Chapter 13

The Two Photochemical Systems; The Red Drop and the Emerson Effect

THE RED DROP; "ACTIVE" AND "INACTIVE" CHLOROPHYLL *a*

In discussing the action spectra of photosynthesis in Chapter 11, we postponed dealing with the decline of the quantum yield (the "red drop"), which occurs above 680 nm in green algae and above 650 nm in the red ones (Fig. 13.1). (A similar drop was found, in our laboratory, also in the action spectrum of the Hill reaction.) Why should the quantum yield of photosynthesis decline in the far red, although quanta are still absorbed there by chlorophyll *a*? The first naive suggestion was that in the far red the quanta simply become "too small" to sensitize photosynthesis. This smallness is, however, irrelevant to our problem, as shown by Fig. 13.2.

Absorption at the red end of the band originates in strongly vibrating molecules in the ground state (arrow A' in Fig. 13.2), while absorption in the peak of the band (arrow A'') originates in the nonvibrating ground state. The former is weak compared to the latter because there are few strongly vibrating molecules at room temperature; but once absorption has occurred, there is no reason why the excited molecules should
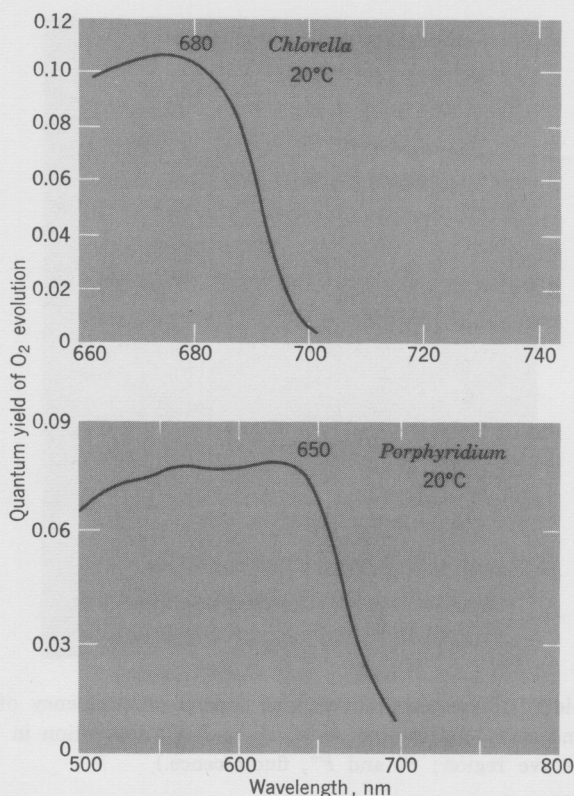
FIG. 13.1 "Red drop" in action spectra of photosynthesis $[\Phi O_2 = f(\lambda)]$ in green and in red algae. (R. Emerson, R. V. Chalmers, and C. Cederstrand, 1957, and M. Brody and R. Emerson, 1959, respectively.)

behave differently from those originated in absorption from weakly vibrating states, for the following reason.

It was noted in Chapter 10 that a molecule lives long enough in the excited electronic state to be exposed (particularly in a condensed medium) to numerous encounters with other molecules. These encounters lead to energy exchange, and quickly (that is, within a period much shorter than the electronic excitation lifetime of more than 10^{-9} sec) equilibrate its "vibrational temperature" with that of the medium (wavy arrows in Fig. 13.2). Thus, by the time the excited molecule participates in a photochemical reaction, it has forgotten, as it were, the vibrational
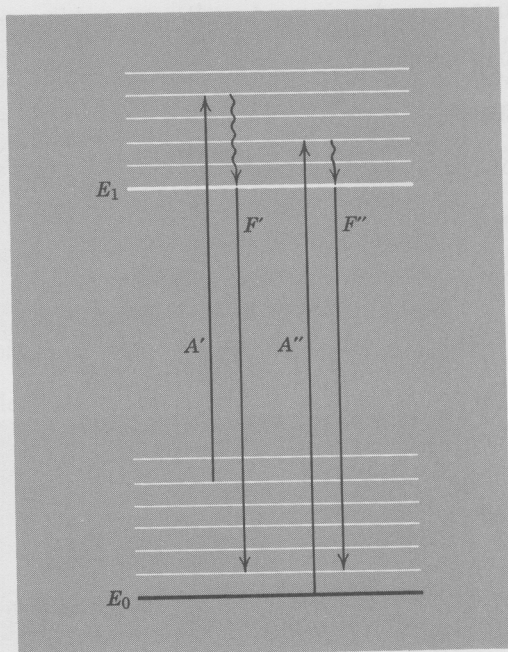
FIG. 13.2 Yield of fluorescence should not depend on frequency of light absorption. (E_0, ground state; E_1, excited state; A' and A'', absorption in the long wave and the short wave region; F' and F'', fluorescence.)

