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Introduction to Photosynthesis

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ABBREVIATIONS

A	Intersystem intermediate
ADP	Adenosine diphosphate
AMP	Adenosine monophosphate
ATP	Adenosine triphosphate
BChl	Bacteriochlorophyll

C550	An absorption change suggested to be due to the primary electron acceptor of pigment system II.
Chl (λ)	Chlorophyll (numbers refer to the location of one of the absorption maxima, in nm)
CF	Coupling factor
Cyt (λ)	Cytochrome (numbers refer to one of the difference absorption bands)
DCMU	3-(3,4-Dichlorophenyl)-1,1-dimethylurea
DCPIP	2,6-Dichlorophenol indophenol
DPC	Diphenyl carbazide
ESR (EPR)	Electron spin resonance (electron paramagnetic resonance)
FRS	Ferredoxin reducing substance
Fd	Ferredoxin
hv	Photon of light
NAD^+	Nicotinamide adenine dinucleotide
$NADP^+$	Nicotinamide adenine dinucleotide phosphate
P430; X	Primary electron acceptor of photosystem I of chloroplasts, with a difference absorption band at 430 nm
P700	Reaction center chlorophyll of pigment system I of chloroplasts
P690	Reaction center chlorophyll of pigment system II
P870 (and P890)	Reaction center chlorophyll(s) of photosynthetic bacteria
PC	Plastocyanin
PEP	Phosphoenol pyruvate
P_i	Inorganic phosphate
PP_i	Inorganic pyrophosphate
PPNR	Photosynthetic pyridine nucleotide reductase
PQ	Plastoquinone
PS I	Photosystem I of chloroplasts (or pigment system I)
PS II	Photosystem II of chloroplasts (or pigment system II)
PSU	Photosynthetic unit
Q	Primary electron acceptor of photosystem II of chloroplasts, quencher of chlorophyll fluorescence
TMPD	*N*,*N*,*N*′,*N*′-Tetramethyl-*p*-phenylenediamine
Tris	Tris(hydroxymethyl)aminomethane
UQ	Ubiquinone
X	Primary electron acceptor of pigment system I
Z	Primary electron donor of photosystem II of chloroplasts

1. GENERAL

Photosynthesis is the process by which chlorophyll-containing plants convert solar energy into photochemical energy. This energy is stored in the form of carbohydrates, providing food for man and all other heterotrophic organisms. In addition, it provides the most vitally needed supply of oxygen. The photosynthetic activity of earlier geologic eras has endowed us with massive fuel deposits. The productive potential of this process has, until recent years, seemed endless. However, the burgeoning human population, with its constantly increasing demands on both present and past products of

photosynthetic activity, threatens our future survival. It is becoming more apparent that a basic understanding of the complexities of photosynthesis is necessary if we are to evaluate its true potential and wisely plan for utilization of this most fundamental metabolic activity of green plants (see Hollaender *et al.*, 1972). An understanding of this process may help us in improving its efficiency and in devising artificial photochemical systems based on it. Moreover, several basic biochemical and biophysical problems (such as mechanisms of excitation energy migration, electron transport, phosphorylation, oxidation of water molecules, and carbon fixation) can be studied with photosynthesizing organisms. The present chapter provides an introduction to the process of photosynthesis, and to the several chapters in this book dealing with the various aspects of the bioenergetics of photosynthesis.

In green plant photosynthesis, CO_2, H_2O, and light energy are the reactants, and O_2 and carbohydrates (CH_2O) are the products; this process takes place in cellular organelles called chloroplasts:

$$CO_2 + H_2O \xrightarrow[\text{chloroplasts}]{\text{light}} O_2 + (CH_2O) + 112 \text{ kcal/mole } CO_2 \qquad (1)$$

Pigment molecules, especially Chl *a*, various enzymes, and electron carriers act in a "catalytic" manner in this reaction. The overall energetics of this reaction may be looked upon in three ways. (1) In photosynthesis, a very stable arrangement of the atoms C, H, and O in CO_2 and H_2O is converted into a much less stable arrangement of the same nuclei and electrons in $(CH_2O) + O_2$; in order to drive this process the necessary energy is provided by light quanta. The total energy (ΔH) stored is 112 kcal/mole, while stored free energy (ΔF) is 120 kcal/mole, the 8 kcal/mole difference being due to the entropy term (ΔS). [$\Delta F = \Delta H - T\Delta S$; $+120 = +112 - (-8)$]. (2) A look at the total bond energy of the reactants and the products involved in the process shows a difference of ~ 105 kcal in going from H_2O and CO_2 to (CH_2O) and O_2:

$$H-O-H + O=C=O \xrightarrow{nh\nu} \begin{matrix} H \\ H \end{matrix}\!\!>\!C=O + O=O \qquad (2.1)$$

$$(2 \times 110 \text{ kcal}) \quad (2 \times 180 \text{ kcal}) \quad (2 \times 90 + 180 \text{ kcal}) \quad (115 \text{ kcal})$$

$$220 \text{ kcal} + 360 \text{ kcal} = 360 \text{ kcal} + 115 \text{ kcal} \qquad (+105 \text{ kcal})$$

This difference is supplied by the light energy ($nh\nu$). (3) Photosynthesis is usually considered as an oxidation–reduction reaction in which four electrons (or four H atoms) are transferred from $2H_2O$ to CO_2, oxidizing the former to O_2 and reducing the latter to (CH_2O) [see Eq. (2)]. The oxidation–reduction potential (E_0') of H_2O/O_2 couple, at pH 7.0 and under normal con-

ditions (1 atm pressure, room temperature), is +0.8 V (with respect to H/H^+ being +0.4 V), whereas that of $CO_2/(CH_2O)$ couple is −0.4 V. Thus the difference in potential (ΔE) is 1.2 V per electron transferred. Since four electrons must be transferred from $2H_2O$ to evolve one O_2 molecule and reduce one CO_2 molecule as:

$$2H_2O \xrightarrow{nh\nu} (4H) + O_2 \tag{2.2}$$

$$CO_2 + 4H \longrightarrow (CH_2O) + H_2O \tag{2.3}$$

the total energy needed is ~ 110 kcal/mole (as $\Delta F = n\mathcal{F}\Delta E = 4 \times 23$ kcal/mole·Volts × 1.2 V, where $\mathcal{F}$ = Faraday's constant).

Thus, the above three ways of looking at the overall energetics of photosynthesis give us approximately the same energy requirement. In terms of quanta of light needed, 120 kcal/mole means a minimum of 3 quanta of red light. However, as we shall discuss later, the mechanism of photosynthesis involves the use of 4 quanta for the accumulation of four positive equivalents (with the production of four reducing equivalents) which are needed for the evolution of one molecule of O_2 from two molecules of H_2O, and the use of another 4 quanta to transfer the four reducing equivalents to the intermediates needed for the reduction of CO_2. This is schematically shown below:

$$4(DA) \xrightarrow{4h\nu} 4(D^+A^-) \tag{3.1}$$

$$4D^+ + 2H_2O \longrightarrow 4D + O_2 + 4H^+ \tag{3.2}$$

$$4A^- + 4X \xrightarrow{4h\nu} 4A + 4X^- \tag{3.3}$$

$$4H^+ + 4X^- \longrightarrow 4XH \tag{3.4}$$

$$4XH + CO_2 \longrightarrow (CH_2O) + H_2O + 4X \tag{3.5}$$

where D = primary electron donor, A = primary electron acceptor, and X = a second primary electron acceptor. Thus, a *minimum* of 8 quanta are needed to evolve one O_2 and reduce one CO_2 molecule.

In addition to green plants, certain species of bacteria (e.g., green and purple) are capable of reducing CO_2 to (CH_2O). The photosynthetic bacteria differ from green plants in that they are incapable of oxidizing H_2O (perhaps due to the lack of enzymes involved in this process); instead, these organisms use substitute hydrogen (electron) donors (H_2A'). Consequently, no O_2 is evolved in bacterial photosynthesis. Van Niel (1935) proposed a general equation which describes both green plant and bacterial photosynthesis as:

$$H_2A' + CO_2 \xrightarrow[\substack{\text{Chl or}\\ \text{BChl}}]{h\nu} (CH_2O) + A' \tag{4}$$

In green plants $H_2A' = H_2O$ and thus $A' = \frac{1}{2}O_2$ (not to be confused with A in Eq. 3). Equation (4) implies that the source of O_2 in photosynthesis is H_2O. This was established by Hill (1939) who discovered that isolated chloroplasts can evolve O_2 when CO_2 is replaced in a reaction mixture by ferric oxalate and ferricyanide. Furthermore, experiments in which ^{18}O-enriched water (or $C^{18}O_2$) was used as substrate (Ruben *et al.*, 1941), or in which the ratio of $^{18}O/^{16}O$ was measured for normal H_2O, CO_2, and O_2 (Vinogradov and Teis, 1941), led to the conclusion that the isotopic composition of O_2 evolved in photosynthesis corresponds to that of H_2O, not of CO_2. These experiments clearly established that O_2 originates in H_2O.

The photosynthetic electron donor (H_2A') is different for the three major groups of photosynthetic bacteria: (1) Nonsulfur purple bacteria (Athiorhodaceae), e.g., *Rhodospirillum rubrum*; here, H_2A' is usually an organic compound. (2) Sulfur purple bacteria (Thiorhodaceae, e.g., *Chromatium* species); here, H_2A' is an inorganic sulfur compound. (3) Green sulfur bacteria (Chloroaceae, e.g., *Chlorobium thiosulfatophilum* and *Chloropseudomonas ethylicum*); these organisms can use either inorganic sulfur compounds or other organic hydrogen donors.

Unlike green plants, bacterial photosynthesis does not result in much energy storage. In fact, the oxidation of H_2S to S results in a net loss of 5 kcal/mole and the conversion of S to sulfate (SO_4^-) leads to a net storage of only 7 kcal/mole; in cases where molecular hydrogen is the donor, 25 kcal/mole are lost.

1.1. Time Sequence

Light absorption is the first act of photosynthesis occurring in 10^{-15} sec (billionth of a millionth of a second). Carbohydrate formation is the last step in photosynthesis; these enzymic reactions may take several seconds. Longer time scales are not unimportant, but they deal with regulation and growth mechanisms. Kamen (1963) has arbitrarily divided the various time scales into different eras.

1. Era of Radiation Physics: 10^{-15} to 10^{-6} sec, during which excitation processes, physical migration, and trapping of excitation energy occur (see Fig. 1):

$$Chl_g + h\nu \longrightarrow Chl_s^* \qquad \text{(Excitation)} \qquad (5.1)$$

$$Chl_s^* \longrightarrow Chl_T \qquad \text{(Intersystem crossing)} \qquad (5.2)$$

$$Chl_s^* + Chl \longrightarrow Chl + Chl_s^* \qquad \text{(Excitation energy transfer)} \qquad (5.3)$$

$$Chl_s^* \longrightarrow Chl_g + h\nu' \qquad \text{(Fluorescence)} \qquad (5.4)$$

$$Chl_s^* + T \longrightarrow T^* + Chl_g \qquad \text{(Trapping of energy)} \qquad (5.5)$$

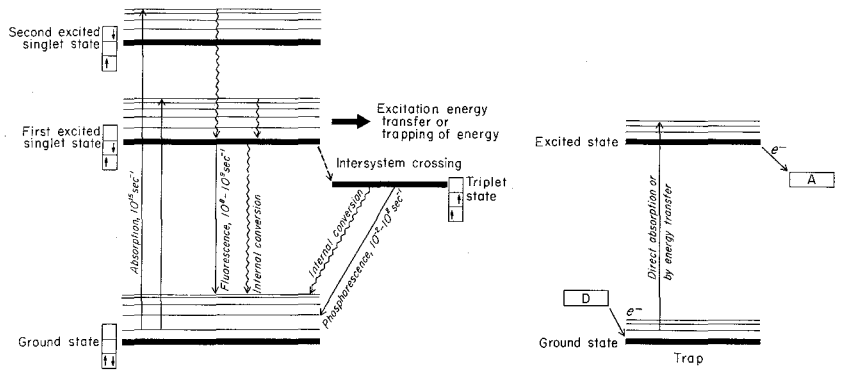

Fig. 1. Energy level diagram for "bulk" chlorophyll (left) and for the trap chlorophyll molecule (right). D: electron donor; A: electron acceptor. Small arrows in the rectangular blocks indicate spins of the electrons in the different levels.

where Chl_g = chlorophyll in ground state, Chl_s^* = chlorophyll in singlet excited state, Chl_T = chlorophyll triplet, and T = energy trap.

2. Era of Photochemistry: 10^{-10} to 10^{-3} sec in which some of the above occurs, as well as the separation of charges, or primary oxidation–reduction reactions. Three alternate ways of expressing the primary redox reaction are shown below:

$$D\cdot T\cdot {}^*A \longrightarrow \begin{cases} D^+\cdot T\cdot A^- & (6.1) \\ D\cdot T^+\cdot A^- \longrightarrow D^+\cdot T\cdot A^- & (6.2) \\ D^+\cdot T^-\cdot A \longrightarrow D^+\cdot T\cdot A^- & (6.3) \end{cases}$$

3. Era of Biochemistry: 10^{-4} to 10^{-2} sec, in which electron carriers and enzymes are involved, leading to oxygen evolution and CO_2 fixation:

$$D^+\cdot T\cdot A^- + \tfrac{1}{2}NADP^+ + H^+ \longrightarrow$$

$$D^+\cdot T\cdot A + \tfrac{1}{2}NADPH + \tfrac{1}{2}H^+ \quad \text{(Reduction of } NADP^+\text{)} \quad (7.1)$$

$$D^+\cdot T\cdot A\cdot + \tfrac{1}{2}H_2O \longrightarrow D\cdot T\cdot A\cdot + \tfrac{1}{4}O_2 + H^+ \quad \text{(Oxygen evolution)} \quad (7.2)$$

$$D^+\cdot T\cdot A^- + ADP + P_i \longrightarrow ATP + D\cdot T\cdot A \quad \text{(Cyclic photophosphorylation; some ATP may also be formed in (7.1) above)} \quad (7.3)$$

$$CO_2 + 2NADPH + 3ATP \longrightarrow (CH_2O) + 2NADP^+ + 3ADP + 3P_i \quad \text{(Carbon fixation)} \quad (7.4)$$

1.2. Membrane Structure and Function

It appears that a highly ordered chloroplast membrane structure is necessary for the separation of positive and negative charges and their stabilization. The structure must somehow prevent the oxidizing and reducing equivalents from recombining to a significant extent, since recombination would result in the loss of energy as heat or light:

$$D^+\cdot T\cdot A^- \longrightarrow D\cdot T\cdot A\cdot + \text{heat} \tag{8.1}$$

$$D^+\cdot T\cdot A^- \longrightarrow D\cdot T\cdot A + h\nu'' \text{ (delayed light emission)} \tag{8.2}$$

Thus, a detailed study of the structure and composition of the chloroplast membrane is necessary for the understanding of the mechanisms of energy coupling. Two approaches have been used to elucidate the structure: (1) a morphological approach using primarily the electron microscope; and (2) a physicochemical approach. The latter can be further subdivided into two approaches: (a) nondestructive—by the use of X-ray crystallography and physical probes (e.g., fluorescent probes), and (b) destructive—by chemical analysis of the various constituents. The relation of chloroplast structure to function, as determined by combined study of electron microscopy and biochemistry, is discussed in Chapter 2 of this volume.

