The Biophysical Problems of Photosynthesis

Electrooptical techniques have brought clarification of physical and chemical events in photosynthesis.

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Contemporary thought on the physical problems of photosynthesis is rooted in two premises. The first of these is the idea that the primary photochemical acts are oxidoreductions mediated by chlorophyll in an electronically excited state. The second is the conception of a photosynthetic unit: a photochemical reaction center coupled with an aggregate of "light-harvesting" pigment. This aggregate, composed of various chlorophylls and accessory pigments (carotenoids and phycobilins), has a size ranging from about 50 to 400 pigment molecules for each reaction center. In the reaction center the central component is a specialized, photochemically active chlorophyll. The energy of light quanta, absorbed anywhere in the ensemble, is delivered efficiently to the reaction center, where the primary oxidoreduction is effected. The primary oxidizing and reducing entities then initiate the biochemical transformations of photosynthesis.

This model of the photosynthetic apparatus has been supported and invested with considerable detail in the past decade through a combination of biochemical and spectrophotometric experiments. I shall trace this development superficially, with no attempt at complete annotation, and then describe current efforts to elucidate the nature of the bacterial photosynthetic system.

The prevailing view of the photochemistry of photosynthesis began with van Niel's revision (*1*) of the overall chemical equation. Instead of

$$\text{Light} + CO_2 + H_2O \rightarrow (CH_2O) + O_2, \quad (1)$$

van Niel suggested

$$\text{Light} + CO_2 + 2H_2A \rightarrow (CH_2O) + H_2O + 2A \quad (2)$$

as a generalized overall equation applicable to photosynthetic bacteria as well as to green plants and algae. Here (CH_2O) represents a subunit of carbohydrate and H_2A is an oxidizable substrate. Green plants and algae can utilize water as the substrate, with O_2 as the product of its oxidation. Photosynthetic bacteria require more easily oxidized substrates: H_2S, H_2, organic acids and alcohols, and so on.

Equation 2 was inspired by a comparison of the photosyntheses of bacteria and green plants. On the basis of further comparisons, especially between photosynthesis and chemoautotrophy, van Niel suggested that the primary photochemistry generates oxidizing and reducing entities which eventually interact with H_2A and CO_2, respectively. This proposal, augmented by current ideas (*2*, *3*) about the involvement of pyridine nucleotide and adenosine triphosphate, is illustrated for bacterial photosynthesis in Fig. 1. The primary oxidant and reductant, generated in a photochemical act sensitized by bacteriochlorophyll (BChl), are left unspecified for the present.

Figure 1 is too simple to delineate the photosynthesis of green plants and algae. In these organisms the cooperation of two distinct photochemical reactions must be invoked.

Cooperation of Two Light Reactions in Photosynthesis

The reduction of CO_2 to the level of sugar and the evolution of O_2 from water are processes that involve the transfer of four electrons per molecule. If the primary photochemical act transfers one electron, this act must then be repeated at least four times to produce one molecule of O_2 (and to reduce one CO_2 molecule to carbohydrate). If the primary act can be driven by the energy of one quantum of light absorbed by chlorophyll, the minimum quantum requirement for photosynthetic oxygen evolution should be 4 $h\nu$ per O_2 molecule. If two quanta must cooperate in the primary act, the quantum requirement should be at least 8 $h\nu$ per O_2 molecule, and so forth. Until recently, therefore, the experimentally established value of about 8 $h\nu$ per O_2 molecule (see *4*) was taken to mean that the primary photochemical act is a "one-electron-transfer" process driven by the cooperative action of two quanta, and investigators such as Franck (*5*) entertained physical mechanisms for this cooperation.

Meanwhile evidence has accrued for a greater complexity in the photochemistry of photosynthesis, at least in green plants and algae. Emerson and Lewis (*6*) had found in 1943 that light of wavelength greater than about 680 mμ ("far-red light") is abnormally inefficient for photosynthesis in the green alga *Chlorella*, even though this light is absorbed by chlorophyll *a*. This observation of the "red-drop" effect was supplemented in 1957 by Emerson's discovery (*7*) of the enhancement phenomenon: the low efficiency of far-red light could be raised by superimposing light of shorter wavelength. Finally, Myers and French (*8*) observed enhancement in *Chlorella* when they used alternating beams of far-red light and shorter-wave light. The enhancing effect of shorter-wave light was thus shown to persist for several seconds after the light had been turned off.

These experiments showed that light has at least two distinct effects that cooperate in the photosynthesis of green plants and algae; with Myers and French's discovery that at least one of the light effects is stable for several seconds, it became difficult to visualize a physical cooperation at the level of excited states in chlorophyll. It was more plausible to suppose that photosynthesis involves the cooperative action of two distinct light reactions that are linked chemically. In 1960 and 1961, six groups of investigators (*9*–*11*) proposed formulations, of the kind sketched in Fig. 2, for the cooperation of two photochemical processes in se-

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ries linked by a chain of electron carriers (cytochromes and quinones). Especially compelling in this connection was Duysens' observation (*9*) that a cytochrome in the alga *Porphyridium* is oxidized by far-red light but is reduced by shorter-wave light.

The two photochemical systems can be disconnected and studied separately (*9, 11, 12*). System 2 can be poisoned selectively by means of dichlorodimethylurea. Electron donors such as reduced indophenol dyes, acting in place of the weak reductant normally generated by system 2, will then support the operation of system 1 to produce strong reductants such as NADPH (the reduced form of nicotinamide adenine dinucleotide phosphate). Conversely, the same dyes in their oxidized forms can be made to react with the weak reductant produced by system 2. Reduction of the dye, coupled with evolution of O_2, then represents a Hill reaction involving system 2 alone. In hydrogen-adapted algae, CO_2 can be reduced through the agency of system 1 alone, with H_2 utilized in place of the weak reductant made by system 2. Finally, the two photochemical systems are displayed separately in mutant algae in which one system or the other is nonfunctional.

Action spectra (*13*) for the enhancement phenomenon and for partial reactions associated with one or the other of the two systems have shown that system 1 is sensitized by a form of chlorophyll *a* having an absorption maximum at 683 mμ (chlorophyll *a 680*) and by carotenoids, while system 2 is sensitized by phycobilins, by chlorophyll *b*, and by a form of chlorophyll *a* having an absorption maximum at 672 mμ (chlorophyll *a 670*). Light of wavelengths greater than 680 mμ is absorbed almost exclusively by chlorophyll *a 680*, accounting for the red-drop effect. Shorter-wave light is absorbed by both types of chlorophyll *a* and by the various accessory pigments.

