Title

The Photosynthetic Cycle

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THE PHOTOSYNTHETIC CYCLE

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Photosynthesis is usually defined as the biochemical reaction

$$CO_2 + H_2O \xrightarrow{nh\nu} (CH_2O)_x + O_2 .$$

This represents the conversion of carbon dioxide and water to carbohydrate and oxygen by green plants in the light. The reaction is separated both chronologically and chemically into two parts: the photolysis of water,

$$H_2O \xrightarrow{nh\nu} 2[H] + 1/2\ O_2 ,$$

and the reduction of carbon dioxide,

$$CO_2 + 4[H] \longrightarrow CH_2O + H_2O .$$

Each of these two reactions represents a complex series of reactions with many steps. The term [H] is used to denote reducing agents generated in the photochemical decomposition of water. These reducing agents probably undergo several transformations before they are used in the reduction of carbon dioxide.

The reactions involved in the reduction of carbon dioxide have been studied and the results of these studies have been reported in a series of papers on "The Path of Carbon in Photosynthesis."[1),2),3)]

The radioactive carbon isotope, C^{14}, was used throughout this investigation. To a lesser extent, radioactive phosphorus, P^{32}, was also employed.

As a result of this work, it is now possible to write the complete path of carbon reduction in photosynthesis, with all intermediates and enzymatic reactions, from carbon dioxide to sucrose. The study of carbon reduction and its relation to respiratory transformations of carbon compounds has provided evidence regarding the nature of the reactions involved in the decomposition of water and the formation of the primary reducing agents and other energy-rich compounds required for carbon reduction.

FIRST PRODUCTS

The methods used in studying the path of carbon in photosynthesis are here described briefly. In nearly all cases the initial condition is an actively photosynthesizing plant in which photosynthesis has been maintained long enough to establish a "steady state." In this steady-state condition the concentrations of various intermediate compounds in the pathway from carbon dioxide to sucrose are constant. The plants commonly used in these experiments are the unicellular green algae, Chlorella or Scenedesmus, but leaves of higher plants are sometimes used.

In the first type of experiment to be discussed, $C^{14}O_2$ is added to the unlabeled CO_2 that the plant has been using. After a measured short period of photosynthesis with $C^{14}O_2$, the plant is killed by sudden treatment with boiling ethanol. All enzymatic processes are thereby quickly halted. Extracts of the plant material are made, concentrated, and then analyzed by two-dimensional paper chromatography and radioautography. The techniques of two-dimensional chromatography and radioautography of plant extracts labeled with C^{14} have been described earlier,[1] as well as the identification of the numerous labeled compounds.[1),4),5),6),7] The radioautographs obtained from experiments of 10-seconds and 60-seconds photosynthesis with $C^{14}O_2$ are shown in Figs. 1 and 2. The 60-second experiment illustrates the importance of various sugar phosphates and acid phosphates in carbon reduction. The 10-second experiment shows the predominance of phosphoglyceric acid at short times. If the percentage of C^{14} in phosphoglyceric acid (PGA) of the total C^{14} incorporated during photosynthesis for various short periods of time is extrapolated to zero time, it is found that at zero time all the C^{14} should be in phosphoglyceric acid. This compound is therefore identified as the first compound into which carbon dioxide is incorporated in photosynthesis.

Fig. 3 shows the distribution of the labeled carbon in the three carbon atoms of the glyceric acid obtained from the phosphoglyceric acid in a 15-second experiment. Half of the C^{14} is in the carboxyl group and the other half is divided equally between the other two carbon atoms. From the same experiment some hexose (fructose and glucose) was obtained and degraded. The distribution of carbon in the two 3-carbon halves of the hexose was found to be very much the same as it is in the three carbons of glyceric acid. This result immediately suggests that the six-carbon piece is made from the two three's by joining the two carboxyl carbon atoms. This is simply a reversal of the well-known aldolase split of fructose diphosphate in the glycolytic sequence, a part of which is shown in Fig. 4. Here the phosphoglyceric acid is reduced with the hydrogen from the photochemical reaction to phosphoglyceraldehyde, which is then isomerized to form dihydroxyacetone phosphate (DHAP). Condensation of phosphoglyceraldehyde with DHAP then results in formation of the hexose, fructose-1,6-diphosphate. Thus, the two carbon atoms which were originally carboxyl-carbon atoms finally fall in the middle of the hexose chain. It is quite clear that there must be some compound that accepts the carbon dioxide to form the glyceric acid. Furthermore, that compound must be regenerated from the PGA (phosphoglyceric acid), triose phosphates, and hexose phosphates, or some other compound formed from them. It is thus evident that there is a cyclic process involved in the reduction of carbon dioxide.

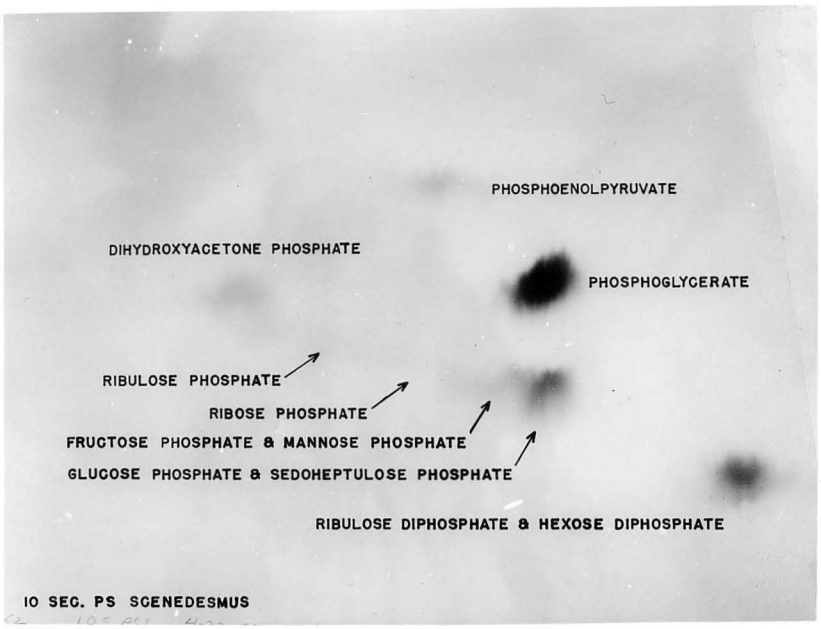


Fig. 1 - Chromatogram of extract from algae, indicating uptake of radiocarbon during photosynthesis (10 seconds).

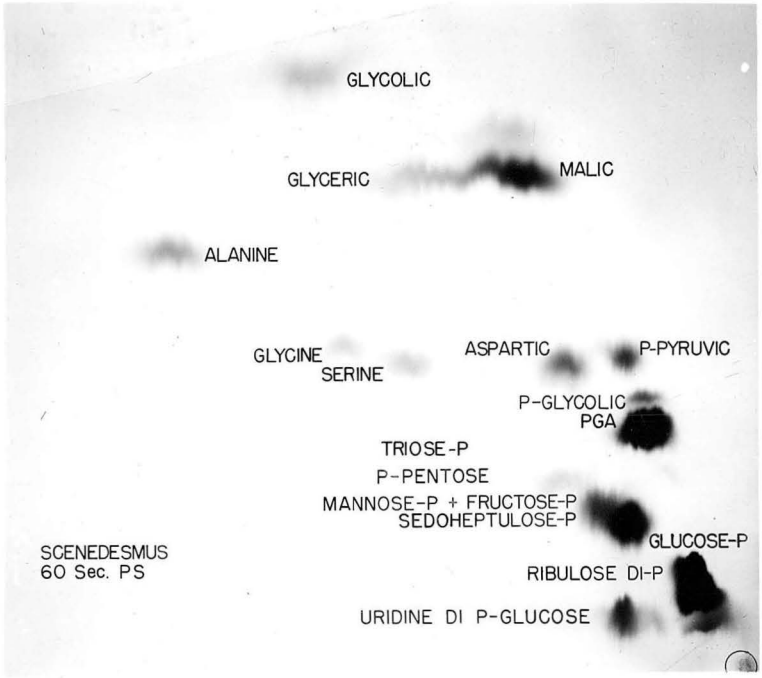


Fig. 2 - Chromatogram of extract from algae indicating uptake of radiocarbon during photosynthesis (60 seconds).