For photophosphorylation to occur, certain particles (CF, ATPase) attached on the outer surface of the chloroplast membranes (thylakoids) are necessary. More recently, the role of membranes has played an important part in the discussion of the mechanism of phosphorylation since it is believed that utilization of a membrane potential and/or a H^+ gradient across the thylakoid membrane provides energy for photophosphorylation (see Chapters 9 and 10 of this volume).

The complete process of photosynthesis, *in vivo*, occurs within the organelle space bounded by the outer envelope of the chloroplasts. All enzymes participating in CO_2 fixation are localized either in the matrix (stroma) of the plastid or are loosely affixed to the lamellar membranes.

1.3. Photosynthetic Units

Emerson and Arnold (1932a), using repetitive bright, short (10^{-5} sec) flashes of light with varying dark periods between the flashes, observed that the number of oxygen molecules evolved per flash increased with increasing duration of dark periods and reached a saturation value at <0.4 sec in *Chlorella* cells at 1°C, and <0.04 sec at 25°C. The half-time was about 0.04 sec at 1°C, thus giving the time needed to complete the dark enzymic reactions of photosynthesis. Interestingly, they found that the reactions of

the carbon fixation cycle are inhibited almost 60% by 1.14×10^{-5} *M* KCN and about 50% by a decrease in bicarbonate ions from 71×10^{-6} to 4.1×10^{-6} moles/liter. However, KCN and bicarbonate ions affected the flash yields (O_2/flash) quite differently: KCN did not affect this yield, whereas a decrease in bicarbonate ions reduced it by 50%. These data suggest a role for bicarbonate ions other than that in the carbon fixation cycle. We shall mention this role later. Using *Chlorella* cells with varying concentrations of Chl, Emerson and Arnold (1932b) noted that the ratio of the maximum number of oxygen molecules evolved per flash to the number of Chl molecules present was 1/2500. Thus, they suggested that for every 2500 molecules of Chl, there is present one "unit" capable of reducing one molecule of CO_2 (or evolving one molecule of O_2, as they measured O_2, not CO_2) each time the unit is activated by light (Table I). In other words, these Chl molecules must "work together to effect the reduction of one CO_2 molecule" (see Emerson, 1937). The current definition of a photosynthetic unit capable of evolving one O_2 refers to the collection of 2500 Chl molecules with its reaction center(s).

The concept of a photosynthetic unit was supported by (1) the arguments of Gaffron and Wohl (1936a, b) (also see Arnold and Kohn, 1934) who calculated that if chloroplasts did not have such units, and if individual Chl molecules had to accumulate the necessary quanta of light to evolve O_2, there would be a lag in O_2 evolution after illumination. Such a lag does not occur, although a small one, seen only with very weak light, has been observed and is due to a different reason; see Chapter 8; (2) O_2 evolution and reduction of an artificial dye (the Hill reaction) requires a minimum size of chloroplast fragments containing hundreds of Chl molecules (Thomas *et al.*, 1953); (3) the discovery of the existence of reaction centers (see Section 2.4 and Chapter 10 of this volume).

The primary photoacts of photosynthesis lead to the transfer of four hydrogen atoms (or electrons) from two H_2O to CO_2 in order to evolve one O_2 molecule. Thus, the original figure of 2400 Chl molecules/O_2 for the PSU means that 600 Chl molecules interact in transferring one H atom. It is now generally believed that each H atom is transferred from H_2O to CO_2

TABLE I
Photosynthetic Unit in Chlorella

Basis	No. of Chl molecules
O_2 evolved	2400
(H) or e^- transferred	600
hv absorbed	300

in two successive photochemical steps (see Sections 1.5 and 3.8 and Chapter 7 of this volume); therefore there must be eight primary photochemical events. The photosynthetic unit size for each of these primary photoacts can be visualized as a group of 250–300 Chl molecules (Table I). Each light quantum absorbed by any Chl molecule of the unit must be transferred through the complex until it reaches the specialized Chl *a* molecules called energy traps or reaction centers. Once the excitation energy reaches the trap, the primary photoact facilitates the transfer of an H atom (or electron) from the donor to the acceptor molecule due to the proximity of the donor and the acceptor molecules to the trap (Fig. 2). The trap molecules first undergo light-induced oxidation and subsequent reduction in the dark, leading to changes in light absorption. Alternatively, trap molecules may undergo reduction in light and oxidation in dark. Since the total number of trap molecules is extremely small (less than 1 % of the total Chl), the magnitude of the light-induced absorption changes due to their redox reactions is too small to be detected by conventional spectrophotometers. However, it can be done by the use of sensitive difference spectrophotometers (see Section 2.4).

The size of a photosynthetic unit is not necessarily uniform for all plants. Schmid and Gaffron (1971) found that PSU varied between 300 and 5000 Chl/CO_2 fixed in various higher plants.

The size of the photosynthetic unit in photosynthetic bacteria is about six times smaller than in green plants. Arnold (cited by Van Niel, 1941) reported that the ratio of P_{max} (maximum number of CO_2 molecules fixed) to the number of BChl molecules present was 1/400. If we divide 400 by the number of quanta used, this number reduces to 50. This is in fair agreement with the ratio of the number of bulk BChl molecules to that of the reaction center (also see Clayton, 1965, p. 17).

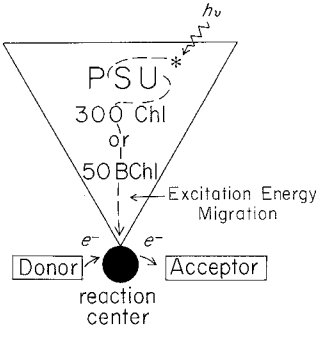

Fig. 2. Diagrammatic sketch of a photosynthetic unit (PSU). Chl: chlorophyll; BChl: bacteriochlorophyll; *hv*: light quantum.

1.4. Overall Quantum Yield; "Red Drop"

Quantum efficiency (the number of O_2 molecules evolved per quantum of light absorbed, or, conversely, the quantum requirement, i.e., the number of quanta required per O_2 evolved) of photosynthesis in green plants was a matter of great controversy between O. Warburg and R. Emerson for many years. Warburg reported values of the quantum requirement ranging from 2.8 to 4 quanta, whereas Emerson found it to be 8 or more quanta (see Emerson, 1958).

The quantum requirement of 4 (claimed by Warburg) does not provide sufficient energy for the hydrogen transfers and the stabilization of the various products. Our recent results (R. Govindjee *et al.*, 1968) confirm Emerson. In view of the present scheme of photosynthesis which involves two light reactions (see Section 1.5), the transfer of four hydrogens will require a minimum of 8 light quanta. A few more quanta may be needed to drive cyclic photophosphorylation. A quantum requirement of 10 would thus allow enough energy for the needed H transfer as well as the stabilization of the various products.

It has been shown that the energy absorbed by the various photosynthetic pigments is transferred to Chl *a*, ultimately reaching the reaction centers where it is converted into chemical energy. If all pigments are equally efficient in transferring energy to the reaction centers, the quantum yield of photosynthesis should remain constant as long as there is absorption by these pigments. Emerson and Lewis (1943) showed that a plot of the quantum yield of photosynthesis as a function of wavelength of light does not remain constant throughout the spectrum. There is a decline in the blue end (where absorption by carotenoids is high), and there is a drop in the red end of the spectrum, well within the red absorption band of Chl *a*. The decline of quantum yield in the red end of the spectrum has since been known as the "red drop" (Fig. 3). This can now be understood in terms of the two light reactions and the two pigment systems hypothesis—in the long-wavelength (red-drop) region, absorption by only one pigment system dominates and this is not enough for efficient photosynthesis (see below).

The quantum yield of bacterial photosynthesis (the number of CO_2 molecules fixed per quantum of light absorbed) is 0.12, the same as that in green plants. Experiments on the quantum yield of bacterial photosynthesis as a function of wavelength did not reveal any "far-red drop" equivalent to the red drop observed in green plants (unpublished experiments of the authors). This indicates the presence of one photosystem or two (or more) with overlapping absorption spectra. [For detailed discussions on photosynthetic bacteria, see Gest *et al.* (1963), Pfennig (1967) and Sybesma (1970).]

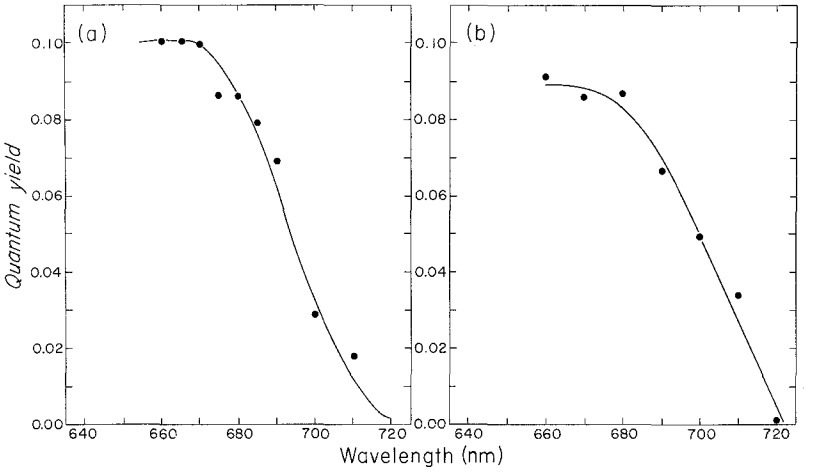

Fig. 3. Quantum yield of oxygen evolution as a function of wavelength. (a) *Navicula minima;* (b) *Chlorella pyrenoidosa.* (After Govindjee, 1960.)

1.5. Enhancement Effect; Two Pigment Systems and Two Light Reactions

Emerson *et al.* (1957) discovered that the "red drop" could be avoided, and the quantum yield increased in this region, if a supplementary light (absorbed by one of the accessory pigments, e.g., Chl *b* in green algae, phycobilins in the blue-green and red algae, and fucoxanthol in the brown algae) was added to the far-red light (absorbed by Chl *a*). This phenomenon has since been known as the "enhancement effect" (the Emerson effect, the Emerson enhancement effect, or the second Emerson effect; see Emerson and Rabinowitch, 1960).

A suggestion was made that simultaneous excitation of Chl *a* and the accessory pigments is necessary for high quantum yields, and Chl *a* alone was inefficient in photosynthesis (Emerson, 1958). In conflict with this idea was the data obtained from the Chl *a* fluorescence studies (Duysens, 1952), which showed that Chl *a* is the prime photochemical sensitizer and the accessory pigments were assigned an indirect role of energy collection and transfer to Chl *a*.

Emerson and Rabinowitch (1960) discarded the idea that Chl *b* directly sensitizes one light reaction. Instead, they suggested that two different forms of Chl *a* sensitize two different light reactions. Govindjee and Rabinowitch (1960a, b) and French *et al.* (1960) showed that the action spectra of the enhancement effect in various organisms, containing different accessory pigments, had peaks representing the effectiveness of the accessory pigment, but *in addition* there was a peak or shoulder around 670 nm which was

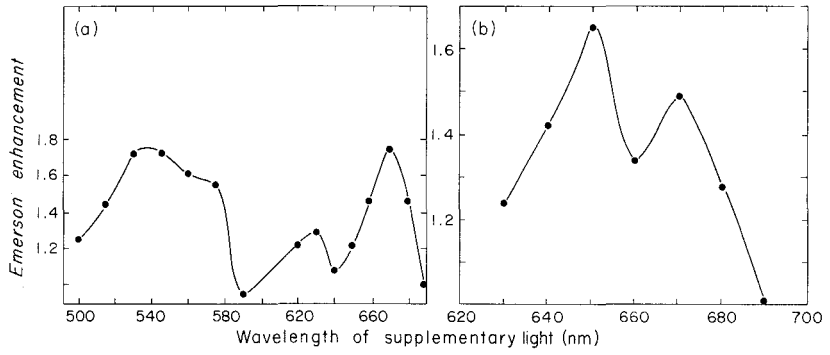

Fig. 4. Emerson enhancement (ratio of the rate of oxygen evolution in combined far-red and short-wavelength lights minus the rate in short-wavelength, and the rate in far-red light alone) as a function of short-wavelength light. (a) *Navicula minima.* (b) *Chlorella pyrenoidosa.* (After Govindjee, 1960.)

clearly in the region of Chl *a* absorption (Fig. 4). On the basis of this, the interpretation of the enhancement effect could be modified, and it was suggested that different forms of Chl *a* exist in both pigment systems. The shorter wavelength forms, e.g., Chl *a* 670 (absorbing around 670), are present in the system containing most of the accessory pigments. Duysen's fluorescence data refer mostly to these forms. The longer wavelength forms (absorbing at wavelengths > 685 nm) are present only in the other system (see review by Govindjee *et al.*, 1973). When only long-wavelength forms are excited, only one pigment system is excited and photosynthesis is low since two photosystems are required. When short-wavelength forms are excited, both pigment systems are excited, and, photosynthesis takes place normally. Excitation with short- and long-wavelength lights leads to a more balanced excitation of the two pigment systems, and photosynthesis is more efficient (enhancement effect).

It was clearly shown by Myers and French (1960a) that the synergistic or the enhancement effect in the quantum yield of photosynthesis could be brought about even when the supplementary light and the far-red light were not given simultaneously, but a few seconds apart. This implied that the product of one light reaction could exist for a few seconds and still be effective in enhancing the rate of another reaction. (See Govindjee, 1963; Fork, 1963; Myers, 1971 for further information on the enhancement effect.)

