BACTERIAL PHOTOSYNTHESIS

FLUORESCENCE COMPOUNDS, PLANT

PHOTOSYNTHESIS

CHLOROPHYLL

BY: DR. GOVINDJEE AND CO-WORKERS

Bacterial photosynthesis

Certain bacteria (green and purple) have the ability to perform photosynthesis. This was first noticed by S. Vinogradsky in 1889 and was later extensively investigated by C. B. Van Niel, who gave a general equation for all bacterial photosynthesis. This is shown in Eq. (1), where $h\nu$ = light, Bchl =

$$H_2A + CO_2 \xrightarrow[\text{Bchl}]{h\nu} (CH_2O) + A \qquad (1)$$

bacteriochlorophyll, (CH_2O) = carbohydrate, and H_2A = externally added H_2 donor. In green plants H_2A is water, except when the plants are adapted to use H_2 as the reductant; this phenomenon, discovered by H. Gaffron, is known as photoreduction. Unlike green plants, photosynthetic bacteria are incapable of evolving oxygen. *See* PHOTOSYNTHESIS.

Generally, the known photosynthetic bacteria can be classified in three major groups:

1. Nonsulfur purple bacteria (Athiorhodaceae). In these bacteria H_2A is usually an organic H_2 donor, such as $CH_3CHOHCH_3$ (propinol); however, they can be adapted to use hydrogen gas as the reductant. They require vitamins for their growth and usually are grown anaerobically in light, but they can also grow aerobically in the dark, indicating they have a mechanism for respiration. They are thus facultative photoheterotrophs; examples of this group are *Rhodospirillum rubrum* and *Rhodopseudomonas spheroides*.

2. Sulfur purple bacteria (Thiorhodaceae). These cannot grow aerobically, and H_2A is an inorganic sulfur compound, such as hydrogen sulfide H_2S; the carbon source can be CO_2. These bacteria are called obligate photoautotrophic anaerobes; an example is *Chromatium* species.

3. Green sulfur bacteria (Chloraceae). These bacteria are capable of using the same chemicals as Thiorhodaceae but, in addition, use other organic H_2 donors. They may then be called photoauto- and photoheterotrophic obligate anaerobes. Two examples of the group are *Chlorobium thiosulfatophilum* and *Chloropseudomonas ethylicum*; the latter uses ethyl alcohol, from which it derives part of its name.

Energetics. The maximum quantum yield of bacterial photosynthesis, as measured by the maximum number of CO_2 molecules reduced (or H_2A molecules oxidized) per quantum of light energy absorbed, is 0.12. In other words, eight quanta of light are required to reduce one CO_2 molecule. This is the same quantum requirement as was found for green-plant photosynthesis and has led to the speculation that the mechanism of the two processes is the same, except that the bacteria lack the enzyme which catalyzes the evolution of oxygen, but instead oxidize the added H_2 donor (H_2A).

Calculation of the energy stored in various bacterial photosyntheses shows that in sulfur bacteria the conversion of H_2S to S leads to a loss of about 5 kcal/mole, and conversion of S to sulfate (SO_4^{--}) leads to a net storage of only 7 kcal/mole. In the case where hydrogen molecules are used as H_2 donors, about 25 kcal/mole is dissipated.

This calculation is made without regard to the possibility of extensive photophosphorylation, that is, the production of adenosinetriphosphate (ATP) from adenosinediphosphate (ADP) and inorganic phosphate (P_i). However, it is important to realize that bacteria are capable of photophosphorylation. Several investigators have suggested that the sole

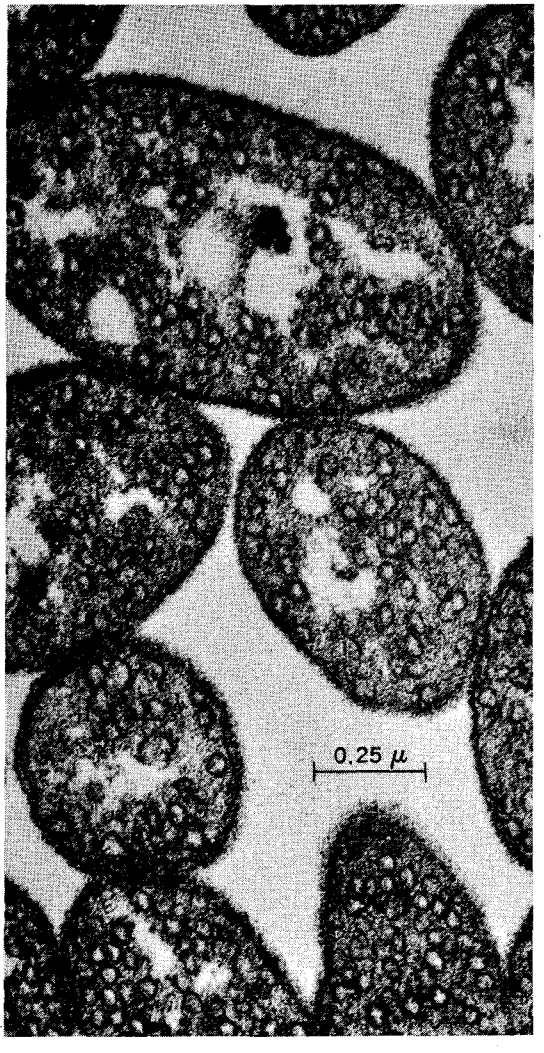


Fig. 1. Electron micrograph of *Rhodopseudomonas spheroides* with very tiny vesiclelike thylakoids. (*From W. Menke, in T. W. Goodwin, ed., Biochemistry of Chloroplasts, vol. 1, Academic Press, 1966*)

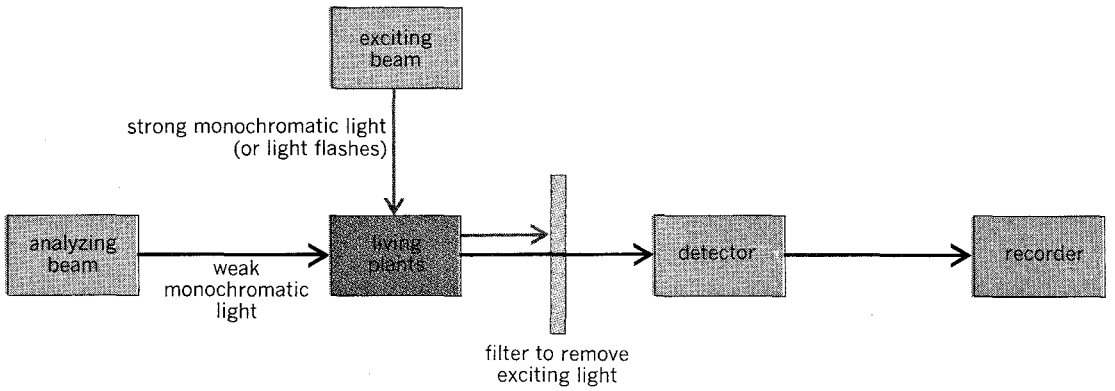


Fig. 2. Principal components of a difference spectrophotometer. (*Govindjee, Transformation of light energy into chemical energy: Photochemical aspects of photosynthesis, Crop Sci., 7:551–560, 1967*)

function of the light reaction in bacteria is to make ATP from ADP and P_i, and the hydrolysis energy of ATP is then used to drive the reduction of CO_2 to (CH_2O) by H_2A. The latter reaction should not require much energy, as has been pointed out above.

Photochemical apparatus. Photosynthetic bacteria do not have specialized organelles set apart in the cell by a membrane, like the chloroplast of green plants. However, it has often been assumed that certain round bodies called chromatophores, which can be easily obtained from bacteria, are these organelles. Although these chromatophores have been prepared in various sizes, they were difficult to observe in whole bacterial cells. It is considered likely that they are artifacts of preparation, though much has been learned about bacterial photosynthesis from biochemical and biophysical studies of these particles. Electron micrographs of photosynthetic bacteria show very tiny spherical sacks with double-layered walls. These structures, named thylakoids, seem to be the photochemical apparatus for bacterial photosynthesis (Fig. 1).

Photosynthetic unit. In Eq. (1) the pigment bacteriochlorophyll was a necessary ingredient for photosynthesis. There seem to be specialized Bchl molecules in bacteria which engage in the primary chemical reactions of photosynthesis, as in green plants.

By using very bright flashes of light, it has been shown that a maximum of one H_2-donor molecule can be utilized for 40–50 Bchl molecules present. Thus, it appears that about 50 Bchl molecules cooperate to perform photosynthesis, and this is the photosynthetic unit of bacteria. It appears that each photosynthetic unit contains one specialized Bchl molecule which engages in chemical reactions. These are called energy traps because energy absorbed within one photosynthetic unit is trapped by them. They may also be called the reaction centers because they are the seats of primary reactions of bacterial photosynthesis.

These traps have been identified as P840 in green bacteria, P870 in *R. rubrum*, and P890 in *Chromatium*. The P stands for pigment and the numerical designation is dependent upon the location, in nanometers (nm), of the maximum decrease in the absorption in the far-red spectrum when illuminated by a bright actinic light. Such identification is carried out with an instrument called the difference (absorption) spectrophotometer (Fig. 2).

In this instrument a weak measuring beam monitors the absorption of the sample; a bright actinic light given at right angles to the measuring beam causes photosynthesis. When photosynthesis occurs and bacteriochlorophyll takes part in it, changes in the absorption take place. These changes are measured as a function of the wavelength of measuring light. In bacteria these changes are located at 840, 870, and 890 nm, depending on the species used.

To determine whether the changes at 840, 870, or 890 nm are due to the existence of energy traps, the criteria for a molecule to be the energy trap must be examined, and these are as follows.

