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PHOTOSYNTHESIS

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ABSTRACT

Some current concepts of the process of photosynthesis in green plants are briefly reviewed. The chloroplast is considered as a highly organized, largely self-sufficient entity. It is capable of photosynthesizing not only carbohydrates, but also fats, proteins, and probably all the other substances required for its replenishment and operation. The conversion of light energy to chemical energy appears to involve intermediates which are paramagnetic. The result of the early stage of photosynthesis is the splitting of water and the production of O_2, TPNH, and ATP, and possible other--as yet unknown-- cofactors. These cofactors are then used to bring about the reduction of CO_2, and other oxides to carbohydrates, fats, proteins, etc. Both stages of photosynthesis appear to require the organization of the intact chloroplast in its natural environment in the cell for maximal in vivo rates of photosynthesis. By means of tracer studies during steady-state photosynthesis in Chlorella pyrenoidosa, rates of specific reaction along several photosynthetic pathways have been measured. The carbon reduction cycle accounts for most of the fixation and reduction of CO_2 during photosynthesis. The greater part of amino-acid synthesis results in the primary formation of alanine, serine, aspartic acid, glutamic acid, and glutamine in the chloroplast.

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Photosynthesis in green plants is the synthesis of organic compounds and the production of oxygen from water, carbon dioxide, light, and inorganic ions. This process has sometimes been defined by the chemical equation

$$CO_2 + H_2O \xrightarrow[\text{green plants}]{\text{light}} [CH_2O] + O_2 \qquad (1)$$

The $[CH_2O]$ moiety, linked together an appropriate number of times, gives various carbohydrated, including sugars and starches. While this chemical equation is correct for the production of sugars, we know now that the process of photosynthesis leads also to production of a number of other organic compounds besides carbohydrates. Among these other products are amino acids, fatty acids, phospholipids, purine and pyrimidine bases, and probably a number of macromolecules including proteins, nucleic acids, and fats. The fact that these compounds must be included among the products of photosynthesis stems from tracer experiments which show that carbon dioxide, once taken up, may be converted directly to these other end products without having first been converted to carbohydrates or sugars. Thus photosynthesis is seen to involve a number of biosynthetic pathways beyond the primary carbon dioxide fixation pathway. It is clear also that the reactants in photosynthesis must include such inorganic ions as nitrate or ammonia, phosphate, sulfate, and other ions. Finally, it must be emphasized that photosynthesis is not merely a photocatalytic process. Rather, it is an energy-storing process in which the light is a reactant in a real sense. It may be viewed as the separation of oxygen from carbon dioxide, hydrogen, nitrogen, and so forth. The production of molecular oxygen and the formation of organic compounds results in a decrease in the total negative free energy of the system, and therefore in a storage of chemical energy.

This very complex series of reactions takes place in a subunit of the photosynthetic cell which is called the chloroplast. The chloroplast, surrounded by a membrane, is an entity within the cell, and is a rather complicated structure. Many details of this structure have been revealed by studies with the electron microscope.[1] Such studies show that the chloroplast is composed of layers, or lamina, which consist of lipids and proteins and other materials. More recently, sublaminar particles, roughly spherical or oblate-spherical, have been the subject of such studies.[2] Attempts to correlate specific biochemical steps with identifiable substructures of the chloroplast appear to be very promising. For some time it has been thought that the laminar structures, which contain arrays of chlorophyll molecules, are responsible for the conversion of light energy, and perhaps for the subsequent splitting of water to oxygen and some hydrogen carrier, or reducing power. It now appears from the studies by Park and Pon that the enzymes of the synthetic pathways which reduce carbon dioxide to organic compounds are also associated with some organized structure of the chloroplast.[2] More and more, it appears that the chloroplast is a highly organized factory at the microscopic level, capable of channeling energy and materials into closely organized amounts of a great variety of finished end products. This channeling of energy and matter

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involves not only the specificity of the individual enzymes, but also the complexity of the geometrical structure of the whole chloroplast. It is important that one keep in mind this structural entity when one considers the individual aspects of photosynthesis one at a time.