In the series formulation of Fig. 2, the functions of carbon assimilation and O_2 evolution are divided, one photochemical system producing a reductant capable of reducing pyridine nucleotide and the other generating a strong oxidant, the precursor of O_2. Eight quanta, each driving a "one-electron-transfer" photochemical act (four in each photochemical system), are sufficient to generate one molecule of O_2 and two of NADPH.

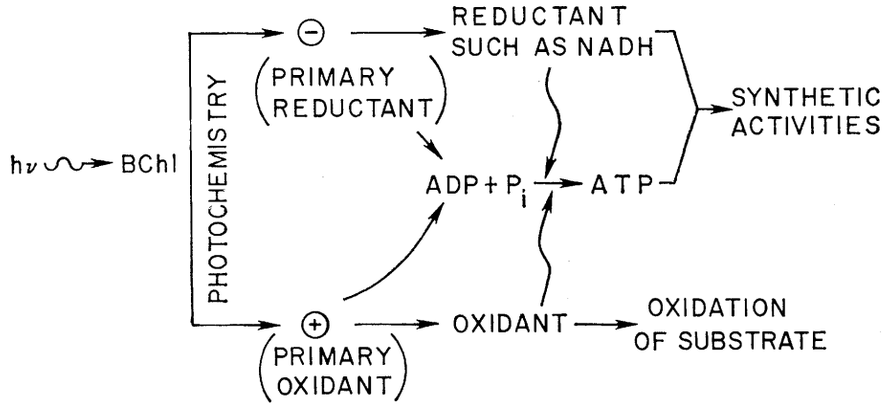

Fig. 1. Bacterial photosynthesis—a modification of van Niel's original formulation.

One molecule of CO_2 can be reduced to carbohydrate, through the Calvin-Benson cycle (*3*), at the expense of two NADPH plus three adenosine triphosphate molecules. The necessary adenosine triphosphate might be formed in a reaction coupled with the interaction of weak oxidant and weak reductant, between the two photochemical systems. Alternatively, some recombination of strong reductant with one or more oxidants (as suggested in Fig. 1) might be needed to give the required amount of adenosine triphosphate. In that case, some quanta are used to drive a cyclic process, and a requirement greater than 8 $h\nu$ per O_2 molecule can be expected.

The position of the photosynthetic bacteria is simple in terms of the foregoing series formulation; bacterial photosynthesis utilizes a single photochemical system analogous to system 1. Lacking a second photochemical system, the bacteria need an oxidizable substrate as a source of electrons to sustain their photochemistry. In one way, however, the bacterial system may differ sharply from the system 1 of green plants and algae. Stanier (*14*) and Gest (*15*) have suggested that bacterial photosynthesis involves extensive recombination of the photochemically produced oxidants and reductants, coupled with phosphorylation and followed by the utilization of phosphate bond energy as a means of generating strong reductants (see also *16*).

There is as yet no evidence that different wavelengths of light can have qualitatively different effects on bacterial photosynthesis.

Several investigators, especially Gaffron (*17*) and Franck (*18*), have found reasons to criticize the series formulation and to propose alternatives. Gaffron, drawing evolutionary arguments for a stronger parallelism between bacterial and green plant photosynthesis, suggested a "parallel formulation" in which each system produces a strong reductant and a strong oxidant (a precursor of O_2). The evolution of O_2 depends on a chemical interaction between the two kinds of strong oxidant through the agency of a manganese-containing enzyme. The bacteria, lacking this enzyme, must employ substrates to dispose of the strong oxidants.

Franck's departure from the series formulation originated in the need to account for certain physical properties of photosynthetic tissues, especially a doubling in the yield of chlorophyll fluorescence which is often observed when the intensity of illumination exceeds saturation for photosynthesis. Franck proposed that the two light reactions of green plant photosynthesis occur not in separate photochemical systems but as alternating events in a single photochemical complex. The complex consists of a special (photochemically active) chlorophyll molecule associated with cytochrome and other electron acceptors and donors. One of the light reactions (corresponding to system 1 in the series formulation) occurs when the cytochrome is in its reduced form. In this reaction, mediated by chlorophyll in its excited triplet state, the cytochrome becomes oxidized and an unspecified strong reductant is formed. Now that the cytochrome is oxidized, the other reaction (corresponding to system 2) occurs: chlorophyll in its excited singlet state brings about the reduction of the cytochrome and the formation of a strong oxidant, a precursor of O_2. This scheme is equivalent to a series formulation in which the two photochemical systems have merged and the chain of

electron carriers connecting them has been eliminated.

Further variations on the theme, including mixed series-parallel networks, are of course conceivable. At present, the series formulation as outlined in Fig. 2 and described in Fig. 3 is generally accepted as a close approximation to reality.

The Photosynthetic Unit

Emerson and Arnold (*19, 20*), on the basis of their study of photosynthesis in intermittent light, concluded that many chlorophyll molecules cooperate to harvest the energy of light quanta and to deliver this energy efficiently to a smaller number of chemical reaction sites. Gaffron and Wohl (*21*) reached the same conclusion by analyzing the kinetics of the onset of photosynthesis in algae exposed to dim light. These considerations led to the concept of a photosynthetic unit: a photochemical reaction center served by an aggregate of chlorophyll and other pigment molecules. The pigment aggregate acts as an antenna, harvesting the energy of light quanta and delivering this energy to the reaction center. The size of the aggregate serving one reaction center can be computed from the results of the experiments with intermittent light. If it is assumed that the photochemical event at the reaction center is driven by the energy of one quantum (as contrasted with a concerted two-quantum reaction), the light-harvesting ensemble is estimated to contain about 300 chlorophyll molecules in the case of green plants and algae (*20, 22*) and about 50 molecules of bacteriochlorophyll in the case of purple photosynthetic bacteria (*23*).

