Title

Photosynthesis

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PHOTOSYNTHESIS

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The process of photosynthesis in plants if often defined by the overall equation

$$CO_2 + H_2O \xrightarrow[\text{green plant}]{\text{light}} \{CH_2O\} + O_2$$

which stated in words says that carbon dioxide and water "under the influence of light" are converted by green plants to carbohydrate and oxygen. Such a definition, when used to introduce the subject of photosynthesis to a general chemistry class provides a much too incomplete description of this important process. How might the definition be improved?

First, the essential energy-storing character of the reaction should be emphasized by showing light energy as a reactant in the equation, thus distinguishing photosynthesis from other photochemical reactions in which the role of light is essentially catalytic. Secondly, in view of present day knowledge of the direct photosynthesis of products other than carbohydrates it should be stated that the reactants include nitrate (or ammonia), sulfate, and phosphate and the products include amino acids, fatty acids, phospholipids and sulfolipids. In addition, a number of other inorganic substances (Fe, Mg, etc.) are required in varying amounts for photosynthesis and may in some cases be incorporated into the products. Finally, if catalysts are to be indicated, then chlorophyll and the many enzymes required should be shown. An equation for the overall process of photosynthesis

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might then be given as

Eq. 1

$$\text{light} + CO_2 + H_2O + (NO_3^- \text{ or } NH_4^+,\ HPO_4^=,\ SO_4^=) \xrightarrow[\text{many enzymes}]{\text{chlorophyll}} O_2 + \underbrace{\{CH_2O\}}_{\text{carbohydrate}} + \begin{pmatrix}\text{amino acids, lipids,}\\ \text{phospholipids,}\\ \text{sulfolipids, etc.}\end{pmatrix}$$

Any generalized formula for photosynthesis is a compromise between simplicity and accuracy, but this one at least hints at the complexity of this important process.

For the purpose of discussion, the photosynthetic reaction may be conveniently broken down into three stages. The first of these is the "photo-" part which consists of the absorption of light by the plant pigments and the conversion of the light energy into the stored energy of new chemical bonds of "high energy" compounds (Eq. 2).

Eq. 2

$$\text{Photo-} \qquad \underset{\text{(light)}}{h\nu} + \text{low energy compounds} \xrightarrow{\text{chlorophyll}} \text{higher energy compounds}$$

An intermediate stage then follows in which the chemical energy of these primary products of the photochemical reaction is utilized in the breaking of the O-H bonds of water and the conversion of two enzymatic cofactors to their more energetic and (for one of them) more reduced forms (Eq. 3).

Eq. 3

$$\text{higher energy compounds} + \text{water} + \text{cofactors} \longrightarrow O_2 + \text{low energy compounds} + \text{high energy cofactors}$$

At the same time molecular oxygen is liberated. We know very little of the detailed mechanism of these reactions and all or part of this so-called

intermediate stage may be in fact a part of the primary photochemical reactions. Indeed, the first stages of photosynthesis may best be considered as the sum of one primary photochemical act in which light energy is absorbed by chlorophyll and a series of non-photochemical steps in which this energy is efficiently transferred through the production of increasingly stable chemical species.

The "-synthesis" stage of photosynthesis is the sum of a great number of enzymatic reactions in which the two cofactors supply the reducing power and energy necessary to bring about the conversion of carbon dioxide (together with nitrate, phosphate, and sulfate) to carbohydrates, amino acids, and lipids (Eq. 4).

Eq. 4

$$\text{-synthesis} \quad CO_2 + \text{high energy (and reduced) cofactors} \; (+NO_3^- \text{ or } NH_4^+, + SO_4^= \text{ etc.}) \longrightarrow \text{reduced organic compounds + low energy (and oxidized) cofactors}$$

Let us consider in somewhat more detail these several stages in photosynthesis. We shall not dwell for long on the primary photochemical reactions for the simple reason that although much experimental data has been obtained regarding this process, there is as yet no clearly detailed theory of the chemical mechanism upon which a majority of the investigators in this field can agree. The best that can be said is that in most plants all light energy which is utilized for photosynthesis appears to be employed in one early stage to raise the energy content of chlorophyll a from its ground state to a higher energy level or "excited state." This is true whether the light energy is absorbed directly by chlorophyll a, or by other

plant pigments in which case the energy appears to be transferred to chlorphyll a.

Then the energy of the excited chlorophyll a must somehow be used for the formation of stable compounds with a high content of energy stored in their chemical bonds. There is no unanimity of opinion as to how this is accomplished. One view of the mechanism is illustrated in general terms by the scheme shown in Fig. 1. In this scheme the energy of the excited chlorophyll is transferred by some mechanism which probably involves its migration from one chlorophyll molecule to another through a closely packed sheet of chlorophyll molecules. This sheet of chlorophyll molecules, which is thought to be only one molecule thick may exist at the interface between lipid and protein layers, the presence of which has been revealed by electron microscope pictures of the chloroplasts. (Chloroplasts are the subcellular units of green cells responsible for photosynthesis.) Eventually (after a millionth of a second or so) the energy packet or "exciton" reaches a point where it is used to supply the energy of ionization of some unknown compound, perhaps chlorophyll itself. For reasons that will be discussed in a moment, this ionization is believed to consist of transfer of electrons in such a way that the resulting ionic species contain "unpaired electrons."[2]

Most electrons in organic molecules are found in pairs, so that the spin of one electron, and its resulting interaction with a magnetic field, is cancelled by the equal and opposite spin of its orbital partner. An unpaired electron does have such an interaction, however, and the energy of its interaction with an externally applied magnetic field may

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[2]Sogo, P. B., N. Pon, and M. Calvin, Proc. Nat. Acad. Sci., 43, 387 (1957).

change if its spin changes in sign. In order for an unpaired electron to change the sign of its spin in a strong magnetic field in the direction of greater energy it must absorb energy, and this it will do if electromagnetic energy of the appropriate wavelength (in the order of 1-5 cm.) is supplied. The characteristic energy absorption can be measured by suitable instruments which provide a sensitive means of detection for unpaired electrons. This phenomenon is known as electron spin resonance, or e.s.r.

By means of e.s.r. measurements with thick pastes of live unicellular algae (microscopic green plants) or chloroplasts obtained from spinach, a strong e.s.r. signal has been observed which increases in the light and decreases in the dark. Moreover, a part of this signal is generated even when the plant material is cooled to the temperature of liquid nitrogen (-196°). At this temperature, the movement of atomic nuclei seems improbable, but the movement of an electron, resulting in ionization could easily occur. The detection of light-induced e.s.r. signals at very low temperatures thus forms the basis for the suggestion that one step in the conversion of the energy of excited chlorophyll to the energy of chemical bonds is an ionization process.