Another phenomenon related to the two "light effect" hypothesis was the discovery by Blinks (1957) of "chromatic transients" in the photosynthesis of the red alga *Porphyra perforata.* He found that when this alga was exposed to alternating red (absorbed by Chl *a*) and green (absorbed by phycoerythrin; see Section 2.1) light of equal steady-state effectiveness,

the rates of O_2 "evolution" were not constant during the transition period: upon going from red to green light, an O_2 gush was observed followed by a decrease and then recovery to steady state, whereas in the transition from green to red light, no O_2 gush was observed, but a decrease in rate and then a recovery. At that time, the reason for this phenomenon was not clear. Blinks proposed two possible explanations: (1) inactivation of some of the Chl to which energy must be transferred from phycoerythrin, and its recovery; (2) altered respiration rates because the platinum electrodes used could not distinguish between light-induced O_2 evolution and uptake. He (1959, 1960) later favored the second explanation. However, Myers and French (1960b) argued against the Blinks hypothesis and suggested that the chromatic transient is closely geared to the photosynthetic sequence. The Blinks effect could, therefore, be another consequence of the two pigment systems—two light reactions hypothesis.

Hill and Bendall (1960) suggested a scheme for photosynthesis which in a highly modified form is now popularly known as the Z scheme. In the original scheme the concept of two light reactions operating in series was proposed, with cytochromes acting as intermediate electron carriers. (Independently, Kautsky *et al.* (1960) had also suggested the existence of two light reactions to explain the kinetics of Chl *a* fluorescence.) The concept of two pigment systems, based on enhancement and other effects, was added later to this scheme. Since cytochromes are thought to be a part of the intersystem chain, one would predict that the cytochromes should be oxidized by one photosystem and reduced by the other. Duysens *et al.* (1961; also see Duysens and Amesz, 1962) indeed showed that, in red algae, the red light (absorbed by Chl *a*) caused an oxidation of Cyt f, and green light (absorbed by phycobilins) caused a reduction of Cyt f. A study of the action spectra of the oxidation and reduction of cytochromes gave an idea of the composition of the two photosystems (see Duysens and Amesz, 1962), since the action spectrum of a photochemical reaction follows the percent absorption spectrum of the active pigment(s).

It has been suggested that for green plants Chl *a* is present in both the systems, the long-wavelength forms of Chl *a* (Chl *a* 680 and longer wavelength forms) being predominant in system I (PS I), which is weakly fluorescent at room temperature. PS I is believed to have a smaller proportion of the accessory pigments and is responsible for the oxidation of cytochromes. The other photosystem (PS II) contains relatively more of short-wavelength forms of Chl *a* (Chl *a* 670) and of the accessory pigments; PS II is believed to be responsible for the photoreduction of cytochromes. (In red and blue-green algae, a larger proportion of Chl *a* is present in PS I.)

After the initial characteristics of the two photosystems were defined, a great deal of evidence was presented confirming their existence, and

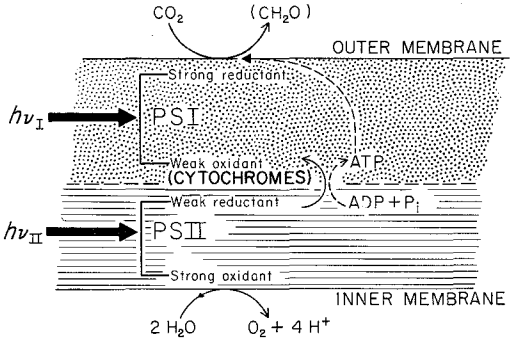

Fig. 5. Diagrammatic sketch of a portion of the thylakoid membrane of grana from higher plants. Pigment system I (PS I), upon light ($h\nu_I$) absorption produces a strong reductant and a weak oxidant. Pigment system II (PS II), upon light ($h\nu_{II}$) absorption, produces a strong oxidant and a weak reductant. Electron flow from the weak reductant to the weak oxidant is coupled to phosphorylation [conversion of adenosine diphosphate (ADP) and inorganic phosphate (P_i) to adenosine triphosphate (ATP)]. With the aid of ATP, the strong reductant (produced by PS I) reduces carbon dioxide to carbohydrate (CH_2O) in the stroma. The strong oxidant (produced by PS II) oxidizes water molecules to molecular oxygen, and H^+ ions are released on the inner membrane.

information was provided for a more detailed picture (see Section 3.1 and reviews by Duysens, 1964; Franck and Rosenberg, 1964; Robinson, 1964; Vernon and Avron, 1965; Witt, 1967; Hind and Olson, 1968; Fork and Amesz, 1969; Boardman, 1970; Goedheer, 1972).

Studies of the two photosystems have led to the hypothesis that PS I produces a strong reductant (for reducing CO_2) and a weak oxidant, and PS II is responsible for the formation of a weak reductant and a strong oxidant (responsible for the oxidation of H_2O to molecular O_2). The transfer of electrons from the weak reductant (produced by PS II) to the weak oxidant (produced by PS I) is coupled to the production of ATP (Fig. 5). As discussed by Arntzen and Briantais (Chapter 2, this volume), the PS I is, perhaps, on the outer side of the thylakoid membrane, and PS II on the inner side. [In red and blue-green algae, however, phycobilins (part of PS II) are contained in "phycobilisomes" that are attached to (or partially embedded in) the outer surface of thylakoids. Thus, the location of PS I and PS II is not yet clear in these algae.]

In photosynthetic bacteria, the Emerson enhancement effect has not been observed (Blinks and Van Niel, 1963). Thus, the existence of two pigment systems in these organisms cannot be supported from this kind of data

1.6. Overall Energetics

Duysens (1958) calculated the maximum efficiency of photosynthesis by applying the second law of thermodynamics. At low light intensities, where the

efficiency is low, maximum efficiency was calculated to be about 70% in green plants. Ross and Calvin (1967) calculated the maximum amount of free energy stored, based on detailed thermodynamic arguments, to be 1.19 and 1.23 eV per photon absorbed by PS I and PS II, respectively, under an illumination of 1 klx of white light. This gives a maximum energy efficiency of about 66–68%, in agreement with Duysen's value. These values place an upper limit on the photosynthetic efficiency. For PS I, a ΔE of 1.0 eV is estimated for oxidizing P700 and reducing "X." If only 1.19 eV of free energy is available, we have only 0.2 eV of extra energy for any other use. The same may be true for PS II. Thus, from this point of view, electron transfer from H_2O to "X," which is estimated to need about 1.6 eV (assuming the Z/Z^+ couple to have an E_0' of + 1.0 V, 0.2 V more positive than the H_2O/O_2 couple, and X/X^- to be about −0.6V), cannot occur with 1 quantum. This is perhaps why plants had to resort to the use of 2 quanta mechanisms.

2. PRIMARY EVENTS

2.1. Light Absorption

The various photosynthetic pigments, mentioned in Section 1, can be classified into three main groups: chlorophylls, carotenoids, and phycobilins. The function of these pigments is to provide the plants with an efficient system of absorbing light throughout the visible spectrum (see reviews by Rabinowitch and Govindjee, 1969; Govindjee and Mohanty, 1972; Govindjee and Braun, 1973). This energy is then transferred to the reaction centers, where it is utilized for the photochemical reactions (see Chapter 3 of this volume). The bulk of the pigments involved in the process of light absorption (and energy transfer) are called the light-harvesting pigments.

2.1.1. Chlorophylls

There are two kinds of Chl in higher plants and green algae: Chl *a* and Chl *b*. These are soluble in organic solvents. Chl *a* is the major pigment and is present in all photosynthetic organisms that evolve O_2 (bacteria do not have Chl *a* and are incapable of evolving O_2). Several forms of Chl *a* have been postulated: Chl *a*660, Chl *a*670, Chl *a*680, Chl *a*685, Chl *a*690, and Chl *a*700–720, the number indicating their respective red absorption maxima (see French, 1971; Fig. 6; and Chapter 12 of this volume). Evidence for the existence of the various forms comes from derivative spectrophotometry, low-temperature absorption measurements, and the action spectra of various photochemical reactions. The short-wavelength Chl *a* forms are fluorescent and are predominantly present in the PS II. The long-wavelength forms are weakly fluorescent and are predominantly present in PS I.

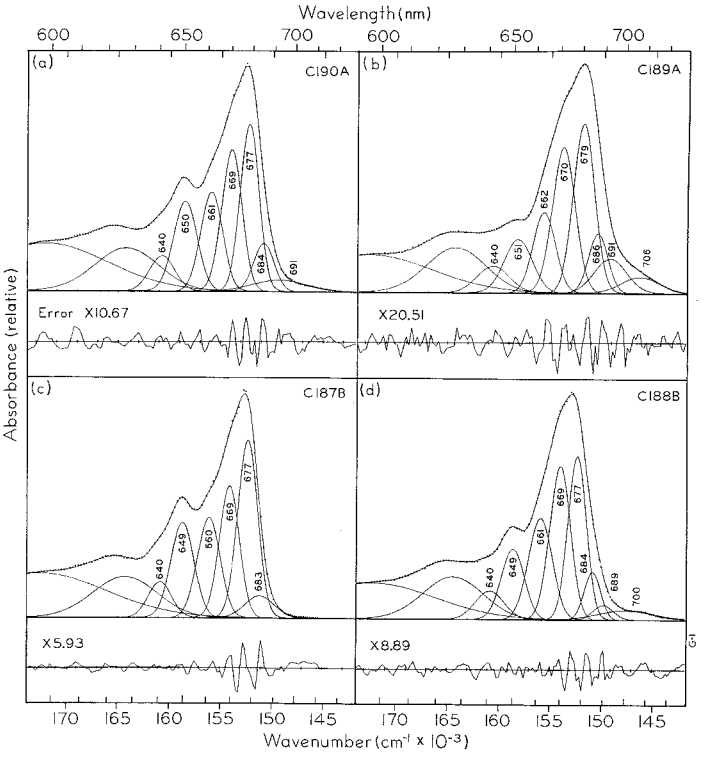

Fig. 6. Curve analyses of the absorption spectra of chloroplast fractions from stroma and grana lamellae measured at −196°C. (a) P-10K: this fraction consists of grana membranes (both pigment systems I and II); (b) P-144K: stroma lamellae containing pigment system I only; (c) F-2D: pigment system II separated from grana; (d) F-1D: pigment system I separated from grana. Note that F-2D (c) does not contain Chl *a* 689–693 and Chl *a* 700–706; the other Chl *a* and Chl *b* forms are present in all samples, although in slightly different proportions. (After R. A. Gasanov and C. S. French, personal communication, 1973.)

Chl *b* is present in all the higher plants and green algae. Its red absorption maximum occurs at 650 nm. Recently it has been shown that there are two forms of Chl *b*—Chl *b*640 and Chl *b*650. The major proportion of Chl *b* is present in PS II.

Apart from the above-listed forms of Chl, there exists a compound related to Chl, labeled Chl *c* in brown algae. The existence of yet another Chl called Chl *d* is doubtful. Its presence has been suggested in some red algae, but never proven to exist *in vivo*.

Chlorophylls *in vivo* are noncovalently bound to protein; upon treatment with organic solvents, the weak interaction is eliminated and the absorption maximum shifts to shorter wavelengths (e.g., from 675 to 660 nm for Chl *a*). (For a review of all aspects of chlorophylls, see Vernon and Seely, 1966.)

2.1.2. Carotenoids

These are the yellow and orange pigments found in almost all the photosynthetic organisms. They are soluble in organic solvents. There are two kinds of carotenoids: (1) carotenes, of which β-carotene is the most common one, are hydrocarbons; they absorb blue light; (2) carotenols are alcohols; these are commonly called xanthophylls. One of the xanthophylls (fucoxanthol), present in diatoms and other brown algae, is thought to be bound to protein *in vivo*. It is generally accepted that most of carotenes are present in PS I and xanthophylls in PS II.

2.1.3. Phycobilins

These water-soluble pigments, present in red and blue-green algae, are open-chain tetrapyrroles. There are two kinds of phycobilins: (1) phycocyanins, which predominate in the blue-green algae and account for the absorption maxima around 630 nm; (2) phycoerythrins, which predominate in the red algae, and absorb around 540 nm (see O'hEocha, 1971). Phycobilins are mainly associated with PS II, but they are present in PS I as well.

A working model for the composition of the two pigment systems in green plants is shown in Fig. 7.

2.1.4. Bacteriochlorophyll

The main light-harvesting pigment of photosynthetic bacteria is BChl—it is a tetrahydroporphyrin. BChl *a*, in organic solvents, has absorption maxima at 360, 590, and 760 nm. *In vivo*, the absorption is red-shifted and often has two or more maxima. For example, the purple bacterium *Chromatium* has absorption peaks at 800, 850, and 870 nm due to bulk BChl *a*. The green bacteria contain *Chlorobium* Chl, of which there are two kinds, with absorption at 725 and 750 nm; in addition they contain a small amount of BChl *a* absorbing at 810 nm (see Olson and Stanton, 1966). Some bacteria, e.g., *Rhodopseudomonas viridis*, contain BChl *b*; in these, the long-wavelength absorption maximum lies at 1025 nm.

