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PHOTOSYNTHESIS

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Photosynthesis, the process whereby all green plants derive their energy for life and thus ultimately make possible the existence of animal life on earth, is commonly schematized as a photolytic dissociation of water molecules coupled with the reduction of free carbon dioxide from the atmosphere (Figure 1). The complex series of reactions occurring during this conversion of light energy into bound chemical energy remained largely unknown until about 1945 because of the lack of any suitable means of following the rapid chemical transformations. Since that time, however, the availability of pile-produced carbon-14, a long-lived radioactive isotope, has made it possible actually to follow the path of carbon atoms from the state of free carbon dioxide through the extremely rapid reactions leading to carbohydrates such as glucose and sucrose. A full description of the experimental details of this method and the results have been reviewed.[1]

PRIMARY QUANTUM CONVERSION

One of the most fundamental unsolved problems in photosynthesis involves the means whereby chlorophyll converts the electromagnetic energy of an absorbed photon into the chemical potential necessary to reduce carbon ([H] of Figure 1) and to oxidize water to molecular oxygen ($[O] \longrightarrow O_2$, Figure 1). A possible approach to this question consists of physical measurements of the early changes induced by light in intact plant material.[2] With this in mind, we have begun an investigation of the light-induced electron spin resonance (ESR)[3] absorption (as a result of unpaired electrons), and the delayed light emission[4] and dielectric loss effects of isolated spinach chloroplasts. These studies have revealed a series of temperature-dependent luminescences (with lifetimes ranging from a tenth of a second to many seconds) which appear to have counterparts in the decay of the unpaired spins (see Table I).

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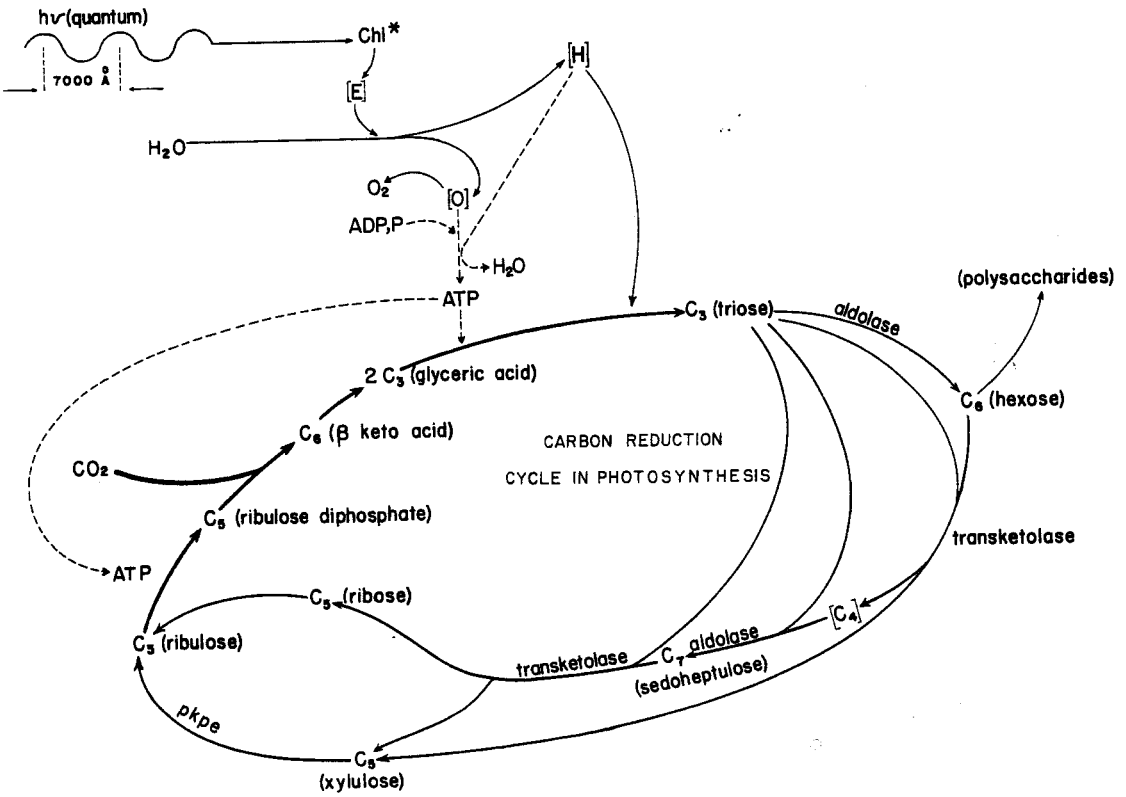


Figure 1. Suggested cyclic scheme for relationships in photosynthesis.

TABLE I

Comparison of ESR and Luminescence Observations on Chloroplasts

Material	T (° C)	Relative ESR Light Signal	Rise Time		Decay Time	
			ESR**	600-800 mμ Luminescence**	ESR**	600-800 mμ Luminescence**
Wet fresh chloro-plasts	25	3	< 0.2 sec*	< 0.1 sec*	< 1 sec (60%) 10 sec (40%)	0.15 sec (6%) 2 sec (94%) 15 sec
	-35	9	< 1 sec* (75) 12 sec (25)	< 0.1 sec*	<1 sec (33%) 10 sec (33%) 3 min (33%)	0.15 sec (75%) 2 sec (25%)
	-140	4	< 1 sec	no signal	~ hrs	no signal
Dried chloro-plasts	25	-	~ min	no signal	~ hr	no signal
	60	-	~ sec	-	~ sec	~ sec

* Excited by wavelengths between 350 and 450 mμ, or 600 and 7000 mμ.