state in which it had been immediately after the excitation and thus also its origin in absorption in a vibrating (or a nonvibrating) ground state.

For this reason, the quantum yield of photosynthesis (as well as that of fluorescence) should be the same throughout an absorption band (that is, there should be no "red drop"), if this band resulted from a single electronic transition in a single type of molecules, differing only by their vibrational state.

Thus, the problem arises: Do all parts of the red band of chlorophyll *a* in vivo really correspond to one and the same electronic transition in one and the same molecule?

Several hypotheses have been proposed as to why this may *not* be true. One suggestion was that the main chlorophyll absorption band, due to single (monomeric) pigment molecules, Chl, may overlap at its

long-wave end with a band due to double (dimeric) molecules, Chl_2, and that the "red drop" occurs when absorption is due to the dimers. This assumption provides a satisfactory explanation of changes that absorption and fluorescence of many dyes undergo in solution with increasing concentration (which favors dimerization). Associated dye molecules usually are nonfluorescent; the coupling of molecules in a dimer seems to permit a more rapid internal conversion of electronic excitation into ground state vibrations. This rapid deactivation may also explain declining photochemical efficiency.

Dimerization has been observed in chlorophyll solutions, although only at relatively high concentrations (about 10^{-3} molar or more). It has been suggested, particularly by A. A. Krasnovsky in Moscow and by S. S. Brody (in our laboratory, and later in New York), that dimerization provides an adequate explanation for the "red drop" in the yields of photosynthesis and fluorescence of chlorophyll in living cells; but this explanation encounters considerable quantitative difficulties.

Alternative (or additional) explanations of the complexity of the red absorption band of chlorophyll a in vivo have been suggested. Chlorophyll a may be present, in vivo, in slightly different chemical forms, with slightly displaced absorption bands and with different photochemical capacities and fluorescence yield. The difference may be in the pigment molecule itself, or in its association with different partners, such as proteins or lipids. The red drop may result from the presence, on the long-wave end of the main absorption band, of an absorption band (or bands) due to a chemically different form (or forms) of chlorophyll a, or to different chlorophyll complexes (rather than to chlorophyll polymers, as in the preceding hypothesis).

If one ascribes the red drop to the overlapping of bands belonging to two forms of chlorophyll a, a short-wave form that is "active" in photosynthesis, and a long-wave "inactive" form, one can derive, from the course of the red drop curve, the shape of the absorption bands of the two components. One arrives, in this way, at the conclusion that—at least in green algae—the "inactive" form is much less abundant than the "active" one. Its absorption peak must lie at about 695 nm (while that of the "active" form is located at about 675 nm). In red algae, on the other hand, most of the chlorophyll a seems to belong to the "inactive" form.

These conclusions bring to mind the analysis of the absorption band

of Chl *a* in vivo into Gaussian components, described in Chapter 9. It suggested the presence, in the red band, of two main components, of about equal intensity, Chl *a* 670 and Chl *a* 680. Some evidence was mentioned also for the existence of a third, relatively weak component, Chl 695 nm (see Fig. 9.7). In green plants, this third component is likely to be the one responsible for the red drop, while the two main components contribute efficiently to photosynthesis and to fluorescence. Again, the situation must be different in red algae, where the larger part of Chl *a* 670 and Chl *a* 680 appears to be "inactive."