Let us consider photosynthesis in two successive stages. First there is a photo stage, in which light is absorbed and converted to chemical energy and in which water is split to produce oxygen and reducing power. At the same time other energetic cofactors such as adenosine triphosphate (ATP) are generated. The second stage, the synthesis stage, then occurs. The energetic and reduced cofactors are used to bring about the reduction of carbon dioxide, as well as nitrates, phosphates, sulfates and other materials, to sugars, fats, proteins, and all the other products of photosynthesis.

The photo stage of photosynthesis is an example of biochemical conversion of one form of energy, in this case electromagnetic, to another form, which in this case is chemical. Such processes have been notoriously resistant to the investigative efforts of scientists. Classical methods of organic chemistry and photochemistry have given us long ago the structure of chlorophyll and provided much information about its photochemistry in solution. An examination of the absorption spectra of chlorophyll in vivo suggests that the in vitro studies may not tell us much about how the energy might be transferred in living organisms. It has been necessary, therefore, to turn our attention to more complex systems, such as occur in solid-state physics or in semi-solid-state living systems. We are studying physical properties in such systems in an attempt to correlate these properties with the effect of light.[3] For example, studies of the production of unpaired electrons by electron-spin-resonance (ESR) techniques have indicated some interesting possibilities for the conversion of energy by way of unpaired electron species and free radicals.[4,5] Studies of photoconductivity in model layered systems may also provide information as to how energy is transferred from one point to another and converted from one form to another.[6]

One theory, based upon the assumption of an orderly array of chlorophyll molecules in the laminar structure[7] and upon the physical-chemical studies[8] suggests that energy conversion is accomplished by the transfer of an electron from the excited chlorophyll molecule to some acceptor substance.

Very careful quantitative studies of the evolution of oxygen during photosynthesis as a function of the wavelength of light, particularly at the longer wavelengths, have produced interesting results during the past year. These studies suggest that light may be used in two different steps. It was found that light of two different wavelengths, one of which was a very long wavelength, could act cooperatively to produce more photosynthesis than could the same amount of light if it were all of the longer wavelength. This suggests that light of the longer wavelength contains enough energy to bring about one kind of step in the primary conversion process but not enough energy to bring about the other step.[9,10] Thus, when light of the shorter wavelength, and hence greater energy, is added, the total result is a greater rate of photosynthesis than could be accomplished if all of the quanta of light were of the longer, and hence less energetic, wavelength. Perhaps two different kinds of electron transfer to or from the pigment molecule are involved.

The transferred electron ultimately must be passed on to an oxidized form of a cofactor, triphosphopyridine nucleotide (TPN^+) to convert it to the reduced form of the nucleotide, TPNH. We do not know how many steps there may be in this electron transport. Plastoquinone and several of the cytochromes that are known to be involved in oxidative electron transport are present in the chloroplasts. These compounds have been found to be either reduced or, in some cases, oxidized during photosynthesis,[11] and quite possible could play a role in electron transport during photosynthesis.

The difficulty is in knowing whether or not these compounds are involved in electron transport between the primary photochemical-conversion act and triphosphopyridine nucleotide or whether they are involved instead in the secondary electron transport between triphosphopyridine nucleotide and either oxygen or some intermediate resulting from the splitting of water.

Another important part of the photo step of photosynthesis is, of course, the splitting of water. The pigment which might transfer an electron to some acceptor must, following this transfer, find another electron, eventually from water, in order to return to its normal state. Kessler has suggested that manganese plays an important role in this process which lies between the splitting of water and the evolution of oxygen.[12] Most theories of photosynthesis postulate some form of peroxide as an intermediate resulting from the primary splitting of water and the final evolution of oxygen. Manganese is believed by Kessler to be involved in the formation of this peroxide.

Recently, Tanner has shown that manganese changes its oxidation state photochemically, as observed from studies of a characteristic manganese ESR pattern.[13] Tanner also sees further evidence for the formation of a free radical during photosynthesis. He has suggested that manganese plays a role in a photochemical reduction of carbon dioxide to glycolic acid, but the results which have so far been published do not seem to require this interpretation.

Moses and Calvin showed that although glycolic acid is synthesized relatively slowly from $C^{14}O_2$, it is very rapidly labeled with tritium during photosynthesis in the presence of tritium-labeled water (T_2O).[14] This result suggests that while glycolic acid may not be formed directly from carbon dioxide, as Tanner purposes, it may nevertheless play an important role in electron transport. Conceivably this role is linked in some way with the role of manganese in the formation of oxygen.