** Instrument limited.

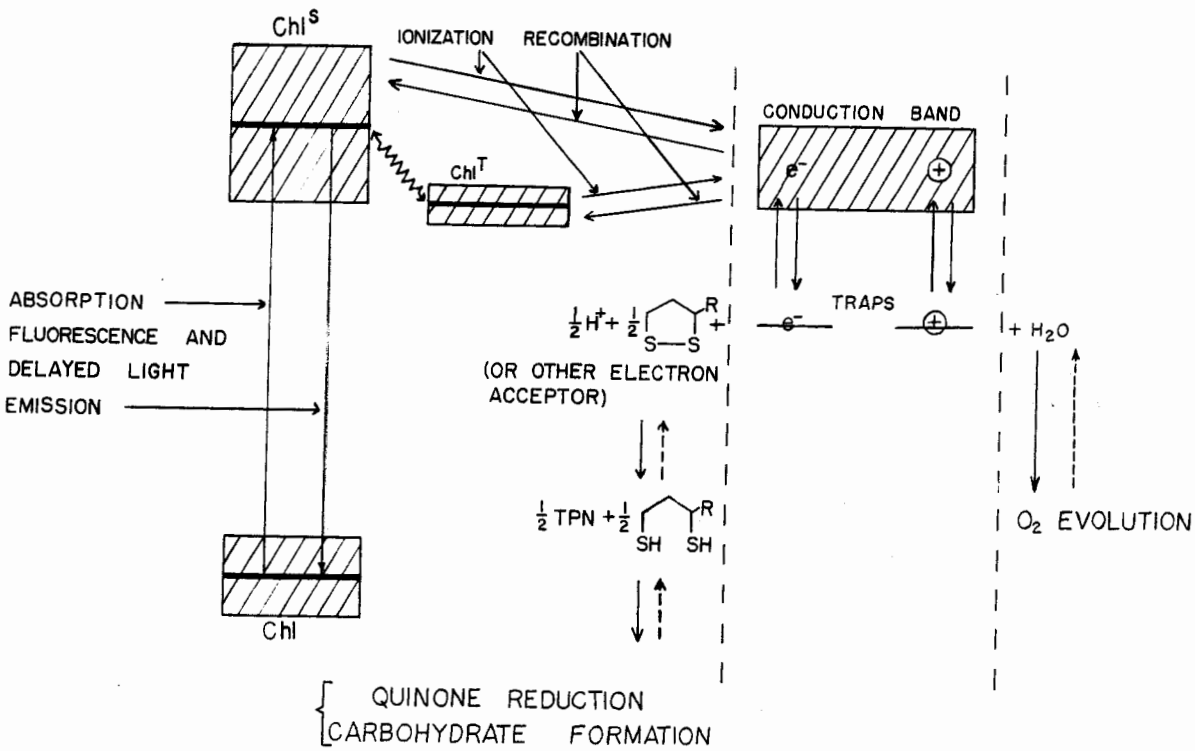
Although promising results have been obtained from the dielectric loss measurements, a more quantitative study is necessary to relate this effect to photosynthesis. The rise time for the production of at least part of the unpaired electrons is independent of the temperature over the range from 25°C to -140°C. A luminescence with a lifetime of a few milliseconds has also been observed which is temperature-independent over this same range. Both the spin resonance and the luminescences are excited by light absorbed by chlorophyll and the luminescence at both room temperature and at -80°C is a result of the transition between the first excited singlet state and the ground state of chlorophyll.[5] Furthermore, drying the chloroplasts causes the luminescence to disappear and results in the appearance of a large nondecaying spin resonance signal and endows the chloroplasts with the property of thermoluminescence.

The above observations have been interpreted in terms of the theory proposed by Katz[6] and by Bradley and Calvin[7] which involves the formation of electrons and holes in the conduction bands of an aggregate of chlorophyll molecules. A schematic representation of these bands is shown in Figure 2. Light is absorbed to produce the transition from the ground state band of an aggregate of chlorophyll molecules to the first excited singlet state band. Singlet state excitons may then undergo one of three competing processes:

(1) They may decay to the ground state via fluorescence emission ($\tau \cong 10^{-9}$ sec).
(2) They may ionize with the formation of electrons and holes in conduction bands ($\tau < 10^{-9}$ sec). Calculations have shown that such a lifetime would permit the exciton to migrate over from 100-1000 molecules.[8]
(3) They may cross over in a radiationless transition into the triplet state in times as short as 10^{-12} sec.

Inasmuch as the band width will be proportional to the square of the transition probability for the ground state to excited state transition,[9] the excited singlet state will be much broader than the corresponding triplet state. Thus, there may be a good deal of overlap between the energy levels of these two states. It is necessary to postulate such overlap in order to provide a relatively temperature-independent pathway between the states to account for the inability to observe triplet state emission, even at -70°C.

