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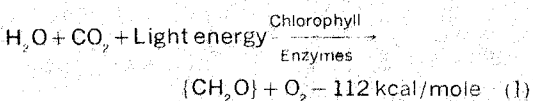
Since fossil fuels (coal, oil, peat) are half-decayed products of plant photosynthesis from past geological ages, it can be said that not only all life energy but also nearly all industrial power, as well as all domestic heat, have their origin in photosynthesis. Exceptions are wind and water power and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

$$H_2O + CO_2 + \text{Light energy} \xrightarrow[\text{Enzymes}]{\text{Chlorophyll}} \{CH_2O\} + O_2 - 112 \text{ kcal/mole} \quad (1)$$

where $\{CH_2O\}$ stands for a carbohydrate (sugar).

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 charac-

teristic of this particular oxidation-reduction is that it goes "in the wrong direction" energetically, 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 absorbed by chlorophyll is converted into chemical energy, in the form of some energy-rich intermediate products 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 transfer of hydrogen atoms, H (or electrons) – not of hydrogen molecules, H_2, or hydrogen ions, H^+ – from an unknown intermediate in phase 2 to some intermediate acceptor capable of reducing carbon dioxide. This is the light phase of photosynthesis. Phase 2 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 may be enzymatic, requires manganous ions, and involves several steps. 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 2, 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
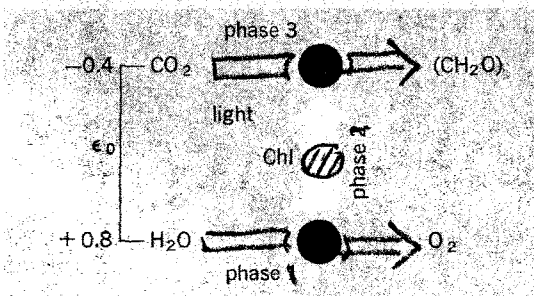

Fig. 1. Schematic illustrations of photosynthesis. Phase 1, the light reaction, is the transfer by light-excited chlorophyll (Chl) of hydrogen (or electrons). Phase 2, oxidation of water, consists of enzymatic reactions converting dehydrogenated water to free oxygen. Phase 3, reduction of carbon dioxide, consists of enzymatic reactions converting carbon dioxide and light-supplied hydrogen to carbohydrates (CH_2O). ϵ_0 is the oxidation-reduction potential of H_2O-O_2 and $CO_2-(CH_2O)$ couples at pH 7.0.

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, V_{max}, determined by the total amount of the 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 substrate before its transformation is completed. This is expressed as Eq. (2). The several enzymes involved in photosynthesis

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

al enzymes involved in photosynthesis thus impose 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 molecule, Z) 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 organelles (called chloroplasts) 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 chloroplast's outer membrane.

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 close 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 with intact membranes which can "use" 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 potential chemical energy ("free energy") are stored. Light is absorbed by matter in the form of quanta or 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 vigorously 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 use of four hydrogen atoms as expressed by Eq. (3). A quantum

$$CO_2 + 4H \rightarrow [CH_4O_2] \rightarrow [CH_2O] + H_2O \qquad (3)$$
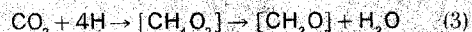
requirement of eight or more would thus permit two quanta to be used for the transfer of each hydrogen atom (or electron) from H_2O to CO_2.

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 nm (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 nm). This effect, known now as the Emerson enhancement effect, suggested that two pigments must be excit-
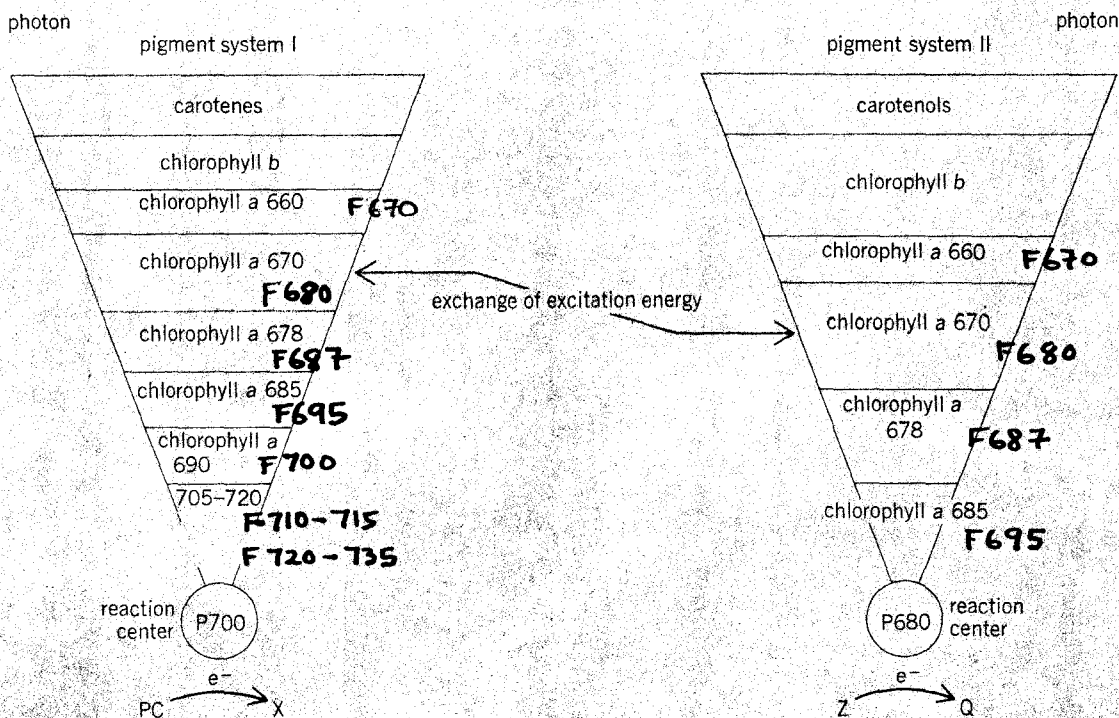
Fig. 2. Distribution of pigments in the higher plants is different in the two photosystems (or pigment systems). Among the carotenoids, carotenes dominate in PSI and carotenols in PSII. Chlorophyll *b* is more abundant in PSII. Chlorophyll *a* is the most important pigment in both systems, but each has a characteristic assortment of spectral forms of this pigment, distinguished by their absorption maxima in the red end of the spectrum, in nanometers (*black*) and fluorescence maxima, also in nanometers (*color*). The reaction-center pigments of the two systems also exhibit different absorption spectra. (*From Govindjee and R. Govindjee: The Primary Events in Photosynthesis, Sci. Amer., 231:68–82, 1974*)