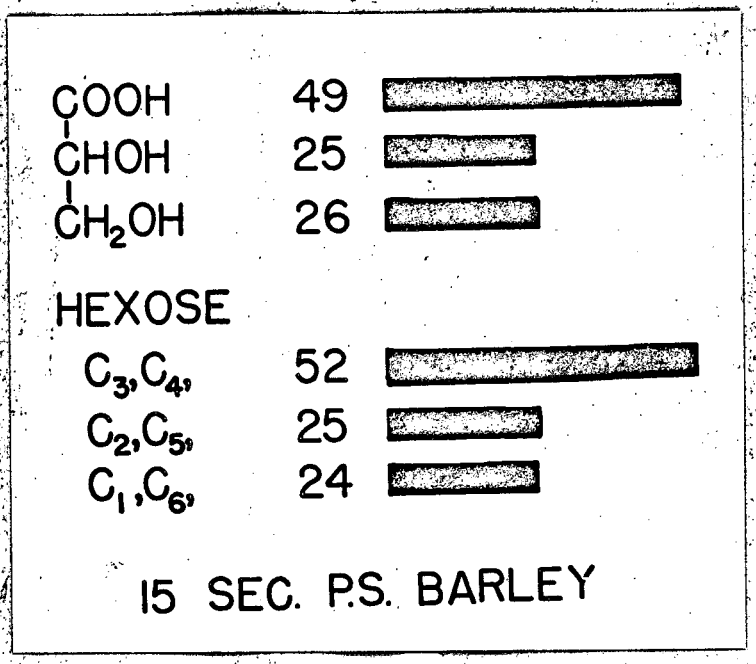


Fig. 3 - Distribution of labeled carbon in photosynthesis experiments

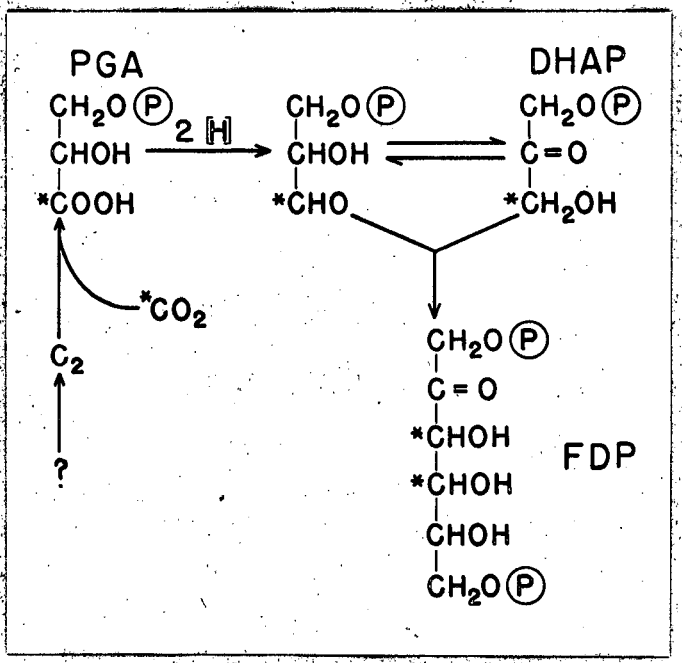


Fig. 4 - Path of carbon from CO_2 to hexose during photosynthesis

Before considering the nature of this cyclic process it is of interest to mention the steps leading from fructose diphosphate to the final product of photosynthesis, sucrose. These steps were identified after the intermediate compounds were isolated by paper chromatography and radioautography. Fig. 5 shows the relationship that was found. Here are shown the phosphoglyceric acid, fructose diphosphate, and the various transformations that lead ultimately to glucose-1-phosphate. This compound reacts with uridine triphosphate, to make uridine diphosphoglucose. Uridine diphosphoglucose (UDPG) is found on the paper, with the glucose moiety labeled after very short $C^{14}O_2$ exposures. UDPG can then react in one of two ways: either with fructose-1-phosphate to form sucrose phosphate which then is phosphatased to sucrose, or directly with free fructose to form sucrose in one operation. However, since one seldom finds any free labeled fructose, the first of these alternatives appears to be the major pathway for green leaves. An enzyme performing the reaction

$$\text{UDPG} + \text{fructose phosphate} \longrightarrow \text{sucrose phosphate}$$

has recently been prepared in a partially purified state by Leloir in Argentina. Fig. 6 shows the structural formula for the UDPG and its reaction with fructose-1-phosphate. This reaction gives uridine diphosphate and sucrose phosphate with the phosphate on the No. 1 carbon atom of the fructose moiety. The phosphate is then removed to give sucrose. This appears to be the common route to sucrose and is therefore one of the major synthetic reactions in agriculture, since sucrose provides the substrate for a wide variety of other transformations.

C_5 and C_7 Sugars

We now return to the problem of cyclic regeneration of the carbon dioxide acceptor. The roles of PGA, triose phosphates, hexose phosphates, UDPG, and sucrose have already been identified. Of the compounds labeled by short periods of photosynthesis, there were left only the seven- and five-carbon sugar phosphates. These were sedoheptulose-7-phosphate (SMP), ribose-4-phosphate (RMP), ribulose-5-phosphate (RuMP) and ribulose diphosphate (RuDP).

An attempt was made to determine the order of occurrence of these compounds by the same technique as was used to identify PGA as the first product of CO_2 fixation.

Since the reactions of carbon reduction are so rapid, a flow system was designed to obtain sufficiently short periods of exposure to $C^{14}O_2$ to permit observation of the relative rates of labeling of the various sugar phosphates.[3] The system used is shown in Fig. 7. A suspension of algae was forced by means of a pump from a transparent tank through a length of transparent tubing into boiling methanol. An aqueous solution of $C^{14}O_2$ was injected at a constant rate into the tubing. The time of exposure of the algae to $C^{14}O_2$ was determined by the rate of flow of algae through the tubing and the length of tubing between the point of injection and the killing with methanol. In this way exposure times ranging from 1 to 20 seconds were obtained. When the radioactivity found in each of the sugar phosphates was extrapolated to zero time of exposure, however, no choice could be made between the pentose, hexose, and heptose phosphates. It appeared that all were formed at the same time. It was necessary, therefore, to turn to degradation studies of these various sugar phosphates labeled in the very short exposures.

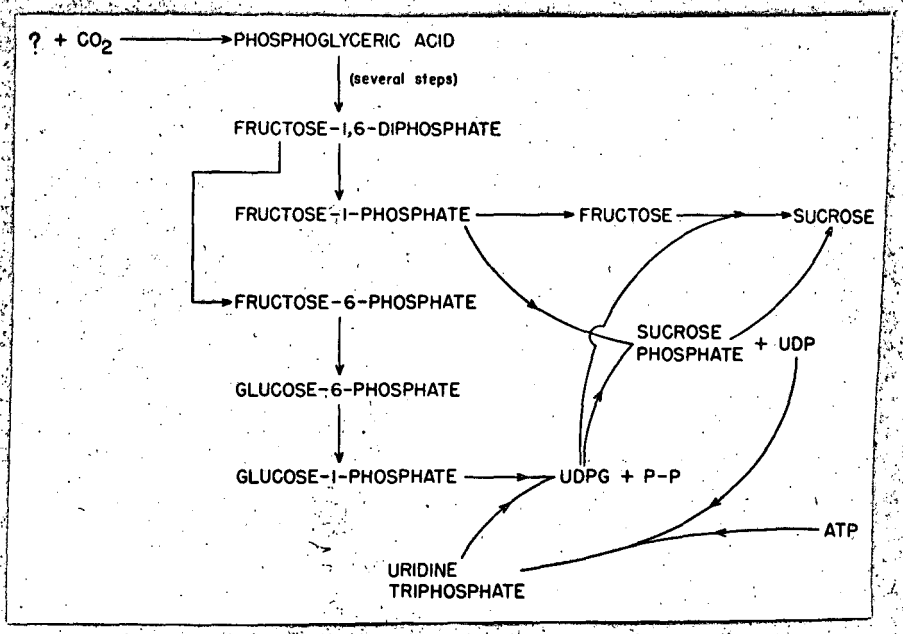


Fig. 5 - Proposed mechanism for formation of sucrose with uridine diphosphoglucose