2.2. Light Emission

2.2.1. Fluorescence

The Chl *a* molecule, upon excitation, by direct light absorption, or through the transfer of excitation energy absorbed in any accessory pigment, goes into the first excited singlet state. The potential energy of the molecule in the excited state can be dissipated in several ways (see Fig. 1). The molecule can return to its ground state through "internal conversion" with loss of the energy as heat; or it can transfer its energy to another molecule until the energy finally reaches the reaction center where it is converted into the

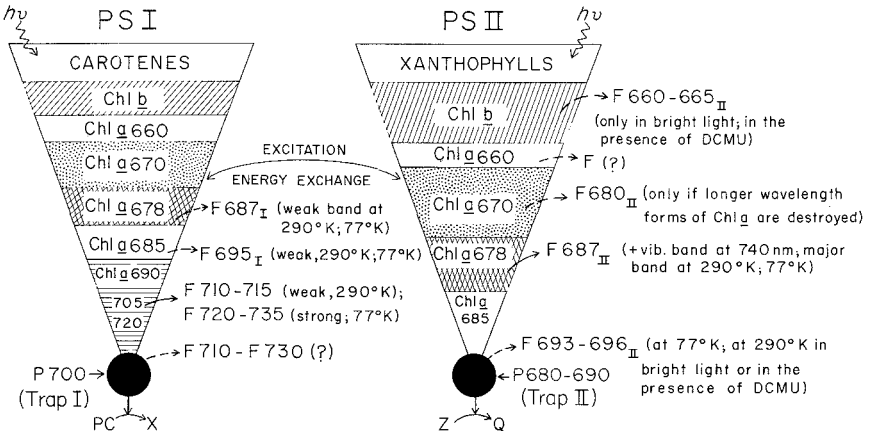

Fig. 7. A working hypothesis for the approximate distribution of the various pigments in the two pigment systems in green plants. PS I: pigment system I; PS II: pigment system II; Chl *b*: chlorophyll *b*; Chl *a*: chlorophyll *a*; the numbers indicate the approximate absorption maxima in the red end of the spectrum. The symbol F followed by numbers refers to the suggested fluorescence emission bands in nm. The conditions under which these bands are observed are listed within parentheses. Z: "primary" electron donor of PS II; Q: primary electron acceptor of PS II; PC: possible electron donor of PS I; X: primary electron acceptor of PS I; P680–P690: energy trap of PS II; P700: energy trap of PS I. DCMU: 3-(3, 4-dichlorophenyl)-1,1-dimethylurea. As shown in Fig. 5, the PS I is assumed to be located on the outerside of the thylakoid membrane and the PS II on the inner side such that excitation energy transfer between them is possible. (We cannot, however, discount the possibility that the two systems may be side by side on the thylakoid membrane.) In diatoms and brown algae, Chl *c* replaces Chl *b*. In red and blue-green algae, phycobilins replace Chl *b*; a large proportion of all Chl *a* forms is in PS I, and a large proportion of phycobilins is in PS II. Phycobilins are located in phycobilisomes; the physical arrangement of pigment systems in these algae is not yet clear.

chemical energy of the reaction products; alternately, the molecule can return to the ground state with the release of a quantum of light. This last process is called fluorescence (see Chapters 5 and 6 of this volume). In solution, Chl has a very high fluorescence yield (about 30%), but *in vivo* the yield is very low (about 3%), as most of the energy is used for photochemistry. The emission spectrum of Chl *a* fluorescence *in vivo* has a main band at 685 nm and a minor band at 740 nm (see Chapter 6). The excitation spectra for the various fluorescence bands indicate that most of the fluorescence at room temperature has its origin in PS II, but fluorescence in the 710–715 nm region originates also from PS I (see Govindjee *et al.*, 1973).

Most of the photosynthetic pigments (with the exception of the carotenoids) are known to fluoresce in solution. *In vivo*, however, Chl *a* fluorescence is dominant because the rest of the pigments transfer their absorbed energy to Chl *a* with a relatively high efficiency. The energy then migrates among Chl *a* molecules until it finally reaches the reaction center (for a theoretical dis-

cussion of energy migration, see Chapter 4 of this volume). Fluorescence from the trap molecules of green plants has not been measured directly, but has been inferred (Krey and Govindjee, 1964, 1966; Govindjee and Yang, 1966; Cho and Govindjee, 1970a,b) from measurements under the conditions when photochemistry is saturated (at high light intensities) or is stopped (at very low temperatures—liquid nitrogen or helium). Under such conditions, the quanta arriving at the reaction site may be given off as fluorescence or transferred back to the bulk pigments, and emitted from there. A fluorescence band at 693–696 nm has been attributed mainly to the reaction center Chl a_{II}. The correlation of various absorption bands with different fluorescence bands is discussed in Chapter 6 of this volume (also see Fig. 7).

The fluorescent property of PS II has been used extensively in studying the reactions of this photosystem. When light is turned on, Chl *a* fluorescence undergoes transient changes with time (see review by Govindjee and Papageorgiou, 1971). These changes have been divided into two types: the fast changes that are completed within a few seconds, and the slow changes that last several minutes. The fast changes are related to the initial photochemical events of PS II. Thus, the rise of fluorescence from a low to a high level (also known as the variable fluorescence, as opposed to the fixed or constant fluorescence observed immediately upon the onset of illumination) is suggested to indicate the reduction of a "primary" electron acceptor Q (the quencher of fluorescence, Duysens and Sweers, 1963). As Q^-* accumulates, the light quanta arriving at the reaction center can no longer drive photochemistry, and are more likely to be given off as fluorescence. When Q^- can be reoxidized by a pool of secondary acceptors or by back-reaction of PS II, fluorescence does not reach a maximum level. Ferricyanide, which can reoxidize Q^-, also lowers the fluorescence level (Malkin and Kok, 1966). When DCMU, which blocks the reoxidation of Q^- by the pool of secondary acceptors, is added, the fluorescence rises quickly to a high level and stays at that level.

Reduction of Q to Q^- occurs at 77°K as shown by the fluorescence rise at that temperature; however, this transient only partially recovers in the dark;

*Recently, Mauzerall (1972), using nanosecond laser flashes, has shown that Q is reduced too slowly (within microseconds) to be able to compete with singlet Chl_S^*, which has a lifetime of about 1 nsec; thus, Q cannot act as the primary electron acceptor of PS II. Butler (1972a) recently suggested from his data at 77°K, that this fluorescence can be governed by the "Z" (the primary electron donor) side of PS II as well. He observed that the fluorescence rise does not parallel the reduction of the primary acceptor of PS II (a component labeled C550; see discussion in Chapters 3 and 6 of this volume), but rather the oxidation of an electron donor, a high potential form of a cytochrome, labeled Cyt b559, which feeds electrons to Z when it is in the reduced state. From this it is suggested that the fluorescence rise is associated with the formation of ZQ^- rather than with Z^+Q^- (if Q can be considered as equivalent to C550). (It remains to be seen whether the same interpretation holds at room temperature where H_2O replaces Cyt b559!) Wraight and Clayton (verbal communication, 1973) have concluded that in bacterial systems, fluorescence should be taken as an indicator of the state of the reaction centers, but not as a quantitative component of a simple theory.

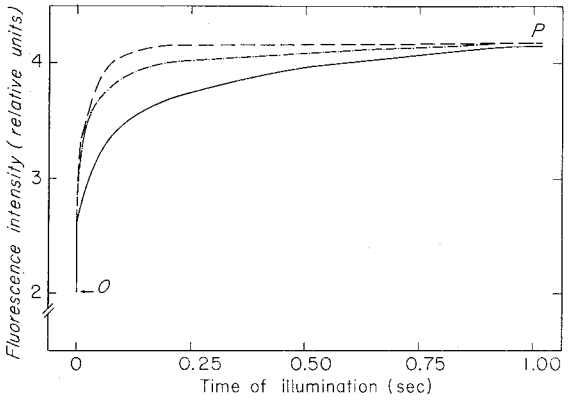

Fig. 8. Fluorescence intensity as a function of time of illumination at 77°K in isolated spinach chloroplasts. (—) first illumination; (– –) second illumination after 5 min darkness at 77°K; (– .) third illumination after 30–60 min darkness at 77°K. Actinic illumination: blue light; filters: C.S. 4-96 and C.S. 3-73; measuring wavelength: 685 nm (half-bandwidth, 6.6 nm). O: constant fluorescence; P: maximum fluorescence. (Unpublished experiments of Govindjee and Barbara Zilinskas Braun, 1972.)

a part of the change is irreversible (Fig. 8). It appears that at 77°K a portion of the primary back-reaction is blocked. Perhaps there are two pathways for the recovery of the primary reactants, one temperature-independent involving a direct reaction of Q^- with Z^+ (the oxidized form of the primary electron donor of PS II, labeled Z) within the reaction center II complex, and the other temperature-dependent via another intermediate.

Slow Chl *a* fluorescence yield changes and those changes which occur in the presence of DCMU may be related to changes in the conformation of the thylakoid membranes that often lead to changes in the "spillover" of excitation energy between PS I and II. These possibilities are discussed by Papageorgiou (Chapter 6 of this volume) and Murakami *et al.* (Chapter 11).

Photosynthetic bacteria also show transient changes in BChl fluorescence. The interpretation of the variable fluorescence in these organisms is, perhaps, the same as that given for the variable fluorescence in green plants, i.e. it represents the reduction of the primary electron acceptor(s). [For details, see Vredenberg and Duysens (1963), Clayton (1966), de Klerk *et al.* (1969), Suzuki and Takamiya (1972), and Malkin and Silberstein (1972).] In photosynthetic bacteria, fluorescence from the reaction center BChl was demonstrated by Zankel *et al.* (1968). An example of fluorescence from a reaction center preparation of *R. rubrum* is shown in Fig. 9.

2.2.2. Delayed Light Emission

Another phenomenon of light emission has been observed in photosynthetic organisms, lasting up to several minutes after the exciting light has

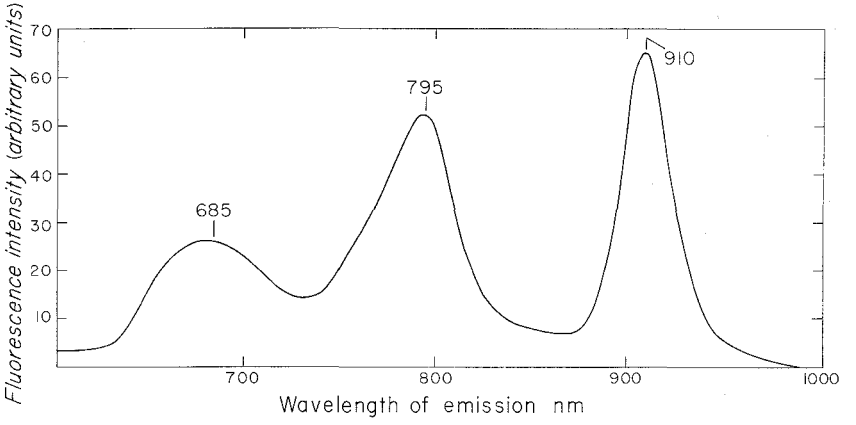

Fig. 9. Fluorescence emission spectrum of dithionite-reduced "large" reaction center preparation from *Rhodospirillum rubrum* (see W. R. Smith, 1972 for the method of preparation) at 77°K. Actinic illumination: broad-band blue light, 10^4 ergs cm^{-2} sec^{-1}; measuring conditions: C. S. 2-58 filter; halfband width, 6.6 nm; photomultiplier, RCA 7102. The peaks at 910, 795, and 685 nm are due to reaction center bacteriochlorophyll, bacteriopheophytin, and solubilized bacteriochlorophyll (?), respectively. (After Govindjee and W. Smith, unpublished observations, 1972.)

been turned off (Strehler and Arnold, 1951). This is known by various names: delayed light, afterglow, luminescence, and delayed fluorescence. The origin of part of the delayed light in green plants has been attributed to a back-reaction between the reduced primary electron acceptor (Q^-) and the oxidized primary electron donor (Z^+, or more oxidized forms of it). Any condition that favors the recombination of Q^- and Z^+ also enhances the delayed-light emission. For a detailed discussion see Chapter 5 of this volume.

2.3. Energy Transfer and Migration

As discussed earlier, the existence of photosynthetic units allows a more efficient use of the absorbed energy. This involves excitation energy migration through a maze of several hundred Chl *a* molecules, until the energy finally reaches the reaction center where it is converted into chemical energy. The process of excitation energy migration through the same kind of molecules involves *homogeneous* energy transfers. In addition, the energy absorbed by pigments other than Chl *a* in any photosystem is also transferred to Chl *a*. This kind of excitation energy transfer between different kinds of pigments is called *heterogenous* energy transfer.

Evidence for the existence of heterogeneous energy transfer comes from fluorescence measurements. Fluorescence bands ascribable to Chl *a* (acceptor) are observed, even when the excitation has been in a spectral region where Chl *a* has relatively very low absorption and where other pigments (donors:

Chl *b*, phycobilins, fucoxanthol, etc.) absorb more strongly. Also, the excitation spectrum (or the action spectrum) of Chl *a* fluorescence should theoretically follow the percent absorption spectrum of Chl *a* if there is no heterogeneous transfer; however, it remains high even in the regions of low Chl *a* absorption. This is particularly striking in the blue-green and red algae, where the action spectrum of Chl *a* fluorescence is very high in the regions of phycocyanin and phycoerythrin absorption. The efficiency of excitation energy transfer from Chl *b* to Chl *a* has been calculated to be almost 100% (Duysens, 1952; Cho and Govindjee, 1970*a*). (Under certain conditions, a small fluorescence from Chl *b* may be observed indicating a less than 100% transfer from Chl *b* to Chl *a*; see Govindjee and Briantais, 1972.)

The efficiency of excitation energy transfer from carotenoids to Chl *a* is poor (10–50% depending upon the organism), except for fucoxanthol which transfers with ~ 70% efficiency (Dutton *et al.*, 1943; Duysens, 1952; Goedheer, 1972). Phycobilins are capable of transferring the absorbed energy to Chl *a* with a very high efficiency (80–90%) (French and Young, 1952; Duysens, 1952; also see Govindjee and Mohanty, 1972) (see Fig. 10).

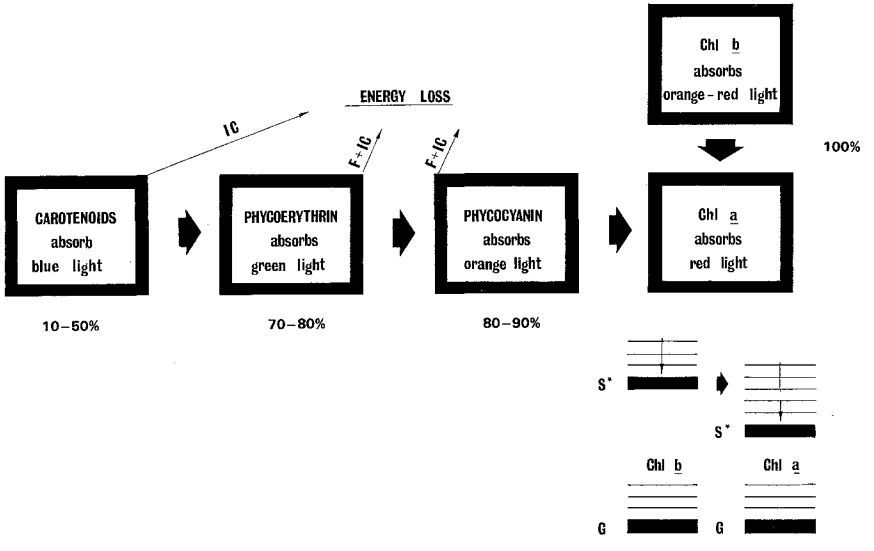

Fig. 10. Diagrammatic scheme for excitation energy transfer. F : fluorescence; IC : internal conversion; Chl *b*: chlorophyll *b*: Chl *a*: chlorophyll *a*; G: ground state; S*: excited singlet state. The numbers, in percent, refer to the efficiency of energy transfer from various pigments to Chl *a*.