Like van Niel's concept of photochemical oxidoreduction, the idea of the photosynthetic unit has become a cornerstone of current descriptions of photosynthesis. The picture has been complicated through the recognition (in green plants and algae) of two distinct light reactions, each served by its own class of light-harvesting pigments. At present there is no way to decide whether each system occupies its own kind of photosynthetic unit, separate from the other system, or whether the two are interlaced morphologically. Each system does appear, however, to have an antenna of light-harvesting pigment, with convergence of energy to photochemical reaction centers. The best evidence for this is the high efficiency with which minor constituents react photochemically at the expense of light absorbed by the major pigments.

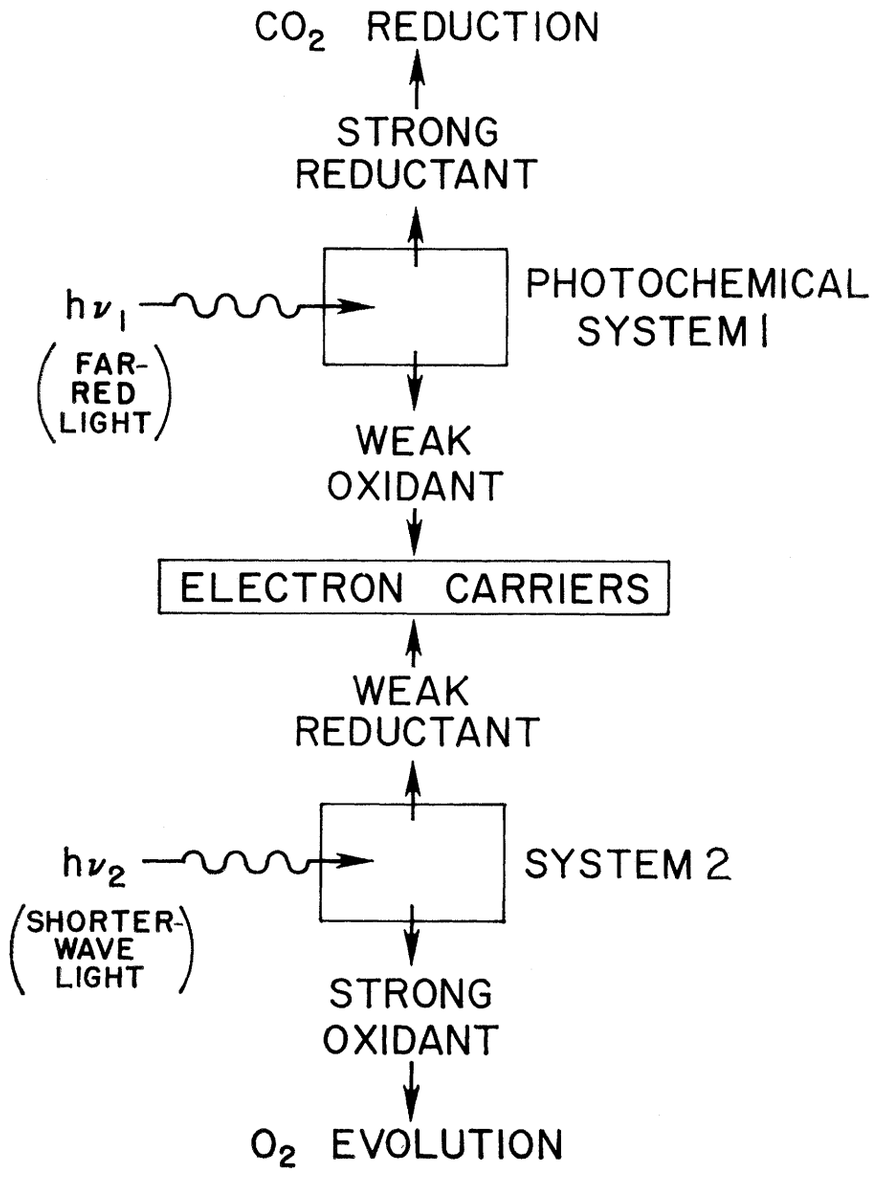

Fig. 2. Outline of a series formulation for green plant photosynthesis.

Spectrophotometry in the Study of Photosynthesis

Photochemical events in photosynthetic tissues are manifested in the form of minute light-induced absorbancy changes having spectra that can often be identified with reactions of known molecules. I shall summarize a few of the basic results of this area of investigation, without attempting to list the many contributions that have resulted from sensitive differential spectrophotometry. Then I shall describe my own investigations with photosynthetic bacteria more extensively.

1) In blue-green algae a cytochrome similar to the cytochrome *f* of higher plants is oxidized by far-red light. The oxidized cytochrome can be reduced, during far-red illumination, by superimposing shorter-wave light. This reducing effect of shorter-wave (system-2) light is abolished by dichlorodimethylurea (*9, 24*).

2) Chloroplasts and algae exhibit a reversible light-induced change that corresponds to the loss of two absorption bands, one centered at 705 $m\mu$ and the other at 430 $m\mu$ (*25*). The hypothetical substance having absorption bands at these wavelengths was termed P700; it was natural to sup-

pose (*25, 26*) that P700 is a special form of chlorophyll *a*. The bleaching of P700 can be caused by oxidation with ferricyanide as well as by illumination; titration shows that oxidized (bleached) P700 has lost one electron and that the oxidation potential is 430 mv (*25*). The molecular ratio of P700 to chlorophyll *a* in chloroplasts is about 1 : 300; this ratio can be increased about tenfold by selective extraction of the major chlorophyll *a* component with acetone (*25*). In chloroplasts and algae, P700 is oxidized by far-red light; the reduction of oxidized P700 is accelerated by shorter-wave light.

These and other observations suggest that the oxidation of P700 is part of the primary photochemical act of the long-wave system 1, with oxidized P700 identified as the primary oxidizing entity. In a closely coupled dark reaction an electron is transferred from cytochrome *f* to oxidized P700, restoring the latter to its reduced form and stabilizing the oxidant in the form of oxidized cytochrome. Eventually the oxidized cytochrome is reduced by the flow of electrons from system 2. The importance of P700 in photosynthesis was shown in a pair of experiments by Kok, Hoch, and Martin (*27*), who used chloroplasts exposed to far-red light in the presence of dichlorodimethylurea. Under these conditions both the oxidation of P700 and the reduction of pyridine nucleotide proceed with a quantum efficiency of 80 percent or more.