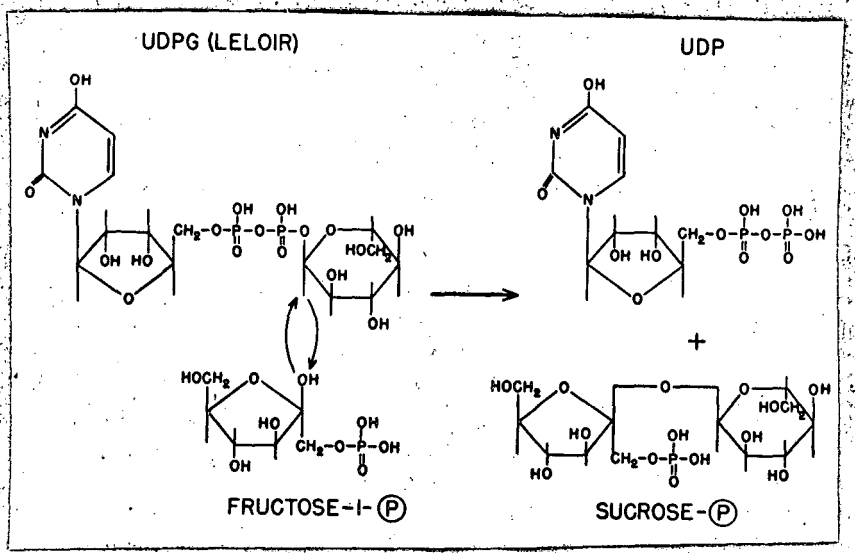


Fig. 6 - Uridine diphosphoglucose reaction with fructose phosphate

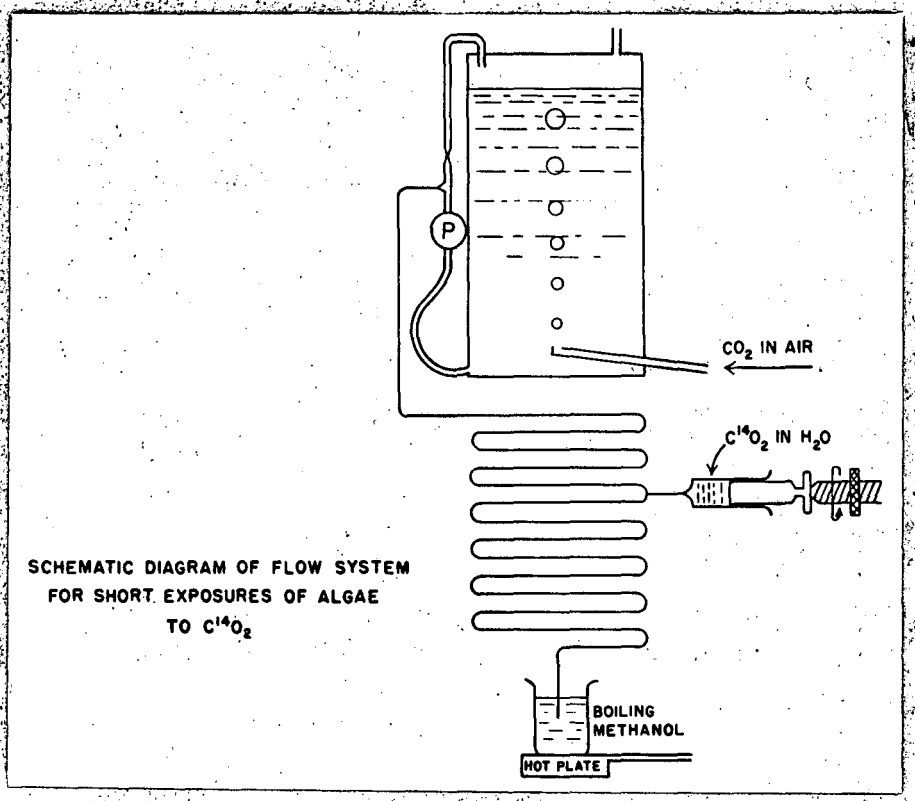


Fig. 7 - Schematic diagram of flow system for short exposures of algae to $C^{14}O_2$

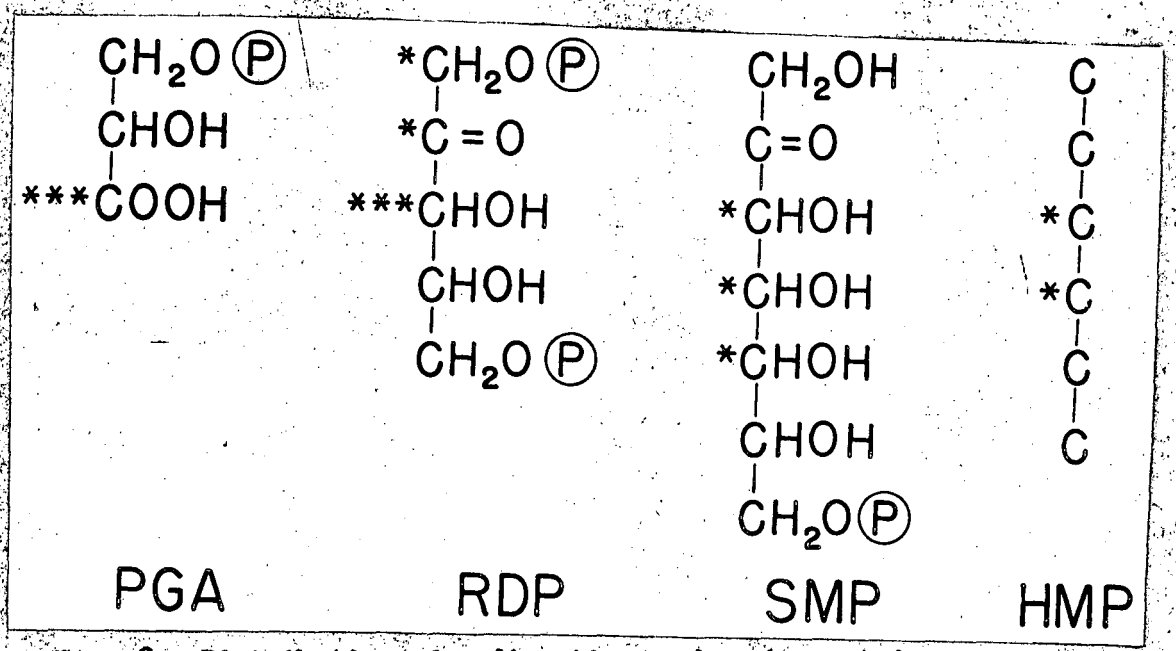


Fig. 8 - Distribution of radioactive carbon in certain sugars

A detailed analysis of the distribution of radioactivity among the carbons of these sugars is shown in Fig. 8. Here, besides PGA, are the five-carbon sugar, ribulose diphosphate (RuDP); the seven-carbon sugar, sedoheptulose phosphate (SMP); and the skeleton of a six-carbon sugar, corresponding either to glucose or fructose (these are the major six-carbon sugars that we find). The stars give some indication of the order of appearance of radioactive carbon in these compounds, and it was from an analysis of these data that it became possible to deduce relationships between the various compounds.

In much the same way as we deduced the relation between the three-carbon PGA and the six-carbon sugars we were able to deduce the relationships between the five-, seven-, six- and three-carbon compounds that are shown here. It is quite clear at a glance that there is no simple structural relationship between the five- and the seven-carbon compounds and the other sugars. At least, there is nothing as simple as the relationship between the three-carbon PGA and the six-carbon hexose. There is no sequence of carbon atoms in the C_5 or C_7 sugars that could be considered as simply the intact C_3 or the intact C_6, respectively. Until we realized that the C_5 might have more than one origin we were not able to deduce a possible route for its formation. This route is shown in Figure 9. By taking two carbons off the top of the C_7 and adding them onto a three-carbon piece labeled as is phosphoglyceraldehyde, we would get two five-carbon pieces - one ribulose and one ribose - with their labeling distributed as shown. The average of their labeling would be the actual one found. This evidence, therefore, indicates that the origin of the ribose and ribulose phosphates is in a transketolase reaction of the sedoheptulose phosphate with the triose phosphate to give the two pentose phosphates. These can be interconverted by suitable isomerization. Thus, the pentoses are formed from heptose and triose.

As was shown earlier, the hexose is formed from two trioses. The question then remains: where does the heptose come from? And here, again, a similar detailed analysis was made of the carbon distribution within the heptose molecule as a function of time. This analysis led to the realization that the heptose must have been made by the combination of a four-carbon with a three-carbon piece. The question arose then: where do the properly labeled four-carbon and three-carbon pieces come from? The four-carbon piece could only come by splitting the C_6 (hexose) into a C_4 and a C_2.

This is accomplished by the transketolase enzyme which removes the two "top" atoms from the fructose molecule and adds them to a molecule of glyceraldehyde-3-phosphate to produce a molecule of ribulose-5-phosphate (RuMP). The four-carbon piece that remains (erythrose-4-phosphate) has the distribution of radiocarbon that is required by the observed labeling in the four "bottom" carbon atoms of sedoheptulose.

The three-carbon piece required for the three "top" carbons of sedoheptulose might be dihydroxyacetone phosphate. In this case the condensing enzyme would be aldolase and the product would be sedoheptulose diphosphate.

Alternately, the three-carbon piece might be obtained by the splitting of hexose by the enzyme transaldolase, which would transfer the three top atoms of fructose-6-phosphate to the four-carbon piece (erythrose phosphate) formed from the four bottom atoms of another fructose molecule. In this case the product would be sedoheptulose-7-phosphate.