At room temperature, the light-induced formation of more than one type of species containing unpaired electrons is indicated by the fact that when the light is turned off, the disappearance of the signal in the dark takes place with more than one characteristic decay time constant. This observation provides evidence for the formation of chemical radicals in which there has been a movement of atomic nuclei.

These radicals may form another stage in the transfer of stored energy of chemical bonds.

Finally, stable chemical compounds of high energy content must be formed. In this scheme they would be formed by the interaction of stable chemical compounds of lower energy with chemical radicals. The resulting chemical compounds may be the cofactors required for the reduction of carbon dioxide and nitrate, or they may be other energy-containing compounds which subsequently give rise to the necessary cofactors by chemical or enzymatic reaction. In addition, molecular oxygen is evolved from the products formed by the breaking of the O-H bond of water.

The two energy-carrying cofactors, triphosphopyridine nucleotide (TPNH) and adenosine triphosphate (ATP) are shown in Figure 2. The requirement for these coenzymes was predicted from studies of the carbon reduction cycle, certain steps of which can only be carried out by the enzymes if the appropriate cofactors are present. TPNH is an electron carrier, transporting electrons obtained by splitting water to the reactions in which they are used for the reduction of carbon dioxide. Each time a molecule of TPNH is oxidized to TPN^+, two electrons are transferred. The energy stored in ATP is stored through the formation of an anhydride from ADP and inorganic phosphate.

An important stochiometric relationship between the formation of ATP, TPNH and O_2 during photosynthesis has been discovered by Arnon and coworkers,[3,4] and is shown in Equation 5:

Eq. 5 $$\text{light} + H_2O + ADP + Pi + TPN^+ \rightarrow 1/2\ O_2 + TPNH + H^+ + ATP$$

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[3]Arnon, D. I., F. R. Whatley, and M. B. Allen, Science, 127, 1026 (1958).
[4]Arnon, D. I., F. R. Whatley, and M. B. Allen, Nature, 180, 182 (1957).

This relationship was discovered in studies with isolated chloroplasts from spinach leaves when oxygen evolution, TPNH formation and ATP formation were measured simultaneously. This stochiometric relationship does not tell us what the mechanism of formation of these substances is but does perhaps hint at a pathway that is simpler than we formerly suspected.

We will now turn to the "synthesis" part of photosynthesis and see how these cofactors which have been formed by means of the light reactions are used. The studies of the pathway of carbon reduction during photosynthesis were in a large part carried out in the Lawrence Radiation Laboratory at the University of California by Professor Melvin Calvin and coworkers.[5] These studies provide an interesting example of the use of radioactive tracer atoms in the elucidation of a complex biochemical pathway. Since carbon dioxide is the sole source of carbon for the photosynthetic reaction, radiocarbon, carbon fourteen, may be introduced very easily into photosynthesizing plants in the form of $C^{14}O_2$ or, for aquatic plants, $HC^{14}O_3^-$ ion.

Let us consider a simple experiment with a suspension of the algae, Chlorella pyrenoidosa, which have been very extensively used in these studies. These green, microscopic unicellular plants, suspended in water containing a few inorganic substances (nitrate, phosphate, etc.) with a stream of $C^{12}O_2$ (ordinary carbon dioxide) photosynthesize at a rapid rate if illuminated from each side in a thin glass vessel. The CO_2 is continually taken up from the solution (where it is in equilibrium with bicarbonate ion) and converted by the photosynthetic plant through a series of biochemical intermediates to various organic products.

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[5] Bassham, J. A., A. A. Benson, L. D. Kay, A. Z. Harris, A. T. Wilson, and M. Calvin, J. Am. Chem. Soc., 76, 760 (1954).

A solution of radioactive bicarbonate, $HC^{14}O_3^-$ is suddenly introduced into the algae suspension. The plant does not distinguish in any important way between the C^{12} and the C^{14} which are chemically identical, and immediately some of the C^{14} is incorporated into the first of the biochemical products leading from CO_2 to end products. As time passes, the C^{14} would be passed on to subsequent intermediates in the chain. After a few seconds exposure to the C^{14} the suspension of algae is run into methanol to a final concentration of 80% methanol. This treatment denatures all the enzymes instantly and freezes the pattern of intermediates toward further change. Now all that remains to be done is to analyze the dead plant material for radioactive compounds to see what are the first products of carbon reduction during photosynthesis.

The first step in this analysis is to prepare an extract of the soluble compounds since the early products of carbon reduction have been found to be simple soluble molecules. This extract is then concentrated and analyzed by the method of two-dimensional paper chromatography.[6]

The first step in this useful method of analysis[7] consists of drying a small quantity of the concentrated extract on the corner of a large sheet of rectangular filter paper. The edge of the paper next to this dried extract is then placed in a trough filled with a suitable chromatographic solvent which travels down the paper by capillarity. As the solvent passes over the dried extract, it dissolves the various compounds and carries them along with it at various rates, depending upon the particular compound.

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[6] Benson, A. A., J. A. Bassham, M. Calvin, T. C. Goodale, V. A. Haas, and W. Stepka, J. Am. Chem. Soc. 72, 1710 (1950).

[7] Block, R. J., E. L. Durrum, and G. Zweig, "Paper Chromatography and Paper Electrophoresis", Academic Press, Inc., New York, 1958, 2nd ed., pp. 710.

The substances are thus separated in a row along the edge of the paper. The paper is dried, turned 90°, and the edge next to the compounds placed in a second solvent where the process of separation is continued with the compounds moving in a direction at right angles to their former direction of travel. Upon drying the paper again, the compounds are found to be scattered about the paper as pure substances, each in its own unique location. The importance of the method for these studies can be seen from the fact that it permits the analysis of a few micrograms or less of dozens of different substances in a single simple operation.

Of these many compounds, those into which the plant incorporated carbon fourteen during its few seconds of photosynthesis with $HC^{14}O_3^-$ are radioactive, and emit the particles resulting from radioactive decay of the C^{14}. In this case these are β^- particles and these may be detected by the fact that they expose x-ray film. Thus, if a sheet of x-ray film is placed in contact with the paper chromatogram, subsequent development of the film will show a black spot on the film corresponding to the exact shape and location of each radioactive compound on the paper. A quantitative determination of the amount of radiocarbon in each compound may then be made by placing a Geiger-Muller tube with a very thin window (to permit the particles to pass through) over the radioactive compound on the paper and counting the emitted particles electronically.

