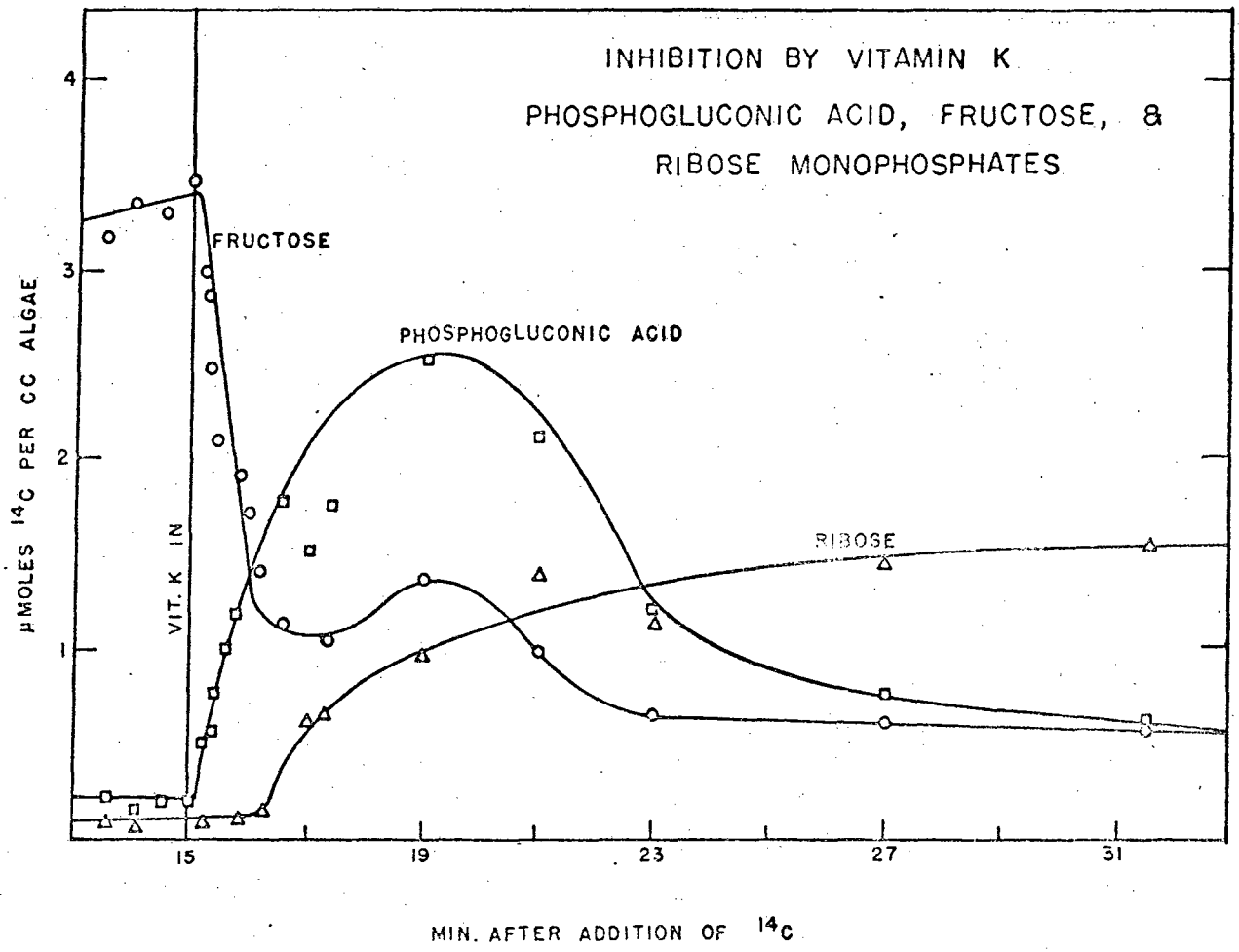
BASSHAM PS OF C COMPOUNDS

FIGURE 3



BASSHAM PS OF C COMPOUNDS

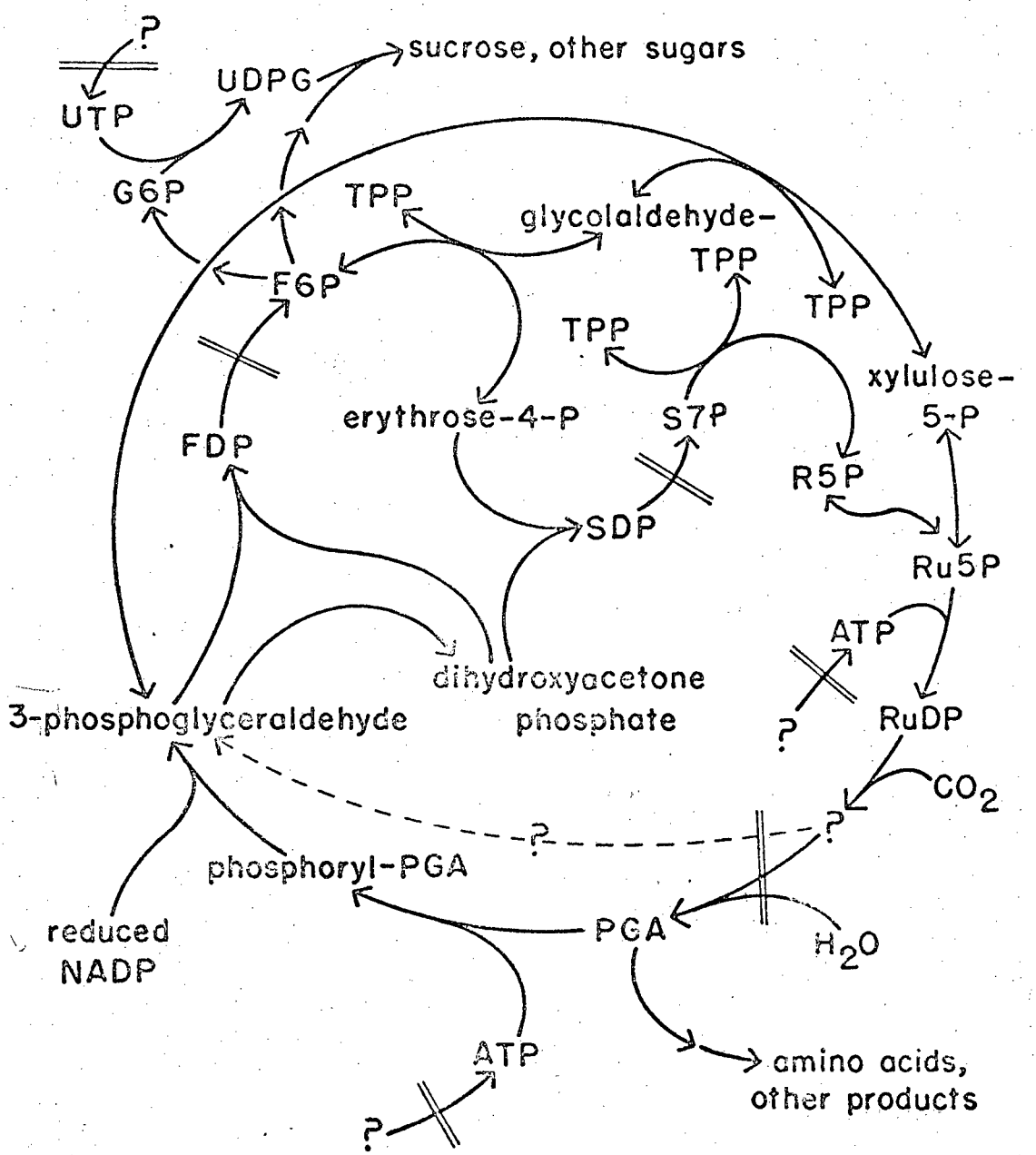