If the triplet state conversion is important in chloroplasts, ionization into the conduction bands may occur from this state. The electrons and holes in the conduction band will migrate and ultimately be trapped at suitable points in the lattice. Characteristic lifetimes of 0.01 to 0.1 second have been observed in many types of experiments on photosynthetic materials.[3,4,7,10] According to the present hypothesis, this would represent the time required to fill the electron and hole traps. If ionization occurs from the singlet state, this time constant may be identified with the lifetime of one of the charge carriers in the conduction band. The hypothesis of such a long carrier lifetime has some support in other systems, for example, in germanium, where the intrinsic hole lifetime



PROPOSED SCHEME FOR VARIOUS PHOTOCHEMICAL PROCESSES IN PHOTOSYNTHESIS

Figure 2. Proposed scheme for various photochemical processes in photosynthesis.

is calculated to be as long as 0.75 second.[11] Experimentally, lifetimes on inorganic semiconductors may range from 10^{-13} seconds to several seconds.[12] No corresponding measurements have been made for organic semiconductors. If, on the other hand, ionization occurs from the triplet state, the 0.01 to 0.1 second time constant may represent either the ionization time constant or a carrier lifetime.

It is not possible, at present, to decide which of the two mechanisms -- direct ionization from the singlet state or ionization from the triplet state -- is operative in chloroplasts. Indeed, it may be that both processes occur simultaneously.

The number of traps in the chloroplast is probably very small, perhaps of the order of one per several thousand chlorophyll molecules. Thus, this scheme leads directly to the idea of a 'photosynthetic unit.'[13] The electrons and holes that are trapped give rise to a spin resonance signal. In the absence of biochemical acceptors, the traps are thermally depopulated and the resultant electrons and holes in the conduction band recombine and a temperature-dependent luminescence results. Such recombination may occur directly into the singlet state or into the singlet state via the triplet state. The long-lived temperature-dependent emissions can be identified with the depopulation of traps of different depths. Further experimentation is in progress to determine the nature of the millisecond decay. This might represent the lifetime of one of the charge carriers in the conduction band or perhaps the lifetime of the triplet state as well as the 0.01 second decay reported by Arthur and Strehler.[10]

At low temperature, the thermal energy is insufficient to excite the electrons and holes out of the traps and enzymatic production and decay of radicals no longer occurs. This results in the disappearance of the luminescence and the appearance of a long-lived ESR signal. The thermoluminescence referred to earlier may be the result of a deepening of the trapping levels due to drying.

The electrons and holes in the traps may also be used up by enzymatic processes. Any reversibility in these enzymatic processes would then lead to a long-lived luminescence which could be classified as a chemiluminescence. It is likely that some of the longer-lived emissions reported by Strehler and co-workers,[14,15] are of this nature. If these enzymatic processes involve free radicals, similar decay times will occur in the spin resonance analysis. We have observed that almost three times as much energy is emitted as light in aged chloroplasts as in fresh chloroplasts, suggesting that these enzymes are easily inactivated. A similar increase in the number of light-induced radicals in aged chloroplasts is found in spin resonance experiments. These observations suggest that enzymatic utilization represents the normal pathway for most of the electrons and holes in the living cell. In this way the light energy could be made available to the photosynthetic mechanism.

The trapped electrons will lead to the production of the active hydrogen ([H] of Figure 1) while the trapped holes will lead to the production of the intermediate oxidant ([O] of Figure 1).

THE PATH OF OXYGEN IN PHOTOSYNTHESIS

The use of isotopic oxygen in tracing the path of the oxygen atom from water to molecular oxygen has been difficult. The half-lives of the radioactive isotopes of oxygen are all very short, so in general it is necessary for oxygen tracer studies to use one of the two naturally-occurring stable isotopes, O^{17} or O^{18}. Both are available in enriched form, and the isotope chosen was O^{18}, which was used as water in an enrichment of 20%.

Tracer Oxygen

The main reason for this choice was that this isotope has a high capture cross-section for protons, yielding F^{18} by the reaction $O^{18}(p,n)F^{18}$. F^{18} has a half-life of 1.8 hours and emits positrons of an energy of 640 KeV. Calculations showed that 0.1 to 1 μg of O^{18} could easily be detected by radioactivity measurements of F^{18} if a 4 MeV proton beam of 1 to 10 μa was allowed to hit the oxygen target for a few minutes. After one hour aging in a bombarded sample of organic material containing enriched O^{18}, interfering radioactivity from carbon, nitrogen and other oxygen isotopes was negligible if protons of an energy of about 4 MeV were used for the bombardment. A qualitative analysis of the oxygen which is incorporated in algae grown in O^{18}-enriched water for short periods of time was attempted.

Ethanol extracts of the algae were made, the extract was concentrated and chromatographed in one dimension, using butanol-propionic acid-water solvent. In order to avoid an impossibly high background resulting from naturally-occurring O^{18} in the filter paper, the spots were transferred onto a tantalum sheet by serrating one edge of the one-dimensional chromatogram and eluting the spots sideways off the paper onto a heated strip of pure tantalum. The pattern of the chromatogram on the paper was thus maintained as a series of drops dried onto the metal strip. A control experiment to test the reliability of this transferring method, using a chromatogram of C^{14}-labeled substances, showed that the pattern of the chromatogram could be reproduced in this way on the metal. The tantalum strip, about 10" long, 2" wide and 0.006" thick, was used as the target, being clamped in a holder in which the metal strip was held as a cylinder. The target strip was rotated in front of the proton beam, while inside the cylindrical strip a jet of air was directed at the target point for cooling. The proton beam was collimated into a cross-sectional area of 3/4" x 1/4". The total bombardment time was two hours with 4.5 ± 0.2 MeV protons. The average current during this time was 2.75 μa. The target was rotated in front of the beam at 160 rpm; as the length of the target was about 10" and the width of the beam 1/4", each spot was exposed to the beam for a total of three minutes.

