Its rate of photorespiration is slower than usual for a C_3 species, and its net photosynthetic CO_2 assimilation is less sensitive to O_2. Other species with characteristics intermediate between C_3 and C_4 plants have been described, and individual C_3 plants with lower-than-usual photorespiration and increased net photosynthesis have also been described. Finding examples of C_3 species and varieties with intermediate rates of photorespiration supports the view that rapid photorespiration is a wasteful process. Presumably decreases in photorespiration might be accomplished by chemical regulation or genetic selection. The application of the technique of making mutant selections from large populations of plant cells and regenerating mutant plants shows promise as a method for obtaining plants of C_3 species with slower rates of photorespiration and increased rates of net CO_2 assimilation. *See Plant respiration.*

Israel Zelitch

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Photosphere

The photosphere of the Sun (the visible surface of the Sun or other stars) is a gaseous layer a few hundred miles thick with an average effective temperature of 9940°F (5780 K), determined from the total radiation per square centimeter. The temperature is maintained by convection, which brings hot material from the opaque solar interior to the surface in the form of rising columns of gas. The convection produces a small-scale granular texture which is visible through a projection telescope. In areas where strong magnetic fields inhibit the convection, the photosphere cools and dark sunspots appear. *See Stellar evolution; Sun.*

John W. Evans

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 by chlorophyll- or bacteriochlorophyll-containing cells. This process requires a supply of energy in the form of light, since its products contain much more chemical energy than its raw materials. This is clearly shown by the liberation of energy in the reverse process, the combustion of organic material with oxygen. *See Chlorophyll; Plant respiration.*

In chlorophyll-containing plant cells and in cyanobacteria, photosynthesis involves oxidation of water (H_2O) to oxygen molecules, which are released into the environment. In contrast, bacterial photosynthesis does not involve O_2 evolution—instead of H_2O, other electron donors, such as H_2S, are used. Both types of photosynthesis are discussed below.

The light energy absorbed by the pigments of photosynthesizing cells, especially by the pigment chlorophyll or bacteriochlorophyll, is efficiently converted 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 ultimate source of all living matter and of all life energy.

Under favorable external conditions, photosynthesis is a remarkably fast process. For example, with an adequate supply of carbon dioxide and light, a green algal 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 (CO_2), the intensity or color of illumination, or the temperature. The rate of photosynthesis depends also on the age, nutrition, and physiological condition of the organism, factors which are much more difficult to define and control precisely.

The total turnover of photosynthesis on Earth has been 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 on 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 annually from the inorganic into the organic state. This corresponds to about 10^{18} kcal (10^{15} kWh) 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. *See Biogeochemistry.*

Plant Photosynthesis

The net overall chemical reaction of plant photosynthesis is shown in Eq. (1), where $\{CH_2O\}$ stands

$$H_2O + CO_2 + \text{light energy} \xrightarrow[\text{enzymes}]{\text{chlorophyll}} \{CH_2O\} + O_2 \quad (1)$$

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 characteristic of this particular oxidation-reduction is that it goes "in the wrong direction" energetically; that is, it converts 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.

Multistage process. Photosynthesis is a complex, multistage process. Its main parts are: (1) 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 (2) the enzyme-catalyzed "dark" (that is, not photochemical) reactions by which these intermedi-

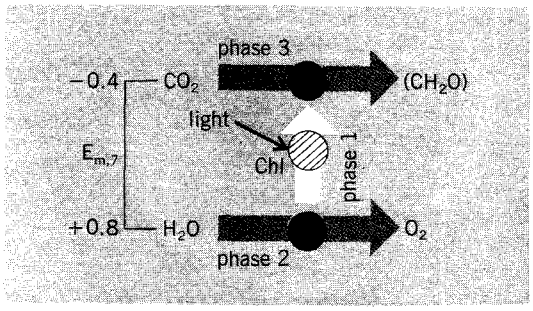


Fig. 1. Schematic illustration of photosynthesis. Phase 1, the light reaction, is the transfer by light-excited chlorophyll (Chl) of electrons. Phase 2, oxidation of water, consists of enzymatic reactions converting dehydrogenated water to free oxygen. Phase 3, reduction of carbon dioxide, consists of enzymatic reactions converting carbon dioxide and light-supplied electrons to carbohydrates (CH_2O). $E_{m,7}$ is the oxidation-reduction potential at pH 7.0.

ates are converted into the final products—carbohydrates and free oxygen. These reactions of photosynthesis can be grouped into three phases (**Fig. 1**). Phase 1 is the transfer of electrons from an unknown intermediate in phase 2 to some intermediate acceptor capable of reducing CO_2. This is the light phase of photosynthesis. Phase 2 is the evolution of oxygen from dehydrogenated water. This is the least-understood aspect of photosynthesis. It requires an oxygen-evolving complex as well as chloride and manganous ions, and involves several steps. Phase 3 is the redution 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.

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 may have various causes, one of which 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. Carbon dioxide 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.)

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; the several enzymes involoved in photosynthesis impose ceilings on the maximum speed at which photosynthesis as a whole can proceed, with each enzyme functioning as a bottleneck of limited capacity in the reaction path. *See Enzyme.*

Hill reaction. Various observations suggest that the immediate action of light in photosynthesis involves the transfer of electrons from the primary reductant (electron donor), the reaction center chlorophyll molecule, to an electron acceptor, the primary oxidant (Fig. 1, phase 1). This is then followed by electron flow from H_2O to the oxidated reaction center chlorophyll molecule (Fig. 1, phase 2), and from the reduced primary acceptor to a molecule of nicotinamide adenine dinucleotide phosphate ($NADP^+$).

The reaction in phase 1 and phase 2 of Fig. 1 resembles the Hill reaction (named after its discoverer, R. Hill) in which illuminated chlorophyll-bearing organelles (chloroplasts) produce oxygen from water without the concomitant reduction of CO_2 but with the reduction of added, less stable oxidants, such as a quinone, ferricyanide, or 2,6-dichloro-phenol indophenol. Since the minimum 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 has been assumed that in the Hill reaction, the primary photochemical apparatus of photosynthesis is preserved more or less intact. In the Hill 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.

Quantum process. 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 group 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. *See Absorption of electromagnetic radiation; Photon.*

The reduction of one molecule of CO_2 to the carbohydrate level requires the use of four hydrogen atoms as expressed by Eq. (2). A minimum quantum

$$CO_2 + 4H \rightarrow [CH_4O_2] \rightarrow [CH_2O] + H_2O \qquad (2)$$

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 Robert Emerson. Emerson had discovered that the "maximum 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 nanometers (the "red drop"). Later, Emerson showed 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 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 (called photosystem I, or PSI, sensitizing reaction I) contains the major part of chlorophyll *a*; the other (called photosystem II, or PSII, sensitizing reaction II) contains some chlorophyll *a* and the major part of chlorophyll *b* or other auxiliary pigments (for example, the red and blue pigments, called phycolibins, in red and blue-green algae, and the brown pigment fucoxanthol in brown algae and diatoms). 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

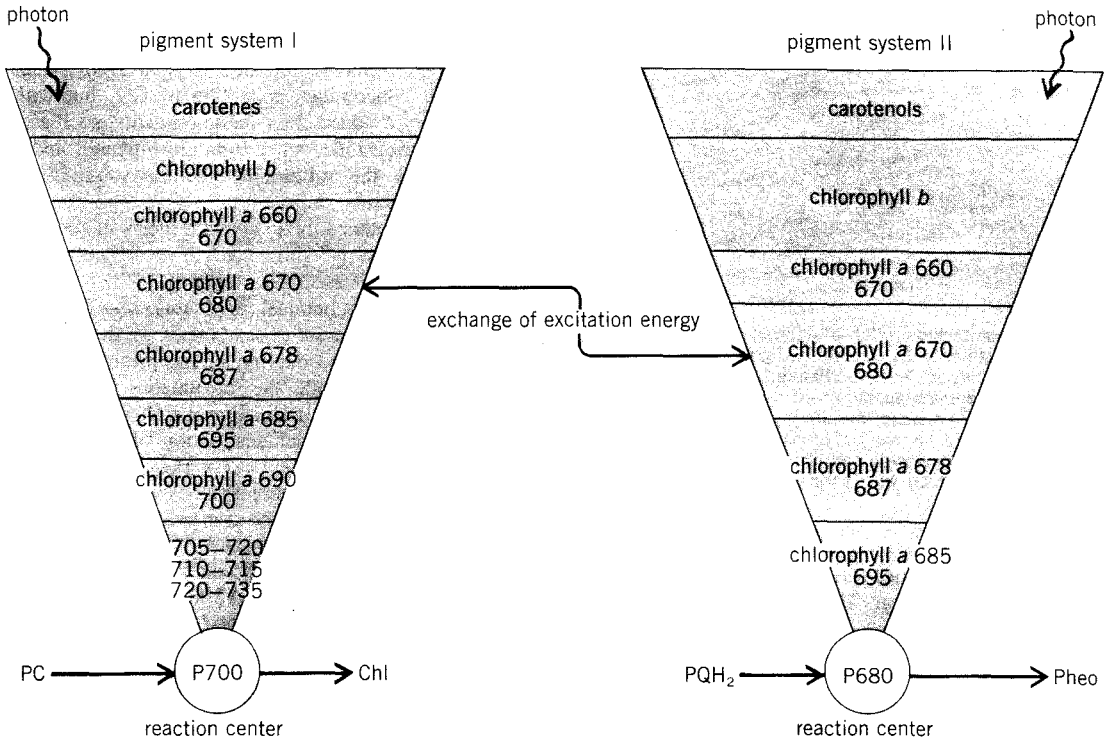


Fig. 2. Working model for the distribution of pigments in the two photosystems of higher plants. Relative abundance is indicated by bandwidth; chlorophyll *b*, for example, is more abundant in PSII. Chlorophyll *a* is the most important pigment in both systems, but each has a characteristic assortment of spectral forms, distinguished by their maxima in the red end of the spectrum, in nanometers (black) and fluorescence maxima (color). In PSI, the primary reaction is the oxidation of P700 (the reaction-center chlorophyll *a* molecule—the primary electron donor) and the reduction of Chl (a chlorophyll *a* molecule acting as the primary electron acceptor, also called A_0); this is followed by the reduction of $P700^+$ by plastocyanin (PC). Photosystem II is similar, but the primary electron acceptor is a pheophytin molecule (Pheo), while the electron donor to $P680^+$ is a plastoquinol (PQH_2) molecule, usually referred to as Z.

molecules of chlorophyll *a* called the reaction centers; the latter (P700 or P680) then enter into the chemical reactions (**Fig. 2**).

