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# The Primary Events of Photosynthesis

*The first steps in photosynthesis are the absorption of light by a pigment molecule and the delivery of the absorbed energy to other molecules capable of entering into chemical reactions*

by Govindjee and Rajni Govindjee

When the sun shines on the leaves of a green plant, some of the radiant energy is utilized to promote chemical reactions, with the ultimate result that water and carbon dioxide are converted into oxygen and organic compounds. Photosynthesis has been summarized in this way since the end of the 18th century. The summary is essentially correct, but it describes the process only in terms of what flows into and out of the plant. Today a more detailed and more precise explanation is sought; we want to know what happens inside the illuminated leaf. It is not sufficient to say that light "promotes chemical reactions." Rather, the molecular mechanism by which light is absorbed and by which its energy is utilized must be identified.

In this article we are concerned mainly with the first steps in photosynthesis: the absorption of light by a specific molecule and the transfer of that energy from one molecule to another, as in a bucket brigade, until it is eventually conveyed to those few molecules that participate in chemical reactions. These initial processes are called the "primary events" of photosynthesis. They are physical in nature, and they must be completed before the chemical activities of photosynthesis can begin.

## Electron Flow in Photosynthesis

Investigations of the primary events have been hindered by the speed with which the events take place and by their complexity and inaccessibility; many can be observed only in the living cell. Most of the experiments intended to explore their sequence have by necessity been indirect. Many of them have been quite ingenious, however, and they have revealed several important characteristics

of the system by which the energy of sunlight is made available to the photosynthetic machinery of the plant.

The major organic products of photosynthesis are carbohydrates: substances, such as sugars and starches, whose composition is some multiple of the empirical formula  $(\text{CH}_2\text{O})$ . Because carbohydrates appear superficially to be compounds of carbon and water, it was thought for many years that photosynthesis consisted in splitting carbon dioxide ( $\text{CO}_2$ ), which would allow the oxygen to escape as a diatomic gas ( $\text{O}_2$ ) and free the carbon to combine with water.

It is now known that this scheme is wrong. Neither carbon dioxide nor water can properly be said to be split or decomposed in photosynthesis. The net effect of the process is instead to transfer hydrogen atoms from water to carbon dioxide; the oxygen evolved comes from the  $\text{H}_2\text{O}$ , not the  $\text{CO}_2$ . Because the process takes place in water solution it is not necessary to actually move a complete hydrogen atom; if an electron is transferred, a hydrogen nucleus, or proton, can be drawn later from the aqueous medium to complete the atom. Chemical processes of this kind, in which electrons are transferred from one molecule to another, are called oxidation-reduction reactions. The molecule that has lost electrons is said to have been oxidized; the

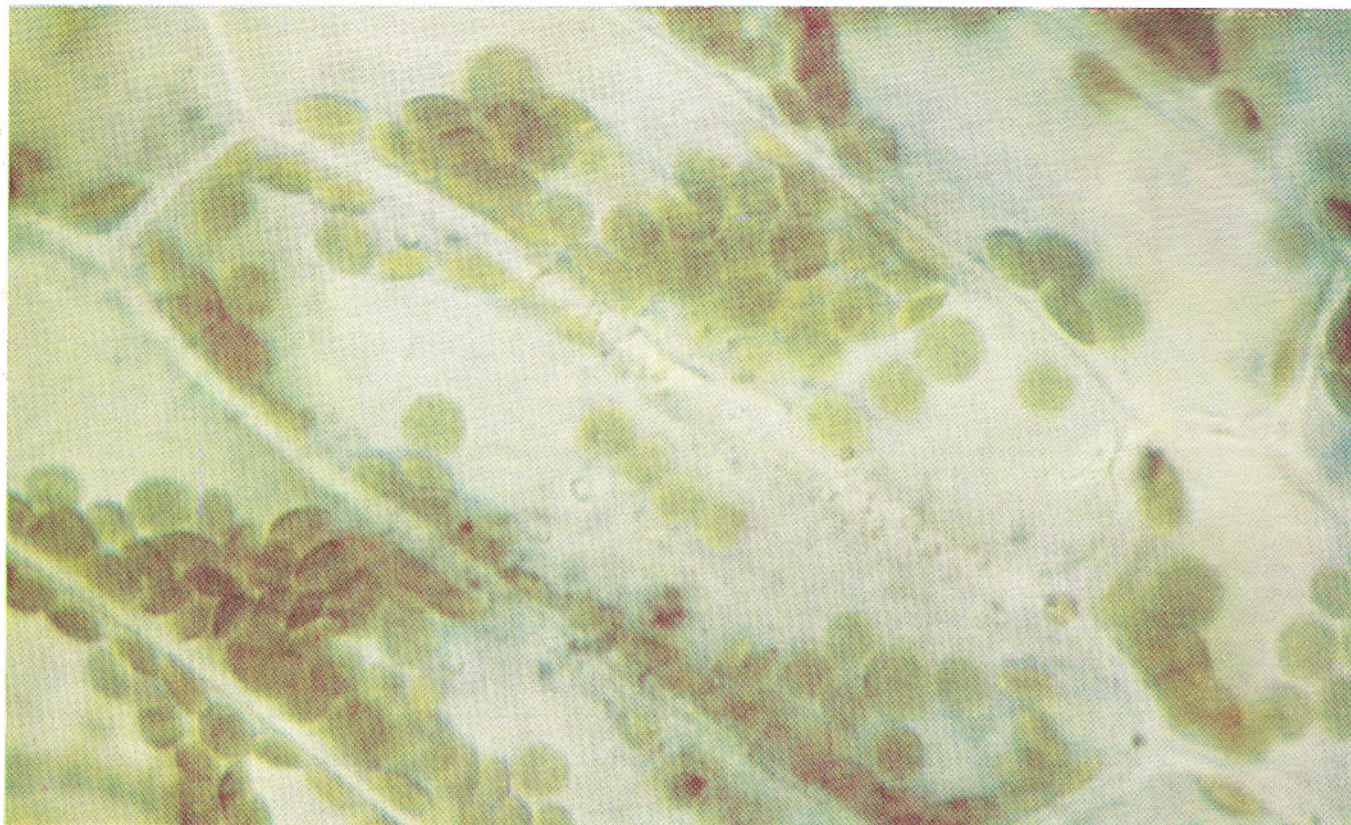
one that has received them is said to have been reduced. Thus in photosynthesis water is oxidized and carbon dioxide is reduced.

Ordinarily, of course, water does not reduce carbon dioxide, and it is not oxidized by it. For the reaction to proceed inside the plant cell, energy must be supplied. The energy requirement of an oxidation-reduction reaction is commonly measured in volts, and the electron transport involved in photosynthesis proceeds against an energy potential of about 1.2 volts.

An electrochemical gradient of 1.2 volts represents a rather large barrier, and there is reason to believe that in photosynthetic organisms it is not overcome by a single quantum of light; it appears instead that two quanta are required for the transport of each electron. This hypothesis is supported by numerous recent experiments; moreover, it accords well with an important observation made almost 20 years ago by the late Robert Emerson of the University of Illinois. Emerson found evidence that there are two pigment systems in plants that preferentially absorb light of slightly different wavelength (or color), implying that electron transport takes place in two main stages and involves two photochemical events [see "The Role of Chlorophyll in Photosynthesis," by Eugene I.

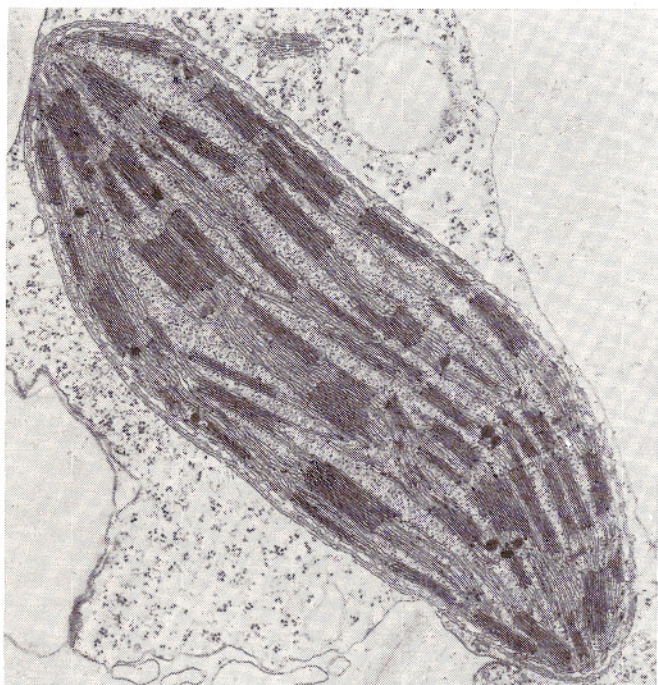
**FLUORESCENCE** of the plant pigment chlorophyll is stimulated by illumination with blue light. The natural green color of the chlorophyll can be seen at the bottom of the container in the photograph on the opposite page. The beam of light, made visible by smoke particles suspended in the air, enters from the top and is absorbed by the pigment, which is in an ether solution. Some of the energy of the absorbed light is dissipated as heat; the rest is reradiated as fluorescence in the red part of the spectrum. Chlorophyll in the leaves of a living plant also fluoresces, but only weakly; most of the energy that in solution is reemitted as red light is applied to the work of photosynthesis in the plant. The photograph was made in the laboratory of Alfred T. Lamme at Columbia University, with chlorophyll extracted from the seaweed *Ulva* by Robert K. Trench of Yale University.





**SITE OF PHOTOSYNTHESIS** in all higher plants and most algae is the chloroplast, an organelle having an intricate internal structure. In the photograph above, made through an optical microscope, the chloroplasts are the small green bodies, and they alone

are responsible for the color of the plant; the cells themselves are translucent. Some algae contain only one chloroplast per cell; higher plants can have as many as 1,000. The photograph was made by D. J. Paolillo of the University of Illinois at Urbana-Champaign.



**SINGLE CHLOROPLAST** is shown in cross section in an electron micrograph made by Paolillo and R. Chollet at the University of Illinois. The most prominent feature of the chloroplast in this view is the network of lamellae, the membranes on which the photosynthetic pigments are located. The lamellae are organized in dense stacks called grana, where many individual vesicular membranes, the thylakoids, are pressed together. The loosely stacked membranes connecting the grana are called stroma lamellae and the space not occupied by membranes is filled with stroma matrix.



