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The Photochemical Stage of Photosynthesis

GOVINDJEE & E. RABINOWITCH
Departments of Botany & Biophysics, University of Illinois, Urbana, Illinois, USA

THE three aspects of photosynthesis, viz. air improvement (i.e. oxygen liberation), energy storage and organic synthesis, are associated with three distinct stages of the overall process. This tripartite nature of photosynthesis is concerned with three stages: (1) production of oxygen molecules by the removal of hydrogen (atoms) from water, (2) the transfer of hydrogen atoms from an intermediate in stage 1 to an intermediate in stage 3, and (3) the conversion of carbon dioxide to a carbohydrate. All these reactions occur within tiny cell organelles called chloroplasts[1]. Stage 1 is the least known part of photosynthesis. It occurs by a non-photochemical reaction and requires at least one manganese-containing enzyme[2,3]. The third stage includes a series of enzymatic reactions. It is the best known part of the overall process — mainly due to the pioneering and extensive studies with the carbon isotope tracer ^{14}C by Benson, Bassham and Calvin (*see* Bassham[4]). The present article deals mainly with the second stage — the energy storage part — the photochemical stage of photosynthesis. This stage is now beginning to yield some of its secrets to biophysical and biochemical research. This is the part of photosynthesis of greatest concern to photochemists and photobiologists because in it the power station function of photosynthesis is fulfilled.

Photosynthesis: An Oxidation-Reduction Reaction

That the photochemical part of photosynthesis is an oxidation-reduction reaction was first recognized by Van Niel[5]. These are reactions in which hydrogen atoms (or electrons) are transferred from a donor (reductant), which is thus oxidized, to an acceptor (oxidant), which is thus reduced. The transfer of hydrogen atom may be replaced by that of an electron. An electron transfer, combined with acquisition or loss of an H^+ ion from the aqueous medium, can become equivalent to the transfer of H-atom. Some stages in a complex redox reaction chain — such as those involved in photosynthesis — may be electron transfers; others, hydrogen atom transfers.

In photosynthesis, hydrogen atoms (or electrons) are moved uphill against the chemical potential with the help of light energy. Light energy is stored in the form of chemical energy. Chloroplasts (the microscopic chlorophyll-bearing particles in photosynthesizing plant cells) act as chemical pumping stations; they can also be described as 'savings banks' of life energy.

A measure of chemical energy stored in the transfer of hydrogen atoms (or electrons) from a certain donor to a certain acceptor is the difference of their oxidation-reduction (or redox) potentials (ΔE_0). The higher (more positive) the E_0 value, the stronger is the oxidant; vice versa, a low (negative) redox potential indicates a strong reductant. (Note that a redox potential always belongs not to an oxidant — or a reductant — as such, but to a certain oxidation-reduction couple; thus, oxygen has a potential of +0·81 eV. when it is reduced to H_2O, but only +0·27 eV. when it is reduced to H_2O_2.)

In ordinary chemistry, when two oxidation-reduction couples are brought together, the one containing the stronger oxidant oxidizes the one containing the stronger reductant. In photosynthesis, however, a weak oxidant (CO_2) oxidizes a weak reductant (H_2O), producing a strong oxidant (O_2) and a strong reductant $(CH_2O)_n$; this energy-consuming reaction is made possible by the massive investment of light energy.

The amount of energy stored in photosynthesis is determined by the difference between the redox potentials of the couple O_2/H_2O (+0·8 eV.) and $CO_2/(CH_2O)$ (−0·4 eV.). In the transfer of a single electron (or H-atom) from water to carbon dioxide, the amount of energy stored is $+0{\cdot}8-(-0{\cdot}4) = 1{\cdot}2$ eV. Now, four electrons (or four H-atoms) are needed to reduce one molecule of CO_2 to a carbohydrate (*see* equation below); the total energy of their transfer is $1{\cdot}2\times4 = 4{\cdot}8$ eV. Energy conversion tables tell us that 1 eV. is equivalent to 23 kcal./mole; therefore, the energy stored in the redox stage of photosynthesis can be also expressed as $4{\cdot}8\times23 = 110$ kcal. (per mole of reduced CO_2). We can thus write the equation