The next stage in the method of radiochromatographic analysis is the identification of the radioactive compounds. This identification is accomplished by a variety of ways. Of these, the most important is by elution or washing of the compound off the paper and the determination of

such chemical and physical properties of the substance as can be measured with a solution of a few micrograms or less of the material. These properties then are compared with those of known compounds. The final check on the identity of the compound is frequently made by placing on the same spot on filter paper the radioactive compound and 10-100 μg of the pure non-radioactive substance with which the radioactive compound is thought to be identical, and chromatographing the two together. A radioautograph is then prepared to locate the radioactive substance after which the paper is sprayed with a chemical spray (for example, ninhydrin for amino acids) which produces a color where the carrier compound is located on the paper. Superposition of the paper chromatogram and the radioautograph (X-ray film) will show an exact coincidence between chemically-developed color on the paper and the black spot on the film if the two substances are identical.

Once the identity of the radioactive compounds formed during a short period of photosynthesis had been established, experiments were performed under a variety of conditions and times of exposure of the algae to radiocarbon. The radioautograph from the experiment with Chlorella described above is shown in Fig. 3. Even after only 10 seconds of exposure to C^{14}, a dozen or more compounds are found. Some of these (the sugar phosphates) are not separated from each other by the first chromatography and must be subjected to further analysis. When the sugar monophosphates are hydrolyzed to remove the phosphate groups and rechromatographed, separate spots are found of triose (dihydroxyacetone), tetrose, pentoses (ribulose, xylulose and ribose), hexoses (glucose and fructose) and heptose (sedoheptulose). The sugar diphosphates are found to include ribulose, fructose, glucose,

and sedoheptulose.

After periods of photosynthesis with C^{14} of less than 5 seconds, 3-phosphoglyceric acid (PGA) was found to be the predominant radioactive product. Chemical degradation of this compound showed that the radioactivity first appears in the carboxyl carbon. Later kinetic studies showed that the rate of incorporation of C^{14} into PGA at very short times was much greater than the rate into any other compound. Therefore, it was concluded that PGA is the first stable product of carbon dioxide reduction during photosynthesis, and furthermore, that carbon dioxide first enters the carboxyl group of PGA, presumably via a carboxyllation reaction.

From this point we borrowed from the already known pathways of the glycolytic breakdown of sugars which lead to PGA as an intermediate. Noticing that the sugar phosphates are important early products of carbon reduction in photosynthesis, we proposed that they are formed from PGA by a reversal of the glycolytic pathway. Degradation of the radioactive hexoses from short experiments showed that they were labeled in the two center carbon atoms (numbers 3 and 4) just as one would expect if two molecules of PGA were first reduced to triose and then linked together by the two labeled carbon atoms to give hexose (Fig. 4).

The hexose and triose phosphates may be converted by aldolase or transaldolase and transketolase enzymes to pentose and heptose phosphates. Degradation of these sugars and comparison of the labeling patterns within the molecules showed that this conversion did occur and in such a way that five molecules of triose phosphate were ultimately converted to three molecules of pentose phosphate.

Other known metabolic pathways leading from PGA (Fig. 4) give rise first to phosphoenolpyruvic acid (PEPA) which then may undergo further transformations as follows: (1) it may be carboxyllated and transaminated to give aspartic acid, (2) it may be carboxyllated and reduced to give malic acid, (3) it may be dephosphoryllated and transaminated to give alanine. All of these compounds are labeled after short exposures of the algae to $HC^{14}O_3^-$ in the light. The carboxyllation of PEPA, while a second point of entry for carbon dioxide during photosynthesis, accounts for only 5-10% of the reduced carbon dioxide under normal conditions in Chlorella, according to kinetic measurements. Since this reaction converts three additional carbon atoms from PGA to malic acid and aspartic acid at the same time, the amounts of these compounds photosynthesized are significant.

The enzyme system which brings about the oxidation of triose phosphate to PGA in the glycolytic pathway was known to produce ATP and TPNH (or DPNH). If PGA is to be reduced to triose phosphate during photosynthesis, it follows that ATP and TPNH must be supplied. We have already seen that these two cofactors are produced as a consequence of the light reaction and the splitting of water. It might be expected that if the light were turned off from plants photosynthesizing in ordinary carbon dioxide at precisely the same time that $C^{14}O_2$ is introduced, PGA would no longer be reduced to sugar phosphates, but would still be formed (if neither TPNH or ATP are required for the carboxyllation reaction) and would still be used in other reactions not requiring these cofactors. In Fig. 5, the radioautograph from just such an experiment, we see that this prediction was correct. Labeled PGA is still formed from $C^{14}O_2$ during 20 seconds in the dark, but only a

very little of the PGA is reduced to sugar phosphates. On the other hand, a large amount of alanine is formed from PGA via PEPA in reactions which do not require ATP or TPNH. The small amount of labeled sugar phosphates which does appear is due to the residual ATP and TPNH which was formed while the light was on but which had not yet been used up when the $C^{14}O_2$ was introduced. Some malic acid is still formed in the dark, indicating the presence of some DPNH, either remaining from the light or derived from some other metabolic reaction.

Thus far we have not discussed the identity of the source of the compound which undergoes carboxyllation to produce PGA. In order to explain its discovery we must turn to another type of experiment with $C^{14}O_2$ and photosynthesizing algae. In these experiments, algae are first permitted to photosynthesize for 20 minutes or more in the presence of a constant supply of $C^{14}O_2$. During this time all environmental conditions are maintained constant (temperature, CO_2 pressure, light intensity, etc.). After about 10 minutes of exposure to $C^{14}O_2$, so much radiocarbon has passed through the various biochemical intermediate compounds on its way to end products that each carbon atom of each intermediate compound contains, on the average, the same percentage of carbon fourteen atoms as the CO_2 which is being absorbed. In other words, the specific radioactivities of all the carbon atoms of all the early intermediates are the same as the specific radioactivity of the entering radiocarbon, which can be measured and is therefore known.

At this point, samples of the algae are removed without disturbing the rest of the algae and these samples are killed and subsequently analyzed by the methods which I have already described. The <u>total radioactivity</u> of

each intermediate is measured, and when this is divided by the known specific radioactivity, the total number of carbon atoms of each intermediate compound in the sample can be calculated. Thus the concentrations of the various intermediates of the actively photosynthesizing system may be determined.

