1967

CHAPTER 12

CHLOROPHYLL FLUORESCENCE AND PHOTOSYNTHESIS

Govindjee, G. Papageorgiou and E. R binowitch

Department of Botany and the Biophysica Division
Department of Physiology and Biophysics
University of Illinois
Urbana, Illinois

I. INTRODUCTION

The physical and chemical processes which lead to the reduction of carbon dioxide and the oxidation of water in plants have been the subject of much study in the last decade (see recent reviews[1,2]). Ten years ago, Robert Emerson[3,4] discovered that the rate of photosynthesis was greater when red light (absorbed mainly by chlorophyll b) and far-red light (absorbed mainly by chlorophyll a) were used simultaneously than when they were used separately (the Emerson "enhancement" effect). To explain it, Emerson suggested that photosynthesis requires two light reactions, sensitized by two pigment systems[5]. It is now widely accepted that two light reactions, arranged in series, are involved in this process[6] (Figure 1). The first light reaction -- arbitrarily called reaction II (by L.N.M. Duysens[7]) -- leads to the reduction of a cytochrome and oxidation of H_2O to molecular O_2; the second light reaction (reaction I), leads to re-oxidation of the cytochrome and reduction of $NADP^+$ (nicotinamide adenine dinucleotide phosphate). Along the electron pathway from H_2O to $NADP^+$, a fraction of light energy is utilized to synthesize ATP (adenosine triphosphate) molecules from ADP and inorganic phosphate. With sufficient NADPH and ATP available, enzymatic reduction of CO_2 to the carbohydrate level (Calvin-Benson cycle) becomes possible.

The experiments of Emerson and Arnold[8] had established that a group of about 2400 chlorophyll a molecules somehow cooperate in evolving one oxygen molecule and reducing one carbon dioxide molecule. Since four hydrogen atoms (or electrons) must be transferred from water to carbon dioxide to achieve this process, about 600 chlorophyll molecules must cooperate in the transfer of one electron. In the two light reaction model of photosynthesis, each electron must be transferred in two steps, so that the group of 600 chlorophyll molecules had to be divided in two parts, probably of 300 molecules each. These groups are usually referred to as "photosynthetic units" (PSU). Photosynthetic units of the pigment system that sensitize light reaction I (PSU_I) and the ones that sensitize light reaction II (PSU_{II}), are spectrally distinguishable.

Each PSU consists of a light collecting part, comprising the majority of the pigment molecules ("bulk") and a "reaction center" or "trap", which participates directly in the primary photochemical reaction. Light energy absorbed by the bulk pigments is funneled (by a resonance transfer mechanism) to the "trap", where the "uphill" primary oxidation-reduction reaction (electron transfer) takes place. The trap in system I has been identified (by Kok[9]) as a chlorophyll a molecule with an absorption peak at 700 mμ on the long-wave side of the absorption band of the "bulk" with a peak at 675 mμ; it is referred to as "P700". The nature of the trap in system II has not yet been established.

We now ask: How are the absorbed light quanta distributed evenly between the two pigment systems? Two different hypotheses have been suggested (see Myers[10]). In one of them, the "spill-over" hypothesis, the excess light energy absorbed in pigment system II can spill over into system I (but not vice versa), leading to balanced excitation of the two systems in the part of the spectrum where more light is absorbed by system II, and to a drop-off of the yield where light is absorbed preferentially in system I. The spill-over is energetically possible because system I absorbs light of longer wavelengths than system II. This hypothesis explains the high quantum yield of photosynthesis at wavelengths below 680 mμ and the "red drop" in the action spectrum of photosynthesis beyond 680 mμ (observed in green plants and algae). In the second, "separate package" hypothesis, excess light quanta absorbed in system II are not transferred to system I. An approximately balanced excitation of both systems is achieved by presence in both systems, of the same pigments in somewhat different proportions. For example, in the green alga Chlorella, system II contains relatively more chlorophyll b, and system I relatively more chlorophyll a. According to this hypothesis, the action spectrum of photosynthesis (and also of chlorophyll a fluorescence) must show drops wherever one of the two systems absorbs more than another. This must cause a "fine structure" in the $\Phi = f(\lambda)$ curve (where Φ stands for quantum yield). Some such structure has been noted in the action spectrum of photosynthesis (see discussion by Myers[10]) and of chlorophyll a (Chl a) fluorescence[11,12]. It has been suggested (see Clayton[13]) that the spill-over may occur only between system II and the trap (P700) in system I, but not the bulk of the latter system. Energy transfer can occur only if P700 is in the reduced, colored state. This means that energy transfer from system II to I is possible only when the electron flow occurs in the same direction; otherwise, P700 becomes oxidized and the energy transfer stops. This hypothesis permits to reconcile certain observations favoring the separate-package hypothesis.

A light quantum absorbed in a pigment molecule in the "bulk" of one of the two pigment systems, faces three alternatives. Within the lifetime of its excited state it may be (a) lost by rapid "internal conversion" into thermal energy; (b) re-emitted as fluorescence or (c) transferred to other pigment molecules. The energy quanta that reach a trap have an additional choice -- to be converted into chemical energy by an "uphill" electron transfer. The efficiency of "quantum conversion" in the traps seems to be close to 100% when the electron transfer chain operates at its highest rate.

The quanta re-emitted as fluorescence are presumed to originate mainly (or exclusively) in the "bulk" of the photosynthetic unit. When the "trap" is less than 100% effective in the conversion of light energy that reaches it into chemical energy, some energy could become available for "trap" fluorescence. However, since the difference in excitation energy between the bulk and the trap at room temperature is not large compared to kT (k = Boltzman constant), the photochemically unused quanta have a good chance to return from the trap to the "bulk" and be emitted there. At very low temperatures, however, the energy barrier opposing the diffusion of quanta back into the bulk becomes prohibitive, and emission from the trap can become significant.

Fluorescence, in photosynthetic organisms, represents a waste of light energy; this waste is, however, quite low since the yield of Chl a fluorescence in living cells is only about 3%. In spite of the low yield of fluorescence, and complexity due to overlapping of the absorption and fluorescence spectra of the various pigments, a systematic analysis of the intensity and spectrum of fluorescence spectrum of fluorescence has yielded important information concerning the composition of the two pigment systems and the primary mechanisms of photosynthesis. The intensity of emission is related to the efficiency of the two postulated primary processes, and its spectrum (and the action spectrum of its excitation) is indicative of the properties of the two postulated pigment systems. Fluorescence provides a non-destructive tool to monitor the transformation of the pigments in photosynthesis.

Prior to the discovery of the two light reactions in photosynthesis, reviews on the relation of chlorophyll fluorescence to photosynthesis were written by Franck[14], Rabinowitch[15] and Weber[16]. More recently, Butler[17], Clayton[13] and Robinson[18] have reviewed the subject in the context of the two-light reactions model. In the following presentation, we shall discuss mainly the complexity of Chl a fluorescence spectrum in vivo, with emphasis on experiments made in our laboratory in Urbana.

The emission spectrum of chlorophyll *a in vivo* has a main band, with a maximum at 685 ± 2 mμ, and a vibrational "satellite" around 740 mμ. These bands originate in a $\pi^* \rightarrow \pi$ transition to the ground state. Excitation in the blue-violet (Soret) band, leading to the second electronic excited state, is followed by radiationless transition to the lowest excited state, which is so fast that no emission originating in the upper state has ever been reported.

II. MULTIPLICITY OF CHLOROPHYLL a FORMS in vivo

The red absorption band of chlorophyll *a in vivo* is broad (half bandwidth, ∿30 mμ) compared to the same band in chlorophyll *a* in organic solvents (half bandwidth in diethyl ether, 18 mμ). This suggests that chlorophyll *a in vivo* is composed of more than one molecular species or complex. Analysis of the absorption bands of chlorophyll *a* in suspensions of chloroplasts or whole algal cells encounter several difficulties: (i) Other pigments besides chlorophyll *a* are present; (ii) Light is strongly scattered by the suspension (Raleigh scattering and selective spectral scattering, see Latimer[19]); this may increase the apparent absorption if measured in an ordinary spectrophotometer, and the absorption spectra may become distorted; (iii) In a suspension, light that is less absorbed has a larger effective path length than the more strongly absorbed light ("detour" effect, see Butler[20]); (iv) A mutual shading of the pigment molecules takes place in colored particles (chloroplasts and grana); while some light may pass the medium without hitting any particle at all ("sieve effect", cf. Rabinowitch[15]); (v) Fluorescence contributes to the "transmitted" light particularly significantly when absorption is strong.

