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THE PATH OF CARBON IN PHOTOSYNTHESIS

Melvin Calvin

Nobel Prize Lecture

December 11, 1961

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THE PATH OF CARBON IN PHOTOSYNTHESIS

Melvin Calvin

Department of Chemistry and Lawrence Radiation Laboratory
University of California, Berkeley 4, California

INTRODUCTION

It is almost sixty years since Emil Fischer was describing on a platform such as this one some of the work which led to the basic knowledge of the structure of glucose and its relatives.[1] Today we will be concerned with a description of the experiments which have led to a knowledge of the principal reactions by which those carbohydrate structures are created by photosynthetic organisms from carbon dioxide and water, using the energy of light.

The speculations on the way in which carbohydrate was built from carbon dioxide began not long after the recognition of the basic reaction and were carried forward first by Justus von Liebig and then by Adolf von Baeyer and, finally, by Richard Wilstatter and Arthur Stoll into this century. Actually, the route by which animal organisms performed the reverse reaction, that is, the combustion of carbohydrate to carbon dioxide and water with the utilization of the energy resulting from this combination, turned out to be the first one to be successfully mapped, primarily by Otto Meyerhof[2] and Hans Krebs.[3]

Our own interest in the basic process of solar energy conversion by green plants, which is represented by the overall reaction

$$CO_2 + H_2O \xrightarrow[\text{Chlorophyll}]{\text{Light}} (CH_2O)_n + O_2$$

began some time in the years between 1935 and 1937, during my postdoctoral studies with Professor Michael Polanyi at Manchester. It was there I first became conscious of the remarkable properties of coordinated metal compounds, particularly metalloporphyrins as represented by heme and chlorophyll. A study was begun at that time, which is still continuing, on the electronic behavior of such metalloporphyrins, It was extended and generalized by the stimulus of Professor Gilbert N. Lewis upon my arrival in Berkeley. I hope these continuing studies may one day contribute to our understanding of the precise way in which chlorophyll and its relatives accomplish the primary quantum conversion into chemical potential which is used to drive the carbohydrate synthesis reaction.

Even before 1940 the idea that the reduction of carbon dioxide to carbohydrate might be a dark reaction separate from the primary quantum conversion act was already extant, stemming most immediately from the comparative biochemical studies of Cornelis van Niel and the much earlier work of F. F. Blackman and its interpretation by Otto Warburg. The photoinduced production of molecular oxygen had been separated chemically and physically from the reduction of carbon dioxide by the demonstration of oxygen evolution by illuminated chloroplasts. This was done by Robert Hill[4] using ferric iron as oxidant in the place of carbon dioxide.

We are thus able to represent in a diagrammatic way (Figure 1) the overall conversion of light energy into chemical energy in the form of carbohydrate and oxygen. The light energy first absorbed by chlorophyll and related pigments is converted into chemical potential in the form of high energy containing compounds, represented by B in Figure 1. These, in turn, lead to the production of oxygen from water and the simultaneous generation of high level reducing agents which can be used, together with whatever collaborators are required, to carry out the carbon dioxide reduction.

One of the principal difficulties in such an investigation as this, in which the machinery which converts the CO_2 to carbohydrate and the substrate upon which it operates are made with the same atoms, namely, carbon and its near relatives, is that ordinary analytical methods will not allow us to distinguish easily between the machinery and its substrate. However, the discovery of the long-lived isotope of carbon, carbon-14, by Samuel Ruben and Martin Kamen in 1940[5] provided the ideal tool for the tracing of the route along which carbon dioxide travels on its way to carbohydrate, represented in Figure 1 by the series of unknown materials, X, Y, Z, etc.

In 1945 it became apparent to us that carbon-14 would be available cheaply and in large amounts by virtue of the nuclear reactors which had been constructed. With the encouragement and support of Professor Ernest O. Lawrence, the Director of the Radiation Laboratory in Berkeley, we undertook to study that part of the energy converting process of photosynthesis represented by the carbon reduction sequence, making use of C^{14} as our principal tool.

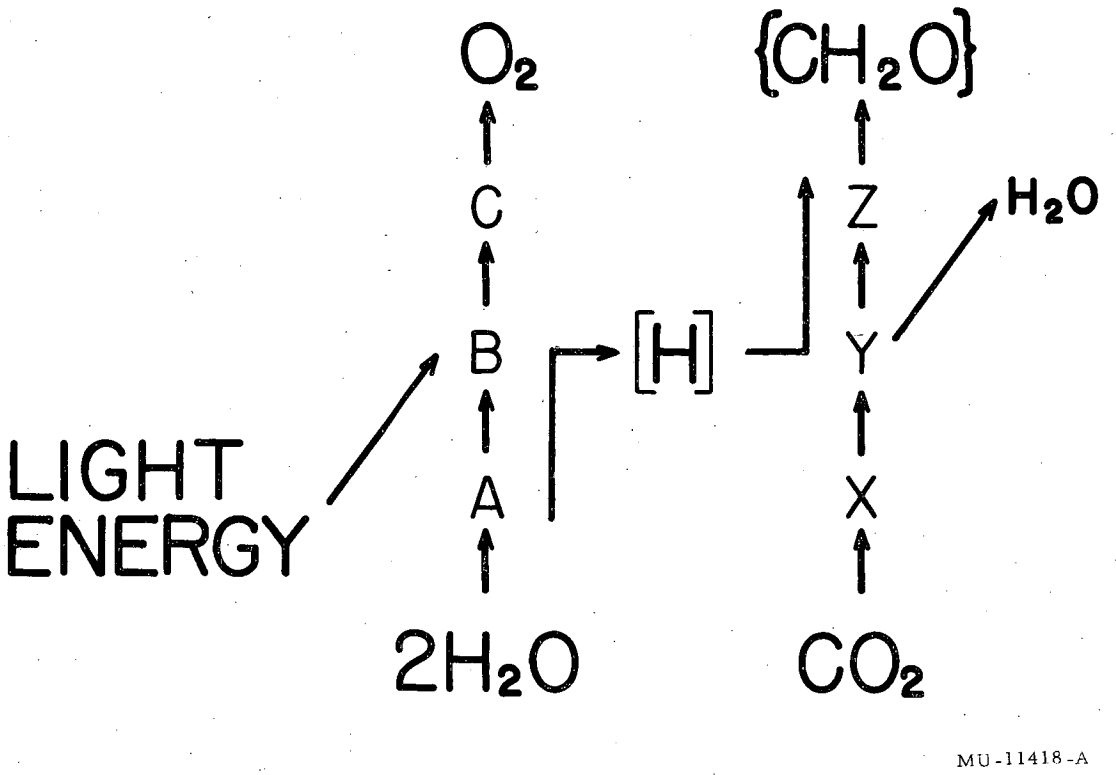

Fig. 1. Elementary photosynthesis scheme.

DESIGN OF THE EXPERIMENT

The principle of the experiment was simple. We knew that ultimately the CO_2 which enters the plant appears in all of the plant materials but primarily, and in the first instance, in carbohydrate. It was our intention to shorten the time of travel to such an extent that we might be able to discern the path of carbon from carbon dioxide to carbohydrate as the radioactivity which enters with the CO_2 passes through the successive compounds on its way to carbohydrate.

Preliminary experiments confirmed the idea that the absorption of CO_2 and its incorporation in organic material was indeed a dark reaction. This was easily established by exposing plants which had first been illuminated in the absence of carbon dioxide so as to store some of the intermediate high energy containing compounds, and then noting that these compounds could be used in the dark to incorporate relatively large amounts of CO_2. However, the products did not proceed very far along the reduction scheme under these conditions, and so we undertook to do the experiment in what we call a steady state of photosynthesis.

Plant Material

As the precision of our experiments increased, the need for more reproducible biological material also increased, and very soon we found it necessary to grow our own plant material in as highly reproducible manner as possible. A very convenient green plant that had already been the subject of much photosynthetic research was the unicellular green alga, *Chlorella*, a photomicrograph of which is shown in Figure 2.

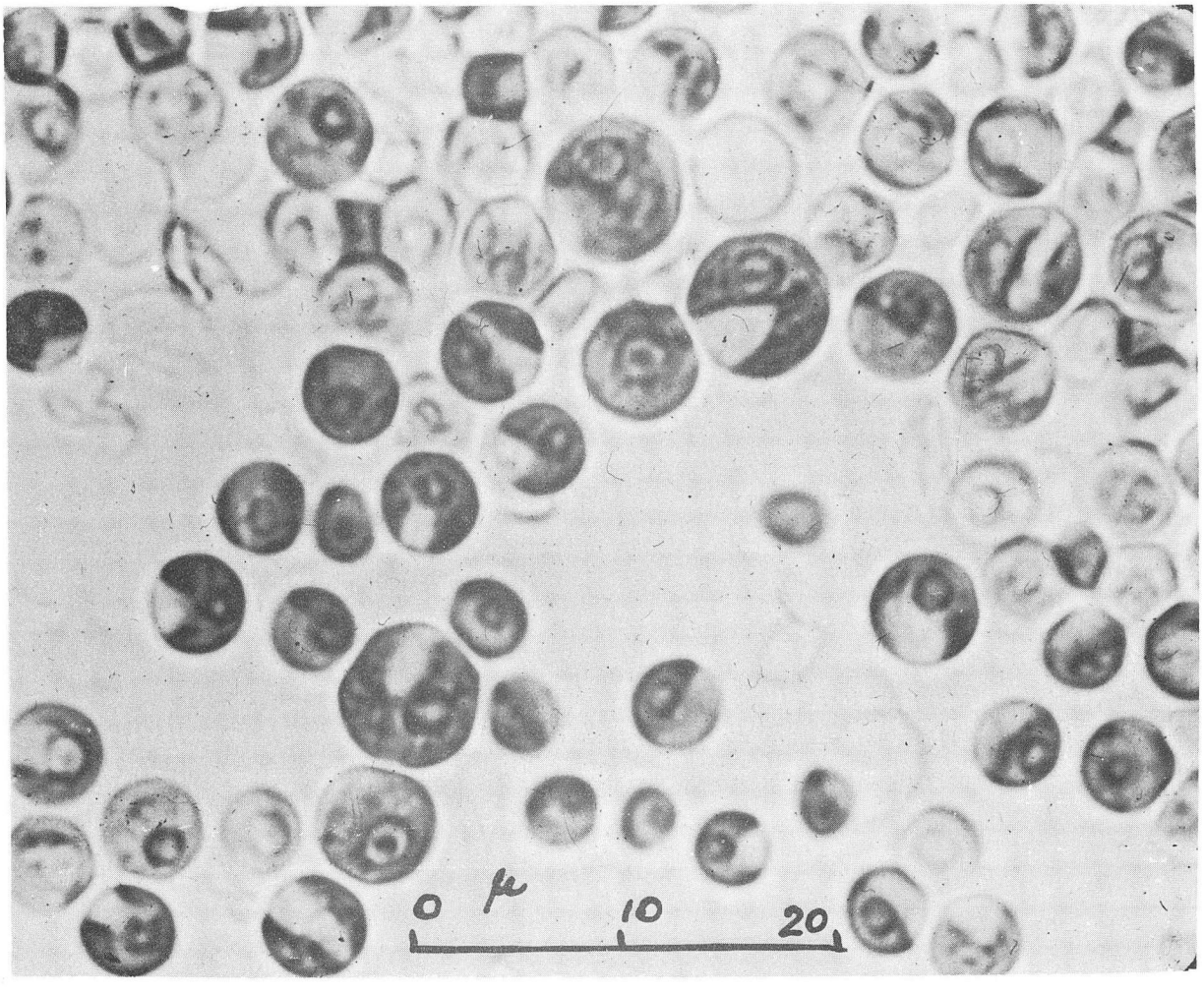

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Fig. 2. Photomicrograph of Chlorella.