3) Several experiments have implicated plastoquinone in the chain of electron carriers connecting systems 1 and 2. Light-induced absorbancy changes in the ultraviolet indicate that plastoquinone is reduced by shorter-wave (system-2) light (*28, 29*) and oxidized by far-red light (*29*) in chloroplasts and algae. The reduction of plastoquinone is inhibited by dichlorodimethylurea. Photosynthetic O_2 evolution (in a Hill reaction) in chloroplasts is abolished by extraction with hydrocarbon solvents (*30*), but is restored if a quinone such as plastoquinone is added to the chloroplasts (*31*).

The foregoing observations are highlights in an assemblage of data that have led to a more specific series formulation, as shown in Fig. 3. In this scheme the reaction center of system 1 is centered in P700, which traps excitation energy absorbed by chlorophyll *a* 680 and engages in photochemical electron transfer.

The Bacterial Photosynthetic Reaction Center

Duysens (*32*) showed that in purple photosynthetic bacteria the absorption spectrum of bacteriochlorophyll changes reversibly upon illumination. Goedheer (*33*) found that a similar change can be produced with ferricyanide. The change has the characteristics of a "one-electron" oxidation with a potential of about 500 mv. The main feature of this change is a bleaching centered at the long-wave absorption maximum of bacteriochlorophyll in vivo—870 mμ in *Rhodopseudomonas spheroides* and 880 to 890 mμ in *Rhodospirillum rubrum* and *Chromatium*. The magnitude of the reversible bleaching is typically about 2 percent of the peak absorbancy. Eventually it became clear that this change reflects alteration of a specific minor constituent rather than a slight change in the major bacteriochlorophyll component. The reactive substance was then named P870 (in *Rhodopseudomonas spheroides*) or P890 (in *Rhodospirillum rubrum* and *Chromatium*), in analogy with the P700 of green plants and algae.

The characterization of P870 and P890 as specific substances came about through the development of techniques for the selective destruction of the major bacteriochlorophyll complement (*34*). This selective destruction could be effected with suspensions of chromatophores (pigmented organelles that contain the entire photochemical apparatus of a blue-green mutant of *Rhodopseudomonas spheroides*. When these chromatophores are treated with a detergent such as Triton X-100 and illuminated for a few hours in the presence of air, the major bacteriochlorophyll component disappears, but the extent of the reversible bleaching at 870 mμ is scarcely diminished. This is illustrated in Fig. 4; note that after photooxidation the only substance absorbing at 870 mμ is P870, as shown by the completeness of the reversible light-induced bleaching at this wavelength. An absorption band at 800 mμ persists after the treatment; it is not known whether this is part of the absorption spectrum of P870 or whether it reflects the presence of a different substance that resisted the treatment. After photooxidation in the presence of detergent the chromatophores could be precipitated with ammonium sulfate and extracted with a mixture of acetone and methanol. The residue, redispersed in water, showed no absorption bands in the visible or near-infrared regions. The organic extract showed only the absorption bands typical of bacteriochlorophyll in vitro, plus

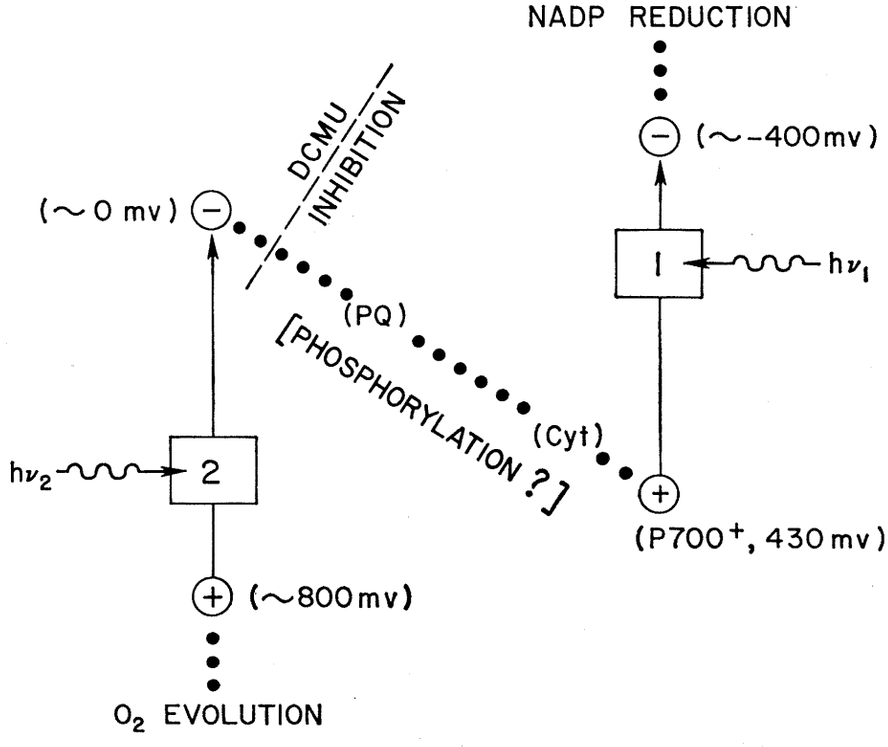

Fig. 3. A series formulation for green plant photosynthesis.

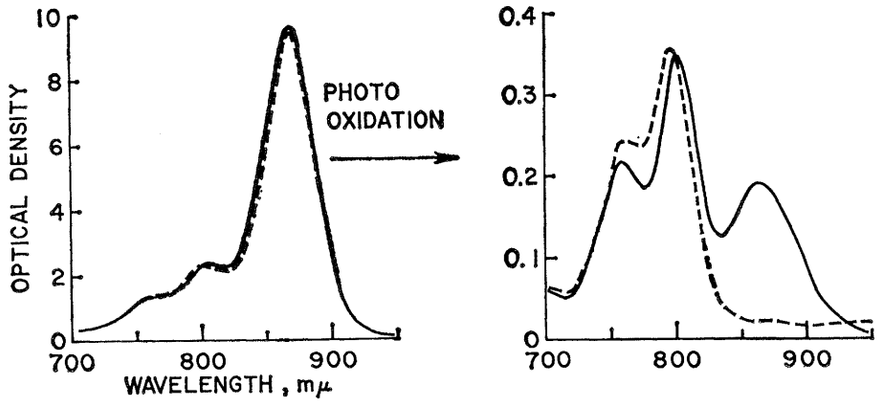

Fig. 4. Absorption spectra of an aqueous suspension of chromatophores from a blue-green mutant of *Rhodopseudomonas spheroides*, (left) before treatment; (right) after photooxidation in the presence of 1 percent Triton X-100. The difference between the solid curve (dark) and the dashed curve (light) shows the reversible light-induced bleaching of P870. Note the different scales for the two ordinates.

the spectrum of a small amount of bacteriopheophytin [note the band at 760 mμ, due to bacteriopheophytin, in the photooxidized chromatophores (Fig. 4, right)]. These experiments indicate that P870 is actually bacteriochlorophyll. The specialized environment of P870 in vivo endows it with photochemical reactivity and incidentally protects it against photooxidative destruction.