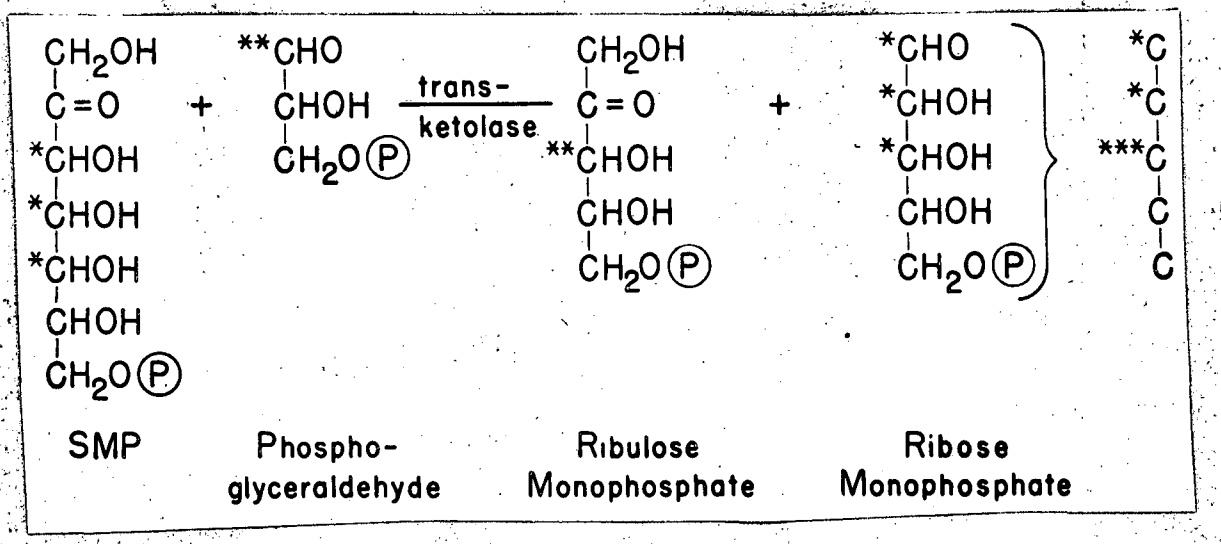


Fig. 9 - Formation of 5-carbon sugars from sedoheptulose phosphate

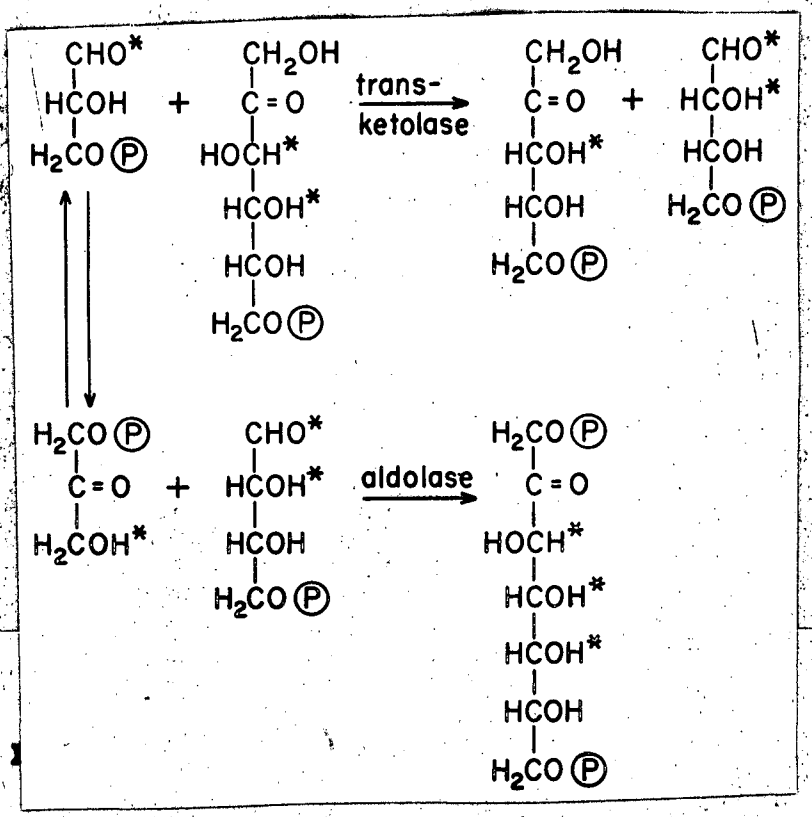


Fig. 10 - Formation of a heptose from triose and hexose

It is not possible at present to choose unequivocally between these two possibilities. However, evidence obtained from degradations from various radioactive sugar phosphates isolated from soybean leaves exposed to $C^{14}O_2$ for a very short time indicate that the proposal requiring aldolase may be correct. These degradation results are shown in Table I.

Table I

Distribution of C^{14} in Sedoheptulose Isolated from Soybean Leaf

	Time of exposure to $C^{14}O_2$	
	0.4 sec.	0.8 sec.
H_2C-OH	0	2
C=O	0	2
HO-C-H	33	39
HC-OH	8	18
HC-OH	49	38
HC-OH	0	2
H_2C-OPO_3H^-	0	2

In either of the alternate sugar rearrangements, carbon atoms No. 4 and No. 5 of sedoheptulose are derived from No. 3 and No. 4 of fructose, respectively. However, in the aldolase version, carbon atom No. 3 of sedoheptulose is derived from carbon No. 1 of dihydroxyacetone phosphate. Alternatively, in the transaldolase version, carbon atom No. 3 of sedoheptulose is derived from carbon No. 3 of fructose and therefore should have the same label at all times. Since the latter condition is not experimentally fulfilled, the aldolase reaction appears to be the correct one. However, it must be noted that this argument rests on the assumption that the concentration of the intermediate erythrose phosphate is small compared with that of fructose-6-phosphate. Also it may be noted that a small amount of labeled sedoheptulose has been obtained from hydrolysis of the sugar diphosphate area, indicating the presence of labeled sedoheptulose diphosphate. The presence of this compound may be accounted for by assuming its formation by aldolase from dihydroxyacetone phosphate and erythrose phosphate. Therefore, this route is tentatively accepted for the formation of sedoheptulose. The described transformation is shown in Fig. 10. Here are shown the two trioses that can make one hexose. One hexose then reacts with another triose to give pentose and tetrose, by means of the action of the enzyme transketolase. Tetrose and triose are then condensed by aldolase to give sedoheptulose. The net result of the reactions shown in Figs. 9 and 10 is the formation of three molecules of pentose from five molecules of triose.

Identification of CO_2 Acceptor

All the results thus far were obtained with the first type of experiment, in which $C^{14}O_2$ was added to plants for a very short period (1 to 60 seconds) before the plants were killed. A second type of experiment was used for the identification of the CO_2 acceptor. In this case once again the starting condition was an actively photosynthesizing algae suspension in "steady-state" condition. In addition, the intermediate compounds were "saturated" with C^{14}. This was accomplished by leaving the plants in contact with an atmosphere of C^{14}-labeled CO_2, maintained at constant specific activity and CO_2 pressure, for more than an hour prior to the start of the experiment. Under this condition, the concentration of each labeled intermediate compound can be determined from the radiocarbon found in that compound on subsequent analysis by chromatography and radioautography.

After this initial C^{14}-saturated steady state was obtained, aliquots of the algal suspension were taken at frequent intervals for analysis. Then some environmental condition such as light was suddenly changed. Aliquots of the algae were taken every two or three seconds for about a minute, and then at less frequent intervals. Analysis of these aliquots showed the way in which the concentrations of the various intermediates varied as a result of the environmental change.

In the first such study[2] the light was turned off. It was found that the concentration of PGA increased very rapidly while that of ribulose diphosphate (RuDP) decreased rapidly. The results of a later, somewhat more refined, experiment are shown in Fig. 11.[8] Here it is seen that the concentration of RuDP decreases to below a detectable amount (<1% of its initial value) in about 30 seconds. These changes in concentrations can be accounted for if we assume the following: the reduction of PGA to triose and the formation of RuDP are reactions requiring light; RuDP is converted to PGA via a carboxylation reaction that does not require light.

These relations are shown in Fig. 12. PGA is reduced to triose phosphate (at the sugar level); the triose phosphate then undergoes a series of rearrangements, such as the ones described earlier, through the hexose, pentose, and heptose, back again to the ribulose-5-phosphate. This is all at the sugar level of oxidation and requires very little energy for its operation. There is then some light requirement for the formation of RuDP from RuMP. The reduction of PGA requires both reducing power, reduced triphosphopyridine nucleotide (TPNH), and adenosine triphosphate (ATP), while the formation of RuDP from RuMP requires ATP, as will be seen later. Both these cofactors are produced at the required rate only when the light is on. Thus, when the light is turned off the rate of formation of RuDP and the rate of reduction of PGA decrease but the rate of carboxylation of RuDP to form PGA continues unaffected except by the concentration of RuDP.

From the above scheme it was possible to predict the result if the light were left on but the CO_2 pressure were suddenly decreased. In that event, the carboxylation of RuDP to form PGA should decrease but the formation of RuDP and the reduction of PGA should be unaffected. Consequently the concentration of RuDP should rise while that of PGA should fall. This experiment was performed[9] and the expected result, shown in Fig. 13, was obtained. When the CO_2 pressure