$$O{=}C{=}O + 2H_2O \rightarrow \overset{H}{\underset{|}{O}} - \overset{H}{\underset{|}{C}} - \overset{H}{\underset{|}{O}} + O_2$$

$$[\rightarrow (CH_2O) + H_2O] + O_2 + 110 \text{ kcal.} \quad \ldots(1)$$

[That the hydrogenation product, $CH_2(OH)_2$, loses a molecule of water and becomes $(CH_2O)+H_2O$ is not important for the energy balance.]

To sum up, the energy-storing stage of photosynthesis is an oxidation-reduction reaction in which about 110 kcal. are stored per four hydrogen atoms transferred from an intermediate (which we shall call ZH) in stage 1 to an intermediate (which we shall call X) in stage 3.

Robert Hill discovered (*see* Hill & Scarisbrick[6]) that upon destruction of the cell, releasing a suspension of whole or fragmented chloroplasts, the capacity of the cell for photo-oxidation of water remains, while its capacity for the photoreduction of carbon dioxide is lost. Substitute oxidants (a ferric salt, a quinone or a dye) have to be supplied to the chloroplast suspension to liberate oxygen in light (so-called Hill reaction). The capacity to reduce CO_2 can be regained, as shown by Thomas and coworkers[7], by adding certain enzymes and cofactors; but even after such reconstruction, the actually observed carbon dioxide-reducing capacity

of the chloroplast preparation is only a few per cent of that of the intact cell.

The photochemical process in photosynthesis is, according to our belief, not a 'photolysis of water' (H_2O + light → [OH] + [H]) — now a widely used formulation; nor is it a 'decomposition of carbon dioxide' (CO_2 + light → C + O_2) as has been often assumed in the past; rather, it is an energy-storing step (or steps) in the transfer of hydrogen atoms (or electrons) from H_2O to CO_2.

The Primary Photochemical Products

The nature of the primary donor (ZH) and primary acceptor (X) and thus also of the primary photochemical products, Z and XH, is not definitely known. There is a great deal of evidence suggesting that X may be that well-known cellular catalyst, NADP (nicotinamide adenine dinucleotide phosphate; formerly called TPN (Triphosphopyridine nucleotide); and that the reduced form of this compound, NADPH, feeds hydrogen into the CO_2-reducing enzymatic reaction sequence. The redox potential of the couple NADP/NADPH (E_0 = −0·32 V.) is not sufficiently negative to reduce the couple $CO_2/\frac{1}{n}(CH_2O)_n$ (E_0 = about −0·4 eV.). However, light is known[8,9] to produce in chloroplasts adenosine triphosphate (ATP) — an ubiquitous cellular energy carrier, able to release about 8 kcal./mole when it is hydrolysed. With the help of ATP as a booster, NADPH can overcome its insufficient reducing power, and initiate the reduction of carbon dioxide; the now widely accepted scheme of photosynthesis suggests that this is what actually happens. Calvin's investigations of the reaction sequence leading from CO_2 to (CH_2O) in photosynthesis suggested that ATP is needed not only in this reduction step, but also in the phosphorylation of ribulose monophosphate to ribulose diphosphate. Whether the yield of ATP-production in light by chloroplasts is high enough to supply the ATP molecules needed according to this reaction scheme is an open question.

Recent evidence has indicated that the first reduced product in photosynthesis may not be NADPH, but a certain iron protein ('ferredoxin') with a somewhat more negative potential (E_0 = −0·42 V.), which then reduces NADP by a secondary, dark reaction[10-12]. Recently, Kok[13] has suggested that 'X' may be a compound with an E_0 value as low as about −0·6 eV.