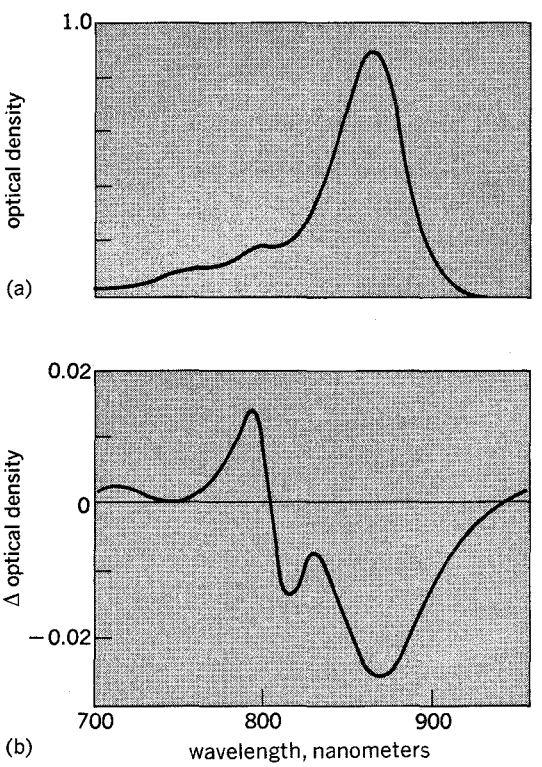


Fig. 3. Light-induced absorption spectrum changes in the chromatophores of the blue-green mutant *Rhodc pseudomonas spheroides*. (*a*) Absorption spectrum. (*b*) Spectrum of the reversible changes. (*R. K. Clayton, Molecular Physics in Photosynthesis, Blaisdell, 1965*)

(1) If a molecule is the energy trap, it is where all the excitation energy absorbed by the photosynthetic unit is funneled and is converted into chemical energy. The expectation is that these molecules undergo oxidation or reduction reaction since this is the essential reaction of photosynthesis. Changes in the absorption of pigment molecules are expected when oxidation or reduction of the molecule occurs. (2) Since energy is utilized very effectively in photosynthesis, the quantum yield (number of trap molecules oxidized or reduced per absorbed quantum) must be very high (closed to 1.0) for the above mentioned reaction. (3) Since the reaction of the trap molecule is the primary light reaction, it should occur at low temperatures as well. (4) Since only one such molecule need be present per photosynthetic unit, their concentration must be about 2% of the total Bchl molecules.

All the above criteria are fulfilled by P840, P870, and P890, and thus it is assumed that they are the energy traps of bacterial photosynthesis. It has further been observed that the decrease in absorption at 840, 870, and 890 nm is an oxidation reaction, because chemical oxidants cause similar changes as the bright actinic light (Fig. 3).

Components of photosynthetic bacteria. These bacteria contain the usual components of living material: proteins, lipids, carbohydrates, deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and various metals. However, the specific components of interest to the electron transport system of bacterial photosynthesis are quinones, pyridine nucleotides, various iron-containing pigments (cytochromes), and especially the photosynthetic pigments which capture light energy.

The bacteria contain special substituted benzoquinones, also called Q coenzymes or ubiquinones. The sulfur purple bacterium *Chromatium* contains a type of Q coenzyme called Q7, while the nonsulfur purple bacterium *R. rubrum* has coenzyme Q9. The nicotinamide adenine dinucleotide (NAD) is the major pyridine nucleotide in bacteria; it is present in large quantities and seems to be active in photosynthesis. Among the various cytochromes, the *c*-type cytochromes *c*552, *c*555, and *cc'* seem to be the important ones for photosynthesis. The numbers 552 and 555 refer to the α bands in the reduced minus oxidized absorption spectra, in nm.

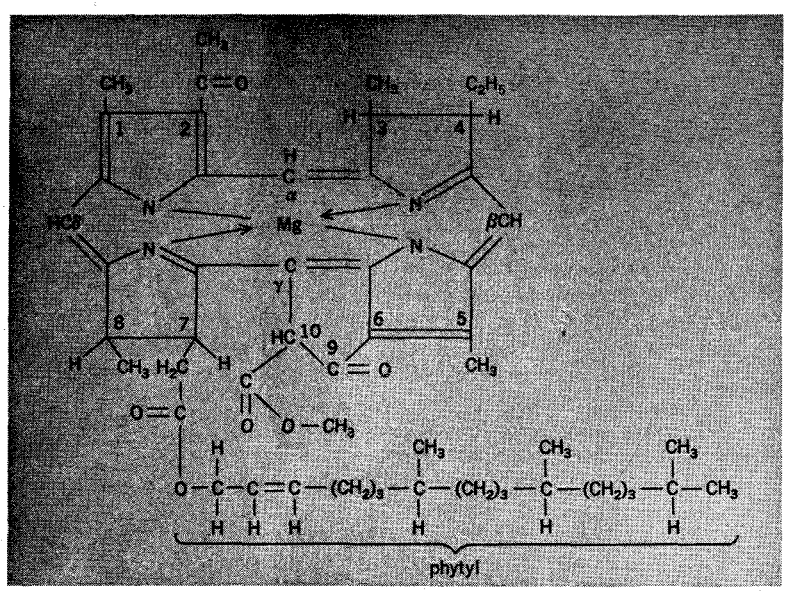


Fig. 4. Structural formula of bacteriochlorophyll.

Pigments. All photosynthetic bacteria, so far as known, contain bacteriochlorophyll, a tetrahydroporphyrin (Fig. 4). The chlorophyll of green plants, by contrast, is a dihydroporphyrin. In diethyl ether, Bchl has absorption maxima at 365, 605, and 770 nm (Fig. 5). A bacteriochlorophyll has been isolated from a strain of *Rhodopseudomonas* which shows absorption maxima at 368, 582, and 795 nm in diethyl ether. The latter Bchl has been called Bchl *b* and the former Bchl *a*. *See* CHLOROPHYLL.

The absorption spectrum of Bchl in the bacterial cell is complex. The infrared band of Bchl *b* has a peak at 1017 nm, but the infrared band of Bchl *a* has a triple-peaked structure with maxima at 800 (B800), 850 (B850), and 890 nm (B890). The relative heights of these peaks are different in different organisms, and even in the same organism they change with the growth conditions (light intensity, CO_2 level, and variations in substrate for growth). There are several explanations for the nature of the Bchl bands in the bacterial cell: different aggregates of Bchl; complexes of Bchl with different kinds of protein; or complexes with carotenoids or cytochromes. It is possible to separate the B890 complex from the B800 and B850 complexes; B800 and B850 behave differently to different treatments.

The green bacteria contain a small amount of Bchl *a*, but they contain large quantities of another type of chlorophyll called chlorobium chlorophyll (chlb chl) (Fig. 6); the latter exists in two forms. One form has an absorption band at 725 nm in the red end of the spectrum, whereas the other form has its red absorption band at 740 nm in the cell.

The second group of pigments is the yellow to red carotenoids. The carotenoids of photosynthetic bacteria are of great variety and include some which are found in green plants, for example, the lycopenes. However, some such as γ-carotene are typical of bacteria. This carotenoid has absorption peaks at 440, 460, and 495 nm in hexane and is found in large quantities in green sulfur bacteria. Another example is spirilloxanthol with absorption peaks at 464, 490, and 524 nm in hexane which is found mainly in purple bacteria. In some bacteria carotenoids function to protect the photooxidation and destruction of bacteriochlorophyll, but they also function in bacterial photosynthesis by trans-

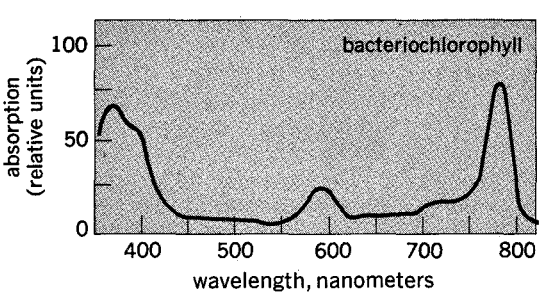


Fig. 5. Absorption spectra of bacteriochlorophyll. *(After J. C. Goedheer, from M. Kamen, Primary Processes in Photosynthesis, Academic Press, 1963)*

ferring their absorbed energy to bacteriochlorophyll. *See* CAROTENOID.

Fluorescence and energy transfer. Light energy absorbed by the various carotenoids is transferred to Bchl with varying efficiency (30–90%). This energy transfer is demonstrated by the method of sensitized fluorescence. When light energy is absorbed by carotenoids, only the fluorescence of bacteriochlorophyll (B890) is observed. By the same method, efficient (almost 100%) energy transfer has been demonstrated from B800 to B850 to B890. In certain cases fluorescence has also been observed from B850, suggesting that the efficiency of energy transfer from B850 to B890 is not always 100%. *See* FLUORESCENCE COMPOUNDS, PLANT.

The lifetime of the excited state of Bchl in the bacterial cell is of the order of 1–2 nanoseconds. The excitation energy must be channeled from the pigments, and ultimately to the energy traps, within this time for photosynthesis to occur.

Mechanisms. The first act of bacterial photosynthesis—like plant photosynthesis—is the absorption of light by various pigments. As discussed above, light energy absorbed by the carotenoids, B800 and B850, is transferred to B890 and finally to the energy traps. Here, the primary reaction occurs, the oxidation of the energy traps. Occasionally, a reduction of some portion of Bchl is observed, but its role is uncertain. Soon after the oxidation of the trap, for example, P890, cytochromes are oxidized and ubiquinones are reduced. The oxidation reduction of cytochromes (cyt) and ubiquinones (UQ) is also measured by the difference spectrophotometer. Quinones show absorbance changes around 260 nm (in the ultraviolet) and cytochromes show several absorbance changes, including those around 552–555 nm. There are controversies about the nature of the primary reactions of bacterial photosynthesis, but the favored hypothesis is one in which UQ, cyt, and P890 exist together in a complex. The early steps in bacterial photosynthesis are suggested to occur as shown in Eq. (2). Here the reduced form is

$$\mathrm{UQ \cdot P890 \cdot cyt} \xrightarrow[(a)]{h\nu} \mathrm{UQ \cdot P890^{*} cyt} \xrightarrow[(b)]{} \mathrm{UQ^{-} \cdot P890^{+} \cdot cyt} \xrightarrow[(c)]{} \mathrm{UQ^{-} \cdot P890 \cdot cyt^{+}} \quad (2)$$

denoted by a negative (−) sign and the oxidized by a positive (+) for a given molecule, and the (*) on P890 denotes the excited state of the trap. Furthermore, reaction (a) represents the excitation of the trap by excitation energy transferred to it (from other pigment molecules) or directly absorbed by it; reaction (b) is the oxidation of P890 to $P890^{+}$, and the reduction of UQ to UQ^{-}; and reaction (c) is the oxidation of cyt to cyt^{+} by $P890^{+}$ which is then restored to P890. The reduced ubiquinone (UQ^{-}) donates the electron for the reduction of CO_2 and UQ is regenerated; the oxidized cytochrome (cyt^{+}) recovers the electron from H_2A, oxidizing the latter to A, and cyt is regenerated.