We developed methods of growing these organisms in a highly reproducible fashion, both in intermittent and continuous cultures, and it is with organisms such as these that most of our work was done. I hasten to add, however, that the essential features of the cycle with which we finally emerged were demonstrated on a wide variety of photosynthetic organisms, ranging from bacteria to the higher plants.

Apparatus

The exposures were initially performed in a simple apparatus (called a 'Lollipop' because of its shape, Figure 3) which contained a suspension of the algae undergoing photosynthesis with normal CO_2. The initiation of our tracer experiment was accomplished by injecting into the nonradioactive carbon dioxide stream, or substituting for it, some C^{14}-labeled CO_2 for a suitable period of time, ranging from fractions of a second to many minutes. At the end of the preselected time period, the organisms were killed by various methods, but principally by dropping the suspension into approximately four volumes of alcohol. This stopped the enzymic reactions and, at the same time, began the extraction of the materials for analysis.

Early Analytical Methods

In the early work, the classical methods of organic chemistry were applied in our isolation and identification procedures, but it soon became apparent that these were much too slow and would require extremely large amounts of plant material to provide us with the identification of specific labeled compounds. Here, again, we were able to call upon

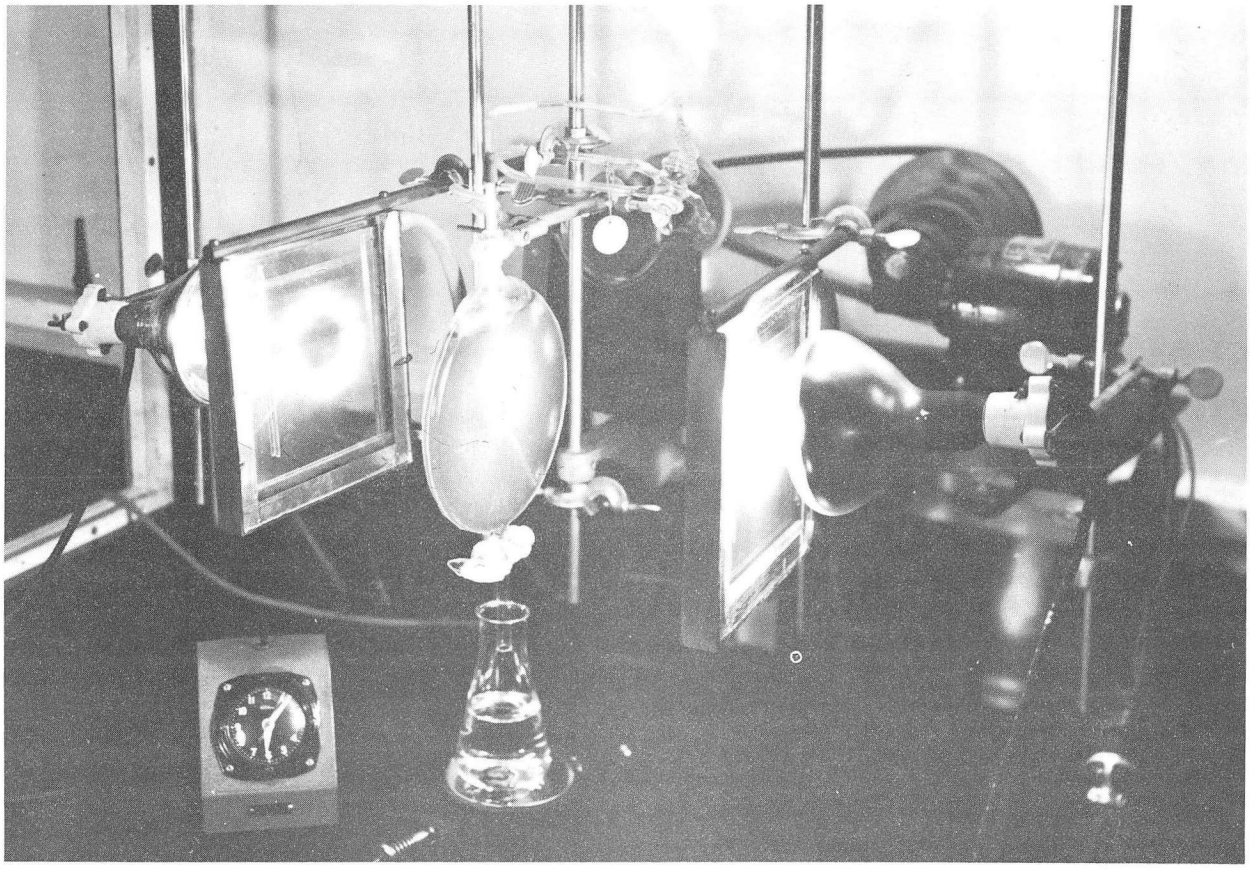

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Fig. 3. 'Lollipop', the apparatus in which the photosynthesis experiments are performed.

our experience during the war years in which we had used ion exchange columns for the separation of plutonium and other radioactive elements. We made use of both anion and cation exchange columns, and soon discovered that the principal compounds in which we were interested, that is, those which became C^{14} radioactive in the shorter exposure times, were, indeed, anionic in character.

Because of the peculiar difficulty we found in eluting the principal radioactive components from anion exchange resins, it became apparent that this radioactive material was a strongly acidic material and very likely had more than one anionic point of attachment to bind to the resin. Among these peculiarities was the fact that an ordinary carboxylic acid could be eluted relatively easily while the prinipal radioactive material would require either very strong acid or very strong base to bring it off. This, taken together with a number of other chromatographic properties, led to the idea that these early products might very well be phosphate esters as well as carboxylic acids.

A more detailed analysis of the precise conditions required to elute the material off the ion exchange columns suggested phosphoglyceric acid as a possibility. To a relatively large amount of algae was added as indicator a small amount of the purified radioactive material obtained from a small sample of algae exposed to radioactive carbon for a few seconds. This led to the direct isolation of slightly over nine milligrams of a barium salt which by classical organic procedures we were able to show to be the barium salt of 3-phosphoglyceric acid.[6]

Paper Chromatographic Methods

About this time Martin and Synge[7] had developed their method of partition chromatography which was particularly adapted for amino acid analysis because of the sensitivity of the colorimetric detection method. We turned to this as our principal analytical tool. It was particularly suited to our needs because, having spread our unknown material from the plant onto a sheet of filter paper by two-dimensional chromatography, we could then find the particular components which we sought, namely, the radioactive ones, without knowing their chemical nature beforehand. This was done by placing the paper in contact with photographic film, thus exposing the film at those points of the paper upon which were located the very compounds in which we were interested.

The result of such an experiment in which the algae were exposed to radioactive CO_2 for thirty seconds under what we then thought were steady state conditions is shown in Figure 4. The blackened areas on the film indicate the presence of radioactive compounds on the paper at those points. Such a chromatogram and film as shown in Figure 4 constituted our principal primary source of information.

It should be noted that this information resides in the number, position and intensity, that is, radioactivity of the blackened areas. The paper ordinarily does not print out the names of these compounds, unfortunately, and our principal chore for the succeeding ten years was to properly label those blackened areas on the film.

The techniques for doing this were many and varied. It was already clear that the coordinates of a particular spot in a particular chromatogram already could be interpreted in terms of chemical structure

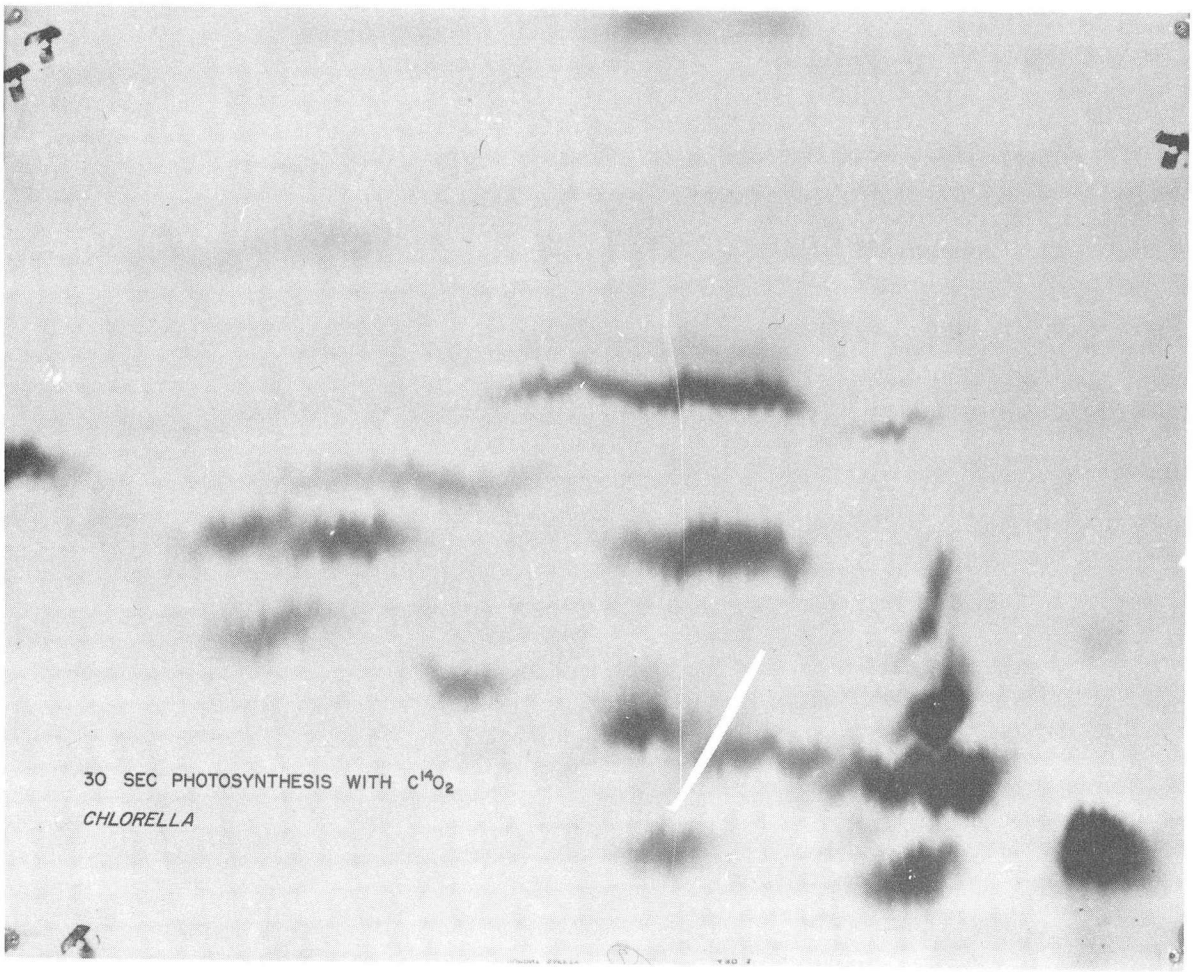

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Fig. 4. Chromatogram of extract from algae indicating uptake of radiocarbon during 30 seconds of photosynthesis, using Chlorella.

in a general way, but this was far from sufficient for identification. Our usual procedure was to seek other properties of the material on the paper, such as fluorescence or ultraviolet absorption if there was enough of it. More commonly it was necessary to elute the material from that part of the paper, as defined by the black area of the film, to perform chemical operations on the eluted material and then rechromatograph the product to determine its fate.