It will be shown later that additional facts, indicating the distribution of photosynthetic pigments between two "pigment systems," which are both active, but play a complementary role in photosynthesis, call for a more sophisticated interpretation of the red drop.

THE EMERSON EFFECT; THE HYPOTHESIS OF TWO PHOTOCHEMICAL REACTIONS AND TWO PIGMENT SYSTEMS

In making a closer study of the "red drop" in the quantum yield of photosynthesis in Chlorella, Emerson and co-workers found, in 1957, that the "inefficient" far-red light (i.e., in green cells, light beyond 680 nm) can be made fully efficient by simultaneous illumination with light of a shorter wavelength—for example, by combining a beam of 700 nm light with a beam of 650 nm light (Fig. 13.3). Oxygen production from the two combined beams was found to be substantially higher than the average of the sum of the production from the separate beams; and since it was the far-red beam that, used by itself, gave a "subnormal" yield, it was natural to interpret the result as evidence of improvement of the photosynthetic efficiency of far-red light (700 nm) by simultaneous illumination with orange-red light (650 nm). This enhancement became known as the Emerson effect. It can be expressed as the ratio of the rate of oxygen evolution (ΔO_2) in the far-red light in the presence of the supplementary beam, and the same rate in the absence of it:

$$E = \frac{\Delta O_2 \text{ (in combined beams)} - \Delta O_2 \text{ (short-wave beam alone)}}{\Delta O_2 \text{ (long-wave beam alone)}} \quad (13.1)$$
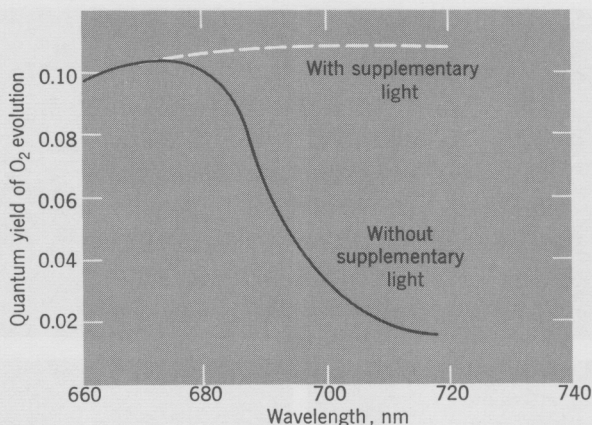
FIG. 13.3 The Emerson effect in *Chlorella*.

Emerson and co-workers studied the action spectrum of this effect; i.e., they made plots of E versus the wavelength of supplementary light (Fig. 13.4). Enhancement proved to be greatest when the largest proportion of supplementary light was absorbed by one of the accessory pigments—chlorophyll b in green algae, chlorophyll c or fucoxanthol in diatoms, and phycobilins in red and blue-green algae. It thus seemed that what was needed to make photosynthesis fully efficient, was the absorption, in addition to a quantum taken up by chlorophyll a, of another quantum taken up by one of the accessory pigments! The red drop takes place in the spectral region where chlorophyll a takes over as the only absorber.

The interpretation of the early drop in red algae appeared particularly easy in this picture. These algae contain no chlorophyll b, and the region of exclusive absorption by chlorophyll a extends in them down to about 650 nm, where the absorption by phycocyanin becomes significant; and this is where the red drop begins!

These observations caused Emerson to suggest tentatively that photosynthesis involves two photochemical reactions—one sensitized by chlorophyll a and one sensitized by accessory pigments.