Two methods that have been used to remedy some of these difficulties are: (a) A diffusing glass (an opal-glass) is placed between the sample and the detector so that almost all forward scattering is collected; (b) An integrating sphere (the Ulbricht sphere) is used, so that almost all scattered energy is collected. Neither of the two methods eliminates the "sieve effect" or the "detour effect".

French[21] constructed a "derivative spectrophotometer", in which the first derivative of the absorbance was plotted automatically as function of wavelength. This procedure accentuates the complex structure of a band, since every inflection in the band envelope appears as a crossing of the abscissa or as a peak. The derivative absorption spectra of various algae obtained in this way were analyzed by Brown and French[22] (also see Brown[23]) into several Guassian components. They were identified as Chl *a* 672, Chl *a* 683 and Chl *a* 694. Cederstrand *et al.*[24]

measured the absorption spectrum of algae in an integrating regular dodecahedron with a photocell on each of the 12 faces: The red band was interpreted as sum of two components, Chl *a* 668 and Chl *a* 683 (again assuming a Gaussian shape of the components). A third band, due to Chl *b*, was located at 650 mμ. The assignment of a Gaussian shape to the bands of individual Chl *a* components is not completely arbitrary, since a Guassian curve matches very closely the red band of chlorophyll *a* in various solvents. Das *et al.*[25,26], have shown that elimination of the so-called "sieve effect" by sonication of Chlorella does not affect the qualitative results obtained with whole cells, but the peaks of the two chlorophyll *a* components are moved (as expected) closer together to 670 and 683 mμ. The half-width of both component is indicated also by observation of preferential photochemical bleaching in this spectral area (Vorobyova and Krasnovsky[30]).

Fractionation of the two (or three) Chl *a* forms has been attempted by breaking the cells mechanically, and solubilizing the pigment complexes selectively by means of detergents (such as digitonin) or by extraction with solvents of varying polarity. The two types of pigment complexes identified in this manner are also Chl *a* 670 and Chl *a* 683, the latter being the more labile form (Allen *et al.*[31], Brown *et al.*[32], Cederstrand *et al.*[33]).

III. FLUORESCENT AND NON-FLUORESCENT (OR WEAKLY FLUORESCENT) FORMS OF CHLOROPHYLL *a*

Duysens[34] and French and Young[35] observed that in red algae, light absorbed by the phycobilins is more efficient in exciting chlorophyll *a* fluorescence than light absorbed in chlorophyll *a* itself. This led Duysens[34] to postulate the existence of two forms of chlorophyll *a* -- one fluorescent and the other non- (or weakly) fluorescent. The fluorescent form must be associated with the phycobilins much more effectively than the "non-fluorescent" one. It is now considered to be part of pigment system II. The "non-fluorescent" form was first assumed to be inactive in photosynthesis, but we now interpret it as belonging to pigment system I, which can perform only light reaction I. A second indication of the existence of two forms of Chl *a* with different fluorescence yields was obtained from measurements by Brody and Rabinowitch[36] of the lifetime, τ, of chlorophyll *a* fluorescence *in vivo* and *in vitro* by a direct flash method. The lifetime was found (in Chlorella) to be 1.5 nsec (assuming a simple exponential decay). If one makes the plausible assumption that the "natural" lifetime (τ_0) of Chl *a* *in vivo* is the same as *in vitro* (15.2 nsec, calculated by integrating of

the area under the band envelope), one calculates $\Phi = 0.10$ from the relation $\tau = \Phi \cdot \tau_o$. However, direct measurements[37] of Φ in Chlorella gave significantly lower values of about 0.03. This discrepancy can be explained if it is postulated that a significant proportion of Chl a *in vivo* does not contribute significantly to fluorescence. The exact proportion of non- (or weakly) fluorescent Chl a could be estimated only if both τ measurements and Φ measurements were done under identical conditions on the same suspension, and if τ_o could be determined directly for chlorophyll in the living cell.

IV. THE "RED DROP" IN FLUORESCENCE YIELD

If the weakly (or non-) fluorescent form of chlorophyll a absorbs more strongly on the long-wave side of the red absorption band than the fluorescent form (as we have reason to believe), a decline ("red drop") in the quantum yield of fluorescence (Φ) plotted as a function of wavelength (λ) can be expected. Indeed, Duysens[34] has noted such decline beyond 680 mμ in green algae. However, Teale[38], who took great precautions to exclude scattering by using a polarizing filter in the path of fluorescence, a suspending medium of high refractive index (concentrated sucrose, ethylene glycol), and a very dilute suspension of Chlorella, could find no red drop up to 690 mμ. We re-investigated the Φ vs λ curve for sonicates of Chlorella (in which the scattering is very much reduced [Das *et al.*[25,26,39]]) and for very dilute suspensions of Chlorella (green alga), both by direct measurements and by computing the Φ vs λ curve according to Stepanov's relation between the absorption and the emission spectrum (Szalay *et al.*[40]). Fluorescence was collected from the same surface on which the exciting light fell, thus reducing its reabsorption. As a result of these studies we are convinced that a "red drop" in the Φ vs λ curve undoubtedly does exist. It begins around 680 mμ in Chlorella.

The fluorescence yield curve [$\Phi = f(\lambda)$] in the phycobilin-containing algae is different because almost all Chl a is present in system I. The system I Chl a, however, is not fluorescent because the long wavelength absorbing form (C700) drains all the excitation and the latter is non- or weakly fluorescent. Because the absorption by system I Chl a extends to shorter wavelengths in these algae, the "red drop" begins earlier. Thus, in the red alga Porphyridium and in the blue-green alga Anacystis, the fluorescence yield begins to decline at about 640 - 660 mμ.

One is naturally tempted to associate the "long-wave" "non-fluorescent" component of Chl a with Cederstrand's Chl a 680, and the "short-wave" fluorescent

component with Chl a 670. However, quantitative analysis makes this interpretation difficult to maintain. It rather suggests that the red drop is associated with a third, minor long-wave component (French's Chl a 694?). This means that both main forms, Chl a 670 and Chl a 680, are present both in PSI and PSII, while Chl a 694 may be present in PSI only, making the whole bulk of Chl a in this system "non-fluorescent" (or rather "weakly fluorescent").

The "red drop" in fluorescence should disappear if the non- (or weakly) fluorescent form of chlorophyll a could be preferentially destroyed. Under aerobic conditions and at low pH, prolonged sonication of Chlorella cells does lead to preferential bleaching of a long-wave form (Chl a 693) of chlorophyll a, as demonstrated by difference between the absorption spectra of the sonicates prepared by sonication in absence (pH = 7.8) and in the presence (pH = 4.5) of air. As expected, the samples deficient in the far-red form of chlorophyll a show a complete absence of the "red drop" in the action spectrum of fluorescence (Das and Govindjee[39]).

V. ANALYSIS OF THE EMISSION SPECTRA AT ROOM TEMPERATURE

A. General

As mentioned above, Chl a *in vivo* fluoresces with a peak at 685 ± 2 mμ and a vibrational band at about 740 mμ. The main red emission band has been investigated intensively in recent years for evidence of its complexity. Due to the experimental difficulties such as the low quantum efficiency of fluorescence in living tissues, the overlapping of the bands, and the polyphasic structure of the material, special techniques must be applied to obtain meaningful data.

B. Matrix Analysis

The intensity of fluorescence emitted at a certain wavelength by a mixture of several fluorescent forms with different position of absorption bands will depend on the wavelength of excitation. If the intensity data for a sufficient number of excitation-emission wavelength pairs are available, one can determine the number of individual fluorescing species. Weber[41] developed for this purpose a matrix method of analysis. The intensity of emission at different wavelengths is determined for fluorescence excited at different wavelengths, and matrices of increasing order are formed. If the 2 x 2 matrixes do not disappear, there must be at least two fluorescent emitters; if the 3 x 3 matrices are significant, at least 3, and so on. Weber's method was applied by Brody and Brody[42] to Euglena, and by Govindjee and Yang[43] to spinach chloroplasts. The analyses showed that at

room temperature there are (at least) two different fluorescent Chl a species. More recently, Williams et al.[11] confirmed this also for Chlorella; they suggested, from consideration of sign and magnitude of different matrix, that only two fluorescent Chl a species seem to be present, and that the one responsible for strongest emission has an emission peak at about 687 mμ, while the less weakly fluorescence component emits around 700 mμ. However, there is as yet no precise way to locate the position of this weak second band fluorescence. Other observations, to be described below, suggest that it may lie around 720 rather than 700 mμ.