**STRUCTURE OF THE LAMELLAE** is visible in an electron micrograph made by C. J. Arntzen, also of the University of Illinois. The specimen was prepared by freezing compressed chloroplast fragments, fracturing the frozen pellet and casting a metal replica of the fractured surface. At lower left is a granum; elsewhere individual thylakoid membranes have been pulled apart longitudinally, revealing interior surfaces covered with grains that resemble cobblestones. There are grains of various sizes and each size may be associated with a different function in the process of photosynthesis.

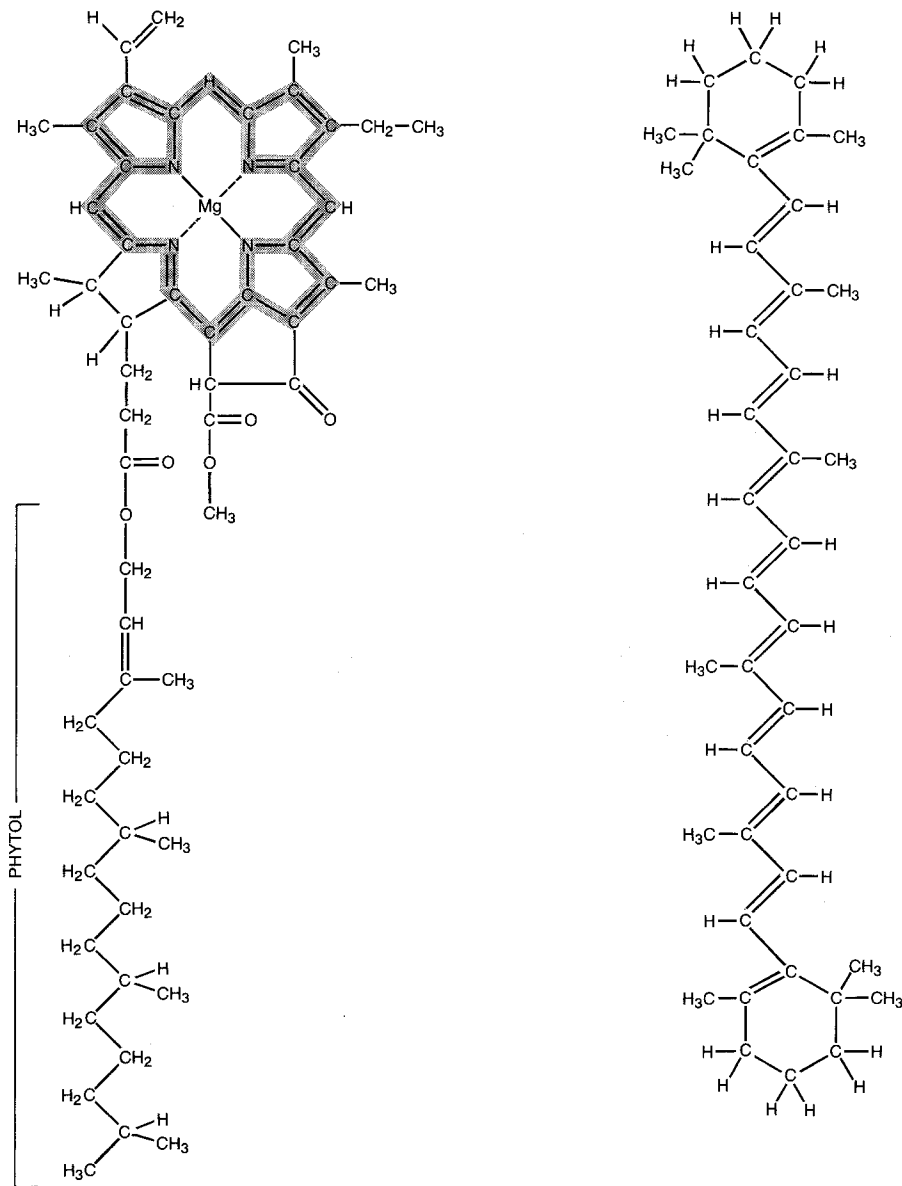
Rabinowitch and Govindjee; SCIENTIFIC AMERICAN, July, 1965].

Not all the molecules associated with the two pigment systems have been identified, nor are all their relations yet clear. Nevertheless, it is possible to draw at least a tentative map of the electron-transport pathway [see illustration on page 8]. The scheme is based on a model proposed by Robert Hill and Fay Bendall of the University of Cambridge [see "The Mechanism of Photosynthesis," by R. P. Levine; SCIENTIFIC AMERICAN, December, 1969].

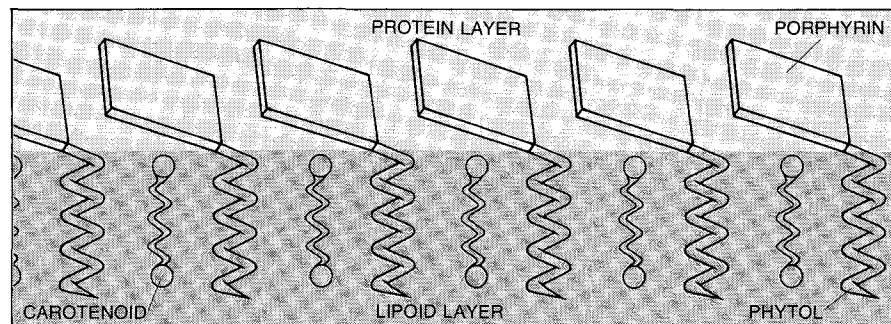
In the modified Hill-Bendall model, light striking the aggregation of molecules designated pigment system II results in the transfer of an electron from a donor called Z to an acceptor called Q. Recent work on the kinetics of oxygen evolution by Pierre Joliot of the Institut de Biologie Physico-Chimique in Paris and Bessel Kok of the Research Institute for Advanced Studies in Baltimore has shown that a molecule of oxygen is evolved after Z has given up four electrons, and thus accumulated four positive charges; Z is eventually restored to neutrality by scavenging four electrons from two water molecules. The oxygen atoms from the water form an O<sub>2</sub> molecule and the four protons enter the solution as positive ions.

From reduced Q the electrons are transferred, with the electrochemical gradient, to pigment system I. Several kinds of molecule serve as intermediaries, including at least one cytochrome (one of the proteins that also serve as electron-carriers in cellular respiration), an unidentified compound B, a plastoquinone and a copper-containing protein, plastocyanin. On absorbing a quantum of light, pigment system I promotes an electron from a "reaction-center" chlorophyll to another acceptor molecule, labeled X. The oxidized reaction center is then reduced by an electron that flows from reduced Q via the intermediates mentioned above. The reduced X then donates the electron to the iron-and-sulfur-containing protein ferredoxin, reducing it. The ferredoxin, with the help of the enzyme ferredoxin-NADP<sup>+</sup> reductase, ultimately reduces nicotinamide adenine dinucleotide phosphate (NADP<sup>+</sup>), a molecule with many roles in metabolism. (In its reduced form it is abbreviated NADPH.) This sequence of events is repeated for each of the four electrons donated by Z, so that eight electron transfers, and eight quanta of light, are required for each molecule of oxygen evolved.

Energetically the transfer of electrons from reduced Q to the oxidized reaction-



**PIGMENT MOLECULES** are distinguished by systems of conjugated, or alternating, single and double bonds. When the pigment absorbs light an electron circulating throughout the system of bonds enters an excited state. In chlorophyll (left) the conjugated bonds (colored band) are in a complex ring called porphyrin. Attached to the ring is a "tail" of phytol, made up of carbon atoms joined mostly by single bonds. Shown is chlorophyll a; other forms differ from it only slightly. In the carotenoid pigments (right) the conjugated bonds are located in a straight chain of carbon atoms that has a cyclic ring at each end.



**ARRANGEMENT OF PIGMENTS** in the lamellar membrane may be governed by the physical characteristics of the molecules. In this speculative model, the phytol tail, which is hydrophobic, or repellent to water, projects into the hydrophobic lipid layer. The porphyrin ring, a hydrophilic, or water-loving, group, associates with the hydrophilic protein layer. Carotenoids are hydrophobic and are probably found in the lipid portion.

center molecule is a downhill process and is coupled with the production of adenosine triphosphate (ATP), the ubiquitous energy-carrying molecule, from adenosine diphosphate (ADP) and inorganic phosphate. The existence of a cyclic flow of electrons around system I has also been documented. The electrons from reduced X, instead of reducing NADP<sup>+</sup>, are cycled back to the reaction center; when this cyclic flow goes via plastoquinone it is coupled with the production of ATP. (Other sites have been implicated in ATP production, but their role is not yet confirmed.) The importance of cyclic electron flow varies from plant to plant, but in most cases its contribution to photosynthesis is rather small. It is ATP and NADPH that effect the reduction of carbon dioxide and mediate its introduction into a carbohydrate cycle [see "The Path of Carbon in Photosynthesis," by J. A. Bassham; SCIENTIFIC AMERICAN, June, 1962].

### Pigment Molecules

The photosynthetic apparatus of the higher plants is organized inside chloroplasts, the cellular organelles that give

plants their characteristic green color. The chloroplasts are complex structures, separated from the cytoplasm of the cell by a membrane and apparently having some autonomy: each has a bit of the genetic material DNA and is able to synthesize some of the proteins it requires independent of the cell nucleus.

