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MODERN TRENDS IN PHOTOBIOLOGY: ENERGY CONVERSION IN PHOTOSYNTHESIS†

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ONE hundred and twenty years ago, a German surgeon, Julius Robert Mayer[1] suggested that light energy is stored in the form of chemical energy when green plants perform photosynthesis. The manner in which light energy is absorbed by the pigments of the chloroplast (the cell organelle where photosynthesis occurs), transferred to the "reaction centers" (the sites where the primary photochemical reactions occur) and stored as chemical bond energy, is the main concern of this discussion. We shall first examine the overall process and its energetics in terms of recent developments in photosynthesis research, and conclude by discussing the mechanism of energy conversion (see recent symposia[2–9], books[10–12], and reviews[13–17].

During 1935-1941, van Niel[18,19], offered an important new formulation of photosynthesis, viz., it is an *oxidation-reduction* reaction; H_2O is oxidized to O_2, and CO_2 is reduced to carbohydrate $[CH_2O]$ (see equation below):

$$CO_2+2H_2O+\text{light energy} \xrightarrow[\text{enzymes}]{\text{pigments}} [CH_2O]$$

$$+H_2O+O_2+ \text{ chemical energy (112 Kcal)}$$

In this process, about 112 Kcal light energy is stored per mole of CO_2 reduced. The chloroplast pigments that absorb the light energy for this process are many and varied. All autotrophic plants contain the yellow-green pigment chlorophyll *a* and certain yellow or orange carotenoids. Photosynthetic bacteria also contain chlorophylls, but they are somewhat different; *e.g.* purple bacteria contain bacteriochlorophyll, and green bacteria contain "Chlorobium-chlorophyll". Chlorophyll *a* is known to exist in several forms* in the living cell. In addition, every photosynthetic organism contains one or more of the "accessory pigments"[16–19]. Some of the accessory pigments found in nature are: the bluish-green pigment chlorophyll *b* (found in all green algae and higher plants), the red pigment phycoerythrin (found in red algae), the blue pigment phycocyanin (found in blue-green and red algae), β carotene (present in all higher plants and algae), fucoxanthol (a carotenoid present only in diatoms and brown algae), and spirilloxanthol (one of several carotenoids found in purple bacteria).

I. ENERGETICS

The reduction of one molecule of CO_2 requires four H-atoms. Since four H-atoms (or electrons, as we cannot distinguish between an electron transfer and an H-atom in an aqueous medium*) are to be transferred from H_2O to CO_2, and since one quantum* of light performs one primary photochemical act (Einstein's law of photochemical equivalency); no less than four quanta of light are required for this process. Recently, it has been shown by several groups of investigators[20–22] that photosynthesis requires two light reactions. Hydrogen atoms (or electrons) are evidently transferred in *two* steps; this would require a minimum of eight quanta for the transfer of four H-atoms (or electrons) from H_2O to CO_2. However, if in one of the two light reactions the primary act is the transfer of two electrons

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* Referred to as Chl *a* 670, Chl *a* 680, and Chl *a* 695 —where Chl *a* stands for chlorophyll *a* and the numbers designate the approximate maxima of their red absorption band in mμ.

* In an aqueous medium, an electron transfer from a compound A^- to B may appear an an H-atom transfer if an H^+ ion (from the medium) is added later, *e.g.*

$$A^-+B \rightarrow A+B^- \text{(electron transfer)}$$
$$B^-+H^+ \text{ (from the medium)} \rightarrow BH$$

On the other hand, an H-atom transfer from XH to Y may appear as an electron transfer, if an H^+ is lost to the medium, *e.g.*

$$XH+Y \rightarrow YH+X \text{ (H-atom transfer)}$$
$$YH \rightarrow Y+H^+ \text{ (to the medium)}$$

*A quantum (or photon) is a unit of light energy; the energy, E, in a quantum is given by the expression hc/λ, where h=Planck's constant, c=velocity of light, and λ =wavelength of light.

at a time by one quantum of light, a minimum of only six quanta would be required per O_2 molecule evolved. That the minimum number of quanta actually required (quantum requirement) for the evolution of one O_2 molecule or for the reduction of one CO_2 molecule is *not* four, but close to eight, has been convincingly shown by several investigators[12,22,23].