The occurrence of two light reactions is now generally accepted; but the assumption of direct sensitization of one of them by accessory pigments runs into difficulties (apart from a certain implausibility of assign-
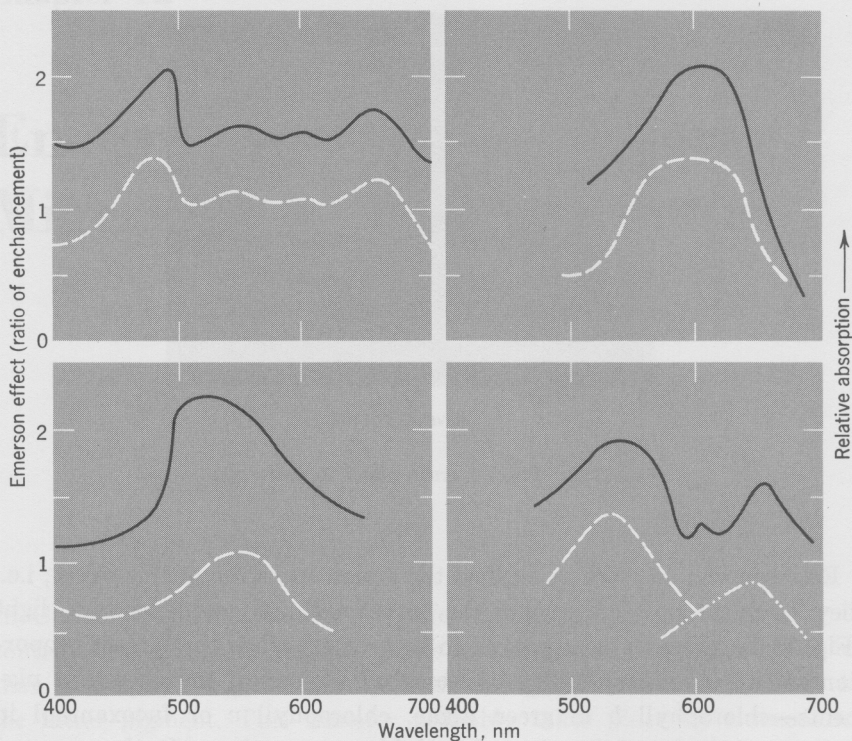
FIG. 13.4   Action spectra of the Emerson effect in different algae. The green alga *Chlorella pyrenoidosa* (top left): the blue-green alga *Anacystis nidulans* (top right), the red alga *Porphyridium cruentum* (bottom left), and the diatom *Navicula minima* (bottom right). In each case, the solid curve shows the action spectrum of the Emerson effect, *i.e.*, the degree enhancement as the wavelength of the supplementary illumination is varied. These curves turn out to be approximately parallel to the absorption curves (broken lines) of the various accessory pigments: chlorophyll *b* in *Chlorella*, phycocyanin in *Anacystis*, phycoerythrin in *Porphyridium*, and fucoxanthol (broken) and chlorophyll *c* (dotted broken) in *Navicula*. (R. Emerson and E. Rabinowitch, 1960.)

ing a common photochemical function to all the, chemically very different, accessory pigments). How can Emerson's suggestion be reconciled with observations of phycobilin-sensitized fluorescence of chlorophyll *a* in red algae (see Chapter 12)? Occurence of sensitized fluorescence clearly shows that energy quanta absorbed by the accessory pigments

are transferred, often very efficiently, to chlorophyll a, causing the latter to emit fluorescence. How could quanta absorbed by an accessory pigment have a different photochemical function from quanta absorbed by chlorophyll a, if they are transferred quantitatively to the latter? This makes no sense!

In 1960, we observed in the action spectrum of the Emerson effect, in addition to peaks attributable to accessory pigments, also a peak at 670 nm, that is, within the main absorption band of chlorophyll a itself (Fig. 13.5). This changed the picture: what now appeared to be necessary for effective photosynthesis, was absorption of one quantum in one form of chlorophyll a and of another quantum in another form of the same pigment! The peaks in the action spectrum of the Emerson effect, corresponding to accessory pigments, could be reinterpreted as due to preferential resonance energy transfer of excitation energy from the accessory pigments to the "active" form of chlorophyll a.