From a succession of such operations the chemical nature of the original material could gradually be evolved, and final identification was usually achieved by co-chromatography of the tracer amount of unknown material with carrier, or macroscopic, amounts of the authentic, suspected compound. A suitable chemical test was then performed on the paper to which the authentic material alone would respond since it was the only material present in sufficient amount. If the response produced by the authentic material coincided exactly with the radioactivity on the paper in all its details, we could be quite confident of the identity of the radioactive compound with the added carrier.

In this way after some ten years of work by many students and collaborators, beginning with Dr. Andrew A. Benson, we were able to place names on a large number of black spots on Figure 4, as shown in Figure 5. It is perhaps worth noting that these two chromatograms are duplicate chromatograms of the same extract and are not identical chromatograms, and the degree of reproducibility of the procedure is thus established.[8]

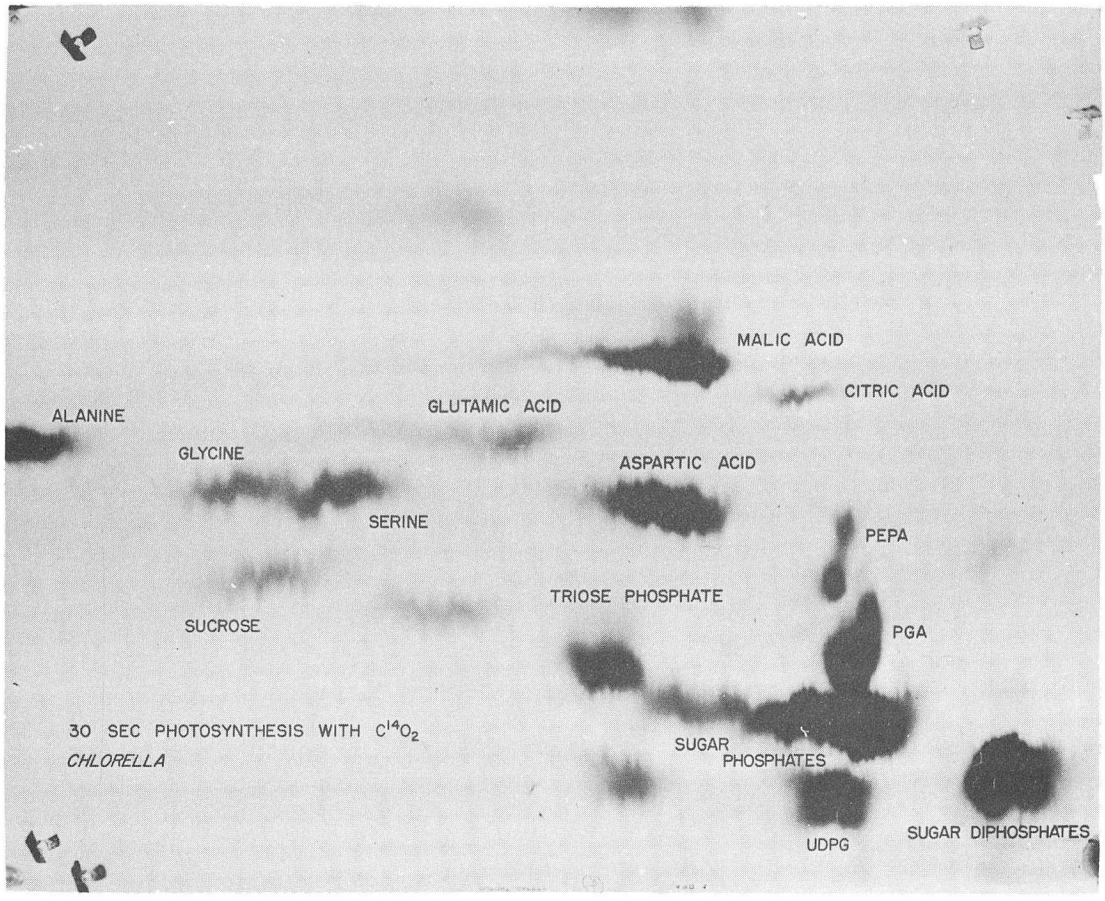

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Fig. 5. Chromatogram of extract from Chlorella indicating uptake of radioactive carbon during 30 seconds of photosynthesis. UDPG = uridine diphosphoglucose; PEPA = phosphoenolpyruvic acid; PGA = phosphoglyceric acid.

DEVELOPMENT OF THE CARBON REDUCTION CYCLE

Phosphoglyceric Acid as the First Product

It was thus already clear that in only thirty seconds the carbon has passed into a wide variety of compounds and that we would have to shorten the exposure time in order to get some due as to the earliest compounds into which CO_2 is incorporated. This we did in a systematic way, and the result of a five second exposure is shown in Figure 6. Here we began to see the dominance of the sugar- and sugar-acid phosphates. In shortening the exposure time still further, it became quite clearly apparent that a single compound dominated the picture in fractions of a second, amounting to over eighty or ninety percent of the total fixed radioactive carbon. This compound was phosphoglyceric acid. That the phosphoglyceric acid was not the result of the killing procedure but was actually present in the living organism is demonstrated by the fact that when CO_2 is fixed by pre-illuminated algae in the dark under conditions in which not much of the phosphoglyceric acid can be reduced to the sugar level, a good fraction of the three-carbon fragment appears as alanine, as is shown in Figure 7. Alanine is a stable compound and is not likely to be formed from precursors by merely dropping the algae into the alcohol, and we can be confident that it was present in the living algae. In addition, a wide variety of killing procedures gave the same result. Thus the presence of phosphoglyceric acid itself in the living plant can be confidently presumed.

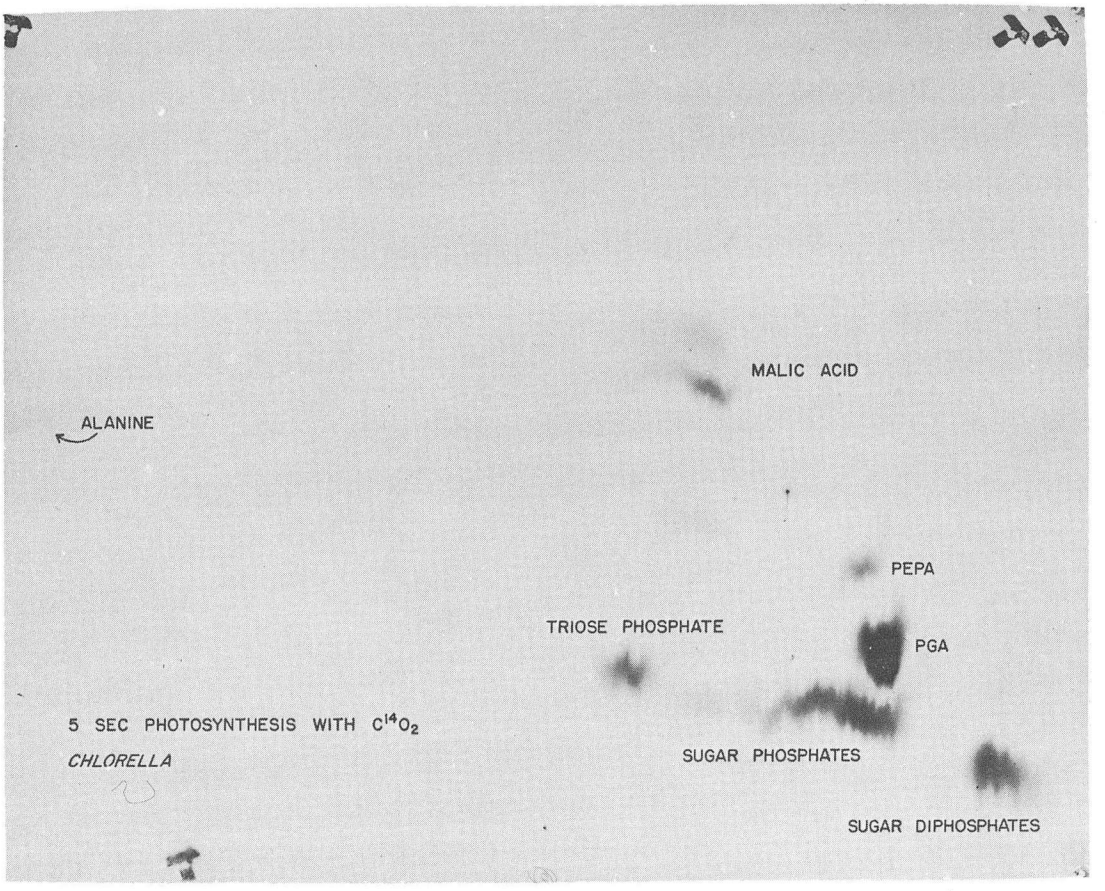

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Fig. 6. Chromatogram of extract from Chlorella indicating uptake of radiocarbon during 5 seconds of photosynthesis.

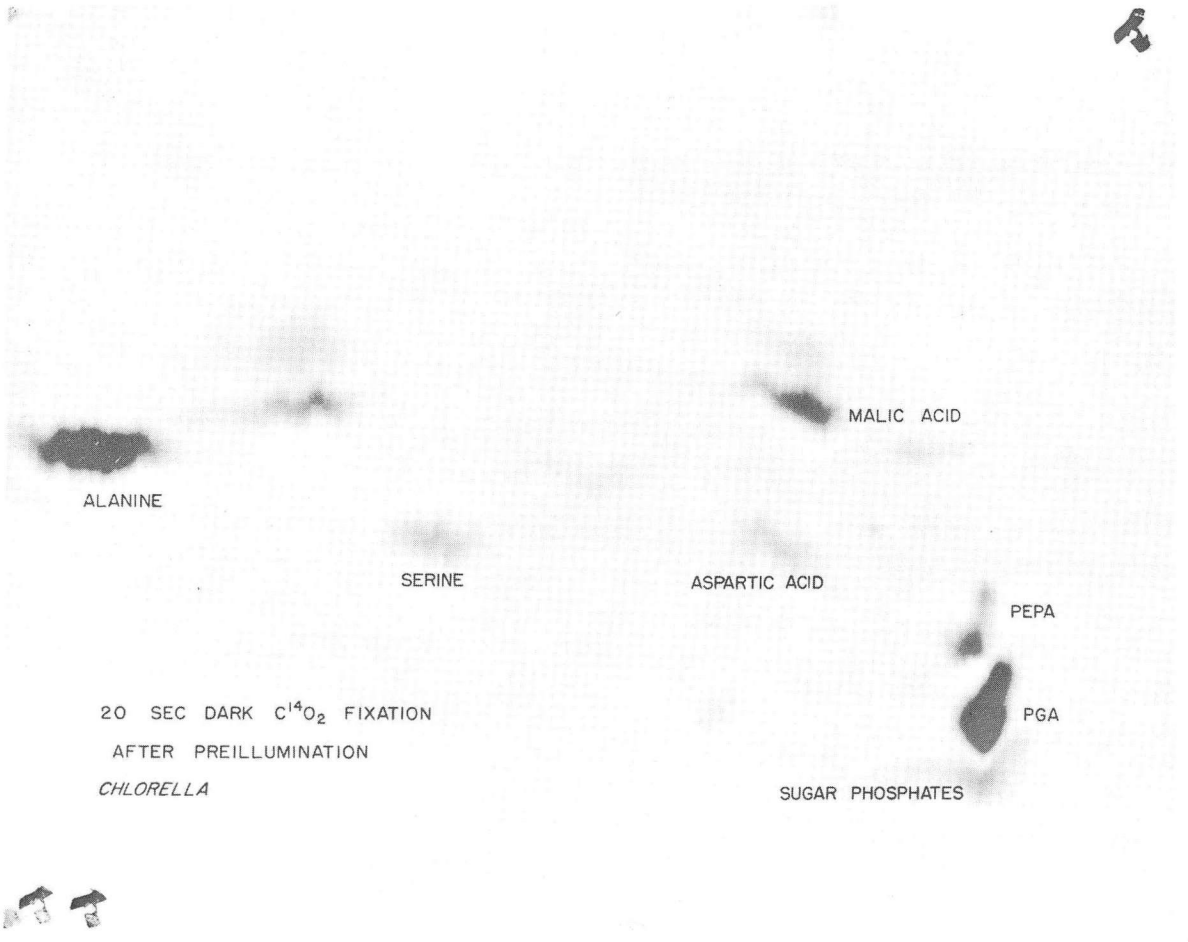

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Fig. 7. Chromatogram of extract from Chlorella indicating uptake of radiocarbon, 20 seconds dark fixation after pre-illumination.