The Two Photochemical Steps

We now ask: What is known about the process by which hydrogen atoms (or electrons) are moved, with the help of light, from the donor, ZH, to the acceptor, X?

An important new development in this field is the finding that this process apparently includes two successive photochemical steps; and that cytochromes (a type of iron-porphyrin-protein complexes well known from their catalytic role in respiration) are the likely intermediates between them.

That two light quanta may be used to move one hydrogen atom in photosynthesis was first suggested by Franck and Herzfeld[14] and elaborated by Rabinowitch[15]; it was then merely a hypothesis, based on Emerson's quantum yield measurements of photosynthesis, which indicated that eight may be the minimum number of light quanta required to transfer four hydrogen atoms needed to reduce one molecule of CO_2 to (CH_2O).

This hypothesis has since received experimental confirmation from several sides.

Red Drop and Emerson Enhancement Effect in Photosynthesis

The first set of these observations pertains to the so-called 'enhancement effect'[16]. Since the measurements of Emerson and Lewis[17], it has been known that the quantum yield of photosynthesis (the number of oxygen molecules evolved per quantum of light absorbed) is approximately constant throughout the spectral region of chlorophyll absorption — except for the far red and the blue end of the spectrum, where it declines. The latter is easily interpreted by the low efficiency of carotenoids as sensitizers for photosynthesis; the red drop is more difficult to explain. In green plants and green algae, this red drop takes place at wavelengths longer than 680 mμ[16,17]. This red drop is clearly seen in the action spectrum of photosynthesis, which is the plot of the quantum yield of a light-produced change as function of the wavelength of light. The red drop occurs in the region where light is absorbed only in chlorophyll *a* and not in chlorophyll *b*. Red marine algae contain only chlorophyll *a*; in addition, they contain a red, water-soluble pigment, called phycoerythrin, which absorbs in the green part of the spectrum; and (in a smaller amount) a blue pigment, called phycocyanin, which absorbs in the orange and red. In red algae, the red drop begins at 650 mμ, that is, in the region where absorption by phycocyanin ends and chlorophyll *a* becomes the only absorbing pigment[18]. It thus appears as if in both cases chlorophyll *a* were an 'inefficient' (or totally ineffective) pigment in photosynthesis — a strange conclusion to make in the face of the fact that chlorophyll *a* is the one common pigment of all photosynthesizing plants!

Emerson and coworkers[16,18-21] made an important discovery. They found that photosynthesis in the region of the red drop can be brought up to full efficiency (i.e. to a quantum yield of the order of $\frac{1}{8}$) by simultaneous illumination with light of a shorter wavelength. For example, the quantum yield of photosynthesis in *Chlorella* in pure 700 mμ light is only 0·06, while that in pure 650 mμ light is 0·12; when the two light beams are given simultaneously, the quantum yield becomes 0·12 for the total light. This has become known as the 'Emerson effect'[21,22].

This observation led Emerson[19] and Emerson and Chalmers[20] to the suggestion that photosynthesis comprises two photochemical processes, of which only one can be brought about by light absorption in chlorophyll *a*, while the other requires light absorption in another pigment — chlorophyll *b* in green algae, phycocyanin or phycoerythrin in red algae, etc. Further development of this point of view led to the generalization

that photosynthesizing cells contain two pigment systems, both of which have to be excited by light absorption for efficient photosynthesis. In the largest part of the visible spectrum, pigments belonging to both systems participate in light absorption, thus making efficient photosynthesis possible; but in the far red, above 680 mμ in green cells, and above 650 mμ in red cells — absorption is limited to one pigment system, and this causes the red drop.

This hypothesis called for a systematic study of the yield of photosynthesis in various combinations of monochromatic light beams. Such studies were begun by Emerson and coworkers[20,21]. A constant beam in the region of the red drop (e.g. at 700 mμ) was combined with a second beam of variable wavelength, and the rate of oxygen liberation was measured, first in each beam separately and then in two of them together. In this way, the action spectrum of the Emerson effect could be determined. In each of the four types of algae studied — red, green, blue-green and brown — the action spectrum of the enhancement effect showed peaks in the region where one or the other of the accessory pigments absorbed most strongly.