II. The "Red Drop", The Emerson Enhancement effect, and two light reactions

One of the most intriguing phenomena in photosynthesis was discovered by Robert Emerson in 1943. He observed that the quantum efficiency* of photosynthesis becomes abnormally low when light is primarily absorbed by chlorophyll *a*. This was an unexpected finding, since fluorescence measurements by several investigators had shown that energy absorbed by other pigments was transferred to chlorophyll *a*. Since it could not matter how the chlorophyll *a* molecule was excited, whether by energy transfer or by photon absorption, this effect—called the "red drop" —was very baffling because chlorophyll *a* was supposed to be the prime sensitizer of photosynthesis; it is the only pigment common to all photosynthetic organisms and since it has the lowest energy level, it is the most logical "energy trap"+. The "red drop" is most dramatic in red and blue-green algae[24]. The cause for the low efficiency of chlorophyll *a* in photosynthesis was discovered by Robert Emerson in 1957; it was suggested that photosynthesis requires two light reactions and that chlorophyll *a* is capable of performing only one of the two required reactions. This conclusion was based on the finding that there is a synergistic effect in the yield of photosynthesis when a certain wavelength of light (which is absorbed primarily in accessory pigments) is combined with far-red light (which is absorbed mainly in chlorophyll *a*). These experiments were made with weak, but *not* catalytic light, under conditions* of maximum quantum yield of photosynthesis. This synergistic effect is often called the *Emerson enhancement effect.* The action spectra of the enhancement effect* were first obtained by measuring enhancement as a function of wavelength of the second short-wave beam when the wavelength of the first beam of light (far-red light, 720 mμ) is kept constant. This characterized one of the two Pigment systems. The other system was characterized by measuring enhancement by varying the wavelength of far-red light with constant short-wave light (green light in Porphyridium and red light in Chlorella) as background. The results obtained by several investigators[25–30] confirmed and extended Emerson's earlier hypothesis that photosynthesis requires *two* light reactions sensitized by *two* pigment systems.

The conclusion that one of the two light reactions can be sensitized by accessory pigments alone, was hard to accept[30], as light absorbed in accessory pigments is transferred with high efficiency (80-100%) to chlorophyll *a*. (This is demonstrated by experiments in which a similar fluorescence yield of chlorophyll *a* is noted whether chlorophyll *a* is excited or an accessory pigment is excited). Our experiments[30] with the green alga *Chlorella pyrenoidosa* and diatom *Navicula minima* on the Emerson enhancement effect as a function of wavelength of light (with far-red light background) showed that light absorbed in one form of chlorophyll *a*—Chl *a* 670 —enhances photosynthesis in far-red light (absorbed in Chl *a* 680). This leads us to believe that for complete photosynthesis, two forms of chlorophyll *a*, Chl *a* 670 (the form that receives energy from accessory pigments), and Chl *a* 680, need to be simultaneously excited. This is a more satisfactory picture, since in it, the high efficiency of energy transfer from accessory pigments to chlorophyll *a* becomes understandable. Whether all the Chl *a* 670 belongs to one pigment system and all the Chl *a* 680 belongs to the other system is not yet clear. A comparison of the action spectrum of enhancement effect[30] in Chlorella with the absorption bands of chlorophyll *b* Chl *a* 670, and Chl *a* 680 (obtained by analysis of the main absorption band into its components) suggests that about 50% of Chl *a* 670 may belong to the same system as does Chl *a* 680. In red algae and blue-green algae, the Emerson enhancement effect shows

*Quantum efficiency (or quantum yield) of photosynthesis is defined as the molecules of O_2 evolved per quantum of light absorbed. It is the inverse of "quantum requirement".

*The "energy traps" are special chlorophyll molecules that receive energy from other pigments, including other chlorophyll molecules, and are responsible for the active primary photochemical reactions (see section IV).

*Maximum quantum yield is obtained when rate of O_2 evolution increases linearly with light intensity.

The enhancement (E) was calculated as

$$E = \frac{RO_2 \text{ (in combined beams)} - RO_2 \text{ (short wave beam)}}{RO_2 \text{ (far-red beam)}}$$

where RO_2 stands for the rate of O_2 evolution; E as a function of wavelength of light is referred to as the action spectrum of the Emerson enhancement effect.

a very small peak due to Chl *a* 670, suggesting that most (but not all) of Chl *a* 670 belongs to the same system as Chl *a* 680.

The above discussion suggests that photosynthesis requires the cooperation of two light reactions that are sensitized by different pigments. Whether this cooperation is physical (*i.e.* interaction between chemical products) in nature is the next question.

Physical vs. Chemical Cooperation:

James Franck[20] in 1958 discussed the implications of the Emerson enhancement effect by suggesting that photosynthesis may require a physical cooperation of two light quanta—one act of absorption raises the electrons first into the singlet* excited state, which may then fall to the triplet state, and another act of absorption raises the electrons in this triplet state to a higher energy state. The idea of physical cooperation was later abandoned because it was discovered that the enhancing effect persists for several seconds. In red algae the O_2 evolution from a flash of green light absorbed in phycoerythrin is enhanced if it is preceded by that of far-red light (absorbed in Chl *a* 680); a product is made in far-red light that has a half-life of approximately 18 seconds. Observation of enhancement in alternating light suggests a chemical interaction of products made by the two lights (see discussion in[31]). For example, one could suggest that light absorbed in phycoerythrin after it is transferred to Chl *a* 670 and finally to an "energy trap" initiates a light reaction, and the products formed by the two reactions somehow chemically cooperate to produce complete photosynthesis. Information concerning the mechanism and the nature of the products, the intermediates and the reactions, are discussed below.