Labeling of Hexose

Among the earliest sugar phosphates which are shown in Figure 6 are the triose phosphate and the hexose phosphate, and thus the succession from phosphoglyceric acid to hexose was immediately suggested. Now, the phosphoglyceric acid being a three-carbon compound (and the hexose with its six carbon atoms) required further examination to determine which of the three carbon atoms were radioactive and in what order they became so. The same information was, of course, required for the hexose as well.

The PGA (phosphoglyceric acid) is readily taken apart following the hydrolysis of the phosphate group, usually with acid, by oxidation, first with periodic acid under conditions which will produce the beta-carbon as formaldehyde and which can be separated as the dimedon compound. The residual two-carbon fragment may be further oxidized with the same reagent, or better with lead tetraacetate, to produce CO_2 from the carboxyl group and formic acid from the alpha-carbon atom. These are separately collected and counted.

We were thus able to show that in the very shortest times most of the radioactivity appears in the carboxyl group ofthe PGA and that radioactivity appears in the alpha- and beta-carbon atoms very nearly equally at later times.

A degradation of the hexose sugar showed that the earliest carbon atoms to be labeled were 3 and 4 (and these approximately equally, although not necessarily exactly so) followed by labeling in carbon atoms 1 and 2, and 5 and 6. The obvious relationship, then, between phosphoglyceric acid and the hexose was the one shown in Figure 8 in which the

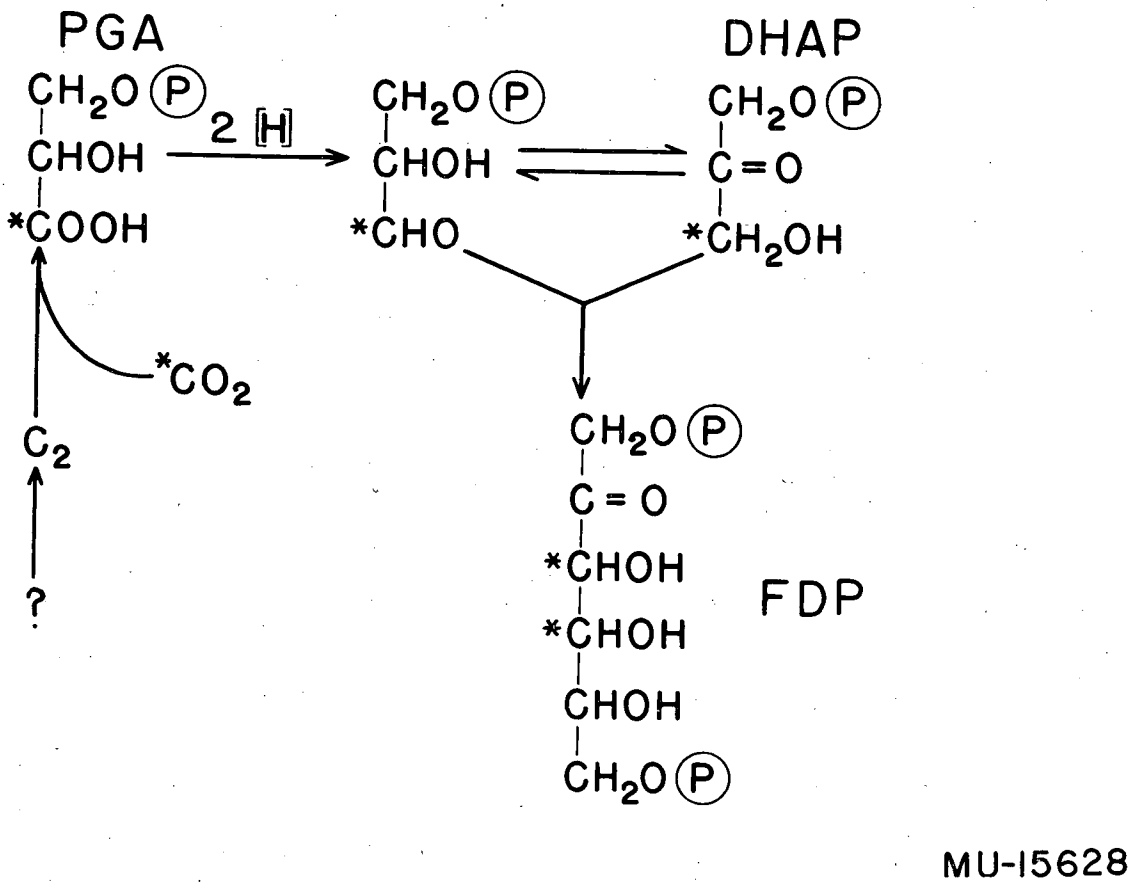

Fig. 8. Path of carbon from carbon dioxide to hexose during photosynthesis.

PGA, following reduction to phosphoglyceraldehyde and its conversion to the ketone, the two are condensed by an aldolase reaction to give fructose diphosphate. This places the labeling of the hexose in the center of the molecule.

It is interesting to note that rather early in the fixation sequence a compound appeared which moved extremely slowly in both solvents (that is, remained near the origin particularly in the acid solvent) and which upon extremely mild hydrolysis produced only labeled glucose. This ease of hydrolysis was even greater than that of glucose-1-phosphate, but the material was not glucose-1-phosphate. It was much later shown to be the nucleoside diphosphoglucose, uridine diphosphoglucose, and its part in the synthesis of sucrose itself deduced from the presence of traces of sucrose phosphate, later searched for and found. The relationship, then, between phosphoglyceric acid and sucrose is illustrated in Figure 9.

Origin of Phosphoglyceric Acid

We now are ready to return to the question of the origin of the PGA itself. Here we were led, by what appeared to be an obvious kind of arithmetic, to seek a compound made of two carbon atoms as a possible acceptor for the radioactive CO_2 to produce the carboxyl-labeled three-carbon compound, phosphoglyceric acid. This search was a vigorous one and extended over a number of years. (Again, a considerable number of students and laboratory visitors were involved.) While free glycolic acid was found under certain very special conditions, these did not

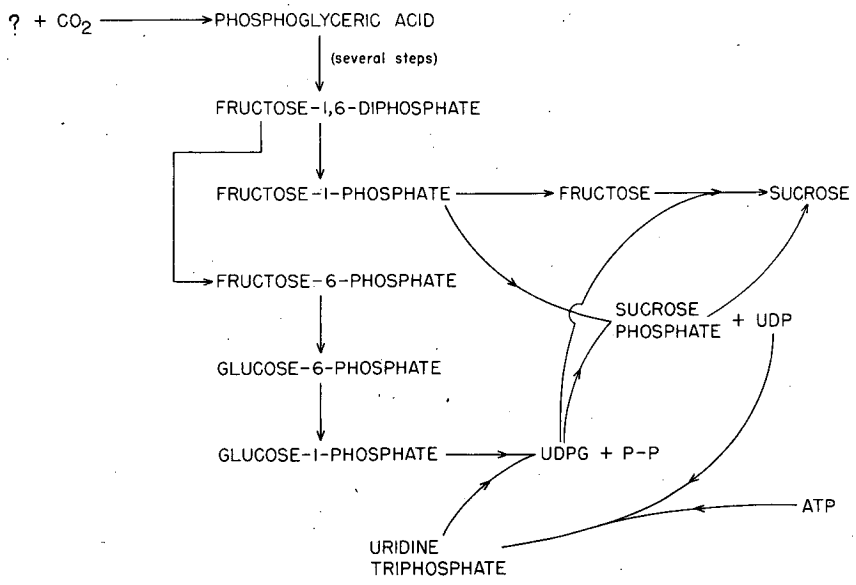

Fig. 9. Relationship between fructose phosphate and sucrose.

correspond to what would be required of the so-called carbon dioxide acceptor. A good many other compounds were identified in the course of this search; particularly among them were a five-carbon sugar, ribulose as its mono- and diphosphate, and a seven-carbon sugar, sedoheptulose as its mono- and diphosphate.

While the relationship of the trioses and hexoses to PGA in a time sequence seemed clear, the sequential relationship of the five-carbon and seven-carbon sugars was not readily determined. In fact, attempts to establish this relationship by ordinary kinetic appearance curves of these two sugars resulted in conclusions on the order of appearance of the pentose, hexose and heptose which varied from day to day, experiment to experiment, and person to person.

Radioactivity Determination of Pentose and Heptose

The distribution of radioactivity in the pentose and heptose was next determined and that distribution is indicated in Figure 10 by the number of asterisks on each atom. Thus the No. 3 carbon atom of the ribulose is the first to be labeled, followed by carbon atoms 1 and 2 and finally carbon atoms 4 and 5. In the sedoheptulose, the center three carbon atoms (Nos. 3, 4, 5) were the first to be labeled, followed by carbon atoms 1 and 2, and 6 and 7. Extremely short experiments, of the order of fractions of a second, did show a low value for carbon atom No. 4 in sedoheptulose.