Considerable overlap of the absorption band of the acceptor molecule, in this case Chl *a*, and the fluorescence band of the donor molecules (Chl *b* or phycoerythrin and phycocyanin), the appropriate orientation and the close distance of these pigments are the important conditions for the high efficiency of energy transfer. The time lag between the emission of Chl *a* fluorescence and the excitation of phycobilins is of the order of 0.3–0.5 nsec, whereas the lifetimes of phycoerythin and phycocyanin fluorescence are 7 and 1.8 nsec, respectively (Brody and Rabinowitch, 1957; Tomita and Rabinowitch, 1962). Thus, the energy transfer from the various pigments to Chl *a* can compete very effectively with the natural fluorescence of the different pigments *in vivo*. Hammond (1973) has recently measured a transfer time of 21 psec (upper limit) from Chl *b* to Chl *a* in the green alga *Chlorella*. [For literature on lifetime of excited states in green plants, see Mar *et al.* (1972), and Briantais *et al.* (1972).]

Evidence for homogeneous energy transfer (migration) comes from the fluorescence studies using polarized excitation light. Since the lifetime of the excited singlet state of Chl *a* is short, the fluorescence emission from Chl excited with polarized light would be highly polarized if it were from the same Chl *a* molecule that absorbed the light. However, experimental measurements show that the fluorescence is almost completely depolarized *in vivo* [Arnold and Meek (1956); for a recent measurement, see Mar and Govindjee (1972a)]. This could indicate that between light absorption and fluorescence emission a quantum is transferred through several molecules, each having a slightly different orientation, thus resulting in a depolarization of the fluorescence. However, most of the depolarization of fluorescence may occur in the first transfer. It may also be pointed out here that if the pigment molecules are highly aligned, information on energy migration cannot be obtained by measuring polarization of fluorescence.

The absorbed energy can migrate from one pigment molecule to another as excitation energy. The excitation energy transfer can take place by the process of exciton migration (see Chapter 4 of this volume). An exciton consists of an "excited" electron and a positive charge or a hole (left by the transfer of the electron to the excited state), the pair moving *together* from one molecule to another. The process of exciton migration involves no charge separation. Alternatively, migration of energy could be similar to that which exists in metals. In this process, electrons and holes can migrate freely (independently of each other) through conductance bands (see Arnold and Azzi, 1968). In this theory, migration of electrons and holes can occur at the same time in opposite directions in the chloroplast membrane. The electrons are finally trapped in an "electron trap," where they cause a reduction and "holes" in a "hole trap" where they cause an oxidation. As stated earlier, this in essence is the goal of the primary photochemical process, i.e., production of an

oxidant and a reductant that would not recombine with each other. However, there is no definite evidence to prove the existence of extensive electron-hole migration in the photosynthetic unit.

Two hypotheses have been proposed for the interaction between the two pigment systems mentioned in Section 1.5. (1) In the *separate package* model, PS I and PS II are physically isolated from each other; there is no exchange of excitation energy between the two systems. (2) In the *spillover* model, light energy absorbed by PS II (and not used by it) spills over to PS I, but not vice versa (see Myers, 1963). More recently a dynamic picture has arisen in which spillover from PS II to PS I is controlled by light and/or concentration of salts (e.g., Mg^{2+}; see discussion by Mohanty *et al.*, 1973, and Chapters 6 and 11 of this volume). Here also there are two different schemes. In the first, Mg^{2+}, Ca^{2+} etc. cause a decrease in the "spillover" of energy from PS II to PS I (Murata, 1969). In the second scheme, these divalent ions cause an increase in the "spillover" of evergy from PS I to PS II (Sun and Sauer, 1971). Present experimental data do not allow us to choose among the two pictures.

There are also two models for excitation energy transfer among units of the same photosystem: the *lake model* and the *puddle model.* In the *lake* model, reaction centers are embedded in a statistical fashion in a lake of bulk pigments; if one reaction center is closed, excitation energy can migrate to another one. In the puddle model, each unit is isolated; there is no excitation energy exchange among the different units (see Robinson, 1967). Perhaps the truth lies somewhere in between (see discussion of a "connected model" by Lavorel and Joliot, 1972).

In photosynthetic bacteria, the evidence for excitation energy transfer is the fact that excitation of carotenoids or BChl in any of the absorption bands results in the fluorescence of the component with its absorption band toward the longest wavelength, i.e., one representing the lowest electronic level (see Olson and Stanton, 1966). However, *Rhodopseudomonas viridis* has the ability for "uphill" energy transfer from BChl *b* (at 1025 nm) to the reaction center P985 (Zankel and Clayton, 1969). (Hammond, 1973, has also recently shown the existence of uphill energy transfer from Chl *a* to phycocyanin in blue-green algae.)

2.4. Reactions at Reaction Centers

Once the energy, by whatever mechanism, reaches the reaction center it is converted into chemical energy with the production of an oxidizing and a reducing equivalent. First, the reaction center Chl (or BChl) goes into the first excited singlet state. Immediately following this event, the primary electron acceptor is reduced and the reaction center becomes oxidized, which in turn receives an electron from the primary electron donor. Thus, the reaction

center is returned to its original state, but the primary electron donor and acceptor are oxidized and reduced, respectively. For a review of primary processes in bacterial photosynthesis, see Clayton (1973).

Duysens *et al.* (1956), using difference spectrophotometry, first reported a light-induced reversible change in absorption of BChl. This change has been attributed to the oxidation of reaction center BChl, since a similar decrease in absorption could be brought about by oxidizing chemicals. The quantum yield of BChl oxidation is high (close to 1.0), indicating that it plays an important role in photosynthesis.

Since this initial discovery, several investigators have looked at the light-minus-dark difference spectra in several bacteria. Parson (1968), using nanosecond laser flashes, showed that the primary photochemical reaction in bacteria was the oxidation of reaction center BChl, with a simultaneous reduction of a primary electron acceptor. It has been shown that the bleaching at 860–870 nm (representing the oxidation of the reaction center BChl) is always accompanied by a blue shift of an absorption band at 800 nm, also due to a BChl. This blue shift in the absorption band accounts for the increase in absorption at 795 nm and a decrease at 810 nm in the difference absorption spectrum (see Chapter 3 of this volume).

Recently, several investigators have isolated pigment–protein complexes from different bacterial species which are free of the bulk of light-harvesting pigments. The absorption spectra of these reaction center preparations have bands at 800 nm and at 870 nm in the near-infrared region. Upon excitation with light, the band at 870 nm bleaches totally, while that at 800 nm shifts to the blue. A detailed discussion of reaction centers is given in Chapter 3 of this volume.

Kok (1956) discovered a light-induced absorbance change at 700 nm in green plants, which he attributed to the redox reactions of Chl *a* molecules (P700), representing the energy trap or the reaction center. Subsequently, he was able to measure the corresponding absorption change in the Soret region at 433 nm, and to prove that the decrease in absorbance at 700 nm was due to oxidation of Chl *a* (Kok, 1961). Since there are two pigment systems (I and II) in green plants, we would also expect two separate energy traps. P700 has been shown to be the reaction center or the energy trap for PS I. The absorbance changes representing the energy trap of PS II remained undetected for a long time despite the efforts of several investigators. Döring *et al.* (1967, 1968, 1969) and Döring and Witt (1972) have reported an absorbance change at 435 nm and 682–690 nm which they attributed to the energy trap of PS II. The 682 nm absorption change is distinct from that of P700 because its half-life is 100 times shorter than that of P700. In addition, the change is present in chloroplast particles enriched in PS II, but not in PS I-enriched particles. (The presence of P680–690 in algae has been inferred from the

appearance of a fluorescence band at 695 nm under conditions when photosynthesis is saturated, or blocked by poisons.) The P680 change can be observed in both wet heptane-treated chloroplasts (which have very little variable fluorescence) and in Tris-washed (0.8 *M*, pH 8.0) chloroplasts (that also have no variable fluorescence) (Govindjee *et al.*, 1970). These data along with those presented by Döring and Witt (1972) show that the P680 change is not an artifact due to fluorescence yield changes (also see Butler, 1972b).

3. SECONDARY EVENTS

3.1. Electron Transfer in Green Plants

An acceptable model of photosynthesis must make provision for the occurrence of two distinct photoreactions, the presence of a chain of electron-transporting intermediates (redox couples), and the presence of a phosphorylating mechanism converting ADP to ATP. In addition, it must provide for the possibility of "artificial" electron transport, in which only a part of the photosynthetic electron transport chain is used. All models proposed, save one, invoke two or more reaction centers communicating with each other by means of electron-transport chains. These are classified as models with their photoreactions coupled *in series* or *in parallel.* One exceptional model has a single reaction center that is capable of two distinct photoprocesses.

3.1.1. Coupling in Series

This is the most widely accepted model. The Hill and Bendall (1960) model (or the Z scheme), briefly discussed here, has been elaborated into a detailed electron path leading from water to $NADP^+$ (for details see Hind and Olson, 1968, and Chapter 7). According to this model (Fig. 11), photoreaction II oxidizes water ($E_0' = +0.8$ V) to free oxygen, and reduces Q ($E_0' \sim$ O V), while photoreaction I reduces a low-potential electron acceptor X($E_0' \sim -0.6$ V) and oxidizes P700 ($E_0' \sim +0.4$ V). Recently, it has been suggested that Q may be equivalent to a component producing an absorbance change at 550 nm, referred to as C550 (see Arnon *et al.*, 1971; Erixon and Butler, 1971), and similarly, X to a component referred to as P430 (see Hiyama and Ke, 1971). Oxidized P700 is reduced by the reduced Q via exergonic electron transport reactions that are coupled to the phosphorylation of ADP (noncyclic photophosphorylation). The carriers catalyzing the *e*-transport reactions are thought to be Cyt b559 (low potential), PQ, Cyt f, and PC in that order. Böhme and Cramer (1972a) have shown that there is a site of phosphorylation between PQ and Cyt f. The low potential electron acceptor (X) for PS I can either reduce $NADP^+$ via FRS(?) and Fd, NADPH entering the carbon fixation cycle, or it can return its electron to an intermediate pool

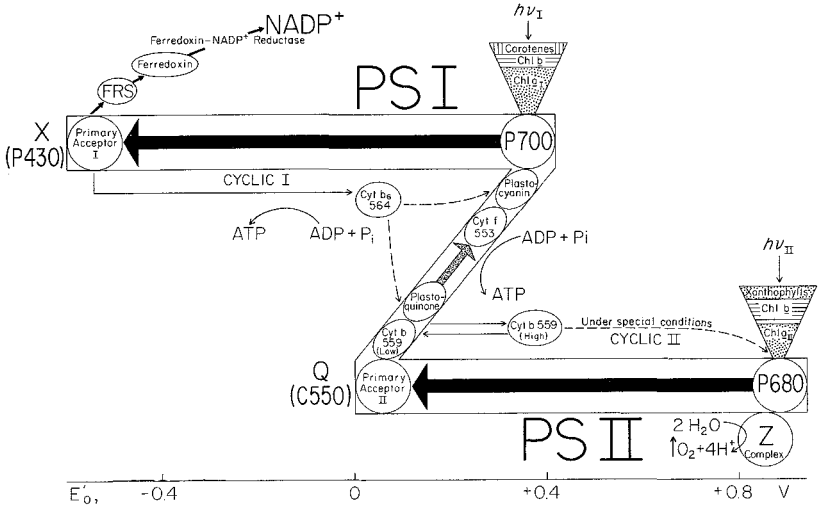

Fig. 11. The Z-scheme for electron flow in photosynthesis. The two bold horizontal arrows represent the two light reactions; all others, dark reactions. Flow of electrons from H_2O to $NADP^+$ (nicotinamide adenine dinucleotide phosphate) is designated as "noncyclic" electron flow, and from the primary acceptor of pigment system I (PS I) to the intersystem intermediates (plastoquinone, or plastocyanin) as "cyclic I". A similar cyclic flow of electrons involving only pigment system II may also exist; this could be designated as "cyclic II". E'_0: oxidation–reduction potential, at pH 7, in volts; Z complex: the electron donor system for pigment system II (PS II); P680: the proposed energy trap of PS II; Chl a_{II}: bulk chlorophyll *a* of PS II; Chl *b*: chlorophyll *b*: Cyt b559: cytochrome b with one of its difference absorption band at 559 nm; Q (C550): electron acceptor for PS II, Q stands for the quencher of Chl a_{II} fluorescence, and C550 for a compound with a difference absorption band at 550 nm, ADP, P_i, ATP; adenosine diphosphate, inorganic phosphate, and adenosine triphosphate, respectively; Cyt f553· cytochrome f with one of its difference absorption bands at 553 nm; P700: the energy trap and the reaction center of PS I; Chl a_I: bulk Chl *a* of PS I: Cyt $b_6$564: cytochrome b_6 with one of its difference absorption band at 564 nm:X (P430): the PS I electron acceptor (pigment with one of its difference absorption band at 430 nm); FRS: ferredoxin reducing substance. (Data of various investigators, too numerous to mention here, are incorporated in this scheme; see text.)

carrier (probably via Cyt b_6, etc.). In the latter instance, electron transport traces a closed circuit utilizing only PS I; it is referred to as cyclic electron transport and the accompanying formation of ATP is designated cyclic photophosphorylation. (See Böhme and Cramer (1972b) for a discussion of the site of cyclic phosphorylation.)

The series model offers an explanation for the synergistic effect of the two photoreactions on the end products of chloroplast photochemistry (O_2 and NADPH). It also explains the antagonistic effect of the two photosystems on the intersystem electron carriers. The series model thus accounts for the enhancement effect observed for both oxygen evolution (discussed above) and

the reduction of $NADP^+$ (R. Govindjee *et al.* 1962, 1964; Govindjee, 1963; Joliot *et al.* 1968; Avron and Ben Hayyim, 1969; Sun and Sauer, 1972) and for the reduction of Cyt f (Duysens *et al.*, 1961; Duysens and Amesz, 1962) and P700 (Kok and Gott, 1960; Kok and Hoch, 1961) by PS II and its oxidation by PS I. Since the electron carrier Q (the quencher of Chl fluorescence) is in the intersystem chain, the two photoreactions are expected to be antagonistic with respect to the Chl *a* fluorescence yield. Indeed, it is known that light absorbed by PS II causes the fluorescence yield of Chl *a* to rise, while light absorbed by PS I has the opposite effect (see Govindjee, *et al.*, 1960; Butler, 1962; Duysens and Sweers, 1963; Munday and Govindjee, 1969; Mohanty *et al.*, 1970).