The conclusion seemed close at hand that of the two postulated pigment systems, one contains chlorophyll *a*, and the other includes all other pigments — which were previously dismissed as accessory, implying their relative unimportance for photosynthesis !

This conclusion was not only startling; it also ran into contradiction with conclusions derived from the study of the fluorescence of plant pigments *in vivo*[23,24]. These studies showed that the fluorescence of photosynthesizing cells is always essentially that of chlorophyll *a*, even when the exciting light is first absorbed by another pigment. Observations of this sensitized chlorophyll *a* fluorescence (i.e. the emission by chlorophyll *a* of light energy first absorbed by another pigment) showed convincingly that light quanta absorbed by chlorophyll *b* are transferred to chlorophyll *a* with practically 100 per cent efficiency; that for phycoerythrin and phycocyanin, the transfer efficiency is only slightly less — of the order of 90 per cent; that for fucoxanthol in diatoms, it is of the order of 80 per cent, etc. We are thus driven to an implausible conclusion that for photosynthesis, the cells need one quantum of light to be absorbed directly in chlorophyll *a*, and one quantum to be absorbed by an accessory pigment, and then transferred, by resonance, to chlorophyll *a* ! How should chlorophyll *a* distinguish between quanta received by direct absorption and quanta received by resonance transfer, and require one of each kind to bring about photosynthesis ?

Two sets of observations helped to clarify this paradox. One was due to the studies by Govindjee and coworkers[22,25-27] and by French and coworkers[28], of the action spectra of the Emerson effect. It was found that this action spectrum contains, in addition to peaks corresponding to the accessory pigments, also a peak or a shoulder at 670 mμ, within the absorption band of chlorophyll *a in vivo*. (This band extends roughly from 660 to 690 mμ.)

This finding suggested that there are two kinds of chlorophyll *a* in green cells — one with an absorption band at 670 mμ, and one absorbing at longer waves. The first form — which was called Chl *a* 670 — must be associated in a common pigment system with the accessory pigments; this is the form that collects excitation energy transferred to chlorophyll *a* by resonance from the accessory pigments, and is the source of sensitized chlorophyll *a* fluorescence. The other form of chlorophyll *a*, absorbing at the longer wavelength, in the region of the red drop belongs to another pigment system (for analysis of absorption bands, *see* Brown and French[29] and Cederstrand[30]).

Since, upon extraction of plant cells, only one chlorophyll *a* is found, the two chlorophyll *a* forms *in vivo* must differ not in their chemical composition, but either in their state of aggregation, or in their association with different partners — proteins, lipoids or other pigments. Which of the factors is most essential remains a controversial subject.

Be this as it may, the new observations suggest that what is needed for photosynthesis is excitation of two types of chlorophyll *a* molecules. One of them can be excited either directly or by resonance transfer of energy from an accessory pigment; while the other has to be excited directly.

This is a much more plausible hypothesis than Emerson's original suggestion that what is needed for photosynthesis is excitation of chlorophyll *a* and of one of the accessory pigments !

According to this picture, a ' balanced ' excitation of the two pigment systems

System I — chlorophyll *a* absorbing in green cells maximally at 680-690 mμ

System II — chlorophyll *a* absorbing in green cells maximally at 670 mμ and accessory pigments (e.g. phycobilins, chlorophyll *b*, etc.)

is needed for effective photosynthesis. According to this hypothesis, monochromatic light should produce photosynthesis with a quantum yield dependent on how well balanced is the excitation of the two systems. The yield should be highest where both systems participate equally in the absorption and dip down whenever one or the other of them receives too much (and the other too little energy); an extreme case of such imbalance is found in the region of the red drop. This hypothesis is generally labelled separate package model[31-33]. This should cause a fine structure of the action spectrum of photosynthesis, which remains to be confirmed and analysed. The dip of the curve at 660 mμ in the action spectrum of photosynthesis in *Chlorella* is one example of such fine structure. An alternative to this hypothesis exists: It postulates resonance energy transfer from pigment system II to pigment system I (but not vice versa !), leading to automatic balancing of excitation between the two systems whenever too much energy is absorbed in system II. This hypothesis is usually labelled spill-over model[31-33]. It would be simpler if this hypothesis were not needed; one could then postulate a spatial separation of the two pigment systems in the chloroplasts.