In summary, a purely hypothetical scheme for the photo stage of photosynthesis, based on the several bits and pieces of evidence, can be presented: Light is absorbed by chlorophyll which becomes excited chlorophyll. This excitation energy is transferred through the array of chlorophyll molecules until chlorophyll, or some associated pigment, transfers an electron to an electron acceptor. This transfer may be of two different types, one of which requires light energy of somewhat shorter wavelength than the other. The electron is then carried by some electron-transporting system, perhaps involving such intermediates as plastoquinone, to the point where it is used to reduce TPN^+ to TPNH. Intermediate stages in the transfer of electrons and the formation of stable compounds may include the formation of charge "traps" and free radicals, which may also exhibit paramagnetism. The pigments that have lost electrons must extract them from water, producing some intermediate hydroxyl radical and eventually peroxide, in a process that seems to require manganese. From this peroxide, oxygen is evolved. Somewhere, during and linked to these processes, adenosine triphosphate (ATP) is formed. Glycolic acid plays a role in electron transport, either in the primary processes of photosynthesis, or perhaps in carrying reducing power from the chloroplasts to the cytoplasm. This role of glycolic acid may be connected in some way with the manganese requirement.

In their studies with isolated chloroplasts, Arnon and co-workers[15, 16] have shown what appears to be a stoichiometric relationship between the production of oxygen, ATP, and TPNH. Since these are the very cofactors that have been postulated as required for the operation of the carbon reduction cycle,[17] it has appeared that these two cofactors were the only link between the photo stage of photosynthesis and the synthesis stage. This conclusion was further supported

by the reports by Trebst that synthesis resembling photosynthesis could be carried out in the dark if one supplied the chloroplasts with reduced TPNH and ATP.[18]

In the past year, however, this agreeably simplified picture has been subjected to some new questions. For one thing, Miyachi, Oh-hama, and Tamiya have reported that when the light is turned off following a period of photosynthesis, the level of TPNH does not decline immediately but, in fact, goes up or stays level for many minutes.[19] Nevertheless, the capacity to fix carbon dioxide drops off, with a half life of about 30 sec, and becomes essentially zero in a minute or two. Moreover, the dark fixation of CO_2 in chloroplasts supplied with ATP and TPNH is disappointingly slow compared with the rate of CO_2 fixation in whole plants in the light. One wonders if some other cofactor may be required for the normal photosynthetic mechanism.

With this doubt in mind, let us turn our attention to the synthesis part of photosynthesis. Here, much more is known about the chemical processes involved, largely as a result of tracer studies with carbon-14 and phosphorus-32. The techniques used so successfully in these studies have been described in detail;[20] I shall summarize them very briefly. Radioactive bicarbonate solution, $HC^{14}O_3^-$, is injected into an actively photosynthesizing suspension of a unicellular algae, Chlorella pyrenoidosa. After only a few seconds of photosynthesis, during which time the plant makes organic compounds from the radioactive bicarbonate, the algae suspension is run into boiling alcohol and killed. All enzymatic reactions stop immediately, and the subsequent labeling of the compounds may be found by analysis. This labeling is indicative of the synthesis of compounds from radiocarbon. For this analysis, the killed algae suspension is extracted and concentrated, and the compounds are separated by two-dimensional paper chromatography. A radioautograph of the paper chromatogram shows which compounds have become labeled. The measurement of the radioactivity in each compound is made with a Geiger counter. A kinetic analysis is then made of the appearance of carbon-14 in each compound as a function of time of photosynthesis in C^{14} by the plant. In some cases, individule compounds were degraded to show the position of the labeled carbon within the molecule.

The results of these studies led to the formulation of the carbon reduction cycle of photosynthesis which is depicted in Fig. 1. The simple essentials of this cycle are as follows. First, carbon dioxide adds to a five-carbon sugar, producing an unstable six-carbon compound which splits into two three-carbon compounds, each of which is an acid. Secondly ATP and TPNH from the light are used in the reduction of the carboxyl group to the corresponding aldehyde. The result is the formation of a three-carbon sugar. Then, five of these three-carbon sugar. Then, five of these three-carbon sugars undergo a series of condensations, dismutations, and rearrangements to produce three five-carbon sugars. Finally, each five-carbon sugar monophosphate is converted with ATP to five-carbon sugar diphosphate, which is the carbon dioxide acceptor. Each of these three five-carbon sugars can add a CO_2 molecule, producing a total of six three-carbon fragments. Therefore, there is a net conversion of three molecules of carbon dioxide to one three-carbon organic compound per turn of the cycle.