This determination of the concentrations of intermediates in vivo is an extremely valuable tool which has many uses, but let us proceed with the experiment which we had started to describe above. Having taken samples of algae for later determination of the concentrations of intermediate compounds, we now turn off the light and proceed to take a series of samples of the algae as rapidly as we can, which is about every three seconds. When the concentrations of intermediate compounds in these samples are determined, any changes resulting from turning off the light will be revealed. The two most striking changes are found to be in the concentration of PGA which increases rapidly (Fig. 6) and in concentration of one particular sugar, ribulose diphosphate, which drops rapidly to zero.

The increase in PGA on turning off the light is expected, since we have seen that cofactors, derived from the light reaction, are necessary for the reduction of PGA. The rapid drop in ribulose diphosphate taken together with the fact that other sugar phosphates do not drop rapidly in concentration at first must indicate that the formation of ribulose diphosphate from other sugar phosphates requires a light-formed cofactor. This conclusion agrees with the fact that the known enzyme which converts ribulose-5-phosphate to ribulose-1,5-diphosphate does in fact require ATP. The drop in ribulose diphosphate, alone among the sugar phosphates means that it is being used up by some reaction which does not require light.

If ribulose diphosphate is used up by some reaction that proceeds in the dark, and if PGA continues to be formed in the dark, could the carboxyllation of ribulose diphosphate to form PGA be the first step in carbon dioxide reduction? To answer this question, another experiment similar to the one just described was performed. This time, however, instead of turning off the light, the light was left on and carbon dioxide was suddenly removed. The result of this experiment (Fig. 7) confirmed the idea of a carboxyllation of ribulose diphosphate, for the concentration of ribulose diphosphate now rose rapidly while PGA dropped rapidly.

The carbon reduction cycle was now complete and is shown in Fig. 8. The methods and reasoning leading to the elucidation of this cycle have been presented more completely elsewhere.[5,8] A complete circuit of the cycle involves the conversion of five molecules of triose phosphate to three molecules of ribulose diphosphate upon which carboxyllation produces six molecules of PGA or its equivalent. There is thus a gain of one three-carbon unit corresponding to the three molecules of carbon dioxide introduced for each turn of the cycle. On the average, five and a fraction of the three-carbon units are reduced to triose phosphate, with five of these going to generate ribulose diphosphate while the fraction is employed (presumably) in the synthesis if sucrose, polysaccharides, glycerol and galactose (constituents if lipids) and other substances. The remainder of the PGA is converted to alanine and serine or, via PEPA, is carboxylated to make four-carbon compounds (Fig. 4).

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[8]Bassham, J. A. and M. Calvin, "The Path of Carbon in Photosynthesis", Prentice-Hall, Inc., Englewood Cliffs, New Jersey, 1957.

An interesting problem remaining at this point is the quantitative determination of the relative importance of the various metabolic pathways discussed. The relative rates of the reactions of the carbon reduction cycle are of course fixed, but it is of interest to compare the rates of introduction of carbon dioxide via the two carboxyllation mechanisms with the externally measured uptake of carbon dioxide. Also it is of interest to ascertain the rates of flow of carbon through such secondary intermediates as alanine and aspartic acid and thus gain some idea as to how much amino acids may be synthesized directly from CO_2, via PGA, without the intermediancy of compounds such as sucrose and polysaccharides, once thought to be the sole products of photosynthesis.

The quantitative determination of the flow of carbon via the several pathways is made as follows: first, algae are allowed to photosynthesize with ordinary CO_2 under constant environmental conditions for a few minutes. $C^{14}O_2$ is then introduced, without any other variation in environmental conditions. These conditions and the specific radioactivity of the $C^{14}O_2$ are kept constant for the duration of the experiment. Samples of algae are taken rapidly following the introduction of C^{14} and then more slowly until sufficient time has passed to obtain radioactivity saturation of the intermediate compounds. During this time CO_2 uptake is continuously measured by an infra-red absorption measuring instrument which monitors the gas bubbling through the algae in a closed system. C^{14} uptake from the gas is measured by means of an ionization chamber. Thus the specific activity of the $C^{14}O_2$ is continually measured. All of the samples are subsequently analyzed by paper chromatography and radioautography. The C^{14} in each compound

in each sample is counted. The growth of radioactivity in each compound from the time of introduction of C^{14} to the time of saturation is thus determined. Typical labeling curves are shown in Figure 9.

As indicated earlier, the concentrations of the intermediates during a period of steady photosynthesis or "steady state" may be calculated from the saturating radioactivity found in the compound and from the specific radioactivity. The concentrations, the growth of radioactivity, and the specific radioactivity of the precursor, all of which are determined in this experiment, may be used in a calculation of the rate of flow of carbon through each intermediate compound. This has been done in one preliminary experiment.

Among the conclusions derived from the calculation of rates of carbon flow through various intermediates and along certain pathways are the following: 1) at least 85% of the assimilated carbon dioxide, as measured externally, is found to enter the reduction pathways via the carboxyl group of PGA. The carbon reduction cycle is therefore by far the most important pathway for the incorporation of carbon dioxide during photosynthesis. 2) Five percent or more of the entering carbon dioxide enters via carboxyllation of PEPA leading to malic acid and aspartic acids and probably to glutamic acid, although the pathway to the latter compound is not completely understood. Altogether, about 20% of all the carbon taken up finds its way into amino acids in photosynthetic reactions under the conditions of this experiment. These amino acids (including alanine and serine) presumably give rise to other amino acids and eventually to protein.

In conclusion, it may be said that through the employment of tracer

elements, particularly carbon fourteen, the pathway of carbon reduction during photosynthesis has been mapped and the resulting pathway has been demonstrated quantitatively to account for most or all of the carbon reduced during photosynthesis. From the nature of this pathway, the requirements for energetic cofactors, derived ultimately from the light reaction, have been established. The detailed mechanism by which these cofactors are formed in the light reaction, as well as the mechanism by which water is split and O_2 is evolved, are not as yet known, but studies with isolated chloroplasts have demonstrated a stochiometric relation between the evolution of oxygen and the formation of both cofactors, ATP and TPNH. Electron spin resonance measurements of plants in the light suggest the possible formation of ions containing unpaired electrons and free radicals in the early stages of the conversion of light energy to the stored energy of chemical bonds.