C. Polarized and Non-Polarized Fluorescence

The existence of a Chl a component absorbing on the long-wave side of the main absorption band was established by R. A. Olson et al.[44] and by Lavorel[45]. A dichroic ratio of more than 4 was observed when the analyzer was set parallel and perpendicular to the lamellar plane of the chloroplast at 695 mμ; at the shorter wavelengths, no dichroism was discernible. The emission spectrum of "polarized" fluorescence showed a maximum at 716-720 mμ, that of "depolarized" fluorescence, around 685 mμ. The intensity of the former was ∿5-10% of that of the latter.

Since the red absorption band of Chl a is due to a $\pi \rightarrow \pi^*$ transition, with the oscillator located on the porphyrin plane (and bisecting rings II and IV), the above experiments suggest that at least a part of the Chl a is either lying flat, or is tilted at a small angle, with respect to the lamellar plane. This orientation can be imposed by the lipoproteidic matrix on which the Chl a molecules are embedded. Possibly, the weakly-fluorescent long-wavelength Chl a form is simply the oriented fraction of Chl a. It thus seems that a minor form of Chl a in system I arrranged in a relatively ordered array; it is the form responsible for the weak, long-wave polarized fluorescence band beyond 700 mμ and for the "red drop" in the overall fluorescence yield in the far-red.

Boardman and Anderson[46] found evidence of partial separation of the two postulated pigment systems by differential centrifugation of digitonin solubilized chloroplast material. Anderson et al.[47] provided further evidence for this separation: The lighter particles, enriched in P700 (i.e., in pigment system I) effectively performed the reduction of $NADP^+$ with 2,6 dichlorophenol indophenol (DCPIPH) (a dye with a normal potential of ∿0.2 eV) as an electron donor, whereas the heavier particles, richer in chlorophyll b (i.e., in pigment system II), effectively reduced DCPIP with H_2O as donor. The degree of polarization of fluorescence was 2.7 in the heavier and 5.4 in the lighter fractions[48,49]. These results are consistent with the conclusions reached above: that a fraction of chlorophyll a in system I is ordered, and emits more strongly polarized fluorescence.

The excitation spectra[45] of the polarized fluorescence in Chlorella have peaks around 700 mμ, 540 mμ and ∿400 mμ, while those of depolarized fluorescence show a "red drop" beyond 680 mμ, again indicating that the oriented Chl a is a part of the system I. Our observations on the red alga Porphyridium confirm these findings[49,50]. The species fluorescing at 685 mμ always follow in its action spectrum closely the absorption spectrum of pigment system II while the action spectrum of polarized fluorescence at 720 mμ follows the absorption spectrum of system I. Vredenberg[51] also suggested that Chl a_1 fluoresces at 720 mμ, but he found no correlation between the kinetics of absorbance changes attributable to P700 and the 720 mμ fluorescence. (However, if P700 sensitizes the reaction in its triplet state, no correlation between fluorescence and photochemical sensitization can be expected.)

VI. FLUORESCENCE AT LOW TEMPERATURES

The sharpening of the emission bands at low temperatures is a useful means to reduce the difficulties arising from the overlapping of the bands at room temperature (see section V, above). In addition, the quantum efficiency of fluorescence is considerably increased at low temperature (both photochemical and internal quenching are slowed down). The low temperature spectra are of particular interest for the identification of the photochemical reaction centers (the traps), since the "trap depth" ($\Delta\lambda \simeq$ 10-15 mμ) increases from 1-2 kT at room temperature, to 5-8 kT at 77°K (liquid nitrogen) and to 100-150 kT at 4°K (liquid helium).

Brody[52] was the first to observe a new emission band, located at 720 mμ (F720), in Chlorella cooled to 77°K. Several investigators[5,42,43,53-57] confirmed its existence, and observed an additional band at 696-698 mμ. An extension of this work to liquid helium temperature (4°K) by Cho et al.[53,58] clearly showed the existence of three fluorescence bands, at 689 mμ (F685), 698 mμ (F696) and 725 mμ (F720) respectively. These bands appear at different locations in different organisms. We will, however, refer to them as F685, F696 and F720.

The overall shape of the red emission band in green cells exhibits a strong temperature dependence caused by different behavior of the three components. Govindjee and Yang[43] studied this behavior in spinach chloroplasts, and Krey and Govindjee[50] in Porphyridium, in the range from 77°K to 273°K. The F696 band appears only in the temperature range between 77°K and 140°K. On warming up (77°K to 140°K), both F696 and F720 decrease in intensity (although at different rates), whereas F685 remains constant. At the lower temperatures (40°K to 4°K), Cho et al.[58] found the F685 band to increase, whereas the F696 band intensity

rose only slightly. These changes can be interpreted as reflecting a reduction in rate of energy transfer from the bulk of Chl a (emission peak at 685 mμ) to the trap in system II (emission peak at 698 mμ) at these low temperatures.

Furthermore, it was found[43,50,53] that excitation in chlorophyll b, in green plants and in phycobilins in red or blue-green algae, leads to a lower F720, but higher F685 and F696 band, whereas excitation in chlorophyll a leads to the opposite result. This suggests that the band F685 and F696 belong to system II, while F720 belongs to system I, in agreement with the conclusions reached in an earlier section.

These conclusions are further confirmed by experiments on particles prepared by solubilization of spinach chloroplasts with detergents (digitonin) and differential centrifugation, with chloroplasts extracted with acetone and methanol of different concentrations, and with Chlorella cells sonicated in air or argon. Particles -- prepared by the digitonin method -- that perform light reaction I according to Boardman and Anderson[46], were found to be richer in F720-emitting material when cooled down to 77°K, whereas particles that perform light reaction II were enriched in F696 producing material (Cederstrand and Govindjee[48]). C. Shimony et al.[59] obtained fractions enriched in F720-emitting material (Chl a in system I) and fractions poorer in F720-producing material (Chl a in system II), from the blue-green alga Anacystis. Thomas and van der Wal[60] reported that chloroplasts extracted with methanol of different concentrations had somewhat different absorption and fluorescence characteristics. Cederstrand et al.[33] studied the absorption and fluorescence characteristics both of chloroplast residues after extraction with aqueous methanol and acetone of different strength, and of corresponding extracts. They found, particularly at low temperatures, an apparent enrichment either of system II or of system I in the residues from extraction with different solvents. For example, chloroplasts extracted with 50% methanol were enriched in F696-emitting material (system II). Very dilute aqueous acetone or methanol apparently extracted whole pigment complexes present in the chloroplasts. Under aerobic conditions and at acid pH, sonication of Chlorella cells[39] leads to preferential bleaching of the weakly (or non-) fluorescent chlorophyll a (Chl a 693); these samples show no F720 band at 77°K, assumed to be due to the long-wave form of chlorophyll a (system I).