The ratio of P870 to total bacteriochlorophyll is about 1 : 50 in most purple bacteria. Two quanta absorbed by bacteriochlorophyll suffice for the oxidation of one molecule of P870 (*35*). The reaction is reversible, even at 1°K (36).

The light-induced oxidation of one or more cytochromes is manifested by absorbancy changes in photosynthetic bacteria and has been studied extensively in Chance's laboratory and elsewhere (*37*). In *Chromatium* this reaction has a quantum efficiency close to 100 percent (*38*); it proceeds, although irreversibly, at liquid-nitrogen temperature (*39*).

The observability of light-induced P870 (or P890) oxidation in purple bacteria is related inversely to the observability of light-induced cytochrome oxidation. In an oxidizing environment sufficient to keep the light-reacting cytochrome oxidized in the dark steady state, a light-induced oxidation of cytochrome is of course not observed. Under these conditions the reaction of P870 is conspicuous. Under reducing conditions the appearance of oxidized cytochrome is prominent during illumination and that of oxidized P870 is suppressed. There are at least two simple ways, illustrated in Fig. 5, to account for these relationships. Duysens, who first observed the light-induced bleaching at 870 to 890 mμ and who first suggested that some form of oxidized bacteriochlorophyll is an intermediate in bacterial photosynthesis, favors the supposition (Fig. 5, top) that oxidized P870 is formed in a primary light reaction and that cytochrome is oxidized (and P870 restored to its reduced form) in a closely coupled dark reaction. The coupling is so close that no accumulation of oxidized P870 is observed as long as the associated cytochrome is in its reduced form. Chance, a pioneer in the observation of light reactions of cytochromes, prefers another picture (Fig. 5, bottom): The oxidation of cytochrome is part of a primary light reaction in which some form of bacteriochlorophyll (perhaps P870) is reduced. The oxidation of P870 is an aberrant process that occurs only if the cytochrome is already oxidized and hence unable to engage in its functionally proper light reaction. The positions of Duysens and of Chance are both compatible with the existing information.

Although the functionality of P870 oxidation remains in some doubt, the presence of P870 in *Rhodopseudomonas spheroides* can be correlated directly with all of the useful photochemistry that this organism carries out. First, many manifestations of photochemical activity are displayed by cells and chromatophores of *Rhodopseudomonas spheroides* in which the major bacteriochlorophyll component has been destroyed or altered chemically (*34, 40*). Among these manifestations are the light reactions of P870, cytochrome, and ubiquinone, the occurrence of a light-induced electron spin resonance signal (*41*), and the ability of chromatophores to sensitize photochemical electron transfer among exogenous substances such as mammalian cytochrome *c* and quinones (*42, 43*).

The opposite situation, in which the main component of bacteriochlorophyll is present but P870 is absent, prevails in a mutant of *Rhodopseudomonas spheroides* isolated by Sistrom (*44*). This mutant cannot grow photosynthetically; when grown aerobically in the dark it is densely pigmented and has the same absorption spectrum (due to bacteriochlorophyll and carotenoids) as the photosynthetically competent parent strain grown under the same conditions. Chromatophores of the mutant, in contrast to those of the parent strain, show no light-induced absorbancy changes whatever. The mutant chromatophores do not display a light-induced electron spin resonance signal,

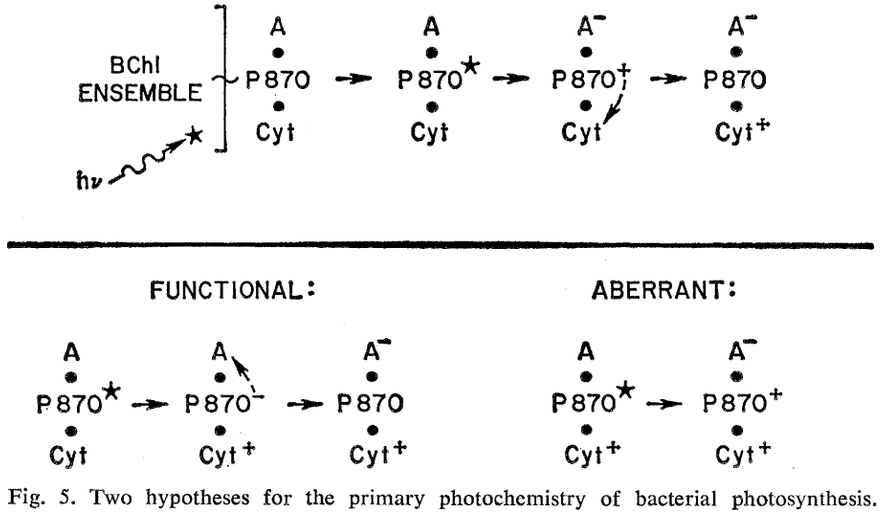

Fig. 5. Two hypotheses for the primary photochemistry of bacterial photosynthesis.

nor do they exhibit transient changes in the intensity of bacteriochlorophyll fluorescence that are associated with photochemical activity in the parent strain. The bacteriochlorophyll in the mutant chromatophores does not sensitize photochemical reactions of exogenous electron donors and acceptors, even though some of these reactions are sensitized by purified bacteriochlorophyll (*43, 45*). In photosynthetically competent strains of *Rhodopseudomonas spheroides* the presence of P870 is shown by the characteristic spectrum of absorbancy changes induced by light or by ferricyanide, and by the selective survival of P870 under photooxidative attack in the presence of detergent. Oxidized P870, present in aged chromatophore preparations, can be detected through the spectrum of absorbancy changes induced by hydrosulfite. The spectrum of the changes caused by hydrosulfite has the same form as that caused by light, but the opposite sign. All of these criteria indicate that the nonphotosynthetic mutant contains neither P870 nor oxidized P870 (*40*). In summary, a genetic mutation in *Rhodopseudomonas spheroides* is characterized by loss of the ability to form P870 and by a complete failure of the photochemistry of photosynthesis. The mutant lacks functioning photosynthetic reaction centers, and the major component of bacteriochlorophyll is photochemically inert.