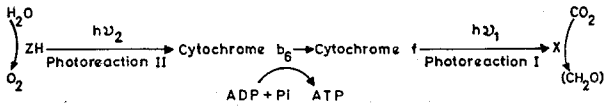
The Role of Cytochromes

The above-described measurements led to the postulation of two photochemical reactions in photosynthesis, brought about by excitation of two forms of chlorophyll *a*; but nothing was said about the nature of these two reactions. Here, a second set of experiments is of great importance, experiments dealing with the transformation of cytochromes in photosynthesis.

Robert Hill and coworkers (*see* Hill[34]) had discovered two types of cytochromes in chloroplasts. One they called cytochrome *f*; it belongs to the type of cytochrome *c* known in respiration. What is most important for us is that it has a positive redox potential, $E_0 = +0{\cdot}42$ eV. The other cytochrome, found in chloroplasts and designated by Hill as cytochrome b_6, belongs to the cytochrome *b* type, and has a potential close to 0·0 eV.

Duysens[35] observed that in illuminated (and, presumably, photosynthesizing) suspensions of algal cells, absorption bands belonging to reduced cytochromes disappear, and bands belonging to oxidized cytochromes appear instead. Upon return to darkness, the change is reversed.

Hill and Bendell[36] made the ingenious suggestion that the two cytochromes, b_6 and *f*, play the role of intermediate carriers in the ' bucket brigade ', carrying hydrogen atoms (or electrons) from ZH to X in a two-step mechanism. The following diagram is a modified version of Hill-Bendell hypothesis:



One photochemical reaction (reaction II) takes a hydrogen atom (or electron) from ZH and transfers it to cytochrome b_6; thus reduced cytochrome b_6 then reacts, in a dark, ' downhill ' reaction, with oxidized cytochrome *f*, reducing the latter. Another photochemical reaction moves the electron from reduced cytochrome *f* to X. The $h\nu_2$ and $h\nu_1$ in the diagram refer to light quanta absorbed in systems II and I respectively. In the downhill part, one ATP molecule can be formed from ADP (adenosine diphosphate) and Pi (inorganic phosphate), storing the released energy, in the same way in which this happens in respiration.

The oxidized Z evolves O_2 from H_2O by a dark reaction (*see* left side of the diagram) and reduced X feeds hydrogen atoms (or electrons) into the ' Calvin sequence ', ultimately reducing CO_2 to the reduction level of a carbohydrate (CH_2O).

This hypothesis was supported by the findings by Duysens *et al.*[37] that in red algae, only light quanta absorbed in ' pigment system II ' (i.e. in phycoerythrin) cause reduction of the cytochrome, while quanta absorbed in system I (i.e. in the longer wave components of chlorophyll *a*) cause its oxidation. The pigment system II can be thus assigned the role of energy supplier for the transfer of hydrogen atoms (or electrons) from ZH to cytochrome b_6. The pigment system I can be similarly assigned the function of sensitizing the hydrogen (or electron) transfer from reduced cytochrome *f* to the acceptor X.

According to this scheme, light absorption in system II must cause a reduction, and light absorption in system I, an oxidation, of all intermediates in the reaction sequence connecting the two photochemical steps.