The peculiar labeling in sedoheptulose and the absence of any single one carbon atom as dominating over the others, clearly indicates that it is not formed by a $C_6 + C_1$ addition, that is, not directly from

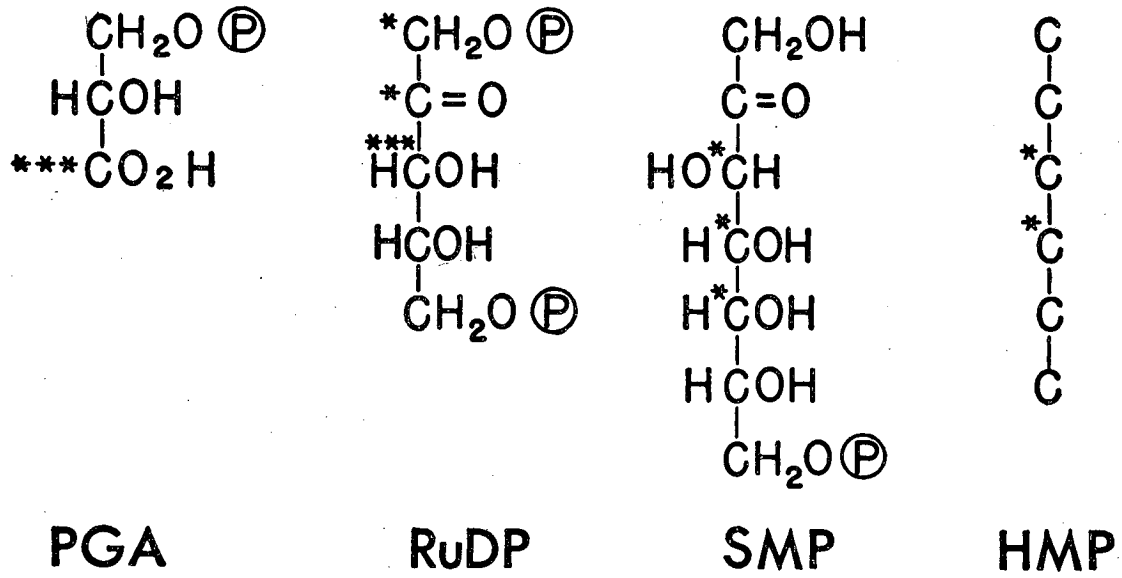

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Fig. 10. Distribution of radioactive carbon in certain sugars.

the hexose by the addition of a single carbon atom. The only other alternatives for the formation of the sedoheptulose are the combinations of $C_5 + C_2$ or $C_4 + C_3$. Again, we call on the apparent nearly equal distribution of the center three carbon atoms of sedoheptulose to indicate that there is no intact element of the five-carbon ribulose present in the sedoheptulose since there is no intact group of five carbon atoms which has the same labeling pattern as we see in the ribulose.

We are therefore forced to seek a $C_4 + C_3$ method for construction of sedoheptulose. The availability of the C_3 fragments is clear enough for the phosphoglyceric acid. The hexose seems, therefore, a possible source of the C_4 fragment which, when combined with a C_3 fragment directly related to PGA, will give rise to a C_7 sugar, sedoheptulose, labeled in the way shown. This can be done by taking a four-carbon sugar made of carbon atoms No. 3,4,5 and 6 (that is, the lower four carbon atoms) of fructose, in which the first two (No. 3 and 4), then, would have the label, and condensing it in an aldol type condensation with the phosphodihydroxyacetone as shown in Figure 11. If the pool sizes of the tetrose and triose are very small it should be possible to arrive very quickly at a heptose in which the center three carbon atoms are very nearly equally labeled, although one might expect some differences in the shortest times, as we have seen. The top carbon atoms of the hexose, Nos. 1 and 2, would be combined with another triose in the same reaction to produce number 3-labeled pentose.

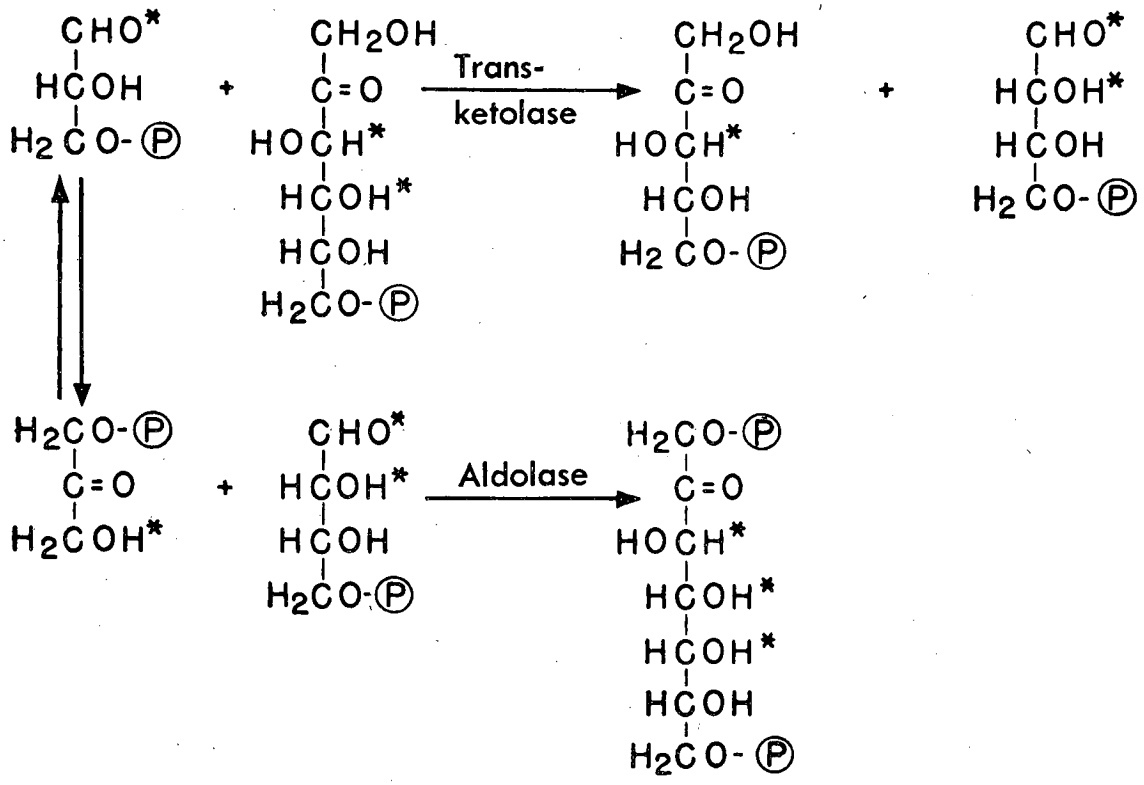

Fig. 11. Formation of a heptose from triose and hexose.

Identification of the Two-Carbon Acceptor

Accepting this as the source of the heptose, we are now left with the problem of the source of the pentose with its peculiar and unsymmetric labeling pattern. Again, we call on our simple arithmetic, and discover that the pentose can be made by losing a carbon from the hexose, or by building it up from smaller fragments, i.e., by adding a C_1 to a C_4 fragment, or by adding a C_2 to a C_3 fragment. Here, again, we can call upon the lack of relationship between the pentose labeling and any five-carbon sequence in the hexose to eliminate from further consideration the construction of the pentose by loss of a terminal carbon from the hexose. Furthermore, of course, this would be a step backwards in our construction program.

We are left, then, with the only remaining alternative for the construction of the pentose, namely, the combination of a C_3 with a C_2 fragment. Again, the source of C_3 fragments is clear enough, but the question of the source of the C_2 fragment to go with it requires some discussion.

At this point it should be remembered that we have already made a C_5 fragment labeled in the No. 3 carbon atom. The ribulose labeling scheme, as shown in Figure 10, indicates that the next label to appear was in carbon atoms No. 1 and 2. Thus it was not until the realization occurred to us that the ribulose which we were degrading and which we obtained from the ribulose diphosphate actually had its origin in two different reactions that it became possible for us to devise a scheme for its genesis.

By taking the two-carbon fragment off the top of the sedoheptulose we could make two five-carbon compounds which, taken together with the five-carbon compound already formed, would produce the labeling scheme finally observed in ribulose diphosphate. This is shown in Figure 12. The enzyme which performs this two-carbon transfer is transketolase and is the same one we have already used to generate the tetrose required for heptose synthesis.

We have thus devised ways of generating from phosphoglyceric acid all of the sugars which appear on our early chromatograms: The triose, the various pentoses, the various hexoses, and the heptose. The earlier failure to succeed in selecting a specific sequence amongst these compounds is now understandable since all of them, that is, pentose, hexose and heptose, appear simultaneously following triose. As yet we had not discovered the compound originally presumed to be the C_2 compound, to which the carbon dioxide may be added in order to produce carboxyl-labeled phosphoglyceric acid.

CARBON-FOURTEEN SATURATION EXPERIMENTS: CHANGES IN STEADY STATE AND TRANSIENTS

In order to do this we devised a different type of experiment. We recognized quite early that most of the compounds which we have so far mentioned, aside from sucrose, saturate with radioactivity very quickly, and yet the amount of these materials present in the plant at any one time is small and does not change. This suggested to us a method for

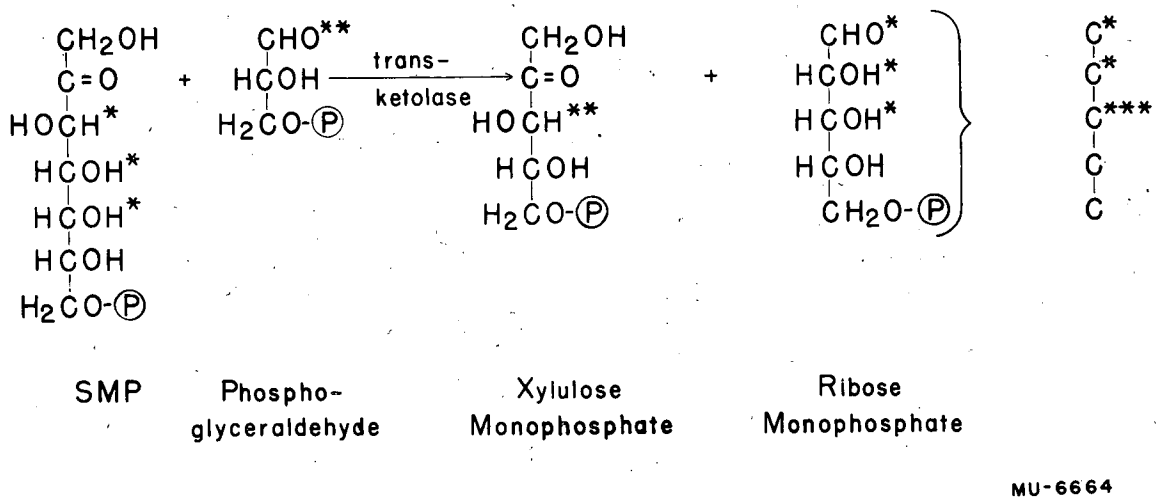

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Fig. 12. Proposed scheme for labeling of pentose.

discovering not only how the light might operate on the PGA, but also how the PGA might arise. We could use the radioactivity saturation levels for these compounds through which carbon was flowing to measure the total amount of active pool size of these compounds in the plant. We could then change one or another external variable and follow the resultant changes in the pool size of these compounds through which we knew the carbon to be flowing.

The first, most obvious and easiest external variable to be changed was the light itself. Figure 13 shows the first set of data taken by Peter Massini which not only demonstrates the early saturation but also the effect of the light on the pool transients.[9] Here you can see that the PGA and the sugar phosphate are indeed very quickly saturated but that the sucrose is not. It is apparent that upon turning off the light there is an immediate and sudden rise in the level of PGA accompanied by a corresponding fall in the level of the diphosphate area which was primarily ribulose diphosphate.

Here we had our first definitive clue as to the origin of the phosphoglyceric acid. It would appear that it came as a result of a dark reaction between the ribulose diphosphate and carbon dioxide. Figures 14 and 15, based upon the work of Dr. James A. Bassham,[10,11] show what can be done with this technique when we know what we are doing. Here you can see the large number of points obtainable and the very much smoother curves with their clear saturation points well defined. On Figure 15 we see again very sharply shown the transient rise in the PGA pool size and the disappearance of ribulose diphosphate when the light is turned off, clearly defining the relationship between these two compounds.