From these experiments it appears that P870 (or P890) is the basis of the bacterial photosynthetic reaction center. P870 is probably bacteriochlorophyll in close association with a cytochrome.

Energy Transfer in the Photosynthetic Unit

Energy absorbed by the major photosynthetic pigments must find its way to the reaction centers, and several mechanisms can be envisioned for this process of energy transfer. The more obvious possibilities include the diffusion of substances, including electric charge in the form of electrons and holes, and the migration of quanta of electronic excitation energy, either singlet or triplet.

A mechanism based on the diffusion of atoms, radicals, or molecules can be ruled out because of the rapid onset of photochemical events (such as the oxidation of bacterial cytochrome) and because energy transfer leading to the oxidation of P870 in chromatophores occurs efficiently at 1°K. Charge migration is also an unlikely mechanism; these is no evidence for the wholesale conversion of primary excitation into separated electrons and holes in the major pigment aggregate. Arnold has amassed considerable evidence that photosynthetic tissues behave like organic semiconductors (*46*), but he now favors the view that the primary flow of energy to reaction centers is in the form of excitation quanta (*47*).

Robinson, McGlynn, and others have shown (*48*) that extensive energy migration can occur in crystals of aromatic compounds through the delocalization of triplet excitation energy (that is, energy in the form of triplet excitons). The triplet-excited state of chlorophyll or bacteriochlorophyll, a conspicuous product of light in vitro (*49*), cannot be observed in photosynthetic tissues even when the reaction centers are saturated with incoming light energy (*50*). It is therefore unlikely that the triplet exciton is important as a carrier of energy in the photosynthetic unit.

The remaining obvious alternative, that delocalized quanta of singlet excitation (singlet excitons) carry energy

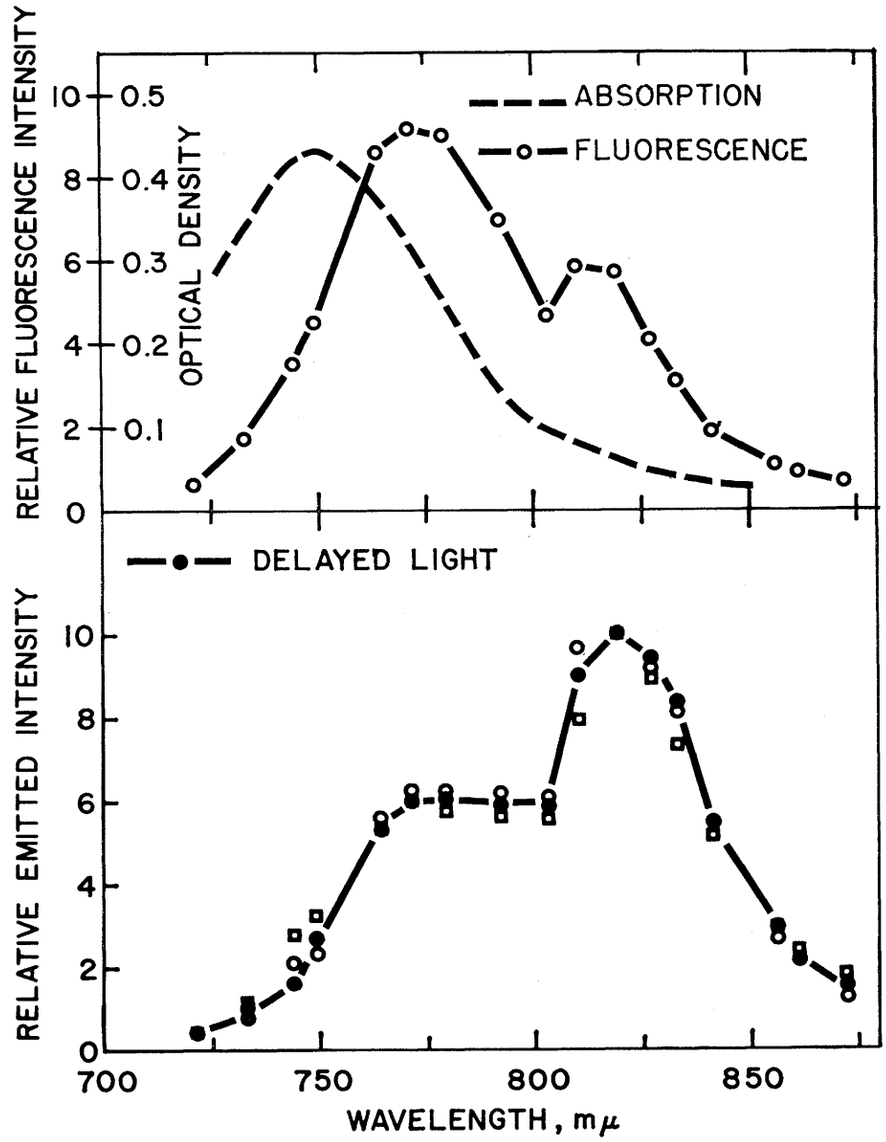

Fig. 6. Spectra of (top) absorption and fluorescence, and (bottom) emission of delayed light in *Chloropseudomonas ethylicum*. The fluorescence spectrum (top, solid curve with circles) pertains to the intensity of fluorescence at the start of illumination; the induction effect (bottom, open circles and squares) is the magnitude of the subsequent change during illumination. The open circle and squares represent two separate experiments.

through the chlorophyll ensemble and to the photochemical reaction centers, appears to be acceptable. Delocalization of singlet quanta in a molecular aggregate depends mainly on electric dipole interactions between the molecules. The strength of these interactions can be estimated for a collection of chlorophyll molecules having a certain mean separation, and the rate of singlet energy transfer (or the extent of delocalization of singlet quanta) can then be computed. Bay and Pearlstein (*51*), relying on morphological evidence (*52*) for the density of chlorophyll in a photosynthetic unit, have made a careful calculation of the rate of singlet energy transfer. They conclude that energy can be delivered efficiently to the reaction centers during the singlet-excitation lifetime of chlorophyll *a*, and their computation of the rate of transfer is in good agreement with the observed lifetime of the excited-singlet state (that is, the fluorescence lifetime). Earlier computations (*53*), which yielded rates of transfer too low to be compatible with efficient utilization of singlet energy, had been in error because they did not allow for the fact that each chlorophyll molecule has more than one nearest neighbor.