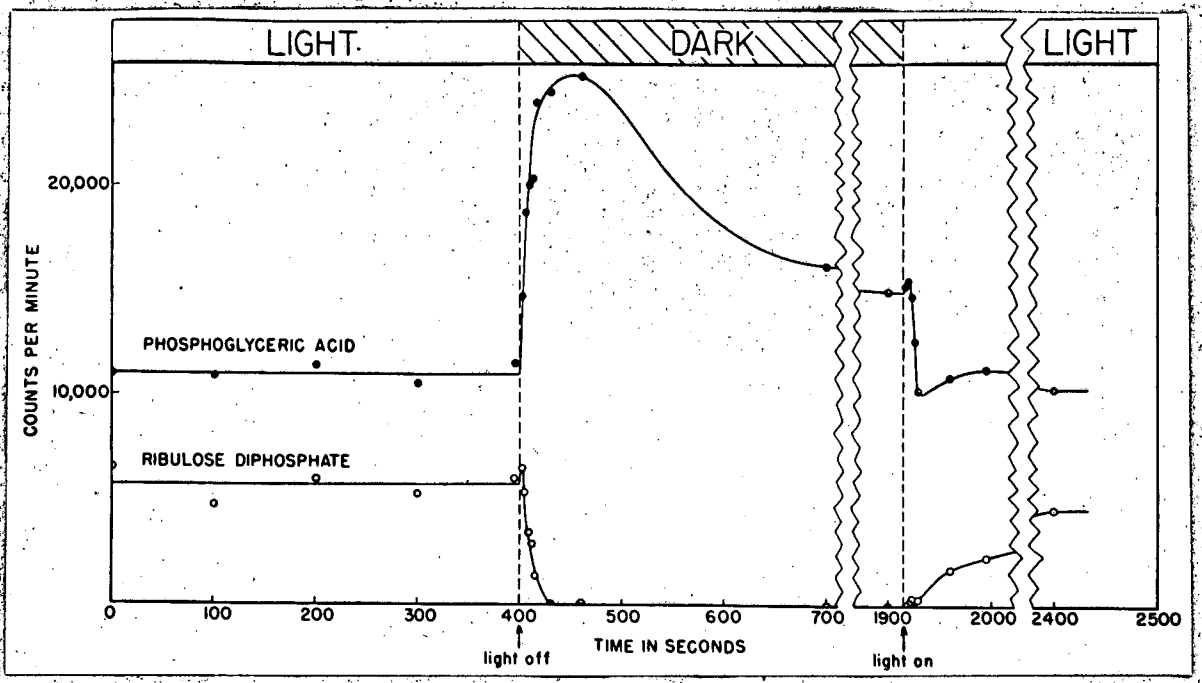


Fig. 11 - Light-dark transients in PGA and RuDP concentrations

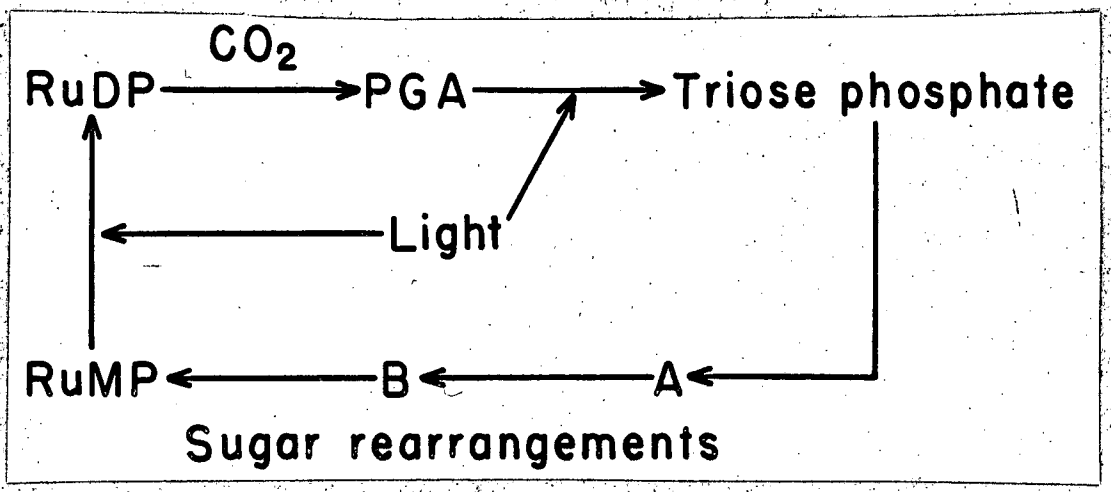


Fig. 12 - Suggested cyclic scheme for relationships in photosynthesis

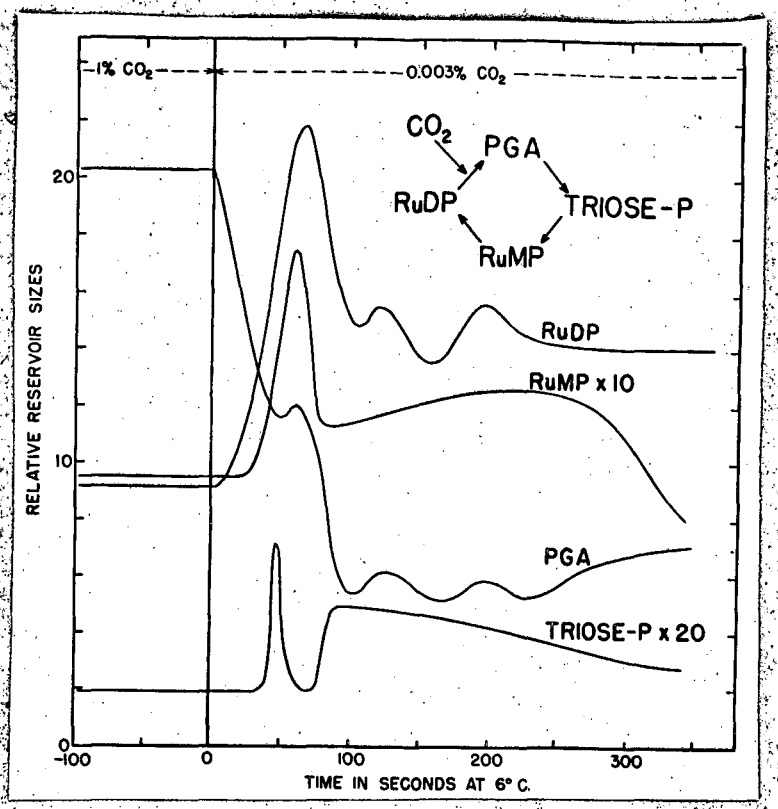


Fig. 13 - Transients in the regenerative cycle

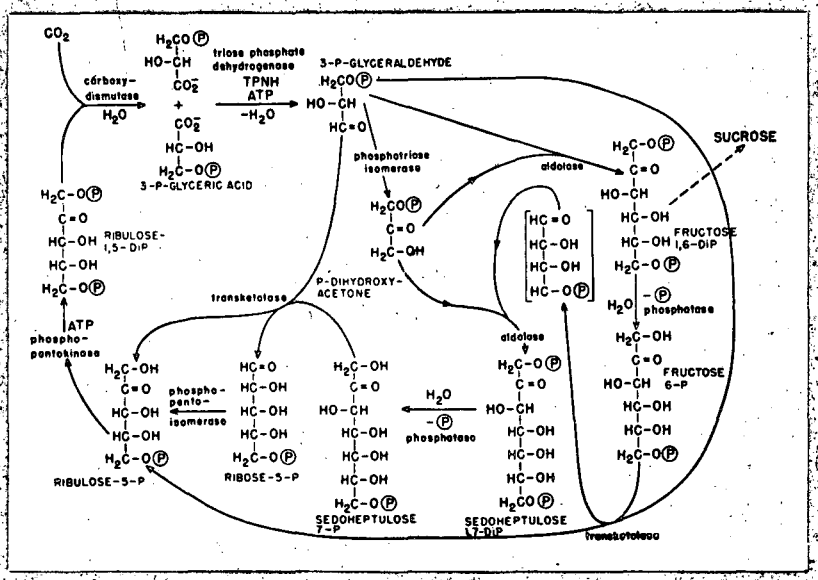


Fig. 14 - The complete photosynthetic carbon cycle

is decreased, the first compound to increase in concentration is RuDP and the second is its immediate precursor, RuMP. Last to rise in concentration is triose phosphate which is one of the precursors of RuMP. The first compound to decrease in concentration is PGA and the second is its immediate product, triose phosphate. Next to decrease is RuMP, and last to decrease is RuDP. These changes provide excellent confirmation for the proposal of the cyclic system.

There remained some question whether the carboxylation of a molecule of RuDP produced two molecules of PGA or whether some other reaction might occur in vitro in which only one molecule of PGA is produced along with a molecule of triose. In order to test this alternative, a rather careful experiment was performed[8] in which the rate of increase of PGA when the light was turned off was compared with the steady-state uptake of CO_2. During the first few seconds after turning off the light, the rate of increase of PGA should approximately equal the rate of its formation during steady-state conditions, provided reduction of PGA could be suddenly halted. The ratio of molecules of PGA increase per second/molecules of CO_2 taken up per second should indicate the number of molecules of PGA actually formed per molecule of CO_2. If this ratio experimentally approached 2 at short times, or even exceeded 1, we would have evidence for the formation of two molecules of PGA for each molecule of RuDP carboxylated. The ratio was calculated from the data shown in Fig. 11 and the measured CO_2 entry rate, and was found to be between 1.5 and 2. Thus, kinetic in vitro evidence is provided for the carboxylation reaction

$$\text{RuDP} + CO_2 + H_2O \longrightarrow 2\ \text{PGA}.$$

THE CARBON-REDUCTION CYCLE

The complete carbon-reduction cycle is shown in Fig. 14. Here are shown all the details, including the intermediate compounds and enzymes required for the various transformations. The net result of each turn of the complete cycle is the introduction of 3 molecules of CO_2 and the carboxylation of 3 molecules of ribulose diphosphate, leading to the formation of 6 molecules of phosphoglyceric acid. These 6 molecules of PGA are then reduced to provide 6 molecules of triose phosphate. Of these, 5 are eventually converted to ribulose diphosphate, thus completing the cycle, while the sixth finds its way ultimately into sucrose and represents the net gain in reduced carbon per turn of the cycle. All the enzymes shown had been previously isolated separately except for the carboxylation enzyme which converts CO_2 and ribulose diphosphate to PGA.

Carboxydismutase

About a year ago, using tracer studies, we sought and found a cell-free preparation, both from algae and from other green plants, that was capable of catalyzing the production of PGA specifically from ribulose diphosphate (RuDP) and sodium bicarbonate. The RuDP used in these experiments was isolated by chromatography from green-plant extracts. The technique was to expose the RuDP and the enzyme preparation to $NaHC^{14}O_3$ and show that carboxyl-labeled PGA was formed (Fig. 15). The traces of malic, citric, and aspartic acids and alanine formed indicate the presence in the preparation of some Krebs-cycle enzymes which could convert some of the PGA initially formed to other compounds.