FIGURE 4



MUB-3685

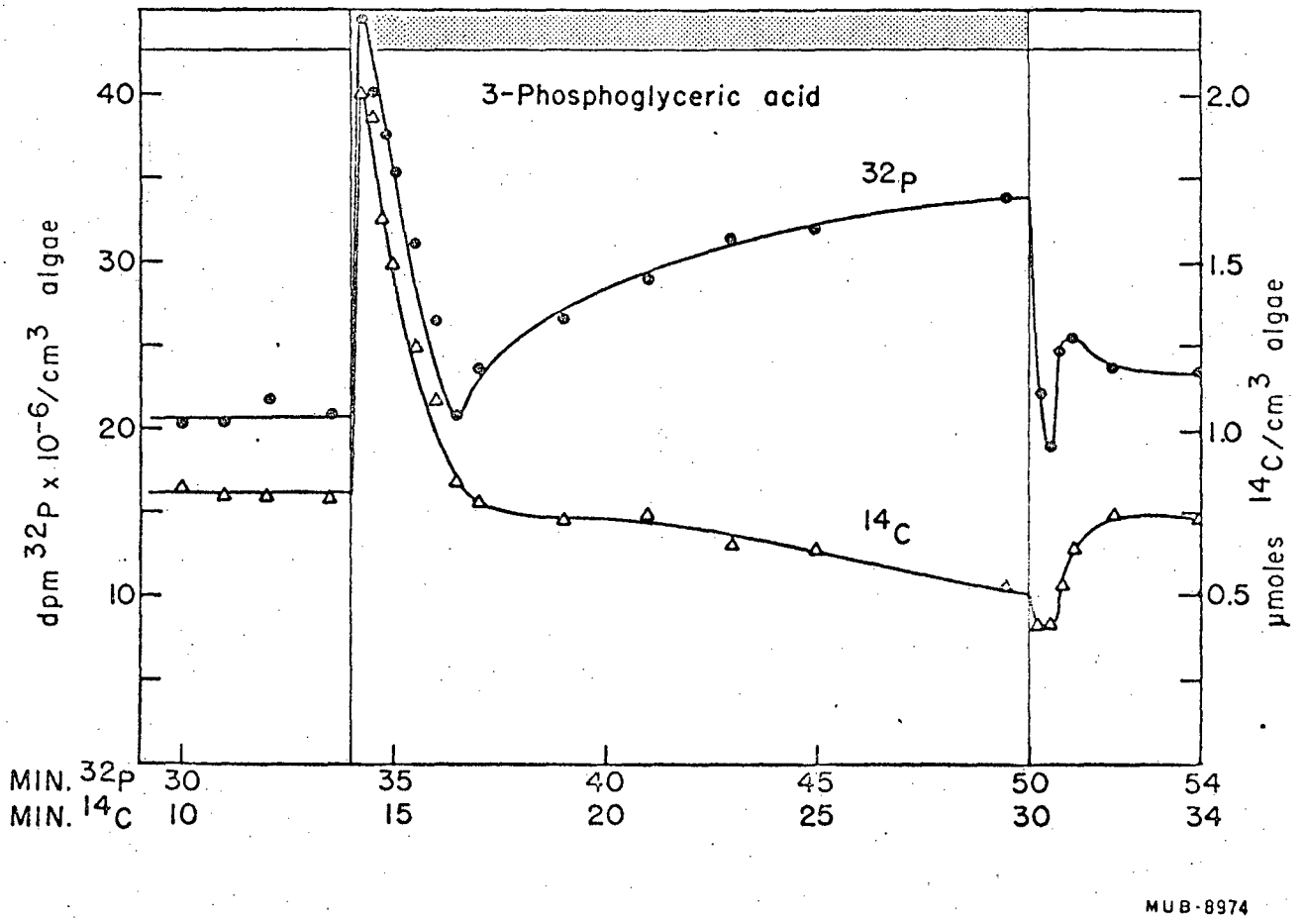
BASSHAM PS OF C COMPOUNDS

FIGURE 5



BASSHAM PS OF C COMPOUNDS