III. The Hill and Bendall Hypothesis and Five Years Later

Robert Hill and F. Bendall proposed in 1960 a working hypothesis that gave an important role in two light reactions to cytochromes (iron-containing tetrapyrrolic-protein compounds). One of the two proposed light reactions is the oxidation of water (redox potential, $E_0{}^+ = +.81$ eV for O_2/H_2O couple) and the reduction of a cytochrome (cytochrome b_6, $E_0 = 0_3$eV for cytochrome b_6/reduced cytochrome b_6 couple) and the other is the oxidation of another cytochrome (cytochrome f; $E_0 = +.37$ eV for cytochrome f/reduced cytochrome f couple), and reduction of an intermediate $E_0 \geqslant -0.4$ eV) that ultimately reduces CO_2. The reduced cytochrome b_6 and oxidized cytochrome f react together and complete the cycle. This reaction—an exergonic reaction—can be coupled with phosphorylation, *i.e.* production of a molecule of ATP (adenosine triphosphate) from ADP (adenosine diphosphate) and inorganic phosphate —an endergonic reaction.

L.N.P. Duysens and co-workers discovered that when red light absorbed in chlorophyll *a* is used to illuminate red algae, an oxidation of a cytochrome is clearly observed; but if a second beam of light—green light absorbed primarily in phycoerythrin—is added, a reduction of the oxidized cytochrome takes place. (These measurements were made with the technique of difference spectroscopy*.) This was the most direct evidence for the role of cytochromes in photosynthesis.

Figure 1 shows the Hill-Bendall hypothesis for photosynthesis which has been modified to incorporate information from the schemes and researches by several groups of investigators. Photosynthesis is shown here as a set of *five* reactions, two of which are the light reactions—I and II—and three are dark reactions. In this scheme, the two light reactions are in series. (An alternate hypothesis in which two light reactions occur in parallel, has also been proposed[21] but we will not discuss it here). Since in the steady state all reactions must operate simultaneously, the numbering is arbitrary. We begin with reaction II (Duysens' terminology), the reaction closely associated with O_2 evolution.

Light Reaction II; Reduction of an —0.16 *e Volt Oxidant* (Y *to* Y^-) *and Oxidation of* +0.8 *e Volt Reductant* (Z *to* Z^+)*:*

*A singlet excited state is one in which the electron in the excited state has the opposite "spin" to that in the ground state, whereas in the triplet state the electron has parallel "spin" to that in the ground state.

*The redox potential measures the relative tendency of an oxidant to act as electron acceptor and the corresponding reductant to act as electron donor with respect to the hydrogen electrode, where the reaction is: $1/2H_2 \rightarrow H^+ + e^-$, the potential of which is arbitrarily set at zero.

*In difference spectroscopy, a change in absorption is caused by strong illumination (actinic light) given at right angles to the measuring light (weak light). By selecting the wavelength of measuring light one can study changes in different compounds, and by changing the wavelength of actinic light, one can excite different pigments. (Rabinowitch introduced this technique to photochemistry; there are several types[32,33] of difference spectrophotometers in use these days).

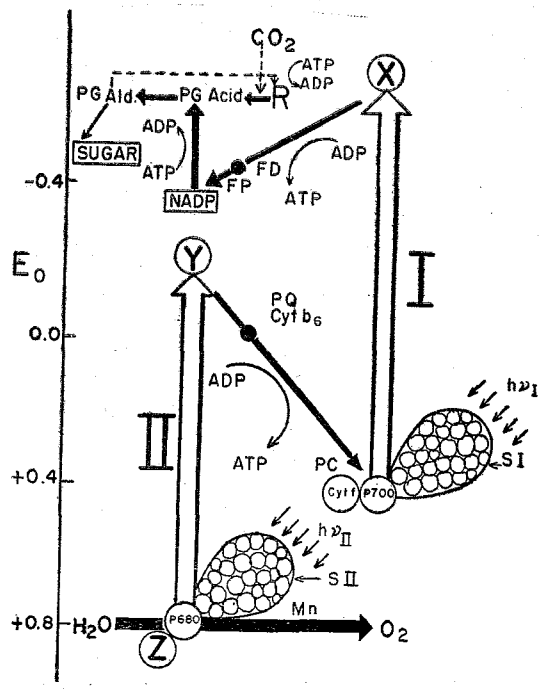


Figure 1. *The Sequential Two Step Model for the Transfer of H-atoms (or Electrons) in Photosynthesis.*