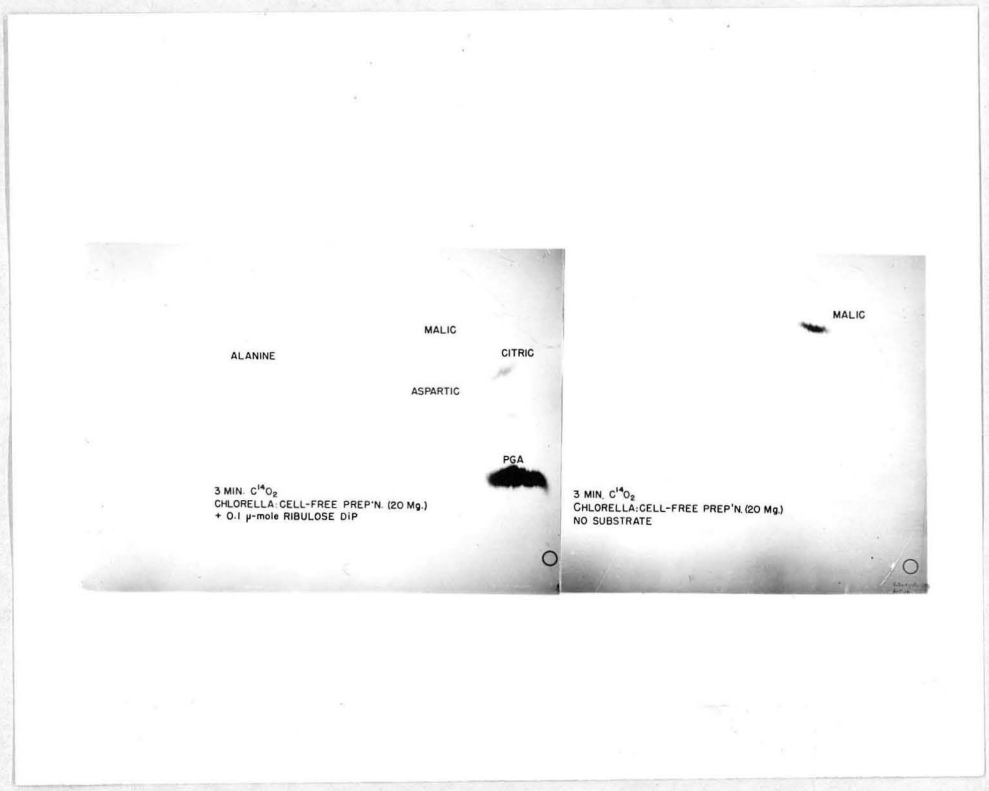


Fig. 15 - Chromatograms indicating formation of carboxyl-labeled PGA

Fig. 16 - Chromatograms showing effect of enzyme action on ribulose diphosphate.

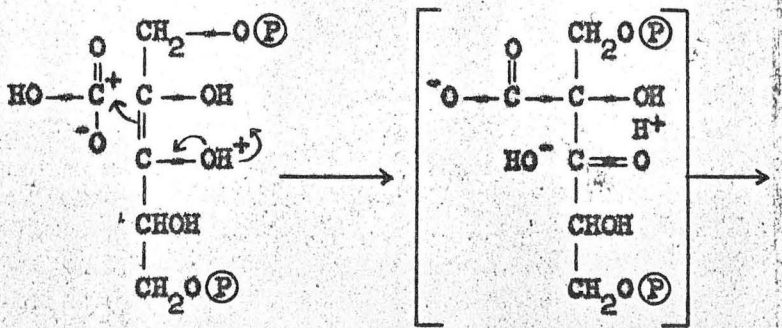
Indeed, upon longer exposure (> three minutes) to these crude preparations, much of the PGA was converted. The formation of a little labeled malic acid in the absence of substrate (RuDP) indicates the presence of pyruvic acid and malic enzyme.

Because in this experiment the tracer was in the CO_2 and not in the RuDP, it did not give direct information about the fate of the five carbon atoms of ribulose. It was therefore necessary to do the experiment with labeled RuDP and unlabeled CO_2. This was not very satisfactory in the first instance when the crude preparation was used. Although labeled PGA was formed, a good many other labeled compounds were formed as well, because of the presence in the preparation of enzymes that could act on ribulose diphosphate and compounds formed from it. In particular there was present a phosphatase which permitted the formation of ribulose-5-phosphate. This compound, in the presence of transketolase and aldolase (and possibly transaldolase), would rapidly find its way into hexose, heptose, and triose. The triose may have given rise to some PGA by oxidation. Although attempts to bypass this difficulty by inhibiting the initial phosphatase reaction on RuDP were partially successful, they were not conclusive, because of the insensitivity of the

$$\text{RuDP} \xrightarrow{HCO_3^-} \text{PGA}$$

system to fluoride ion (F^-). It was therefore necessary to proceed with the attempt to free the preparation from any other enzymes capable of acting upon RuDP except the one(s) required for the PGA-forming reaction (from CO_2). This was accomplished first from neutral extracts of New Zealand spinach (Tetragonia expansa), and later from extracts of sonically ruptured algae. The enzyme appears in the protein fraction, salted out of neutral extracts, between approximately 0.3 and 0.4 of saturation with $(NH_4)_2SO_4$. The results of an early experiment with such a preparation acting on labeled RuDP are shown in Fig. 16.[10] Here the fate of the ribulose carbon is clearly its conversion to PGA when both enzyme and $NaHCO_3$ are present. There appears to be some sugar monophosphate present in all the experiments, partly because of its presence in the original RuDP sample and perhaps partly because of the presence of some residual phosphatase in the enzyme preparation. Later experiments have given preparations that convert essentially all of the ribulose carbon into PGA and nothing else.

It thus appears that the original formulation of the reaction is at least a likely one,



$$2\ \begin{matrix}CH_2\text{—}O\text{Ⓟ}\\ |\\ O\text{Ⓟ}\end{matrix}\text{—}CHOH\text{—}CO_2^- + H^+.$$

Because the carboxylation reaction takes place at the expense of the oxidation of carbon atom No. 3 of the ribulose to the carboxyl level, the name "carboxydismutase" suggests itself as uniquely descriptive. It is interesting to note that the enzyme is not readily demonstrated in animal tissues (rat liver), and that it can be obtained from spinach in association with the highly organized intact chloroplasts,[11] from which it is extremely easily separated. It does not appear to be especially sensitive to vereene, o-phenanthroline, or cyanide, but it is sensitive to p-chloromercuribenzoate, an inhibition that is reversed by cysteine.

Chemical Requirements to Run the Cycle

We now have the cycle in its details (Fig. 14), and we now know precisely what reagents are required to make the cycle turn. It can be seen that the requirement for the reduction of a PGA molecule to a triose is one molecule of triphosphopyridine nucleotide (TPNH) and one molecule of adenosine triphosphate (ATP). The only other energy requirement comes at the point of conversion of RuMP to RuDP, where another molecule of ATP is used. A calculation of energetic compounds needed per CO_2 molecule entering will show that the net requirement for the reduction of one molecule of CO_2 to the carbohydrate level is four equivalents of reducing agent, or four electrons, and three molecules of ATP. The four electrons are supplied by two molecules of TPNH. All these required cofactors must be made ultimately by the light through the conversion of the electromagentic energy in some way. It must be emphasized that in this requirement for reducing carbon there is no particular requirement for a photochemical reaction other than the production of the two reagents. If we could supply those two things from some other source than the photochemical reaction, we should be able to make this whole sequence of operations function. We have reason to believe that this is indeed being done by the use of the required collection of enzymes. But a suitable situation exists in nature also. The situation is such that we must have simultaneously a high level of this particular reducing agent - which we now know can be triphosphopyridine nucleotide (TPN) - and ATP at the same time and the same place.

Running the Cycle Without Light

There is one known system in nature, aside from the green plants, in which that situation occurs. This situation exists in one of the photosynthetic purple bacteria that does not make oxygen, but does reduce carbon dioxide with molecular hydrogen. Figs. 17 and 18[12] show that it is possible to have the reduction of CO_2 take place either through the agency of light or through the agency of a chemical oxidation system. The organism is the purple bacterium, Rhodopseudomonas capsulatus. The initial slope corresponds to the reduction of carbon dioxide in the light. In this case both hydrogen - as the reducing agent - and light are required. As soon as the light is turned off, the reduction of carbon dioxide stops. Fig. 18 shows the same organism. This is a dark fixation. Here it is exposed only to helium and hydrogen, and there is an initial fixatbn which immediately saturates and stops. When oxygen is then admitted to the system, the fixation again continues in the same way as it does with light. The intermediates in the dark are very much the same as in the light. The hydrogen presumably provides the reducing power that is needed. The oxygen is required to oxidize some of that hydrogen to make ATP, and the two together can make the carbon dioxide cycle function. This suggests that a prime function of the light,