Duysens and Amesz[38] concluded, from quantitative studies of these phenomena, that the several pigments are not neatly divided between the two pigment systems; rather, some chlorophyll *a* is present also in system II, and some accessory pigments (particularly, phycoerythrin in red algae) in system I. This problem as well as the earlier mentioned one of possible excitation energy transfer by resonance from system II into system I are now under intensive study in several laboratories. The role of cytochrome b_6 has not been clearly established.

Photosynthetic Units and P700

Twenty or more enzyme molecules are likely to be involved in photosynthesis. Each is a protein with a molecular weight of 10^5-10^6, and requires a volume of the order of 10^{-18} to 10^{-19} cm.^{3}. That means that a total volume of about 10^{-17} cm.^{3} may be required to accommodate a single set of photosynthetic enzymes. A typical chloroplast has a total volume of the order of 5×10^{-11} cm.^{3}; thus, even if it were tightly packed with nothing but photosynthetic enzymes (which it is not !), it could not hold more than, say, 5×10^6 such sets. On the other hand, a chloroplast may contain — in fact, it must contain, in order to absorb significant amounts of light ! — as much as 10^9 chlorophyll molecules. Obviously, several hundred pigment molecules must share a single set of enzymes — a common enzymatic ' conveyor belt '. This calculation provides an *a priori* justification of the hypothesis of a ' photosynthetic unit '. This concept was proposed by Gaffron and Wohl[39] to explain the results of Emerson and Arnold[40,41] on the maximum yield of photosynthesis in intense light flashes. This yield was found to be about one O_2 molecule per flash per 2400 chlorophyll molecules, meaning that one H-atom (or electron) could be transferred from ZH to X per flash per 600 chlorophyll molecules (since four H-atoms are involved in the release of one O_2 molecule). This figure could be interpreted as measure of the content in chloroplasts of a ' yield-limiting ' enzyme — an enzyme that can be put to work only once during the practically instantaneous flash. (Other experiments suggested that this enzyme requires about 10 millisec. to complete its action and become available for the next flash). One molecule of this enzyme must be present (at least in normal green cells) per 600 chlorophyll molecules. If two pigment systems of equal size are postulated, one molecule of the limiting enzyme must be associated with 300 pigment molecules.

Since then, much evidence has accumulated, confirming the existence in chloroplasts of units of about 300 chlorophyll molecules, somehow associated with a single molecule of an enzyme (or rather, with the entrance to a single enzymatic conveyor

belt). The pigment molecules belonging to a unit are packed so densely that excitation of anyone of them by light is easily transferred, by resonance, to its neighbour — the picture reminding one of a pinball table on which a steel ball is running about, lighting one electric light after another. The energy can thus migrate through the unit, until it arrives at an enzymatic centre. This energy migration is a quantum-mechanical phenomenon; this means that the excitation energy cannot be divided between two or several molecules, as in the case of resonating mechanical vibrators — bells or tuning forks. Rather, what diffuses through the unit is the probability of finding the energy quantum as a whole in different pigment molecules. When the probability of finding it at the entrance to an enzymatic conveyor belt becomes significant, the quantum is in fact trapped there, and utilized to inject an H-atom (or an electron) on to this belt (or to unload an H-atom or an electron from it).

The pigment molecules immediately adjoining the enzymatic centre can be expected to differ somewhat from those in the bulk of the unit, since they are the only ones likely to participate chemically in the oxidation-reduction process (all others serving as merely physical 'energy suppliers' for the centre).

Kok[42] found evidence that 'pigment system I' contains a minor component with an absorption band at 700 mμ (he called it 'pigment 700', or P700), which, similar to the cytochromes, shows evidence of reversible oxidation and reduction in photosynthesis. The absorption band of P700 lies on the long-wave side of that of the bulk of chlorophyll *a* in system I (680-690 mμ); i.e. its excitation energy quantum is slightly smaller: this makes P700 suitable to act as a trap, catching the energy quanta migrating through the unit. The concentration of P700 (about 1/300 of that of chlorophyll *a*) was also found to be 'just right' for the purpose assigned to it — it corresponds to one molecule of P700 per unit. (Incidentally, the same is roughly correct also for the number of cytochrome *f* molecules present!) Kok[43] was able to determine also the oxidation-reduction potential of P700, and found $E_0 = -0{\cdot}45$ eV. — slightly above that of cytochrome *f*; this, too, is just right for a pigment that is supposed to recover the electron (or H-atom), which it had lost by transfer to the acceptor (X) in the primary photochemical step, from cytochrome *f*. We are thus justified in placing P700 in the key position assigned to it.