(The scale on the left margin is for the oxidation-reduction potential (E_0) of the intermediates). The two light reactions (I and II) are represented by the long vertical arrows. The primary H-donor of light reaction II is Z and primary H-acceptor is Y, whereas the primary H-donor of light reaction I is "P700" (special chlorophyll *a* molecules absorbing at 700 mμ) and the primary H-acceptor is X (the light reactions are oxidation-reduction reactions). The reaction I is sensitized by light ($h\nu_I$) absorbed in pigment system I (S_I; containing mainly chlorophyll *a*—Chl *a* 680); the "P700" being the energy trap for this reaction. The reaction II is sensitized by light ($h\nu_{II}$) absorbed in pigment system II (S_{II}; containing accessory pigments and some short-wave form of chlorophyll *a*); the hypothetical energy trap is P680 (see bottom of the figure). The evolution of oxygen (O_2) is by a reaction of oxidized Z(formed by light reaction II) with H_2O; this requires Mn containing enzymes and can occur in dark (see lower horizontal arrows). The transfer of H-atoms (or electrons) from the reduced Y to P700 is by a set of dark reactions (see slanting arrow in the center of the figure. The intermediates in this downhill process are plastoquinone (PQ), cytochrome b_6(Cyt b_6), plastocyanin (PC) and cytochrome f(Cyt f). This transfer is coupled with phosphorylation-production of adenosine triphosphate (ATP) from adenosine diphosphate (ADP). The transfer of H-atoms from the reduced X to nicotinamide adenine dinucleotide phosphate (NADP) occurs via ferredoxin (FD) (see slanting arrow in the upper part of the figure). A reductase called ferredoxine-NADP reductase, which is a flavoprotein (FP) is required for the transfer of H-atoms from FD to NADP. It is suggested here that phosphorylation may also be coupled to the transfer of hydrogen atoms from X to FD. The reduced NADP is used to reduce Phosphoglyceric acid (PG acid) to phosphoglyceraldehyde (PG Ald). Sugar is produced from PG Ald. (see left hand corner of the figure. Ribulose diphosphate (R) is the primary acceptor of carbon dioxide (CO_2). The ATP's produced in the earlier reactions are also utilized in the Calvin cycle.

Light absorbed by accessory pigments (such as chlorophyll *b* in green plants, and phycobilins in red and blue-green algae) is ultimately transferred to certain* chlorophyll *a* molecules—referred to as P680, which is assumed to be in a favourable condition for acting as an "energy trap" in a "reaction center". The evidence for an "energy trap" for reaction II is rather weak; its existence is suggested by a difference fluorescence band at 693 mμ in *Porphyridium* and *Anacystis* observed in our laboratory[34] and by the occurrence of an emission band at 696 mμ appearing at —196°C. The "beetle-shaped" structure in figure 1, labelled SII, represents the group of pigments that collect and transfer energy to the "trap" for reaction II; SII stands for pigment system II. The evidence for the participation of chlorophyll *b* and Chl *a* 670 comes from the study of the action spectra of the Emerson enhancement effect, and from observations of reaction II when reaction I is poisoned, or saturated with light.

At the energy trap—P680—the following set of reactions is suggested:

$$P680 + h\nu_{II} \rightarrow P680^*$$

$$P680^* + Z + Y \rightarrow [Y^-] + P680 + Z^+$$

$$Y^- + (\text{Cyt } b_6)\ Fe^{3+} \rightarrow Y + (\text{Cyt } b_0)\ Fe^{2+}$$

In the above set of equations, P680 is the assumed energy acceptor in system II; (*indicates that the molecules are in the excited state); $h\nu_{II}$ is light quantum absorbed in system II; Z is the primary reductant of photosynthesis (its identity is not yet known); Y is the unknown primary oxidant of reaction II. It may have an oxidation-reduction potential of —0.16, ~~as suggested by some recent experiments of B. Kok.~~ Ferricytochrome b_6, (Cyt b_6) Fe^{3+}, is assumed to be reduced in dark by Y to ferrocytochrome b_6, (Cyt b_6) Fe^{2+}. This set of reactions achieves the oxidation of the primary reductant, Z, and reduction of cytochrome b_6. The oxidation-reduction (O–R) potential of Z/Z^+ couple should be above +0.81 electron volts to allow Z^+ to evolve O_2 from H_2O by a dark reaction. The O–R potential of the couple ferricytochrome b_6/reduced ferrocytochrome b_6 is around 0.0 volts. This set of reactions is represented by the lower long vertical arrow in figure 1.

We assume that for light reaction II, the energy trap is a pigment molecule that has an absorption band near 680 mμ; P stands for pigment and 680 denotes the absorption maxima, in mμ. For light reaction I, there is another energy trap called "P700".