Data were accumulated during 1966–1968, suggesting that bacterial photosynthesis has two light reactions. It was found by S. Morita that the action spectra (effectiveness as a function of the wavelength of light) of the oxidation of different cytochromes (cyt 552, cyt *cc'*, and cyt 555) in *Chromatium* strain D are different. The most outstanding difference was between cyt 552 and cyt 555. Light absorbed by B800 and B850 compared to that absorbed in B890 was very efficient for the oxidation of cyt 555, whereas light absorbed by all three forms of Bchl was almost equally effective for cyt 552. These experiments suggest the operation of two pigment systems, at least, in bacteria.

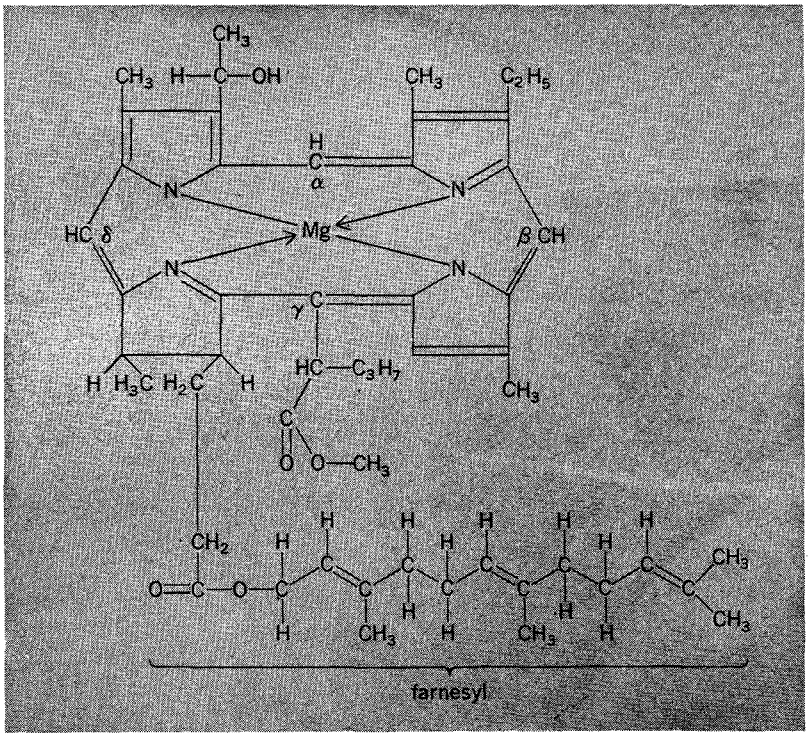


Fig. 6. Structure of chlorobium chlorophyll.

It was proposed by M. A. Cussanovich and coworkers that the light reactions for bacterial photosynthesis are (1) a cyclic reaction, that is, the electron leaves a molecule and returns to it, which involves phosphorylation; and (2) the reduction of pyridine nucleotide (NAD) at the expense of the added H_2 donor—H_2A in Eq. (1). For *Chromatium* the hypothesis is shown in Fig. 7. The first light reaction is the oxidation of P890 and reduction of UQ (Q7 coenzyme). UQ^{-}, instead of reducing CO_2, transfers its electron to cyt *cc'*. The oxidized $P890^{+}$ oxidizes cyt 553 which, in turn, does not oxidize H_2A but oxidizes the reduced cyt *cc'*, thus completing the cycle. Some of these reactions release energy and this is coupled to phosphorylation. Thus, the available energy is utilized for the synthesis of ATP from ADP and P_i. The second light reaction in this hypothesis is the reduction of another Bchl molecule named P905. In this case, however, $P905^{-}$ is formed which donates its electron to pyridine nucleotide (NAD). The P905 regains its lost electron from cyt 552, oxidizing the latter; the oxidized cyt 552 regains its electron from the ultimate H_2 donor (H_2A), oxidizing it to A. There is some doubt as to the assignment of P905 to the trap. C. Sybesma and C. F. Fowler gave evidence for the two light-driven reactions in *Rodospirillum rubrum*. In *R. rubrum* the cyclic reaction uses P890 (+P800) as its trap, and the noncyclic one uses P'890—a somewhat different

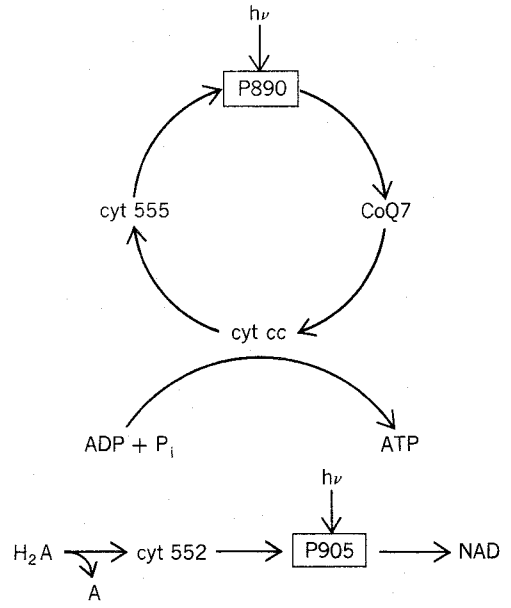


Fig. 7. Schematics of a hypothesis of two light reactions for bacterial photosynthesis. (*From M. A. Cussanovich, R. G. Bartsch, and M. D. Kamen, Light-induced electron transport in Chromatium strain D, pt. 2: Light-induced absorbance changes in Chromatium chromatophores, Biochim. Biophys. Acta, 153:397–417, 1968*)

P890. The cytochromes and quinones involved in *R. rubrum* differ from those in *Chromatium*.

The reduced pyridine nucleotide made in the second light reaction and the ATP in the first light reaction are then utilized to convert CO_2 into carbohydrates. The pathway of carbon involves either the reversal of the Krebs cycle or the Calvin cycle with some modifications. *See* BACTERIAL METABOLISM. [GOVINDJEE]

Bibliography: R. K. Clayton, *Molecular Physics in Photosynthesis*, 1965; M. A. Cusanovich, R. G. Bartsch, and M. D. Kamen, Light-induced electron transport in *Chromatium* strain D, pt. 2: Light-induced absorbance changes in *Chromatium* chromatophores, *Biochem. Biophys. Acta*, 152:397–417, 1968; H. Gest, A. San Pietro, and L. P. Vernon (eds.), *Bacterial Photosynthesis*, 1964; M. Kamen, *Primary Processes in Photosynthesis*, 1963; E. N. Kondratseva, *Photosynthesizing Bacteria*, 1965; S. Morita, Evidence for three photochemical systems in *Chromatium* D, *Biochim. Biophys. Acta*, 153:241–247, 1968; C. Sybesma and C. F. Fowler, Evidence for two light-driven reactions in the purple photosynthethic bacterium *R. rubrum*, *Proc. Nat. Acad. Sci.*, 61:1342–1348, 1968.

Photosynthesis

Literally, synthesis of chemical compounds in light. The term photosynthesis, however, is used almost exclusively to designate one particularly important natural process of this type: the manufacture in light of organic compounds (primarily certain carbohydrates) from inorganic materials, with simultaneous liberation of oxygen, by chlorophyll-containing plant cells. This process requires a supply of energy in the form of light, since its products (carbohydrates and oxygen) contain much more chemical energy than its raw materials (water and carbon dioxide). This is clearly shown by the liberation of energy in the reverse process, the combustion of organic material with oxygen. *See* PLANT RESPIRATION.

The light energy taken up by the pigments of the photosynthesizing cells, especially by the green pigment chlorophyll, is efficiently converted by photosynthesis into stored chemical energy. Together, the two aspects of photosynthesis—the conversion of inorganic into organic matter, and the conversion of light energy into chemical energy—make it the fundamental process of life on Earth. It is the unique source of all living matter and of all life energy on this planet.

Since fossil fuels (coal, oil, peat) are half-decayed products of plant photosynthesis from past geological ages, it can be said that not only all life energy but also nearly all industrial power, as well as all domestic heat, have their origin in photosynthesis. Exceptions are wind and water power and nuclear power.

The elements carbon, oxygen, and hydrogen are exchanged through photosynthesis and respiration in an endless cycle between the organic and the inorganic worlds. However, one ingredient of photosynthesis—light energy—is not regenerated in this cycle. Therefore, life on Earth can be maintained only by the constant supply of solar energy and its utilization through plant photosynthesis.

Speed of photosynthesis. Under favorable external conditions, photosynthesis is a remarkably fast process. With an adequate supply of carbon dioxide and light, a green cell will produce as much as 30 times its own volume in oxygen every hour. The rate of photosynthesis can be varied by varying the supply of carbon dioxide, the intensity or color of illumination, or the temperature. In addition to these easily controllable external conditions, the rate of photosynthesis depends also on the age, nutrition, and physiological conditions within the organism, factors which are much more difficult to define and control precisely.

Turnover of photosynthesis on Earth. The total turnover of photosynthesis on Earth has been roughly estimated in two ways: by averaging the yields of organic matter per unit area of field, forest, steppe, and ocean; and by determining the average utilization of incident solar energy by vegetation-covered areas (which is of the order of 1% if the whole solar spectrum is taken into consideration, or 2% if only visible light is considered). Both procedures lead to numbers of the magnitude of 10^{11} tons of carbon transferred from the inorganic into the organic state each year. This corresponds to about 10^{18} kcal (10^{15} kwhr) of light energy stored annually. The estimate is rough, mainly because of uncertainty as to the average rate of photosynthesis in the world's oceans.

The overall reaction. The net overall chemical reaction of photosynthesis is shown in Eq. (1),

$$H_2O + CO_2 + \text{Light energy} \xrightarrow[\text{Enzymes}]{\text{Chlorophyll}} \{CH_2O\} + C_2 - 112\ \text{kcal/mole} \quad (1)$$

where $\{CH_2O\}$ stands for a carbohydrate (sugar). All oxygen liberated in photosynthesis originates in water, and none in CO_2, as shown by experiments with isotopic tracers in which tracer oxygen was found in liberated O_2 when it was incorporated into water but not into CO_2. These experiments were made by S. Ruben, M. D. Kamen, and coworkers in 1941.