The excitation spectra of F720, F696 and F685 fluorescence bands in Chlorella at very low temperatures (4-77°K) provided the following information[53]: (a) there are two excitation peaks, around 670 mμ and 680 mμ, respectively, which

must be due to the two forms of chlorophyll a identified in the absorption spectra (see Cederstrand et al.[24] and Das et al.[25,26]); excitation spectra of both F720 and F696 show these peaks, confirming the earlier suggestion that both system I and system II contain both components. (It was not possible to extend the measurement of the excitation spectrum of F685 to the 680 mμ region). The exact location of the 670 and 680 mμ peaks in the action spectra are different for F720 and F696, suggesting some differences in the environment of these Chl a components in the two systems. (b) The ratio of fluorescence intensity excited at 440 mμ (Chl a absorption) to that excited at 480 mμ (Chl b absorption)is greater for F720 than for F685 and F696. This confirms that F720 is excited more effectively by absorption in chlorophyll a (i.e., preferentially in pigment system I) and F685 and F696 by absorption in chlorophyll b (i.e., preferentially in pigment system II). Near identity of the excitation spectra for F685 and F696 confirms our previous suggestion[49] that F696 belongs to the same system as F685 (system II). Williams et al.[11] concluded from action spectra that the "F700" band, observed in Chlorella fluorescence in vivo at room temperature, belongs to system I; this shows that it is not identical with the low-temperature F696 band. Perhaps it is identical with the low-temperature F720 band. (it was said before that the designation of the band by Williams et al. as "F700" was very approximate; also, this band may shift considerably with temperature½). (c) The highly efficient energy transfer from chlorophyll b to chlorophyll a appears temperature-independent. This is suggested by the constancy of ratio of fluorescence excited at 440 mμ (absorption in Chl a) to that excited at 480 mμ (absorption in Chl b), when the temperature was varied from 4°K to 77°K. This applies to all three emission peaks (F685, F696 and F720).

VII. LIFETIMES OF THE EXCITED STATES

The decay period of Chl a fluorescence in vivo has been estimated from flash fluorometry (Brody and Rabinowitch[36], Tomita and Rabinowitch[61]) and phase fluorometry (Dmietrievsky, et al.[62] and Butler and Norris[63]) to be in the range of 1-2 nsec; these authors assumed that the decay of chlorophyll a fluorescence in vivo follows a simple first order exponential function. Under conditions of interrupted photochemical de-excitation, such as low temperature[63] and DCMU [DCMU = (3,4-dichloro)-3 phenylo-1,1-dimethyl urea] poisoning[64] the lifetimes were somewhat longer, corresponding to increased quantum yield of fluorescence under these conditions. Murty and Rabinowitch[65] employed shorter flashes and a faster detecting system is trace the decay curve of Chl a fluorescence in various algae.

Plotting the logarithm of the fluorescence intensity against time, gave not a single straight line, but a curve with two linear portions. From their slopes two time constants were calculated -- one corresponding to a decay period τ_1 = 1,2 nsec, and the other to a decay period of τ_2 = 4-5 nsec. This experiment suggests the existence of two components of Chl *a* fluorescence decaying with different rates. The authors suggested as one of the alternatives that the faster-decaying component originates in pigment units with a higher Chl *a* concentration, in which the excitation is rapidly transferred to the reaction site, lowering the quantum yield of fluorescence. A similar conclusion has been reached by Pearlstein[66] on theoretical grounds.

According to Robinson[18] the more rapid photochemical deexcitation of the Chl *a* singlets in comparison with the slower rates of intersystem crossing renders the participation of the Chl *a* triplets in the primary reactions unlikely. Assuming that at 77°K the Chl *a* singlets can either fluoresce (with a measured lifetime of 3.1 nsec) or cross over to the triplet state, an intersystem crossing rate of 0.26 $nsec^{-1}$ can be calculated on the basis of the τ_o value of 15.2 nsec. This rate represents an upper limit, since at room temperature at least, other competing deexcitation processes will reduce it further. The rate of chemical deexcitation *in vivo* and at room temperature, however, calculated on the basis of the 0.03 quantum yield is much higher ($\sim$6 $nsec^{-1}$) than the upper limit of the rate of intersystem crossing, so that the latter cannot compete favorably with the former. The low fluorescence yields indicate a fast chemical turnover, with the participation of the singlet states of the reaction centers. This concept is by no means universally accepted. For example, Franck and Rosenberg[67] suggested that the reaction I proceeds through the triplet state.

VIII. VARIATION OF THE YIELD OF FLUORESCENCE WITH LIGHT INTENSITY

There are several pathways for the dissipation of energy by a population of excited chlorophyll *a* molecules including radiative de-excitation (fluorescence), photochemical quenching, non-radiative de-excitation (internal conversion into heat energy) and transfer to a fluorescent or to a non-fluorescent species. The following equation can be written for the yield (Φ) of Chl *a* fluorescence:

$$\Phi = \frac{k_F}{k_F + k_R + k_C\,[A]} \qquad (1)$$

where k_F is the rate of radiative de-excitation, k_C the rate of a bimolecular quenching process, [A] the concentration of the quenching partner and k_R the sum

of non-radiative and non-photochemical de-excitation rates. Since the availability of the photochemical quencher [A] is limited by a sequence of dark enzymatic reactions in photosynthesis, it is expected that at saturating light intensities, the quantum yield of fluorescence will reach an upper limit corresponding to [A] = = 0, while at low intensities a lower limit will be established corresponding to a constant value [A] = A_o.

In pure dilute chlorophyll *a* solutions fluorescence intensity (F) is proportional, within wide limits, to the incident intensity (I); *i.e.*, the quantum yield (Φ_f) of fluorescence is independent of light intensity. Chlorophyll *a* fluorescence *in vivo* shows, however, a dependence of Φ on I, as expected from equation (1). This was cleraly shown, among others, by Franck[14], Wassink (see 15) and by Brugger[68], who plotted F as a function of I. They found that the slope of this curve at "high" intensities (*i.e.*, in light strong enough to "saturate" photosynthesis), is twice that at low intensities [where the quantum yield (Φ_p) of photosynthesis is maximal and constant]. Latimer *et al.*[37], who measured absolute quantum yields of fluorescence in the green alga, Chlorella, confirmed these findings, showing that the quantum yield of Chl *a* fluorescence increased from about .025 to .05 with increasing intensity of exciting light. (The quantum yield of photosynthesis [O_2 evolution] is known to be about 0.12 at low intensities[69] and declines steadily at high intensities). More recently, Krey and Govindjee[50] measured the function F = f(I) in the red alga *Porphyridium cruentum*. They could confirm the non-linearity of the F = f(I) curve only in the case when excitation took place in the red pigment phycoerythrin (*i.e.*, in system II), while no dependence of Φ on I could be observed when excitation took place in chlorophyll *a* itself (*i.e.*, in system I). This difference can be understood if the reaction centers of system I operate in triplet state, while those in system II operate in the singlet state, because only in the second case is there a competition between sensitization and fluorescence.

IX. CHANGES IN Φ WITH TIME AND THE TWO-LIGHT EFFECT; THE ACTIVATION REACTION

The yield of chlorophyll fluorescence *in vivo* undergoes complex but reproducible changes with time; not only the intensity but also the spectral composition of the fluorescence is altered.

When dark-adapted Chlorella cells are exposed to strong light, the fluorescence intensity rises instantaneously to an initial level, which is independent of photochemical processes. It then rises to a level which remains constant for a

brief period, in the millisecond range, and then rises again to a peak (reached after 0.25 to 1 second). Within ∿1-2 seconds it decreases to an almost steady level; another peak is observed after about 30-50 seconds, after which the fluorescence declines to its final steady level. The exact shape of the F = f(t) curve, and the time of occurrence of the various characteristic points , varies with the conditions of the experiment.

To explain the induction curve of fluorescence, Kautsky et al.[70] suggested that its yield depends on the presence of the oxidized form of a redox intermediate (A) which quenches the fluorescence. This quencher is reduced by the first light reaction [$A + e \xrightarrow{h\nu} A^-$] and reoxidized by a dark reaction [$B^+ + A^- \longrightarrow B + A$] with a second light reaction [$B + X \xrightarrow{h\nu} B^+ + X^-$] completing the sequence.

According to Kautsky, the fluorescence plateau reached in the millisecond range is due to the removal of the quencher (A) by a first order photochemical process. However, the rate of the regeneration of the dark reaction becomes significant when the concentration of B^+ is increased by the second light reaction; this causes the first plateau in the induction curve. The renewed rise to the peak reached after about 0.25 seconds, follows as B^+ is sued up. Kautsky et al. did not explain the decay of this peak.

Lavorel[71] suggested that the fluorescence yield is a sum of two contributions: one, invariant with time, is independent of any photochemical events; the other, time-dependent one, is due to a photoactive form of Chl a. With an experimental setup in which an algal suspension flowed at controlled speed through a capillary, a segment of which was illuminated, he succeeded in recording the spectra of the two components of fluorescence. The variable fluorescence contained relatively more of the 685 mμ fluorescence than the 717 mμ species. This was confirmed by Rosenberg et al.[72] and by J. Munday[73].