The mechanism by which excitation energy is trapped at photochemical reaction centers is still a matter of speculation. The P700 of system 1 in green plants is a natural sink for singlet excitation energy since it absorbs at slightly greater wavelengths than the light-harvesting chlorophyll *a 680*. Trapping by P700 could thus be a localizing transfer based on dipole interaction; this is also the mechanism whereby accessory pigments such as carotenoids can transfer excitation energy to chlorophyll or bacteriochlorophyll. The P870 or P890 of purple bacteria is not an energy sink relative to the long-wavelength absorption band of bacteriochlorophyll in vivo, so there is no reason to expect that delocalized singlet energy will be concentrated or trapped as such by P870. At best the singlet energy absorbed by bacteriochlorophyll might be shared by P870 to an extent commensurate with the relative abundance of these pigments. In the purple bacteria we must therefore envision a different trapping mechanism, such as the efficient conversion of the excited-singlet state to a metastable state in P870. The proximity of cytochrome, containing both a heavy atom and an extensive delocalized pi electron orbital similar to that of chlorophyll, could facilitate a transition from the excited-singlet to the triplet state in P870. Alternatively, the association of P870 with electron donor and acceptor molecules could promote a conversion of the excited-singlet state to a charge-transfer state. Such a state could lead directly to oxidized and reduced donor and acceptor molecules in their ground states.

Significance of Emitted Light Accompanying Photosynthesis

Fluorescence and emission of delayed light by chlorophyll and bacteriochlorophyll have been studied extensively as indices of energy conversion during photosynthesis. I shall define fluorescence as emission that accompanies the direct return from primary excited-singlet states to ground states. The term *primary* is used in order to exclude the delayed regeneration of singlet states that gives rise to delayed emission. Strehler and Arnold (*54*) discovered delayed-light emission in *Chlorella* while attempting to detect adenosine triphosphate with the firefly luminescence technique. The delayed emission has the same spectrum as chlorophyll or bacteriochlorophyll fluorescence (*55, 56*); it therefore reflects a delayed restoration of the pigment to its lowest excited-singlet state. This restoration can occur long after the initial singlet excitation has subsided; the lifetime of chlorophyll fluorescence in vivo is about 10^{-9} second (*57*), but delayed light can be detected an hour after a flash of exciting light (*58*).

In all kinds of photosynthetic organisms both the fluorescence and the delayed emission show induction effects: changes in the quantum yield of the emission during the first few seconds of illumination. In green plants and algae the typical response to constant illumination is an initial increase in the intensity of emission followed by a decline to a lower steady-state value. Aside from these induction effects, the fluorescence yield in the steady state depends on the intensity of illumination, increasing when the intensity becomes sufficient to saturate the photosynthetic apparatus with light energy.

Interpretations of variations in the fluorescence have played a major role in the development of theories for the mechanisms of photosynthesis. In particular, the doubling of the fluorescence yield that comes with light saturation in *Chlorella* was the point of departure for most of Franck's theories during the past decade (*18, 59*), providing the basis for postulating an alternation of "singlet" and "triplet" photochemistry. More recently, the induction effects have been related to the saturation of energy sinks in photosynthetic units—that is, to the bleaching of P870 or P890 in purple bacteria (*60*) and to the saturation of the hypothetical reaction center of system 2 in green plants and algae (*29, 61*).

The induction effects might disclose the fate of excitation energy in photosynthesis, but their interpretation has involved certain assumptions. Franck assumed, for example, that the fluorescence is emitted by a specialized chlorophyll at reaction centers and not by the light-harvesting component. Nearly everyone has assumed that the fluorescence is indeed fluorescence—that is, that it reflects the direct decay of primary singlet excitation. As we shall see, these assumptions are open to question. It is important that both the source and the nature of the emission be clarified to provide a sound basis for interpreting the significance of emitted light.

The intensity of delayed-light emission diminishes with time after a flash of exciting light. This decay has been measured in *Chlorella* over a range from about 10^{-4} second to 1 hour (*58*). The decay kinetics indicate that energy is detained, prior to the reappearance of the excited-singlet state, in a great many metastable states having different energies somewhat below the excited-singlet level. This tends to rule out the triplet state of chlorophyll, and Arnold has suggested that the delay mechanism involves photoionization, trapping of the separated electrons and holes, and then untrapping and recombination. A quantum of singlet energy is regenerated when the electrons and holes in the chlorophyll aggregate recombine. The mechanism for producing delayed light cannot take place in the major pigment aggregate without the help of photochemical reaction centers. Sistrom's mutant of *Rhodopseudomonas spheroides*, which possesses a normal complement of the major pigment but lacks functioning reaction centers, is more strongly fluorescent than the parent strain but emits no delayed light (*62*). Furthermore, the emission of delayed light (in contrast to fluorescence) is ex-

tremely sensitive to poisons of photosynthesis in algae as well as in purple bacteria (*63*). Even though the mechanism of delayed-light emission requires the functioning of reaction centers, the emission comes from chlorophyll in the light-harvesting aggregate. This is shown by the spectrum of delayed light emitted by green photosynthetic bacteria (*56*). In these bacteria (specifically in *Chloropseudomonas ethylicum*), the major pigment is chlorobium-chlorophyll, absorbing at 750 mμ. A lesser component, identified as bacteriochlorophyll (*64*), absorbs at 810 mμ, and the ultimate energy sink appears to be a pigment (P840) absorbing at 840 mμ (*65*). The existence of P840 is inferred from a reversible light-induced bleaching at this wavelength. Both fluorescence and delayed light are emitted by chlorobium-chlorophyll and bacteriochlorophyll, as shown by emission bands at 770 and 820 mμ, but not by P840 (see Fig. 6). The delayed-light spectrum differs from the fluorescence spectrum, showing a greater proportion of emission at 820 mμ than at 770 mμ.