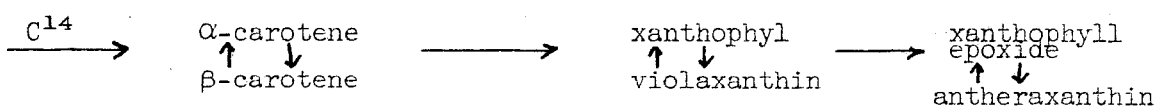
A radioautograph was then taken of the bombarded tantalum strip, using X-ray film, in order to locate the positions of the F^{18} derived by bombardment from the O^{18}. The eluted chromatogram showed three peaks of radioactivity: these were not unequivocally identified but appeared to correspond with the mono- and diphosphates and phosphoglyceric acid areas on a paper chromatogram as they usually appear in $C^{14}O_2$ fixation experiments.

To test the sensitivity of the O^{18} analysis, Chlorella was grown for 2-1/2 days in O^{18}-enriched water. The algae were washed twice with distilled water and five samples were evaporated as spots on the area of the target sheet. The amounts of the algae in the different spots were 85,15,7.5,4 and 2.5 μg and the amounts of O^{18} were 5,1,0.5,0.2 and 0.1 μg, respectively. A control sample of algae, not treated with O^{18}, was similarly evaporated onto the target sheet; the amounts of algae in the three spots produced were 40,4 and 2 μg. After bombardment and radioautography, the spot containing 2 μg of algae and 0.1 μg of O^{18} could still be detected against the background. The radioautographs of the untreated algae show that 40 μg gave rise to a very slight dark spot; this was probably due to the F^{18} formed from the 0.2% of O^{18} in the ordinary oxygen of the algae. The radioautographs of the two smaller amounts of algae showed no dark spot at all. The method thus appears to have the necessary sensitivity and specificity for this sort of tracer study.[16]

Carotenoid Participation in Oxygen Transfer

Using a completely different technique, some progress has been made in a study of the biosynthesis of the photosynthetic pigments, with particular emphasis on the role, if any, of carotenoids for oxygen transfer within the photosynthetic mechanism. The algae were incubated in the presence of radioactive carbon dioxide, both in the light and in the dark, for varying amounts of time. Column chromatography, using polyethylene, cellulose or magnesium oxide as adsorbents, was used for the separation of the pigments; their concentration was determined spectroscopically. Further separation of each fraction was achieved by two-dimensional paper chromatography with suitable solvents.

It was found that chlorophyll a became radioactive considerably faster than chlorophyll b. From the data obtained, it would appear that the path of carbon through the carotenoids of the algae is as follows:



Most of the pigment concentrations showed little difference between the light-treated and the dark-treated algae. However, there was a marked increase in the concentration of violaxanthin in the dark, which is reversed in the light, suggesting that violaxanthin may be involved in the transfer of oxygen. A similar result was observed by D. I. Sapoznikov.[17]

THE PATH OF HYDROGEN IN PHOTOSYNTHESIS

Photosynthesis involves two main processes: the photolytic splitting of water, followed by the utilization of the active hydrogen so produced to reduce the incoming carbon dioxide, with the concomitant release of molecular oxygen. Work has now been started in this laboratory to investigate the pathways of hydrogen in photosynthesis.

An early program to use deuterated cells to follow the path of hydrogen was abandoned when it was found that such cells showed distinct pathological characteristics and that the quantities required for the detection apparatus (nuclear magnetic resonance) would involve the use of very large amounts of cell material and would necessitate the isolation of intermediates on a large scale. In addition, deuterium is a stable species, and the techniques which had been used so successfully to follow the path of carbon by making use of the radioactivity of C^{14} could not be applied in studies utilizing deuterium.

There remained the possibility of using the radioactive isotope of hydrogen, tritium, to follow the uptake of hydrogen from radioactive water by cells actively carrying out photosynthesis. Many difficulties had to be overcome, not the least of which was the very weak energy of the β-particles emitted by tritium, and the large dilution of the radioactive tracer by the relatively enormous amounts of water which are inevitably present in any biological system. The energy of the β-particles emitted by tritium averages about 18 KeV, as compared with about 150 KeV for those emitted by C^{14}. This means that the penetrating power of the particles is very much diminished as compared with those from carbon, and their ability to pass through paper and affect photographic film is correspondingly reduced.

Detection of the radioactive materials on paper chromatograms would become considerably more difficult for this reason, and to compensate in part for the weaker radiation, larger amounts of the radioisotope would have to be used. In systems in which the cells are supplied with radioactive carbon dioxide, the system can be so arranged that the supply of unlabeled carbon dioxide to the cells is reduced to a minimum, keeping the specific activity of the added tracer high. This is not possible with water; the only hope is to use cell suspensions much more concentrated than those used for the carbon studies, in order to achieve a more favorable substrate-to-cell ratio. This, in turn, presented new difficulties; as the cell concentration in the suspension was increased, the optical density of the suspension rose very considerably and the amount of light passing through the suspension was correspondingly reduced.

The problem was finally solved in two ways. The conventional 'lollipops' were abandoned in favor of small cylindrical vessels with flat bottoms, of such a size that 1 ml of liquid in them formed a layer on the bottom 1 mm thick. The vessels were shaken for the incubation period over a bank of fluorescent lights and the cell concentration was increased from the usual value of a 1% suspension (1 ml of wet-packed cells/100 ml of suspension) to a concentration of 12 to 25%. The second modification from the carbon work was to increase the dose of labeled tracer added from about 20 μc/ml in the carbon work to a specific activity in the tritium studies of one curie of tritiated water/ml. In this way, some effort was made to overcome the disadvantages of the very weak radiation. Owing to the health hazards of working with such high specific activities of radioactivity, all the operations up to the stage of chromatography were performed in 'glove boxes', through which a rapid draught of air was maintained by a suction fan, and the whole system was very carefully monitored to ensure that no radioactivity escaped from the confines of the box and the venting system.