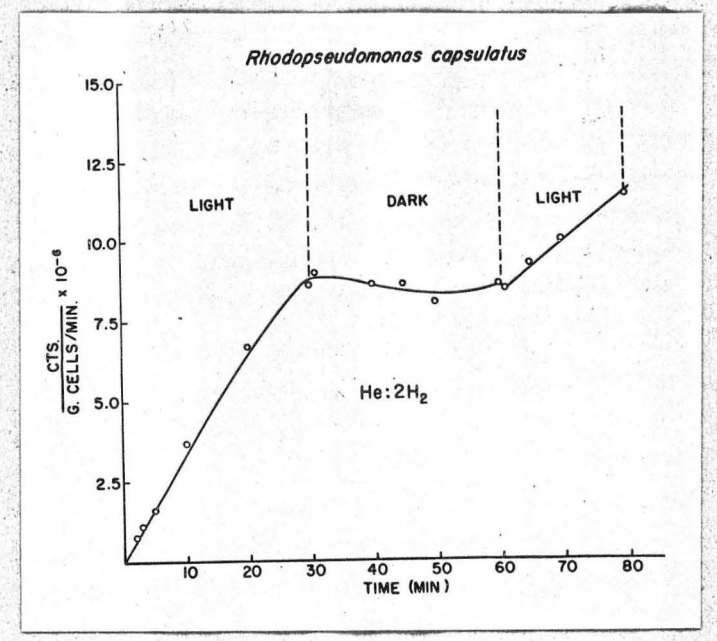


Fig. 17 - Photoreduction of CO_2 by purple bacteria

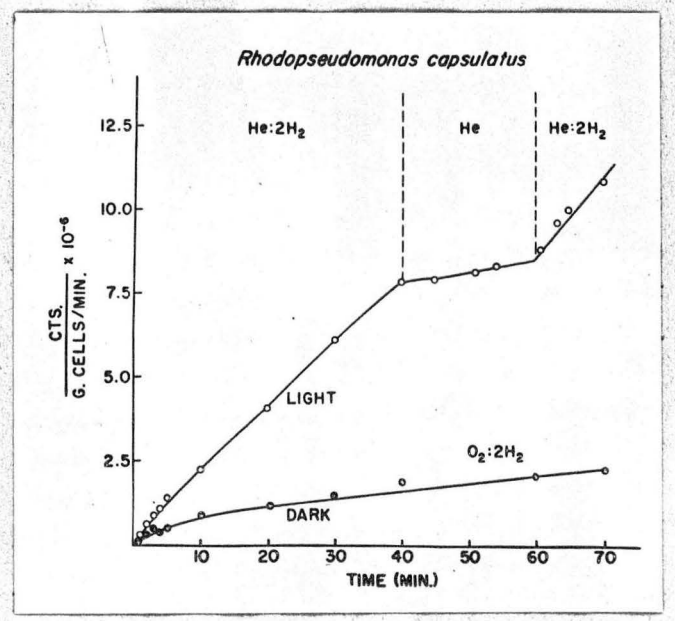


Fig. 18 - Chemical reduction of CO_2 by purple bacteria

in this case where hydrogen is the reducing agent, is to supply the oxidizing agent necessary for the production of the required ATP.

Quantum Requirements

In order to estimate what a minimum quantum requirement for photosynthesis may be, on the basis of the information we have so far accumulated about the detailed chemistry of the process, at least one assumption is necessary. This is related to the mode of interaction of electromagnetic radiation and matter. It is that a single quantum can excite not more than a single electron. Another assumption about the behavior of the excited electron is required, namely, that it does not by some chemical (or physical) dismutation process give rise to more than one equivalent of reducing power at the potential of TPNH. And if that is the case, inspection of the requirements mentioned above allows one to predict what the minimum quantum requirement for such an operation would be. Four electrons are needed for the reduction, and three molecules of ATP.

Something about the various ways in which ATP can be produced is already known. For example, during the transfer of two electrons from DPNH to an atom of oxygen, two or three molecules of ATP can be produced. Therefore, one can suppose that when all the energy for the operation of this cycle comes from light, the minimum quantum requirement must be six or seven. That is, four electrons are needed for the reduction and two or three more for the three molecules of ATP that are required. However, it should be possible to find conditions under which the quantum requirement for the reduction of CO_2 and the evolution of oxygen would be as little as four, provided there were some other source besides the light for the three molecules of ATP. These conditions have been realized.[13] The quantum-requirement determination was carried out by use of an apparatus in which one could measure directly, without any ambiguity, the production of oxygen by a direct measurement of a unique quality of the oxygen, paramagnetism, rather than merely by a gas pressure. Also it was possible to measure directly the amount of carbon dioxide absorbed by measuring a property of the CO_2 in the gas phase, in this case its infrared spectrum.

As a result of these measurements, it was found that the quantum requirement ranged experimentally from 7.4 at high light intensities, where photosynthesis exceeded respiration by a factor of 12, to 4.9 at low light intensities, where photosynthesis and respiration were nearly equal. At zero light intensity the value of the quantum requirement extrapolated to four.[14] This result indicates that some of the ATP requirement of photosynthesis can be met by reactions of respiration which produce ATP but that the four electrons of reducing agent must be supplied by the light reaction or, with special organisms, by externally-supplied reducing agents.

QUANTUM CONVERSION

So far only the reduction of carbon has been considered. Since this seems to be quite a separate system from the oxygen-evolution reaction, it might appear that one should not expect to learn much about the photoproduction of the electrons and the ATP from studying the carbon reduction. But there must be a connection between the two. By suitable observations it is possible to see at least one point at which the carbon-reduction cycle makes contact directly with the photochemical apparatus. This is shown in Fig. 19. Here the cycle is

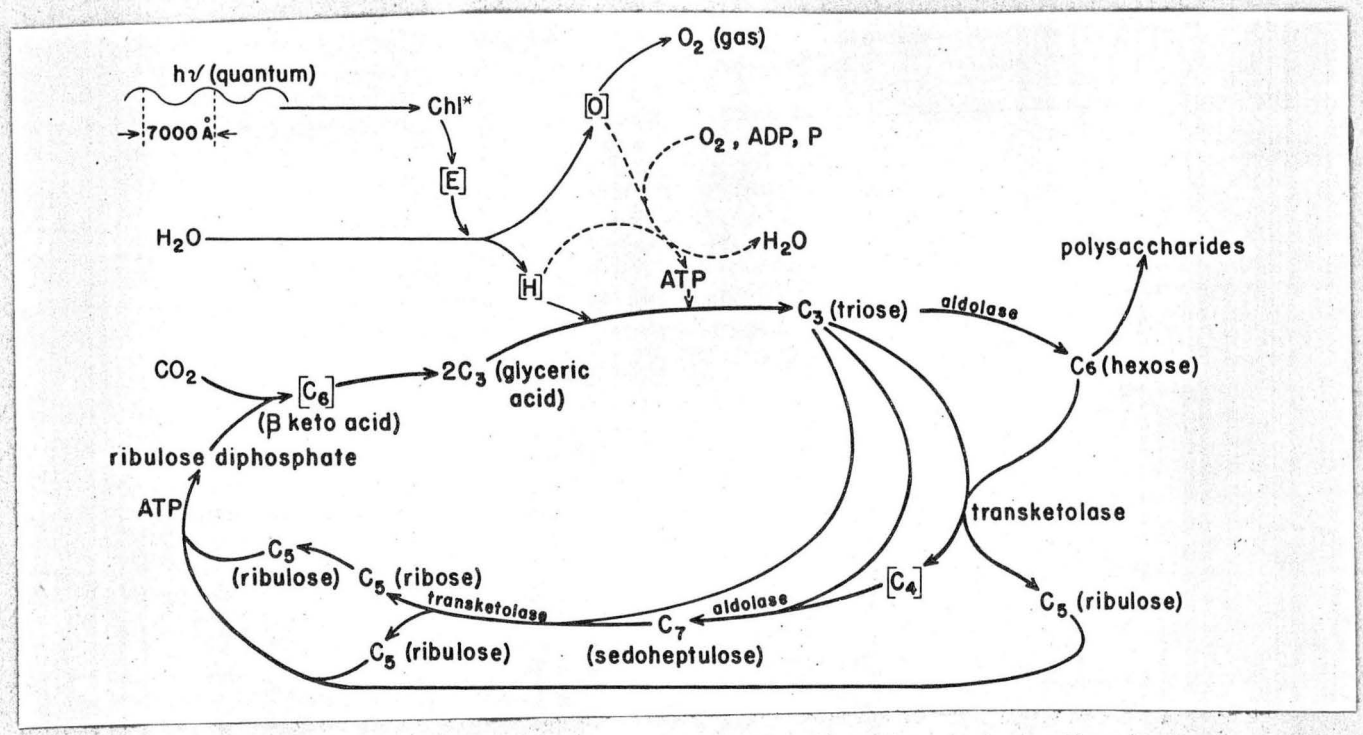


Fig. 19 - Proposed cycle for carbon reduction in photosynthesis

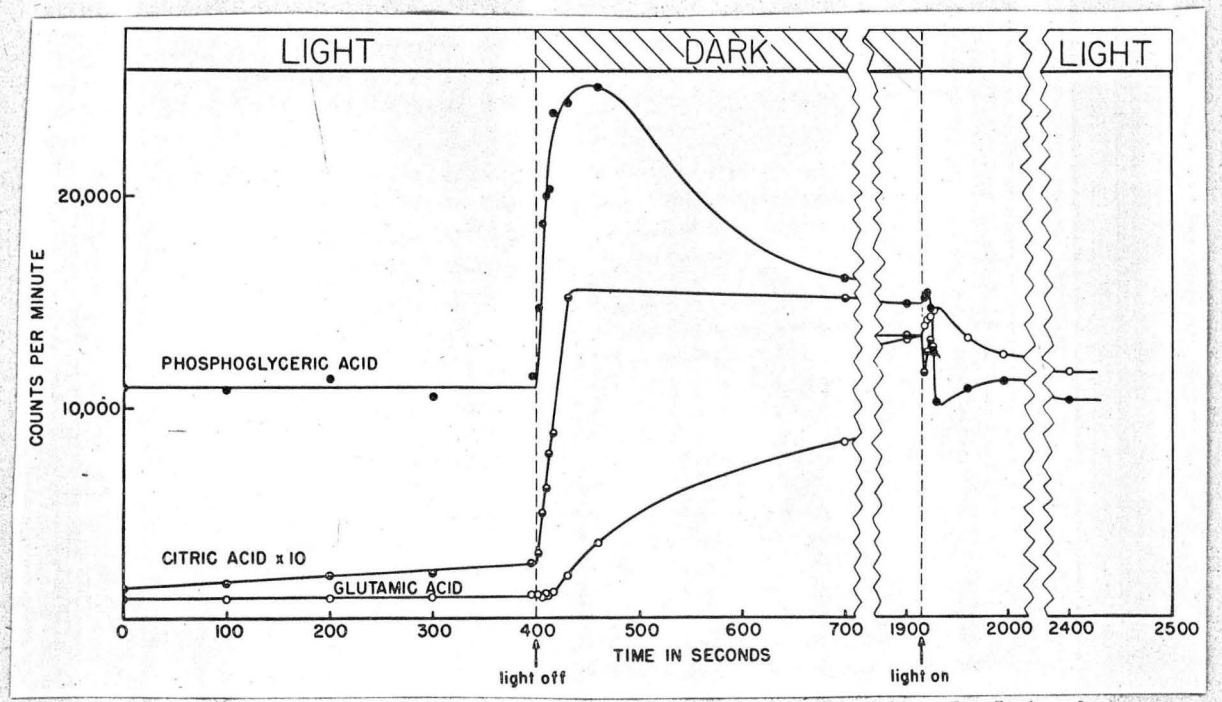


Fig. 20 - Light-dark transients in PGA, citric acid and glutamic acid concentrations

show again. The quantum is first absorbed by chlorophyll and converts water into something that makes a reducing agent [H] and some oxidizing agent [O]. The reducing agent can reduce the glyceric acid to triose. Some of the reducing agent must be used to make ATP, with oxygen or the intermediates on the way to oxygen, because that is necessary for the cycle to run. What we wish to consider now is this point of contact, [H], between the photochemical apparatus and the carbon cycle and what information about the quantum conversion we can gain from this study.