Hill and F. Bendall proposed that one of these reactions is the transfer of an electron from some intermediate in the conversion of water to oxygen to cytochrome b_6 (shown later to be a plastoquinone molecule), while the other is the transfer of an electron from cytochrome *f* to an intermediate in the conversion of CO_2 to carbohydrate. The intermediate transfer of hydrogen (or electron) from reduced plastoquinone to cytochrome *f* can occur without energy input because the former is a stronger reductant than the latter. Experimental evidence for the existence of two pigment systems and the key role of plastoquinone and cytochrome *f* in this sequence has been provided. However, the specific role of cytochrome b_6 has not been confirmed.

Photosynthesis is conceived of as a set of at least five reactions, two of which are light reactions (PSI and PSII) and three of which are dark reactions (**Fig. 3**). The PSII reaction is 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 a plastoquinone (an oxidation-reduction catalyst). Light absorbed by the major part of the accessory pigments 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 P stands for pigment and the numerical designation gives the location, in nanometers, of the maximum decrease in the absorption in the red or the near-infrared region of the spectrum when the pigment is illuminated by a bright actinic light. The primary light reaction (**Fig. 3**) is suggested to be an electron transfer from P680, within about a picosecond, to the electron acceptor pheophytin (Pheo), powered by excited P680. The P680 recovers by accepting an electron from a plastoquinol, Z, within 20–400 nanoseconds, as shown in reactions (3).

$$P680 + h\nu\ II \rightarrow P680^* \quad (3a)$$
$$Pheo + P680^* \rightarrow Pheo^- + P680^+ \quad (3b)$$
$$Z + P680^+ \rightarrow Z^+ + P680 \quad (3c)$$

The components of reactions (3), that is, P680, pheophytin, and Z, and the next intermediate Q (a bound plastoquinone), are located, in all likelihood, in a chlorophyll *a*–protein complex having a molecular weight of 47,000–51,000 daltons. The oxidation product, the strong oxidant Z^+, is utilized to oxidize water and liberate O_2 and proton (Fig. 3).

The PSI reaction is the one most closely associated with the reduction of $NADP^+$. Light absorbed by most of the chlorophyll *a* molecules is ultimately transferred to the reaction center chlorophyll *a* and energy trap of PSI, the P700. The primary light reaction of PSI is oxidation of P700 and the reduction of an electron acceptor chlorophyll a (Chl *a*) within a few picoseconds, as shown in reactions (4).

$$P700 + h\nu_I \rightarrow P700^* \quad (4a)$$
$$Chl\ a + P700^* \rightarrow Chl\ a^- + P700^+ \quad (4b)$$

The three dark reactions, mentioned above, are (1) the electron flow from Chl a^- to $NADP^+$ via an in-

termediate A_1, the iron sulfur centers (F_X, F_B, F_A), and ferredoxin (Fd); (2) the electron flow from $Pheo^-$ to P700 via several electron carriers (plastoquinones Q_A, Q_B, and PQ, Rieske iron sulfur center, cytochrome *f*, and a copper protein plastocyanin); and (3) the electron flow from H_2O to oxidized plastoquinol Z^+.

O_2 evolution. The mechanism of O_2 evolution is the least-known part of the photochemical process. All oxygen liberated in photosynthesis originates in water. Based on the measurements of the amount of O_2 evolved in single brief (10 microseconds) saturating light flashes, it has been suggested that four oxidizing equivalents must accumulate on the O_2-evolving complex before it can oxidize H_2O to O_2. It appears as if an oxygen "clock" exists in which the state (S) of the O_2-evolving complex undergoes the sequential reaction (5) where the subscripts on S represent the oxidizing equivalents accumulated on the complex.

$$S_0 \rightarrow S_1 \rightarrow S_2 \rightarrow S_3 \rightarrow S_4 \quad (5)$$
$$\uparrow \qquad\qquad \downarrow O_2 \qquad\qquad \uparrow$$

Manganese (Mn) is required for O_2 evolution. Plants grown in a manganese-deficient medium lose their capacity to evolve O_2. It has been speculated that the charge accumulator of the reaction mechanism may be a manganese complex, as manganese is known to exist in several valence states. In addition to manganese, chlorine ions also function at the O_2-evolving site, though the mechanism of action is not known. The O_2-evolving complex is composed of at least four proteins having molecular weights of 34,000, 33,000, 24,000 and 18,000 daltons. Further research is needed to understand the biochemical mechanism of O_2 evolution.

Photophosphorylation. Chromatophores from photosynthetic bacteria and chloroplasts from green plants, when illuminated in the presence of adenosinediphosphate (ADP) and inorganic phosphate, use light energy to synthesize adenosinetriphosphate (ATP); about 10 kcal of converted light energy is stored in each molecule of the high-energy phosphate, ATP. This photophosphorylation could be associated with some energy-releasing step in photosynthesis, such as the electron flow from PSII to PSI. When phosphorylation is associated with noncyclic electron flow from H_2O to $NADP^+$, it is called noncyclic photophosphorylation. *See Adenosinediphosphate (ADP); Adenosinetriphosphate (ATP).*

There is also evidence to suggest that light reaction I (PSI) can be reversed, at least in isolated chloroplasts; electrons, instead of going to $NADP^+$, may simply return to an intermediate (such as a cytochrome, plastoquinone, plastocyanin, or P700) and thus close 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 added cofactors and ADP and inorganic phosphate (P_i), leads to the production of ATP and has been termed cyclic phosphorylation.

It has been shown that light produces a high-energy state, and that the actual phosphorylation occurs in the 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

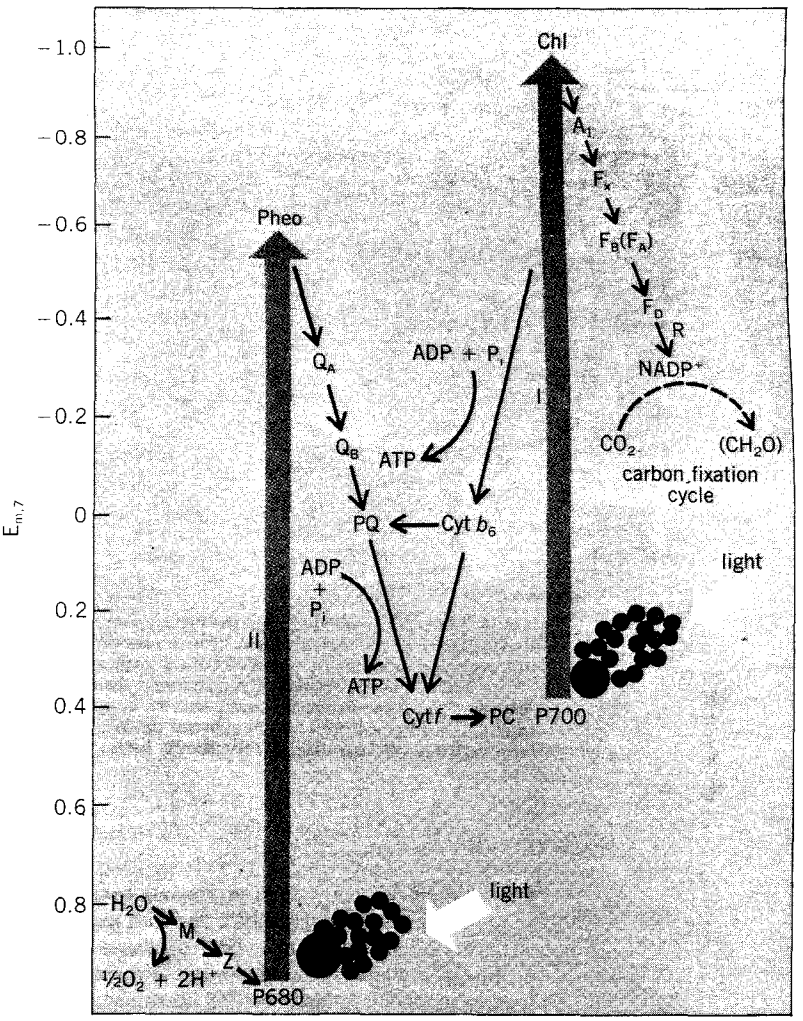


Fig. 3. Schematic representation of electron flow and chemical reactions in the two light steps in photosynthesis. Z (a plastoquinol) is the electron donor to the oxidized reaction-center chlorophyll *a* P680 of photosystem II (PSII), and M (a manganese-containing protein) is the charge accumulator that leads to O_2 evolution. Pheo (pheophytin) and Chl (chlorophyll *a*) are the primary electron acceptors of PSII and PSI, respectively. Q_A and F_X (an iron sulfur center) are the stable electron acceptors of PSII and PSI, respectively. Q_B is another bound plastoquinone, while PQ is plastoquinone, cyt is cytochrome, and PC is plastocyanin. A_1 is an electron acceptor of PSI, F_B and F_A are iron sulfur centers, FD is ferredoxin, and R is the FD-$NADP^+$ reductase. The pigment-containing antenna units I and II involved in the process are indicated by solid circles. $E_{m,7}$ is the oxidation reduction potential at pH 7.0.

these experiments have been interpreted in terms of a hypothesis by P. Mitchell, in which light produces a H^+ ion gradient (ΔpH) across the lamellar membranes in the chloroplasts, and the energy dissipation of this H^+ ion gradient via the coupling factor (or ATP synthase, a protein complex present in the lamellae) leads to phosphorylation. In addition, an electric field ($\Delta\Psi$) generated across the thylakoid membrane as a result of the initial light-induced charge separation can also drive photophosphorylation. Reagents that dissipate the electric field or the H^+ gradient also inhibit phosphorylation. The two together are referred to as proton motive force (**Fig. 4**).