The experiments were performed essentially in the same way as the carbon experiments described in a later section. The cells were aerated with a constant stream of air containing CO_2 and at a certain time the one-curie sample of tritiated water was added. After the desired time of incubation in the light, the cells were killed and extracted with ethanol. The ethanol extracts of the cells were evaporated to dryness and redissolved in ordinary distilled water four times in order to wash out any exchangeable tritium present in the compounds extracted from the cells, leaving the tritium present only in nonexchangeable positions. The final residue was dissolved in water and chromatographed in the usual way, and the chromatograms were exposed to X-ray film to find the locations of the radioactive materials. Similar experiments were performed with cells exposed to tritium oxide in the dark.

This work is still in the preliminary stages, but the results so far have shown that tritium is incorporated into a number of compounds in the course of three minutes; these substances appear to be the same ones as those containing C^{14} after the cells are exposed to $C^{14}O_2$, though the relative distribution of activities is quite different with the two tracer substances.

A positive confirmation of the nature of the substances incorporating tritium from water is still in progress, but there is, nevertheless, good reason for supposing that label appears in the amino acids glutamic acid, aspartic acid, alanine and serine plus glycine (the latter two amino acids are not well separated by the chromatographic techniques in use for this work), in the organic acids glycolic acid, malic acid, citric acid, fumaric acid and succinic acid, and in phosphoenolpyruvic acid, phosphoglyceric acid (PGA) the sugar monophosphates and diphosphates, and possibly also in uridine diphosphoglucose.

While cells incorporate C^{14} from $C^{14}O_2$ during photosynthesis for short periods mainly into the sugar phosphates and into sucrose (these two groups of substances frequently account, after three minutes, for 75 to 85% of the total labeled substances present in the ethanol and water extracts of the cells), tritium is found after three minutes largely in glutamic, aspartic and malic acids, and alanine, which together contain about 63% of the soluble tritium fixed (excluding glycolic acid -- see below). The amount in the sugar phosphates is very much smaller (about 28%) and none appears in sucrose. Most significant is the amount appearing in glycolic acid. This substance is quite volatile, yet even after the cell extract had been evaporated to dryness four times, glycolic acid remains the most radioactive spot on the chromatogram. It seems probable that the overwhelmingly greatest quantity of tracer is incorporated into glycolic acid. The significance of this is not yet known. This is a preliminary conclusion as to the pattern of tritium incorporation and a final conclusion is dependent on the confirmation of the identity of the compounds involved. Although larger amounts of tracer are incorporated in the light than in the dark nevertheless considerable amounts are also incorporated in the dark (far greater, in proportion, than with carbon) and the identity of the compounds in which tritium appears in the light and in the dark is similar.

The interpretation of these results is likely to be a complicated matter. While the tritium may be incorporated by genuine biochemical reactions into many compounds under investigation, it is also possible that tritium may originally enter some substances by nonspecific exchange reactions, and, subsequently, be relocated into nonexchangeable positions. Further, tritium, incorporated by a genuine biochemical reduction into a nonexchangeable position, may later be moved to an exchangeable one and hence be lost in the repeated evaporations. If the latter are not performed, it is probable that every compound on the paper which contains exchangeable hydrogen atoms will show radioactivity, whether or not it had played any part in the metabolic reactions under examination. In spite of all these difficulties, it seems likely that the use of tritium will eventually enable us to learn something of the path of hydrogen, although not with the ease and completeness which our studies with C^{14} have taught us the path of carbon in photosynthesis.

THE PATH OF CARBON IN PHOTOSYNTHESIS

The essence of the experimental procedure employed in C^{14} tracer studies is to maintain plant material, either an alga or a leaf from a higher plant, in controlled, constant conditions under which photosynthesis is allowed to continue in the presence of carbon-14 for varying lengths of time. The cells are then killed rapidly and the soluble components are extracted, and assimilated radioactive carbon is analyzed by means of chromatographic separation of the soluble components of the plant material. Not only is it determined which compounds contain the carbon-14, but by degradation of these isolated compounds it can be determined exactly which carbon atoms within the molecule have become labeled.

By variation in one or more of the experimental conditions, such as light intensity, temperature, CO_2 concentration, length of exposure to $C^{14}O_2$, etc., it was possible to study the interrelationships of many of the suspected intermediates involved in the photosynthetic fixation of carbon dioxide. The first stable compound into which C^{14} was fixed was seen to be the three-carbon sugar acid, 3-phosphoglyceric acid (PGA). This compound is then reduced to triose phosphate, two molecules of which combine by a reverse aldolase reaction, to form fructose diphosphate. From fructose diphosphate, fructose monophosphate is formed by the loss of a phosphate group, and glucose phosphate produced by the action of phosphoglucoisomerase. Sucrose, starch and other polysaccharides are built up from units of glucose formed in this way from carbon dioxide.

However, of the three carbon atoms in each molecule of phosphoglyceric acid, only one is derived in the first place from carbon dioxide, and for some time a search was made for the two-carbon fragment which was presumed to combine with CO_2 to form PGA. Eventually, it was discovered that the initial carboxylation reaction of ribulose diphosphate (a five-carbon sugar phosphate) is followed by a dismutation and hydrolysis of the product to produce two molecules of PGA. In time it was possible to work out the whole sequence of sugar rearrangement reactions by which new ribulose diphosphate was continuously synthesized from triose phosphates for this purpose, and a cyclic relationship among the sugar phosphates and PGA emerged.