FIGURE 6

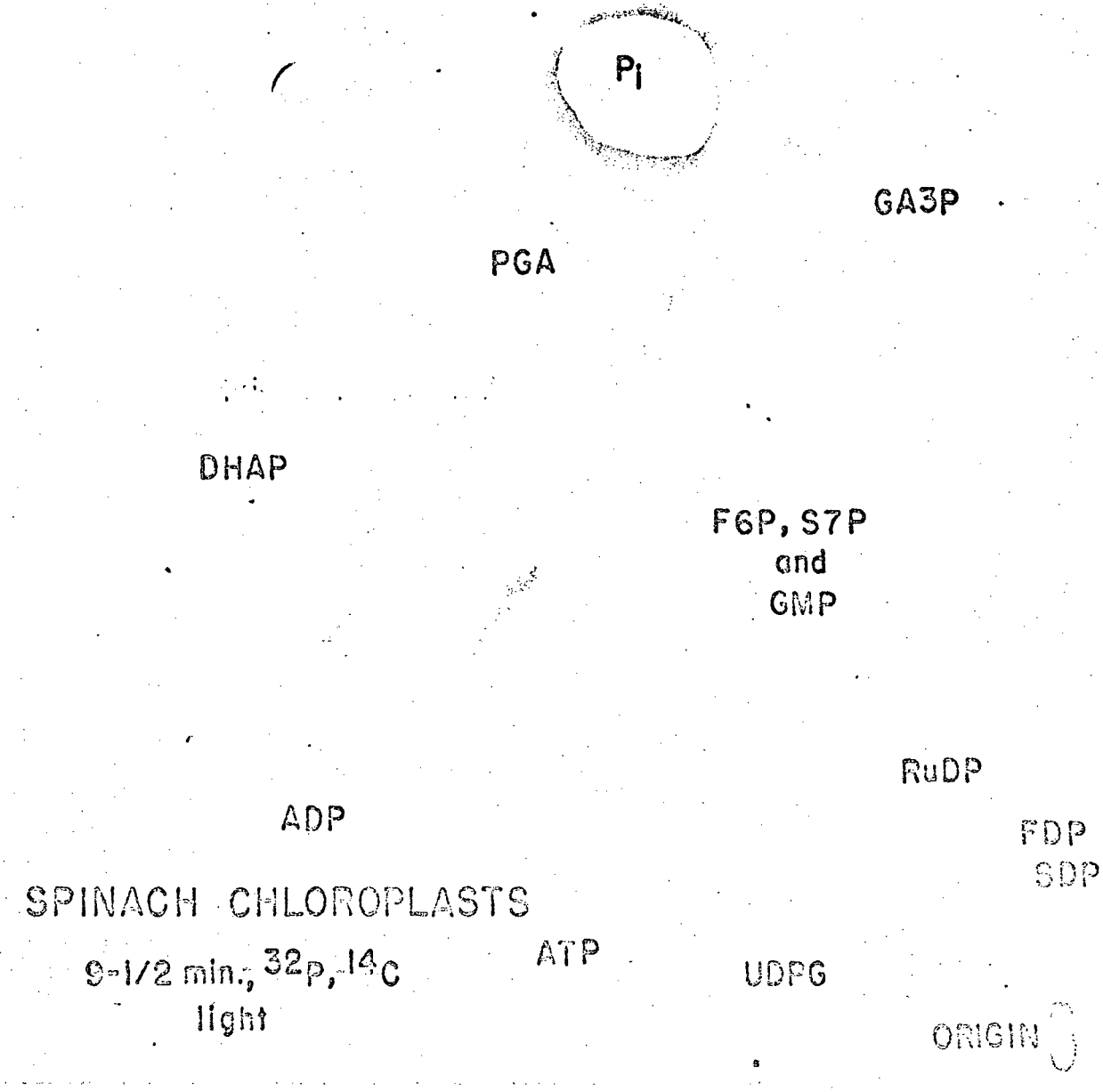


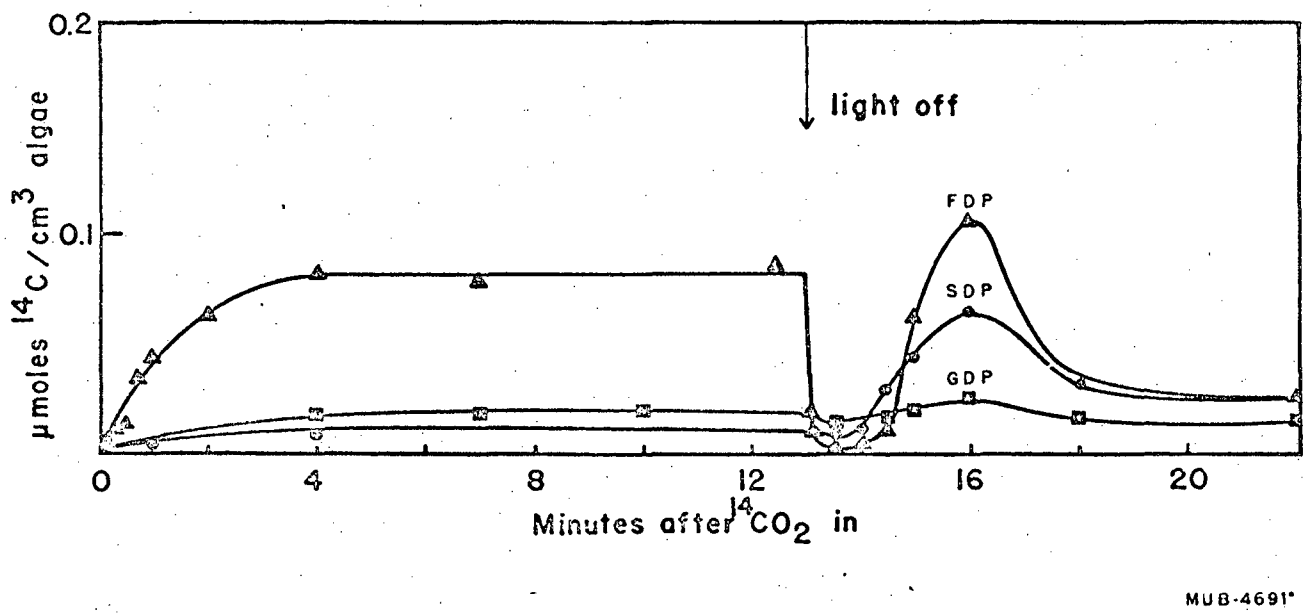
BASSHAM PS OF C COMPOUNDS

FIGURE 7