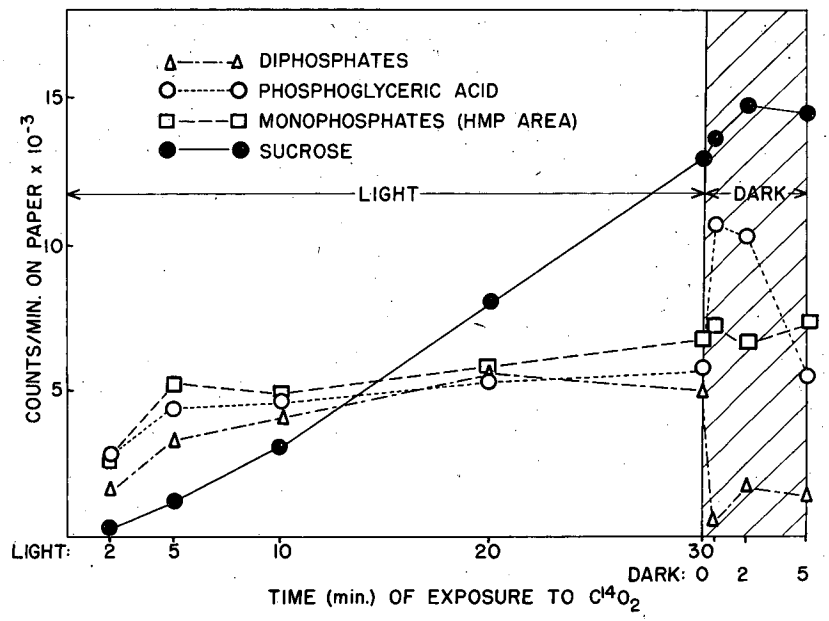


Fig. 13. Effect of light and dark on activities of phosphates and sucrose.

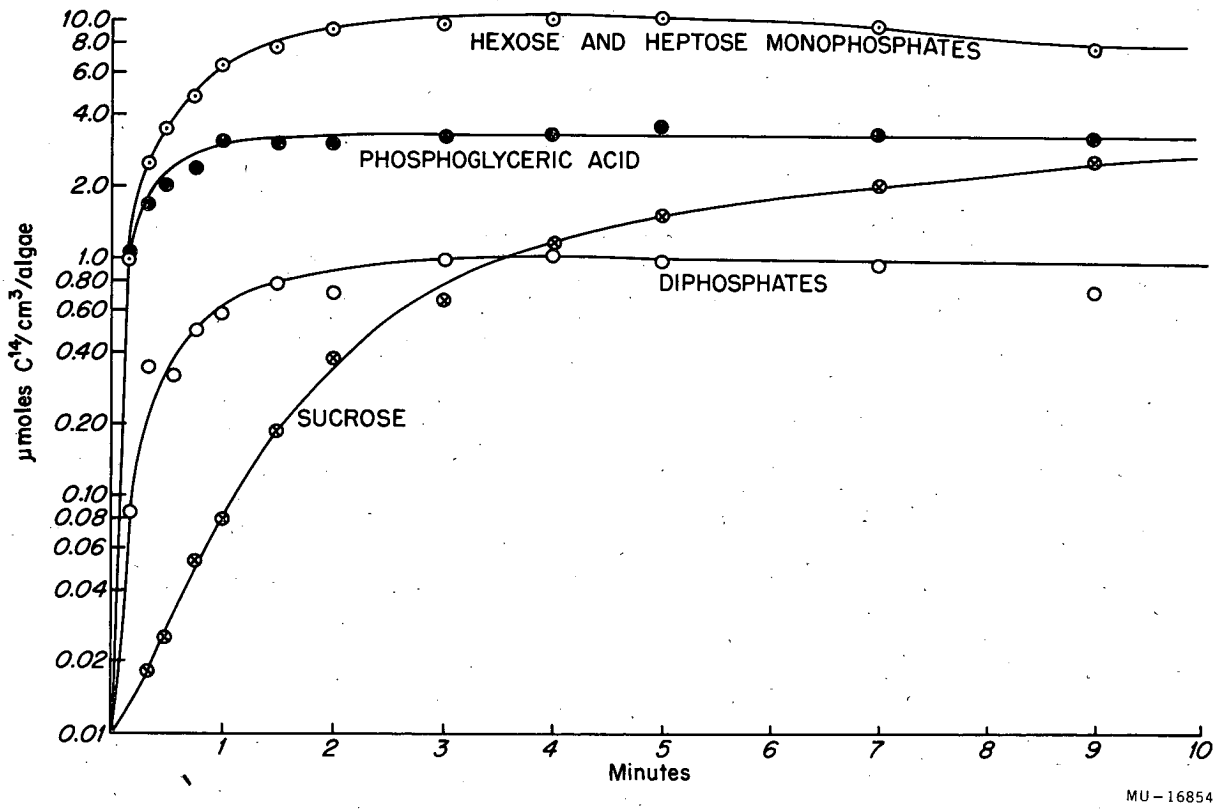


Fig. 14. Appearance of carbon-14 in phosphoglyceric acid and sugar phosphates vs. time of photosynthesis with $C^{14}O_2$.

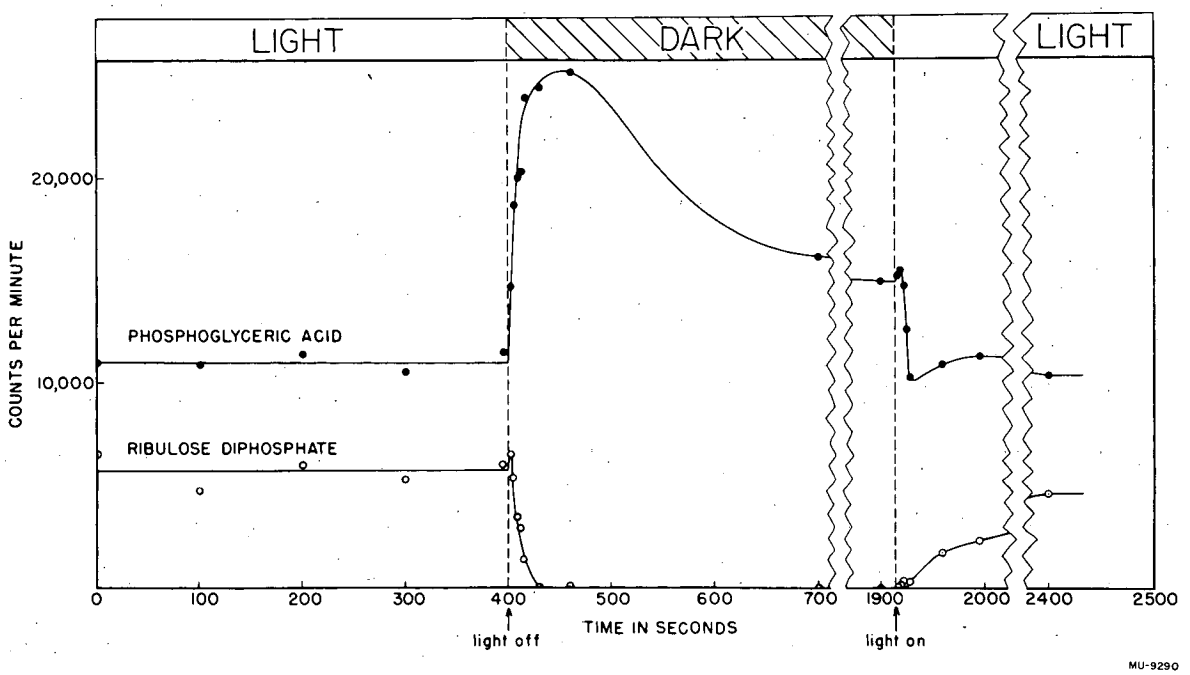
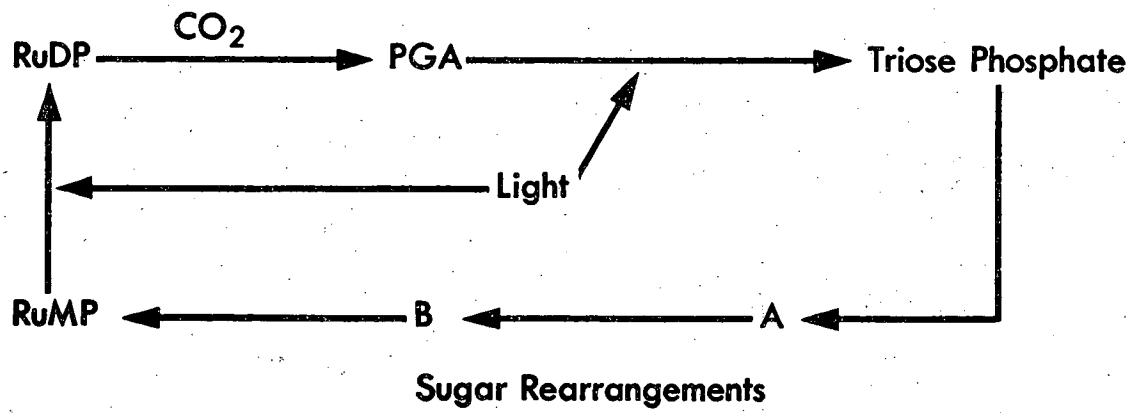


Fig. 15. Light-dark changes in concentrations of phosphoglyceric acid and ribulose diphosphate.

We can now formulate the cyclic system driven by high energy compounds produced in the light, acting upon phosphoglyceric acid which, in turn, is made as a result of a reaction between ribulose diphosphate and carbon dioxide as shown in Figure 16. The triose phosphate then undergoes a series of condensations and sugar rearrangements, represented by the letters A and B and including the pentose and heptose rearrangement which we have just discussed, leading back again to a ribulose monophosphate which is then phosphorylated to ribulose diphosphate, thus completing the cycle.

By turning off the light we have, in effect, blocked the easy conversion of phosphoglyceric acid to triose without any reduction in the rate of formation of PGA from ribulose diphosphate. This should result in an immediate rise in the amount of PGA and an immediate fall in the amount of ribulose since it no longer can be produced in the dark.

Such a scheme allows us to predict another transient, namely, the one which would result if, in the presence of light, that is, in the presence of the high energy compounds required to drive the cycle, we suddenly diminished the availability of carbon dioxide, putting a block between ribulose and the phosphoglyceric acid. The prediction, which resulted here in the first transients, would be the accumulation of ribulose and the disappearance of PGA. These two transients would then make their way back through the cycle, the fall in PGA in a clockwise direction, the rise in ribulose in a counterclockwise direction, thus making for oscillating transients. The result of such an experiment performed by Dr. Alexander T. Wilson is shown in Figure 17.[12] Here it is quite clear that the sudden reduction in the CO_2 results in a



MU-25046

Fig. 16. Formation of five-carbon sugars from ribulose diphosphate.