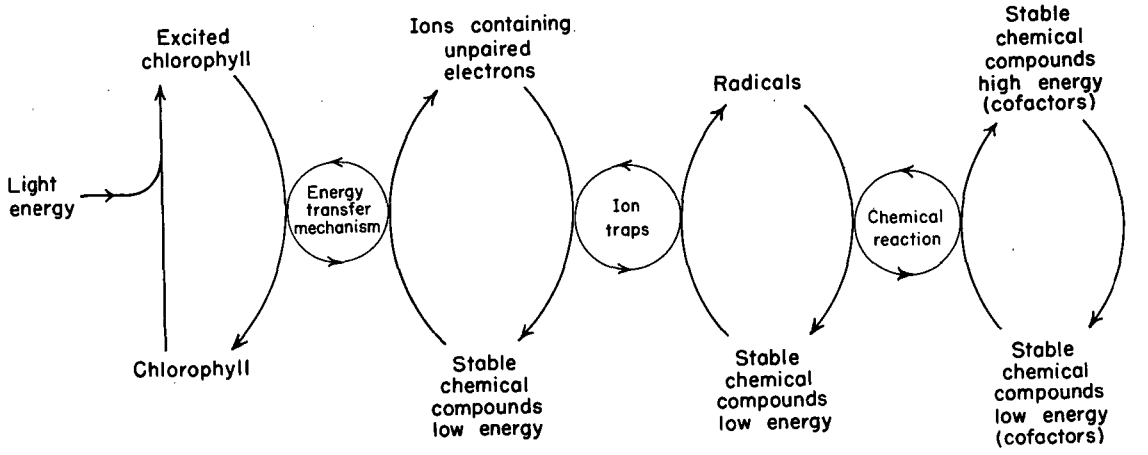
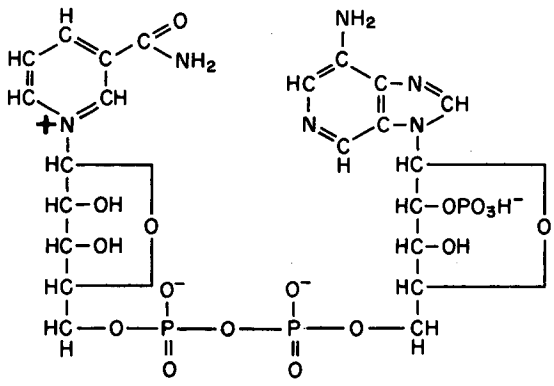
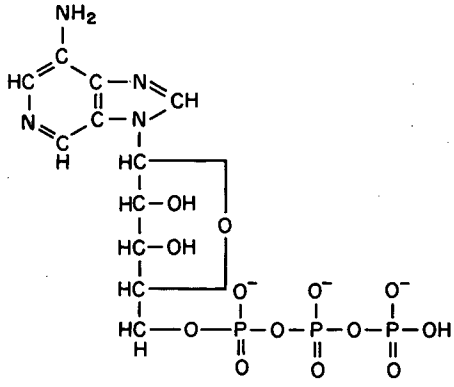


Fig. 1. Mechanism of Conversion of Light Energy to Chemical Energy.

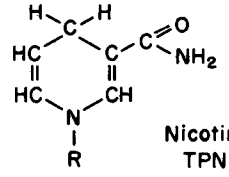


Triphosphopyridine nucleotide (oxidized form) (TPN^+)



Adenosine triphosphate (ATP)

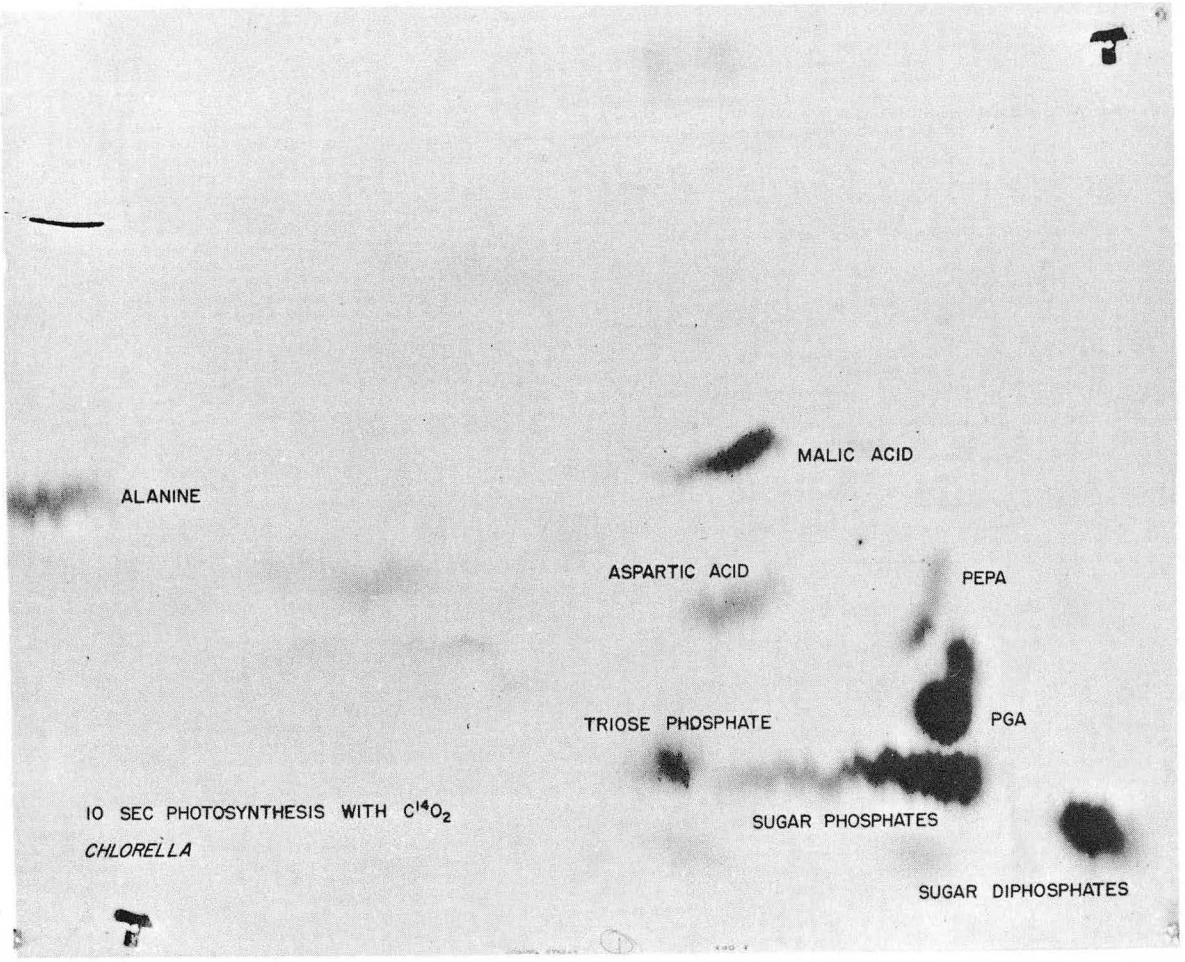
In Adenosine diphosphate (ADP), terminal phosphate is replaced by −OH.