Two light reactions were postulated in Kautsky's scheme, but he reported no attempts to separate them by exciting with light of different wavelengths. The first observation that the yield of fluorescence can be modified by adding far-red light (absorbed in system I) to short-wave light (absorbed in system II) was made by Govindjee et al.[74], in experiments analagous to those by which the photosynthetic enhancement effect was demonstrated. They found that fluorescence excited with red or blue light (absorbed in system II) was lowered (quenched) when far-red light (which by itself produced no fluorescence) was added. Butler[75,76] used strong red (system II) and far-red (system I) actinic light to establish a steady state concentration of the postulated redox intermediate, and followed with a weak exciting beam to observe the fluorescence. (The actinic light was eliminated during the

fluorescence measurement). Butler confirmed the antagonistic effects of the two lights on the chlorophyll *a* fluorescence, in whole leaves. The quenching of fluorescence by far-red light was maximal at 705 mμ[77]. This maximum depended, however, on intensity of the actinic lights; it is shifted to longer wavelengths at the higher light intensities. This band may be due to the same long-wave Chl *a* form that is responsible for 720 mμ emission band at 77°K and 740 mμ band of the polarized emission.

Another method employed to establish a steady photochemical state before measuring fluorescence is to excite fluorescence with a weak modulated beam, superimposing on a strong constant background light (Duysens and Sweers[78]). The two-light effect on fluorescence was confirmed by this method. Duysens and Sweers[78] proposed that in reaction II -- sensitized by light absorbed in pigment system II -- the reductant (indirectly, water), reduces an intermediate they called (Q); we will continue to use Kautsky's notation, A, for the sake of uniformity in this presentation. The oxidation-reduction potential (E_o) of this intermediate has been estimated to be +180 mV, over a pH range from 6 to 9 by Kok and Owens[79]. If A is associated with the trap, and the reaction can occur in the singlet excited state of the latter, it must cause a quenching of fluorescence of chlorophyll *a* in system II. The steady state fluorescence yield of system II is governed by the proportion of oxidized "traps:, *i.e.*, of traps associated with A (as contrasted to those associated with A^-), since only the oxidized form can be utilized for the photochemical reaction. At very high intensities of light absorbed in system II, there is a preponderance of A^- (since light reaction I is unable to re-oxidize A at a high enough rate). The increase in fluorescence at the higher light intensities is due to this shift in system II. This has been confirmed by observations of Butler and Bishop[80] on the excitation spectra of fluorescence of the green alga, Scenedesmus, and by Krey and Govindjee[50,81] on the emission spectra of the red alga Porphyridium. System I, upon excitation, depresses the fluorescence yield because A^- is oxidized to A. Duysens and Sweers postulated an additional dark back reaction via another intermediate (A'). Their schene is very similar to that of Kautsky:

$$\begin{array}{ccc} H_2O & & \\ \downarrow & A \underset{h\nu_I}{\overset{h\nu_{II}}{\rightleftharpoons}} A^- & (2) \\ O_2 & A' & \end{array}$$

The fluorescence rise is attributed to the removal of A by the light reaction II. On poisoning with DCMU, this rise can be observed in "pure" form, without subsequent decline. This indicates that DCMU inhibits the reactions which regenerate A. The brief plateau (in the millisecond range) is explained (as in Kautsky's scheme) by the fact that oxidation of A^- by system I is indirect, involving one or more additional redox intermediates. Finally, the decay from the peak (at about 0.25 secs) is due to both system I action and the dark back reaction via A'. The latter was suggested to account for the higher steady state fluorescence value at the end of the decay period as compared with the first plateau in the millisecond range. Since both A^- and A' do not quench the Chl *a* excited singlets, one can assume that the higher yield at the steady state is due to the fact that part of the quencher A exists in the non-quenching form A'. Since A is the primary electron acceptor, the rate of O_2 evolution will depend upon its concentration, and an inverse linear relationship should exist between it and the fluorescence yield. This prediction has been substantiated by the experiments of Delosme *et al.*[82]

Kautsky estimated the relative concentration of the fluorescence quencher as [A]:[Chl] ≈ 1:400, while Duysens' estimate gave [A]:[Chl a_2] ≈ 1:150. A somewhat higher ratio was found by Malkin and Kok[83]. These figures indicate that the "quencher" is present in amounts approximately equal to the number of "reaction centers". In all likelihood, Kautsky's A (Duysens' Q) is not plastoquinone, because the latter is known to be present in much larger quantities. Plastoquinone is an intermediate which participates in the electron transfer chain between the two photosynthetic systems, operating at a site close to system II.

After a dark period, simultaneous recordings of the fluorescence kinetics and oxygen evolution rate performed by Joliot[84] and Delosme *et al.*[82] show that the inverse relationship is not valid over the entire fluorescence rise curve. During the first phase ("activation phase") fluorescence and oxygen evolution rate increase in parallel, while during the second one ("complementary phase"), they are anti-parallel (McAlister and Myers[85] has first observed this phenomenon with the techniques available in 1940). The duration of the activation phase is inversely proportional to the intensity of the exciting light. In the complementarity phase, a linear relationship exists between fluorescence yield (F/I) and the yield of O_2 evolution (V/I) of the form:

$$\frac{F}{I} = A\frac{V}{I} + b \qquad (3)$$

This relationship between the yields is independent of light intensity since the constants "a" (which is negative) and "b" are independent of it. To explain his kinetic results, Joliot[84] postulated (i) that the initial fluorescence variations originate in pigment system II and (ii) that a photochemical quencher designated by him as E (perhaps identical with A) is activated during this period. Schematically, the reaction sequence given by Joliot can be rewritten as follows:

$$A_iH \underset{k_1}{\overset{k_2 I}{\rightleftarrows}} AH \underset{BH}{\overset{B}{\rightleftarrows}} A \overset{k_2 I}{\rightarrow} A^* \qquad \begin{matrix} O_2 \\ H_2O \end{matrix} \qquad (4)$$

In this scheme, B is the form of another intermediate and is produced by light reaction I. Both A_iH and A can quench the fluorescence, while AH does not. In the very beginning of the excitation period, the photochemical quencher is assumed to exist in the form A_iH. During the activation phase, it is transformed to AH with paral-el increase of the fluorescence intensity (due to removal of A_iH), and in the rate of oxygen evolution (due to the production of A*). At the end of the activation phase, the form A_iH is consumed and only the forms A (a quencher) and AH (a non quencher) are present. In the "complementary" phase the transformation of AH to A and the subsequent photochemical reaction results in a decrease in fluorescence and increase in oxygen evolution.

Recently, Munday[86] has shown that preillumination with far-red light (system I), even if followed by a brief dark period (1/2 second), affects the induction of fluorescence by largely eliminating the .25 second peak. These experiments indicate the presence of a "pool" of intermediate (B) formed by far-red light as suggested earlier by French[87] on the basis of flashing-light experiments on O_2 evolution and as suggested recently by Kok et al.[88] and Witt et al.[89] Munday[86] also found that after far-red preillumination, the steady state fluorescence at about 2-3 seconds is slightly higher than the brief plateau in the millisecond range. Joliot's reaction sequence can explain the above-mentioned results. Prolonged far-red light treatment converts BH to B, so that when system II begins to operate, the non-quencher AH is rapidly transformed to the quencher A, by the dark bimolecular reaction, thus causing a delay in the rise and a reduction of the height of the 0.25 second peak. The hump mentioned in reference (85) must be the well-established first phase of the fluorescence rise (the "activation phase").

Experiments on O_2 evolution in flashing light support the idea of an activation reaction in photosynthesis. Allen and Franck[90] reported that no oxygen is produced in algae by a single short light flash (∿1 msec). However, when two brief flashes with a spacing of a few seconds, or a longer flash (∿25 msec) were given, or when weak preillumination was provided before the flash, O_2 was evolved. Whittingham and Brown[91] and Whittingham and Bishop[92] confirmed and extended these observations. Joliot[93], who developed a very sensitive O_2 electrode, provided more precise data on this phenomenon, which can also be ascribed to the need for an activation reaction.