BASSHAM PS OF C COMPOUNDS

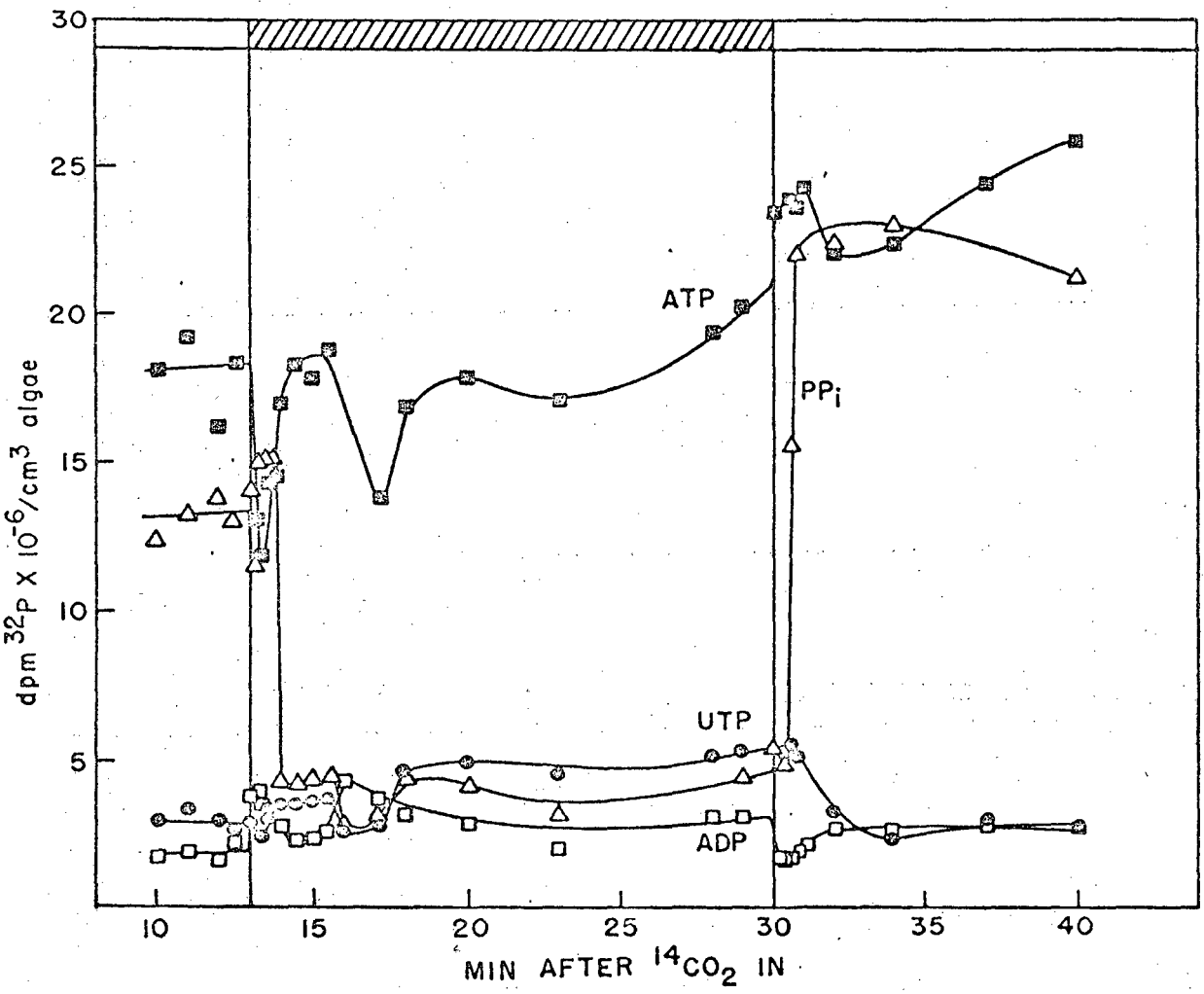
FIGURE 8





BASSHAM PS OF C COMPOUNDS

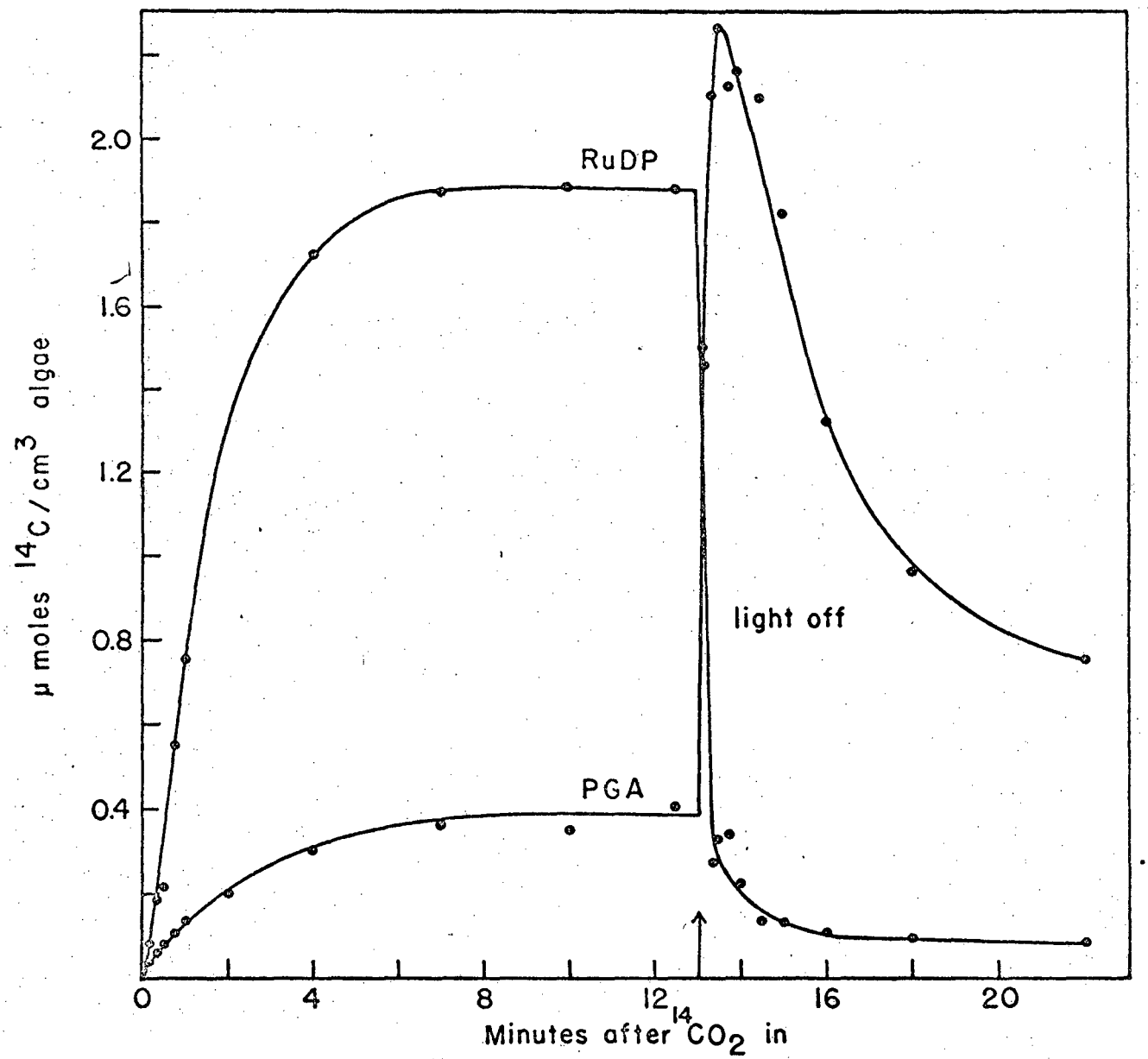