ed 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 (photosystem I, PSI, sensitizing reaction I) contains the major part of chlorophyll a: the other (photosystem II, PSII 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 PSII also contains some chlorophyll a. (In green cells and diatoms there is a preponderance of a spectral form with an absorption band at 670 nm, chlorophyll a 670.) It appears that efficient photosynthesis requires the absorption of an equal number of quanta in PSI and in PSII: 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 (Fig. 2).

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.

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 PSI and PSII 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 Hill and Bendall, or just one, and whether an iron-containing enzyme (bound 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: the reaction center chlorophyll a P700, discovered by B. Kok: the reaction center chlorophyll a P680, discovered in H. T. Witt's
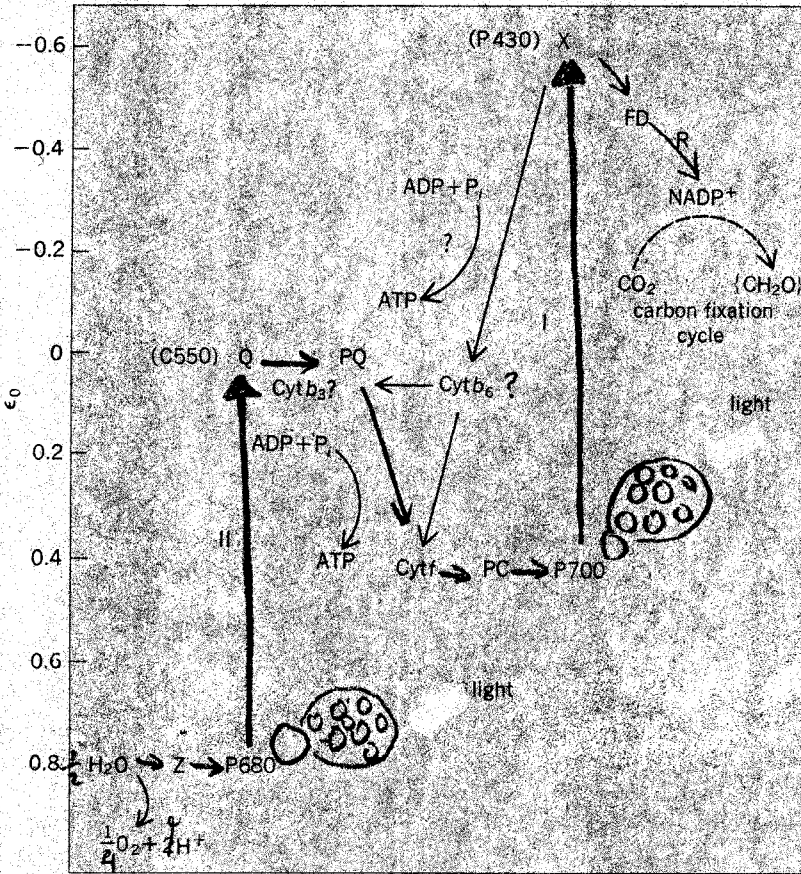
Fig. 3. Two light steps in photosynthesis (compare with Fig. 1). Z is the (unknown) donor of hydrogen atoms (or electrons). Q (C550) and X (P430) are the primary acceptors of electrons of pigment systems II and I, respectively. P_i is inorganic phosphate, ADP is adenosinediphosphate, and ATP is adenosinetriphosphate (high-energy phosphate). P680 is the reaction center of pigment system II; P700 is the reaction center for system I. Cyt is 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. The roles of Cyt b_3 and of the phosphorylation step in the cycle around PSI have not been established and are indicated by question marks.

laboratory: Q and X that act as the primary electron acceptors of PSII and PSI, respectively; and Z, the electron donor to P680$^+$ of PSII.