The common feature of the three reaction schenes (Kautsky, Duysens and Joliot), proposed to account for the cause of induction in fluorescence in vivo, is a photochemical substrate which can be directly reduced by system II, and indirectly reoxidized by system I, both processes proceeding with a quantum efficiency of unity. This picture permits an explanation of the antagonistic effect of lights absorbed in system II and system I, respectively, and of complementarity between fluorescence intensity and the rate of oxygen evolution. However, so far no chemical identification of the postulated primary photochemical reactants has been possible.

X. VARIATIONS IN FLUORESCENCE: CHANGES IN INTENSITY AND SPECTRA

Light-induced variations in the quantum yield of the Chl a fluorescence have been employed in the study of the complexity of Chl a in vivo (see sections VIII and IX).

Butler and Bishop[80] compared the excitation spectra of an intact leaf after red and far-red preillumination and observed that it was the system II fluorescence that increased at high light intensities; while a band at 705 mμ was virtually independent of the preillumination of the sample. Difference emission spectra of Porphyridium, constructed by Krey and Govindjee[81] by subtracting the emission spectrum obtained in low light (below the saturation of photosynthesis) from the spectrum obtained in high light,exhibits a band at 693 mμ, in addition to the main 685 mμ band (system II). The increase in 685 mμ fluorescence confirms Butler's conclusions. The 693 mμ band was new, however. From its spectral location, the expected Stoke's shift, and the consideration that when photosynthesis is saturated, some emission from the traps may become possible, the 693 mμ band was tentatively assigned to the trap in system II.

An alternative means of modifying the quantum yield of the fluorescence in a living cell, is the interruption of the photosynthetic electron flow by selective poisons, such as certain derivatives of phenyl-urea, and o-phenanthroline. Poisoning with DCMU results in a rapid reduction of oxygen evolution (although the possibility of its complete abolition is questionable) and an increase of the fluorescence yield by a factor of more than two. Under conditions of interrupted photochemical quantum conversion one may expect that previously non-fluorescent species, such as energy traps, may also emit some fluorescence, although most of the excess fluorescence must originate in the "bulk" as pointed out in the "Introduction". Indeed, "difference spectra" obtained by comparison of DCMU poisoned with non-poisoned Porphyridium cells[50] reveal the existence of a large 685 mμ band (bulk) and an additional band at 692 mμ, which, for the above-stated reasons, may also originate in the trap of system II.

The occurrence of an effective energy transfer from accessory pigments to chlorophyll *a* is well known[34,35,61,94-96]. M. Brody and Emerson[97] and Brody and Brody[98] reported changes in the quantum yield of O_2 evolution and of fluorescence in Porphyridium induced by prolonged preillumination which they ascribed to changes in the efficiency of energy transfer from phycoerythrin to chlorophyll *a*. Such modification could be achieved by growing Porphyridium in light of different color and intensity, or by preilluminating the cells for 20 minutes with light of different wavelengths. Ghosh and Govindjee[99] obtained similar results with the blue-green alga Anacystis; they suggested that decreased efficiency of energy transfer from phycocyanin to "fluorescent" chlorophyll *a* in system II may mean a more effective transfer to the weakly fluorescent form of the same pigment in system I.

Papageorgiou and Govindjee[100] studied the effect of prolonged illumination on normal and DCMU-poisoned Anacystis; they found that not only the quantum efficiency of fluorescence changes in a specific way, but also the spectral distribution of fluorescence is altered, the effect being largest around 695 mμ. In order to interpret their kinetic data, the authors assumed that light-induced conformation changes may occur and affect both the rate of energy transfer (from phycobilins to chlorophyll *a*) and the quantum yield of chlorophyll *a* fluorescence.

The effects of prolonged illumination on the quantum efficiency of fluorescence were also studied in Chlorella[101]. Under constant exciting light conditions, the fluorescence intensity, after the initial induction effects are over in about 1 second, rises for 30-40 secs; this rise is followed by a slower decline to a

a lower steady level than that observed after 2-3 seconds. The rates of change, as well as the final established level of the yield, depend on light intensity, the pH of the medium, the integrity of the electron transport chain and phosphorylation. In whole cells, poisoning by DCMU and o-phenanthroline abolishes the transients and inhibits electron transport in photosynthesis. When phosphorylation is uncoupled from the electron transport by NH_4Cl and FCCP (p-trifluoromethoxy-phenylhydrazone ketomalonyldnitrile), the fluorescence transients are also affected. FCCP, which is known to uncouple phosphorylation and increase the electron transport in the Hill reaction, may affect differently the whole cells. In whole cells, where CO_2 is the oxidant and ATP is needed in the Calvin cycle, uncoupling of the phosphorylation would reduce the rate of the Calvin cycle, thus reducing rather than accelerating the electron transport. An increased fluorescence may then be expected. We found that in whole Chlorella cells FCCP, in a concentration of 5×10^{-5}M, eliminates completely the decay after the 30-40 sec fluorescence peak and the fluorescence remains high. The dependence of the long-time light-induced fluorescence changes on the same parameters (pH, uncouplers of phosphorylation, etc.) which affect also the light-induced conformational (volume and scattering) changes in chloroplasts, may indicate a close relationship of fluorescence changes with energy preserving processes such as phosphorylation. Closer analysis of this subject cannot be undertaken without further data on short-time induction and observations on isolated chloroplasts.

XI. FLUORESCENCE INTENSITY AND PARTIAL REACTIONS

Prior to the discovery of the two light reactions, Lumry *et al.*[102] studied the relationship of fluorescence and the Hill reaction. (The Hill reaction is the reduction of an added oxidant [other than CO_2] and the simultaneous oxidation of O_2O to molecular O_2). Quenching of fluorescence was observed when Hill-oxidants were added to chloroplasts. Kok[56] observed quenching of Chl *a* fluorescence in chloroplasts when $NADP^+$ and ferredoxin were added; this quenching was reversed by DCMU. Further addition of DCPIP (2,6 dichlorophenol indophenol) plus ascorbate did not cause renewed quenching. These experiments show a clear relationship between fluorescence yield and the efficiency of electron transport.

Yang and Govindjee[103] observed a strong quenching of chlorophyll a_2 fluorescence (685 mμ) when PMS (phenazine meto sulfate) was added to DCMU-treated spinach chloroplasts. (Excitation was at 610 mμ to avoid absorption by PMS.) PMS is known to accelerate "cyclic" phosphorylation (*i.e.*, phosphorylation coupled with reversal

of light reaction I), which is sensitized by system I. These results can be explained if we suggest that under these conditions, "spill-over" of excess energy takes place from system II to system I. However, a direct reaction of PMS with (PSU_{II}) cannot be excluded.

XII. CONCLUDING REMARKS

Absorption and fluorescence spectrophotometry have been used successfully in the study of the photosynthetic pigments *in vivo*. Chlorophyll *a* is the most important pigment since it is present in all the green plants and all the algae and a special "complex" of chlorophyll *a* -- designated as P700 -- participates in the primary oxidation reduction reaction of photosynthesis. Although only one form of chlorophyll *a* can be identified in the organic solvent extracts, the evidence presented in this review suggests that several modifications of it exist *in vivo*; they may be different holochromic forms or different aggregates of chlorophyll *a*. Figure 2 shows a probable distribution of the different forms of Chl *a* (and Chl *b*) in the two pigment systems of photosynthesis in higher plants and green algae. In the red and blue-green algae, Chl *b* is replaced by the phycobilins and most of the Chl *a* is in the pigment system I.

Some of the chlorophyll *a* forms are designated as Chl *a* 670, Chl *a* 680, Chl *a* 695 and P700. These forms (see Table 1) have been identified mainly from the absorption spectra, the difference absorption spectra, the action spectra of the light reactions and the action spectra of chlorophyll *a* fluorescence. It is generally believed that the two pigment systems (I and II) contain both chlorophyll *b* and chlorophyll *a* (Chl *a* 670 and Chl *a* 680) but in different proportions. (In the phycobilin-containing algae, most of the Chl *a* 670 and Chl *a* 680 belong to pigment system I).