This generalization appeared more plausible than Emerson's original
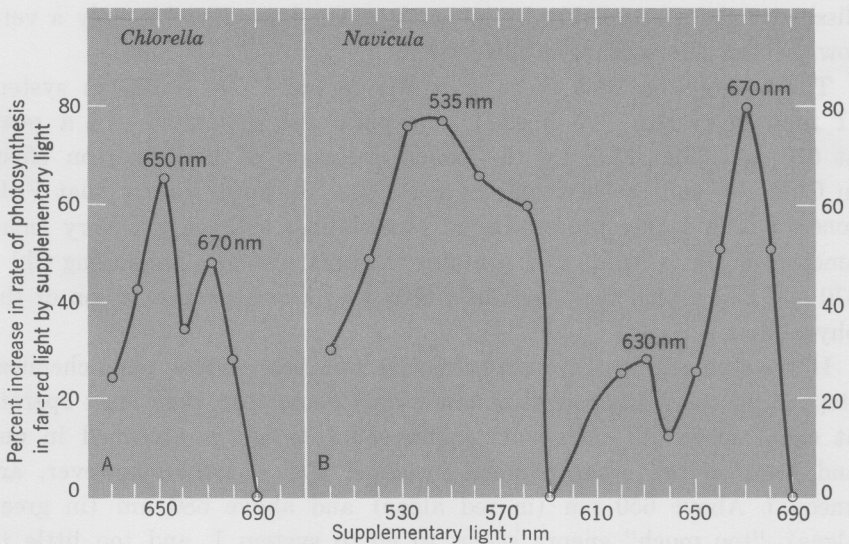

FIG. 13.5 Details of action spectra of the Emerson effect in a green alga (*Chlorella*) and in a diatom (*Navicula*), showing a peak at 670 nm; the peak at 650 nm is due to Chl b, at 535 nm to fucoxanthol, and at 630 nm, to chlorophyll c. (Govindjee and E. Rabinowitch, 1960.)

one. It required, however, giving up the above-suggested simple explana-
tion of the early red drop in red algae. In the spectrum of the Emerson
effect in these algae, only peaks due to phycobilins were observed, and
no peak at 670 nm; and yet there is no evidence of a deficiency of
Chl *a* 670 in these organisms.

A provisional picture, taking into account all results, can be attempted
along the following lines:

All photosynthetic pigments are distributed in two "pigment systems"
(Fig. 13.6), which, following a suggestion by Duysens, we will call "sys-
tem I" (PSI) and "system II" (PSII). System II is (relatively)
strongly fluorescent; system I only very weakly fluorescent. System II
contains, in green plants, both Chl *a* 670 and Chl *a* 680, with a slightly
larger proportion of the former, and no Chl *a* 695. System I also contains
both Chl *a* 670 and Chl *a* 680, but somewhat more of the latter; most
importantly, it contains Chl *a* 695 (perhaps, 10% of total chlorophyll
a in this system). The latter drains, by resonance transfer, practically
all quanta from Chl *a* 670 and Chl *a* 680 in this system and effectively
dissipates them—probably by internal conversion—allowing only a very
low yield of fluorescence in this system.

The assumption that there is relatively more Chl *a* 670 in system
II than in system I is needed to explain the appearance of a peak
at 670 nm (Fig. 13.5) in the action spectrum of the Emerson effect
in Chlorella and in Navicula. In red algae, we must assume that PSII
consists in a larger proportion of phycobilins, with only a very small
amount of Chl *a*, while PSI contains the bulk of Chl *a* (including Chl *a*
670 and Chl *a* 680, and also Chl *a* 695) and a smaller proportion of the
phycobilins.