For each complete turn of the cycle, one molecule of CO_2 is fixed, and one molecule of ribulose diphosphate is resynthesized ready for the next carboxylation. After six turns of the cycle a six-carbon sugar (hexose) such as glucose or fructose is formed, and after a further six turns, two monosaccharides combine to form a disaccharide, sucrose. This cyclic arrangement known as the 'photosynthetic carbon cycle', is shown in its present form in Figure 3.

New Intermediates in the Photosynthetic Cycle

In the past two years, we have obtained some evidence for the presence in the algae for an intermediate which had been postulated in earlier versions of the cycle, but which had not yet been identified. This intermediate is the β-keto acid which is believed to be the first product of the carboxylation of ribulose diphosphate and which gives rise to two molecules of PGA (Figure 4). Very small traces of this β-keto acid diphosphate (2-carboxy,3-ketopentitol-1,5-diphosphate) have been tentatively identified in extracts prepared from algal cells. The compound is very unstable, and most of the amount originally present in the plants is probably decomposed by the extraction and analytical techniques, particularly chromatography in acid solvents. However, enough of this substance has been obtained to provide a tentative identification consistent with the structure of the proposed β-keto acid.[18]

In addition to this keto acid, another keto acid has been found in much larger (though still rather small) amounts. The latter compound is much more stable than the β-keto acid, and has proved to be an isomer of the latter, i.e., the γ-keto acid, 2-carboxy,4-ketopentitol-1,5-diphosphate. We are not yet certain of the exact stereochemical configuration of either of these two compounds, and it cannot be said as yet that this γ-keto acid is a biochemical intermediate of metabolism rather than an artifact of our analytical procedures.[18]

A second intermedite, proposed some years ago as playing a part in the cycle, but which had remained undetected, is erythrose phosphate. Recently, we have found a weakly radioactive spot on our chromatograms which appears to correspond satisfactorily, after removal of the phosphate group, with authentic tetrose. However, although this is probably indeed the missing tetrose, this work has not yet been fully confirmed, and the unknown substance may turn out to be something else. It is, incidentally, no coincidence that the substances we are now finding are present either in very minute quantities or are very unstable, or both. Obviously, such compounds are ordinarily the last members of a biochemical sequence to be discovered, and their presence may well go undetected until their existence is predicted by hypotheses based on the study of more easily detected substances.

Carboxydismutase Reaction

A study of the carboxydismutase enzyme system is being carried out *in vitro*. *Tetraselmis* leaves are generally used as the source of the enzyme which is obtained from the leaves by acetone powders, isolated chloroplast fractions,[19] or directly from crude extracts. Further purifi-

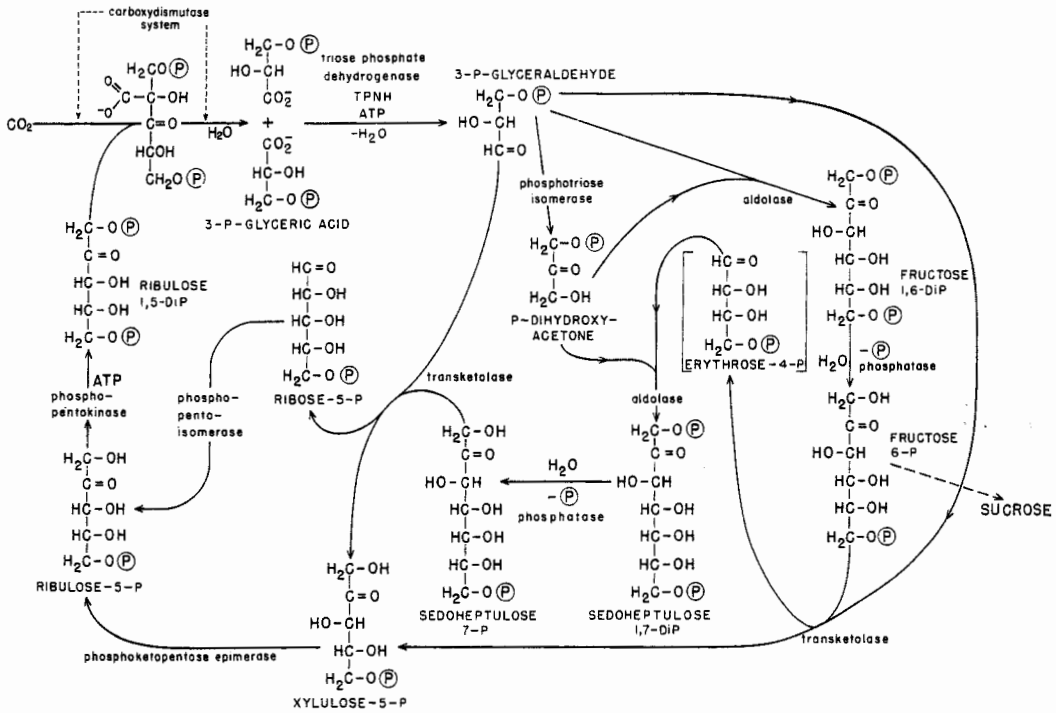


Figure 3. The Photosynthetic carbon cycle.