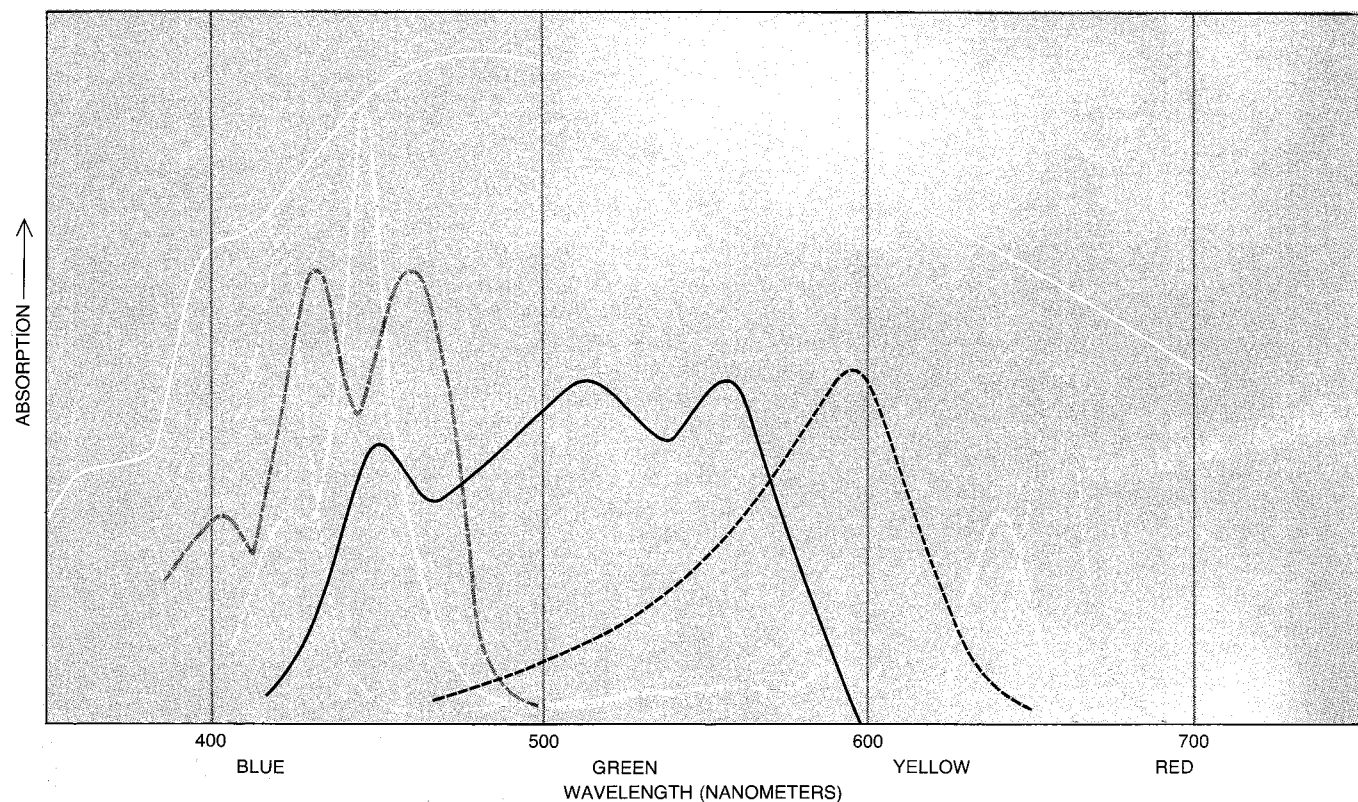
Inside the chloroplast is an elaborately folded network of membranes termed lamellae. In chloroplasts from most plants the lamellae form structures called grana, which are separated by a material known as stroma. The grana appear to be dense stacks of membranous sacs, which are the basic units of the lamellae. These sacs, called thylakoids, contain lipids, proteins and pigments. It is on and perhaps between the thylakoids that the business of photosynthesis is transacted.

A further level of structural organization can be inferred in the chloroplast, even if it cannot be resolved with certainty in the electron microscope. Experiments performed more than 40 years ago by Emerson and William A. Arnold, who is now at the Oak Ridge National Laboratory, suggested that a minimum of 2,400 chlorophyll molecules are required to evolve one molecule of oxygen.

An aggregate of this size, they proposed, should be considered the ultimate photosynthetic unit. We now know that eight photochemically driven electron transfers are required to generate one molecule of oxygen, so that a smaller unit is plausible, one containing 300 pigment molecules. This is the modern photosynthetic unit, the smallest unit capable of photochemical action.

Embedded in the thylakoid membranes are the pigment molecules that initiate the process of photosynthesis. Pigments are substances that by definition strongly absorb visible light. Most absorb only in certain regions of the spectrum and transmit light of all other wavelengths; as a consequence they appear colored. For example, chlorophyll, the most important plant pigment, absorbs both the longer and the shorter waves in the visible spectrum: red and orange and blue and violet. The transmitted wavelengths, chiefly the yellow and green in the middle of the visible spectrum, combine to yield the green of grass and trees [see illustration below].

The majority of organic molecules absorb most strongly in the ultraviolet; the various pigments of the chloroplast ab-



CHLOROPHYLL a  
 CHLOROPHYLL b  
 CAROTENOIDS  
 PHYCOERYTHRIN  
 PHYCOCYANIN  
 SOLAR SPECTRUM

**ABSORPTION SPECTRUM** of the photosynthetic pigments measures the amount of light they absorb at various wavelengths. The chlorophylls have two absorption bands, one in the blue and one in the red. They are green because they transmit the intermediate wavelengths. The carotenoids absorb shorter wavelengths and appear yellow or red. Phycoerythrin, which absorbs blue through yellow light, is red and phycocyanin, absorbing long waves, appears blue. Together the pigments absorb most of the light in the solar spectrum.

sorb at the longer wavelengths of visible light because they have chains or rings of carbon atoms connected by "conjugated," or alternating, single and double bonds. In chlorophyll the system of conjugated bonds is located in a ring, and it encompasses nitrogen atoms as well as carbon atoms [see top illustration on page 5]. The ring structure is one of the class of compounds called porphyrins, which are found widely in both plants and animals. In chlorophyll the central cavity of the ring is occupied by a magnesium atom; the function of the magnesium is not yet fully understood. Porphyrin rings in the blood protein hemoglobin and in the cytochromes contain an atom of iron instead of magnesium.

Attached to the porphyrin ring in chlorophyll is a long hydrocarbon "tail," the phytol chain. It consists of carbon atoms linked together, but only one of the bonds is double; for this reason the phytol chain does not appear to play an important part in determining the chlorophyll absorption spectrum. Its function may be to anchor the molecule in the thylakoid membrane. The phytol chain is hydrophobic, that is, it repels water but has an affinity for oils and fats; the porphyrin ring, on the other hand, is hydrophilic, being drawn to water. It has therefore been proposed that the phytol portion of the molecule may project into the lipid layer of the thylakoid membrane, securing the porphyrin at a particular location in the protein part of the membrane [see bottom illustration on page 5].

Three major varieties of chlorophyll are distinguished by small differences in structure in one region of the porphyrin ring. Chlorophyll *a*, the most abundant form, found in all higher plants and in all algae, has a methyl group ( $-\text{CH}_3$ ) at that position. In chlorophyll *b*, which is found only in the higher plants and in the green algae, the methyl group is replaced by an aldehyde group ( $-\text{CHO}$ ). Finally, in bacteriochlorophyll, the form of the pigment found in photosynthetic bacteria, a single bond is substituted for one of the double bonds in the conjugated ring, and two hydrogen atoms are added.

In addition to these structurally distinct molecules, various spectral forms of chlorophyll have been detected in living cells: they can be distinguished only by differences in their absorption spectra. C. Stacy French of the Carnegie Institution of Washington's Department of Plant Biology has detected forms of chlorophyll *a* whose maximum absorption of red light is at wavelengths of 660,

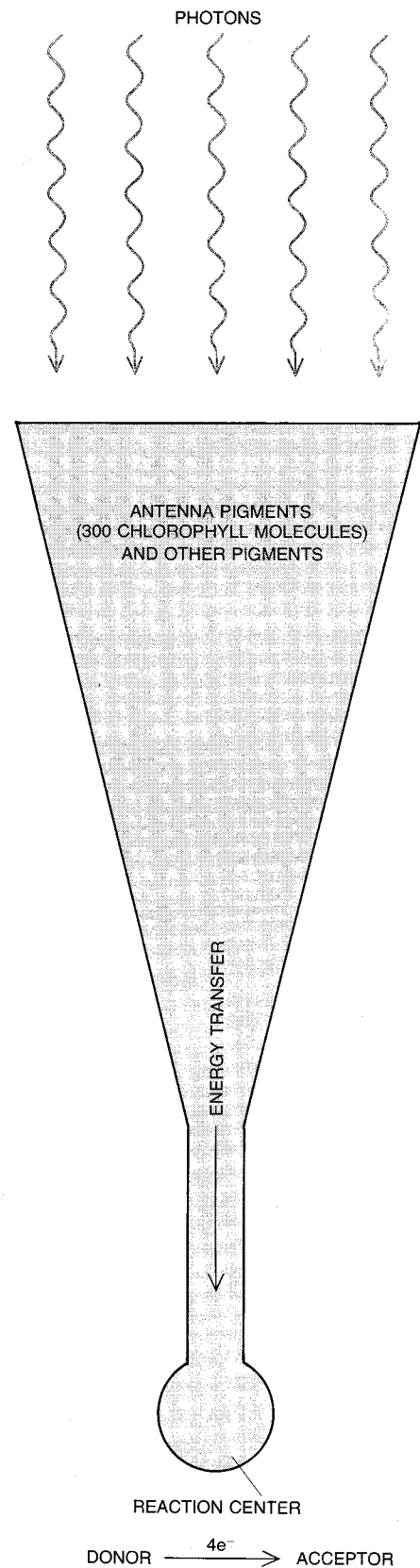
670, 680, 685, 690 and 695 to 720 nanometers. The variations are probably produced by chlorophyll molecules in different environments; they may be aggregated differently with other chlorophyll molecules, or they may be associated with proteins.

Although the chlorophylls are undoubtedly the most important plant pigments, they are not the only ones present and they are not the only ones that participate in light absorption. The carotenoids, found in bacteria, algae and higher plants, absorb mainly blue wavelengths and are yellow, orange or red in color. (Their name derives from the same root as "carrot," and they give the carrot its orange color.) They consist of long chains of carbon atoms linked by conjugated single and double bonds and bearing a six-carbon ring at each end [see top illustration on page 5]. The carotenoids are divided into two classes: the carotenes, which are hydrocarbons and therefore consist of hydrogen and carbon only, and the carotenols, which are alcohols and ketones and contain oxygen as well as carbon and hydrogen. The final category of pigments is the phycobilins, named for their resemblance to pigments in the bile of animals. They include the red phycoerythrins and the blue phycocyanins and are found only in the blue-green and red algae. The molecules of both types have an open ring related to the porphyrin structure of the chlorophylls, but in the phycobilins the ring is bound to a protein component.

### Light Absorption

When a photon is absorbed by an atom or molecule, its effect is to change the configuration of the electronic charge associated with the valence, or outer, electrons surrounding the atomic nucleus (or nuclei). Because the new configuration has more energy than the "ground," or lowest, state, the atom or molecule is said to be in an excited state. The transition from the ground state to an excited state can take place only under certain conditions prescribed by the laws of quantum mechanics. The electrons can occupy only specified, distinct energy states; regions between states are forbidden to them. Moreover, the energy of the absorbed photon must exactly match the energy of the transition. Since the energy of a photon is inversely proportional to its wavelength, only certain wavelengths can be absorbed by a particular atom or molecule [see top illustration on page 9].