Photosynthetic unit. The concentration of the special chlorophyll *a* molecules (P700 or P680) that engage in the chemical reactions is one in several hundred chlorophyll molecules; energy absorbed by other pigments is effectively transferred to these spe-

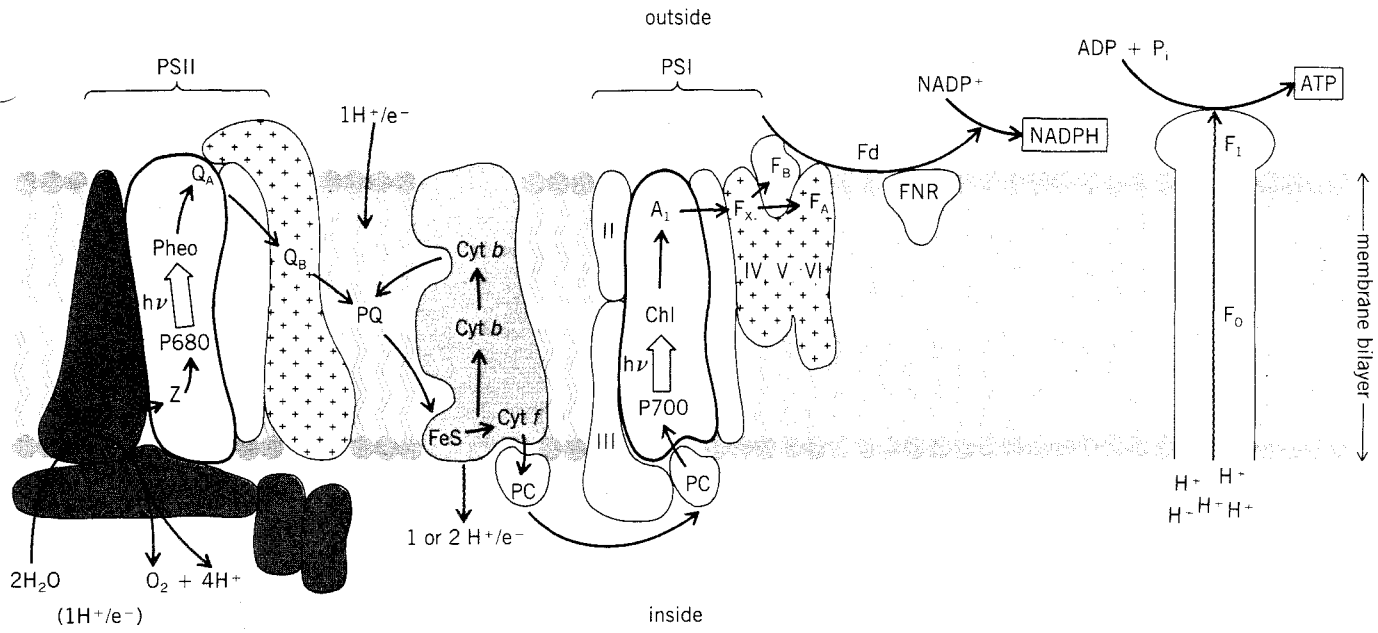


Fig. 4. Special representation of the electron and proton transport mechanisms for plant photosynthesis, located on the inner membrane of a chloroplast. The shapes of the proteins are largely hypothetical. The shaded proteins are the intrinsic and extrinsic polypeptides of the oxygen-evolving complex. The proteins containing P680 and P700 are PSII and PSI reaction center complexes, respectively. Abbreviations as in Fig. 3. FeS = Riese iron sulfur center. The iron sulfur centers F_X, F_B, and F_A are on complexes IV, V, and VI, respectively. Complex I is PSI, and the functions of complexes II and III are unknown. The coupling factor protein has two components: F_0 (embedded in the membrane) and F_1 (extrinsic, and active in ATP synthesis). FNR stands for ferridoxin – $NADP^+$ reductase.

cial molecules (energy traps or reaction centers). The groups of antenna (bulk) molecules with their energy traps are often referred to as photosynthetic units.

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 s at 1°C (33°F). This, then, is the minimum time required for the efficient utilization of the products from the light reactions. Emerson and Arnold further observed that under optimal conditions the maximum yield from a single flash was one O_2 molecule per 2500 chlorophyll molecules present. 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, the ratio of one O_2 per 2500 chlorophylls means one photoact per 300 chlorophyll molecules. By using spinach grown in moderate to high light intensity, it has been shown that there is one active reaction center II (P680) and one active reaction center I (P700) per a total of 600 chlorophyll molecules present. If these chlorophyll molecules are equally divided in photosystems I and II, there is one reaction center per 300 chlorophyll molecules in each system. This is the commonly accepted size of one photosynthetic unit in higher plants and algae.

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 prokaryotic algae such as green *Prochloron* and the blue-greens), as well as all higher plants, contain pigment-bearing organelles called chloroplasts. In the leaves of the higher land plants, these are usually flat ellipsoids about 5 micrometers in diameter and 2.3 micrometers in thickness; 10–100 of them may be present in an average cell of leaf parenchyma. Under the electron microscope all chloroplasts show a layered structure with alternate lighter and darker layers roughly 0.01 μm in thickness. *See* CELL PLASTIDS.

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

Two main types of chloroplasts are known. In lamellar chloroplasts, the layered structure extends more or less uniformly through the whole chloroplast body. In granular chloroplasts, this structure is emphasized in certain cylindrical sections (the grana) and is less pronounced in the area between them (the stroma region). When such granular chloroplasts are permitted to dry out and disintegrate, stacks of disks break off the structure and appear as cylindrical grana in the electron microscope. The photochemical apparatus is less complex in blue-green algae.

The unit of photochemical apparatus in both advanced and primitive plants may be a lamella consisting of two submembranes forming a saclike disk

called a thylakoid. The light reactions of photosynthesis are intimately associated with this membrane. The fine structure of thylakoid membranes is far from clear; various-sized particles and surfaces have been observed. It has not been possible to prove the correlation of the 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.

Accessory pigments. In addition to chlorophyll *a*, the one pigment present in all photosynthetically active plants, there are other chlorophylls, such as chlorophyll *b* in the green algae and higher plants. In brown algae, chlorophyll *c* replaces chlorophyll *b*. There are also 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 and in algae. (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 special granules called phycobilisomes in red algae (Rhodophyta) and blue-green algae (Cyanophyta or cyanobacteria); the red pigment prevails in red algae and the blue pigment prevails in blue-green algae. Another phycocyanin called allophycocyanin is also present in blue-green algae. *See* CAROTENOID; FLUORESCENCE COMPOUNDS (PLANT); PHYCOBILIN.

Light absorbed by accessory pigments does contribute to photosynthesis. This is known from measurements of the so-called action spectra of photosynthesis. In such measurements, photosynthesis is excited by monochromatic light, and the production of oxygen per incident quantum of light is measured as a function of wavelength. The observed spectral variations in the yield of photosynthesis can be related to the proportion of light absorbed at each wavelength by the different pigments in the cells. Measurements of this kind have led to the conclusion that quanta absorbed by carotenoids are 50–80% as effective as 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 that supplies light energy to photosynthesis about as effectively as chlorophyll *a*. The red and blue pigments of the Rhodophyta and Cyanophyta are also highly effective. They can be 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 (**Fig. 5**). Observations of the action spectrum of chlorophyll *a* fluorescence in different plants have suggested close parallels 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. Excitation of chlorophyll fluorescence by light quanta absorbed by phycoerythrin requires transfer of the excitation energy from the excited phycoerythrin molecule to a nearby chlorophyll molecule (as in acoustic resonance, where striking one bell causes another nearby bell to ring). Therefore, light quanta absorbed by accessory pigments, such as carotenoids and phycobilins, may contribute to photosynthesis by being transferred to chlorophyll *a*. 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 *a*, which has a very weak absorption in the green region of the spectrum.

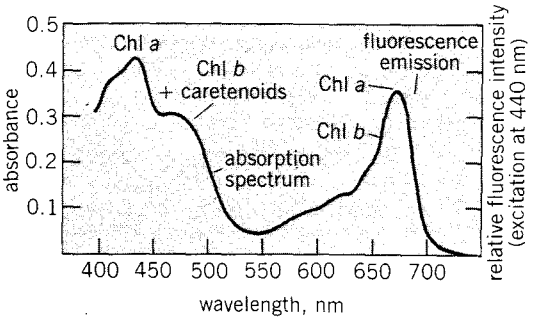


Fig. 5. Absorption spectrum of a corn (*Zea mays*) leaf. Pigments responsible for specific bands are shown. Also shown is the fluorescence emission of chloroplasts from a corn leaf.

If excitation energy can be transferred efficiently in the chloroplasts from accessory pigments to chlorophyll *a*, there is a good probability that a similiar transfer occurs also between different chlorophyll *a* molecules themselves. Excitation-energy transfer among chlorophyll *a* molecules or among phycobilin molecules, and 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* can be demonstrated. The most widely accepted hypothesis, Förster's hypothesis, is that energy transfer is preceded by thermal relaxation in the donor molecules. The efficiency of energy transfer depends upon three basic factors: orientation of acceptor molecules with respect to the donor molecule; overlap of the fluorescence spectrum of the acceptor molecule with the absorption spectrum of the acceptor molecule; and the distance between the two molecules. The function of most of the pigments (including most of the 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; Fig. 2). Energy is thus trapped and used for photochemistry.

Chemical role of chlorophyll. The question arises as to how does the chlorophyll *a* molecule, utimately 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, H_2O, to a reluctant acceptor (perhaps $NADP^+$). It has been shown that chlorophyll acts as a typical oxidation-reduction catalyst—that is, by being itself first oxidized and then reduced. Support for this concept is provided by observations of reversible photochemical oxidation and of reversible photochemical reduction of chlorophyll in solution, and the comparison of these data with those in chloroplasts. Studies of changes in the absorption spectrum of photosynthetic cells in light show that a small fraction of a special form of chlorophyll *a* (P700), absorbing maximally at 700 and 430 nm, is in an oxi-

dized state during illumination. This is the reaction center of PSI. The reaction center of PSII (P680) has been suggested also to undergo oxidation-reduction. Furthermore, a chlorophyll *a* molecule appears to be chemically reduced when P700 is oxidized to $P700^+$ in PSI, as noted earlier [see Eq. (4)]. The detailed chemical nature of P700 and P680 remains unknown. There is a good possibility that these components, although similar to chlorophyll *a*, are chemically distinct entities. *Govindjee; R. Govindjee*

Carbon Dioxide Fixation

The light-dependent conversion of radiant energy into chemical energy as ATP and reduced nicotinamide adenine dinucleotide phosphate (NADPH) serves as a prelude to the utilization of these compounds for the reductive fixation of CO_2 into organic molecules. Such molecules, broadly designated as photosynthates, are usually but not invariably in the form of carbohydrates such as glucose polymers or sucrose, and form the base for the nutrition of all living things, as well as serving as the starting materials for fuel, fiber, animal feed, oil, and other compounds used by people. Collectively, the biochemical processes by which CO_2 is assimilated into organic molecules are known as the photosynthetic dark reactions, not because they must occur in darkness, but because light—in contrast to the photosynthetic light reactions—is not required.