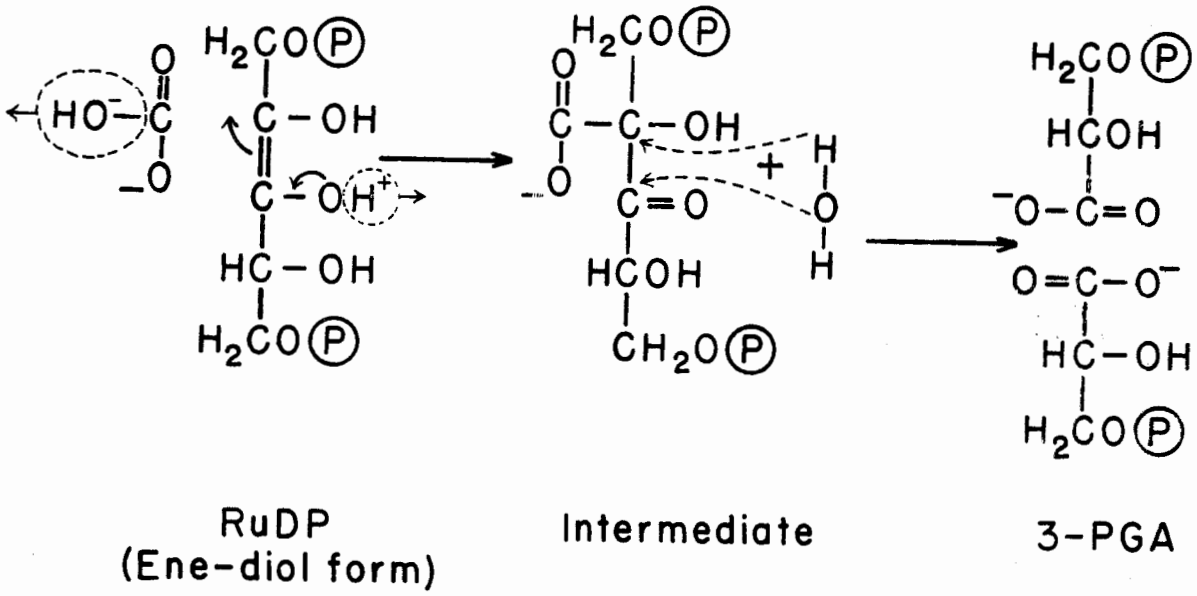


Figure 4. The carboxydismutase reaction.

cation has been effected by ammonium sulfate precipitation and by paper electrophoresis. In no case has there been any apparent separation of the enzyme functions which catalyze the formation of the ene-diol form of ribulose diphosphate, 2-carboxy,3-ketopentitol diphosphate, or the final product of the reaction, 3-phosphoglyceric acid. Although the enzyme has been found to be associated with isolated chloroplasts, there is no guarantee that the enzyme resides entirely in these particles, as at least 5% of the enzymatic activity can be washed free from the chloroplasts by sodium chloride within one hour. The enzyme is either located on the periphery or is specifically adsorbed onto the chloroplast. Preparations of chloroplasts in nonaqueous solvents, such as hexane and carbon tetrachloride,[20] have shed no new light on this matter.

One aspect of the reactions undergone by carbon in photosynthesis which deserves further careful examination is whether or not there exists some 'active' organic complex of CO_2 which might serve as the substrate for the carboxylation of ribulose diphosphate, in much the same fashion as 'active' acetate is transferred by acetyl Coenzyme A. There is some evidence indicating that the enzymatic carboxylation of ribulose diphosphate to yield PGA may not be rapid enough to account for the required rate in the intact plant if free CO_2, or an inorganic species thereof, is the actual substrate for this reaction. Thus, on the basis of in vitro enzymatic tests, Weissbach, et al.[21] and Pon[22] have reported that the turnover rate for this reaction is slower than would be required in the intact plant, assuming that the estimates for the total enzyme concentration are approximately correct. Also, Racker[23] has reported the unusually high value of 10^{-2} for the Michaelis constant of the CO_2 substrate.

Although there is no direct evidence at the present time indicating such an 'active' CO_2 transferring system, work by Metzner, et al.[24] has recently indicated that there may be very unstable complexes of carbon dioxide formed which are too unstable to survive the methods of isolation and thus are not usually observed. Metzner, et al. found that when cells are killed with acetone at -40°C and the extract is subsequently allowed to warm up to room temperature, a considerable fraction of the activity found in the cold extract is lost on warming. However, the total fixed activity in cold ethanol- or cold acetone-killed cells, including the unstable activity, is not significantly greater than the stable radioactivity on killing with boiling ethanol. All the measurements on which these results depend were made by combusting the samples in tubes which were sealed before the samples were allowed to warm up. The resulting $C^{14}O_2$ was assayed in an ionization chamber.

If there exists any transferring mechanism for 'active' CO_2 it might be expected that when the photosynthetic cycle is inhibited, then more 'active' CO_2 might be available for other carboxylation reactions. It is interesting to note that when the photosynthetic uptake of CO_2 in an alga such as Chlorella is inhibited by heavy water (D_2O),[25] much more activity than usual is found in citrulline, which can arise from ornithine by carboxylation and amination. Whether or not this conjecture has any validity remains to be seen.