The photochemical reaction in photosynthesis belongs to the type known as oxidation-reduction, with CO_2 acting as the oxidant (hydrogen or electron acceptor) and water as the reductant (hydrogen or electron donor). The unique characteristic of this particular oxidation-reduction is that it goes "in the wrong direction," converting chemically stable materials into chemically unstable products. Light energy is used to make this "uphill" reaction possible, and a considerable part of the light energy utilized is stored as chemical energy, namely, 112 kcal per mole, or 44 g, of reduced carbon dioxide, as indicated in Eq. (1).

Multistage process. From an enormous amount of research by plant physiologists, biochemists, photochemists, and biophysicists, it is known that photosynthesis is a complex, multistage process. Its main parts are the primary photochemical process in which light energy taken up by chlorophyll is converted into chemical energy, in the form of some energy-rich intermediate products

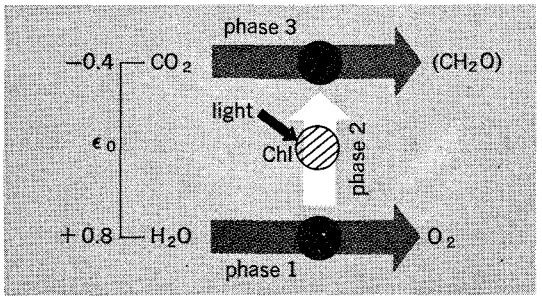


Fig. 1. Schematic illustration of photosynthesis. Phase 1, oxidation of water, consists of enzymatic reactions converting dehydrogenated water to free oxygen. Phase 2, the light reaction, is the transfer by light-excited chlorophyll (Chl) of hydrogen (or electrons). Phase 3, reduction of carbon dioxide, consists of enzymatic reactions converting carbon dioxide and light-supplied hydrogen to carbohydrates (CH_2O). ϵ_0 is the oxidation-reduction potential of H_2C-O_2 and $CO_2-(CH_2O)$ couples at pH 7.0.

and enzyme-catalyzed "dark" (that is, not photochemical) reactions by which these intermediates are converted into the final products—carbohydrates and free oxygen. These reactions of photosynthesis can be grouped into three phases, as shown in the scheme of Fig. 1. Phase 1 is the evolution of oxygen from dehydrogenated water by a series of dark reactions. This is the least-known aspect of photosynthesis. About all that is known is that it is enzymatic, requires manganous ions, and may involve several steps. Phase 2 is the transfer of hydrogen atoms, H (or electrons)—not of hydrogen molecules, H_2, or hydrogen ions, H^+—from an unknown intermediate in phase 1 to some intermediate acceptor capable of reducing carbon dioxide. This is the light phase of photosynthesis. Phase 3 is the reduction of CO_2 by a series of dark reactions. The use of radioactive carbon (carbon-14) as a tracer has given considerable insight into the nature of these reactions. This phase, like phase 1, occurs at a more or less constant level of energy.

Saturation: light and dark reactions. If the rate of photosynthesis is plotted as a function of light intensity, a curve results which shows first a proportional increase, then a gradual saturation. This saturation could be due to various causes. One is the limitation of CO_2 supply from the outside. Further increase of light intensity becomes of no use when all CO_2 molecules reaching the cell are used up as fast as they arrive. CO_2 concentration can thus act as a limiting factor. (The same principle applies to the effect of increasing CO_2 concentration in weak light when the reaction is light-limited.)

The concept of limiting factors was introduced into photosynthesis by F. Blackmann in 1905. It is not a special characteristic of photosynthesis but applies to all chemical systems in which one or several reactants must be continuously supplied from the outside to keep the reaction going. (Light can be considered as a reactant in photochemistry.)

When the supply conditions for CO_2 and light are most favorable, the rate of photosynthesis still shows saturation. This is generally attributed to the need for the completion of photosynthesis of at least one (and more likely, several) light-independent enzymatic reactions. An enzyme-catalyzed reaction has a certain maximum rate, E_0/t, determined by the total amount of its catalyst (enzyme) available in the cell, E_0, and its turnover time, t, which is the average time the enzyme molecule must work at a given temperature on a molecule of the reaction substrate before its transformation is completed. This is expressed as Eq. (2). The several enzymes involved in photosynthesis thus impose

$$V_{max} = E_0/t \qquad (2)$$

ceilings on the maximum speed at which photosynthesis as a whole can proceed, each enzyme functioning as a bottleneck of limited capacity in the reaction path. The enzyme which imposes the most effective (lowest) ceiling seems to be involved in the liberation of oxygen rather than in the reduction of CO_2, since the same saturation rate is observed also in the Hill reaction (see below).

The Hill reaction. Various observations suggest that the immediate action of light (the primary photochemical process) in photosynthesis involves the transfer of hydrogen (or electrons) from water (or from a large molecule, ZH) to an acceptor X (primary oxidant).

This conclusion is made plausible by consideration of the Hill reaction (named after its discoverer, R. Hill). This reaction is a process in which illuminated chlorophyll-bearing fragments of plant cells produce oxygen from water without concomitant reduction of CO_2 but with the reduction of added, less stable oxidants, such as a quinone or ferricyanide, or a dye, such as 2,6 dichloro-phenol indophenol. Since the quantum requirement (number of quanta required to evolve one oxygen molecule) and other kinetic characteristics of the Hill reaction prove to be similar to those of photosynthesis, it can be assumed that in the Hill reaction the primary photochemical apparatus of photosynthesis is preserved more or less intact. In this reaction, however, the coupling of the primary photochemical process with the enzymatic mechanism which brings about the reduction of CO_2 is easily impaired by the mechanical destruction of the cell.

In 1954, with the use of C^{14} as a radioactive tracer, it was observed that certain organic compounds containing the tracer and having reduction levels up to that of sugars are formed by illuminating whole or fragmented chloroplasts in the presence of C^{14}-labeled CO_2, provided certain auxiliary substances are supplied. This suggests that the coupling of the photochemical apparatus with the CO_2 enzymatic system is not entirely lost by the mechanical destruction of the cells; or, at least, that this coupling can be partially restored by the addition of these compounds. It has been shown that it is possible to obtain whole chloroplasts which can "fix" CO_2 as the oxidant with very high efficiency (up to 60% of that in leaves). However, this lasts only a few minutes.

Quantum process of photosynthesis. In photosynthesis, the energy of light quanta is converted into chemical energy. In the conversion of 1 mole of CO_2 and 1 mole of H_2O into 1 mole of carbohydrate groups, $\{CH_2O\}$, and 1 mole of oxygen, according to Eq. (1), about 112 kcal of total energy, or, under natural conditions, about 120 kcal of poten-

tial chemical energy ("free energy") are stored. Light is absorbed by matter in the form of quanta of energy (photons). A 2% energy-conversion yield means that an average of considerably over 100 quanta are absorbed by the pigments under natural conditions to bring about the reduction of one molecule of CO_2. *See* ABSORPTION OF ELECTROMAGNETIC RADIATION; PHOTON.

Under natural conditions, CO_2 supply is not always adequate, while light supply may be overabundant for most effective utilization. Furthermore, not all plant cells are in the most productive physiological state. By using turbulently flowing suspensions of microscopic unicellular algae in CO_2-enriched water, a utilization of up to 7% of absorbed visible sunlight has been obtained in large-scale experiments. Under still more favorable small-scale laboratory conditions (very weak illumination and very effective CO_2 supply to the algae by strongly stirred carbonate–bicarbonate buffer solutions), up to 30% of absorbed light energy could be converted into stored chemical energy, corresponding to a quantum requirement of about eight quanta per molecule of O_2 evolved. (These measurements were made by R. Emerson and several other investigators.) This is a very high efficiency, not matched by any known photochemical reaction.

The reduction of one molecule of CO_2 to the carbohydrate level requires the transfer of four hydrogen atoms as expressed by Eq. (3). A quantum

$$CO_2 + 4H \rightarrow [CH_4O_2] \rightarrow [CH_2O] + H_2O \quad (3)$$

requirement of eight or more would thus permit two quanta to be used for the transfer of each hydrogen atom (or electron).

Two-quanta hypothesis. A specific mechanism in which two quanta are used to transfer one hydrogen atom in photosynthesis was suggested by experiments of Emerson in 1956–1958. Earlier (1943), Emerson had discovered that the "quantum yield of photosynthesis" (number of O_2 molecules evolved per absorbed quantum), while constant at the shorter wavelengths of light (red, orange, yellow, green), declines in the far-red above 680 mμ (the "red drop"). Thirteen years later, Emerson found that this low yield could be enhanced if both chlorophyll *a* and *b* are simultaneously excited (only chlorophyll *a* absorbs above 680 mμ). This effect, known now as the Emerson enhancement effect, suggested that two pigments must be excited to perform efficient photosynthesis and thus indicated involvement of two light reactions in photosynthesis, one sensitized by light absorption in chlorophyll *a*, and one by absorption in another pigment (for example, chlorophyll *b*). Experiments by others enlarged Emerson's observation by suggesting that plants contained two pigment systems. One (system I, sensitizing reaction I) contains the major part of chlorophyll *a*; the other (system II, sensitizing reaction II) contains the major part of chlorophyll *b* and other auxiliary pigments (for example, the red and blue pigments, called phycobilins, in red and blue-green algae, and the brown pigment fucoxanthol in brown algae and diatoms). Experimental data suggest that system II contains also some chlorophyll *a* (in green cells and diatoms there is a preponderance of a special form with an absorption band at 670 mμ, chlorophyll *a* 670). It appears that efficient photosynthesis requires the absorption of an equal number of quanta in system I and in system II; and that within both systems excitation energy undergoes resonance migration from one pigment to another until it ends in special molecules of chlorophyll *a* called the reaction centers; the latter then enters into the chemical reactions.