The model of photosynthesis (or electron transport) shown in Fig. 3 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 electrons 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. 3). 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 plastoquinone. Light absorbed by the major part of the accessory pigments (PSII, or unit II, such as chlorophyll b in

green plants, and phycobilins in red and blue-green algae) is ultimately transferred to a chlorophyll a molecule (P680), which is assumed to be in a favorable position to act as an "energy trap" (or reaction center). The primary light reaction (lower vertical arrow, Fig. 3) is suggested to be an electron transfer from P680 to the electron acceptor, Q ($\epsilon_0 \approx 0v$), powered by excited P680 as shown in Eqs. (4). The P680 recovers by accepting an electron from Z (Eq. 5):

$$P680 + h\nu \, II \rightarrow P680^*$$
$$Q + P680^* \rightarrow Q^- + P680^+ \qquad (4)$$

$$Z + P680^+ \rightarrow Z^+ + P680 \qquad (5)$$
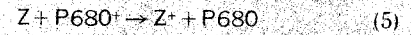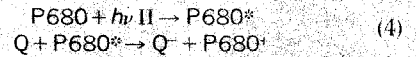
The oxidation product, the strong oxidant Z$^+$, is then utilized to oxidize water and liberate O_2 and H$^+$ ions by dark reactions (bottom arrow, Fig. 3).

The chemical nature of the weak reductant Q$^-$ is unknown, but its existence has been inferred from the quenching effect of PSI light on the chlorophyll fluorescence of PSII. Q is the quencher of chlorophyll fluorescence; in light reaction II it is converted to Q$^-$ (the nonquencher of chlorophyll fluorescence). Light reaction I (discussed below) indirectly restores Q, causing quenching of the fluorescence. It has been shown that under favorable conditions an absorbance change at 550 nm (labeled C550)—which is, perhaps, due to a shift in absorption band of a carotene complex—can serve as an excellent indicator of changes in Q. In addition, data have accumulated which suggest that Q$^-$ may be a semiquinone with an absorption change around 320 nm and equivalent to another component labeled X-320 in Witt's laboratory.

The reduced Q(Q$^-$) transfers its electrons to other electron carriers. It is not clear whether Q$^-$ transfers its electrons 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 $\epsilon_0 \approx 0v$—participate in the electron transport chain, but whether there are parallel paths or one path is under investigation. Most researchers are not sure about the role of b_3. However, it does act as an electron donor to P680 at 77 K.

The electrons are then transferred to c-type cytochrome (cytochrome f) and then to a copper protein, the plastocyanin, 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 PSI, known as P700. Here again, the possibility of parallel paths exists. One suggested path is summarized in Eq. (6). Enough energy is released in the "down-

$$Q \rightarrow B \text{ (or R)} \longrightarrow PQ \rightarrow$$
$$\text{Cytochrome } f \rightarrow PC \rightarrow P700 \quad (6)$$
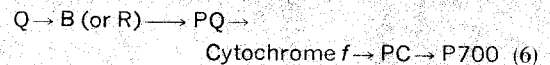
hill" reaction from Q to PC so that phosphorylation (see below) be coupled with it. In addition to the clarification of the role of cytochrome b_3, the chemical nature of a new component labeled B (or R) between Q and PQ needs to be investigated. This component has been shown by kinetic measurements to accumulate two electrons before transferring them to the plastoquinone pool. It has been suggested that Q, B, and PQ are all different populations of plastoquinones.

Another light reaction (I) is needed to transfer

electrons from P700 to the level of $NADP^+$, nicotinamide dinucleotide phosphate. The pigment system that sensitizes reaction I is mainly composed of long-wave forms of chlorophyll a and P700, but accessory pigments also contribute their energy to this reaction (Fig. 2). 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. (7a). 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. (7b).

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

$$X + P700^* \rightarrow X^- + P700^+ \qquad (7b)$$
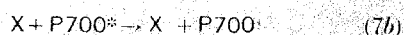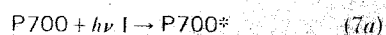
The final result of the set of reactions in light reaction I is the oxidation of cytochrome f (and other intermediates) and the reduction of $NADP^+$ (nicotinamide adenine dinucleotide phosphate). Thus, a reductant strong enough to reduce CO_2 (or an intermediate containing a COOH group) and an oxidant 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 transfers 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. This "R" should not be confused with the "R" mentioned above.

The chemical nature of X has not yet been definitely established. Electron-spin resonance studies indicate that it is an iron-sulfur protein. Optical studies show that it has an absorbance band at 430 nm and, thus, is often referred to as P430. There are strong indications that it may be a bound ferredoxin.

The chemical nature of the reaction centers P680 and P700 is also not definitely established. Chlorophyll a is definitely a component of these centers. There are strong indications, based on optical and electron-spin resonance studies, that these are dimers of chlorophyll a.
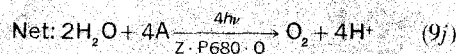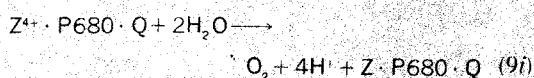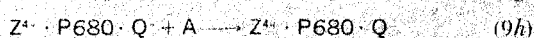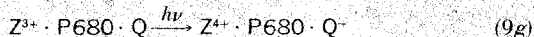
Two products, adenosinetriphosphate (ATP) and reduced $NADP^+$ (NADPH), are needed for the reduction of CO_2 to carbohydrate (CH_2O). For a discussion of how ATP is produced see the section on photophosphorylation; for a discussion of the path of carbon in photosynthesis see the section on carbon dioxide reduction.

By treatment of chloroplasts with detergents, such as digitonin or triton, 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.