This three-carbon compound may then be used for subsequent steps in the synthesis of specialized and products. For example, two three-carbon sugars may be condensed to make one six-carbon sugar, and two six-carbon sugars may react together to form one molecule of sucrose. Alternatively, a large number of six-carbon sugars (such as glucose) may be linked together to form a complex polysaccharide, for example starch or cellulose.

Another biosynthetic pathway leading from the cycle begins with phosphoglyceric acid (PGA), the three-carbon acid compound formed as the product of the

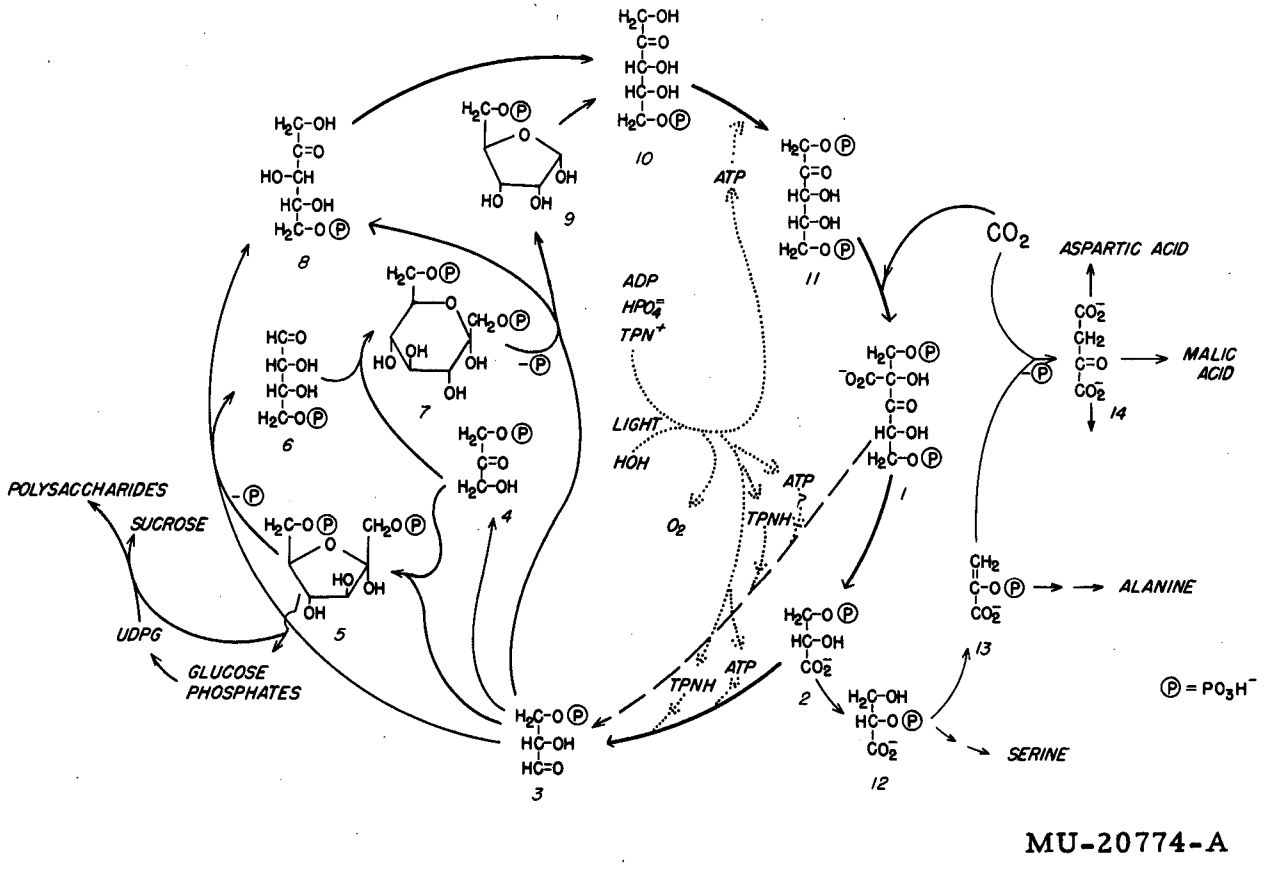

Fig. 1. The carbon reduction cycle of photosynthesis.