In atoms these restrictions are quite

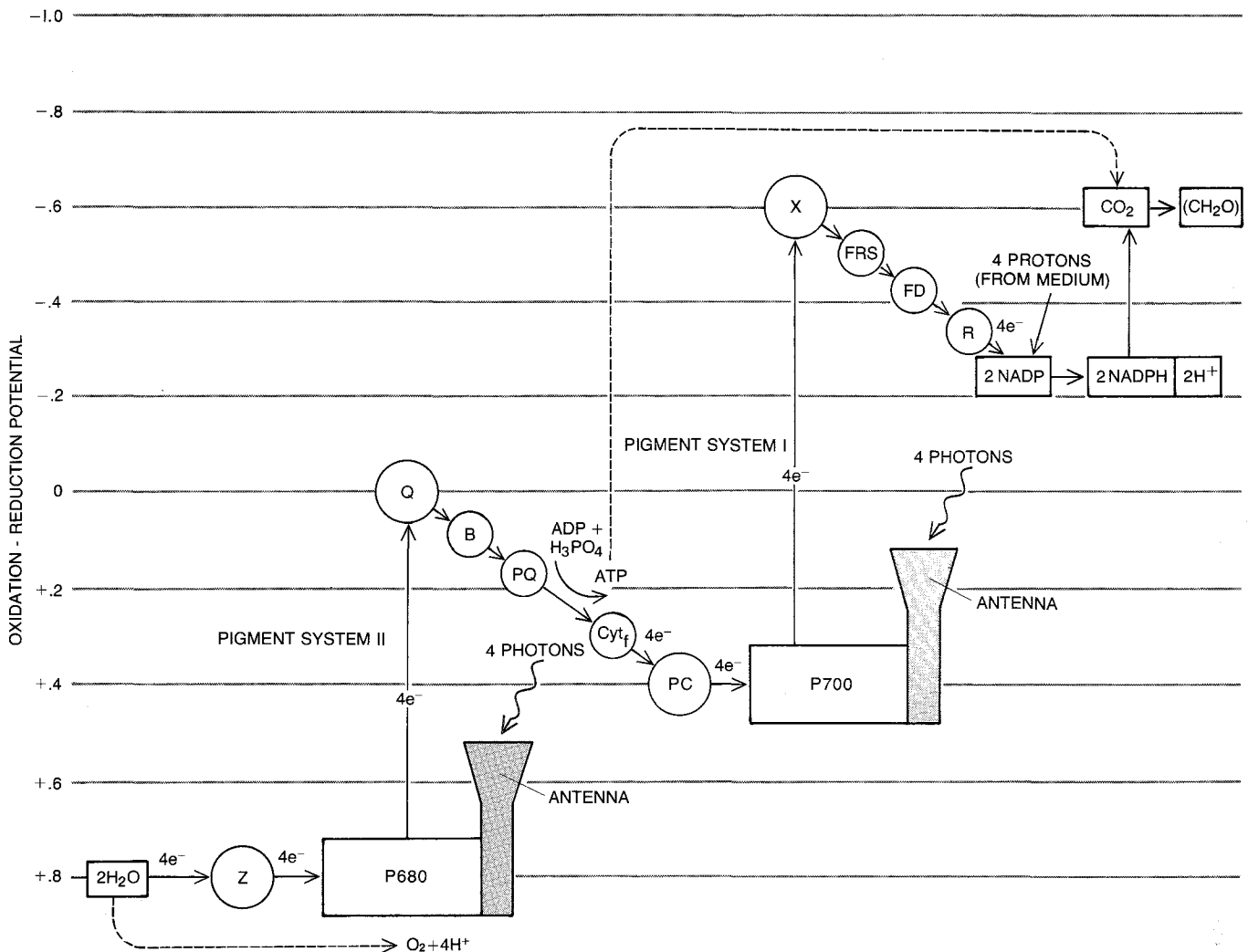


**PHOTOSYNTHETIC UNIT** is thought to consist of about 300 chlorophyll molecules; in the higher plants and algae that seems to be the minimum number necessary to bring about the transport of a single electron. The unit includes the bulk pigments, which serve as an energy-gathering "antenna," and a reaction center, where energy is trapped and utilized to promote chemical reactions.

confining, since the permitted energy levels are few and transitions between them are large. As a consequence the absorption spectra of atoms usually consist of a relatively few narrow lines. In molecules, however, and particularly in pigment molecules, a number of factors tend to broaden the lines into bands. For one thing, the conjugated bonds in pigments bring about a great proliferation of allowed states because each double bond adds a pair of electrons shared by the conjugated system as a whole. The

effect of a large system of conjugated bonds is therefore to decrease the transition gap between the ground state and the first, or lowest, excited state and to create many additional excited states just above the first. It is this effect that is responsible for shifting the absorption spectrum of pigment molecules from the ultraviolet region of the spectrum into the visible region; because of the smaller transition to the first excited state pigments are able to absorb photons of lower energy and hence greater wavelength.

Two other factors also increase the number of possible quantum states in molecules: the vibrational and the rotational energy of the molecules. These motions are also confined to discrete energy levels, but the levels are much more closely spaced, so that the ground state and each of the excited states broaden into a manifold of substates. In approximate terms the transition between the ground state and the first excited state in chlorophyll *a* represents an energy difference of between one and two electron



**FLOW OF ELECTRONS** from water to carbon dioxide proceeds against an electrochemical gradient of 1.2 volts and requires two photochemical events. Four electrons must be transferred, one at a time, to liberate a molecule of oxygen and reduce a molecule of  $\text{CO}_2$  to carbohydrate. The process begins with the absorption of a photon by the antenna of pigment system II. The energy of excitation is conveyed to a chlorophyll molecule in the reaction center of the photosynthetic unit; the molecule is designated P680 because one of the bands in its absorption spectrum is at 680 nanometers. The excited P680 transfers an electron to the acceptor Q, and subsequently recovers an electron from the donor Z. After Z has given up four electrons it regains them by oxidizing two molecules of water. From Q the electron is passed through a series of carrier molecules, including B, which has not been identified, plastoquinone (PQ) and cytochrome *f* (*Cyt<sub>f</sub>*), to plastocyanin (PC). Plastocyanin injects the electron into pigment system I. The reac-

tion-center chlorophyll of pigment system I, designated P700, is excited through its own antenna pigments, and promotes an electron to the acceptor X. Finally, the electron is passed through ferredoxin reducing substance (*FRS*), ferredoxin (*FD*) and the enzyme ferredoxin-NADP<sup>+</sup> reductase (*R*) to nicotinamide adenine dinucleotide phosphate (NADP<sup>+</sup>), which is thereby reduced to NADPH. NADPH is the primary product of these reactions. In addition, during two of the electron transfers adenosine triphosphate (ATP) is generated from adenosine diphosphate (ADP) and inorganic phosphate. One site for ATP generation is between plastoquinone and cytochrome *f*; the other is associated with system II but has not been located. The NADPH and the ATP drive the process by which  $\text{CO}_2$  is incorporated into carbohydrates (multiples of the unit  $\text{CH}_2\text{O}$ ). There is also a cyclic system of photosynthesis, in which electrons pass from P700 to X and then return through various electron carriers to P700; in this system only ATP is produced.



volts. (An electron volt is the energy acquired by an electron when it is accelerated through a potential of one volt.) The vibrational substates are separated by about .1 electron volt and the rotational substates by about .01 electron volt.

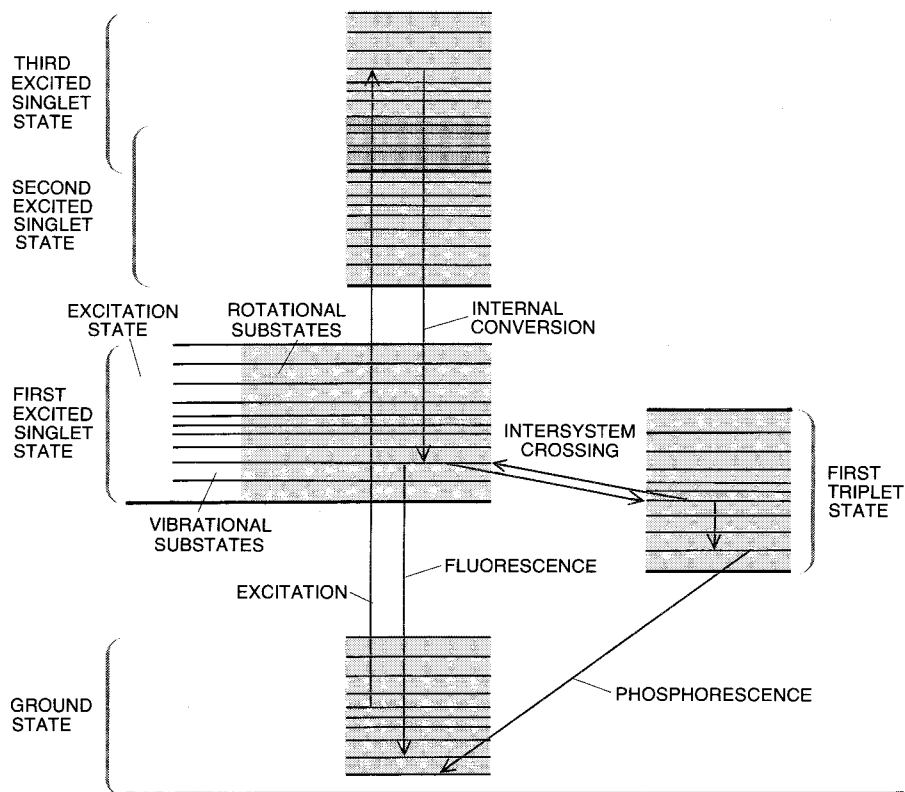
When a photon of appropriate energy strikes a molecule of chlorophyll *a*, the pigment enters an excited state almost instantaneously, within  $10^{-15}$  second. This is an extremely brief interval; in a vacuum light travels only about three ten-thousandths of a millimeter in  $10^{-15}$  second.

There are two main excited states available in chlorophyll *a*. The absorption of red light (with a wavelength of about 680 nanometers) raises the molecule to the lowest and most important of the levels, called the first excited singlet state. Blue light (with a wavelength of about 440 nanometers) promotes the molecule to the third excited singlet state. (Transition to the second excited singlet state, between the first and the third, is weak.) The upward transition may begin from any of the various vibrational and rotational substates of the ground state and end on any of the substates of the excited state.

Having reached an excited state, the chlorophyll molecule cannot immediately and directly apply its energy to the tasks of biochemistry, nor is useful work the only possible outcome of the absorption of a photon. The excited molecule can give up its energy in any of a number of ways, all of which conform to the same laws of quantum mechanics that govern light absorption.