O_2 evolution. All oxygen liberated in photosynthesis originates in water and none in CO_2, as shown by experiments with isotopic tracers in which tracer oxygen, O^{18}, was found in liberated O_2 when it was incorporated into water but not when it was incorporated in CO_2. These experiments were made by S. Ruben, M. D. Kamen, and coworkers in 1941. There ha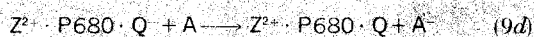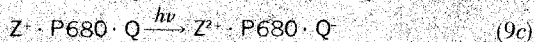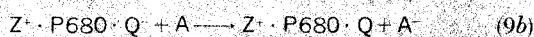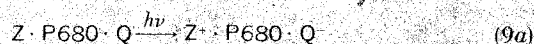s been some criticism of these experiments, because the experimental conditions were such that a considerable amount of exchange reaction between H_2O and CO_2 must have taken place. These results, however, were confirmed in 1975 by A. Stemler under conditions which minimized the exchange reaction.

The mechanism of O_2 evolution is the least-known part of the photochemical process. However, based on the measurements of the amount of O_2 evolved in single brief (10 μsec) saturating light flashes, Kok and P. Joliot have suggested that four oxidizing equivalents must accumulate on Z before it can oxidize H_2O to O_2. Because the pattern of O_2 evolution follows a period of 4, that is, the maxima occur on every fourth flash, reaction mechanism (9) has been proposed (A refers to the plastoquinone pool, whereas other symbols have the same meaning as described earlier).

$$Z \cdot P680 \cdot Q \xrightarrow{h\nu} Z^+ \cdot P680 \cdot Q^- \qquad (9a)$$

$$Z^+ \cdot P680 \cdot Q^- + A \longrightarrow Z^+ \cdot P680 \cdot Q + A^- \qquad (9b)$$

$$Z^+ \cdot P680 \cdot Q \xrightarrow{h\nu} Z^{2+} \cdot P680 \cdot Q^- \qquad (9c)$$

$$Z^{2+} \cdot P680 \cdot Q^- + A \longrightarrow Z^{2+} \cdot P680 \cdot Q + A^- \qquad (9d)$$

$$Z^{2+} \cdot P680 \cdot Q \xrightarrow{h\nu} Z^{3+} \cdot P680 \cdot Q^- \qquad (9e)$$

$$Z^{3+} \cdot P680 \cdot Q^- + A \longrightarrow Z^{3+} \cdot P680 \cdot Q + A^- \qquad (9f)$$

$$Z^{3+} \cdot P680 \cdot Q \xrightarrow{h\nu} Z^{4+} \cdot P680 \cdot Q^- \qquad (9g)$$

$$Z^{4+} \cdot P680 \cdot Q^- + A \longrightarrow Z^{4+} \cdot P680 \cdot Q \qquad (9h)$$

$$Z^{4+} \cdot P680 \cdot Q + 2H_2O \longrightarrow$$

$$O_2 + 4H^+ + Z \cdot P680 \cdot Q \quad (9i)$$

$$\text{Net: } 2H_2O + 4A \xrightarrow[Z \cdot P680 \cdot Q]{4h\nu} O_2 + 4H^+ \qquad (9j)$$
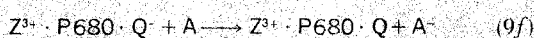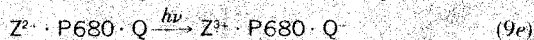
Further analysis of O_2 evolution in single brief flashes of light and in low-intensity continuous light preceded by varying number of light flashes suggests that in darkness $Z^+ \cdot P680 \cdot Q$ is stable. In dark-adapted samples, about 80% of reaction-center complex is in this state and about 20% in the $Z \cdot P680 \cdot Q$ state. Such a situation would lead to O_2 evolution in the third flash followed by a period of 4, as actually observed. The oscillations in O_2 evolution in a series of flashes is found to damp out after 10–15 flashes. In order to quantitatively explain all the data, one has to suggest a certain number of "double hits," that is, two light reactions in the same flash, and a certain number of "misses," that is, inability of the centers to proceed forward. Research suggests that there is more than one component between H_2O and P680 and, therefore, the charge accumulator (Z, above) should be distinguished from Z, the electron donor to $P680^+$. Govindjee and R. Govindjee propose that these compounds be distinguished by subscripts as follows:

$$\left. \begin{array}{c} H_2O \\ O_2 \end{array} \right\} \quad Z_3 \rightarrow Z_2 \rightarrow Z_1 \rightarrow P680$$
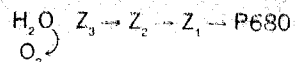
where Z_3 is the charge accumulator (Z in Eq. 9).

and Z_1 is the electron donor to P680$^+$, and Z_2 is another intermediate between Z_3 and Z_1 which is responsible for an electron spin resonance signal labeled II vf in K. Sauer's laboratory.

It has also been shown that Mn is required for O_2 evolution. Plants grown in an Mn-deficient medium lose their capacity to evolve O_2. Reconstitution by adding external Mn, however, has not yet been shown to restore O_2 evolution, although light-induced electron flow from Mn to an externally added Hill oxidant is restored. O_2 evolution can only be restored by growing the Mn-deficient plants in an Mn medium. It has been speculated that the charge accumulator (Z_3) may be an Mn complex, as Mn is known to exist in several valence states. Further research is needed to understand the biochemical mechanism of O_2 evolution. Research by T. Wydrzynski, Govindjee, and coworkers has shown that water-proton relaxation rate may be an excellent monitor of changes in redox state of manganese during O_2 evolution.