Hill and F. Bendell proposed in 1960 that one of these reactions is the transfer of hydrogen (or an electron) from some intermediate in the conversion of water to oxygen to a cytochrome (specifically, cytochrome b_6), while the other is the transfer of hydrogen (or electron) from another cytochrome (specifically, cytochrome *f*) to an intermediate in the conversion of CO_2 to carbohydrate. The intermediate transfer of hydrogen (or electron) from cytochrome b_6 to cytochrome *f* can occur by a dark reaction because the former is a stronger reductant than the latter. The "bucket brigade" for the transfer of hydrogen can thus be represented as Eq. (4), where *hν* represents a light quantum.

$$\begin{array}{l} H_2O \xrightarrow[\text{System II}]{h\nu\ \text{II}} \text{Cytochrome } b_6 \rightarrow \\ O_2 \\ \text{Cytochrome } f \xrightarrow[\text{System I}]{h\nu\ \text{I}} NADP^+ \begin{array}{l} CO_2 \\ \{CH_2O\} \end{array} \end{array} \quad (4)$$

The designation of the first light reaction in the sequence as II and of the second as I is somewhat confusing but is widely used. Experimental evidence for the existence of two pigment systems and the key role of cytochromes in this sequence was provided in 1961 by L. N. M. Duysens and his coworkers: An antagonistic effect of light absorbed in system II and system I on the oxidation-reduction state of the cytochrome was demonstrated. For example, in the red alga *Porphyridium*, green light absorbed by the red pigment phycoerythrin causes the reduction of a cytochrome which has been oxidized by red light absorbed by chlorophyll *a*.

Whether two cytochromes are involved, as suggested by Eq. (4), or just one, and whether $NADP^+$ or an iron-containing enzyme (ferredoxin) or some other compound (X) is the immediate acceptor of hydrogen in light reaction I are the subjects of much research. In addition, the possible role of other experimentally identified oxidation-reduction catalysts such as plastiquinones and a copper-containing protein, plastocyanin, has been investigated in various laboratories. The tentative positions of these electron carriers and others which have not yet been chemically identified but have been observed only kinetically (or spectroscopically) are discussed below. Examples of these carriers are: a special form of chlorophyll *a* (P700), discovered by B. Kok; Q and X that act as the primary electron acceptors of systems II and I, respectively; and ZH, the primary electron donor of system II.

The model of photosynthesis (or electron transport) shown in Fig. 2 is a relatively simple version of the ideas expressed. Research in several laboratories has confirmed, in general, the basic mechanism proposed by Hill, that is, the operation of two light reactions in series with a large number of electron carriers intervening the transfer of elec-

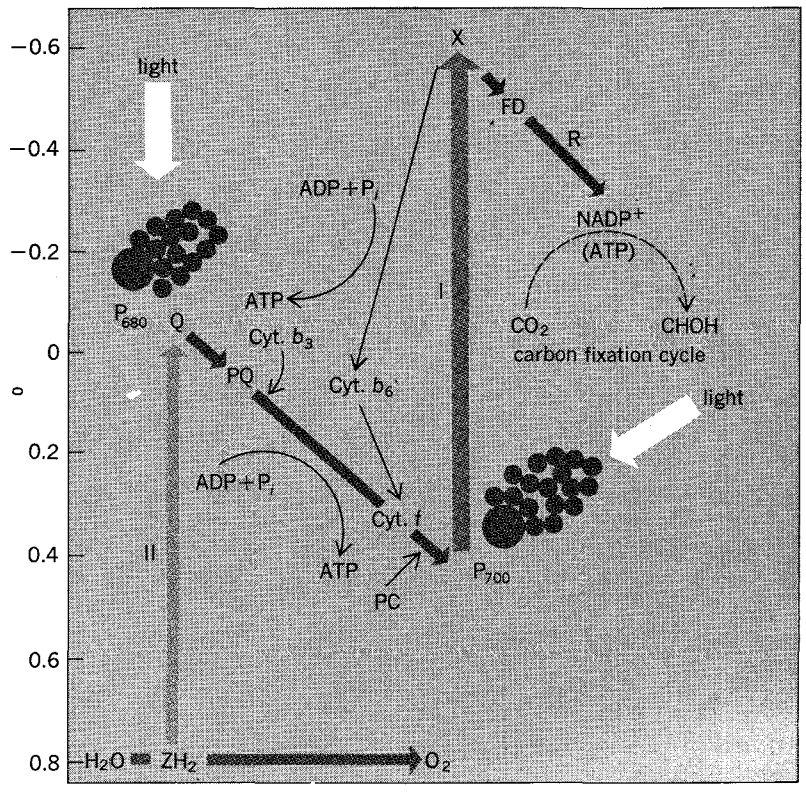


Fig. 2. Two light steps in photosynthesis (compare with Fig. 1). ZH is the (unknown) primary donor of hydrogen atoms (or electrons). Q and X are the primary acceptors of electrons of pigment systems II and I, respectively. P_i is inorganic phosphate, ADP is adenosinediphosphate, and ATP is adenosinetriphosphate (high-energy phosphate). P680 is the "trap" of pigment system II; P700 is pigment 700, the energy trap for system I. Cyt is for cytochrome, PQ is plastoquinone, and PC is plastocyanin. $NADP^+$ is nicotinamide adenine dinucleotide phosphate, FD is ferredoxin, and R is the FD–$NADP^+$ reductase. The two photosynthetic units I and II involved in the process are indicated by solid circles. Both units contain all the pigments but in different proportions; the long-wave form of chlorophyll *a* predominates in unit I and the accessory pigments in unit II. The light energy absorbed in unit I is delivered by transfer to a molecule of P700; by analogy, the energy absorbed in II is supposed to be delivered to a molecule of the pigment P680. ϵ_0 is the oxidation reduction potential at pH 7.0.

trons from reaction II to reaction I.

Photosynthesis is conceived of as a set of at least five reactions, two of which are light reactions (I and II) and three of which are dark reactions (Fig. 2). The starting point is reaction II, the one most closely associated with O_2 evolution. The final result of this set of reactions is the oxidation of water to O_2 and the reduction of cytochromes. Light absorbed by the major part of the accessory pigments (pigment system II, or unit II, such as chlorophyll *b* in green plants, and phycobilins in red and blue-green algae) is ultimately transferred to hypothetical chlorophyll *a* molecules (P680), which are assumed to be in a favorable position to act as an "energy trap" (or reaction center). Evidence for the existence of such reaction centers in system II was obtained during 1967–1968 by G. Döring and coworkers in Berlin. The primary light reaction (lower vertical arrow, Fig. 2) is suggested to be an electron (or hydrogen atom) transfer from the unknown H donor, ZH ($\epsilon_0 \geq +0.8\ \nu$), to the unknown H acceptor, Q ($\epsilon_0 \approx 0\nu$), sensitized by excited P680 as shown in Eqs. (5).

$$\begin{aligned} &P680 + h\nu\ II \rightarrow P680^* \\ &ZH + Q + P680^* \rightarrow Z + QH + P680 \end{aligned} \qquad (5)$$

The oxidation product, the strong oxidant Z, is then utilized to evolve oxygen from water by a dark reaction (bottom horizontal arrow, Fig. 2). The chemical nature of the weak reductant QH is unknown, but its existence has been inferred from the quenching effect of system I light on the chlorophyll fluorescence of system II. Q is the quencher of chlorophyll fluorescence; in light reaction II it is converted to QH (the nonquencher of chlorophyll fluorescence). Light reaction I (discussed below) indirectly restores Q, causing quenching of the fluorescence.

The reduced Q(QH) transfers its electrons to other electron carriers. It is not clear whether QH transfers its electrons first to a plastoquinone (PQ) or to a cytochrome, b_3 (not b_6 as originally proposed by Hill). There are suggestions that both PQ and cytochrome b_3 – both of which have $\epsilon_0 \approx 0\ \nu$ – participate in the electron chain, but whether there are parallel paths or one path is under investigation.

The electrons are then transferred to a copper protein, the plastocyanin, and then to a *c* type cytochrome (cytochrome *f*), both of which have ϵ_0 values close to each other. In some cases, the role of plastocyanin and cytochrome *f* seems to be reversed, but the electrons are in both cases transferred to the well-known energy trap of system I, known as P700. One suggested path is summarized in Eq. (6). Enough energy is released in the "down-

$$\begin{aligned} QH \rightarrow PQ \rightarrow \text{Cytochrome } b_3 \longrightarrow \\ \text{Cytochrome } f \rightarrow PC \rightarrow P700 \end{aligned} \qquad (6)$$

hill" reaction from cytochrome of *b* type (b_3) to cytochrome *f* that phosphorylation (see below) may be coupled with it.

Another light reaction (I) is needed to transfer electrons from P700 to the level of CO_2. The pigment system that sensitizes reaction I is mainly composed of long-wave forms of chlorophyll *a* and P700, but some accessory pigments also contribute their energy to this reaction. Light quanta directly absorbed by P700 molecules and, more importantly, those transferred to them from other pigments excite the P700 molecules as shown in Eq. (7*a*). There are very few P700 molecules because there is only one P700 molecule per 300 chlorophyll molecules.

The primary light reaction is suggested to be a photooxidation of P700 ($\epsilon_0 \cong +0.4\ \nu$) and a reduction of an unknown intermediate X ($\epsilon_0 \approx -0.6\ \nu$). This is expressed in Eq. (7*b*).

$$P700 + h\nu I \rightarrow P700^* \qquad (7a)$$

$$X + P700^* \rightarrow X^- + P700^+ \qquad (7b)$$

The final result of the set of reactions in light reaction I is the oxidation of cytochrome *f* (and other intermediates) and the reduction of $NADP^+$ (nicotinamide adenine dinucleotide phosphate). Thus, a reductant strong enough to reduce CO_2 (or an intermediate containing a COOH group) and an oxidant strong enough to oxidize the reductant, produced by light reaction II, is made available. It has been suggested that the path of electrons from X to $NADP^+$ follows that shown in Eq. (8). The

$$X \rightarrow FD \xrightarrow{R} NADP^+ \qquad (8)$$

reduced X, which has been named ferredoxin reducing substance (FRS) by A. San Pietro, trans-

fers electrons to the iron-containing enzyme ferredoxin (FD), previously known as photosynthetic pyridine nucleotide reductase, and then to $NADP^+$. The transfer to $NADP^+$ is catalyzed by an enzyme (R) which has many names, the most commonly accepted being ferredoxin-$NADP^+$ reductase.

Two products, adenosinetriphosphate (ATP) and reduced $NADP^+$ (NADPH), are needed for the reduction of CO_2 to carbohydrate (CH_2O). For a discussion of the path of carbon in photosynthesis, see the section on carbon dioxide reduction.

By treatment of chloroplasts with detergents, such as digitonin or triton X, it has been possible to physically separate the two pigment systems which perform the two light reactions. Work on mutant algae lacking individual electron carriers has provided further support for the mechanism described above.