### The Fate of Excitation Energy

For the purposes of photochemistry the most important energy level in chlorophyll *a* is the first excited singlet state. Molecules elevated to higher excited states return to the first excited singlet state rapidly (in from  $10^{-14}$  to  $10^{-13}$  second), so that they reside in the higher state too briefly to enter into any competing processes. The return to the first excited state is achieved through the small transitions separating the vibrational and rotational sublevels. Each of these transitions is so small that the wavelengths associated with them are not perceived as radiation, only as heat. By the same mechanism a molecule that has been raised to one of the higher sublevels of the first excited state will quickly decay to one of the lower substates. This process is called internal conversion; since it results in the degradation of the received energy to heat, it contrib-

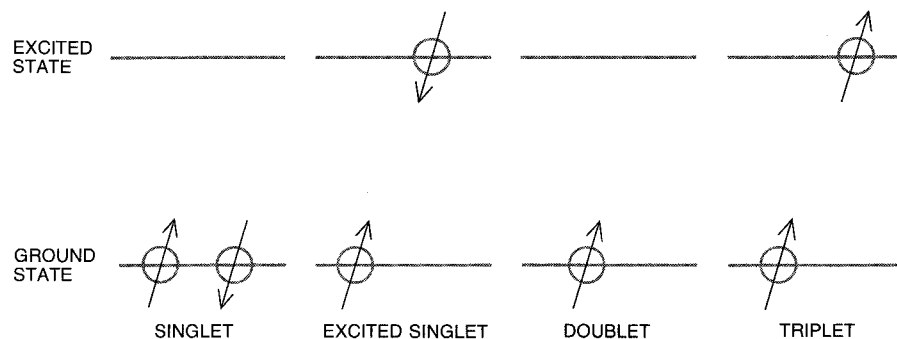


**EXCITATION OF AN ELECTRON** in a pigment molecule promotes the electron to one of a few discrete energy states; intermediate levels are forbidden. Superimposed on the permitted energy states are substates representing the vibrational and rotational energy of the molecule; the substates also are discrete, but they are closely spaced. When an electron absorbs a photon it is first elevated to an energy level called an excited singlet state. Depending on the energy of the photon, the electron may reach the first or some higher excited singlet state; it quickly subsides to the first, however, by dissipating part of its energy as heat in a process called internal conversion. In the first excited singlet state the molecule can utilize its energy in a chemical reaction, lose energy by internal conversion, reradiate it as fluorescence or enter another excited state, the triplet state. Because the triplet state is longer lived than the excited singlet states, light is emitted by molecules in the triplet state only after a delay and is called phosphorescence instead of fluorescence.

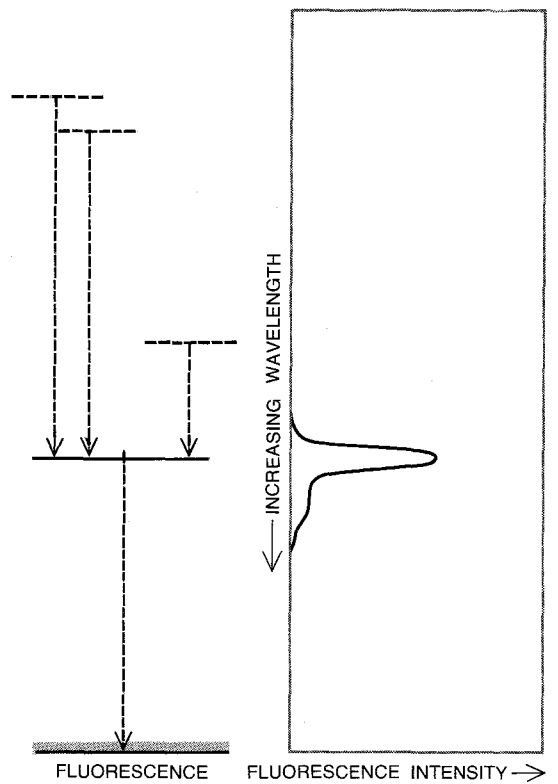
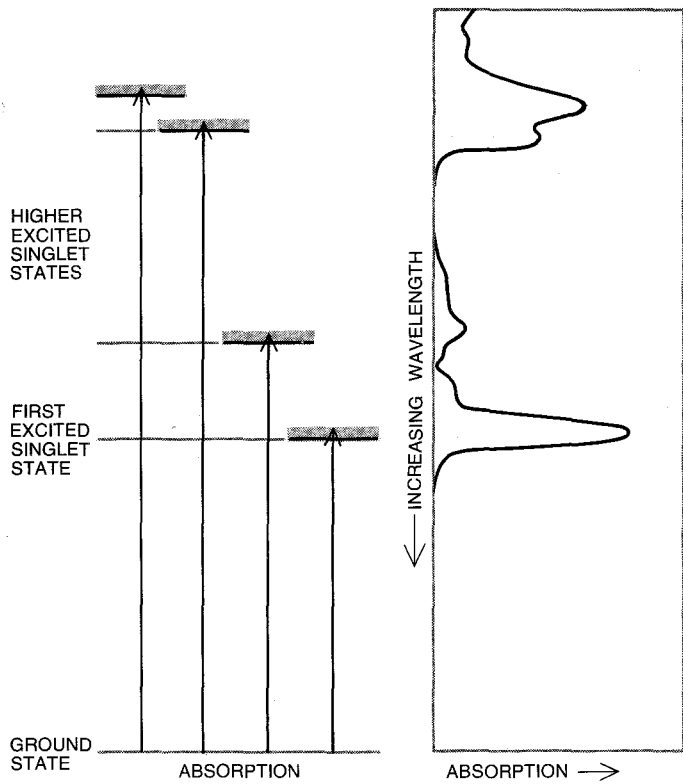
utes only to the kinetic motion of the molecules, not to photochemistry.

Because of internal conversion a high-energy photon of blue light is of no more use to a plant than a relatively low-energy red one. The squandering of the energy of short-wavelength light, however,

is less extravagant than it may at first seem. Although individual photons of short wavelength are the most energetic, most of the energy of the solar flux is distributed in the yellow and orange and beyond. There are simply greater numbers of photons at longer wave-

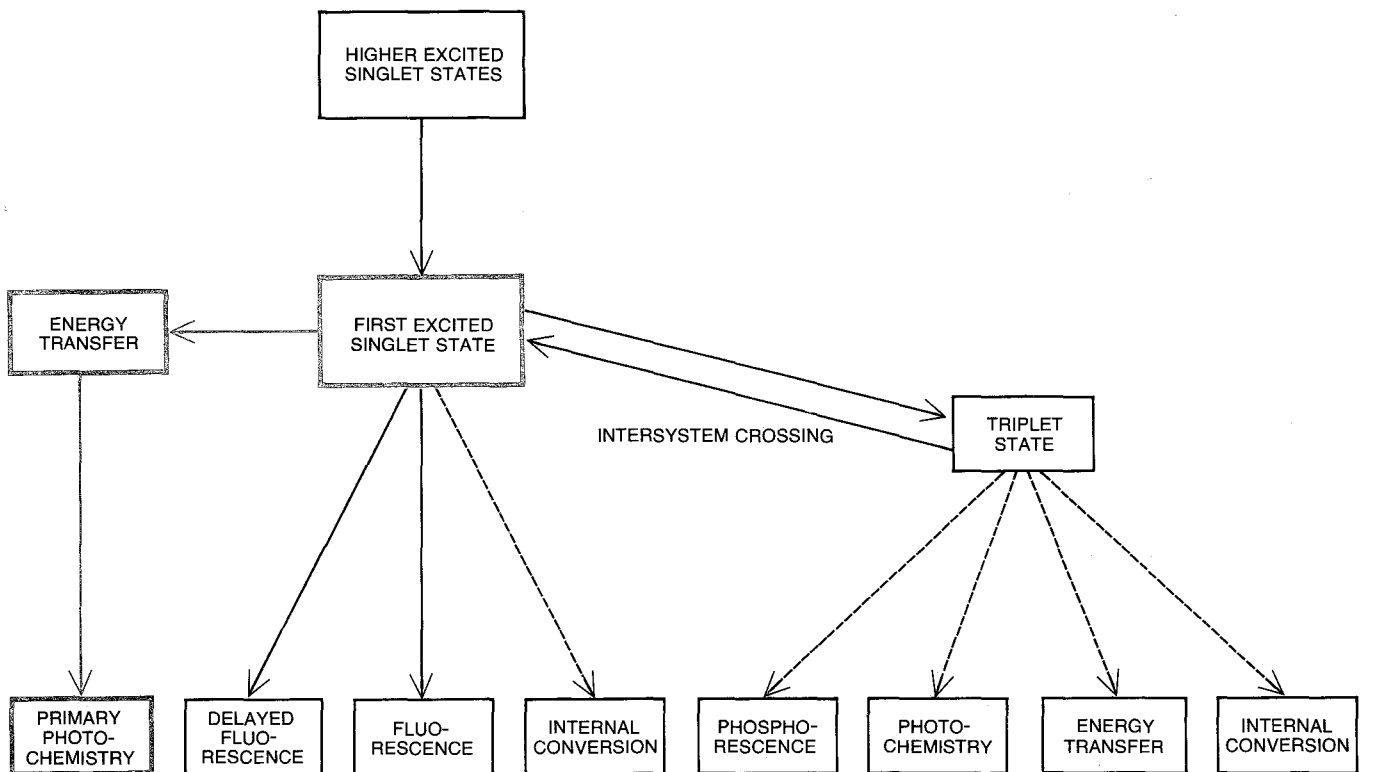


**SINGLET AND TRIPLET STATES** are defined by the "spin" of the outer electrons in an atom or molecule. In this diagram the arrows represent the spin axis. If the spins are antiparallel, the molecule is in a singlet state; if they are parallel it is in a triplet state. The doublet state requires an unpaired electron. "Singlet," "doublet" and "triplet" refer to the number of ways the electrons can orient themselves with respect to a magnetic field.



**ENERGY TRANSITIONS** associated with light absorption and fluorescence by chlorophyll are constrained by the requirement that the molecule occupy only discrete energy states. Only photons whose energy corresponds to the energy difference between the ground state and an excited state can be absorbed; since the energy of a photon is inversely related to its wavelength, the selective absorption of photons is reflected in the absorption spectrum of the

pigment (*diagram and graph at left*). Even though chlorophyll absorbs light in several parts of the spectrum, it fluoresces only in the red (*diagram and graph at right*). The fluorescence is from the first excited singlet state; molecules in higher states decay to this one by internal conversion. Fluorescence is at longer wavelengths than the lowest-energy absorption band because molecules relax to a low substate of the first excited singlet state before fluorescing.



**FATES OF EXCITATION ENERGY** are of varying probability and of varying importance to the living plant. The pathway indicated in color is the only one known to contribute to photosynthesis.