In 1974 A. Stemler, Govindjee, and coworkers demonstrated that the presence of bicarbonate ions (HCO_3^-) is necessary for the recovery of reaction centers during O_2 evolution in chloroplasts incapable of CO_2 fixation. Further studies have shown that HCO_3^- is needed for electron flow from Q$^-$ to A (see Eq. 9).

Photophosphorylation. In 1954 A. Frenkel working with chromatophores from photosynthetic bacteria and D. I. Arnon and coworkers using chloroplasts 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. 3, the reduction of cytochrome f by plastoquinone [Eq. (10)].

$$PQ\,(reduced) + ADP + P_i$$
$$+ Cytochrome\,f \rightarrow PQ + ATP$$
$$+ Cytochrome\,f\,(reduced) \qquad (10)$$
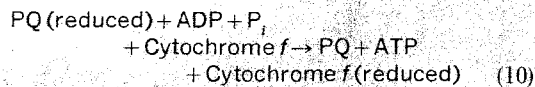
This is analogous to the way in which ATP is produced in respiration. This site has been termed site I. A second site of phosphorylation has recently been proposed to lie somewhere between H_2O, and PQ and has been termed site II. The two sites of phosphorylation are slightly different in their pH dependence and other requirements. Site II phosphorylation occurs only in phase 2 (Fig. 1), that is, oxidation of H_2O resulting in the release of H$^-$ on the inside of the lamellar membrane occurs. If non–H$^+$ ion–producing artificial electron donors are used instead of H_2O, site II of phosphorylation is inhibited, but site I continues to operate. 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, at least in culture; that is, electrons, instead of going to NADP$^-$, may simply return to any of the intermediates mentioned above (such as cytochromes, plastoquinone, plastocya-

nin, or P700), thus closing the cycle. It has been suggested that cytochrome b_6 may be an intermediate in this "back reaction" of PSI. This type of electron flow, mediated by phenazine methosulfate, leads to the production of ATP and ADP from and inorganic phosphate (P_i) and has been termed cyclic phosphorylation. The existence and role of such phosphorylation in living material needs further study.

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 and then transferred to an alkaline medium in the presence of ADP and P_i, 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 across the lamellar membranes in the chloroplast, and the energy dissipation of this H$^+$ gradient via the "coupling factor" (or ATP synthase, a protein complex present in the lamellae and associated with phosphorylation) is used to drive phosphorylation. W. Junge and Witt have shown that phosphorylation in short saturating light flashes is related to the electric field generated across the thylakoid membrane as a result of the initial light-induced charge separation. This may occur within <20 nanoseconds (nsec). Reagents which dissipate the electric field or $\Delta\psi$, for example, valinomycin, also inhibit phosphorylation. However, under steady-state conditions phosphorylation is not affected by valinomycin. It is possible that under flash conditions phosphorylation is related to $\Delta\psi$, and under steady-state conditions ΔpH. The ΔpH can also lead to membrane potential. The two together are referred to as proton motive force (pmf). Experimental evidence exists for the creation of both ΔpH and $\Delta\psi$ in chloroplasts as well as in chromatophores. The relative importance of ΔpH and $\Delta\psi$ in these two systems may, however, be different. In addition, these pigment-bearing particles also show light-induced conformational changes. The generally accepted picture, based on Mitchell's hypothesis, is shown in Eq. (11). Another theory, the conformational

$$Light \rightarrow Electron\,transport \rightarrow$$
$$Proton\,motive\,force \rightarrow ATP \qquad (11)$$

theory, however, considers the conformation of proteins to be of primary importance in the storage of energy. However, it has not been possible to show that such changes occur in a short time. A third theory is the well-known chemical theory in which a chemical intermediate precedes ATP synthesis. Such an intermediate has never been isolated. Thus, it appears best to continue to use Eq. (11) as a reasonable hypothesis for ATP synthesis. *See* ADENOSINEDIPHOSPHATE (ADP); ADENOSINETRIPHOSPHATE (ATP).

Photosynthetic unit. 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. They found that the yield of a single flash was maximum when the interval between the consecutive light flashes was at least 0.04 sec at 1°C. This, then, is the minimum time required for the efficient utilization of the products from the light reactions. Emerson and Arnold further observed that, when the dark period between the flashes was more than 0.04 sec, the maximum yield from a single flash was one O_2 molecule per 2500 chlorophyll molecules. Since a minimum of eight quanta are required to evolve one O_2 molecule, it can be envisioned that the absorption of eight quanta of light by a group of 2500 chlorophyll molecules results in the evolution of one O_2 molecule. However, it is now known that the two light-reaction mechanisms of photosynthesis require the transfer of four electrons through two light reactions for every molecule of oxygen evolved. Thus there are at least eight photoacts leading to the evolution of one O_2 molecule. Therefore, this ratio of one O_2 per 2500 Chl should be one photoact per 300 chlorophyll molecules. This is the commonly accepted size of one photosynthetic unit in higher plants and algae.

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, as discussed earlier.

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, called grana, and is less pronounced in the area between them, called the stroma region (Fig. 4). 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. See CELL PLASTIDS.