The route by which CO_2 is assimilated was studied for over a century when it was discovered that photosynthesis leads to the accumulation of sugars and starches. Details of the assimilation of CO_2 were worked out in the 1950s, when the availability of paper chromatographic techniques and $^{14}CO_2$ allowed M. Calvin, A. A. Benson, and J. A. Bassham to develop the outlines of the reductive pentose phosphate cycle, now usually called the C_3 cycle. The C_3 cycle forms the primary, or basic (with other, feeder pathways occurring in some plant types), route for the formation of photosynthate from CO_2.

C_3 photosynthesis. The essential details of C_3 photosynthesis can be seen in **Fig. 6**. Three molecules of CO_2 combine with three molecules of the five-carbon compound ribulose bisphosphate (RuBP) in a reaction catalyzed by RuBP carboxylase to form three molecules of an enzyme-bound six-carbon compound. These are hydrolyzed into six molecules of the three-carbon compound phosphoglyceric acid (PGA), which are phosphorylated by the conversion of six molecules of ATP (releasing adenosinediphosphate or ADP, for photophosphorylation via the light reactions). The resulting compounds are reduced by the NADPH formed in photosynthetic light reactions to form six molecules of the three-carbon compound phosphoglyceraldehyde (PGAL). One molecule of PGAL is made available for combination with another three-carbon compound, dihydroxyacetone phosphate, which is isomerized from a second PGAL (requiring a second "turn" of the Calvin-cycle wheel) to form a six-carbon sugar. The other five PGAL molecules, through a complex series of enzymatic reactions, are rearranged into three molecules of RuBP, which can again be carboxylated with CO_2 to start the cycle turning again.

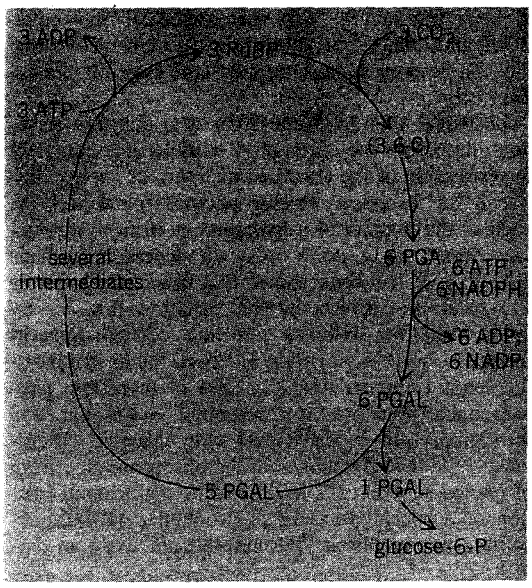


Fig. 6. Schematic outline of the Calvin (C_3) carbon dioxide assimilation cycle.

It should be noted that the enzyme that incorporates CO_2 into an organic compound, RuBP carboxylase, can comprise up to half of the soluble protein in C_3 chloroplasts, and most likely is the most abundant protein found in nature. RuBP carboxylase has 8 large polypeptide subunits and 8 small subunits. Interestingly, the small subunit polypeptide is produced (as a larger, precursor form) in the cytoplasm from mRNA encoded in the nucleus. The precursor polypeptide is then transported across the chloroplast membrane (the mature form of this polypeptide cannot be transported in this manner), processed into the shorter, mature polypeptide, and combined with large subunits (encoded in the chloroplast DNA and produced in the stroma) to form the mature enzyme.

The net product of two "turns" of the cycle, a six-carbon sugar (glucose-6-phosphate) is formed either within the chloroplast in a pathway leading to starch (a polymer of many glucose molecules), or externally in the cytoplasm in a pathway leading to sucrose (condensed from two six-carbon sugars, glucose and fructose). This partitioning of newly formed photosynthate leads to two distinct pools; starch is stored in the photosynthesizing "source" leaf cells, and sucrose is available either for immediate metabolic requirements within the cell or for export to "sinks" such as developing reproductive structures, roots, or other leaves. Factors within the photosynthesizing cell, such as energy requirements in different compartments (such as mitochondria, cytoplasm, and chloroplasts) of the cell, along with energy needs of the plant (such as increased "sink" requirements during different developmental stages) and external, environmental factors (such as light intensity and duration) ultimately regulate the partitioning of newly formed photosynthetic product (PGAL) into starch or sucrose.

This profound control of photosynthate partitioning is accomplished primarily through the regulation of the enzymes which convert PGAL to sucrose in the cytoplasm. Under conditions where sink demand is low (and sucrose is not transported through the phloem away from source leaf cells), metabolic effectors accumulate in the cytoplasm which lower the activities of the sucrose-forming enzymes. This results in a condition that reduces PGAL export from the chloroplast, and hence, more PGAL is retained in the chloroplast for starch formation. Also, under conditions which cause low chloroplast PGAL levels (such

as low light), PGAL transport out of the chloroplast is restricted, resulting in decreased substrate for sucrose formation, increasing the relative amount of starch production. The energy status of the cell affects sucrose formation (and therefore, photosynthate partitioning) because cytoplasmic uridine triphosphate (used in the formation of sucrose) levels are dependent on ATP generation, and also because PGAL export to the cytoplasm is coupled obligatorily to inorganic phosphate (formed when ATP is metabolized in the cytoplasm) import into the chloroplast.

Photosynthetic induction phenomenon. Carbon dioxide assimilation in plants does not begin immediately upon illumination; there is a lag of several minutes before assimilation attains a rapid rate. This lag is called photosynthetic induction, and is not limited by light reactions because high levels of ATP and NADPH are found almost immediately. Two possible limiting mechanisms have been considered: the buildup of intermediates and the activation of enzymes involved in assimilation reactions.

D. Walker concluded that induction represents the time needed to build up the intermediates of the C_3 cycle, in an autocatalytic manner, from newly formed photosynthate to concentrations sufficient for assimilation to proceed at a rate controlled by the prevailing environment (CO_2 levels, temperature, light intensity, and other factors). The autocatalytic nature of the cycle can be best understood by considering that the net product of one "turn" of the cycle (representing three carboxylations), a PGAL molecule, can be fed back into the cycle, and that the rate of carboxylation during this lag phase is dependent on the level of newly formed RuBP. The level of RuBP would double after five carboxylations. This would increase the RuBP level so that the next five carboxylations would occur in a shorter amount of time, resulting in an exponential increase in the rate of photosynthesis until factors other than intermediate levels become limiting.

Not only is the cycle autocatalytic, but the initial carboxylation catalyst, RuBP carboxylase, as well as glyceraldehyde-3-phosphate dehydrogenase and the enzymes sedoheptulose and fructose biphosphate phosphatase, requires activation. These catalysts are inactivated in the dark and activated in the light. Several conditions are required for activation, including high concentrations of Mg^{2+}, CO_2, and a reductant and high pH. These conditions are facilitated by light-dependent processes but are reversed in darkness. This regulatory mechanism conveniently allows for the synthesis pathway to be "shut off," preventing a futile cycle during the night, when starch reserves are mobilized to meet cell energy requirements via intermediates which, if C_3 cycle enzymes were activated, would be reconverted to starch. It is uncertain whether the rates of light-activated enzyme catalysis are comparable with those of the induction phenomenon, but buildup of intermediates, together with activation and inactivation of catalysts, offers an explanation and working hypothesis for the induction phenomenon.

C_4 photosynthesis. Initially, the C_3 cycle was thought to be the only route for CO_2 assimilation, although it was recognized by plant anatomists that some rapidly growing plants (such as maize, sugarcane, and sorghum) possessed an unusual organization of the photosynthetic tissues in their leaves (Kranz morphology). The researches of H. Kortschak and coworkers in Hawaii and of M. D. Hatch and R. C. Slack in Australia demonstrated that plants having the Kranz anatomy utilized an additional CO_2 assimilation route now known as the C_4-dicarboxylic acid pathway (**Fig. 7**). Carbon dioxide enters a mesophyll cell, where it combines with the three-carbon compound phosphoenolpyruvate (PEP) to form a four-carbon acid, oxaloacetic acid, which is reduced to malic acid or transaminated to aspartic acid. The four-carbon acid moves into bundle sheath cells, where the acid is decarboxylated, the CO_2 is assimilated via the C_3 cycle, and the resulting three-carbon compound, pyruvic acid, moves back into the mesophyll cell and is transformed into PEP, which can be carboxylated again. The two cell types, mesophyll and bundle sheath, are not necessarily adjacent (sedges are an example), but in all documented cases of C_4 photosynthesis the organism had two distinct types of green cells. As depicted in **Fig. 8** extensive transport of metabolites must occur between the two cell types in C_4 plants. It is unknown how this directional transport is facilitated, although the presence of plasmodesmata forming a cytoplasmic continuum between the two cell types may be involved.

C_4 metabolism is classified into three types, depending on the decarboxylation reaction used with the four-carbon acid in the bundle sheath cells.

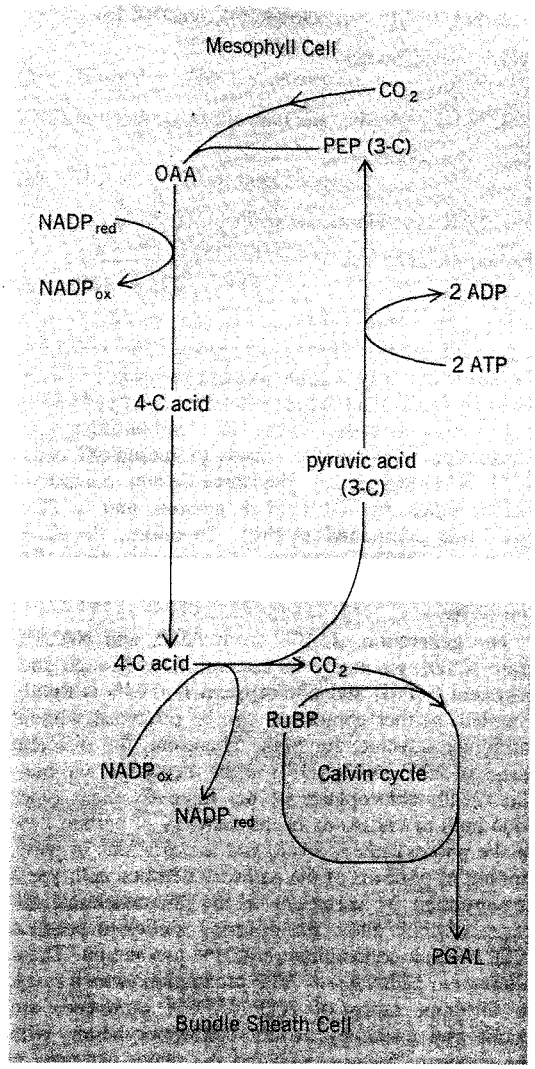


Fig. 7. Schematic outline of the Hatch-Slack (C_4) carbon dioxide assimilation route in two cell types of a NADP-ME-type plant.