A quantum of 680 mμ light has enough energy (1.8 eV) to transfer H-atoms from water to CO_2; but the transfer that is achieved by this reaction stores about +0.8 eV, *i.e.* about 50% of the quantum energy as chemical bond energy; the rest is wasted.

There is enough cytochrome b_6 in chloroplasts to perform these reactions, but so far, no direct evidence has been provided for its participation. However, evidence has accumulated for the reduction of plastoquinone (PQ) by reaction II. Duysens suggests that reaction II reduces a compound Q to QH in order to explain the quenching of fluorescence[35,36] by far-red light absorbed in system I upon excitation of fluorescence by light absorbed in system II. Whether the primary reaction in system II is reduction of Duysens' "Q" (or plastoquinone) or of cytochrome b_6 is not yet clear. Since all these oxidants have a potential of about 0.0 eV, if any of them is reduced, others can be reduced by dark reactions. It is also not definite whether "Y" is different from the abovementioned primary oxidants.

Dark Reaction : Evolution of O_2

This set of reactions leading to evolution of O_2 can be represented as:

$$Z^+ + H_2O \xrightarrow{\text{Mn Enzyme}} Z + 1/4\ O_2 + 1/2\ H_2O$$

This reaction is represented by the lowest dark horizontal arrow in figure 1.

According to this formulation, evolution of oxygen is not a light reaction; in dark, however, all Z remains in the reduced form, the oxidant Z^+ is produced only by light reaction. It should be possible to evolve O_2 by just one light reaction (II), at least temporarily, as this will stop as soon as all the oxidant for reaction II is used up. Such an O_2 evolution has been observed. For continuous O_2 evolution, the other light reaction is necessary to oxidize the reductant produced by reaction II.

The evolution of O_2 may occur in a series of steps. The mechanism is a complete mystery as yet. All we know[21] is that somewhere a Mn enzyme is needed for the evolution of O_2.

Dark Reaction: Phosphorylation and Reduction of Cytochrome f

The weak reductant, Y (or the reduced plastoquinone, or Duysens' "Q" or a reduced cytochrome b_6, whatever it may be), can react with cytochrome f (+.37 eV), reducing the latter and getting itself oxidized. There is enough energy released in this downhill reaction that phosphorylation can be coupled with it. Recently evidence has been provided for this coupling. The electron (or hydrogen atom) transfer may involve other electron transport carriers, *e.g.*, the copper protein *plastocyanin*[37] (PC) has been recently implicated[37]. The set of reactions is shown by the slanting down arrow in the middle of figure 1. This set of reactions can be represented as:

where iP=inorganic phosphate, ADP= adenosine diphosphate, ATP=adenosine triphosphate, (Cyt b_6) Fe^{2+} is reduced cytochrome b_6, and (Cyt f) Fe^{3+} is oxidized cytochrome f.

Light Reaction I : Reduction of an —0.6 eV Oxidant, X, and Oxidation of a +0.4 eV Reductant

Light primarily absorbed by chlorophyll *a*—in particular Chl *a* 680—is transferred to a special form of chlorophyll *a*—P700, discovered by Kok by difference spectroscopy[38]. This is the "energy trap" for light reaction I. There is one "P700" (and one cytochrome f molecule) per 300 chlorophyll molecules. (On the contrary, Franck and Rosenberg[13] have suggested that there may be only one "reaction center", capable of performing *both* light reactions—once in the singlet excited state and another time in the triplet state. In their hypothesis, the reaction center is a chlorophyll molecule complexed with a cytochrome.) The pigment system for reaction I is labelled SI in figure 1. The nature of the pigments sensitizing this reaction have been found by action spectra measurements, by poisoning or saturating reaction II, by providing external sources of H-atoms (O–R potential +0.4 eV), or by the use of H-adapted algae or mutants of algae. The primary reaction is a photooxidation of P700. The reactions represented by the upper long vertical arrow and the upper slanting down arrows in figure 1 is :

$$P700 + h\nu_f + X \rightarrow X^- + P700^+$$

$$P700^+ + (\text{Cyt f})\ Fe^{2+} \rightarrow (\text{Cyt f})\ Fe^{3+} + P700$$

$$X^- + FD \rightarrow \text{reduced (FD)} + X$$

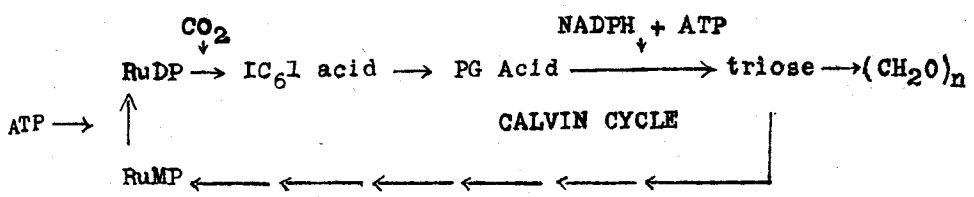
$$(\text{Cyt } b_6)\ Fe^{2+} + (\text{Cyt f})\ Fe^{3+} + ADP + iP \xrightarrow{\text{"Phosphorylation"}} ATP + (\text{Cyt f})\ Fe^{2+} + (\text{Cyt } b_6)Fe^{3+}$$

$$\text{reduced (FD)} + NADP \xrightarrow{\text{FD-NADP reductase}} NADPH + (FD)$$