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 chloroplasts have been observed also in blue-green algae (and much smaller lamellar particles in bacteria). The unit of photochemical apparatus may be a lamella consisting of two submembranes forming a saclike disk called a thylakoid. It was suggested earlier that the outer one contains the PSI, and the inner one the PSII. Newer experiments suggest that these two systems may transverse the entire membrane and there may be a vectorial arrangement of electron transport components in them. For example, it has been suggested that in PSII the oxygen-evolving system is on the inner (locular) side of the membrane, and Q, the primary electron acceptor, is close to the outer (stromal) side of the membrane. In PSI, plastocyanin, cytochrome f, and P700 are suggested to be closer to the inner side, and X, ferredoxin, and ferredoxin-NADP$^+$ reductase on the outer side. Such a picture is only tentative and is based partly on immunochemical data.

The fine structure of thylakoid membranes is far from clear. Various-sized particles and surfaces have been observed by the use of freeze-etch and freeze-fracture techniques. It has not been possible to correlate fine structure with function except in the case of coupling factor. These particles are found on the top of the thylakoid membrane in the unstacked regions. Their removal stops phosphorylation activities. They can be reconstituted with the membrane with the restoration of phosphorylation activities.

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 mμ^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-
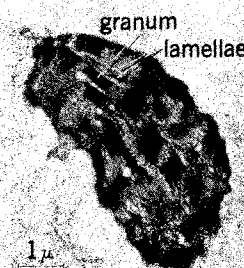

Fig. 4. Electron micrograph of a cross section of a chloroplast from mesophyll cells of corn (*Zea mays*), fixed with osmic acid. (*After A. Vatter*)

granum
lamellae
1μ

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.

However, the picture of chlorophyll molecules distributed in uniform monolayers on interfaces between proteinaceous and lipoidic lamellae is, perhaps, grossly oversimplified. There is considerable evidence that not all chlorophyll a molecules in the cell are in the same physical 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 (Fig. 2). 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. See CHLOROPHYLL.

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 photosynthetic bacteria. (2) The phycobilins, or "vegetable bile pigments," are chemically related to animal bile pigments. The phycobilins are either red (phycoerythrin) or blue (phycocyanin). 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. Another phycocyanin called allophycocyanin is also present in blue-green algae. See CAROTENOID; FLUORESCENCE COMPOUNDS (PLANT).

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 phycobilisomes.

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 excited by monochromatic light, and the production of oxygen 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 physiological status of the algae and the color of the light to which they have become adapted. The primary function of all these pigments is to harvest the light energy and transfer it to reaction-center chlorophyll molecules.

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 a fluorescence in different plants have suggested close parallelism with the action spectrum of photosynthesis. In other words, fluorescence 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. This mechanism is also called heterogeneous energy transfer. By this mechanism red algae, growing relatively deep under the sea where only green light penetrates, can supply the energy of this light to chlorophyll which has a very weak absorption in the green region of the spectrum.

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. This is called homogeneous energy transfer. 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. Excitation-energy transfer among chlorophyll a molecules or among phycobilin molecules can be shown by the depolarization of fluorescence when these pigments are excited by polarized light. Excitation-energy transfer from accessory pigments (donor molecules) to chlorophyll a (acceptor molecules) or from various short-

wavelength forms of chlorophyll a to the long-wavelength forms of chlorophyll a is shown by sensitized fluorescence technique. The most widely accepted hypothesis is that proposed by T. Förster. In it, energy transfer is preceded by thermal relaxation in the donor molecules. The efficiency of energy transfer depends upon three basic factors: orientation of acceptor molecule with respect to the donor molecule; overlap of fluorescence spectrum of the donor molecule with the absorption spectrum of the acceptor molecule; and the distance between the two molecules (energy transfer is proportional to R^{-6}, where R is the distance). Other mechanisms have not yet been ruled out. The function of most of the pigments (including most of chlorophyll a molecules) is to act as an antenna, harvest the energy, and transfer to very few (1 in 300) reaction-center molecules (P700 and P680, depending upon the pigment system; see in Fig. 2). Energy is trapped and used for photochemistry as noted earlier.

If PSI and PSII are separated in space, there is also the possibility of excitation-energy transfer among such units. Energy transfer can also occur in units of the same type. It has been shown that excitation-energy transfer among units of the same type or different types need not be constant; it can vary depending upon various physical and chemical factors.

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 other 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 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 inter-system en-

zymatic chain in photosynthesis thus represents a downhill electron transfer similar to that in respiration (Fig. 3). At the two ends, however, the oxidation of water and the reduction of NADP$^+$ must involve the loss and the acquisition of hydrogen atoms — that is, of electrons and protons.
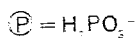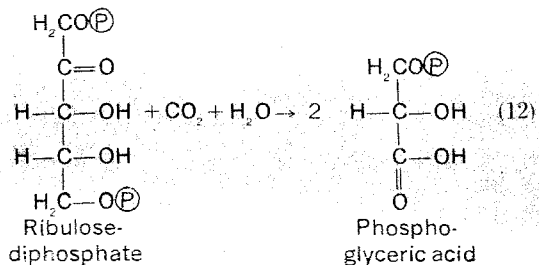
Chemical role of chlorophyll. Unless a solid-state picture of the primary photochemical process in photosynthesis is assumed, the question arises: How does the chlorophyll a molecule, ultimately in possession of the absorbed quantum of energy, utilize it for an energy-storing photochemical process, such 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 an oxidized state during illumination. This is the reaction center of PSI. The reaction center of PSII (P680) has been suggested to undergo oxidation-reduction [see Eqs. (4) and (7)].