Nicotinamide portion of TPNH (reduced TPN^+)

MU-17251

Fig. 2. Formulae of TPNH and ATP.



ZN-2146

Fig. 3. Radioautograph of Chlorella Extract after 10 seconds Photosynthesis with $C^{14}O_2$.

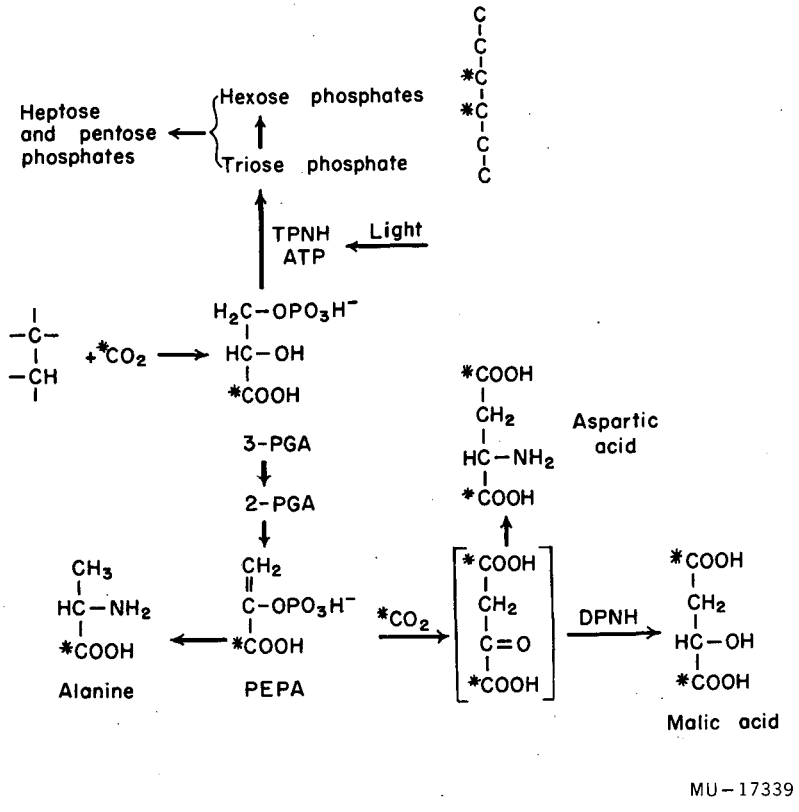
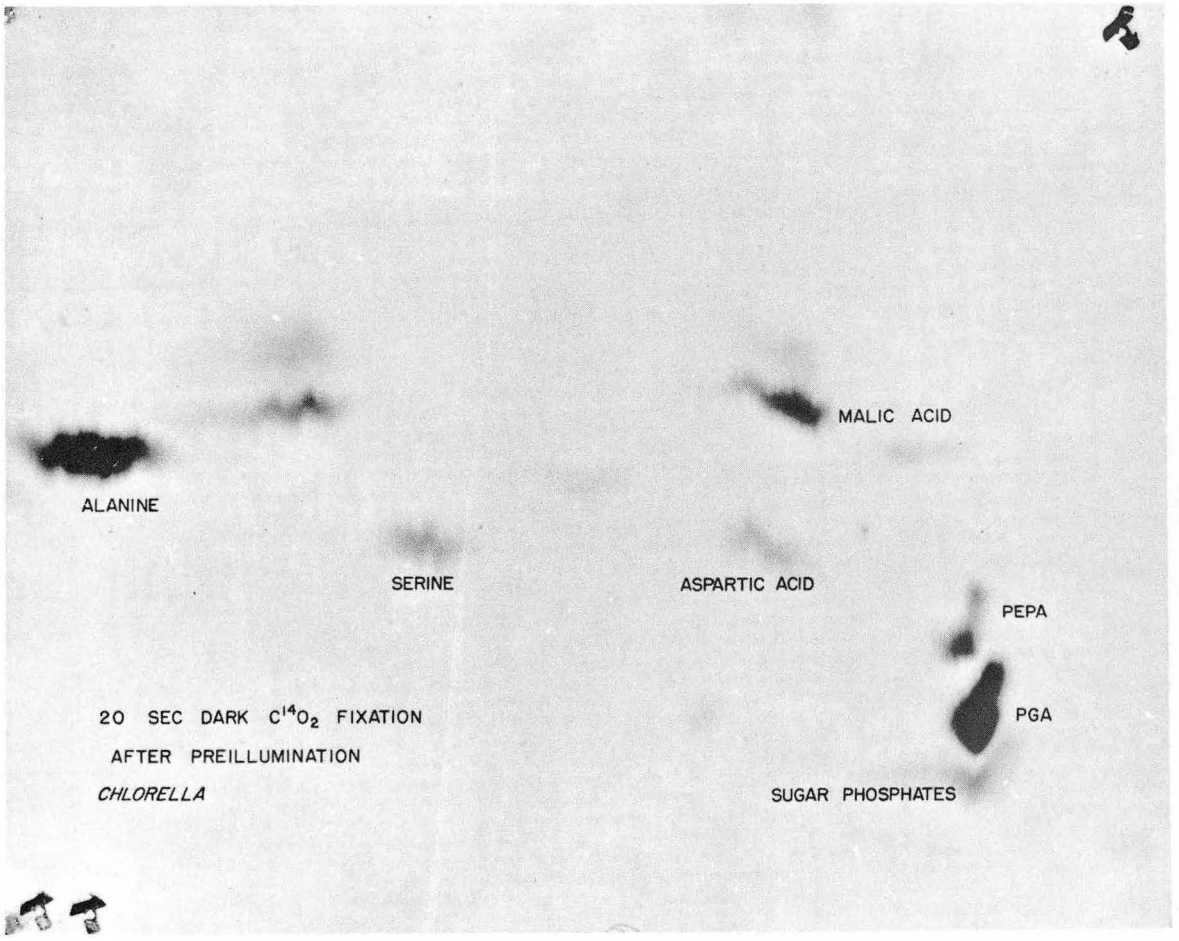


Fig. 4. Pathways of $C^{14}O_2$ Incorporation in Early Stages of Photosynthesis.