carboxylation reaction, and proceeds via phosphoenolpyruvic acid and a second carboxylation to a four carbon carboxylic acid, malic acid. Phosphoenolpyruvic acid (PEPA) may also be converted to alanine, forming an amino acid. This amino acid is then used to form other amino acids, which are used ultimately in the synthesis of proteins and enzymes.

By careful quantitative studies with carbon-14 during steady-state photosynthesis, it is possible to measure the relative importance of various biosynthetic pathways. First, algae are allowed to photosynthesize with ordinary CO_2 under constant environmental conditions for a few minutes. Radioactive carbon dioxide 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 pressure is continuously measured by an infrared-absorption measuring instrument which monitors the gas bubbling through the algae in a closed system. The level of $C^{14}O_2$ in 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 Fig. 2.

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 these experiments--may be used in a calculation of the rate of flow of carbon through each intermediate compound.

Among the conclusions derived from the calculation of rates of flow of carbon through various intermediates and along certain pathways are the following:[21]

(1) At least 70 to 80% 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 per cent or more of the entering carbon dioxide enters via carboxyllation of PEPA leading to malic acid and aspartic acids and other compounds.

(3) There is a very active synthesis of several important amino acids. During photosynthesis the rates of synthesis of alanine, serine, aspartic acid, glutamic acid, glutamine and other amino acids, have been measured. The synthesis of these amino acids accounts for about 30% of all of the carbon taken up during steady state photosynthesis and about 60% of all of the ammonia uptake. Furthermore, the synthesis of these amino acids reaches a maximal rate very quickly after exposure to carbon-14 and at a time when the only possible precursors are intermediates for the carbon reduction cycle in the choloroplasts. From these facts it may be concluded that the synthesis of these amino acids takes place in the chloroplasts directly from intermediates of the carbon reduction cycle. Since so much of the total ammonia uptake and carbon uptake may be accounted for by these synthetic pathways, we can say that protein synthesis takes place in the chloroplasts via the free amino acids.

During steady-state studies we have also studied the rate of appearance of carbon-14 in PGA and compared that rate of appearance with the rate of appearance of carbon-14 in ribulose diphosphare (RuDP) the carbon dioxide acceptor. We