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 of 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. (11).

$$RH + CO_2 \rightarrow RCOOH \qquad (11)$$
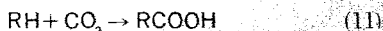
By the use of C^{14} tracer, it has been found that the compound RH is a pentose, that is, a sugar with only five carbon atoms instead of the six present in the more common hexoses. The pentose involved is called ribulose, or more precisely, a phosphate ester of this sugar, ribulose diphosphate (RuDP). It is still uncertain whether the carboxylation of this compound is normally accompanied by hydrolytic splitting, giving rise to two molecules of phosphoglyceric acid (PGA), as indicated in Eq. (12). This reaction is catalyzed by an enzyme called carboxydismutase or carboxylase.

$$
\begin{array}{l}
H_2CO\circledP \\
\quad | \\
\quad C=O \\
\quad | \\
H-C-OH \;+\; CO_2 + H_2O \rightarrow \\
\quad | \\
H-C-OH \\
\quad | \\
H_2C-O\circledP
\end{array}
\qquad
2 \;\;
\begin{array}{l}
H_2CO\circledP \\
\quad | \\
H-C-OH \\
\quad | \\
C-OH \\
\quad \| \\
O
\end{array}
\quad (12)
$$

Ribulose-diphosphate   Phospho-glyceric acid

$$\circledP = H_2PO_3{}^-$$

208 PHOTOSYNTHESIS

The alternative to Eq. (12) is the formation of an intermediate: 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 light 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.

The next step after PGA formation is its reduction to phosphoglyceraldehyde as shown in Eqs. (13). This reaction is assumed to take place in two

$$\text{(13a)}$$

Phospho-
glyceric
acid
(PGA)

Diphospho-
glyceric
acid

Diphospho-
glyceric
acid

Phospho-
glyceraldehyde
(TP)

$$\text{(13b)}$$
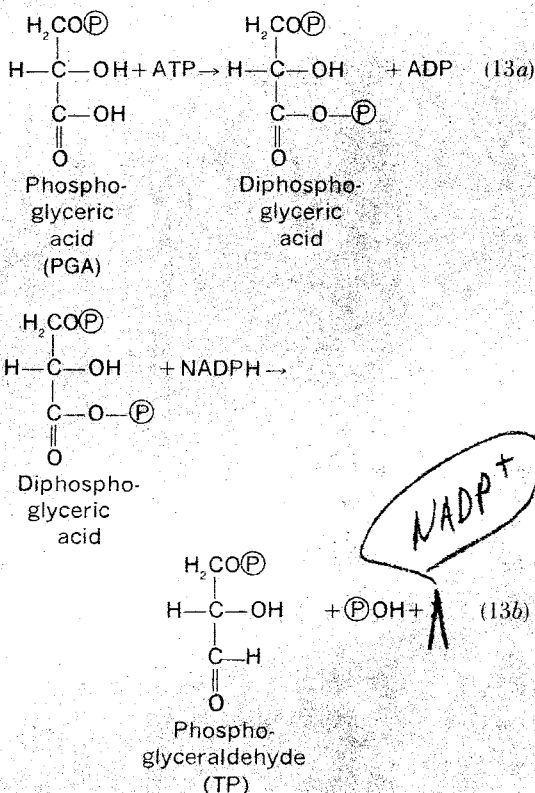
steps: (1) phosphorylation of PGA to diphosphoglyceric acid; and (2) reduction of the latter to phosphoglyceraldehyde (or triose phosphate, TP). These reactions are catalyzed by an enzyme called triose phosphate dehydrogenase. The strong reductant (NADPH) and ATP must be supplied by the primary photochemical 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 corre-

sponding 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. (14). One molecule of each of these

$$\text{(14)}$$

TP

Dihydroxyacetone
phosphate (DHAP)
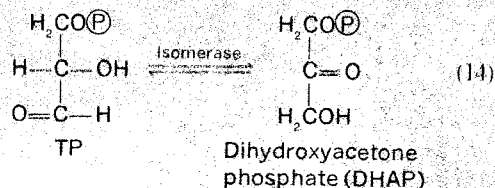
two intermediates combines (under the action of an enzyme, aldolase) to form a molecule of fructose diphosphate as in Eq. (15). The fructose di-

$$\text{(15)}$$

TP   DHAP

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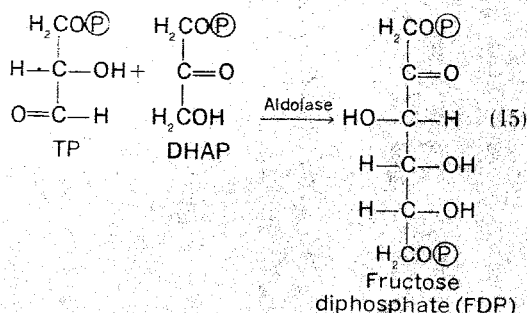
phosphate (FDP) thus formed loses a phosphate group, by the action of a phosphatase enzyme, to form fructose monophosphate (FMP). With the help of an isomerase, the latter isomerizes partially to glucose monophosphate. Once glucose and fructose molecules are available, higher molecular carbohydrates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

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 aldehyde, TP, and (3) regeneration of RuDP by mutual conversion of carbohydrates. As mentioned previously, the reductant generated by the light reactions (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 production 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.