1. NADP-ME type (sorghum), reaction (6):

$$NADP^+ + \text{malic acid} \xrightarrow{\text{NADP-malic enzyme}} \text{pyruvic acid} + CO_2 + NADPH \quad (6)$$

2. NAD-ME type (*Atriplex* species), reaction (7):

$$NAD^+ + \text{malic acid} \xrightarrow{\text{NAD-malic enzyme}} \text{pyruvic acid} + CO_2 + NADH \quad (7)$$

3. PCK type (*Panicum* species), reaction (8):

$$\text{Oxaloacetic acid} + ATP \xrightarrow{\text{phosphoenolpyruvate carboxykinase}} PEP + CO_2 + ADP \quad (8)$$

In addition to differing decarboxylation reactions, the particulars of the CO_2 fixation pathway in NAD-ME and PCK plant types differ from those depicted in Fig. 8 with respect to the three-carbon compound transported from bundle sheath to mesophyll cells. With NAD-ME types, the three-carbon compound can be either pyruvic acid or alanine, and in PCK types this compound is PEP. Therefore, the three variations in the C_4 pathway necessarily predicate different energy (ATP and NADPH) usage in the two cell types.

The generation of ATP from ADP, and NADPH from NADP via noncyclic electron flow through photosystem I (PSI) and photosystem II (PSII) is tightly coupled: neither compound can be produced without sufficient substrate for both. Therefore, the different usage of ATP and NADPH in the mesophyll and bundle sheath chloroplasts of the three C_4 plant types (due both to variations in the pathway of carbon flow in the photosynthetic cycle and to variations in partitioning of portions of the pathway between cell types) is supported by variations in the photochemical apparatus which allow for differing ability to produce ATP without concomitant NADPH production. These alternative pathways of ATP production (which result in different ratios of ATP:NADPH produced) are cyclic and pseudocyclic photophosphorylation, with the cyclic pathway considered the major pathway of uncoupled ATP production in chloroplasts, and the pseudocyclic pathway possibly acting as a "fine-tuning" modulator.

Variations in the photochemical apparatus which indicate enhanced cyclic photophosphorylation capacity (utilizing only PSI) are a high chlorophyll *a*/*b* ratio, low Chl/P700 ratio, and a low PSII reaction. These characteristics are found in bundle sheath chloroplasts of NADP-ME–type plants, indicating that the primary function of the photochemical apparatus in these chloroplasts is the generation of ATP. NADPH is supplied via the decarboxylation of malic acid to support the C_3 cycle activity (PGA conversion to PGAL) in these chloroplasts. Assays of chlorophyll *a*/*b* ratio, Chl/P700 ratio, and PSII activity indicate that NAD-ME mesophyll chloroplasts also have a primary role of cyclic photophosphorylation, while NAD-ME bundle sheath chloroplasts have a primary role of noncyclic electron flow. In PCK-type plants, mesophyll chloroplasts appear to have a photochemical apparatus similar to C_3 chloroplasts, while bundle sheath chloroplasts appear to have a low PSII activity. The enhanced ability of PCK bundle sheath chloroplasts to produce ATP via cyclic photophosphorylation supplies the extra ATP needed to convert pyruvic acid to PEP. These variations in the C_4 pathway and photochemical apparatus among the C_4 plant types demonstrate the close relationship that has evolved between light reactions and the biochemical processes of carbon dioxide assimilation, and show the highly integrated cooperation between the cell types involved.

Functions of C_4 cycle. The concentration of CO_2 in air is about 0.03% by volume, a concentration that does not fully saturate the C_3 cycle when it is operating at capacity. It would be necessary to have about 0.1% CO_2 to saturate the C_3 cycle, which can only be achieved under controlled conditions (CO_2-enriched greenhouses or growth chambers). Leaf photosynthesis in C_4 plants, however, is fully saturated at air CO_2 concentrations. Thus, C_4 photosynthesis may be considered to be an evolutionary adaptation to current-day CO_2 levels in air. During the C_4 cycle, CO_2 is continually fed via biochemical reactions in mesophyll chloroplasts to RuBP carboxylase in bundle sheath chloroplasts so that air CO_2 is not rate-limiting. Apparently the spatial compartmentalization of portions of CO_2 assimilation into two cell types not only allows C_4 plants to assimilate air CO_2 rapidly, but also partly explains other physiological characteristics and responses to the external environment of C_4 plants. These include their high efficiency of water use (as water vapor exits through the same stomatal pores through which CO_2 enters the leaf, and since the C_4 plant is more efficient at fixing CO_2 than C_3 plants, more CO_2 is incorporated per unit water lost), their greater efficiency of nitrogen usage (as RuBP carboxylase is produced only in bundle sheath cells in C_4 plants, only 10–35% of the leaf nitrogen is tied up in this enzyme, as opposed to 40–60% in C_3 plants), and their high rates of sugar formation which can facilitate the rapid growth rates seen in such C_4 plants as maize, sugarcane, sorghum, and crabgrass. Other differences in response to the environment between C_3 and C_4 plants is that C_4 plants exhibit a nonsaturating response curve of leaf photosynthesis to light levels found in nature, and tolerate more salinity and higher temperatures than do C_3 plants.

The higher energy requirements of C_4 plants are also reflected by the fact that quantum yields of photosynthesis for C_3 plants are higher than for those possessing the auxiliary C_4 system. At 2% oxygen partial pressure and 30°C (86°F), quantum yield for C_3 plants is about 0.073 mole CO_2 assimilated per absorbed einstein of light, while for C_4 plants the quantum yield is 0.054. However, at normal O_2 partial pressures (21% O_2) quantum yields are almost indentical. This is due to the presence of high photorespiration in C_3 plants, and thus represents a net quantum yield rather than a true photosynthetic yield. *See* PHOTORESPIRATION.

CAM photosynthesis. Under arid and desert conditions, where soil water is in short supply, transpiration during the day when temperatures are high and humidity is low may rapidly deplete the plant of water, leading to desiccation and death. By keeping stomata closed during the day, water can be conserved, but the uptake of CO_2, which occurs entirely through the stomata, is prevented. Desert plants in the Crassulaceae, Cactaceae, Euphorbiaceae, and 15 other families have evolved, apparently independently of C_4 plants, an almost identical strategy of assimilating CO_2 by which the CO_2 is taken in at night when the stomata open; water loss is low because of the reduced temperatures and correspondingly higher humidities. Although these succulent plants with thick, fleshy leaves were known since the nineteenth century

as being unusual, the biochemical understanding of the process did not occur until the 1960s and 1970s when the details of C_4 photosynthesis were being worked out. First studied in plants of the Crassulaceae, the process has been called crassulacean acid metabolism (CAM).

In contrast to C_4, where two cell types cooperate, the entire process occurs within an individual cell; the separation of C_4 and C_3 is thus temporal rather than spatial. At night, CO_2 combines with PEP through the action of PEP carboxylase, resulting in the formation of oxaloacetic acid and its conversion into malic acid. The PEP is formed from starch or sugar via the glycolytic route of respiration. Thus, there is a daily reciprocal relationship between starch (a storage product of C_3 photosynthesis) and the accumulation of malic acid (the terminal product of nighttime CO_2 assimilation (Fig. 8).

As in C_4 plants, there may be variations in the decarboxylase which provides the CO_2 for assimilation via the C_3 cycle. In some CAM plants (pineapple) phosphoenol carboxykinase (PCK) is involved, while in others (cactus) the decarboxylase in the NADP-malic enzyme (NADP-ME type) is involved. The role of NAD-ME is uncertain, although it has been found in some CAM plants. The **table** summarizes some physiological differences between C_3, C_4, and CAM plants.

Other CO_2 assimilation mechanisms. Both the C_4 cycle and CAM involve the synthesis of oxaloacetic acid, which is also one of the intermediates in the tricarboxylic acid (TCA) cycle of respiration. In the late 1960s a light-driven reversal of the TCA cycle was discovered by B. Buchanan, D. Arnon, and coworkers in Berkeley. This CO_2 fixation cycle, called the reductive carboxylic acid cycle, results in the net synthesis of pyruvic acid via the reversal of the three decarboxylation steps in the TCA cycle (pyruvic to acetyl coenzyme A, isocitric to α-ketoglutaric, and succinyl CoA to succinic acids). The pathway has been detected in the photosynthetic bacteria. *See* *Krebs cycle*.

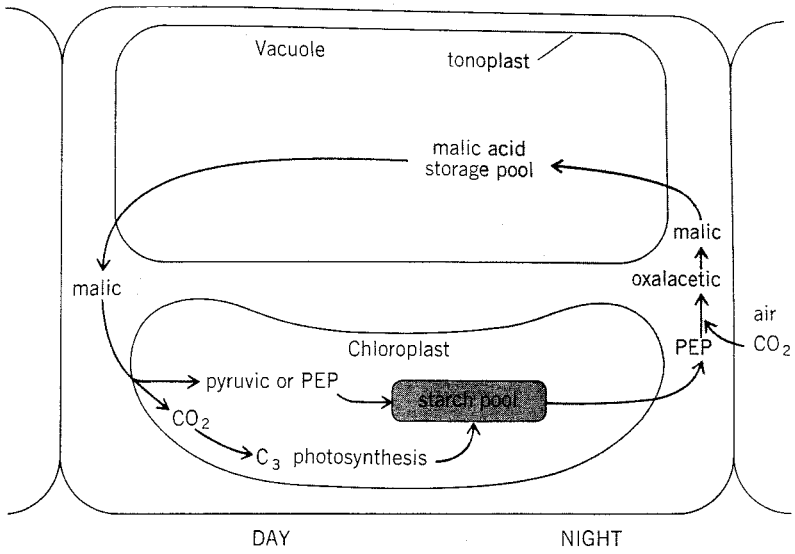


Fig. 8. Scheme for the flow of CO_2 within a single crassulacean acid metabolism cell over a day, showing initial dark CO_2 fixation, malic acid storage in the vacuole at night, followed by decarboxylation and the C_3 cycle the next day.