It has been suggested that X may be ferredoxin (FD) (previously known as photosynthetic pyridine nucleotide reductase PPNR). However, it has recently been suggested[39], on the basis of reduction rates of different viologen dyes having different oxidation-reduction potentials, that chloroplasts may produce a reductant X^- with an oxidation-reduction potential of as low as —0.6 eV (ferredoxin has O—R potential of only —0.4 eV). X^- can reduce ferredoxin by a dark reaction, and the latter can reduce NADP (nicotinamide adenine dinucleotide phosphate) with the help of a flavoprotein enzyme now called "ferredoxin—NADP reductase" (see reference 3). There is enough energy available in the oxidation of "X" by ferredoxin that it could be coupled with another phosphorylation. However, as yet, there is no experimental evidence for it. By the above set of reactions, cytochrome f is oxidized to complete the cycle, and reduced NADP (referred to as NADPH) is produced.

Dark Reaction: Reduction of CO_2

This is the set of dark reactions that accomplishes the reduction of CO_2. It is the well-known cycle discovered by Calvin, Benson, Bassham and coworkers at Berkeley. We will not discuss this aspect in detail here (see references[40]). The reduced NADP, the end product of light reactions of photosynthesis, is used to reduce an acid [phosphoglyceric acid, (PG Acid) or a C_6 acid made from addition of CO_2 to a ribulose diphosphate, RuDP, producing an aldehyde, phosphoglyceraldehyde, or triose) which, by further reactions, makes carbohydrates $[(CH_2O)_n]$. The cycle is continued by triose molecules undergoing a series of conversions which result in ribulose monophosphate (RuMP) which, upon phosphorylation, produces the acceptor of CO_2—the ribulose diphosphate. These reactions are summarized below (see also top part of figure 1):



born in the experiments of Robert Emerson and William Arnold in 1932. They discovered that when bright and short (10^{-5} sec.) flashes of light are given to Chlorella, a maximum of one O_2 molecule is produced per flash per 2400 chlorophyll molecules present in the algae. Since four H-atoms (or electrons) are moved from H_2O to CO_2 to produce one O_2 molecule, about 600 chlorophyll molecules must cooperate to transfer one H-atom. As this H-transfer occurs in two steps, and two reactions are involved, at least 300 chlorophylls must cooperate in one primary reaction of photosynthesis. This is then the size of our "conceptual" photosynthetic unit. On the average, there must be one "reaction site" per 300 chlorophyll molecules.

It has been pointed out that in weak light, when photosynthesis is efficient (*i.e.* one O_2 molecule is evolved per eight quanta of light absorbed), on the average, one chlorophyll molecule absorbs light every eight minutes or so, and this means that for the same molecule to absorb eight quanta of light, it would take about one hour; but we know that when plants are transferred from darkness to light, O_2 evolution starts right away. One can then assume that quanta absorbed in different chlorophyll molecules are somehow assembled in one spot. This was the beginning of the concept of energy transfer in photosynthesis[41].

Jan B. Thomas and co-workers[42] discovered that the ability of fragments, made from chloroplasts, to evolve O_2 remains constant with decreasing size of the fragments until the size becomes so small that each particle holds less than 300 chlorophyll molecules; this is when rate of O_2 evolution suddenly declines. This, too, suggests a photosynthetic unit of about 300 chlorophyll molecules.

The surface of the chloroplast membrances is not smooth but shows a "cobblestone" appearance[43]. Recently, such particles seen on electron micrographs have been named

IV. Photosynthetic Unit

The concept of "photosynthetic unit" comprising several hundred pigment molecules cooperating to perform photosynthesis, was

"quantasomes"[44]. Calculations suggest that one quantasome may carry about 150-200 chlorophyll molecules. It is likely that these structural units (quantasomes) may be the photosynthetic units—perhaps there may be

two kinds of quantasomes, one for system I and the other for system II!

A physical separation of two pigment may be possible by the method of solubilization with a detergent followed by fractionation by differential centrifugation. Chloroplast fractions prepared by the detergent solubilization method show very different fluorescence characteristics (at —196°C) in fractions supposed to perform reactions I or II; the fractions performing system II have a prominent fluorescence peak at 696 mμ, while the system I fractions have a prominent peak at 720 mμ, both at —196°C.