Carbon dioxide fixation in C_4 plants. In 1969 M. D. Hatch and C. R. Stack showed that in maize, sugar cane, and several other plants containing dimorphic (mesophyll and bundle sheath) chloroplasts the path of carbon is somewhat different: The primary acceptor of CO_2 is phosphoenolpyruvic acid; this carboxylation, catalyzed by phosphoenolpyruvate carboxylase, leads to the formation of a 4-carbon dicarboxylic acid such as oxaloacetic acid in the mesophyll cells. Plants fixing CO_2 in this manner are called C_4 plants. Oxaloacetate is reduced to malate or malic acid with the help of NADPH in most plants studied (malate formers). In some plants — the aspartate formers — aspartate may be produced instead of malate. Malate is rapidly transported to the bundle-sheath cells, where it is converted into pyruvate and CO_2 (decarboxylation) with the simultaneous reduction of $NADP^+$ to NADPH. CO_2 is fixed into sugars via the Calvin cycle, and phosphoglyceric acid is generated from ribulose 1,5-diphosphate. Thus, malate transfers the CO_2 and the reducing power from the mesophyll to the bundle-sheath cells.
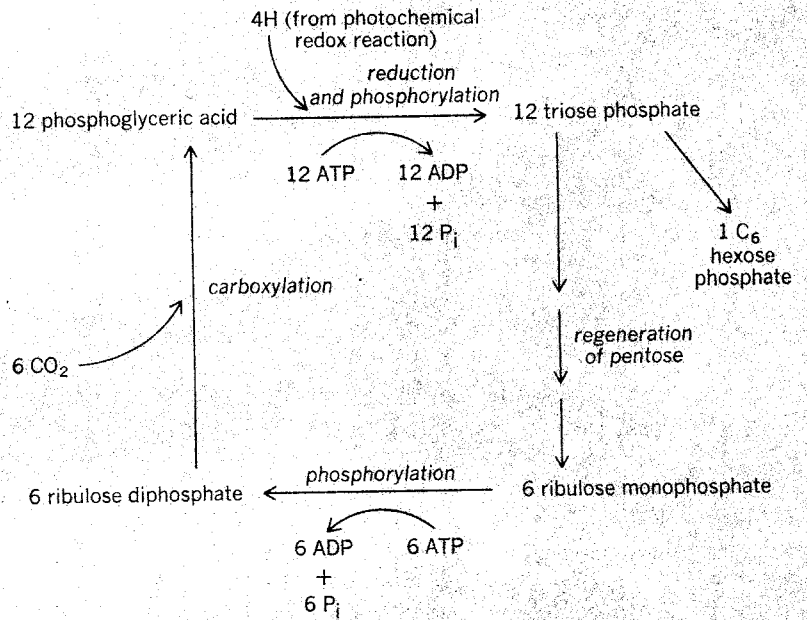

Fig. 5. Condensed version of the Calvin cycle.

Pyruvate is translocated back to the mesophyll cells, where it is converted into phosphoenolpyruvate (with the help of ATP) to continue the cycle. The above cycle is reminiscent of Crassulacean acid metabolism (CAM), with the difference that CAM plants fix CO_2 into malate at night, and refix CO_2 into phosphoglyceric acid during the day. The temporal specialization in CAM plants is replaced by spatial specialization in C_4 plants. An evaluation of taxa showing C_4 pathways suggests that this pathway must have originated independently and in parallel in various species.

Improving efficiency of photosynthesis. In view of the tremendous increase in human population on Earth and limited resources available to feed all its inhabitants, it is necessary to exploit ways to increase the efficiency of photosynthesis, which in turn may lead to increased production of food. Basic research has revealed several possible approaches to this problem. These include attempts to reduce wasteful respiration losses, particularly of photorespiration (release of CO_2 in light). Low photosynthetic efficiency of many plants in bright light is due to the release of photorespiratory CO_2 leading to loss of carbon. Hence, the slowing of photorespiration by biochemical or genetic means should lead to increases in net photosynthesis and yield. Several laboratories are now attempting to use tissue cultures to obtain desirable mutants.

In nature, plants have adapted their photosynthetic machinery to the environment they grow in. Scientists must begin to utilize the adaptive mechanisms of these plants and diversify agriculture to grow nonconventional plants to feed animals as well as humans.

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

210 PHOTOTRANSISTOR

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. 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 "easy" conditions of abundant energy supply? [GOVINDJEE; R. GOVINDJEE]

Bibliography: J. Berry, Adaptation of photosynthetic processes to stress, *Science*, 188:644–650, 1975; R. K. Clayton, *Molecular Physics in Photosynthesis*, 1965; H. Gaffron, Energy storage in photosynthesis, in *Plant Physiology: A Treatise*, vol. 1B, 1960; Govindjee (ed.), *Bioenergetics of Photosynthesis*, 1975; M. D. Kamen, *Primary Process in Photosynthesis*, 1963; B. Kok and A. T. Jagendorf (eds.), *Photosynthetic Mechanisms of Green Plants*, 1963; J. M. Olson (ed.), *Energy Conversion by the Photosynthetic Apparatus*, 1966; E. I. Rabinowitch, *Photosynthesis and Related Processes*, 3 vols., 1945–1956; E. I. Rabinowitch and Govindjee, *Photosynthesis*, 1969; J. B. Thomas and J. H. C. Goedheer (eds.), *Currents in Photosynthesis*, 1966; L. P. Vernon and G. R. Seely (eds.), *The Chlorophylls: Physical, Chemical and Biological Properties*, 1966; I. Zelitsch, Improving the efficiency of photosynthesis, *Science*, 188:626–633, 1975.