In most photosynthetic bacteria, the C_3 cycle is functional despite some differences in detail. The green sulfur bacteria, however, carry out C_3 photosynthesis poorly or not at all. *Chlorobium thiosulfatophilum*, lacking the key enzyme RuBP carboxylase, utilizes a reductive carboxylic acid cycle in which reduced ferredoxin drives the TCA cycle in reverse, resulting in carboxylation reactions much like those of the cycle discovered by Buchanan and Arnon.

Heterocysts of blue-green algae do not have a func-

Some characteristics of the three major plant groups

Characteristics	C_3	C_4	CAM
Leaf anatomy in cross section	Diffuse distribution of organelles in mesophyll and palisade cells with less chloroplasts in bundle sheath cells if present	Layer of bundle sheath cells around vascular tissue with a high concentration of chloroplasts; layers of mesophyll cells around bundle sheath	Spongy, often lacking palisade cells; mesophyll cells have large vacuoles
Theoretical energy requirement for net CO_2 fixation (CO_2:ATP:NADPH)	1:3:2	1:5:2	1:6.5:2
Carboxylating enzyme	RuBP carboxylase	PEP carboxylase, then RuBP carboxylase	Darkness:PEP carboxylase; light: mainly RuBP carboxylase
CO_2 compensation concentration, ppm CO_2	30–70	0–10	0–5 in dark
Transpiration ratio, g H_2O/g dry weight increase	450–950	250–350	50–55
Maximum net photosynthetic rate, mg CO_2/(dm^2 leaf)(h)	15–40	40–80	1–4
Photosynthesis sensitive to high O_2	Yes	No	Yes
Photorespiration detectable	Yes	Only in bundle sheath	Difficult to detect
Leaf chlorophyll *a*/*b* ratio	2.8 ± .4	3.9 ± .6	2.5–3
Maximum growth rate, g dry wt/(dm^2 leaf)(day)	0.5–2	4–5	0.015–0.018
Optimum temperature for photosynthesis	15–25°C (59–77°F)	30–40°C (86–104°F)	About 35°C (95°F)

tional C_3 cycle because, in contrast to the normal cells of these algae, the heterocyst cell (implicated in nitrogen fixation) lacks the key enzyme RuBP carboxylase. It has been suggested that CO_2 fixation in heterocysts may be via PEP carboxylase as in C_4 and CAM photosynthesis. Guard cells in C_3 plants which regulate the opening of stomatal pores for gas exchange in leaves also lack RuBP carboxylase and apparently use PEP carboxylase exclusively to fix CO_2.

Martin Gibbs; Gerald A. Berkowitz

Bacterial Photosynthesis

Certain bacteria 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 bacterial photosynthesis. This is shown in reaction (9).

$$2H_2A + CO_2 + \text{light} \xrightarrow[\text{enzymes}]{\text{bacteriochlorophyll}} \{CH_2O\} + 2A + H_2 \quad (9)$$

Photosynthetic bacteria cannot use water as the hydrogen donor and are incapable of evolving oxygen. The prokaryotic cyanobacteria (also called blue-green algae) are excluded in this discussion of bacterial photosynthesis since their photosynthetic system closely resembles that found in eukaryotic algae and higher plants discussed above. Photosynthetic bacteria can be classified in four major groups.

1. Nonsulfur purple bacteria (Rhodospirillaceae). In these bacteria, H_2A is usually an organic H_2 donor, such as succinate or malate; however, these bacteria can be adapted to use hydrogen gas as the reductant. They require vitamins for their growth and usually grow anaerobically in light, but they can also grow aerobically in the dark by using respiration to utilize organic compounds from the environment. They are thus facultative photoheterotrophs. Examples of this group are *Rhodospirillum rubrum* and *Rhodopseudomonas sphaeroides*.

2. Sulfur purple bacteria (Chromatiaceae). 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 vinosum*.

3. Green sulfur bacteria (Chlorobiaceae). These bacteria are capable of using the same chemicals as Chromatiaceae but, in addition, use other organic H_2 donors. They may then be called photoautotrophic and photoheterotrophic obligate anaerobes. An example of the green sulfur bacteria is *Chlorobium thiosulfatophilum*.

4. Green sliding bacteria (Chloroflexaceae). These are primarily photoorganotrophic bacteria which can grow under anaerobic conditions in light by photosynthesis or in aerobic conditions in the dark by using respiration to utilize organic compounds from the environment. They are thermophilic bacteria found in hot springs around the world. They also distinguish themselves among the photosynthetic bacteria by possessing mobility. An example is *Chloroflexus aurantiacus*.

Bacteria are capable of photophosphorylation, which is the production of adenosinetriphosphate (ATP) from adenosinediphosphate (ADP) and inorganic phosphate (P_i) using light as the primary energy source. Several investigators have suggested that the sole function of the light reaction on bacteria is to make ATP from ADP and P_i. The hydrolysis energy of ATP can then be used to drive the reduction of CO_2 to carbohydrate by H_2A in reaction (9).

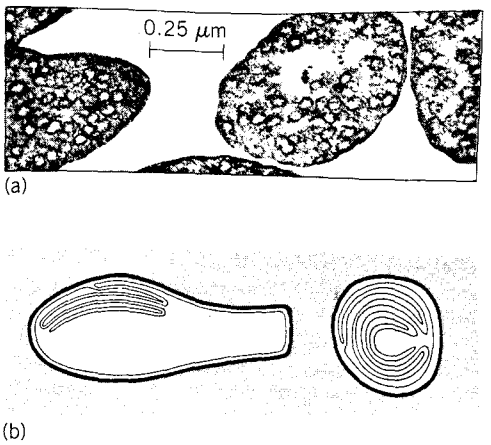


Fig. 9. Photosynthetic bacteria. (*a*) Electron micrograph of *Rhodopseudomonas sphaeroides* with vesiclelike invaginations (*from T. W. Goodwin, ed., Biochemistry of Chloroplasts, vol. 1, Academic Press, 1966*). (*b*) Pictorial representation of a stacked invagination in a photosynthetic bacterium; at left is a longitudinal section and at right is a transverse section (*after R. Whittenbury and A. G. McLee, Archiv für Microbiologie, 59: 324–334, 1967*).

Photochemical apparatus. Photosynthetic bacteria do not have specialized organelles such as the chloroplasts of green plants. Electron micrographs of certain photosynthetic bacteria show tiny spherical sacs, with double-layered walls, as a result of invaginations which form stacks of membranes (**Fig. 9***a*). Other photosynthetic bacteria have invaginations which form stacks of membranes (Fig. 9*b*). These structures, called chromatophores, contain the photosynthetic apparatus and can be easily isolated by mechanical disruption of bacteria followed by differential centrifugation. Isolated chromatophores are the basic preparation for biochemical and biophysical studies of bacterial photosynthesis.

Reaction centers. The pigment bacteriochlorophyll (Bchl) is a necessary ingredient for bacterial photosynthesis. There are specialized Bchl molecules in bacteria which engage in the primary chemical reactions of photosynthesis. In addition to these specialized molecules, there are 40–50 Bchl molecules referred to as antenna, whose sole function is to harvest light energy and transfer it to reaction center molecules. This is similar to the photosynthetic unit of plants. Each reaction center contains a special pair (dimer) of Bchl molecules that engage in chemical reactions after they trap the absorbed light energy. They are also called the energy traps of bacterial photosynthesis.

The energy trap in *Rhodopseudomonas sphaeroides* had been identified as P870. Such identification is carried out with a difference (absorption) spectrophotometer. In this instrument a weak monochromatic measuring beam monitors the absorption of the sample; a brief but bright actinic light given at right angles to the measuring beam initiates photosynthesis. When photosynthesis occurs, changes in absorption take place. **Figure 10** shows the absorption spectrum of reaction centers isolated from *R. sphaeroides*. These changes are measured as a function of the wavelength of measuring light. A plot of the change induced in *R. sphaeroides* reaction centers by an ac-

tinic light flash, as a function of the wavelength of measuring light, is the difference absorption spectrum (**Fig. 11***b*). This spectrum is due largely to the photooxidation of the Bchl dimer, P870.

If P870 is the energy trap, then the following criteria must be met: (1) It must undergo a reduction or oxidation reaction, since this is the essential reaction of photosynthesis. The decrease in absorption at 870 nm (Fig. 10) is an oxidation reaction since chemical oxidants cause a similar change. (2) The quantum yield (number of trap molecules per absorbed photon) must be very high (close to 1.0) for this photooxidation. (3) The primary light reaction should occur at very low temperatures, down to 1 K (−460°F or −273°C). (4) The above photochemical reaction should be extremely fast, that is, picosecond to nanosecond range.

All the above criteria are fulfilled by P870, and thus it is the reaction center of bacterial photosynthesis in *R. sphaeroides*. Among other reaction centers which have been identified and studied extensively are P890 in *Chromatium vinosum*, P960 in *R. viridis*, and P840 in *Chlorobium*. Each species of bacteria has only one type of reaction center, unlike plants, which utilize both PSI (P700) and PSII (P680) reaction centers. Reaction centers can be isolated from chromatophores as a pure protein, which has been important in providing a well-defined system in which primary reactions of bacterial photosynthesis can be studied. A milestone in bacterial photosynthesis was reached in the early 1980s by the crystallization of *R. viridis* reaction centers. These crystals were adequate to obtain 0.3-nm resolution of the molecular structure of the reaction center.