FIGURE 9



MUB-7268

BASSHAM PS OF C COMPOUNDS

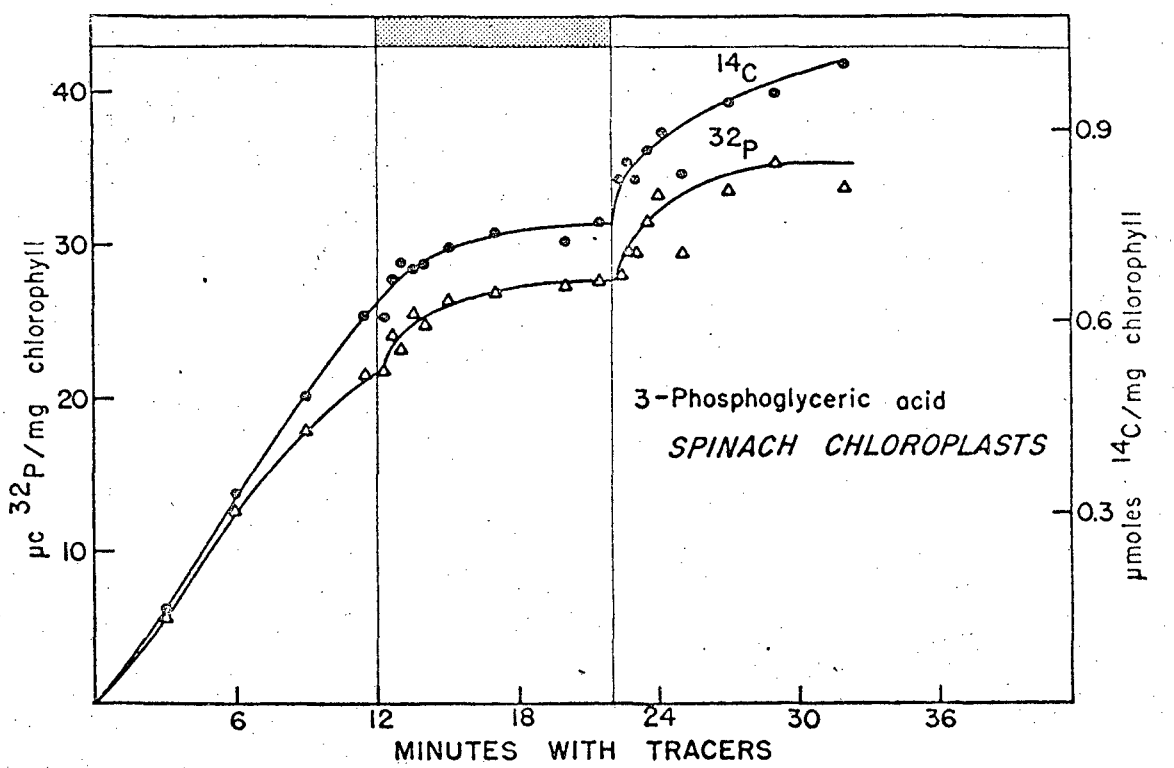
FIGURE 10



MUB-4694