A number of studies strongly support the series formulation. Some of these follow. (1) DCMU was shown to inhibit or block the electron transport from Q to other intersystem electron carriers (see Duysens, 1972). By adding DCMU and artificial electron donors, such as $DCPIPH_2$ and $TMPDH_2$, one could study PS I and its characteristics independently of PS II (Hoch and Martin, 1963). Similarly, reactions of PS II (electron flow from H_2O to certain Hill oxidants) have been studied by adding ferricyanide, quinones, or DCPIP, although these acceptors can also accept electrons from PS I under certain conditions (e.g., see Govindjee and Bazzaz, 1967; Selman and Bannister, 1971). Certain treatments, such as washing with 0.8 *M* Tris (at pH 8.0) (see Yamashita and Butler, 1968), irradiation with ultraviolet light (Jones and Kok, 1966) or heating chloroplasts to about 50°C for 5 min (see Yamashita and Butler, 1968; Katoh and San Pietro, 1968), result in the loss of the oxygen-evolving capacity; however, substitute electron donors (e.g., DPC and hydroxylamine) can reduce the electron acceptor and the reduction of Hill oxidants or $NADP^+$ can then be achieved. Thus, electron-flow separate from oxygen evolution can also be studied. (2) Removal of one or more components of the system results in loss of photochemical activity in partial reactions described above. Restoration of activity by the addition of the appropriate component(s) has elucidated the relative position of some of the electron carriers. Evidence from removal of carriers that block complete noncyclic electron flow without interfering with the independent activity of either photosystem strongly implicates requisite link between the two photosystems. (3) Isolation and characterization of mutants lacking one or the other electron carrier has been very helpful in determining the location of the various electron carriers in the chain (see review by Levine, 1969). (4) Finally, detergent or mechanical fractionation of chloroplasts followed by differential centrifugation has led to the physical separation of two kinds of particles that are enriched in one or the other photosystem (see reviews by Boardman, 1970; Park and Sane, 1971; and Chapter 2 of this volume).

3.1.2. Parallel Coupling

Photosynthetic models in this classification invoke the formation of a strong reductant ($E_0 < -0.4$ V) by one or both photoreactions. This does not conflict with the energy content of a red quantum since a 700-nm photon has enough energy (1.77 eV) to span the 1.2 eV potential differences between water and $NADP^+$ (however, see Section 1.6). Although the models discussed below have not been supported by experiments as the series model has been, they will be mentioned here for completeness and for the sake of keeping an open mind toward future developments.

Gaffron (1962), in an attempt to account for the quantum requirement of 8 in bacterial photosynthesis and the photoreduction of carbon dioxide by hydrogen gas in algae, developed a scheme in which each photoreaction oxidizes cytochromes (denoted here as Y′ and Z′) and produces a low potential reductant.

$$Z' + H_2O \xrightarrow{h\nu_{II}} Z'O + 2(H) \tag{9.1}$$

$$Y' + H_2O \xrightarrow{h\nu_{I}} Y'O + 2(H) \tag{9.2}$$

The oxidized cytochromes, then, react with a Mn-containing enzyme MnE (absent from photosynthetic bacteria) releasing oxygen as follows:

$$Z'O + MnEO \longrightarrow Z' + MnE + O_2 \tag{9.3}$$

$$Y'O + MnE \longrightarrow Y' + MnEO. \tag{9.4}$$

In photosynthetic bacteria, hydrogen donors could donate electrons to oxidized cytochromes, or there may be a cyclic reaction. Another model of parallel coupling was advanced by Govindjee *et al.* (1967; also see Hoch and Owens, 1963). In this model, photoreaction II produces a weak reductant, while photoreaction I supplies the ATP, or a high-energy precursor of it, by means of a cyclic electron transport. The weak reductant is assumed to reduce Fd, or another low potential intermediate, by a reverse electron flow powered by the energy-rich product of photoreaction I. In contrast to this model, McSwain and Arnon (1968) proposed that photoreaction II directly reduces Fd, while a photoreaction I-supported cyclic electron flow supplies the ATP required for CO_2 fixation. This model is based on the observation that enhancement could be demonstrated in isolated spinach chloroplasts in their laboratory only with CO_2 as the terminal electron acceptor, but *not* with $NADP^+$.

The existence of an enhancement effect in isolated chloroplasts with $NADP^+$ as the oxidant was, however, confirmed by Sun and Sauer (see their

1972 paper) in the presence of magnesium salt but not in its absence. Arnon *et al.* (1971) could not find this critical role of Mg salts. Sane and Park (1971) observed that when PPNR was added, enhancement was observed, but when Fd was used, it was not. We recall that in the early measurements of the enhancement effect in $NADP^+$ reduction R. Govindjee *et al.* (1962, 1964) had used PPNR and Mg^{2+} salts in their experiments. McSwain and Arnon (1972) recently confirmed the earlier work, and further showed that addition of ATP eliminated enhancement in CO_2 fixation with intact chloroplasts. Furthermore, they obtained a protein factor which, when added along with Fd, allows one to observe enhancement in $NADP^+$ reduction as with PPNR. The role of Mg^{2+} is still uncertain.

Knaff and Arnon (1969) proposed two sequential photoreactions, II_a and II_b, which are driven by the pigments of PS II. Photoreaction II_b oxidizes water and reduces an unidentified electron carrier, denoted from its difference absorption maximum as C550. Photoreaction II_a (that does not include P700) reduces Fd and oxidizes the copper protein PC. Electrons are transported from C550 to PC along a chain of carriers which includes PQ and Cyt b559. This transport is in the direction of the electrochemical gradient and it includes one phosphorylation site. In addition to the photoreactions II_a and II_b, photoreaction I carries out a cyclic electron transport through a chain of carriers that includes Chl *a* (P700), Fd, Cyt b_6, and Cyt f. There are two photophosphorylation coupling sites in this cyclic transport, one between Fd and Cyt b_6 and another between Cyt b_6 and Cyt f. Specific aspects of this model have been criticized by Esser (1972) and Bazzaz and Govindjee (1973), among others, on several grounds (also see Chapter 2 of this volume).

The parallel coupling model by Arnold and Azzi (1968) resembles the models of Govindjee and Arnon (*vide supra*) in the sense that it visualizes a Fd-reducing PS II and an auxiliary PS I-driven cyclic electron transport. The absorption of a photon by PS II results either in a reduced trap A and a Chl *a* cation, or in an oxidized trap B and a Chl *b* anion, as follows (absorption of 2 quanta in PS II are needed):

$$\text{Chl } a + \text{A} \xrightarrow{h\nu_{II}} \text{Chl } a^+ + \text{A}^- \tag{10.1}$$

$$\text{Chl } b + \text{B} \xrightarrow{h\nu_{II}} \text{Chl } b^- + \text{B}^+ \tag{10.2}$$

The ionic chlorophylls (holes and electrons) are discharged by recombination that leads to delayed light emission (hence the dependence of its intensity on the square of the excitation intensity) or to heat loss. The reduced energy trap A^- reduces Fd, while the oxidized trap B^+ decomposes water.

In general, models in which the two photoreactions of photosynthesis function with some degree of independence (e.g., the parallel coupling models)

have the advantage that they do not require the synchronization of the primary photoacts for the sake of efficient photosynthesis. Such parallel models are supported by the recent work of Rurainski and Hoch (1972), who find that the addition of increasing concentration of $MgSO_4$ leads to a decreased turnover of P700 and an increased rate of $NADP^+$ reduction, i.e., there is no correlation between the number of electrons passing through P700 and those which end up in $NADP^+$; in fact, a competition is observed. On the other hand, it is difficult to adapt such models to the massive biochemical and biophysical evidence obtained in support of the series model (discussed in this and other chapters in this book).

3.1.3. The United Reaction Center

Franck and Rosenberg (1964) visualize a single reaction center performing both the photoreactions of photosynthesis. Two groups of light-collecting chlorophylls, one of which is amorphous and one semicrystalline, supply the excitation to this center. The latter group donate triplet excitation quanta, which are used for the reduction of an electron acceptor X, and the oxidation of a Cyt. This process is equivalent to photoreaction I of the series model. The amorphous Chl population supplies singlet excitation quanta, by which the reduction of the previously oxidized Cyt and the oxidation of another intermediate Z is effected. Oxidized Z extracts electrons from water to release oxygen, while reduced X provides the electrons for the reduction of carbon dioxide. These reactions are envisioned as follows:

$$X + 2Cyt \xrightarrow{Chl_T} X^{2-} + 2Cyt^+ \tag{11.1}$$

$$Z + 2Cyt^+ \xrightarrow{Chl_S^*} Z^{2+} + 2Cyt \tag{11.2}$$

$$Z^{2+} + H_2O \longrightarrow Z + 2H^+ + \tfrac{1}{2}O_2 \tag{11.3}$$

This model is now of historical importance only, since it does not provide for the chain(s) of electron transport carriers known to exist. It is mentioned here because of the possibility that PS I may operate via triplet state and PS II by singlet state; this, of course, is speculation. [See a recent review on the role of triplets by VanderMeulen and Govindjee (1973).]

3.2. Electron Transfer in Photosynthetic Bacteria

Electron flow in bacteria is thought to follow a cyclic pathway. Parson (1968) showed by means of laser flashes that in *Chromatium* chromatophore preparations, the light-induced oxidation of P890 was severalfold faster than the oxidation of a Cyt labeled C555. He also showed that the rate of rereduction of oxidized P890 after the flash corresponded to the rate of oxidation of

C555. He demonstrated that the primary photochemical reaction in bacteria is the oxidation of P890.

Following the primary event, i.e., the photooxidation of the reaction center BChl (P840, P870, or P890 depending upon the organism) and the reduction of the primary electron acceptor (X), the electrons then trace a cyclic pathway back to the oxidized reaction center via several intermediates (Fig. 12). Phosphorylation has been shown to be coupled to this electron transport. ATP (or the high-energy intermediate) thus produced supplies the energy for the reduction of NAD^+ by a reversed electron flow, coupled to substrate (i.e., external H donor) oxidation.

The identity of the intermediates involved in the electron transport is not definitely known except for the cytochromes. Through the use of absorption difference spectroscopy, light-induced oxidation of three cytochromes (C555, C552, and CC′) has been revealed by Olson and Chance (1960) in *Chromatium*. C552 photooxidation was shown to occur at low light intensities; it is auto-oxidizable under aerobic conditions. The rereduction of C552 is dependent upon the presence of oxidizable substrate (Olson and Chance 1960; Morita *et al.* 1965). The photooxidation of C555 and CC′ occurs at high light intensities, independent of C552. C555 and CC′ were linked with the cyclic electron transport (mentioned above) and C552 with a separate (?) noncyclic electron transport linked with substrate oxidation.

Morita (1968) showed that the action spectra for the oxidation of the C555 and C552 in *Chromatium* were different, suggesting the possible involvement of more than one light reaction. Cusanovitch and Kamen (1968a,b) confirmed these observations and titrated the midpoint oxidation–reduction potentials (E_0') for the various cytochromes in *Chromatium*. C555 has an E_0' (at pH 7.5) of $+0.32$ V, CC′ of $+0.18$ V, and C552 of slightly below 0.0 V. They also found that the light-minus-dark difference spectra in the near infrared region were dependent upon the ambient redox potential of the chromatophores. They suggested that the two electron transport systems were linked with different reaction centers P890 and P905 (the latter based on the presence of an increase in absorption at 905 nm in the light-minus-dark difference spectrum at low redox potentials).

Sybesma and Fowler (1968) and Fowler and Sybesma (1970) reported that in *Rhodospirillum rubrum*, C428 (C552) was oxidized at low light intensities and its rereduction was extremely slow in the absence of a substrate; however, it could be speeded up upon the addition of malate. Oxidation of C422 (C555) was shown to occur at high light intensity. They found that the action spectra for the oxidation of C422 and C428 were different. Thus they also suggested two electron transport pathways: a noncyclic pathway linked with substrate oxidation via C428, and a cyclic pathway linked with C422 and associated with phosphorylation. Whether the suggested noncyclic and cyclic electron

transport pathways are mediated by different reaction centers is not certain. It has been shown that the photooxidation of both C552 and C555 is linked to the same reaction center P890 (Parson and Case 1970; Case *et al.* 1970; Dutton, 1971) in *Chromatium*, *Rhodopseudomonas viridis*, and *Rhodopseudomonas gelatinosa*. Such evidence is not available for *R. rubrum*; however, attempts at isolation of reaction center fractions from this organism has resulted in only one kind of preparation—that of P870 (Smith, 1972).

NAD^+ photoreduction in bacteria has been suggested (Olson and Chance, 1960; Bose and Gest, 1962; Keister and Yike, 1967; Jones and Vernon, 1969) to occur via a reversed electron transport with the help of ATP (or high-energy intermediate) produced in a light-induced cyclic electron flow. Nozaki *et al.* (1961) suggested a direct reduction of NAD^+ by a noncyclic electron transport. R. Govindjee and Sybesma (1970, 1972) have proposed two different pathways for NAD^+ reduction in *Rhodospirillum rubrum*. They suggested that succinate-supported NAD^+ reduction occurs by a reversed electron flow and NAD^+ reduction with $DCPIPH_2$ occurs directly via a noncyclic electron flow. R. Govindjee *et al.* (1974) showed that with the latter condition, low potential viologen dyes are capable of reacting with this system as inferred from light induced absorption changes of P870.

Hind and Olson (1968) also proposed a scheme of electron transport in bacteria in which there are two separate light-induced electron transport pathways. One is cyclic and is coupled to phosphorylation, thus reducing NAD^+ with the help of ATP, and another noncyclic pathway linked with substrate oxidation and direct NAD^+ reduction via Fd and flavoproteins. However, the existence of the noncyclic pathway remains to be proven (see Fig. 12 for a general scheme).

The nature of "X," the primary electron acceptor, in photosynthetic bacteria and PS I in green plants is under active investigation (see Chapter 3 of this volume for progress in this area). Dutton and Leigh (1973) have coined the term photoredoxin for the reduced form of "X" which exhibits an ESR signal centered at $g = 1.82$; it has an E_0' (at pH 7.0) of -50 mV in *R. spheroides* and -130 mV in *Chromatium*. On the basis of the EPR studies, Loach and Hales (personal communication, 1973) found that the primary electron acceptor in the photoreceptor complex preparations (from purple photosynthetic bacteria) has a midpoint potential of -0.37 V. Loach found a similar midpoint potential for quenching phototrap activity in chromatophores and whole cells when they were kept under very dark conditions and subjected to a single short pulse of light.