Arnold and Davidson (*58*) have suggested that much of the so-called fluorescence of chlorophyll is actually a short-lived component of the delayed emission. This "fast" component, corresponding to the detention of singlet energy in metastable states for periods of less than 10^{-7} second, might have an intensity comparable to that of genuine fluorescence, as defined earlier. My experience with green bacteria (*56*) indicates that this is actually the case, and that the fluorescence-induction effect is a manifestation of "fast" delayed emission. First, that part of the fluorescence comprising the induction effect in *Chloropseudomonas ethylicum* shows the same kinetic pattern as the delayed emission. Second, the delayed light and the "induction component" of fluorescence have identical spectra (Fig. 6, bottom) distinct from the spectrum of the fluorescence intensity at the start of illumination (Fig. 6, top). Finally, the delayed emission and the fluorescence-induction effect show similar dependence on the intensity of the exciting light, varying roughly as the square of the exciting intensity in dim light and reaching saturation in bright light. In contrast, the initial intensity of fluorescence varies linearly with the intensity of the exciting light.

In purple bacteria the delayed light is about 100-fold weaker, relative to fluorescence, than it is in green bacteria or algae. Furthermore, the delayed light bears no resemblance, in its kinetics and in its dependence on external parameters, to the fluorescence-induction effect. Thus it is unlikely that the observed fluorescence of bacteriochlorophyll in purple bacteria includes an appreciable component of delayed emission.

The source of fluorescence in *Rhodopseudomonas spheroides* is not P870. This pigment, examined in isolation from the major bacteriochlorophyll component (see Fig. 4, right), is not fluorescent unless its photochemical activity has been suppressed. The fluorescence in healthy cells of purple bacteria should therefore be attributed to the light-harvesting bacteriochlorophyll and not to the reaction-center pigment.

Being assured as to the genuineness and the source of bacteriochlorophyll fluorescence in purple bacteria, one can, hopefully, make a meaningful interpretation of the induction effect.

Vredenberg and Duysens (*60*) first reported that the intensity of bacteriochlorophyll fluorescence rises in *Rhodospirillum rubrum* cells during constant illumination. The increase parallels the bleaching of P890, suggesting that P890 is a sink for singlet excitation energy in the major bacteriochlorophyll aggregate. As P890 becomes bleached it loses its property of being an energy sink; less energy is trapped, and more emerges as fluorescence. Vredenberg and Duysens assumed that fluorescent de-excitation, radiationless de-excitation, and trapping by P890 all obey first-order kinetics with respect to the concentration of excitation quanta, and that the rate of trapping by P890 is proportional to the concentration of unbleached P890. The fluorescence yield is then predicted to be

$$\phi_f = \frac{k_f}{k_f + k_d + k\,[P]}, \qquad (3)$$

where k_f, k_d, and k are first-order rate constants for fluorescence, radiationless dissipation, and trapping, respectively, and $[P]$ is the concentration of unbleached P890. According to this equation, the reciprocal of the fluorescence yield should change during illumination to an extent proportional to the absorbancy change at 890 mμ. Adherence to this relationship was reported, at least for the difference between light and dark steady states. The data do not appear to be smooth enough to afford a reliable test during the actual change.

These phenomena can be studied in chromatophores, with the advantage that the data are less "noisy" because the preparations are not turbid. The changes in fluorescence and absorbancy can be measured accurately throughout the transition between light and dark steady states. I find that chromatophores from *Rhodospirillum rubrum* show a fluorescence change related to the bleaching of P890, and in some cases the relationship is in fair agreement with Eq. 3. Usually it is not, and sometimes the change in fluorescence intensity, rather than the change in its reciprocal, is proportional to the change in absorbancy at 890 mμ. Often the relationship describing the differences between steady states is not the same as that describing the transition from one steady state to another. These deviations are not surprising. A strict adherence to Eq. 3 requires that the population of chromatophores be homogeneous with regard to the efficiency of energy transfer and also that a quantum of excitation in the bacteriochlorophyll ensemble be delocalized over a region occupied by many P890 molecules. If, on the other hand, each photosynthetic unit contains one molecule of P890 and is isolated from neighboring units with regard to energy transfer, Eq. 3 does not hold. Analysis of this case predicts that the change in fluorescence intensity (and not the change in its reciprocal) will be proportional to the absorbancy change.

Adherence to Eq. 3 is closest in whole cells and in fresh chromatophore preparations that have been sheltered from exposure to oxygen. The slightest mistreatment leads to deviations from Eq. 3 that can be attributed to changes in the range and efficiency of energy transfer. The model implied by Eq. 3, with many molecules of P890 lying within a single domain of energy transfer, thus seems appropriate for healthy photosynthetic tissues in the bacteria.

Conclusions

A reasonable framework for thought about the physical problems of photosynthesis is given by the series formulation shown in Fig. 3 in conjunction with the idea that the photochemical reaction centers are served by aggregates of light-harvesting pigments. The main physical questions appear to be

the following. How does energy, absorbed by the major pigments, become localized efficiently at the reaction centers? What molecular species are involved in the primary photochemistry? How do these molecules convert electronic excitation energy into a stable separation of oxidants and reductants?

Some progress has been made toward isolating and describing a reaction center in photosynthetic bacteria. With better chemical characterization and with improved differential spectrophotometry (incorporating better time resolution), the roles of P870 and cytochrome in this system will, hopefully, be clarified.

Interpretations of fluorescence and of delayed-light emission have played a large part in shaping theories of the photosynthetic mechanism. New evidence indicates that the emission traditionally regarded as fluorescence includes a significant component of delayed light. To make matters worse, the most interesting part of the "fluorescence," the time-varying part, is the most likely to be contaminated with delayed-light emission. The experimental basis of theories drawn from the nature of the fluorescence thus needs to be reexamined.

In photosynthetic bacteria, the fluorescence and delayed-light emission can be distinguished and observed separately. These bacteria therefore offer the best prospect for obtaining an understanding of the meaning of emitted light and knowledge of the details of energy flow from major pigments to photosynthetic reaction centers.

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