BASSHAM PS OF C COMPOUNDS

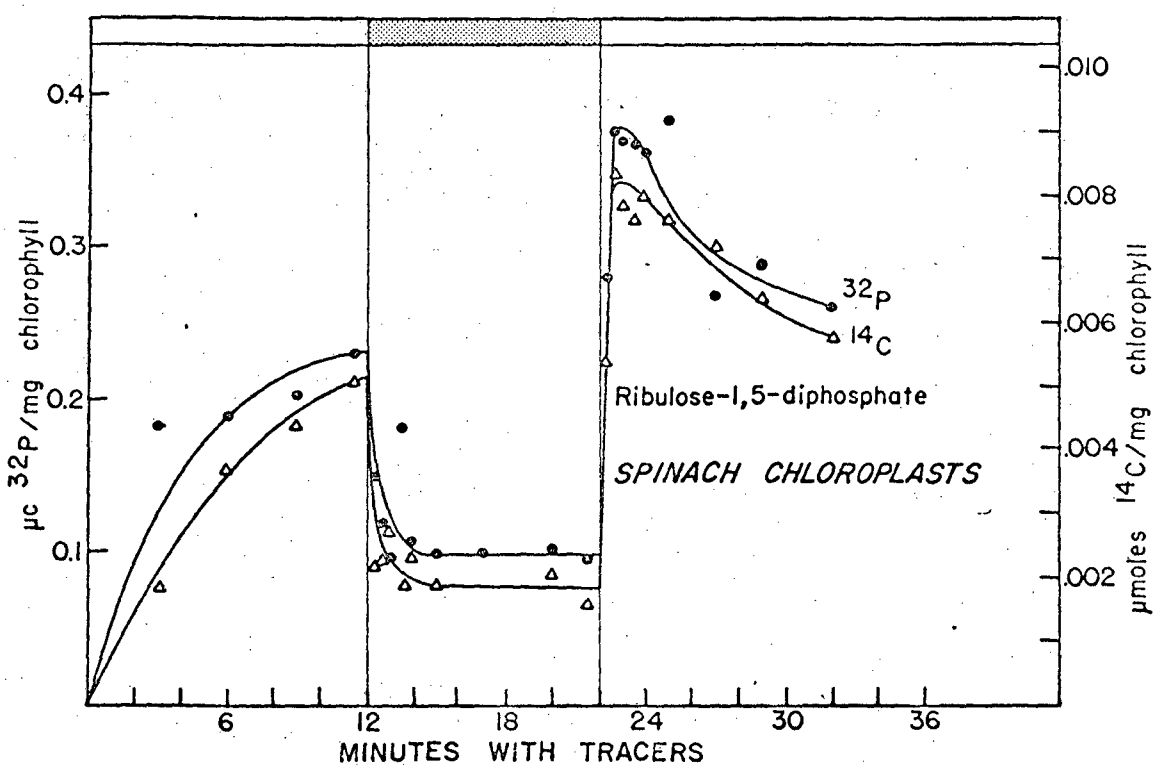
FIGURE 11



MUB-9701

BASSHAM PS OF C COMPOUNDS

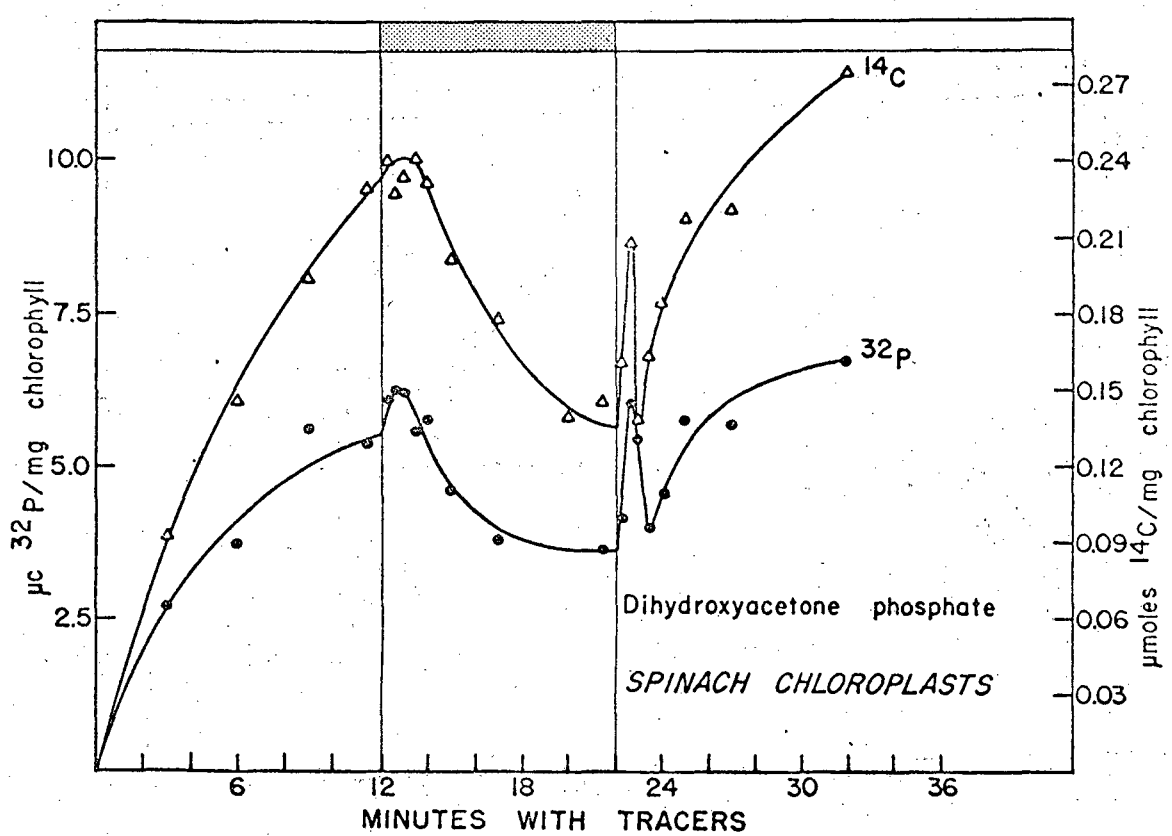