If the two pigment systems sensitize two consecutive photochemical
steps in photosynthesis, as it is now widely postulated, they must operate
at equal speed. At each wavelength, some energy is absorbed in one
and some in the other pigment systems. The amounts, however, are
unequal. Above 650 nm (in red algae) and above 680 nm (in green
algae), "too much" energy seems to go to system I, and too little to
system II, causing the "red drop" in the yield of photosynthesis. The
Emerson effect reveals improvement of this distribution by simultaneous
illumination with a second light beam. The fact that photosynthesis does
occur, albeit with low efficiency, throughout the region of the red drop
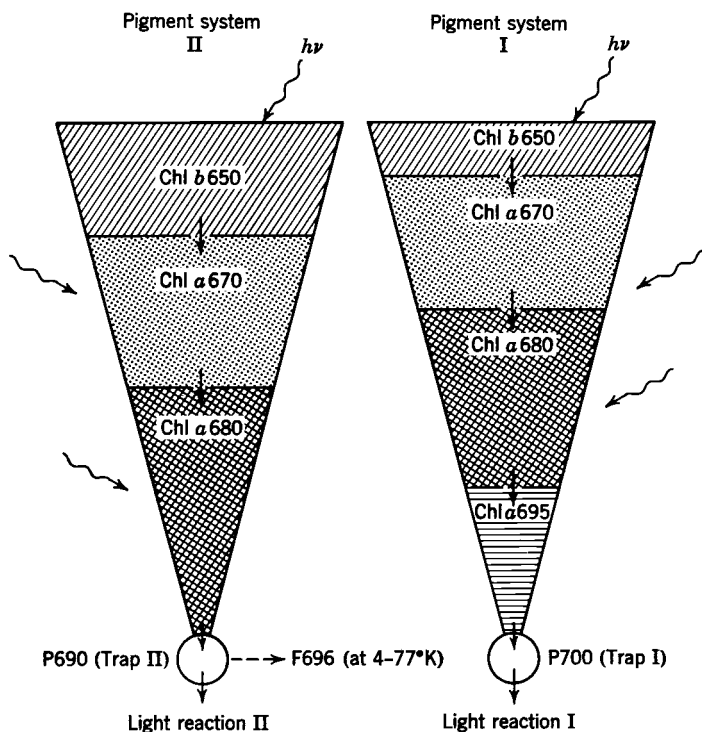
FIG. 13.6 A working hypothesis for the distribution of the chlorophylls in the two pigment systems in the higher plants and the green algae. The two systems seem to contain both chlorophyll a (Chl a) and chlorophyll b (Chl b 650), but in different proportions. (In the red and the blue-green algae, the phycobilins replace Chl b.) It is suggested that the long-wave form of chlorophyll a (Chl a 695) is present only in the pigment system I. The two "bulk" chlorophylls a (Chl a 670 and Chl a 680) are almost equally distributed in the two systems, but there is relatively more Chl a 670 in system II. (In the red and the blue-green algae, a much larger proportion of Chl a is in pigment system I.) The energy trap (Trap I) of the system I is P700 and the Trap II is P690.[1] (Govindjee, G. Papageorgiou, and E. Rabinowitch, 1967).

(up to 720 nm), shows that both photochemical reactions can take place there, although one proceeds so slowly as to become a bottleneck for the whole process. At the shorter waves, both photochemical systems are excited at a much better balanced rate.

Duysens pointed out that if the two light reactions, sensitized by

[1] Witt and co-workers located this band at 690 nm in chlorella and at 682 nm in "system II particles" (compare p. 192).
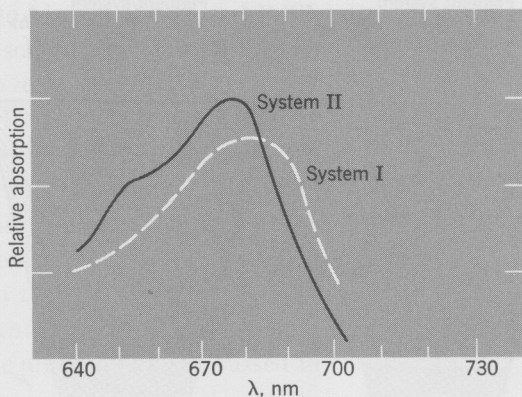
FIG. 13.7 Absorption spectra of two pigment systems derived from the data of C. S. French and co-workers (1960), on action spectra of photosynthesis in *Chlorella* cells "saturated" with light absorbed preferentially in PSI (far-red light) and in PSII (near-red light). The difference may be explained by the presence of more Chl *b* in PSII and of more Chl *a* 695 in PSI.