Photophosphorylation. A. Frenkel working with bacterial material and D. I. Arnon and coworkers using chloroplast material from green plants showed that these pigment-bearing particles, when illuminated in the presence of ADP (adenosinediphosphate) and inorganic phosphate, use light energy to synthesize ATP; the particles store about 10 kcal of converted light energy in each molecule of the high-energy phosphate ATP. This photophosphorylation could be associated with some energy-releasing step in photosynthesis. A possible location of this step is shown in Fig. 2, the reduction of cytochrome *f* by reduced cytochrome b_3 [Eq. (9)].

$$\text{Cytochrome } b_3 \text{ (reduced)} + \text{ADP} + P_i + \text{Cytochrome } f \rightarrow \text{Cytochrome } b_3 + \text{ATP} + \text{Cytochrome } f \text{ (reduced)} \quad (9)$$

This is analogous to the way in which ATP is produced in respiration. The ATP produced in the light stage of photosynthesis apparently is needed to make some later, enzymatic reactions (such as the reduction of a carboxylic acid by reduced $NADP^+$) run in the needed uphill direction.

Evidence has accumulated that light reaction I can be reversed; that is, instead of going to $NADP^+$ and then to the Calvin cycle, electrons may simply return to any of the intermediates mentioned above (such as cytochromes, plastoquinone, plastocyanin, or P700), thus closing the cycle. It has been suggested that cytochrome b_6 may be an intermediate in this "back reaction" of system I. This type of reaction leads to the production of ATP from ADP and inorganic phosphate (P_i) and has been termed cyclic phosphorylation by Arnon.

It has been shown by A. Jagendorf that light produces a high-energy state, and the actual phosphorylation occurs in dark. Furthermore, if chloroplasts are first suspended in an acidic medium, phosphorylation occurs without the need of light. All these experiments have been interpreted in terms of a hypothesis by P. Mitchell, in which light produces a H^+ ion gradient on the lamellar membranes in the chloroplast, and the energy of this gradient is used to drive phosphorylation. *See* ADENOSINEDIPHOSPHATE (ADP); ADENOSINETRIPHOSPHATE (ATP).

Photosynthesis in flashing light. In the section on the two-quanta hypothesis, mention was made that there are special chlorophyll *a* molecules which engage in the chemical reactions. Their concentration seems to be one in several hundred chlorophyll molecules, and energy absorbed by other pigments is effectively transferred to these special molecules (energy traps or reaction centers). These hypothetical groups of molecules with their energy traps are often referred to as photosynthetic units.

In 1932 Emerson and W. Arnold showed how the light reaction in photosynthesis can be separated from the dark reaction by the use of brief, intense light flashes, separated by intervals of darkness of variable duration. The main conclusion was that the maximum yield of O_2 from a single flash is about 1 molecule of oxygen per 2500 molecules of chlorophyll present in the cell. This can be interpreted as meaning that the cells contain 1 molecule of the rate-limiting enyzme per 2500 chlorophyll molecules; but if the same enzyme has to work n times for the liberation of one molecule of O_2, this ratio must be reduced to $2500/n$; for example, if $n=8$, the ratio becomes about 1:300.

The use of flashing light, with varying flash intensity and duration, variable flash grouping, and varying dark intervals, is one of the most important approaches to understanding the way in which different factors affect the overall rate of photosynthesis through their effects on different steps in the reaction sequence. Monochromatic flashes have been used to gain understanding of the mechanism of the two light reactions. By using these techniques, it has been shown that the evolution of O_2 molecules requires the accumulation of oxidizing power.

Photochemical apparatus. The primary photochemical stage of the photosynthetic process appears to be closely associated with certain structural elements found in plant cells. All algae (except the primitive blue-green algae), as well as all higher plants contain pigment-bearing intercellular bodies called chloroplasts. In the leaves of the higher land plants, these are usually flat ellipsoids about 5000 mμ (0.005 mm) in diameter and 2000–3000 mμ in thickness; 10–100 of them may be present in an average cell of leaf parenchyma. *See* LEAF (BOTANY).

In algae the number and shape of chloroplasts are much more variable; for example the much-studied green unicellular alga *Chlorella* contains only one bell-shaped chloroplast.

All chloroplasts fixed (solidified, usually by osmic acid) and sliced show under the electron microscope a layered structure with alternate lighter and darker layers roughly 10 mμ in thickness. It is generally assumed that these layers differ in their proportion of proteidic and lipoidic (fatlike) substances.

Two main types of chloroplasts are known. In some, the layered structure extends more or less uniformly through the whole chloroplast body (lamellar chloroplasts). In others, this structure is emphasized in certain cylindrical sections and is less pronounced between them (Fig. 3). When such granular chloroplasts are permitted to dry out and disintegrate, stacks of disks break out of the structure and appear as cylindrical grana in the electron micrograph.

In photosynthesizing bacteria and in the lowest truly photosynthetic plants (blue-green algae, Cyanophyta), the photochemical apparatus is more primitive. However, lamellae similar to those in

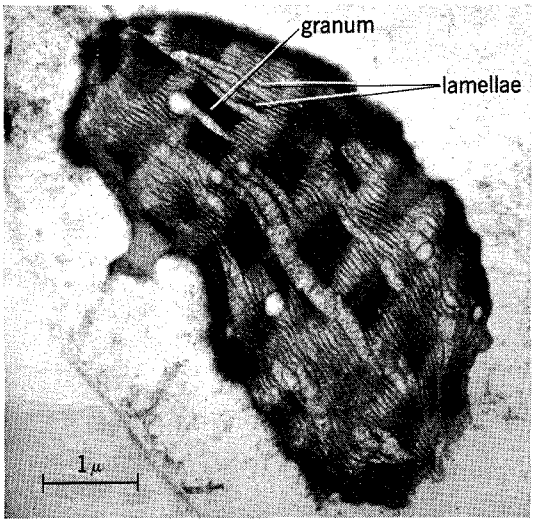


Fig. 3. Electron micrograph of a cross section of a chloroplast of corn (*Zea mays*), fixed with osmic acid, and sliced. It shows lamellae and cylindrical grana formed by their local reinforcement. Chloroplasts of some other species show lamellae only. (*After A. Vatter*)

chloroplasts have been observed also in blue-green algae (and much smaller lamellar particles in bacteria). The true unit of photochemical apparatus may be a lamella consisting of two membranes. It has been suggested that the outer membrane contains the pigment system I, and the inner membrane the pigment system II. Evidence of a "cobblestone" appearance of the lamellae in higher plants has been noted on electron micrographs. These "cobblestones" could be perhaps identified with photosynthetic units containing about 300 chlorophyll *a* molecules; the way in which these units are attached to the membranes remains uncertain. The "cobblestones" have been given the name quantasomes to indicate that they are the most probable sites of the light reaction in photosynthesis (Fig. 4).

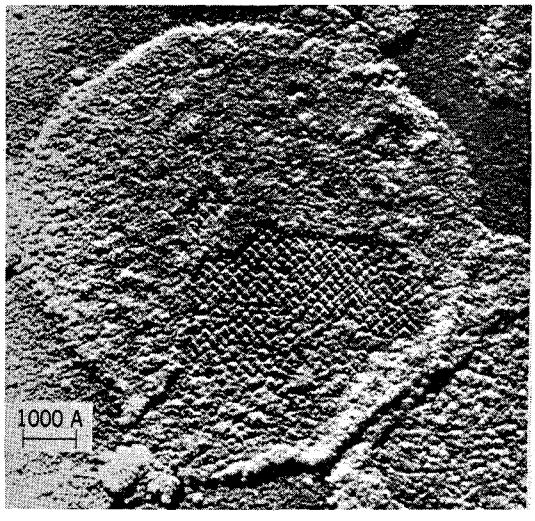


Fig. 4. Membranes containing chlorophyll taken from a spinach chloroplast. This chromium-shadowed preparation shows that the membrane is composed of a highly ordered array of units called quantasomes. (*After R. B. Park, courtesy of Science, 144(3621), 1964*)

Distribution of chlorophyll. It is generally assumed that chlorophyll molecules, which give the green color to plants because they absorb blue and red light and transmit green light, are located at the interfaces between the proteidic and the lipoidic layers of the chloroplasts, perhaps forming one-molecule-thick cohesive layers (monolayers). Estimates suggest that the total area of such interfaces in a chloroplast is just about adequate to accommodate all the chlorophyll molecules present, allowing about 1 $m\mu^2$ for each molecule.

What could be the purpose of a laminar structure "painted over," as it were, with monolayers of pigments? Two hypotheses are offered, both of which may be correct. One is that the two-dimensional, laminar structure creates the best conditions for easy access of the reaction substrates to the chlorophyll molecules and also for rapid removal of the reaction products. Photosynthesis is by far the fastest metabolic process in the cell, and easy supply and removal of reactants may be an important advantage. (In terms of number of molecules transformed per unit volume per unit time, photosynthesis can be 10 or 20 times faster than respiration.) The other hypothesis places emphasis on the possibility of excitation-energy migration in the pigment layer. The absorption of a photon activates a single chlorophyll molecule into a short-lived, energy-rich excited state. To minimize the danger of the dissipation of this excitation energy before it can be used for photochemical purposes, it may be advantageous to permit the energy to move around and to jump from one chlorophyll molecule to another to increase the chance of its encounter with the reaction substrates. Such a mechanism of resonance-energy migration is in fact postulated in some theories of the primary photoprocess in photosynthesis. It is a plausible but by no means a proven concept.

However, the picture of chlorophyll molecules distributed in uniform monolayers on interfaces between proteinaceous and lipoidic lamellae may be oversimplified. There is considerable evidence that not all chlorophyll *a* molecules in the cell are in the same state. These kinds of chlorophyll differ in the positions of their absorption bands and in their capacity for fluorescence, and they may have different functions in photosynthesis. Only a small fraction of chlorophyll *a* seems to be closely associated with the primary photochemical process, while the rest serves primarily as its energy supplier. These differences in function must be somehow associated with the spatial arrangement of chlorophyll molecules in the layered structure, but just how is as yet unknown.

Accessory pigments. An interesting problem is also the location in the chloroplasts and the function in photosynthesis of so-called accessory pigments – that is, pigments other than chlorophyll *a*, the one pigment present in all photosynthetically active plants. In the first place, there are other chlorophylls, such as chlorophyll *b* in higher plants and green algae, and chlorophyll *c* in brown algae. Then there are nonchlorophyllous pigments belonging to two groups: (1) The carotenoids, so called because of similarity to the orange pigment of carrots, are a variable assortment of pigments found in all photosynthetic higher plants, algae,

and even bacteria. (2) The phycobilins, or "vegetable bile pigments," are chemically related to animal bile pigments. The phycobilins are either red (phycoerythrins) or blue (phycocyanins). Both types are present in red algae (Rhodophyta) and blue-green algae (Cyanophyta), the red pigment prevailing in the first group of organisms and the blue pigment prevailing in the second. *See* CAROTENOID.