Although isolated reaction centers are able to absorb light and convert it to chemical energy, the antenna pigment system in chromatophores (or in whole cells) absorbs most (>90%) of the light. The antenna funnels this energy to the reaction center. Antenna

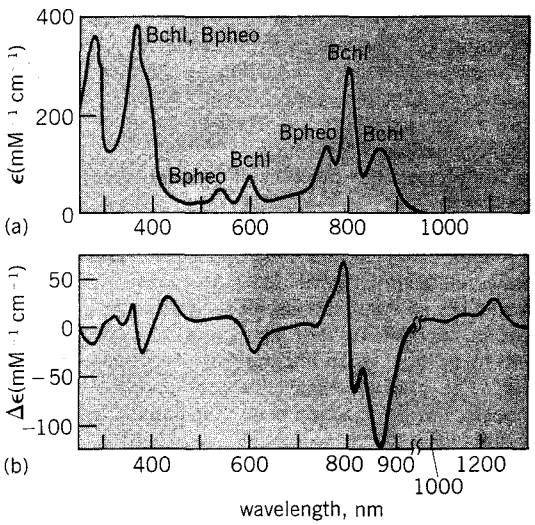


Fig. 10. Plots of (*a*) absorption spectrum and (*b*) the light-induced absorption changes in it, as occurring in reaction centers isolated from carotenoidless mutant R-26 of *Rhodopseudomonas sphaeroides*. In *a*, bands attributed to bacteriochlorophyll and bacteriopheophytin are labeled Bchl and Bpheo, respectively. The ordinate in *a* is the millimolar extinction coefficient; in *b*, it is the differential extinction coefficient. (*After R. K. Clayton, Photosynthesis: Physical Mechanisms and Chemical Patterns, Cambridge University Press, 1980*)

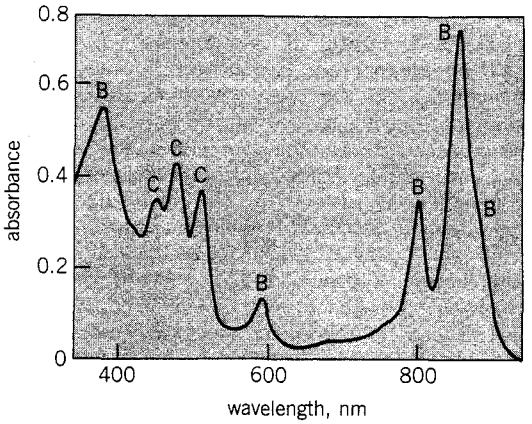


Fig. 11. Absorption spectrum of chromatophores from the bacterium *Rhodopseudomonas sphaeroides*. Absorption bands attributed to Bchl *a* are labeled as B, and those attributed to carotenoids as C. (*After R. K. Clayton, Photosynthesis: Physical Mechanisms and Chemical Patterns, Cambridge University Press, 1980*)

Bchl molecules are bound to protein in a specific manner; this binding and pigment-pigment interactions modify the properties of the pigment and define the absorption maxima and the width of the absorption band. An example is B800 (B represents Bchl, and the number indicates the wavelength of the absorption peak in nanometers) found in *R. sphaeroides* (Fig. 11).

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, and various iron-containing proteins (cytochromes, ferrodoxins, Rieske iron sulfur centers, and others) in addition to the photosynthetic pigments which capture light energy.

In contrast to plastoquinones found in plants, bacteria contain substituted benzoquinones called ubiquinones (UQ or coenzyme Q) and substituted naphthoquinones called menaquinones (MK or vitamin K_2) which act as electron acceptors. The purple bacteria have a pool of UQ (about 25 UQ per reaction center) which mediates transfer of electrons and protons between protein complexes in the chromatophore membrane. However, MK is found only in some bacteria, usually in a smaller quantity (about 1–2 MK molecules per reaction center) than the more plentiful UQ. Menaquinone's function is probably limited to electron transfer within the reaction center. In contrast to plants which contain $NADP^+$, the major pyridine nucleotide in bacteria is nicotinamide adenine dinucleotide (NAD); it is present in large quantities and seems to be active in photosynthesis. Among the various cytochromes, the *c*-type cytochromes and the *b*-type cytochromes are the important ones for bacterial photosynthesis.

Pigments. Most photosynthetic bacteria contain Bchl *a*, a tetrahydroporphyrin. The chlorophyll of green plants, by contrast, is a dihydroporphyrin. In diethyl ether, Bchl *a* has absorption maxima at 365, 605, and 770 nm. The infrared band of various antenna Bchl *a* has maxima at 800 (B800), 850 (B850), or 890 nm (B890). These antenna absorption bands in

the bacterial cell are due to the formation of complexes of Bchl *a* with different proteins.

The reaction center protein from *R. sphaeroides* binds four Bchl *a* and two bacteriopheophytin (Bph; similar to Bchl but does not contain magnesium). Two of the Bchl form the energy trap P870. Another Bchl and a Bph are involved in the transfer of electrons within the protein. The function for the remaining "voyeur" Bchl and Bph is unknown.

The bacterium *R. viridis* utilizes only an antenna with an infrared band at 1015 nm. The isolated Bchl from this species has absorption maxima at 368, 582, and 795 nm in diethyl ether, and has been designated Bchl *b*. The reaction center of *R. viridis*, P960, uses Bchl *b* and Bph *b* much in the same way as P870 in other bacteria utilize Bchl *a*.

The green bacteria contain a small amount of Bchl *a*, but they contain large quantities of another type of chlorophyll called chlorobium chlorophyll; the latter exists in two forms. In the cell, the red absorption band is at 725 or 740 nm; the names Bchl *c* and Bchl *d* are assigned, respectively. The Bchl *a* has been shown to be associated with the reaction center, while the Bchl *c* acts as antenna.

The second group of pigments is the carotenoids, which have absorption peaks from 450 to 550 nm. The carotenoids of photosynthetic bacteria are of great variety and include some which are found in green plants, for example, the lycopenes. However, some are typical only of bacteria: γ-carotene, which is found in large quantities in green sulfur bacteria, and spirilloxanthol, which is found mainly in purple bacteria. Carotenoids function to prevent photooxidation and destruction of antenna bacteriochlorophyll. They also function in bacterial photosynthesis by transferring their absorbed energy to bacteriochlorophyll.

Transfer of excitation energy. Light energy absorbed by the carotenoids is transferred to Bchl with varying efficiency (30–90%), as demonstrated by the method of sensitized fluorescence. When light energy is absorbed by carotenoids, only the fluorescence of bacteriochlorophyll (B875) is observed. By the same method, efficient (almost 100%) energy transfer has been demonstrated from B800 to B850 to B875. The high (almost 1.0) quantum yield of P870 oxidation, when bacteria are excited in the antenna pigments, is a clear demonstration of an extremely efficient excitation energy transfer by antenna pigments and trapping in reaction centers. *See Fluorescent compounds (plant).*

The lifetime of the excited state of antenna Bchl in the bacterial cell is of the order of 1–2 nanoseconds. The excitation energy must be channeled from the antenna pigments to the energy traps within this time for efficient photosynthesis to occur. In reaction center preparations, it takes only 4 picoseconds to create a definitively stable charge separation (see below) after the absorption of light. Moreover, the lifetime of the physical state or states preceding P870 oxidation is <3 ps. Thus, it appears that within a few picoseconds of receiving excitation energy, the reaction center has converted the absorbed light energy into chemical energy. In principle, similar reactions must occur in plant photosynthesis.

Mechanisms of electron transport. The first act of photosynthesis is the absorption of light by various pigments. As discussed above, light energy absorbed by the carotenoids B800 and B850 is transferred to B875 and finally to the reaction centers, where the primary reaction occurs: the oxidation of the reaction center Bchl dimer leads to bleaching of P870 and reduction of an acceptor. In the current model, P (short for P870 and so on) is oxidized to P^+ and an intermediate "I" is reduced to I^- within a few picoseconds; in all likelihood, I is a Bph molecule. The reduced I^- transfers the electron to an iron-quinone complex, reducing the primary quinone (Q_A) to a semiquinone within 100–200 ps. For most bacteria Q_A is ubiquinone, though for those containing both menaquinone and ubiquinone the menaquinone functions as Q_A. Although an iron atom is in this complex, and is within 0.5–1.0 nm of the quinone, its presence is not necessary for the reduction of Q_A, nor does the iron undergo redox changes. The function of this nonheme iron in the reaction center is unknown. In plant photosynthesis, PSII contains Q_A, which is a bound plastiquinone; the function of the iron there is also unknown.

The photooxidized donor Bchl dimer, P^+, can be re-reduced by a cytochrome *c* in 1–30 microseconds, thus oxidizing the cytochrome. In *R. sphaeroides* and a number of other species, this cytochrome is soluble cyt c_2. In other bacteria the cytochrome that donates electrons to P^+ is an integral part of the reaction center. The photochemical reactions and the electron transfers in the reaction center are summarized in reaction (10).

$$PIQ_A \xrightarrow{h\nu} P^*IQ_A \xrightarrow{4\ ps} P^+I^-Q_A \xrightarrow{200\ ps} P^+IQ_A^- \xrightarrow[1\text{–}30\ \mu s]{\text{cyt } c\ \text{cyt } c^+} PIQ_A^- \qquad (10)$$

After this set of reactions, the electron is transferred from Q_A^- to Q_B (a bound UQ) producing $Q_AQ_B^-$. In a subsequent absorption of a photon, the $Q_A^-Q_B^-$ state is created, which is followed by electron transfer from Q_A^- to Q_B^- forming Q_BH_2 with the uptake of two protons. The bound quinol (Q_BH_2) is re-

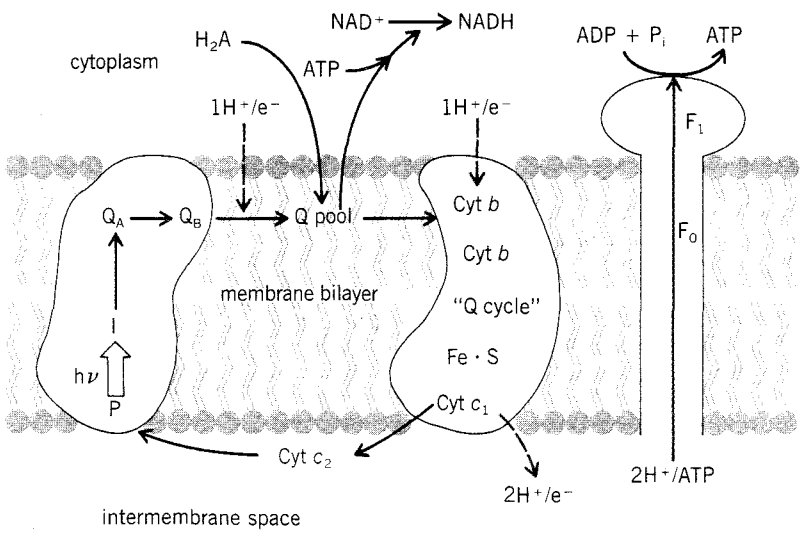


Fig. 12. Electron and proton transport for purple photosynthetic bacteria. For details and explanation of symbols see the text. The shapes of the proteins are largely hypothetical, as in the equivalent diagram for plant photosynthesis (Fig. 4).