The presence of Fd has been shown in several photosynthetic bacteria. However, there is no conclusive evidence for its role in their electron transport pathway. Phosphorylation and cyclic electron transport have been reported in *R. rubrum* chromatophores from which Fd was absent (Horio *et al.*, 1968).

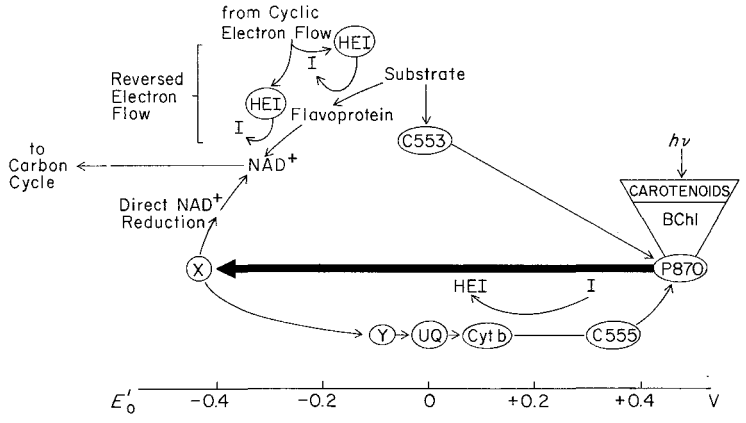

Fig. 12. A hypothetical scheme for electron flow in bacterial photosynthesis. NAD^+ (nicotinamide adenine dinucleotide) reduction may occur by reversed electron flow (see top left of scheme), or possibly by a direct reduction. E_0': oxidation–reduction, potential, at pH 7.0, in volts; P870: reaction center bacteriochlorophyll; BChl: bulk bacteriochlorophyll; X: primary electron acceptor; Y: secondary electron acceptor; UQ: ubiquinone; Cyt b: two kinds of cytochrome b; C555: cytochrome c with one of its difference absorption band at 555 nm; I: intermediate; HEI: high energy intermediate; C553: cytochrome c with one of the difference absorption band at 553nm. Whether two separate pigment systems (with their own reaction centers) sensitize two separate light reactions remains to be proven. Results of several experiments suggest the idea of a direct reduction of NAD^+. Such a direct NAD^+ reduction would be easier, if there was an electron acceptor (X) with more negative E_0' than -0.3 V. (However, an electron acceptor having a more positive E_0' than -0.3 V could also reduce NAD^+ directly under conditions when it is largely in the reduced state.)

Trebst *et al.* (1967) reported that Fd is not required for NAD^+ reduction in *R. rubrum*. Fd-linked NAD^+ reduction by molecular H_2 in *Chromatium* has, however, been reported by Weaver *et al.* (1965).

3.3. Oxygen Evolution in Green Plants

Although photosynthetic oxygen evolution was discovered 200 years ago by Priestley (see R. Hill, 1972), the oxygen evolving side of PS II is still not very well elucidated. An unknown component Z is the "primary" electron donor. Z is linked with the oxidation of H_2O through one or several steps. The nature of Z and the other components involved is not known. As noted earlier, this system is heat-labile, sensitive to ultraviolet radiation, and is (perhaps reversibly) impaired by treatment with a high molarity Tris at pH 8.0. After any of the above treatments, the oxygen-evolving capacity of the organism is lost, but substitute electron donors such as DPC or hydroxylamine can replace H_2O. We know that Mn^{2+} (see Cheniae, 1970), Cl^- (Izawa *et al.*, 1969), and bicarbonate ions (Stemler and Govindjee, 1973, 1974) are in some way involved in the oxidation of water to molecular O_2. A protein (enzyme) is

implicated, but has never been isolated and purified (see Braun and Govindjee, 1972).

An important piece of information about the O_2-evolving system was provided by Allen and Franck (1955). They showed that after a period of dark adaptation, no O_2 was evolved by algae in the first flash of light; however, O_2 was evolved if this flash was preceded by weak light or another flash. Joliot *et al.* (1969) and Forbush *et al.* (1971) found that after dark adaptation the first flash of light yields no O_2, the second flash shows some, the third flash has a high yield, the fourth flash a little less, the fifth even less, then the sixth flash yields more, and the seventh flash again represents a maximum (see Fig. 1 in Chapter 8 of this volume). In this way, they found a periodic oscillation in the O_2 yield from a series of flashes, the yield reaching a peak with a period of 4. Thus, the 3rd, 7th, 11th, and so forth, flashes represented yield maxima. These oscillations eventually damped out with time, and the sequence could be repeated again by dark-adaptation of the system.

Kok *et al.* (1970) proposed a mechanism in which a light-activated accumulation of four positive charges was necessary before a molecule of oxygen could be evolved. In order to explain the occurrence of the maxima at the 3rd, 7th, and 11th flashes, they suggested that in the dark there are two stable species, such as Z and Z^+ (Z being the primary electron donor).* Thus, the Z^+ to Z^{4+} reaction needs 3 more quanta to enable the evolution of O_2; thereafter, it returns to the Z state and must build up four positive charges before the sequence is repeated (also see Mar and Govindjee, 1972b). Details of O_2 evolution are discussed by Joliot and Kok in Chapter 8 of this volume.

The basic idea is that four oxidizing equivalents must accumulate before O_2 can be evolved as follows:

$$Z\cdot P680\cdot Q \xrightarrow{h\nu} Z^+\cdot P680\cdot Q^- \tag{12.1}$$

$$Z^+\cdot P680\cdot Q^- + A \longrightarrow Z^+\cdot P680\cdot Q + A^- \tag{12.2}$$

$$Z^+\cdot P680\cdot Q \xrightarrow{h\nu} Z^{2+}\cdot P680\cdot Q^- \tag{12.3}$$

$$Z^{2+}\cdot P680\cdot Q^- + A \longrightarrow Z^{2+}\cdot P680\cdot Q + A^- \tag{12.4}$$

*There is some confusion regarding the nomenclature of the "primary" electron donor that donates electrons to the primary electron acceptor. The P680 itself is, in all likelihood *the* primary electron donor. For purposes of present discussion, the intermediate that donates electrons to $P680^+$ is referred to here as the primary electron donor. There are at least two intermediates between H_2O and P680—a primary donor, and a secondary donor. Unfortunately, no standard nomenclature has been accepted for these unknown intermediates. Some call it Z_1 and Z_2 (e.g., see Braun and Govindjee, 1972); Lavorel (see Chapter 5 of this volume) has used Y and Z, Papageorgiou (Chapter 6) "Z" and "S", and Avron (Chapter 7) "Z" and "E", respectively. The reader is warned that he should not transform "Z" of one author to that of another without a careful check.

$$Z^{2+}\cdot P680\cdot Q \xrightarrow{h\nu} Z^{3+}\cdot P680\cdot Q^{-} \tag{12.5}$$

$$Z^{3+}\cdot P680\cdot Q^{-} + A \longrightarrow Z^{3+}\cdot P680\cdot Q + A^{-} \tag{12.6}$$

$$Z^{3+}\cdot P680\cdot Q \xrightarrow{h\nu} Z^{4+}\cdot P680\cdot Q^{-} \tag{12.7}$$

$$Z^{4+}\cdot P680\cdot Q^{-} + A \longrightarrow Z^{4+}\cdot P680\cdot Q + A^{-} \tag{12.8}$$

$$Z^{4+}\cdot P680\cdot Q + 2H_2O \longrightarrow O_2 + 4H^{+} + Z\cdot P680\cdot Q \tag{12.9}$$

$$\text{Net: } 2H_2O + 4A \xrightarrow{Z\cdot P680\cdot Q + 4h\nu} O_2 + 4A^{-} + 4H^{+} \tag{12.10}$$

In order to explain all their kinetic data and those of Joliot, Kok and coworkers postulated the following: (1) after a dark period, the initial states are $Z^{+}\cdot P680\cdot Q$ (75%) and $Z\cdot P680\cdot Q$ (25%); (2) there is a small possibility of "misses," i.e., the reaction will not proceed even though a PSU receives light; (3) in the light flashes used for their experiments, there was a small probability of "double hits," i.e., the reaction center moved two steps, instead of one, with one flash. With these and other assumptions (discussed by Joliot and Kok, Chapter 8, this volume), all the kinetic data were explainable. Much further work needs to be done to understand fully the mechanism of O_2 evolution.

As noted earlier, no oxygen evolution occurs in photosynthetic bacteria, as they are unable to use H_2O as an electron donor.

3.4. Photophosphorylation

Photophosphorylation was first clearly demonstrated by Arnon *et al.* (1954) and Frenkel (1954) in chloroplasts and in photosynthetic bacteria, respectively. In the Z scheme, ATP can be produced either during the exergonic flow of electrons in the noncyclic electron transport chain or via cyclic electron flow (see Section 3.1). Details of photophosphorylation are discussed by Jagendorf (Chapter 9 of this volume) and by Witt (Chapter 10).

It is generally accepted that at least one site of photophosphorylation is between PQ and Cyt f. If ADP or NH_4Cl is added to chloroplasts, PQ oxidation and Cyt f reduction is accelerated, i.e., electron flow from PQ to Cyt f is increased when ADP phosphorylation is allowed to occur, or when electron flow is uncoupled from phosphorylation with NH_4Cl. These and other data indeed suggest that one of the sites of phosphorylation is between PQ and Cyt f (see Böhme and Cramer, 1972a). From similar and other experiments, Böhme and Cramer (1972b) have further shown that electron flow from reduced Cyt b_6, that occurs via PQ, to Cyt f (cyclic) also contains an energy-coupling site; it was tentatively suggested that this coupling site is common to

noncyclic electron flow. Further work is needed to establish other (suggested) site(s) of phosphorylation.

The mechanism of phosphorylation is not well understood. There are two major competing hypotheses: (1) chemical and (2) chemiosmotic (see Chapter 9 and 10 of this volume). In the former an energy-rich chemical intermediate is made and its deenergization is coupled to ATP production, whereas in the latter hypothesis, energy for ATP production is obtained by the discharge of membrane potential and/or H^+ ion gradient created across the chloroplast membrane by the electron flow. No definite conclusions can be made yet. However, the experiments of Jagendorf and Uribe (1966), in which they produced ATP in darkness from an artifically created H^+ gradient, and of Witt and coworkers (see Junge *et al.*, 1969), in which they show that an absorption change of 515 nm (probably due to a change in membrane potential) decays faster in the presence of the uncoupler gramicidin or ADP + P_i, support Mitchell's chemiosmotic theory. [However, for different considerations, see Weber (1972, 1973).]

3.5. Carbon Fixation

After the elegant work of Benson, Bassham and Calvin (see Bassham and Calvin, 1957, and Gaffron, 1960), the path of carbon is shown as possessing the following basic features: (1) the carboxylation of a five-carbon (C_5) sugar phosphate (ribulose 1,5-diphosphate) with the production of 2 molecules of 3 phosphoglyceric acid; (2) the conversion of phosphoglyceric acid to triose phosphate (the simplest sugar) with the aid of reducing power (NADPH) and ATP made by the "light reactions" of photosynthesis; and (3) the interconversion of sugars, with the result that if one starts with 6 molecules of CO_2, 1 molecule of hexose is formed, and 6 molecules of ribulose 1,5-diphosphate are regenerated to carry on the cycle (often called the Calvin cycle; see Fig. 13). There is an additional input of an ATP molecule in the conversion of ribulose monophosphate to ribulose 1,5-diphosphate. The net reaction (for 1 glucose molecule) is:

$$6CO_2 + 12NADPH + (12H^+) + 18ATP \longrightarrow$$

$$C_6H_{12}O_6 + 12NADP^+ + 18ADP + 18P_i + 6H_2O \qquad (13)$$

Recently (see review by Hatch and Slack, 1970) it has become clear that there is another pathway for CO_2 fixation, called the C_4 pathway, found in certain plants containing dimorphic (mesophyll and bundle sheath) chloroplasts. This mode is visualized by some workers as follows (see Fig. 14). In mesophyll cells, CO_2 is fixed by its addition to PEP, making oxalacetic acid. The latter is reduced to malate (in most C_4 plants called "malate formers") with the help of NADPH (produced in the light reactions). Malate is then

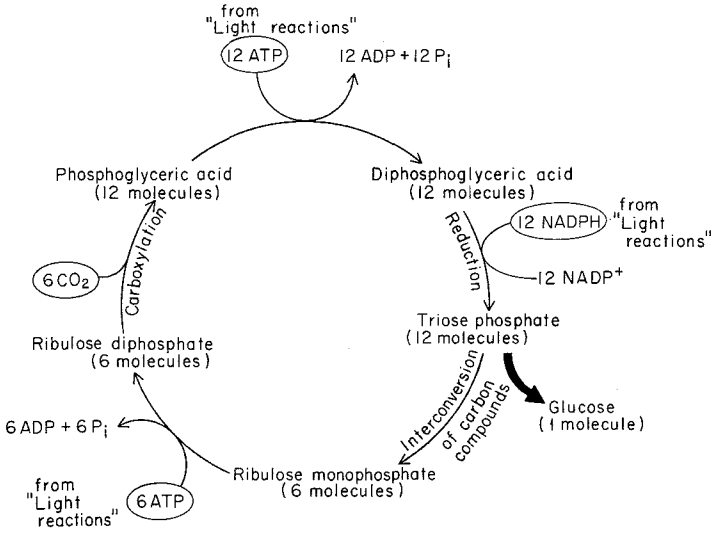

Fig. 13. A simplified diagram of the Calvin–Benson–Bassham cycle for the path of carbon fixation in photosynthesis. (See Fig. 11 for abbreviations.)