In green plants and green algae, fluorescence measurements (see Table 2) at room temperature have revealed the presence of at least two emission bands at 685 mμ (with its satellite band at 740 mμ) and at 720 mμ. We have observed an additional band at 695 mμ in the blue-green and the red algae under a variety of conditions. At low temperatures (4°K to 140°K), however, three bands are clearly observed in all the organisms so far studied; these bands are at 685, 696 and 720 mμ. The exact locations of these bands differ with different organisms. The 685 mμ and the 696 mμ bands originate in the pigment system II. A minor part of the 685 mμ band may also come from the pigment system I. The 720 mμ band, however, originates in the pigment system I.

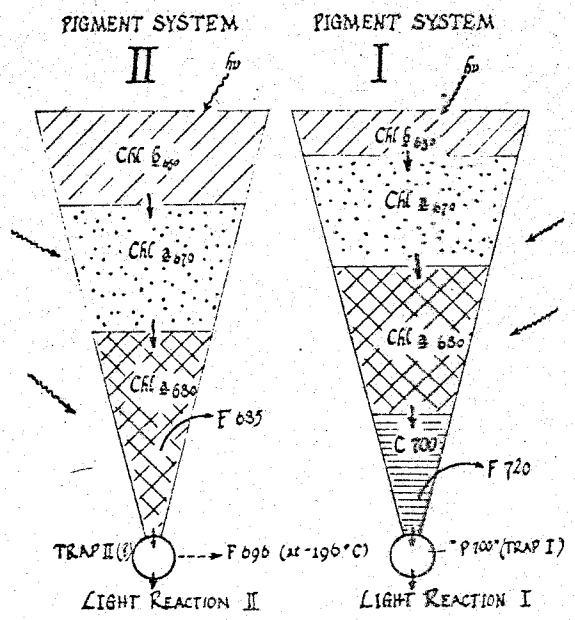

FIG. 2

A working hypothesis for the distribution of the chlorophylls in the two pigment systems in the higher plants and the green algae. The two systems seem to contain both chlorophyll a (Chl a) and chlorophyll b (Chl b 650) but in different proportions. (In the red and the blue-green algae, the phycobilins replace Chl b). It is suggested that the long-wave form of chlorophyll a (C700) is present only in the pigment system I. The two "bulk" chlorophylls (Chl a 670 and Chl a 680) are almost equally distributed in the two systems. (In the red and the blue-green algae, a larger proportion of Chl a is in pigment system I). The energy trap (TRAP I) of the system I is P700 but the Trap II (?) has not been identified. However, it has been suggested that the new emission band that appears at 696 mμ (F696) when plants are cooled to 77°K originates in the trap II. At room temperature most of the main fluorescence band at 685 mμ (and its 740 mμ "satellite") originates from the pigment system II whereas a band at 720 mμ originates in the pigment system I.

TABLE 1

In Vivo Chlorophyll *a* Absorption Bands

Band Maximum mμ	Methods of Observation	Ref.
668-672	Derivative Absorption Spectrophotometry	21-23
678-683	Direct Analysis of Absorption Spectra	24-26
	Action Spectra of Emerson Enhancement	27-29
	Fractionation and Separation of the Pigment Complexes	31-33, 46
	Excitation spectra of Chlorophyll *a* Fluorescence at Low Temperature	53-57
694-705*	Derivative Absorption Spectrophotometry	21-23
	Chlorophyll *a* Dichroism	44
	Action Spectra of the Quenching of Fluorescence by Far-red Light	76
	Action Spectra of Chlorophyll *a* Fluorescence at 77°K	17,43
	Action Spectra of the Polarized Fluorescence	45
P700	Difference Absorption Spectrophotometry	9

*This band may be composed of more than one species.

TABLE 2

In Vivo Chlorophyll *a* Fluorescence Bands

Band Maximum mμ	Methods of Observation	Ref.
683-687	Emission Spectra at Room Temperature	--
	Emission Spectra at Low Temperature	5,42,43, 50,52-58
	Induced Variations in the Quantum Yield of Fluorescence	50,71,72 78,100
693-696*	Emission Spectra at Low Temperature	5,42,43, 50,52-58
	Induced Variations in the Quantum Yield of Fluorescence	50,81,86 100
716-720*	Emission Spectra at Room Temperature (by Matrix Analysis)	11,43,51
	Emission Spectra at Low Temperature	5,42,43, 50,52-58
	Emission Spectra of Polarized Fluorescence	44,45

*These bands may be composed of more than one species.

REFERENCES

1. L.P. Vernon and M. Avron, Annl. Rev. Biochem., 34, 269 (1965).

2. G.W. Robinson, Annl. Rev. Physic. Chem., 15, 311 (1964).

3. R. Emerson,R. Chalmers and C. Cederstrand, Proc. Natl. Acad. Sci., U.S., 43,133 (1957).

4. R. Emerson and E. Rabinowitch, Plant Physiol., 35, 477 (1960).

5. Govindjee, Natl. Acad. Sci. - Natl. Res. Council, 1145, 318 (1963).

6. E. Rabinowitch and Govindjee, Sci. Amer., 213, 74 (1965).

7. L.N.M. Duysens and J. Amesz, Biochim. Biophys. Acta, 64, 243 (1962).

8. R. Emerson and W. Arnold, J. Gen. Physiol., 15, 391 (1932).

9. B. Kok, Biochim. Biophys. Acta, 21, 399 (1956).

10. J. Myers, Natl. Acad. Sci. - Natl. Res. Council,1145,

11. P. Williams, N.R. Murty and E. Rabinowitch, data to be published, 1967.

12. G. Papageorgiou and Govindjee, unpublished observations.

13. R.K. Clayton, The Chlorophylls, Physical, Chemical, and Biological Properties, (L.P. Vernon and G.R. Seely, eds.), Academic Press, 1966, p. 610.

14. J. Franck, Photosynthesis in Plants, (J. Franck and W.E. Loomis, eds.), Iowa State College Press, Ames, Iowa, 1949, p. 293.

15. E.I. Rabinowitch, Photosynthesis and Related Processes, Volumes I and II (parts 1 and 2), Interscience Publishers, N.Y., 1945, 1951, 1956, chapters 23,24 and 37c.

16. G. Weber, Comparative Biochemistry of Photoreactive Systems, (M.B. Allen, ed.), Academic Press, N.Y., 1966, p. 49.

17. W.L. Butler, Current Topics in Bioenergetics, Volume I, (D.R. Sanadi, Ed.), Academic Press, N.Y., 1966, p. 49.

18. G.W. Robinson, Brookhaven Symposia in Biology, 19, 16 (1966).

19. P. Latimer, Ph.D. thesis, University of Illinois, Urbana, 1956.

20. W.L. Butler, Optical Soc. Amer., 52, 292 (1962).

21. C.S. French, in Photobiology, Proceedings of the 19th Annual Biology Colloquium, Oregon State College, 1958, p. 52.

22. J. Brown and C.S. French, Plant Physiol. 34, 305 (1959).

23. J. Brown, Photochem. and Photobiol., 2, 159 (1963).

24. C.N. Cederstrand, E. Rabinowitch and Govindjee, Biochim. Biophys. Acta, 126, 1 (1966).

25. M. Das, E. Rabinowitch, L. Szalay and G. Papageorgiou, J. Phys. Chem., in press, 1967.

26. E. Rabinowitch, L. Szalay, M. Das, N.R. Murty and Govindjee, Brookhaven Syposia in Biology, 19, 1 (1966).

27. Govindjee and E. Rabinowitch, Science, 132, 355 (1960).

28. Govindjee and E. Rabinowitch, Biophys. J. 1, 73 (1960).

29. C.S. French, J. Myers and G.C. McCloud, in Comparative Biochemistry of Photoreactive Systems, (M.B. Allen, ed.), Academic Press, N.Y., 1960, p. 361.

30. L.M. Vorogyova and A.A. Krasnovsky, Biokhimiya, 21, 126 (1956).

31. M.B. Allen, J.C. Murchio, S.W. Jeffrey and S.A. Bendix, in Studies on Microalgae and Photosynthetic Bacteria, Japanese Society of Plant Physiologists, The University of Tokyo Press, Tokyo, 1963, p. 407.

32. J. Brown, C. Bril and W. Urbach, Plant Physiol. 40, 1086 (1965).

33. C.N. Cederstrand, E. Rabinowitch and Govindjee, Biochim. Biophys. Acta, 120, 247 (1966).

34. L. N.M.Duysens, Ph.D. Thesis, University of Utrecht, The Netherlands, 1952.

35. C.S. French and V.M.K. Young, J. Gen. Physiol. 35, 873 (1952).

36. S.S. Brody and E. Rabinowitch, in Proceedings of the First National Biophysics Conference, Yale University Press, 1959, p. 110.