ZN-2145

Fig. 5. Radioautograph of Chlorella Extract after 20 seconds with $C^{14}O_2$ in the Dark.

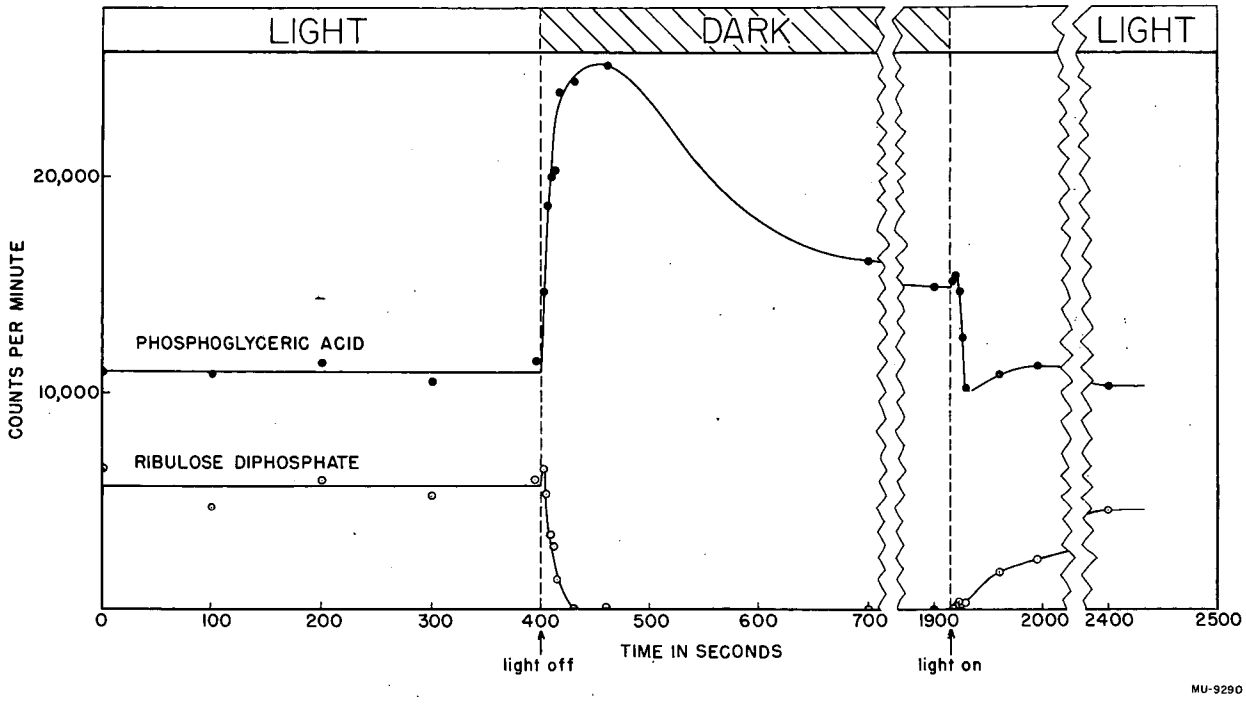


Fig. 6. Light to Dark Concentration Changes in PGA and Ribulose Diphosphate.

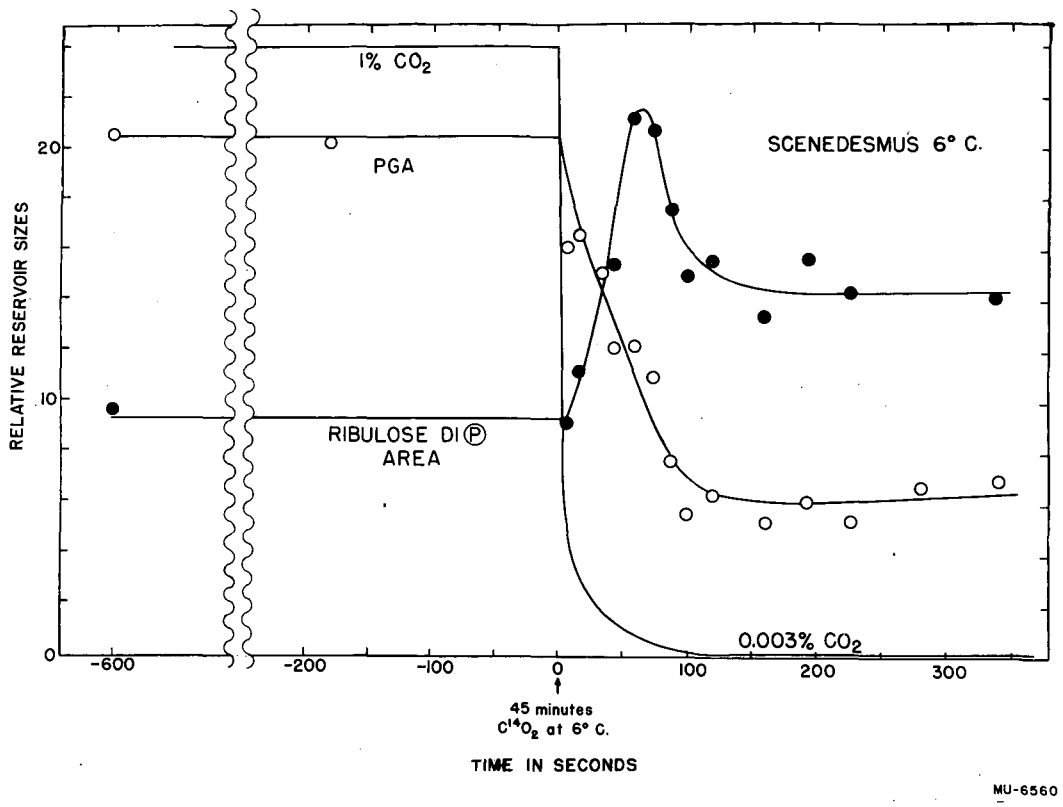


Fig. 7. High CO_2 to Low CO_2 Concentration Effect on PGA and Ribulose Diphosphate Concentrations.