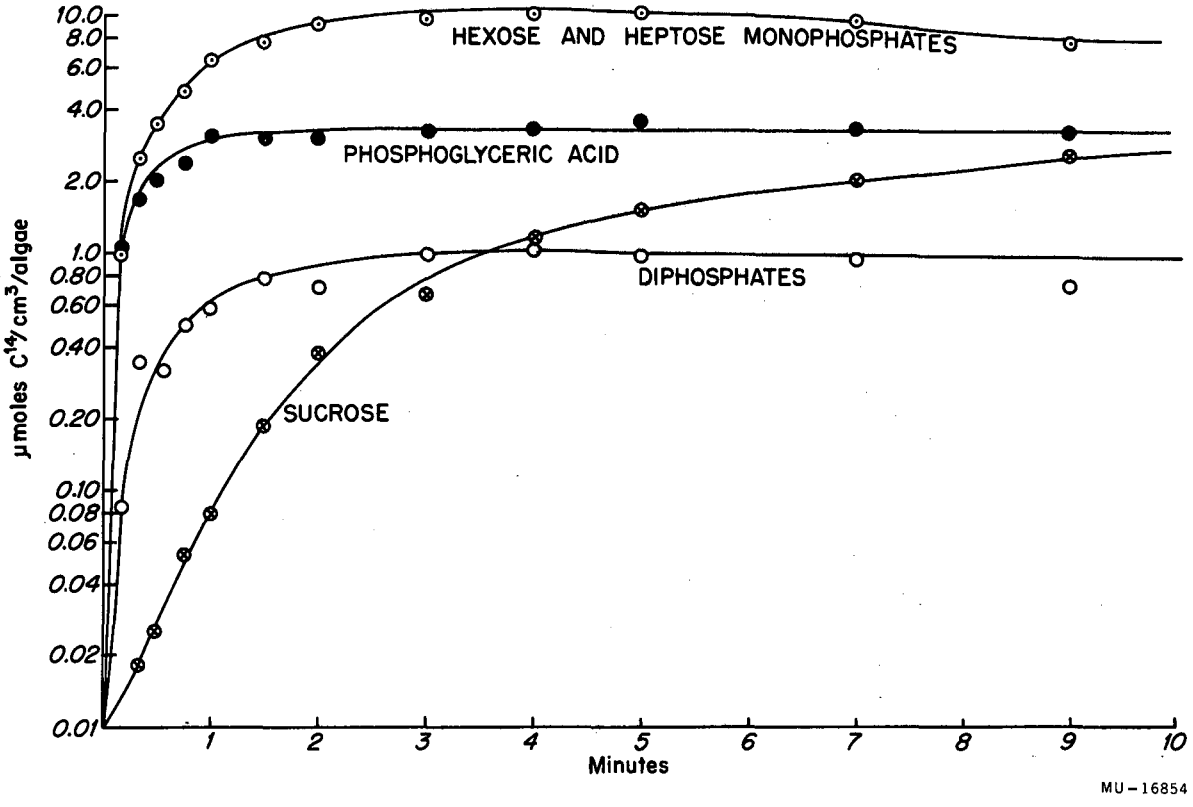

Fig. 2. Rates of labeling of several compounds during steady-state photosynthesis in Chlorella.

know the rate of uptake of carbon-14 from the medium, and we can calculate the rate of flow of carbon-14 through the carboxylation reaction and through the carboxyl carbon of PGA. Knowing that the carboxyl carbon of PGA must become saturated with radiocarbon in a few seconds after the beginning of exposure to $C^{14}O_2$, we can calculate the rate of appearance of carbon-14 in the alpha- and beta-carbons of PGA and compare that with the carbon-14 in RuDP, which must be the precursor to the alpha- and beta-carbons of PGA. It turns out that the alpha- and beta-carbons of the PGA are labeled too rapidly for them to be derived from all five of the carbon atoms of RuDP. Therefore, probably the carboxylation product, the unstable six-carbon compound, splits to two three-carbon fragments, only one of which is PGA.

It is known that in the isolated enzyme systems and in some isolated chloroplasts the carboxylation reaction results in two molecules of PGA. Thus it appears that in the living cell the biosynthetic pathway may be slightly different than it is in isolated, or broken, systems. This is not surprising when we remember the highly organized, complex structure of the chloroplasts. Rather, it suggests that in the intact chloroplast in the living cell reducing power can be transferred from the primary photo reaction to the synthetic reaction by some mechanism that is different from the one mediated by free reduced TPN. This transfer could result in a reductive split of the unstable six-carbon carboxylation product, giving one molecule of PGA and one molecule of triose phosphate. This possibility is indicated by the dotted line in the cycle shown in Fig. 1.

Once again it would appear that the simple assumption that only TPNH and ATP are formed by the photo reaction and required by the carbon reduction cycle may have been an oversimplification of the true situation. No doubt carbon reduction pathways very similar to those of photosynthesis can function with only these two known cofactors, and such has been demonstrated with isolated chloroplasts.[18] However, it is important to note that the rate of synthesis of carbon compounds in isolated chloroplasts is only a very small fraction (2 to 5%) of the rate that would be measured in healthy, active, living whole cells.

In conclusion, it may be said that through the employment of tracer elements, particularly carbon-14, 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 reduction during photosynthesis. From the nature of this pathway, the requirements for energetic cofactors, derived ultimately from the light reaction, have been suggested. Studies with isolated chloroplasts show that these cofactors are generated in the light. 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 known as yet.

The possibility exists that other, as yet unidentified cofactors, are formed in the light and required for normal rates of carbon-compound synthesis.

The chloroplast appears to be a self-sufficient factory, capable of making the great variety of compounds required for its own operation, as well as organic molecules for "export" to the rest of the cell or plant.

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