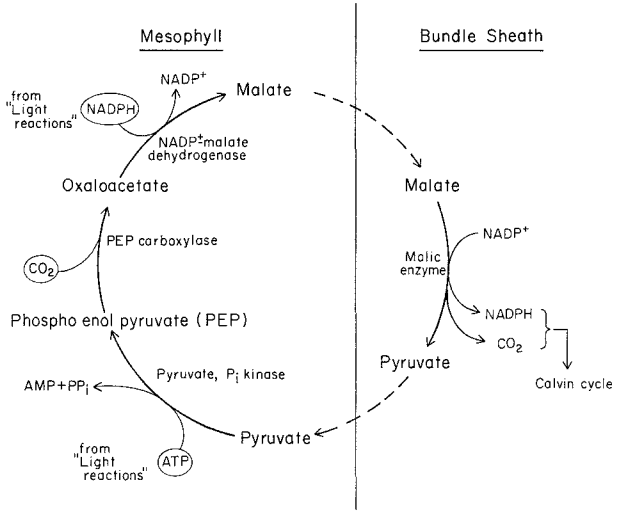

Fig. 14. A simplified diagram of the Hatch and Slack pathway for carbon fixation in plants with agranal (bundle sheath) and granal (mesophyll) chloroplasts.

translocated to the bundle sheath cells where it is converted into pyruvate and CO_2; simultaneously, $NADP^+$ is reduced to NADPH. Thus malate acts in transferring both the carbon and the reducing power from the mesophyll to bundle sheath cells. (There is also the possibility that bundle sheath cells make some of their own reducing power, and utilize CO_2 *per se* if it reaches them.) Bundle sheath cells then catalyze the usual Calvin cycle. Pyruvate is translocated back to the mesophyll cells where, with the help of an additional ATP molecule, it is converted to PEP to continue the cycle. Thus, the Hatch and Slack cycle requires an additional ATP. The above is a very simplified picture. The details of the pathway of carbon in C_4 plants (called "aspartate formers") that produce aspartate (instead of malate) is not well known yet. The path of carbon is discussed at length by Bassham (1965) and Hatch (1970) (also see Hatch *et al.*, 1970; Zelitch, 1971; Gibbs, 1971).

Almost all the photosynthetic bacteria have the ability to fix carbon by the reductive pentose (or Calvin) cycle. Evans *et al.* (1966), however, found that the green bacterium *Chloropseudomonas thiosulfatophilum* is also capable of reversing the citric acid (or Krebs) cycle. This reversal is accomplished by using reduced Fd and ATP made by light reactions. Several photosynthetic bacteria, when grown photoheterotrophically, instead of autotrophically, can obtain reducing equivalents by the oxidation of organic compounds via the citric acid cycle. Others produce it by the oxidation of externally added H_2 donors via the electron transport chain discussed above (noncyclic or by combination of cyclic with reversed electron flow). The reducing equivalents thus produced are then used for the reduction of carbon compounds (see discussion in Gregory, 1971).

Often, photosynthetic bacteria are capable of evolving hydrogen, using the hydrogenase enzyme. It is this reaction that may be of significance to the solution of our energy crisis, if one can efficiently couple the production of reducing equivalents from H_2O by green plants with bacterial hydrogen evolution (see Hollaender *et al.*, 1972).

4. SUMMARY

Photosynthesis is an oxidation–reduction process in which light energy is converted into chemical energy. In green plants, this amounts to the reduction of CO_2 into (CH_2O) and the oxidation of H_2O to molecular O_2. In photosynthetic bacteria various H donors replace H_2O, and no O_2 is evolved.

Several hundred pigment molecules (e.g., Chl *b* and Chl *a* in a green plants, or BChl in photosynthetic bacteria) somehow cooperate to perform photosynthesis. This collection of pigment molecules, along with other necessary

components, is called a photosynthetic unit (PSU). In green plants, there are two pigment systems, and thus two types of PSU's. Each PSU has its own energy trap or reaction center (P700 in PS I and P680 in PS II). Certain photosynthetic bacteria possess at least one type of reaction center, labeled P870. Light energy absorbed by any of the pigment molecules in a PSU is probably transferred, as an "exciton" to the reaction centers where the primary light reaction occurs. The primary light reaction is essentially the oxidation of the reaction center and the reduction of a primary electron acceptor.

In green plants, one light reaction (I) leads to the oxidation of a cytochrome (f) and reduction of pyridine nucleotide. The other light reaction leads to the oxidation of H_2O to molecular O_2 and reduction of certain intermediate(s). The transfer of electrons from the latter to Cyt f completes the chain; this step is exergonic and is coupled to ATP production (noncyclic phosphorylation). A cyclic flow of electrons around reaction I can also lead to ATP production (cyclic photophosphorylation). ATP and reduced pyridine nucleotide produced by the two light reactions are sufficient to run the carbon fixation cycle that leads to the production of organic matter (CH_2O) from CO_2.

In photosynthetic bacteria, there is at least one light reaction that runs in a cyclic fashion producing ATP or a high-energy intermediate. By utilizing these high-energy compounds, bacteria reduce NAD^+ with external hydrogen donors (e.g., succinate), by reversed electron flow (i.e., against a potential gradient). There are, however, some indications that NAD^+ may be reduced directly by a noncyclic pathway. Whether a separate reaction center is involved here is not yet established. Photosynthetic bacteria fix carbon by various pathways.

APPENDIX I. PHOTOSYNTHESIS LITERATURE

Extensive literature exists in the area of photosynthesis research. A discussion of earlier literature appears in the most thorough and detailed treatise on photosynthesis by Rabinowitch (1945, 1951, 1956). These books are still a gold mine of information and ideas. Several more recent small books of varying emphasis and/or depth are also available. The primary photochemical reactions are discussed by Kamen (1963); the biophysical aspects of photosynthesis by Clayton (1965); carbon fixation by Bassham and Calvin (1957) and Calvin and Bassham (1962); physiological aspects by Heath (1969); and general aspects of photosynthesis by Gaffron (1960), Fogg (1968), Rabinowitch and Govindjee (1969), Zelitch (1971) and Gregory (1971). Mention should also be made of the small books by Hill and Whittingham (1953) and Rosenberg (1965).

Several valuable multiauthor books or collections of papers have appeared, many of which are proceedings of conferences held on photosynthesis.

The following is a partial list of such volumes edited by Franck and Loomis (1949), Danielli and Brown (1951), Gaffron *et al.* (1957), Fuller (1959), Allen (1960), Ruhland (1960), McElroy and Glass (1961), Ashida (1963), Gest *et al.* (1963), Kok and Jagendorf (1963), Tamiya (1963), Wurmser (1963), Krogmann and Powers (1965), Goodwin (1966), Thomas and Goedheer (1966), Olson (1967), San Pietro *et al.* (1967), Shibata *et al.* (1968), Metzner (1969), Hatch *et al.* (1970), Gibbs (1971), Forti *et al.* (1972), and Jacobi (1972). Reference is also made to three special issues of journals on photosynthesis, edited by Brown (1959), Pearlstein (1971), and Govindjee (1972).

A very valuable book on methods edited by San Pietro (1971, 1972), is available from Academic Press. Spectroscopy of chlorophyll is summarized in a book by Gurinovich *et al.* (1968), and all aspects of chlorophylls by Vernon and Seely (1966).

APPENDIX II. CONSTANTS, CONVERSION FACTORS, AND EQUATIONS

A. Physical Constants

Avogadro's number	N	6.023×10^{23} molecules $mole^{-1}$
Boltzmann's constant	k	1.3805×10^{-16} erg deg (Kelvin)$^{-1}$
Electronic charge	e	1.6021×10^{-19} C or 4.8030×10^{-10} esu
Faraday's constant	$\mathscr{F}e$	96,494 C g $equiv^{-1}$
Gas constant	R	8.314 J deg (Kelvin)$^{-1}$ $mole^{-1}$ or 1.98 cal deg (Kelvin)$^{-1}$ $mole^{-1}$
Gravitational constant	g	980.665 cm sec^{-2} or 1.01325×10^6 dyn cm^{-2}
Planck's constant	h	6.62×10^{-27} erg sec or 1.58×10^{-34} cal sec
Velocity of light *in vacuo*	c	2.997×10^{10} cm sec^{-1} or 186,000 miles sec^{-1}

B. Conversion Factors

1 cal = 4.184×10^7 ergs or 4.184 J (abs.) or 2.612×10^{19} eV or 2.106×10^{23} cm^{-1}.

1 cm^{-1} = 1.986×10^{-16} ergs or 1.986×10^{-23} J (abs.) or 4.747×10^{-24} cal or 1.240×10^{-4} eV.

1 einstein cm^{-2} sec^{-1} = 12×10^{14} ergs cm^{-2} sec^{-1} or $12 \times 10^7/\lambda$ W cm^{-2}.

I erg = 10^{-7} J (abs.) or 2.389×10^{-8} cal or 6.242×10^{11} eV or 5.034×10^{15} cm^{-1}.

1 eV = 1.602×10^{-12} erg or 1.602×10^{-19} J (abs.) or 3.829×10^{-20} cal or 8066 cm^{-1} or photon of 1240 nm wavelength.

1 J (abs.) = 10^7 ergs or 0.2389 cal or 6.242×10^{18} eV or 5.034×10^{22} cm^{-1}
Temperature (T) in °Kelvin = temperature (t) in °Celsius (c) plus 273.16°C
1 W cm^{-2} = 10^7 ergs cm^{-2} sec^{-1} or 8.3×10^{-9} (λ) einsteins cm^{-2} sec^{-1} (λ in nm).
At any one wavelength, 1 W cm^{-2} = $5.75 \times 10^5 \times V_{rel}$ f-c (see Fig. 3–3 for V_{rel} in R. K. Clayton, 1970).
To change some of the above units from molecular to molar units, multiply by 6.023×10^{23}. For example, 1 eV = 3.829×10^{-20} cal or $3.829 \times 10^{-20} \times 6.0223 \times 10^{23}$ cal $mole^{-1}$ or 23.06 kcal $mole^{-1}$.
Energy of one quantum (λ in nm) = $2 \times 10^{-9}/\lambda$ erg or $1{,}240/\lambda$ eV. The energy of 1 einstein (1 mole of quanta) is the above numbers multiplied by 6.023×10^{23}.

C. Equations

$E = hv = hc/\lambda$, where E = energy, h = Planck's constant, v = frequency, c = velocity of light, and λ = wavelength of light.

$$\Phi_f = k_f/(k_f + k_d + k_p)$$

where Φ_f = quantum yield of fluorescence, k_f = rate constant (k) for fluorescence, k_d = k for radiationless, and k_p = k for photochemical deexcitation.

$$\Phi_f = \tau/\tau_0$$

where τ = measured lifetime of excited state and τ_0 = intrinsic lifetime when all deexcitation is by fluorescence.

$$1/\tau_0 \approx 3 \times 10^{-9}\, km^2\, \Delta k\, \varepsilon m$$

where km (cm^{-1}) = wavenumber at the peak, Δk (cm^{-1}) = half-bandwidth of the absorbance band, and εm (M^{-1} cm^{-1}) = extinction coefficient* at the absorption peak.

OD = $\log I_0/I = \log\ 1/T = -\log\ T = -\log\ (1-A) = \varepsilon cd$, where OD = optical density or absorbance, I_0 = incident intensity, I = transmitted intensity, T = fractional transmission, and A = fractional absorbance, ε = extinction coefficient, c = concentration, and d = optical pathlength.

$$\Delta c\ (\text{moles sec}^{-1}) = \Delta T/2.3T\,(d)\,(10^3)\,\Delta\varepsilon$$

*Extinction coefficient, an important property of a molecule, refers to the cross section of absorption (units of area). Therefore, the proper unit is cm^2 $mole^{-1}$ of the material. However, one often uses the unit M^{-1} cm^{-1} for convenience in certain types of calculations. The two units are, however, equivalent as: M^{-1} cm^{-1} = $moles^{-1}$ liter cm^{-1} = $moles^{-1}$ (10^3) cm^3 cm^{-1} = 10^3 $moles^{-1}$ cm^2 = 10^3 cm^2 $moles^{-1}$, or cm^2 $mmoles^{-1}$.

if $\Delta OD \ll 1$ and $\Delta T \ll T$, where Δc = change in molar concentration, ΔT = change in transmission, T = fractional transmission, d = optical path length and $\Delta\varepsilon$ = differential extinction coefficient. This equation is applied to light-induced absorption changes of various components in photosynthesis.

$$\Phi_p = \frac{\#\text{ moles sec}^{-1}\text{ transformed}}{\#\text{ einsteins absorbed sec}^{-1}} = \frac{\Delta c\ (\text{moles sec}^{-1})}{I_0\,\text{A (einstein sec}^{-1})} = \frac{\Delta\text{OD sec}^{-1}}{10^3\, I_0\, A\, \Delta\, \epsilon},$$

where Φ_p = quantum yield of a photochemical reaction, ΔOD is change in absorbance and other terms have the same meaning as described above.

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Note added in proof: During the time this volume has been in press many new papers have been published that have provided a better understanding of the process of photosynthesis. Some examples are: (1) Picosecond laser technology has permitted the measurement of lifetime of excited states in picosecond time scale [Seibert, M., and Alfano, R. R. (1974). *Biophys. J.* **14**, 269], and of the time needed (7 ± 2 psec) for excitation energy transfer plus primary oxidation reduction reaction at the reaction centers in photosynthetic bacteria [Netzel, T. L., Rentzepis, P. M., and Leigh, J. (1973). *Science* **182**, 238]. (2) Studies of structure–function relationships have lead to a tentative, but detailed, picture of how various components may be embedded in or located on the thylakoid membrane [Trebst, A. (1974). *Ann. Rev. Plant. Physiol.* **25**, 423]. (3) A light-induced electrical signal, perhaps reflecting membrane potential, which can be correlated with phosphorylation events has been measured [Witt, H. T., and Zickler, A. (1973). *FEBS Lett.* **37**, 307]. (4) The essential role of bicarbonate ions in O_2 evolution steps has been firmly established. It was shown that HCO_3^- accelerates the recovery of the reaction center II complex following light reactions so as to allow it to operate again [Stemler, A., Babcock, G. T., and Govindjee (1974). *Proc. Nat. Acad. Sci., U.S.*, in press]. (5) A phosphorylation step associated with system II has been shown to occur [Trebst, A., and Reimer, S. (1973). *Biochim. Biophys. Acta* **325**, 546; Ouitrakul, R., and Izawa, S. (1973). *Biochim. Biophys. Acta* **305**, 105]. (6) The role of C550 as a primary electron acceptor has now been challenged by several research workers—it may still be an indicator of PS II reactions. (7) Witt's group in Berlin has suggested that most of the electron

flow may bypass cyt f. For an up-to-date knowledge, the reader should consult the recent issues of various journals including *Annual Review of Plant Physiology, Archives of Biochemistry and Biophysics, Biochemical Biophysical Research Communications, Biochemistry, Biochimica Biophysica Acta* (Bioenergetics), *Biophysical Journal, FEBS Letters, Journal of Biological Chemistry, Journal of Theoretical Biology, Photochemistry and Photobiology, Photosynthetica, Plant and Cell Physiology, Plant Physiology, Plant Science Letters,* and *Proceedings of National Academy of Sciences, U.S.A.*