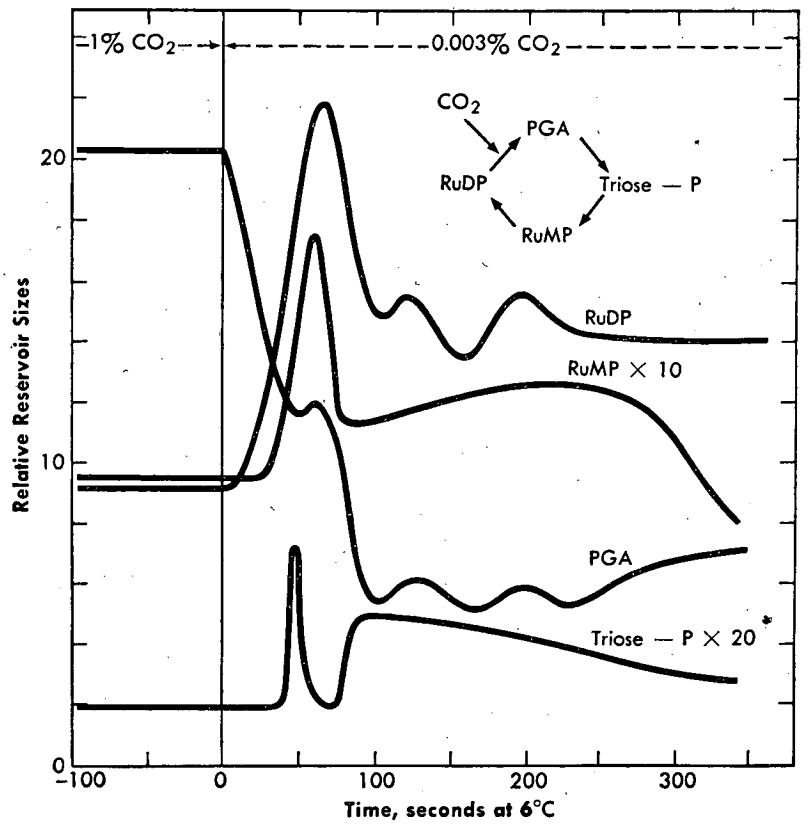


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Fig. 17. Transients in the regenerative cycle.

drop in the size of the PGA pool associated with a rise in the ribulose. It is interesting to note that the triose is the last to rise and the first to fall, as predicted from its position in the cycle.

THE PHOTOSYNTHETIC CARBON CYCLE

We can now arrange all of the individual steps that we have separately discussed in a sequence to produce the photosynthetic carbon cycle as shown in Figure 18. One compound is shown therein as an intermediate lying between ribulose diphosphate and phosphoglyceric acid. This is a branched chain alpha-hydroxy-beta-keto sugar acid. As yet this compound has not been isolated as a separate entity. The enzyme system catalyzing the reaction of ribulose diphosphate to PGA has been isolated and purified, but as yet we have been unable to break its reaction down into two steps.[13] If this intermediate is present, it is present in extremely small amounts as the free compound, if any at all, and its hydrolysis to produce two molecules of phosphoglyceric acid takes place extremely rapidly in the isolated enzyme system and even in the living plant itself.

The mechanism for the carboxydismutase (the enzyme responsible for the formation of PGA from ribulose diphosphate) reaction is formulated in Figure 19. Here the intermediate is split by hydrolysis to two molecules of phosphoglyceric acid. However, in our earlier work the possibility of a reductive fission at the same point to give one molecule of triose and one molecule of phosphoglyceric acid was considered. It was rejected in favor of the hydrolytic splitting because of our failure

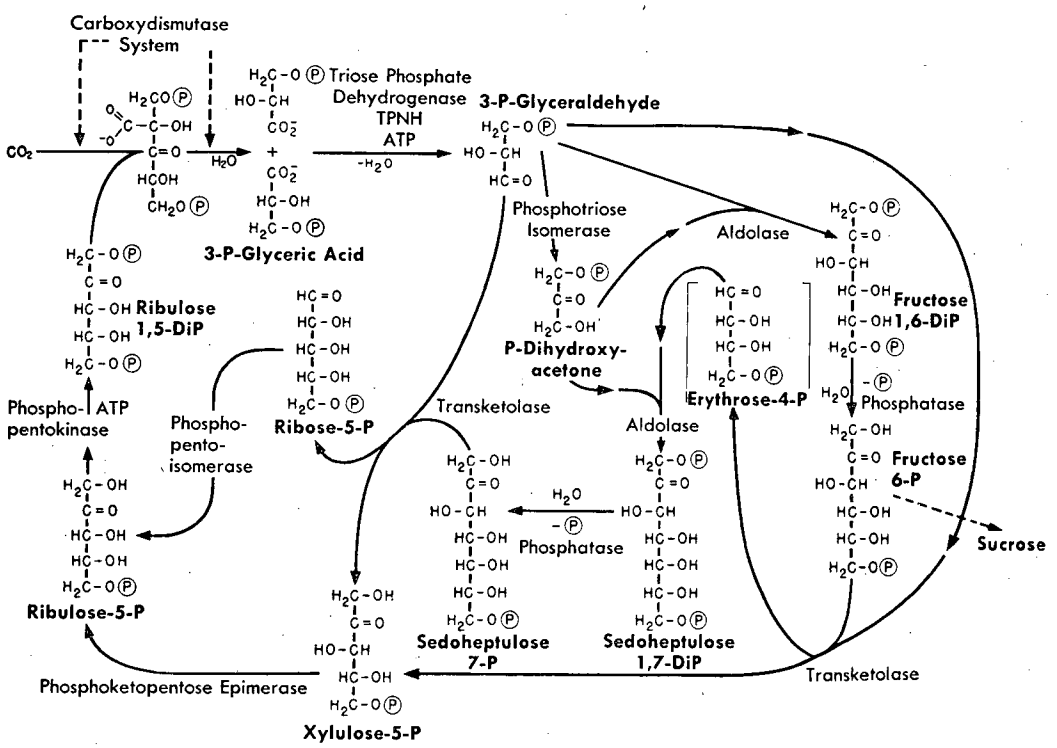


Fig. 18. The photosynthetic carbon cycle.

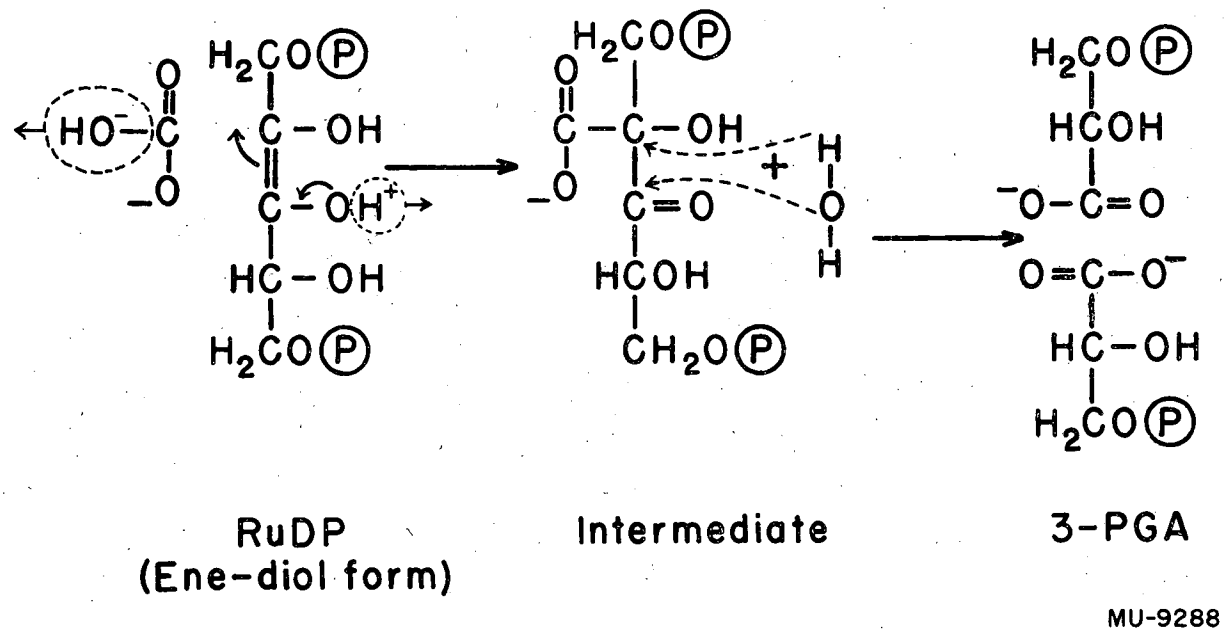


Fig. 19. Mechanism of the carboxydismutase reaction.

to find any evidence of the intermediate. However, more recent, very careful kinetic analysis of the carbon flow rates by Dr. James A. Bassham[11,14] has suggested that the reductive split may indeed participate in the reaction to some extent while the light is actually on. This path is indicated in Figure 20 by the dotted line from the presumed C_6 keto acid.

QUANTUM CONVERSION IN PHOTOSYNTHESIS

As you can see from the various levels of the schematic drawing of the photosynthetic carbon cycle (Figure 18 and 20), the energy required to drive the synthetic sequence from carbon dioxide to carbohydrate and the many other reduced carbon materials which can be derived from the cycle is delivered to it in the form of a number of compounds of relatively high chemical potential in the aerobic aqueous system in which the plant operates. The particular ones with which we can actually drive the photosynthetic carbon cycle in the absence of light but in the presence of all the initial enzymes and substrates[15] are triphosphopyridine nucleotide (TPNH) and adenosine triphosphate (ATP) whose structures are shown in Figure 21. The primary quantum conversion of the light absorbed by chlorophyll will result in materials which can ultimately give rise to such substances as these.

That light energy might be readily converted into chemical potential as ATP independent of CO_2 reduction and its re-oxidation was clearly indicated in whole cell experiments of Goodman and Bradley[16] and first demonstrated as independent of oxygen by Frenkel[17] with bacterial chroma-