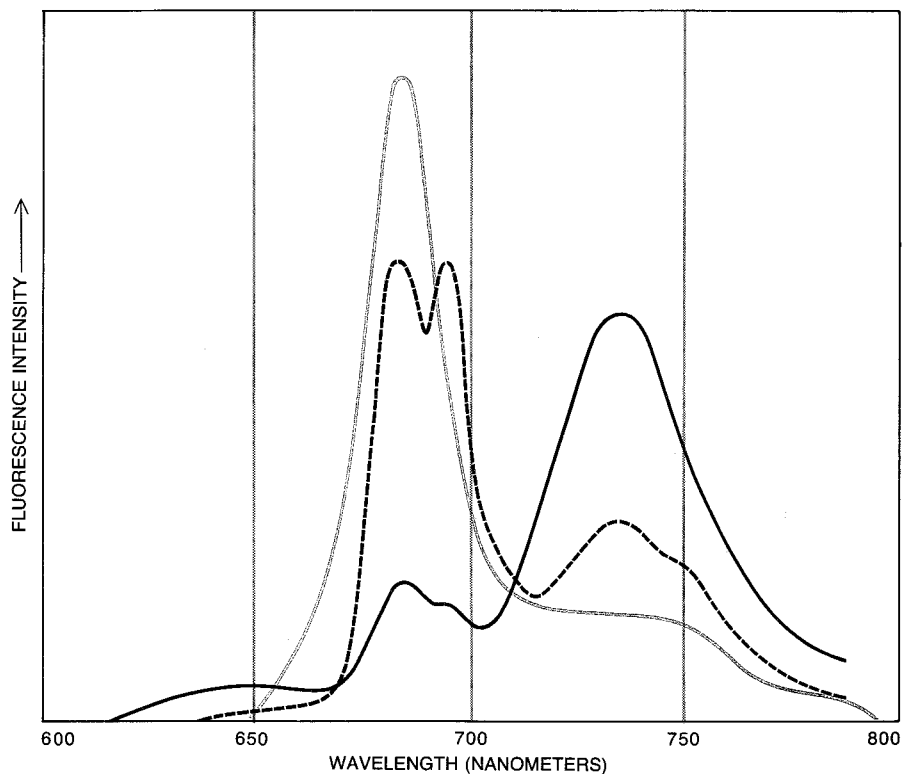
Transitions shown in solid black lines take place in the plant but do not result in useful chemical reactions. Transitions indicated by broken lines have not been observed in living cells.

lengths, and their aggregate energy is much greater.

Once an excited chlorophyll molecule has subsided to one of the lower sublevels of the first excited singlet state, several pathways are open to it. Through further internal conversion it can dissipate its remaining energy of excitation as heat; it can enter another excited state, of a somewhat different character, called the triplet state; it can return to the ground state by emitting a quantum of light as fluorescence; it can transfer its energy to an appropriate neighboring molecule, or it can enter into a chemical reaction, such as those characteristic of pigment systems I and II. The last process is obviously the most important, but it is by no means the only one that takes place.

Singlet and triplet states are distinguished by a quantum number called the total spin number, which has to do with the directions in which the electrons in a molecule "spin." In singlet states the spins of a pair of electrons are antiparallel, or opposite; in triplet states the spins are parallel. Doublet states, which are also found in some photosynthetic pigments, occur when there are unpaired electrons. The triplet state is so named because electron pairs spinning in parallel can align themselves in three ways with an external magnetic field; moreover, they superimpose three additional energy sublevels on the quantum state of the molecule. In the singlet state all orientations with respect to an external field are equivalent, and no additional sublevels are introduced [see bottom illustration on page 9].

The triplet state is metastable, that is, it is stable for a comparatively long time. Events that can reverse the spin of an electron are relatively uncommon, and transitions between the ground state and the triplet state are therefore rare. The triplet state can be readily entered, however, from an excited singlet state. There is considerable evidence that molecules in the triplet state are present in the chloroplast. An analysis made in our laboratory at the University of Illinois suggested that they are present, but in low concentration; our data suggest that at any given moment less than one chlorophyll molecule in 10 million is in the triplet state. J. S. Leigh, Jr., and L. P. Dutton of the University of Pennsylvania School of Medicine have found evidence for the triplet state of bacteriochlorophyll in photosynthetic bacteria, but only at low temperature and only when the photochemical oxidation-reduction processes driven by the pigments were blocked.

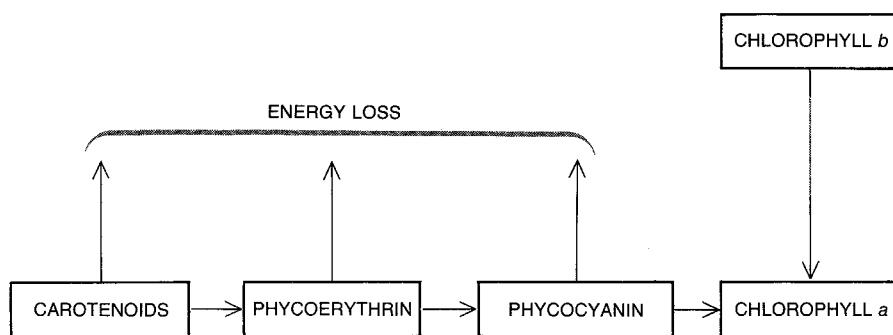


**FLUORESCENCE EMISSION SPECTRA** can discriminate between pigment molecules serving different functions. Intact chloroplasts at room temperature (*colored line*) have a strong fluorescence peak at about 685 nanometers and a much smaller peak at about 735 nanometers. At low temperature a more complex pattern emerges. Pigment system I (*solid black line*) fluoresces most strongly at 735 nanometers, with minor bands at 684 and 695. In pigment system II (*broken black line*) peaks at 685 and 695 nanometers are more important.

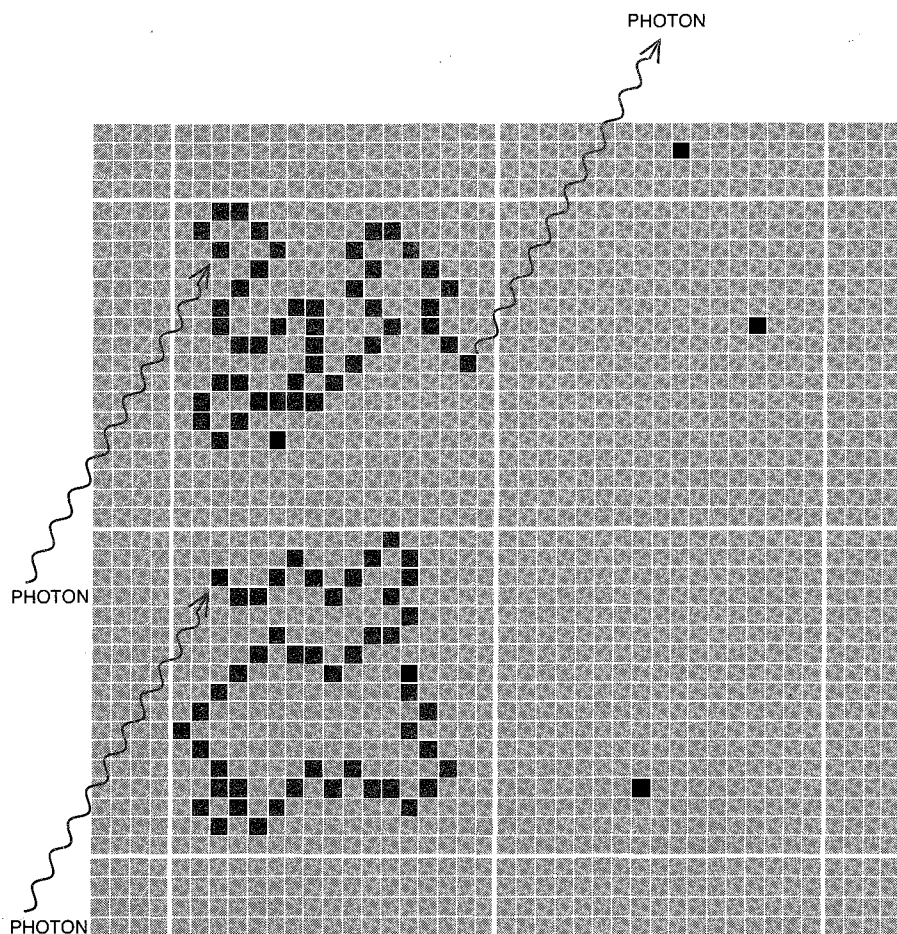
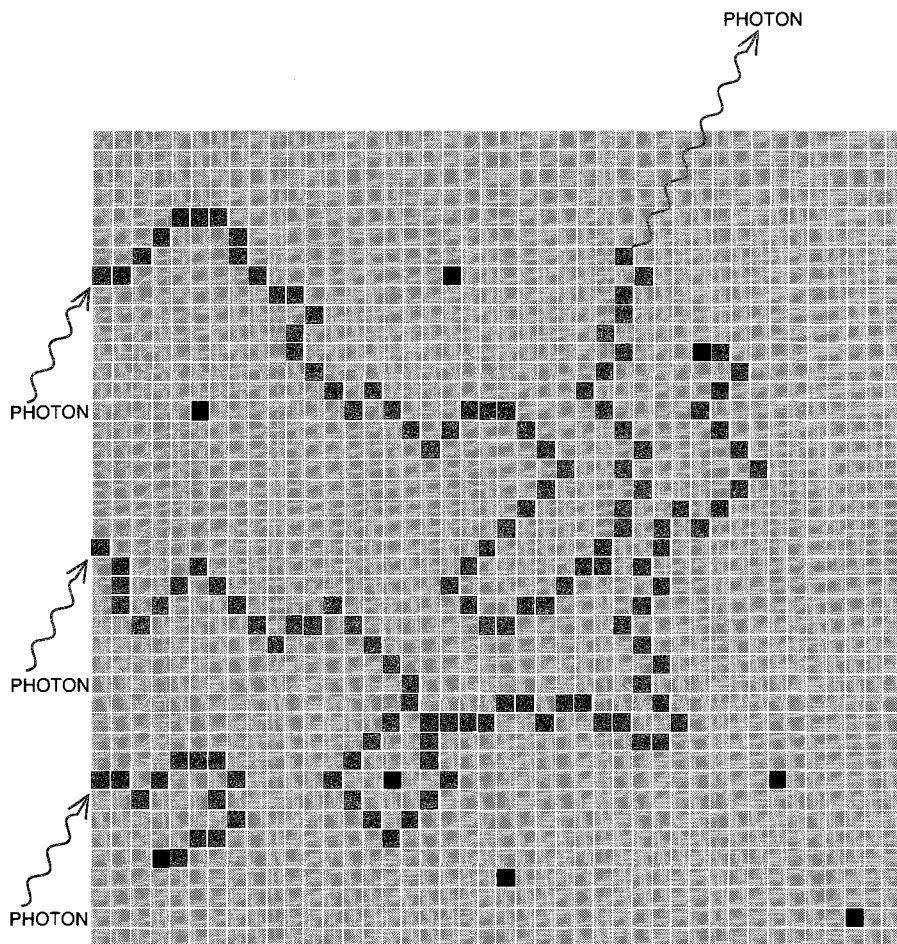
Even if the triplet state is present in illuminated plants, there is no evidence that it forms a part of the main photosynthetic pathway; there are many other ways in which its energy could be dissipated, such as internal conversion and a return directly to the ground state with the emission of light. The latter effect is termed phosphorescence: it is character-

ized by light emitted after a delay and at a considerably longer wavelength than the light absorbed. A molecule in the triplet state can also return to the first excited singlet state if it absorbs a small quantum of energy; it then has before it all the possible fates of other molecules at that energy level.