Light Inhibition of TCA-Cycle Incorporation

An experiment was carried out in which a steady state was examined and the changes induced by a sudden change of conditions were observed. Fig. 20 shows the result of this experiment. Here is the same type of experiment as before, but with the examination directed toward different substances. Attention is focused on glutamic acid and citric acid, and it will be seen that while the light is on, the rate of formation of radioactive glutamic acid and radioactive citric acid is quite low. But immediately after the light is turned off, the rate of formation of these labeled acids is increased manyfold. Glutamic and citric acids are two compounds very closely related to the respiratory cycle known as the Krebs cycle, and Fig. 21 describes in schematic terms the metabolic relationships leading to the experimental facts we have just seen. Here is shown the photosynthetic cycle and the Krebs (tricarboxylic acid) cycle. The glutamic acid and citric acid are in or related to the Krebs cycle. The photosynthetic cycle does not contain either glutamic or citric acid but does form PGA and sugars. Eventually these direct products of the photosynthetic cycle have to become carbohydrates, proteins, and fats, and ultimately they will get back into the tricarboxylic acid cycle. That is the major route in the light. But immediately after the light is turned off a direct connection between the two cycles is apparently made which allows the PGA to be transformed directly into the compounds of the tricarboxylic acid cycle. Fig. 22 shows the details of that mechanism. Carbon can enter the tricarboxylic acid cycle via acetyl Coenzyme A, condensing with oxalacetic acid to give citric acid, thence continuing around this cycle and via a side reaction to glutamic acid. The question is: how is glyceric acid converted to acetyl Coenzyme A? This must happen rapidly in the dark, but not very rapidly in the light. Fortunately we have some idea how acetyl-CoA may be formed from glyceric acid, and Fig. 22 shows this. The glyceric acid is dephosphorylated to form pyruvic acid; the pyruvic acid then reacts with an enzyme system, of which thioctic acid is a coenzyme, to form acetyl-thioctic acid and carbon dioxide. The acetyl-thioctic acid then undergoes a thiol ester interchange with CoA to form reduced thioctic acid and acetyl-CoA, which then goes on into the citric acid cycle, Fig. 23.[16]

How does light affect these reactions? The conversion of PGA to citric acid provides for the entrance of carbon into the tricarboxylic acid cycle, and if somehow this pathway is closed by reduction of the level of the disulfide, the rate of transfer of radioactive carbon from the photosynthetic cycle to the citric acid cycle will be reduced. This suggests that the light shifts the equilibrium from the disulfide to the dithiol form of thioctic acid by inducing reaction with something other than pyruvic acid, perhaps ultimately water. In the dark, oxidation converts the dithiol form to the disulfide, which can again catalyze the oxidation of pyruvic acid to CO_2 and acety-CoA. This system is like a valve that is closed by light, and that controls the flow of carbon from

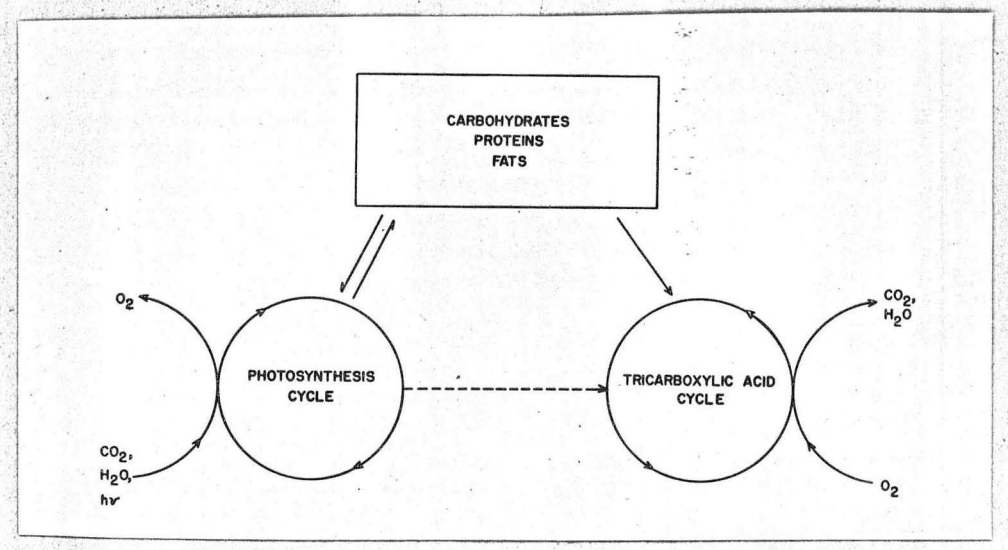


Fig. 21 - Schematic relationships between the photosynthetic cycle, the tricarboxylic acid cycle, and storage products in the plant

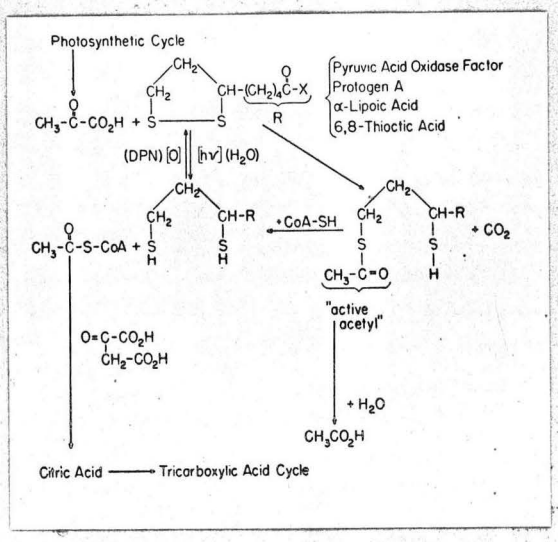


Fig. 22 - Mechanism of photochemical control of the relationships between the photosynthesis cycle and the tricarboxylic acid cycle

the photosynthetic cycle directly into the tricarboxylic acid cycle. It suggests further that the disulfide may be closely allied to, if not identical with, the electron acceptor from the photochemical act. Actually a number of experiments have been performed that indicate that this may be so.[17),18),19)]

The proposed relations between the photosynthetic carbon reduction cycle, the photochemical reactions, and the Krebs cycle are shown in Fig. 23. It is suggested that the required ATP is generated by reactions coupled with the oxidation of TPNH or DPNH through the cytochrome system.

It can be seen that the use of radioactive elements, employed as tracers, have made possible the elucidation of the path of carbon reduction in photosynthesis. In addition, information gained from the study of the path of carbon in photosynthesis and its relation to reactions of respiration has provided the basis for proposals regarding the energy transport from the primary photochemical act.

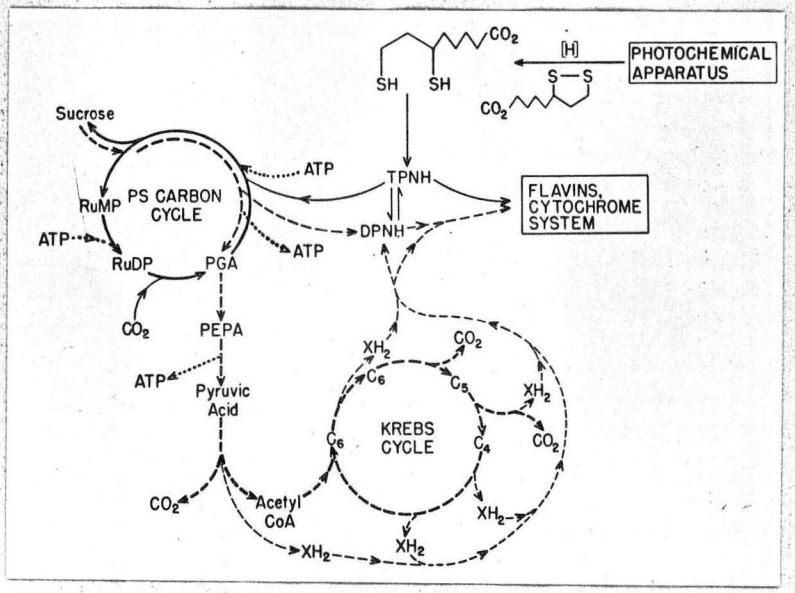


Fig. 23 - Diagram of the suggested nature of the photochemical apparatus and its relationship to other functions

- - - - Oxidative, or respiratory, pathways
———— Reductive, or photosynthetic, pathways

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