FIGURE 12



MUB-9704

BASSHAM PS OF C COMPOUNDS

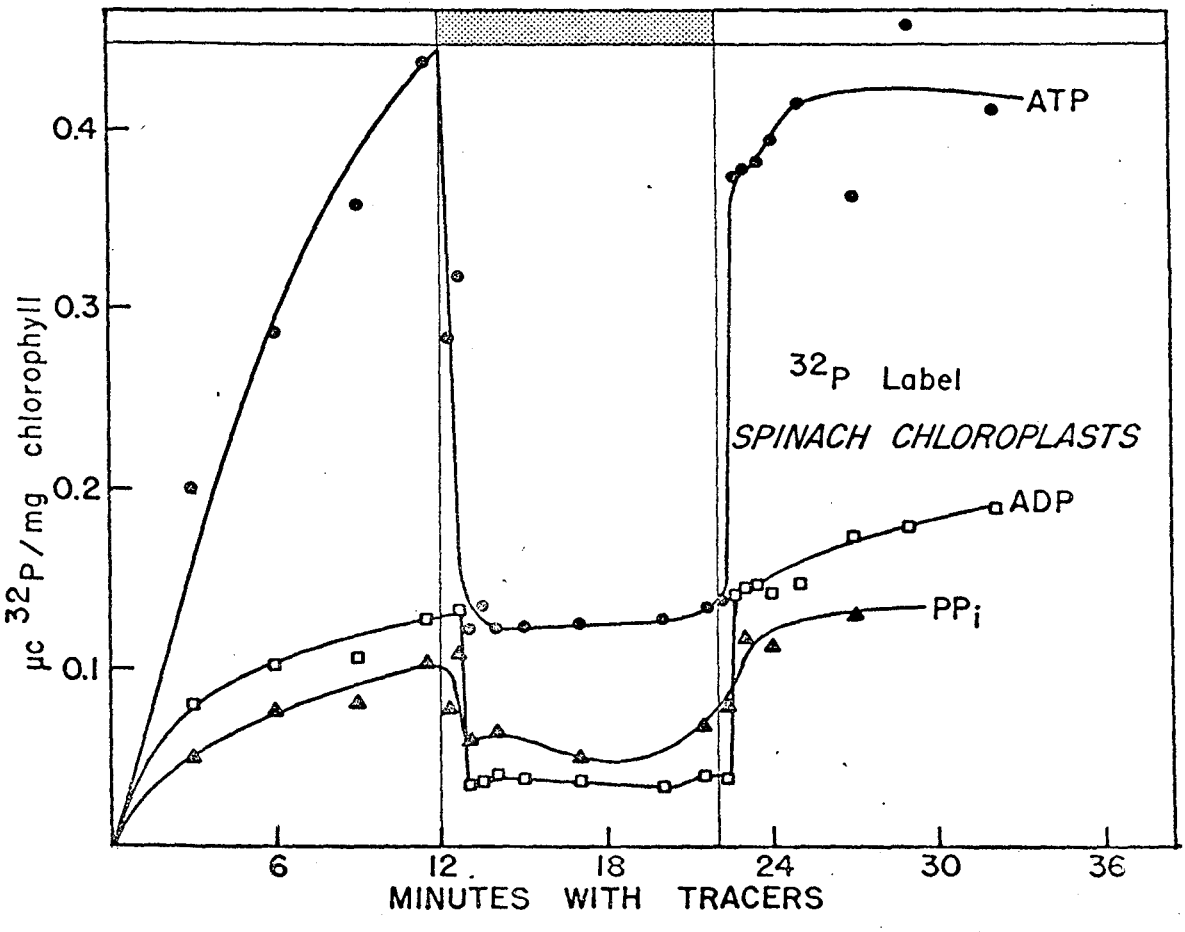
FIGURE 13



MUB-9705

BASSHAM PS OF C COMPOUNDS

FIGURE 14



MUB-9702

BASSHAM PS OF C COMPOUNDS

FIGURE 15

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