Light emitted during a transition from



**TRANSFER OF ENERGY** from one kind of pigment to another follows an established sequence. The carotenoids, which absorb blue light, pass their energy of excitation on to phycoerythrin. Energy received in this way, as well as the energy of green light absorbed directly by phycoerythrin, is transferred to phycocyanin. Phycocyanin absorbs orange light, and passes the accumulated energy to chlorophyll *a*. In each of these transitions energy is lost by internal conversion, and in some of the transfers energy is also dissipated as fluorescence. Chlorophyll *b* contributes its energy directly to chlorophyll *a* without loss.



a singlet state directly to the ground state is termed fluorescence instead of phosphorescence. Ordinarily there is little delay between absorption and re-emission, and the wavelength of the fluorescent light is only slightly longer than that of the light absorbed. The small increase is caused by the loss of energy through internal conversion.

### Fluorescence

Fluorescence has provided some of the most valuable techniques available for the investigation of photosynthesis, but for the plant it is an entirely wasteful phenomenon. Ordinarily, however, the magnitude of the effect is rather small. For every 100 photons absorbed, only from three to six are reemitted. For this reason fluorescence in living plants is faint and can rarely be detected by the unaided eye. The fluorescence of chlorophyll in solution, on the other hand, can be quite bright, since the process that would normally drain away most of the energy of excitation—photosynthesis—is disrupted.

Chlorophyll fluorescence is invariably red, even if the exciting light is blue or green or yellow. The fluorescent light is emitted in a transition from the first excited singlet state, and molecules promoted to higher states by more energetic photons merely dissipate part of their energy as heat before fluorescing [see top illustration on page 10]. At room temperature the fluorescence spectrum consists of a major band at 685 nanometers and a minor band at about 740 nanometers. When the spectrum is measured at low temperature, bands appear at 685, 695 and 720 nanometers. Pigment system II is primarily responsible for the bands at 685 and 695 nanometers, and

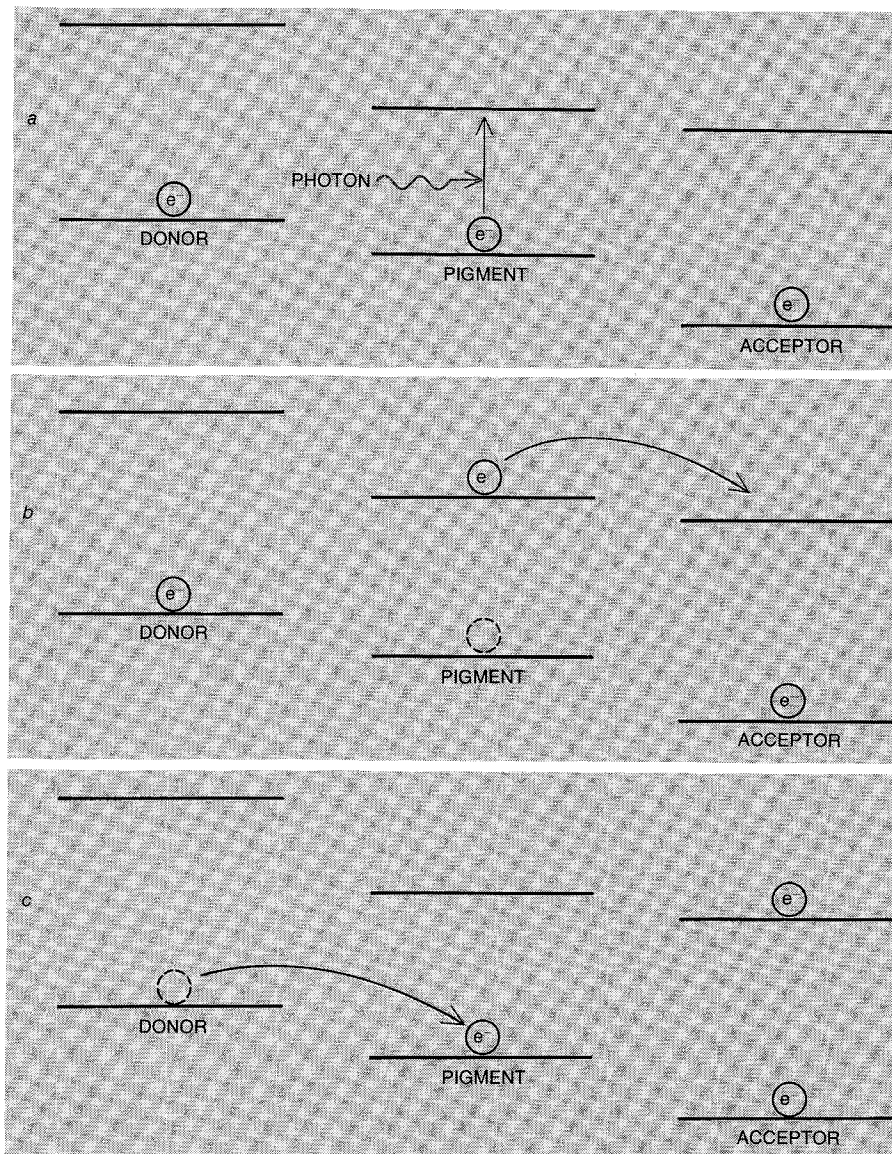
**FLOW OF ENERGY** through chlorophyll molecules in the thylakoid membrane is thought to be rapid and efficient, but undirected. In the diagrams at left each square represents a pigment molecule; there is one reaction center (*black squares*) for approximately 300 antenna molecules. In the “lake model” (*top*), energy absorbed by any molecule can wander through the entire mass of pigments until it is trapped in a reaction center or until it is reemitted. In the “isolated puddle model” (*bottom*), a single reaction center is committed to each aggregate of about 300 chlorophyll molecules. The energy again meanders randomly, but only within the confines of the 300-molecule unit. The mechanism of energy transfer, which probably involves resonance between nearby molecules, operates with perfect efficiency.

pigment system I for the 720-nanometer band.

In living cells the intensity of chlorophyll *a* fluorescence changes as a function of time. This effect, discovered by the late Hans Kautsky of the University of Heidelberg, consists of a fast change, completed about two seconds after the plant is illuminated, and a slower fluctuation that achieves a steady state only after several minutes. Both temporal patterns could reflect the shifting relation between pigment systems I and II. The fast change is sensitive to the rate of electron flow through the transport system and might be explained as indicating that pigment system II briefly gets ahead of system I, and wastes the surplus energy in fluorescence.

The slow change is thought to be related to temporary alterations in the thylakoid membrane. One hypothesis suggests that if the two pigment systems are spatially close together when the light is first turned on, energy from pigment system II, which is highly fluorescent, might "spill over" into the weakly fluorescent system I, thereby diminishing the overall light emission. If the two systems were then separated, the spill-over would be eliminated and the fluorescence would increase. The conformational change in the thylakoid membrane required by this theory might be caused by the movement of ions. An alternative explanation postulates changes in the density or spacing of chlorophyll molecules in pigment system II. Separating the molecules from one another could be expected to decrease internal conversion and thus to increase fluorescence.

The lifetime of an excited state in a fluorescent molecule is an important parameter in photosynthesis, since it gives an indication, albeit an indirect one, of how much time is available for the energy of excitation to reach a chemically active molecule. The lifetime is defined as the time required for fluorescence to decay to  $1/e$  of its maximum intensity ( $e$  is approximately 2.7). As we have mentioned, the lifetime of the higher singlet states is extremely brief, from  $10^{-14}$  to  $10^{-13}$  second; these states, however, do not enter into photochemistry. The first excited singlet state is much longer lived. Its lifetime was first measured by Seymour S. Brody at the University of Illinois and by Aleksander N. Terenin and his colleagues at Leningrad State University. New measurements in our laboratory of the lifetime of chlorophyll *a* in living cells, made with photosynthesis stopped by inhibitors or by low temperature, or made in light so bright



**TRANSPORT OF ELECTRONS "uphill," against an electrochemical gradient, in the reaction center of the chloroplast takes place in three stages. On absorbing a photon (a), an electron in a pigment molecule is promoted to an excited state. The electron can then be transferred to the acceptor molecule (b); because the pigment is in an excited state this process is "downhill," that is, it is favored by the electrochemical gradient. In the final step (c), the pigment regains an electron from a donor molecule, another downhill process.**

it saturates the photochemical system, yield a lifetime of about  $2 \times 10^{-9}$  second. Michael Seibert of the General Telephone and Electronics Research Laboratories and R. R. Alfano of the City College of the City University of New York have recently employed a laser and an instrument capable of marking extremely brief intervals in measuring fluorescence lifetimes in chloroplasts. At the resulting low average intensities they discovered two fluorescing species, one with a lifetime of less than  $10 \times 10^{-12}$  second, the other with a lifetime of about  $300 \times 10^{-12}$  second. From the emission spectra they have suggested that the two species are molecules of chlorophyll *a* in

different environments, the more rapidly decaying one making up part of pigment system I, the other contributing to pigment system II.

### The Transfer of Energy

Most of the pigment molecules in the chloroplast do not take part directly in the chemical processes of photosynthesis. Of the 300 molecules in the basic photosynthetic unit, almost all are thought to serve merely as "antenna" molecules. (The metaphor is not far-fetched; after all, the molecules are tuned to receive signals of a particular wavelength.) The antenna pigments

transfer the energy they absorb to a reaction center, which might consist of a single specially deployed molecule of chlorophyll *a*. This molecule then introduces the energy into the oxidation-reduction cycle.