Experiments by E. Gant and S. F. Conti have established that phycobilins are located in special granules associated with the lamellae of the chloroplasts: These granules have been called phycobilins.

In 1884, T. W. Engelmann suggested that all these pigments contribute to photosynthesis. Later it was concluded that only light absorbed by chlorophyll was of importance. However, it is now clear that light absorbed by accessory pigments does contribute to photosynthesis. These conclusions are derived from measurements of the so-called action spectra of photosynthesis obtained primarily by Emerson and by F. T. Haxo and L. R. Blinks. In such measurements photosynthesis is produced by monochromatic light, isolated by means of a spectrophotometer, and the production of oxygen (or consumption of) either per absorbed quantum of light (the quantum yield) or per incident quantum of light is measured as a function of wavelength. The observed spectral variations in the quantum yield of photosynthesis can be related to the proportions of light absorbed at each wavelength by the different pigments in the cells. Measurements of this kind led to the conclusion that quanta absorbed by carotenoids are 50–80% less effective than those absorbed by chlorophyll *a* in contributing energy to photosynthesis. An exception is fucoxanthol, the carotenoid that accounts for the color of brown algae (Phaeophyta) and diatoms (Bacillariophyta). This pigment supplies light energy to photosynthesis about as effectively as the green pigment. The red and blue pigments of the Rhodophyta and Cyanophyta are also highly effective, as effective as chlorophyll or somewhat less, depending among other things on the history of the algae, particularly the color of the light to which they have become adapted.

Energy transfer between pigment molecules. Chlorophyll *a* in plant cells is weakly fluorescent; this means that some of the light quanta absorbed by it (up to 6%) are reemitted as light. Observations of the action spectrum of chlorophyll fluorescence in different plants have suggested close parallelism with the action spectrum of photosynthesis. In other words, fluorescence of a form of chlorophyll *a* in the plant can be excited also by light absorbed by the accessory pigments, with the probability of this sensitized fluorescence closely paralleling that with which the same light is used for photosynthesis. Excitation of chlorophyll fluorescence by light quanta absorbed by phycoerythrin requires transfer of the excitation energy quantum from the primarily excited phycoerythrin molecule to a nearby chlorophyll molecule (as in acoustic resonance, where striking one bell causes another nearby bell to ring). Therefore it can be suggested that light quanta absorbed by accessory pigments, such as carotenoids and phycobilins, contribute to photosynthesis by being transferred to chlorophyll *a*. By this mechanism red algae, growing relatively deep under the sea where only blue-green light penetrates, can supply the energy of this light to chlorophyll which does not itself absorb it.

If excitation energy can be transferred efficiently in the chloroplasts from accessory pigments to chlorophyll, there is a good probability that a similar transfer can and does occur also between different chlorophyll molecules themselves. If this happens repeatedly during the lifetime of excitation, the excitation energy can migrate as much over considerable distances in the chloroplast. As suggested in the section on distribution of chlorophyll, this migration of excitation energy may have advantages from the point of view of efficient utilization of absorbed light quanta for photosynthesis.

Electron transfer in chloroplasts. It has also been suggested that absorption of a light quantum in the dense layer of chlorophyll molecules may lift an electron into a state in which it will be able to move through the lamella. This is comparable to photoconductivity, a phenomenon known to occur in certain insulating crystals which become electric conductors when irradiated with light. In this way, an electron may become spatially separated from the positive chlorophyll ion and may then act as a reductant at some location in the chloroplast structure (addition of an electron is equivalent to reduction—compare, for example, the conversion of ferric ion, Fe^{3+}, to ferrous ion, Fe^{2+}), while the positive ion may act as an oxidant by taking an electron away from a substrate in another place. Thus the oxidation and the reduction products of the light reaction will be spatially separated, and the danger of their recombination, with the loss of stored energy, reduced.

This picture of photosynthesis as a process typical of a solid, crystalline medium rather than of a solution is a tempting one; it has been supported by certain experiments on chloroplast films. However, other considerations do not support it, such as the similarity of the shape of the absorption band of chlorophyll in the living cell with its shape in solution and the fact that electrons cannot remain free in the presence of water. Perhaps the solid-state theory applies only to very small regions in the chloroplasts or grana, containing 10 or 100 pigment molecules.

The concept of electron transfer is used in a somewhat different sense in the theory of photosynthesis. In the section on the two-quanta hypothesis, the scheme of photosynthesis was discussed in which two cytochromes were involved as intermediates between the two photochemical steps. Cytochromes are known to be oxidized by conversion of their iron atoms from the Fe^{2+} to the Fe^{3+} state by loss of an electron. The intermediate enzymatic state in photosynthesis thus represents a downhill electron transfer similar to that in respiration. At the two ends, however, the oxidation of water and the reduction of carbon dioxide must involve the loss and the acquisition of hydrogen atoms—that is, of electrons and protons. It remains uncertain where in the sequence of reactions the hydrogen transfer is replaced by electron transfer, and again by hydrogen transfer—in particular, whether the two primary photochemical

reactions themselves result in the one or the other.

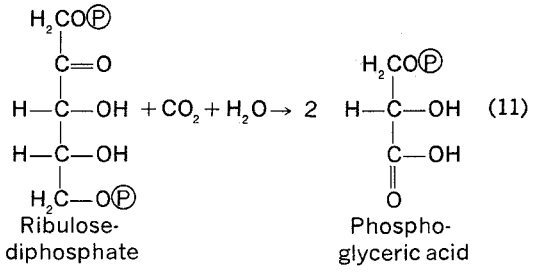
Chemical role of chlorophyll. Unless a solid-state picture of the primary photochemical process in photosynthesis is assumed, the question arises: How does the chlorophyll *a* molecule, ultimately in possession of the absorbed quantum of energy, utilize it for an energy-storing photochemical process, such as the transfer of a hydrogen atom from a reluctant donor (perhaps water) to a reluctant acceptor (perhaps $NADP^+$)? It has been suggested that chlorophyll acts as a typical oxidation-reduction catalyst—that is, by being itself first oxidized and then reduced, or vice versa, with the difference that it uses its excitation energy either in one or in the other, or in both, of these steps. Support for this plausible hypothesis is provided by observations of reversible photochemical oxidation and of reversible photochemical reduction of chlorophyll in solution. Studies of changes in the absorption spectrum of photosynthetic cells in light suggest that a small fraction of a special form of chlorophyll *a* (P700), absorbing maximally at 700 and 430 mμ, is in a changed state during illumination. This is the reaction center of pigment system I. The reaction center of system II (P680) has not yet been shown to undergo oxidation-reduction, but it must aid in the transfer of electrons from water to cytochrome [see Eq. (5)].

Carbon dioxide reduction. Since 1939, knowledge of the conversion of CO_2 into organic molecules, such as glucose, has been much advanced by the application of radioactive tracers, particularly of C^{14} by M. Calvin, A. A. Benson, J. A. Bassham, and coworkers. It was long assumed that the molecule CO_2 is not reduced photochemically as such but is first incorporated into a larger molecule.

The process is now generally assumed to occur by way of carboxylation, that is, by formation of an organic acid from a hydrogen-containing organic molecule as shown in Eq. (10).

$$RH + CO_2 \rightarrow RCOOH \qquad (10)$$

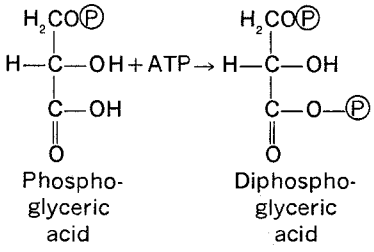
By the use of C^{14} tracer, it has been found that the compound RH is a pentose, that is, a sugar with only five carbon atoms instead of the six present in the more common hexoses. The pentose involved is called ribulose, or more precisely, a phosphate ester of this sugar, ribulose diphosphate (RuDP). It is still uncertain whether the carboxylation of this compound is normally accompanied by hydrolytic splitting, giving rise to two molecules of phosphoglyceric acid, as indicated in Eq. (11). This reaction is catalyzed by an enzyme called carboxydismutase.

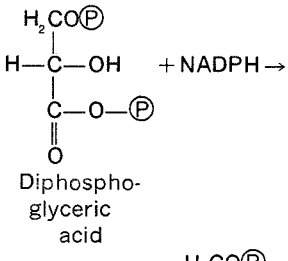


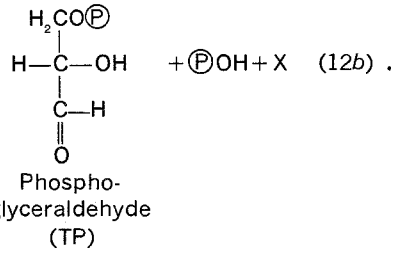
$$\text{℗} = H_2PO_3^-$$

The alternative to Eq. (11) is the formation of a single molecule of an acid with a six-membered carbon chain. Phosphoglyceric acid has been found by some workers to be the main C^{14}-containing product after very brief (1–10 sec) photosynthesis of algae in C^{14}-tagged carbonate. However, in these experiments, algae were killed at the end of exposure by dropping them into boiling alcohol, and it has been suggested that this may have caused the decomposition of a 6-carbon acid into two molecules of phosphoglyceric acid.

If phosphoglyceric acid is the normal intermediate in photosynthesis, it is reasonable to postulate that the next step after its formation is its reduction to phosphoglyceraldehyde as shown in Eqs. (12). This reaction is assumed to take place in two







steps: (1) phosphorylation of phosphoglyceric acid to diphosphoglyceric acid; and (2) reduction of the latter to phosphoglyceraldehyde (or triose phosphate, TP). These reactions are catalyzed by an enzyme called triose phosphate dehydrogenase. This strong reductant (NADPH) must be supplied by the primary photochemical process.