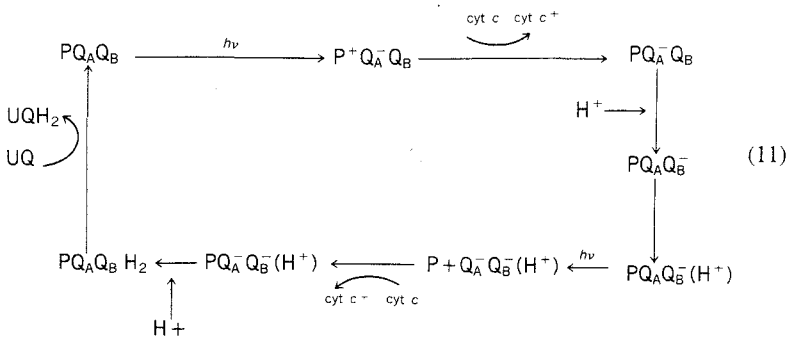
placed by a UQ molecule. This cycle is known as the two-electron gate and is summarized in reaction (11) [omitting the early photochemical steps illustrated in reaction (10)]. The same cycle occurs in photosystem II of plants, except that the electron donor to P^+ is plastoquinol Z, and Q_BH_2 is another plastoquinol instead of ubiquinol. The molecular detail is so similar in plants and bacteria that many of the herbicides which act to inhibit PSII electron transfer from Q_A^- to Q_B are also potent inhibitors of electron transfer from Q_A^- to Q_B in photosynthetic bacteria.

The mechanism of proton uptake in the two-electron gate is not completely known. The first proton does not bind directly to the semiquinone (Q_A^- or Q_B^-), but instead it binds to a protonatable amino acid of the reaction center. The net result from the absorption of two photons is the formation of a quinol in the membrane, the oxidation of two cyt *c*, and the removal of two protons from the cytoplasm of the bacterial cell.

The doubly reduced ubiquinone (QH_2, quinol) through a cyclic pathway serves to re-reduce the oxidized cytochrome (cyt c^+). This cyclic reaction (**Fig. 12**) is coupled to the production of ATP via the creation of a proton gradient (more accurately a proton motive force) across the membrane. Just as in plants, the proton motive force (which includes two components: a membrane potential, and a proton gradient) is used to drive ATP synthesis. Protons move down the potential gradient through the ATPase to contribute energy to drive the ADP + $P_i \rightarrow$ ATP reaction. This overall mechanism is consistent with P. Mitchell's chemiosmotic theory. The quinol produced by the two-electron gate mechanism binds to the cytochrome *b-c* complex (an integral membrane protein) which contains two *b*-cytochromes, a *c*-cytochrome, a Rieske iron-sulfur center, and two quinone binding sites. Plants also contain a similar complex, where cytochrome *b* is replaced by cytochrome b_6, and cytochrome *c* is replaced by cytochrome *f*. The mechanism is strikingly similar, on a molecular level, to that of noncyclic electron transfer from photosystem II to plastocyanin via the plastoquinone pool and the cytochrome *b-f* complex. The path of the electrons and protons through this complex is still a matter of controversy. In all likelihood, it includes a pathway called a Q-cycle by Mitchell; this cycle incorporates two different redox-linked pathways for the electrons. For each quinol oxidized by this complex, two cyt *c* are reduced, two protons are removed from the quinol, an additional two protons are removed from the cytoplasm, and these four protons are released into the intermembrane space. Absorption of two photons leads to the translocation of four protons across the membrane. Data on the bacterial ATPase suggest that $2H^+$ are needed to make an ATP (Fig. 12).

The mechanism described here for the generation of ATP from light energy is largely from studies on *R. sphaeroides* and is generally valid for other purple photosynthetic bacteria.

The mechanisms for oxidizing the reduced substrate H_2A [reaction (9)] are known in much less detail than those for photophosphorylation. Most substrates feed electrons into the quinone pool, and the resulting quinol can be used by the cytochrome *b-c* complex. An example of this is succinate, which reduces quinone via a succinate dehydrogenase. In bacteria which have a low potential cytochrome *c* bound



(11)

to the reaction center (such as cyt c_{551} in *Chromatium vinosum*), it has been hypothesized that electrons from some substrates can be fed into the reaction center through this cytochrome. The electrons for the reduction of NAD^+ in purple photosynthetic bacteria are from the quinone pool, but these electrons require additional energy gained perhaps from the hydrolysis of ATP.

Alternatively, especially in green bacteria, the primary stable acceptor of electrons in the reaction center may not be a quinone but an acceptor with a negative enough oxidation-reduction potential to directly reduce NAD^+. In the green bacterium *Prosthecochloris aestuarii*, this electron acceptor has been shown to be an iron-sulfur (Fe·S) center instead of a quinone. The midpoint redox potential of this Fe·S center is much lower than that for the quinone acceptor in the purple photosynthetic bacteria. This Fe·S center can then directly reduce a ferredoxin, and this can drive the $NAD^+ \rightarrow$NADH reaction. The reduced ferredoxin may also feed electrons into a cytochrome *b* complex from which a soluble cyt *c* could be reduced, thus allowing cyclic electron transfer to occur. This scheme is very reminiscent of photosystem I driven reactions in plant photosynthesis. However, not all green photosynthetic bacteria follow the above pattern, but, instead, they resemble more the purple photosynthetic bacteria.

The reduced pyridine nucleotide NADH and the ATP made in the light reactions are then utilized to convert carbon sources into carbohydrates. The pathway of carbon involves a reversal of either the Krebs cycle or the Calvin cycle with some modifications. *See* BACTERIAL PHYSIOLOGY AND METABOLISM.

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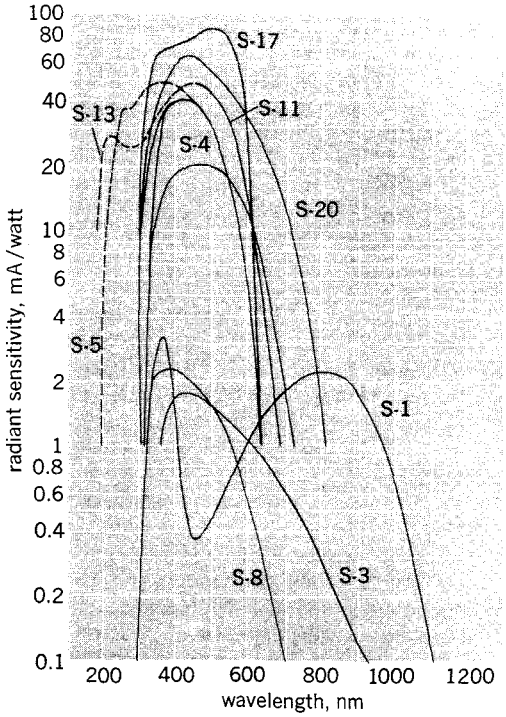
Phototransistor

A semiconductor device with electrical characteristics that are light-sensitive. Phototransistors differ from photodiodes in that the primary photoelectric current is multiplied internally in the device, thus increasing the sensitivity to light. For a discussion of this property SEE TRANSISTOR.

Some types of phototransistors are supplied with a third, or base, lead. This lead enables the phototransistor to be used as a switching, or bistable, device. The application of a small amount of light causes the device to switch from a low current to a high current condition. SEE PHOTOELECTRIC DEVICES.

W. R. Sittner



Curves of the average spectral sensitivity characteristics of some typical phototubes.

Phototube

An electron tube comprising a photocathode and an anode mounted within an evacuated glass envelope through which radiant energy is transmitted to the photocathode. A gas phototube contains, in addition, argon or other inert gas which provides amplification of the photoelectric current by partial ionization of the gas. The photocathode emits electrons when it is exposed to ultraviolet, visible, or near-infrared radiation. The anode is operated at a positive potential with respect to the photocathode. SEE ELECTRICAL CONDUCTION IN GASES; ELECTRON TUBE.

Characteristics. A phototube responds to radiation over a limited range of the spectrum that is determined by the photocathode material. Radiant sensitivity, shown in the **illustration** as a function of wavelength, is the photoelectric current emitted per unit of incident monochromatic radiant power. Sensitivity on the short-wavelength side of the curves is limited by the transmittance of the glass envelope. Electron affinity of the photocathode determines the long-wavelength threshold of sensitivity. SEE PHOTOEMISSION.

Typical phototube characteristics are summarized in the **table**. Quantum efficiency, or photoelectron yield, is the number of electrons emitted per incident photon. It is tabulated at the wavelength of maximum response. For photometric applications a useful parameter is luminous sensitivity: the photoelectric current per lumen incident from a specified source of light. A source commonly used is a tungsten-filament lamp operated at a color temperature of 4700°F (2870 K). SEE INCANDESCENCE; LUMINOUS FLUX; PHOTON.

Average cathode characteristics

Spectral sensitivity characteristic*	Cathode material	Wavelength of maximum response, nm	Peak radiant sensitivity, mA/W	Peak cathode quantum efficiency, %	Luminous sensitivity, μA/lumen†	Remarks
S-1	Cs_2O, Ag	800	2.2	0.3	25	
S-3	Rb_2O, Ag	420	1.8	0.5	6.5	
S-4	Cs_3Sb	400	40	12.4	40	
S-5	Cs_3Sb	340	49	17.8	40	Ultraviolet transmitting window
S-8	Cs_3Bi	365	2.3	0.8	3	
S-10	Bi, Ag, O, Cs	450	20.3	5.6	40	Semitransparent
S-11	Cs_3Sb	440	48	13.5	60	Semitransparent
S-13	Cs_3Sb	440	47	13.2	60	Semitransparent; ultraviolet transmitting window
S-17	Cs_3Sb	490	85	21.4	125	Semitransparent, on reflecting substrate
S-20	(NaKCs)Sb	420	64	18.8	150	Semitransparent

*These characteristics, shown in the figure, refer to typical phototubes rather than to photocathodes.
†Light source is a tungsten-filament lamp operated at a color temperature of 4700°F (2870 K).