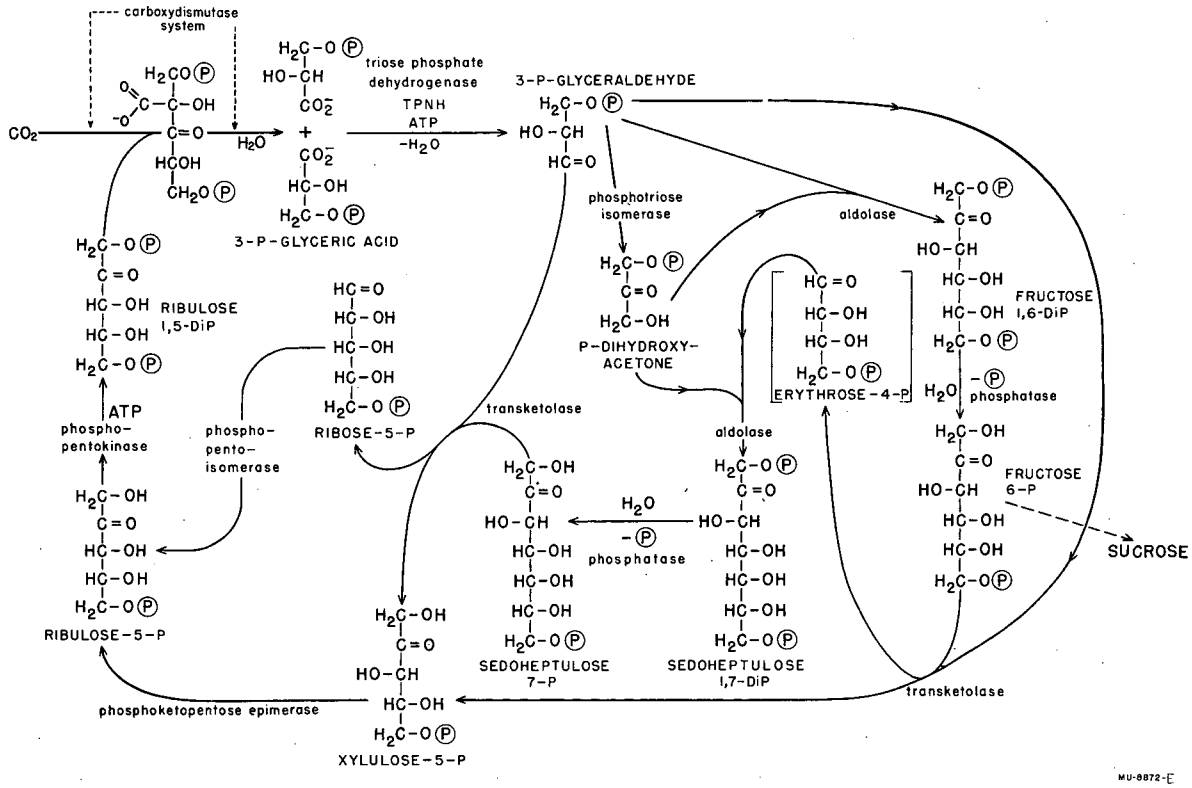


Fig. 8. The Carbon Reduction Cycle of Photosynthesis.

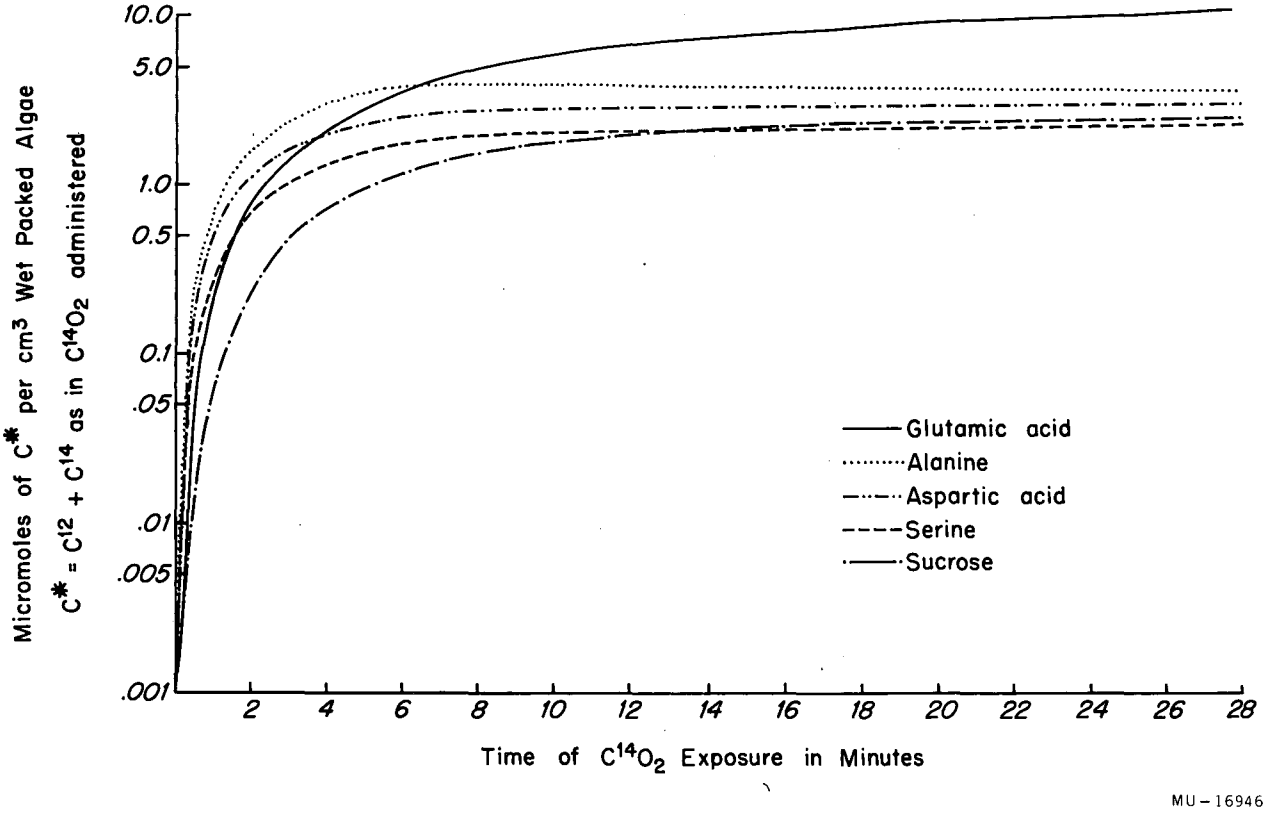


Fig. 9. Typical Labeling Curves of Biochemical Intermediates in Photosynthesis.