It might be supposed that there would be a central form of 'active' CO_2 which could be used for any of the half-dozen or more carboxylation reactions which are known to occur. However, most of these carboxylations can occur equally easily in the light or in the dark, while Metzner, et al.[24] obtained evidence suggesting that an 'active' CO_2 was formed in the light and not in the dark. It is therefore possible that this labile compound inferred by Metzner, et al. is not a central form available for one of a number of functions, but may be a large pool of the unstable β-keto acid mentioned earlier. Following our usual techniques of extraction and chromatography, no more than a minute trace of this β-keto acid has been dtected, but it has, as one would expect on chemical grounds, been found to be a very unstable compound, and far larger amounts of it may be present in the living cell. This would account for the formation of 'active' CO_2 in the light but not in the dark, as the presence of the β-keto acid would be dependent on the pool of ribulose diphosphate and this, in turn, is known to be formed by a light-dependent reaction.[1]

Biosynthetic Pathways

The prime function of the photosynthetic carbon cycle is that of fixing and reducing carbon dioxide to organic material of a form which can be passed on to the relevant sites in the plant for use as a building material for all those hundreds or thousands of constituents which make up living cells. The production of polysaccharides and sucrose from glucose derived from the cycle has already been mentioned. Another connection of the cycle with further synthetic activities is by the conversion of PGA to pyruvic acid (Figure 5). From here, many important reactions can proceed. Transamination yields alanine; serine, glycine and cystine are also believed to be derived from pyruvic acid. Carboxylation, followed by transamination, produces aspartic acid and its family of amino acids: threonine, isoleucine, methionine and possibly leucine. From pyruvate, carbon can enter the tricarboxylic acid cycle, either by decarboxylation of pyruvate to yield acetate, or carboxylation to form oxalacetate. The acetate and oxalacetate can then condense to form citrate and later α-ketoglutarate, from which glutamic acid is derived by transamination. Glutamic acid can give rise to two further amino acids which are found in protein: arginine and proline. In addition, glycine and glutamic acid are used in the synthesis of N-formylglycinamidine, a precursor of nucleic acid; and glycine, with succinic acid, leads through δ-aminolevulinic acid to the porphyrins, chlorophyll, cytochromes, etc. In another direction, PGA may be reduced to phosphoglyceraldehyde and then isomerized to dihydroxyacetone phosphate; the latter is reduced to glyerol phosphate and a connection made with the routes to fat metabolism.

The interconnection between some of these activities is illustrated in experiments in which the pattern of CO_2 incorporation of cells suspended in distilled water is compared with those supplied with a readily assimilable source of nitrogen, such as an ammonium salt. When cells are not given an external supply of nitrogen, some carbon from CO_2 appears in alanine, aspartic acid, and, to a lesser extent, in serine and glycine, and large quantities show up in sucrose and starch, the latter acting mainly as storage products of carbon and energy. However, when the cells are also

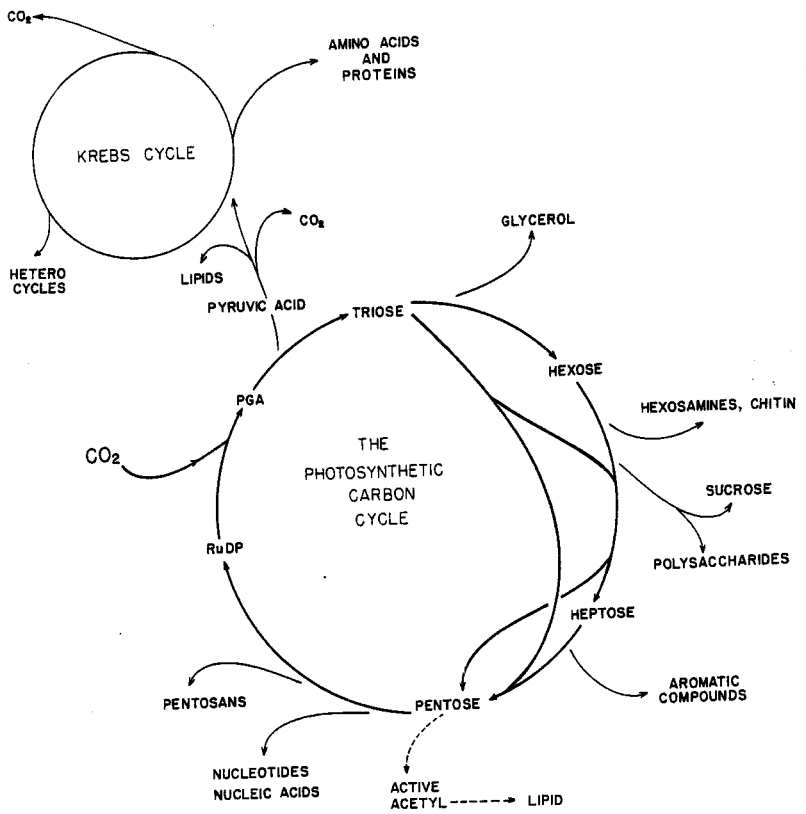


Figure 5. Biosynthetic pathways

supplied with nitrogen, the amounts of carbon appearing in sucrose are noticeably reduced and, instead, considerably larger amounts show up in the amino acids, including glutamic acid, glutamine, and sometimes citrulline, and in their associated organic acids, malic, fumaric, and citric acids. Cells supplied with nitrate rather than ammonia show a similar, though less marked effect, and it is well known from other studies that ammonia is much more rapidly assimilated than is nitrate.[20]

Thus, from the point of view of a photosynthetic organism, the carbon cycle is, in a sense, the focal point, not only for the initial uptake of carbon (one of the basic raw materials), but is also a central junction and connection between carbohydrate, fat, amino acid and protein metabolism. The net result of photosynthetic activity is the uptake of carbon dioxide from the atmosphere, its reduction by means of the energy obtained from sunlight, and its utilization in many facets of organic biosynthesis.