If such a system is to operate efficiently, some method of rapidly transferring the energy of excitation from one molecule to another must be provided. That energy is indeed transferred is suggested by a simple observation: green leaves contain chlorophyll *b*, which is fluorescent in isolation, yet in living cells only the fluorescence of chlorophyll *a* can be detected. Evidently the accessory pigments transfer their energy to chlorophyll *a* so efficiently that their own fluorescence is quenched. The effect can be demonstrated directly in monomolecular layers of mixed chlorophyll *a* and *b*; even when the mixture is illuminated at a wavelength that will stimulate only chlorophyll *b*, only chlorophyll *a* fluorescence is observed.

Among the accessory pigments a sequence of energy-carriers has been worked out. It begins with the carotenoids, then passes through phycoerythrin, phycocyanin and allophycocyanin

(another phycobilin) to chlorophyll. In plants that have no phycobilins, energy can be transferred directly from the carotenoids to the chlorophylls, but when the phycobilins are present they are invariably utilized in sequence. Each transfer from one kind of pigment to another involves a modest loss of energy as fluorescence or heat [see *bottom illustration on page 11*].

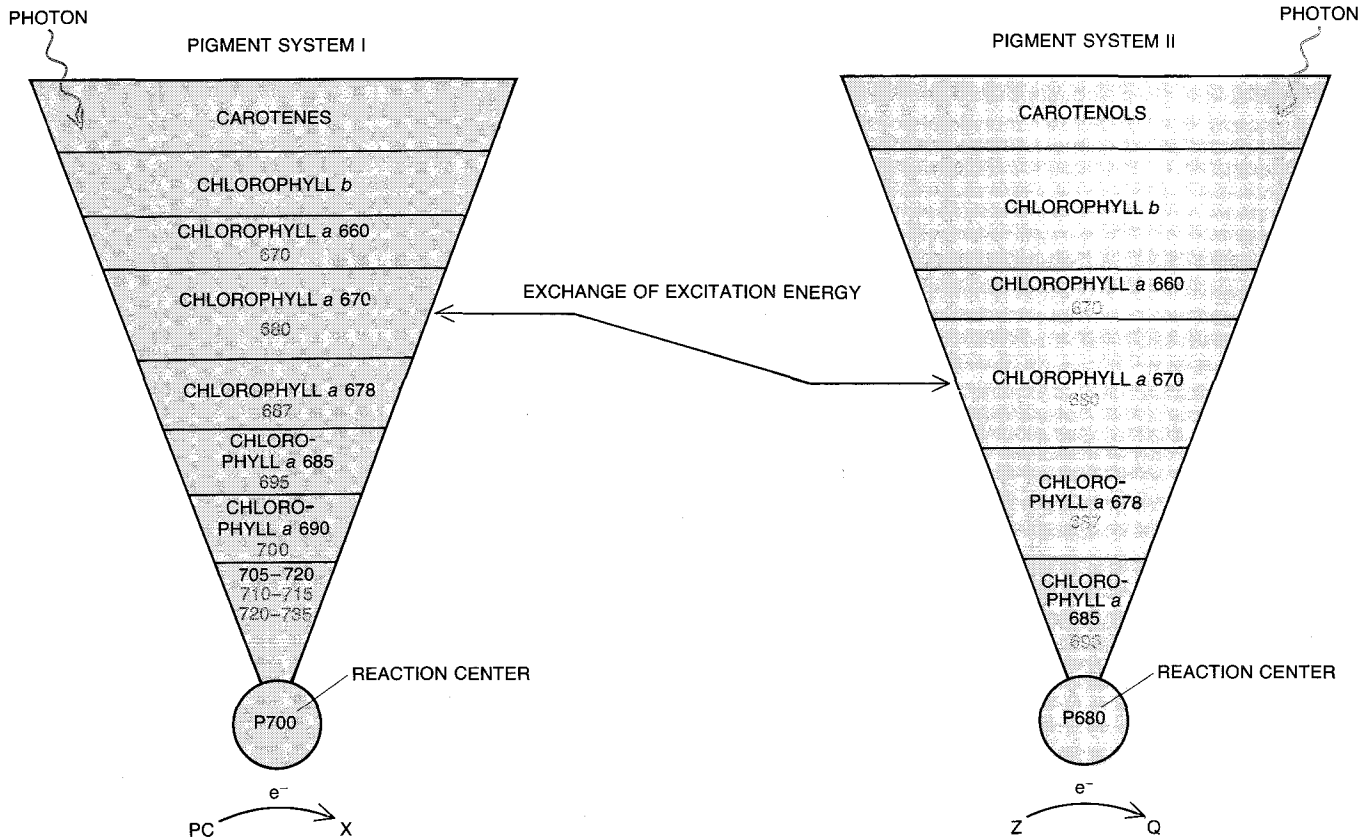
Once the excitation energy has been passed to a chlorophyll *a* molecule it can migrate through the entire population of chlorophyll *a* molecules without loss. That the migration does take place was first shown by Arnold and E. S. Meek at Oak Ridge in 1956. Arnold and Meek illuminated chloroplasts with plane-polarized light and observed the resulting fluorescence. If the exciting energy had been retained by each molecule during its fluorescence lifetime, the polarization would have been maintained in the fluorescent light; it was in fact destroyed entirely, indicating extensive energy migration.

One possible explanation of the mechanism underlying this migration is called the Förster resonance theory, formulated in 1948 by the late Theodor

Förster of the Technical University in Stuttgart. The theory holds that a molecule must first subside to the lowest vibrational sublevel of the first excited singlet state before its energy can be transferred; once this requirement is met the molecule is coupled to its neighbors by a resonance fundamentally dependent on their distance and orientation and on the overlap between the fluorescence spectrum of the donor and the absorption spectrum of the acceptor.

Faster mechanisms, in which the molecule need not first relax to its lowest vibrational state, have been proposed. They cannot yet be excluded, but calculations based on measurements of fluorescence lifetime support Förster's theory. An upper limit to the time required for energy transfer has been provided by an experiment devised by Peter M. Rentzepis of Bell Laboratories. Working with bacterial photosynthetic systems that had been stripped of all antenna pigments, so that only the reaction centers remained, he observed an energy exchange in  $6 \pm 2 \times 10^{-12}$  second.

The path taken by the migrating excitation energy in the antenna pigments is thought to resemble a "random walk"



**DISTRIBUTION OF PIGMENTS** in the higher plants is different in the two pigment systems. Among the carotenoids, carotenes dominate in system I and carotenols in system II. Chlorophyll *b* is more abundant in pigment system II. Chlorophyll *a* is the most im-

portant pigment in both systems, but each has a characteristic assortment of forms of this pigment, distinguished by their absorption spectra (*black*) and fluorescence spectra (*color*). The reaction center pigments of the two systems also exhibit different spectra.

that continues until the energy quantum happens to encounter an available reaction center, or until it is reradiated by fluorescence. The reaction centers themselves might be dispersed randomly in a monolithic bed of bulk pigment, so that any quantum of energy could wander throughout the mass and in principle could eventually reach any of the reaction centers. Alternatively, each reaction center might be surrounded by, and served exclusively by, a fixed aggregate of pigment molecules. These two hypotheses are called the "lake model" and the "isolated puddle model." The actual situation appears to be a hybrid of the two, calling for a "connected puddle model" [see illustrations on page 12].

### The Reaction Center

The elaborate system of pigmentation in plant cells is intended solely to convey the energy of light quanta to reaction centers where the central events of photosynthesis take place. Learning the nature and the exact business of the reaction center has been an intractable problem in the study of plant metabolism, but some of the molecules active there have now been at least labeled spectrally if not identified chemically. Louis N. M. Duysens of the University of Leiden discovered the first of these molecules when he observed a light-induced change in the absorption spectrum of photosynthetic bacteria at 890 nanometers; the change was later shown to be produced by bacteriochlorophyll in the reaction center. Kok identified a similar change in the absorption of green plants at 700 nanometers; this light-in-

duced absorption change is now known to be caused by the reaction-center molecule of pigment system I, designated P700. Exposure to light has been demonstrated to oxidize P700. The analogous molecule for pigment system II was discovered by G. Doring and Horst T. Witt of the Technical University in West Berlin and is labeled P680 or P690; whether or not it is a direct party to an oxidation-reduction process has not been definitely determined.

These specialized pigment molecules appear to stand at the culmination of the physical, energy-gathering stage of photosynthesis, and at the beginning of the chemical, energy-storing phase. There are a number of ways of determining whether or not they do mark that intersection. The chemical processes they participate in should be the first to take place after a brief flash of light, and they should be in the main pathway of photosynthesis. From experiments on the inhibition of photosynthesis by low temperature we know that the reactions should also continue even at very low temperatures (below 77 degrees Kelvin, or 77 degrees Celsius above absolute zero). These tests have been made and the evidence that has accumulated is encouraging. P700 is in fact oxidized by light at 77 degrees Kelvin (although the pigment cannot be fully reduced at that temperature). The absorption change associated with P680 or P690 has been observed at low temperatures. A series of calculations, beginning with early measurements made by Kok, show that the oxidation of P700 is a highly efficient reaction, and therefore it must be part of the main pathway. (If it was not, it would compete successfully with the main course of events.)

Finally, if P680 and P700 are the active molecules of the reaction center, they must be capable of initiating the sequence of events that leads to the transfer of electrons. They could do so, when they are in the photoexcited state, by donating one of their own electrons to the acceptor molecule, then recovering it when in the ground state from the donor [see illustration on page 13]. In the case of pigment system II, for example, P680, after absorbing a photon, would transfer one of its valence electrons to the molecule labeled Q and in the process would return to the ground state. It could then accept an electron from the donor molecule Z. This hypothesis implies that the reaction-center pigments bear a positive charge during the electron transfer, and the presence of chlorophyll ions in the chloroplast has indeed been demonstrated by electron-spin-resonance techniques that monitor unpaired electrons.

If this sequence of events is correct, some means must be provided to prevent Z and Q from reacting with each other to annul the charges developed on them. A direct reaction between them is thermodynamically the most favorable of all reactions, and it is known to take place, although only to a minor extent. (It is responsible for the delayed light emission discovered in 1951 by Arnold and Bernard L. Strehler at Oak Ridge.) The back-reaction between Z and Q must not be allowed to dissipate a significant amount of the absorbed energy, however, if photosynthesis is to achieve useful results. What mechanism intervenes to separate them is for now entirely unknown. That is only one of several mysteries, however, that remain to be solved by further study of photosynthesis.

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