V. The Three Events: Light Absorption, Exciton Migration and Primary Reactions

First Event: Light Absorption

The first act of photosynthesis is the absorption of light by accessory pigments or chlorophylls. The transition of a valence electron from its ground state to an excited state occurs within 10^{-15} sec. This transition is governed by the well-known Franck-Condon principle (*i.e.*, the transition probability is greatest when change in internuclear separation is minimal) and by the rules of quantum mechanics, including the requirement that the spin of the electrons is not altered. The absorption of light is a quantized process.

The Life Time of Excited State

Two groups of investigators[45] report the lifetime of chlorophyll *a* in solution to be about 6.0×10^{-9} sec. (10^{-9} sec.=a billionth of a second) and that in living cells to be 1.2 to 1.6×10^{-9} secs. This is a very short time. The excited chlorophyll molecule can lose its excitation by many ways (*a*) by fluorescence (fast emission), (*b*) complete internal conversion to heat, (*c*) energy transfer to another molecule, (*d*) a photochemical reaction, (*e*) transfer to a triplet state (or another low lying metastable state) which may have a greater life time ($=10^{-3}$ secs.). A metastable state may, in turn, lose energy in a photochemical reaction, by heat loss, by "phosphorescence", by delayed light emission (by kicking up of the electron to singlet excited state by means of thermal quanta and loss from the singlet excited state) and by energy transfer through triplet-triplet interaction. For photosynthesis, we are interested in the reactions that lead to energy transfer, ultimately to the energy trap (or reaction center), where the primary reactions of photo-synthesis occur.

Second Event: Exciton Migration

That light energy absorbed by accessory pigments is transferred to a form of chlorophyll *a* is shown by the discovery of "sensitized fluorescence" of chlorophyll *a*. When chlorophyll *b* is excited, fluorescence from chlorophyll *b* is absent, but what is observed is the fluorescence of chlorophyll *a*, suggesting that energy has been transferred from *b* to *a*. The mechanism of such a transfer is probably the one called resonance transfer or exciton* migration.[46] The probability of resonance transfer depends upon the extent of overlap of the fluorescence band of the donor molecule with the absorption band of the acceptor molecule, and the distance between the donor and the acceptor molecule; if the distance is r, the probability of transfer goes with r^{-6} in one theory. By this mechanism, the energy is transferred from accessory pigments that have higher excited states to chlorophyll *a*, that has a lower excited state.

The energy transfer between molecules of the same kind, *i.e.*, between chlorophyll *a* molecules, is demonstrated by the high efficiency of the primary reaction when chlorophyll *a* is excited, and by the fact that when polarized light is used to excite chlorophyll *a*, there is an almost complete depolarization of fluorescence. This can be explained if we assume energy transfer between chlorophyll *a* molecules.

The mechanism of energy transfer between chlorophyll *a* molecules may be different than that from chlorophyll *b* to *a*. It has been suggested that because of the ideal resonance, the probability of this transfer must be proportional to r^{-3} where r is the distance between the donor and the acceptor molecule, rather than to r^{-6} (see discussion in references [2,14,43,47].

Electron Transfer

Several investigators have suggested an alternative picture for the transfer of energy. This assumes that light causes the separation of "electrons" and "holes" and the two move independently of each other; electrons reduce CO_2 in one spot and "holes" react with H_2O and evolve O_2 in another. This picture has several difficulties, if it is assumed that this is the main mechanism. The quantum yield of photoconductivity (a necessary concommitant of such a mechanism) is much too low ($=10^{-3}$). Also this picture gives no explanation of sensitized fluorescence *in vivo*.

*Exciton is an electron in the excited state coupled strongly with its hole.

It has been proposed[2,5] that there is energy migration or exciton migration followed by a "hole migration"—the latter occurring after the "energy trap" has first received an energy quantum by energy migration, and has reacted with an electron acceptor, thus becoming a "hole".

Distribution of Quanta in Two Systems

It is not yet clear how energy absorbed by any pigment molecule is channeled to perform light reactions I or II. There are two hypotheses. In one, called "spill-over" hypothesis, whenever system II is excited, energy "spills-over" to system I, and there is thus a balanced excitation of both systems. (This is possible because system II absorbs on the short-wave side of system I.) Whenever system I absorbs light, energy is not transferred to system II because of the lower excitation level of system I. In the alternate "separate package" hypothesis, light energy is transferred within the two systems I and II, but not between them, as it is assumed that they are specially separated; each system contains both chlorophylls and accessory pigments, but in widely different proportions. (These two hypotheses have been recently discussed by several investigators[25,31].)