The situation is less clear in pigment system II, containing accessory pigments and Chl *a* 670. One is tempted to search there, too, for a small proportion of a special form of chlorophyll *a*, with an absorption band at slightly longer wavelengths than that of bulk (perhaps, in the neighbourhood of 680 mμ), which could serve as a trap for migrating energy; one would expect this component to be reversibly reducible and oxidizable, so as to be able, after having received an H-atom (or electron) from ZH in a photochemical reaction, to transfer it to cytochrome b_6 or some other intermediate by a dark, enzymatic reaction. We may call this hypothetical energy trap 'pigment 680' or P680. So far, no convincing evidence has been found for the existence of such a component in system II in algae. Krey and Govindjee[44] have observed a new fluorescence band in red algae at 693 mμ upon excitation with saturating light absorbed primarily in system II. This band may arise from our hypothetical P680.

Two other interesting components have been found in chloroplasts—one called plastoquinone[45], a naphthaquinone related to vitamin K, and one called plastocyanin, a copper-protein complex[46]. Both are redox catalysts with potentials close to those of cytochromes b_6 and *f* respectively; they are also present in concentrations of the order of 1/300 of that of chlorophyll — suggesting that they, too, may be intermediate catalysts in the photochemical reaction chain. Recent experiments[47,48] have implicated that both plastoquinone and plastocyanin play an important role in the electron transfer in photosynthesis.

Nothing in the suggested scheme of the photochemical reaction in photosynthesis is established beyond doubt; it is merely an attempt to penetrate analytically into the inner sanctum of photosynthesis — its primary light reaction sequence (as contrasted to the enzymatic follow-up reactions).

We have already mentioned two alternatives — either spatially separate pigment packages, containing pigment systems I and II respectively, or a close association of units of two kinds, permitting energy transfer from system II to system I (but not vice versa!) by resonance (so-called spill-over model).

Another alternative was proposed by Franck and Rosenberg[49]. They suggested that the two photochemical redox steps may take place in one and the same reaction centre or energy trap, containing a chlorophyll *a* and a cytochrome molecule. In one light reaction, the cytochrome is reduced by electron transfer from ZH to the cytochrome; in another light reaction, the same cytochrome molecule is oxidized, giving its extra electron to an acceptor (X). The salient point in Franck's theory is that one photochemical step is brought about by chlorophyll *a* in the singlet, short-lived state (the state in which the valence electrons have opposite spins; lifetime, 10^{-8} sec.), because the cytochrome is associated with the reaction centre and available for immediate reaction; while the other step involves the reduction of a free-swimming acceptor molecule X, and therefore occurs after a delay, during which the excited chlorophyll *a* molecule is transferred into a metastable triplet state (with the two electrons having parallel spins; lifetime, several milliseconds or more). The experimental foundation of this picture is the approximate doubling of the fluorescence yield of chlorophyll upon light saturation of photosynthesis; this was interpreted by Franck as suggesting that of the two steps in photosynthesis one competes with fluorescence — i.e. occurs in the singlet excited state — and the other does not — i.e. occurs in the triplet excited state.

We cannot discuss here in more detail the relative advantages of the two pictures, and the possibility of combining them. What seems significant is the considerable degree of agreement between them, particularly in the assumption of two successive photochemical oxidation-reduction steps.

Many workers in photosynthesis have been now brought to this picture by their own observations[50-52]; it is unlikely that it should not contain some elements of truth.

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