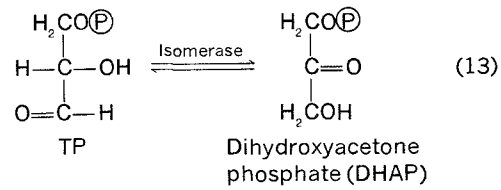
The pyridine nucleotide $NADP^+$ serves as a mediator between the primary photochemical oxidation-reduction and the enzymatic reduction of CO_2 in photosynthesis. It has been proved that $NADP^+$ can be reduced to NADPH by illuminated chloroplast suspension in the Hill reaction; however, this is not in itself convincing proof of the postulated participation of this compound in photosynthesis because many different oxidants can be reduced under the same conditions. However, it has been shown that $NADP^+$ occurs in high enough concentration in chloroplasts to serve as a reductant in photosynthesis and that it does undergo photochemical changes in living matter.

One difficulty arises: NADPH is not a strong enough reductant to reduce phosphoglyceric acid to phosphoglyceraldehyde (or, more generally, to reduce any carboxyl group, RCOOH, to the corresponding aldehyde, RCHO). In fact, the reverse reaction, oxidation of NADPH by glyceraldehyde,

liberates a considerable amount of energy. In respiration, this reaction is coupled with the conversion of ADP and inorganic phosphate into ATP, an energy-storing reaction in which the oxidation energy is neatly preserved in a so-called high-energy phosphate bond, a widely used biological energy currency. It has been suggested that in photosynthesis the reverse happens—that is, the reduction of phosphoglyceric acid to phosphoglyceraldehyde by NADPH is made possible by coupling it with the energy-supplying conversion of ATP back into ADP and inorganic phosphate.

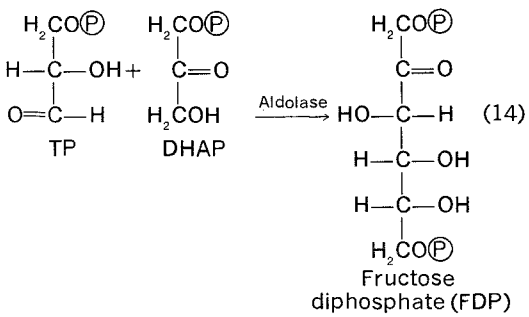
This is the most common version of the mechanism of photosynthesis at present. Since glyceraldehyde has the reduction level of a carbohydrate ($C_nH_{2n}O_n$, with H:O=2:1), its enzymatic conversion to sugars, for example, to a hexose (as final product) or a pentose (as CO_2 acceptor, thus closing the cycle), can be accomplished without further need for light energy by enzymatic reactions of the kinds well known from different metabolic pathways. However, experience shows that one ATP molecule is needed for the conversion of ribulose monophosphate (RuMP) into RuDP.

The specific mechanism by which TP is converted in photosynthesis into hexose phosphates has been established by tracer studies. Phosphoglyceraldehyde first undergoes partial isomerization (with the help of an enzyme, isomerase) into dihydroxyacetone phosphate. This is another three-carbon sugar phosphate, which contains a ketone (C=O) group instead of an aldehyde (CHO) group. An equilibrium is established with 60% glyceraldehyde and 40% dihydroxyacetone phosphate, as shown by Eq. (13). One molecule of each of these



(13)

two intermediates combines (under the action of an enzyme, aldolase) to form a molecule of fructose diphosphate as in Eq. (14). The fructose di-



(14)

phosphate (FDP) thus formed loses a phosphate group, by the action of a phosphatase enzyme, to form fructose monophosphate (FMP). With the help of an isomerase, the latter isomerizes partially to glucose monophosphate. Once glucose and fructose molecules are available, higher molecular carbohydrates such as sucrose or starch can be formed.

For the continuous operation of the carbon cycle, RuDP must be regenerated for the carboxylation reaction. When the cycle rotates six times, one hexose molecule is formed. From 6 molecules of RuDP, 6 molecules of CO_2, 12 molecules of NADPH, and 12 molecules of ATP, 12 TP molecules are formed; 2 of these give 1 molecule of sugar, but the other 10 molecules regenerate RuDP (C_5 compound) by a very involved mechanism. A key role is played here by vitamin B (thiamine), which has the capacity of transferring C_2 groups from one sugar to another. Thiamine is used in two ways: It takes a first C_2 group from a C_6 sugar to form a C_4 sugar (erythrose); and a second one from a C_7 sugar (sedoheptulose), formed by condensation of this erythrose (C_4) with a triose (C_3). This gives a pentose (C_5) molecule; other pentoses are formed by addition of the two C_2 groups to triose molecules. These pentoses may be either ribose, xylose, or ribulose. Ultimately they are all converted into ribulose.

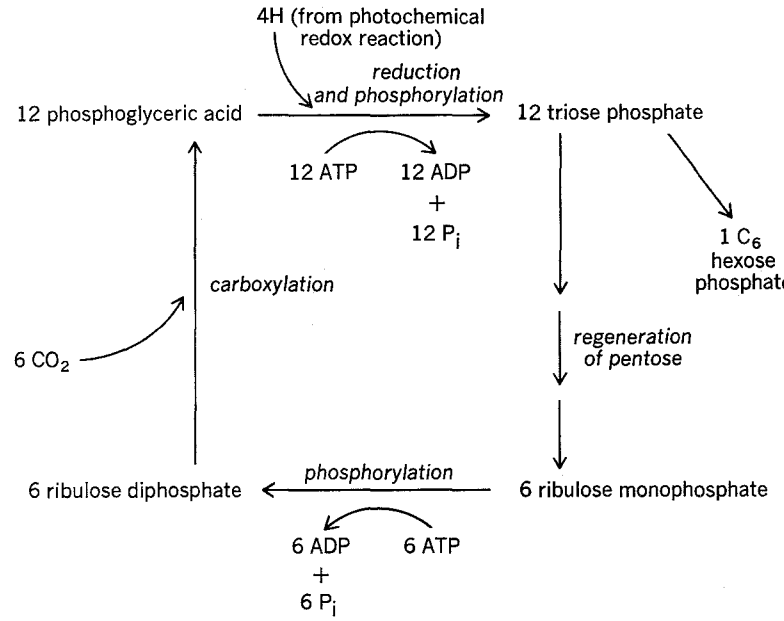


Fig. 5. Condensed version of the Calvin cycle.

Figure 5 is a condensed version of the Calvin cycle, an elaboration of the upper arrow of Fig. 1 where each arrow represents a sequence of several reactions. The three major reactions are: (1) carboxylation of RuDP (such as by the addition of CO_2 to the 5-carbon keto sugar phosphate) and formation of phosphoglyceric acid (PGA), a 3-carbon acid; (2) reduction of PGA to a 3-carbon aldose, TP, and (3) regeneration of RuDP by mutual conversion of carbohydrates. As mentioned previously, the reductant generated by the light reactions (presumed to be NADPH) is utilized for the reduction steps while ATP, another product of the light reaction, is utilized at two sites. These are (1) in the phosphorylation of the 5-carbon keto sugar, RuMP to RuDP, and (2) in the phosphorylation of PGA to diphosphoglyceric acid prior to its reduction.

It has long been suspected that direct photosynthesis of compounds other than carbohydrates can take place in photosynthetic systems. It has been shown, for example, that under certain conditions, 30% or more of the photosynthate in one green algae appears in the form of amino acids. Under other conditions, when algal cells are exposed to

CO_2 for only 1–2 min, as much as 30% of the fixed carbon is found in lipid-like substances.

In 1969 M. D. Hatch and C. R. Stack showed that in corn and several other plants the path of carbon is somewhat different: The primary acceptor of CO_2 is phosphoenolpyruvic acid; this carboxylation leads to the reaction formation of a 4-carbon compound (oxaloacetate). The latter feeds the carboxyl group to the carbon cycle and produces pyruvate that, after phosphorylation, regenerates phosphoenolpyruvate.

Bacterial photosynthesis and chemosynthesis. Certain species of pigmented bacteria, some green (containing a green pigment called bacterioviridin, or chlorobium-chlorophyll), some purple or red (containing bacteriochlorophyll and carotenoids), are able to synthesize organic matter from CO_2 in light. Since the main absorption band of bacteriochlorophyll is located in the near-infrared while that of chlorophyll is in the red, purple bacteria can live also in invisible, infrared light. In contrast to green plants and algae, these organisms cannot use water as a source of hydrogen for the reduction of CO_2 and can survive only under conditions providing other hydrogen sources, such as hydrogen sulfide or other sulfur compounds, free molecular hydrogen, or organic compounds. In the last case, the bacteria destroy one kind of organic matter to synthesize another.

Because hydrogen is bound nowhere as strongly as in water, these types of photosynthesis store little if any light energy. They do not have the same significance as plant photosynthesis in the transformation and storage of cosmic energy on Earth. In fact, all they can do is utilize, in light, chemical energy already available in the form of unstable hydrogen compounds. In most cases they use light energy merely or mainly as chemical activation energy as it is also used in most photochemical reactions in the test tube.

It has been suggested that bacterial photosynthesis also involves two light reactions, but the details are very different. Eight quanta seem to be required for the reduction of one CO_2 molecule in bacteria. *See* BACTERIAL PHOTOSYNTHESIS.

It is unknown whether bacterial photosynthesis is an earlier mode of life, preceding plant photosynthesis, or a later form of life into which plant photosynthesis has degenerated in chemical surroundings providing certain sources of hydrogen. In any case, bacterial photosynthesis is bound to remain limited to a few natural habitats, such as stagnant canal waters or volcanic sulfur springs.

For the sake of completeness, mention should be made also of chemosynthetic bacteria, cells which can achieve the conversion of CO_2 to organic matter with the help of hydrogen donors similar to those utilized by photosynthetic bacteria, but without the help of light. They simply burn chemical fuel by a mechanism permitting them to salvage some combustion energy to reduce CO_2. In the simplest case, that of so-called hydrogen bacteria, the cells oxidize one part of molecular hydrogen to water and use some of the liberated energy to transfer another part of the hydrogen to CO_2. Whereas photosynthetic bacteria can live anaerobically, the chemosynthetic ones require oxygen to keep their energy-liberating process in operation. Some chemosynthetic organisms have developed wherever oxidizable material is present in nature, be it coal, oil, free hydrogen, sulfur compounds, ammonia, nitrite, or ferrous salts. Again the question can be asked: What is the evolutionary role of the chemosynthetic way of life? Is it a predecessor of photosynthesis, or is it degradation of photosynthesis under especially "easy" conditions of abundant energy supply? [GOVINDJEE]

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