37. P. Latimer, T.T. Bannister and E. Rabinowitch, in Research in Photosynthesis, (H. Gaffron, et al eds.), Interscience, N.Y., 1957, p. 107.

38. F.W.J. Teale, Report to U.S. Army Research and Development in Europe (DA-91-508-EUC-281) 1959.

39. M. Das and Govindjee, data to be published, 1967.

40. L. Szalay, E. Rabinowitch, N.R. Murty and Govindjee, Biophys. J. 7, 137 (1967).

41. G. Weber, Nature, 190 , 27 (1961).

42. S.S. Brody and M. Brody, Nat. Acad. Sci. - Nat. Res. Council, 1145, 455 (1963).

43. Govindjee and L. Yang, J. Gen. Physiol. 49, 763 (1966).

44. R.A. Olson, W.H. Jennings and W.L. Butler,, Biochim. Biophys. Acta, 88, 318 (1964); Biochim. Biophys. Acta, 88, 331 (1964).

45. J. Lavorel, Biochim Biophys. Acta, 88, 20 (1964).

46. N.K. Boardman and J. Anderson, Nature 203, 166 (1964).

47. J.M. Anderson, D.C. Fork and J. Amesz, Biochem. Biophys. Res. Comm., 23, 874 (1966).

48. C.N. Cederstrand and Govindjee, Biochim. Biophys. Acta, 120, 177 (1966).

49. Govindjee, in Currents in Photosynthesis, (J.B. Thomas and J.H.C. Goedheer, eds.), Ad Donker, Rotterdam, The Netherlands, 1966, p. 93.

50. A. Krey and Govindjee, Biochim. Biophys. Acta, 120, 1 (1966).

51. W. Vredenberg, Ph.D. Thesis, University of Leiden, The Netherlands, 1965.

52. S.S. Brody, Science 128, 838 (1958).

53. F. Cho and Govindjee, Paper presented at the annual meeting of the Biophysical Society held at Houston, Texas, February 1967; data to be published, 1967.

54. F.F. Litvin, A.A. Krasnovsky and G.T. Rikhireva, Dokl. Akad. Nauk. SSSR, 135, 1528 (1960).

55. J. Bergeron, Natl. Acad. Sci.-Natl. Res. Council, 1145, 527 (1963).

56. B. Kok, Natl. Acad. Sci.-Natl. Res. Council, 1145, 45 (1963).

57. J.H.C. Goedheer, Biochim. Biophys. Acta, 88, 304 (1964); Biochim. Biophys. Acta, 102, 73 (1965).

58. F. Cho, J. Spencer and Govindjee, Biochim. Biophys. Acta, 126, 174 (1966).

59. C. Shimony, J. Spencer and Govindjee, Photosynthetica, (in press, 1967).

60. J.B. Thomas and U.P. Van der Wal, Biochim. Biophys. Acta, 79, 490 (1964).

61. G. Tomita and E. Rabinowitch. Biophys. J. 2, 483 (1962).

62. O.D. Dmietrievsky, V.L. Ermolaev and A.N. Terenin, Dokl. Akad. Nauk SSSR, 114, 751 (1957).

63. W.L. Butler and K. Norris, Biochim. Biophys. Acta, 66, 72 (1963).

64. A. Müller and R. Lumry, Proc. Natl. Acad. Sci., U.S. 54, 1479 (1965).

65. N.R. Murty and E. Rabinowitch, Biophys. J., 5, 655 (1965).

66. R.M. Pearlstein, Proc. Natl. Acad. Sci., U.S., 52, 824 (1964).

67. J. Franck and J. Rosenberg, J. Theoret. Biol. 7, 276 (1964).

68. J.E. Brugger, in Research in Photosynthesis, (H. Gaffron et al, eds.), Interscience Publishers, N.Y., 1957, P. 113.

69. R. Emerson, Annl. Rev. Plant Physiol. 9, 1 (1958).

70. H. Kautsky, W. Appel and H. Amann, Biochem. Z., 332, 277 (1960).

71. J. Lavorel, in La Photosynthese, Coll. Intern. C.N.R.S., 119, 159 (1963); Biochim. Biophys. Acta, 60, 510 (1962).

72. J.L. Rosenberg, T. Bigat and S. Dejaegere, Biochim. Biophys. Acta, 69, 9 (1964); J.L. Rosenberg and T. Bigat, Natl. Acad. Sci.-Natl. Res. Council 1145, 122 (1963).

73. J.C. Munday - unpublished observations.

74. Govindjee, S. Ichimura, C. Cederstrand and E. Rabinowitch, Arch. Biochem. Biophys., 89, 322 (1960).

75. W.L. Butler, Plant Physiol. Suppl., 36 IV (1961).

76. W.L. Butler, Biochim. Biophys. Acta, 64, 309 (1962).

77. W.L. Butler, Biochim. Biophys. Acta, 66, 275 (1963).

78. L.N.M. Duysens and H.E. Sweers, in Studies on Microalgae and Photosynthetic Bacteria, Japanese Society of Plant Physiologists, University of Tokyo Press, Tokyo, 1963, p. 353.

79. B. Kok and O. Owens, cited by B. Kok and G.M Cheniae, in Current Topics in Bioenergetics, (D.R. Sanadi, ed.), Vol. 1, Academic Press, N.Y., 1966, p. 1.

80. W.L. Butler and N.I.Bishop, Natl. Acad. Sci.-Natl. Res. Council, 1145, 91 (1963).

81. A. Krey and Govindjee, Proc. Natl. Acad. Sci., U.S., 52, 1968 (1964).

82. R. Delosme, P. Joliot and J. Lavorel, C.R. Acad. Sci., Paris, 249, 1409 (1959).

83. S. Malkin and B. Kok, Biochim Biophys. Acta, 126, 413 (1966).

84. P. Joliot, Biochim. Biophys. Acta, 102, 116 (1965).

85. E.D. MacAllister and J. Myers, Smith Inst. Misc. Collections, 99, 1 (1940).

86. Govindjee, J.C. Munday and G. Papageorgiou, Brookhaven Symposia in Biology, 19, 434 (1966).

87. C.S. French, in Currents in Photosynthesis, (J.B. Thomas and J.H.C. Goedheer, eds.), Ad Donker, Rotterdam, The Netherlands, 1966, p. 285.

88. B. Kok, S. Malkin, O. Owens and B. Forbush, Brookhaven Symposia in Biology, 19, 446 (1966).

89. H.T. Witt, G. Döring, B. Rumberg, P. Schmidt-Mende, U. Siggel and H.H. Stiehl, Brookhaven Symposia in Biology, 19, 161 (1966).

90. F.L. Allen and J. Franck, Arch. Biochem. Biophys. 58, 510 (1955).

91. C.P. Whittingham and A.H. Brown, J. Expt. Botany, 9, 311 (1958).

92. C.P. Whittingham and P.M. Bishop, Natl. Acad. Sci.-Natl. Res. Council, 1145, 371 (1963).

93. P. Joliot, Brookhaven Symposia in Biology, 19, 418 (1966).

94. H.J. Dutton, W.M. Manning and B.B. Duggar, J. Phys. Chem., 47, 308 (1943).

95. E. C. Wassink and J.A.H. Kersten, Enzymologia 12, 3 (1946).

96. W. Arnold and J.R. Oppenheimer, J. Gen Physiol. 33, 434 (1950).

97. M. Brody and R. Emerson, J. Gen Physiol., 43, 251 (1959).

98. S.S. Brody and M. Brody, Arch. Biochem. Biophys., 82, 161 (1959).

99. A.K. Ghosh and Govindjee, Biophys. J., 6, 611 (1966).

100. G. Papageorgiou and Govindjee, Biophys. J., in press, 1967.

101. G. Papageorgiou and Govindjee, data to be published, 1967.

102. R. Lumry, B. Mayne and J.D. Spikes, Disc. Faraday Soc., 27, 149 (1959).

103. L. Yang and Govindjee, data to be published, 1967.