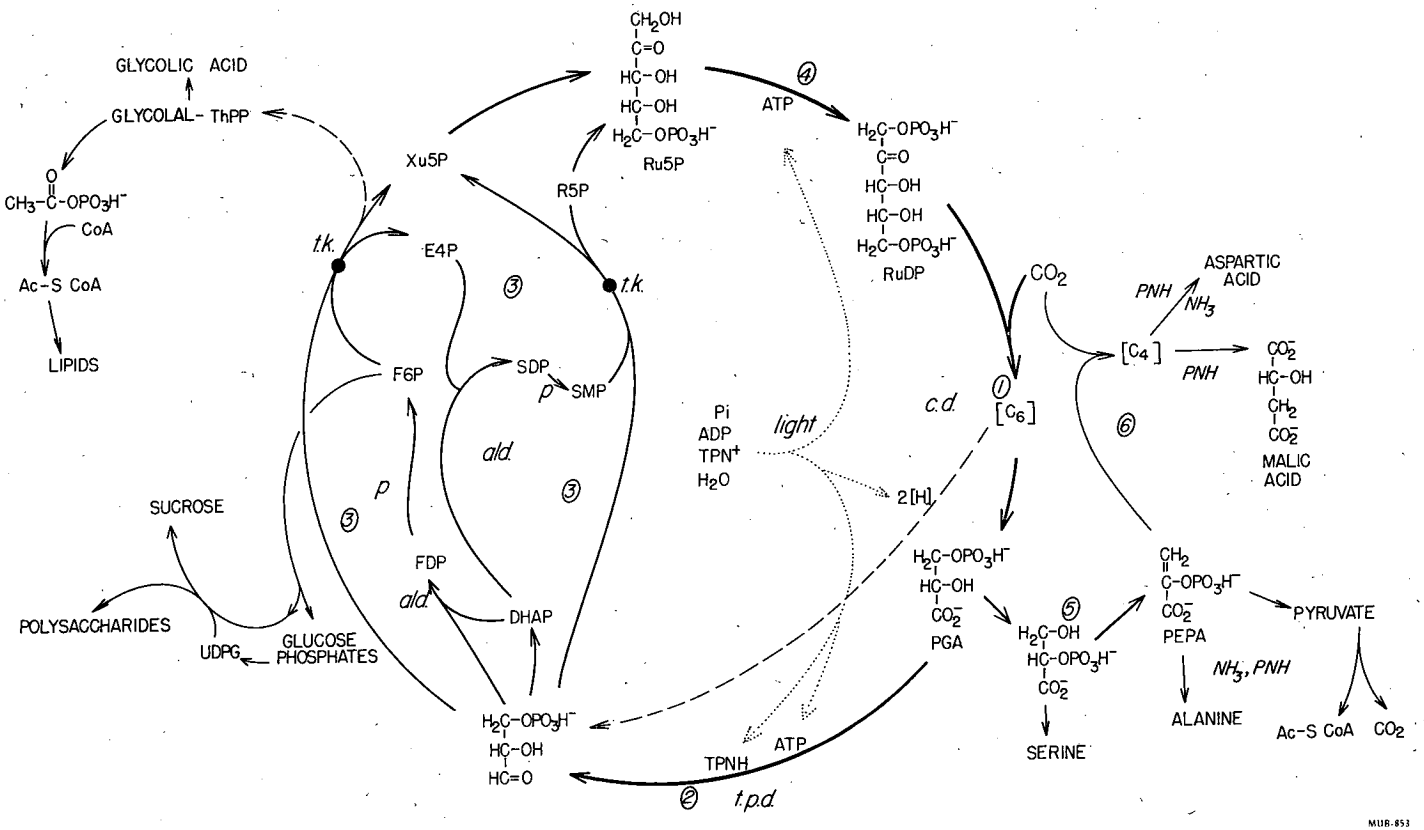
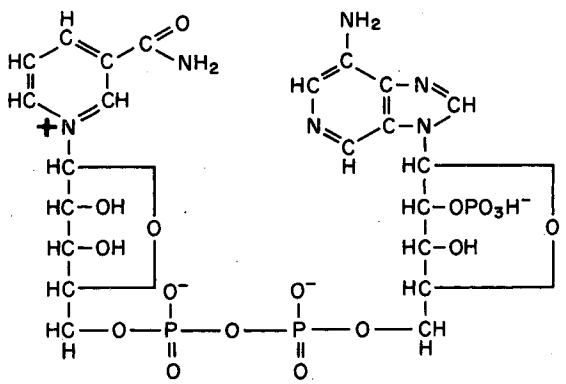
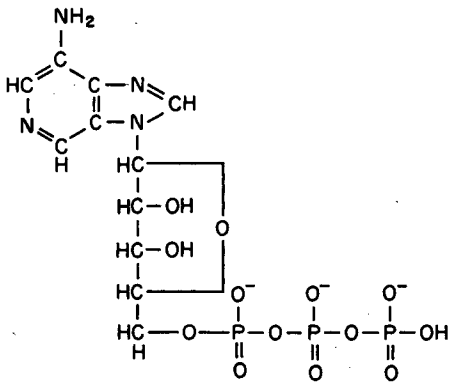


Fig. 20. The photosynthetic carbon cycle and its relation to quantum conversion and to succeeding biosynthesis.

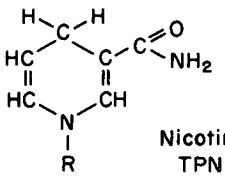


Triphosphopyridine nucleotide (oxidized form) (TPN^+)



Adenosine triphosphate (ATP)

In Adenosine diphosphate (ADP), terminal phosphate is replaced by –OH.



Nicotinamide portion of TPNH (reduced TPN^+)

MU-17251

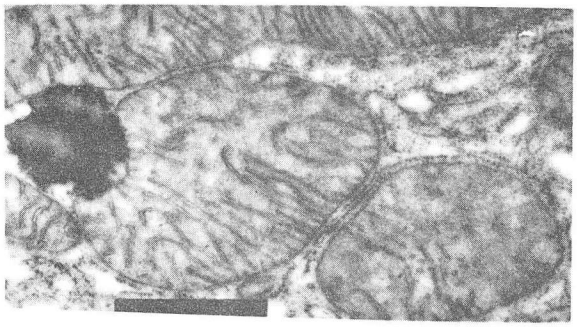
Fig. 21. Structure of TPNH and ATP, and two compounds which are used to drive the photosynthetic carbon cycle in the absence of light and the presence of enzymes.

tophores. A corresponding anaerobic demonstration of ATP production by green plant chloroplasts was made by Arnon.[18] The ability of chloroplasts to photoreduce pyridine nucleotide was demonstrated by Ochoa and Visniac.[19] But the precise nature of the primary quantum conversion act whose products ultimately give rise in a dark reaction to ATP and TPNH is still a matter of speculation.[20,21]

The apparatus which performs the quantum conversion act in the plant, together with all of the carbon reduction enzymes we now know, can be isolated from the intact chloroplasts in the higher plants. The carbon reduction enzymes are very easily washed off the chloroplasts by water, leaving behind only the chlorophyllous quantum conversion equipment. This has a highly ordered structure in which the lamellae are alternating electron-dense and electron-thin materials, as has been shown in many electron micrographs, a few examples of which are shown in Figure 22 for various organisms

The next level of structure within the lamellae is only now beginning to be visible to us, and an example is shown for a spinach chloroplast in Figure 23.[22] Here we can see the lamellae on its flat side showing a granular structure, made up of fairly uniform oblate spheroids which we have called Quantosomes; this work was performed by Drs. Roderic B. Park and Ning G. Pon. Within these Quantosomes the chlorophyll itself is highly organized, as we have been able to demonstrate, particularly by electric dichroism experiments performed by Dr. Kenneth H. Sauer.

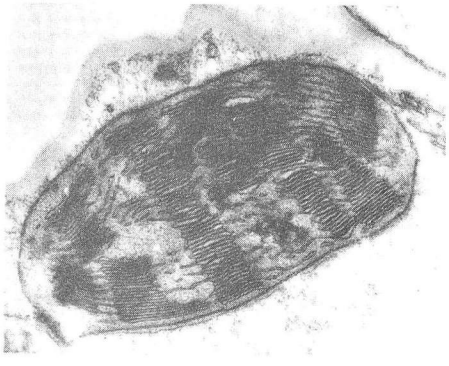
We are now in the midst of trying to determine precisely what happens after the chlorophyll has absorbed the quantum and has become



MITOCHONDRIA FROM GUINEA PIG PANCREAS

CHLOROPLAST WITH SEVERAL MITOCHONDRIA.
CHLAMYDOMONAS (SAGER)

ENTIRE CELL OF *ANABENA* SHOWING
LAMELAE (VATTER)

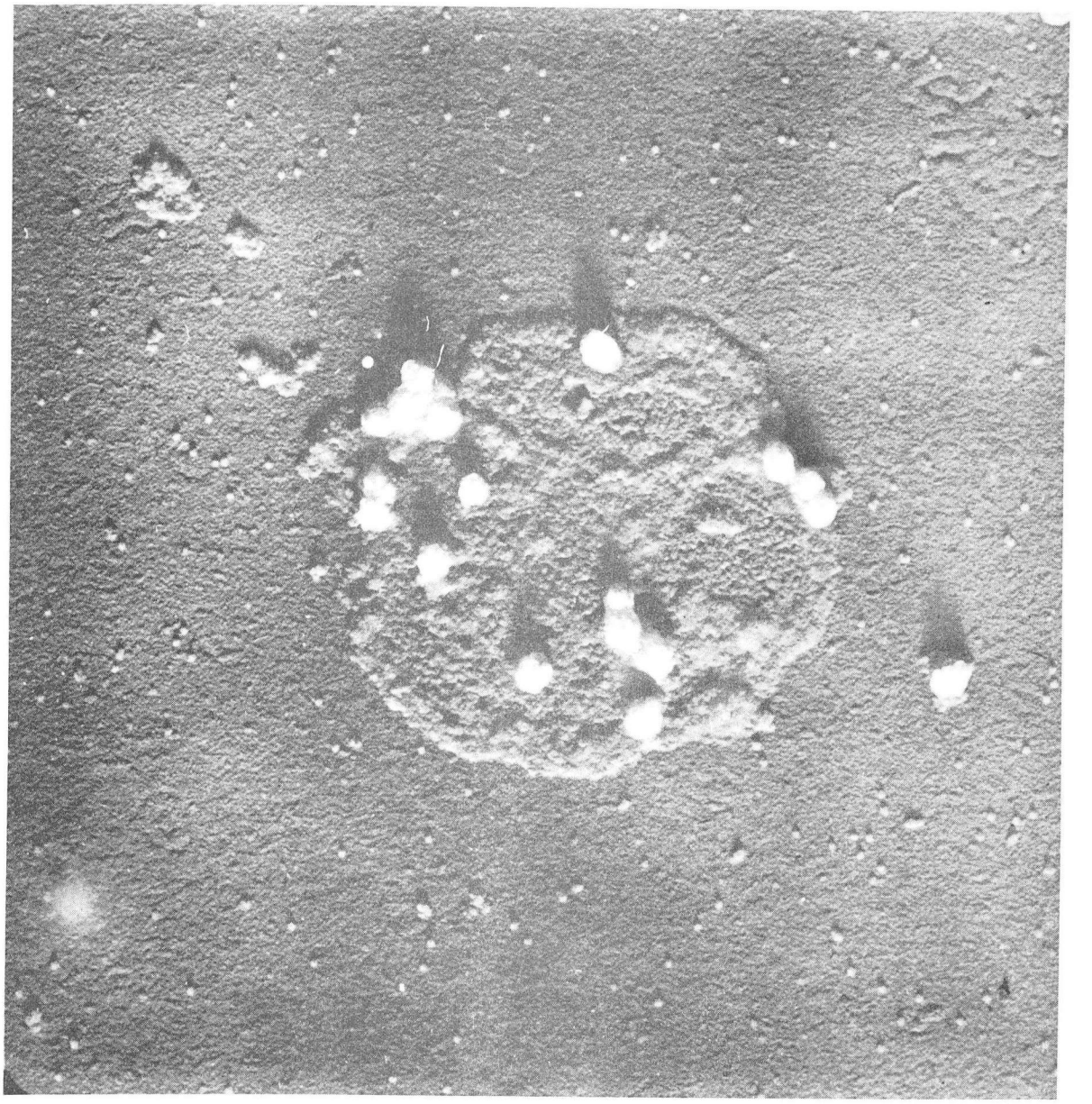


Tobacco chloroplasts

Maintained 24-36 hours in the dark before fixing with permanganate. Weier.

ZN-2982

Fig. 22. Electron micrographs of chloroplasts from a unicellular green alga, from a blue-green alga, from tobacco, and mitochondria from guinea pig pancreas.



ZN-2981

Fig. 23. Quantosomes from spinach chloroplasts; 880 Å diameter PSL (polystyrene latex) marker spheres.

an excited chlorophyll molecule, a problem that involves the physicist and physical chemist as well as the organic and biochemists. The determination of the next stage in the energy conversion process is one of our immediate concerns. Either it is an electron transfer process, and thus comes close in its further stages to the electron transfer processes which are being explored in mitochondria,[23] or it is some independent non-redox method of energy conversion.[20] This remains for the future to decide.

Chemical biodynamics, involving as it does the fusion of many scientific disciplines, will play a role in this problem, as it has in the elucidation of the carbon cycle. It can be expected to take an increasingly important place in the understanding of the dynamics of living organisms on a molecular level.

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