The Third Event: Primary Reactions

At the energy traps, there is separation of oxidants (positive charge) and reductants (negative charge). The primary light reactions of photosynthesis are oxidation-reduction reactions involving electron transfers or hydrogen-atom transfers. The primary reactants (oxidants and reductants) have been characterized by absorption and fluorescence spectroscopy; their biochemical characterization must await further studies (see section III). The reactions following these events, such as the formation of adenosine triphosphate and the reduction of pyridine nucleotide, are secondary reactions.

VI. Summary

Photosynthesis is a unique process on earth, in which energy of sunlight is massively converted into chemical energy. All life draws upon this energy source; it is a one-way traffic. Photosynthetic plants contain "photosynthetic units". Each unit is composed of several hundred pigment molecules (accessory pigments and chlorophylls) and one or two reaction centers (or energy traps). Light energy absorbed by any molecule in the photosynthetic unit is ultimately transferred to the "energy traps" by energy transfer or exciton migration (electron transfer may also be involved). The primary reactions are oxidation-reduction reactions (electron or H atom transfers) and they occur at the energy traps. Recent experiments, beginning with the discovery of the "enhancement effect" (discovered by the late Robert Emerson at Urbana), have led to a picture that suggests the operation of *two* light reactions in photosynthesis. In one hypothesis discussed here, reaction II is (the ultimate) oxidation of water to oxygen molecules, and (the ultimate) reduction of cytochrome b_6, reaction I is the (ultimate) reduction of carbon dioxide via reduced nicotinamide adenine dinucleotide phosphate and oxidation of cytochrome f; the two reactions (I and II) occur in series, and the cycle is completed by the reduction of cytochrome f oxidized by reaction I by chrochrome b_6 reduced in reaction II; the production of adenosine triphosphate, which is a dark reaction, is perhaps coupled to this exergonic reaction. Another site for the production of adenosine triphosphate may be when the primary acceptor (X) of reaction I (in its reduced form) reduces nicotinamide adenine dinucleotide phosphate in another exergonic reaction. The primary oxidants and reductants of the two reactions are still not definitely known.

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The attached reprint entitled "Modern Trends in Photobiology: Energy Conversion in Photosynthesis" has many errors. (The proofs were not sent to the author.)

The errors are listed below (see Errata). The pages referred to in the errata are those in the reprint; the page 1 is page 468 of the article, page 2 is page 469 of the article, and so on.

ERRATA

(1) The equation (Cyt b_6) Fe^{2+} + (Cyt f) Fe^{3+} + ADP + iP⟶ATP + (Cyt f)Fe^{2+} + (Cyt b_6)Fe^{3+} does not belong on the last-but-one line on page 5. It should follow line 16, column 2, p. 5.

(2) The second major error is in the lay-out of footnotes. The same symbols have been used on the same column on the same page. These errors are clearly seen on pages 1-4.

(3) The third major error concerns the elimination of certain references by the editorial board. At many places references to the original discoveries have disappeared; this is very embarrassing to the author.

(4) Other specific errors are listed below:

Page 1, column 1, line 18: the end of parenthesis is not shown.

Page 1, column 1, footnote, line 4: "This articles was..", should be "This article was...".

Page 1, column 2, line 1: only reference 16 should be referred to here.

Page 1, column 2, line 20: semicolon should be replaced by a comma.

Page 1, column 2, footnote, line 6: "an an H-atom transfer..." should read "as an.."

Page 3, column 2, line 7: "Eo = 0_3eV" should read "Eo = 0.0 eV".

Page 3, column 2, line 21: "L.N.P. Duysens" should be "L.N.M. Duysens".

Page 4, legend of figure 1, line 22: "arrows" should be "arrow".

Page 4, column 2 (see equation, line 3): (Cyt b_0)Fe^{2+} should be (Cyt b_6)Fe^{2+}.

Page 4, column 2, lines 37-38: comma after -0.16 should be a period. Change -0.16 eV to -0.2 eV. Delete "as suggested... B. Kok". Insert the following explanation for Y/Y^- having Eo = -0.2 eV: "Light reaction I overcomes a potential of 1.0 eV (Eo of $P700/P700^+$ = +0.4 eV and of X/X^- = -0.6 eV). It is suggested that light reaction II has the same efficiency; it also overcomes a potential of 1.0 eV (Eo of H_2O/O_2 = +0.8 eV; thus Eo of Y/Y^- should be -0.2 eV)."

Page 6, column 1, line 32: delete the square bracket.

Page 6, column 1, line 36: "or triose)" should read "(or triose)".

Page 8, column 1, lines 23-24: "specially" should read "spatially".

Page 9, reference no. 16: "The Major"..." instead of "The Mayor...".

Page 9, reference no. 23: "Berlin" instead of "Verlin".

Page 9, reference no. 30: (a) is not necessary.

Page 9, reference no. 46: Forster should have umlaut on "o".