the two pigment systems, occur in series, one way to determine the absorption spectrum of each system is by measuring the action spectra of photosynthesis in the presence of strong (but not saturating) light belonging to the other system. The rate of the overall reaction is then determined by that of the limiting light reaction. When excess light goes to system I, the rate (and thus the action spectrum) is determined by system II, and vice versa. Such measurements had been made by French and co-workers in California; the result is shown in Fig. 13.7. This figure confirms that both pigment systems contain several forms of chlorophyll; system II seems to be much richer in Chl *b*, and somewhat richer in Chl *a* 670; while system I contains relatively more Chl *a* 680, and all of Chl *a* 695.

This is one observation—we will quote others later—suggesting that a simple identification of Chl *a* 670 as sensitizer of one photochemical reaction in photosynthesis, and Chl *a* 680 as sensitizer of the other (a tempting and simple suggestion, which we ourselves had made at first), cannot be true.

That the two light reactions are linked together by a relatively slow dark reaction first became clear from experiments in which the Emerson

effect was observed to occur even when the two light beams entered the reaction vessel at right angles to each other, thus striking a given algal cell at different times (with an interval depending on the intensity of stirring). J. Myers and C. S. French noted that the Emerson effect could be observed also using alternate flashes of two beams, with intervals up to several seconds between them. In the red alga *Porphyridium*, oxygen evolution by a green flash (absorbed by phycoerythrin, belonging mainly to system II) was enhanced when it was preceded by a red flash, absorbed by chlorophyll *a* in system I, suggesting that the latter left a long-lived intermediate. One half of this intermediate appeared to survive for as long as 18 seconds (see Fig. 13.8). On the other hand, oxygen evolution from a red flash was not enhanced when it was preceded by a green flash, suggesting that green light absorbed in PSII produced no long-living intermediates.
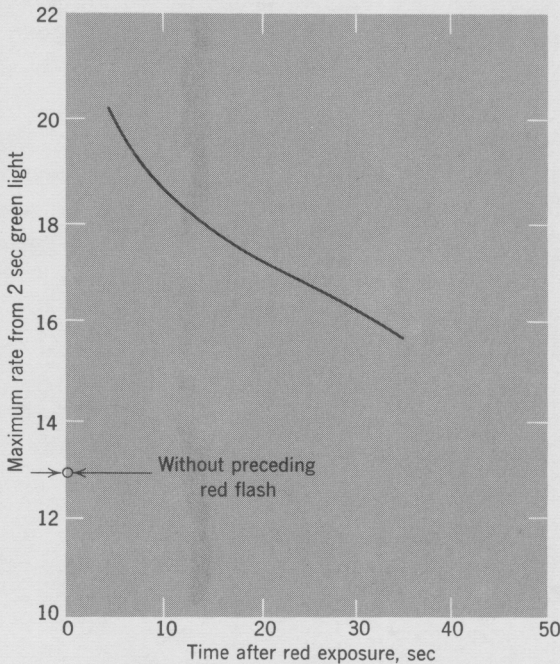

FIG. 13.8 Enhancement of oxygen yield (in red algae) from a green flash by preliminary exposure to a red flash, as function of time between the two flashes. (C. S. French, 1963.)

We are thus justified in postulating that the two photochemical primary processes in photosynthesis occur in series, with a dark reaction involving the products of the two photochemical reactions linking them together.

We shall discuss, in Chapters 14–16, other evidence that supported the conclusion that photosynthetic pigments are organized into two systems with different photochemical functions. Here, we summarize the mechanism of photosynthesis as derived from red drop and enhancement studies (Fig. 13.9).

If we assume that two separate photosystems sensitize two consecutive light reactions in photosynthesis, we must ask: How are the absorbed light quanta distributed equally between the two pigment systems permitting them to run at equal speed—as this is needed for photosynthesis
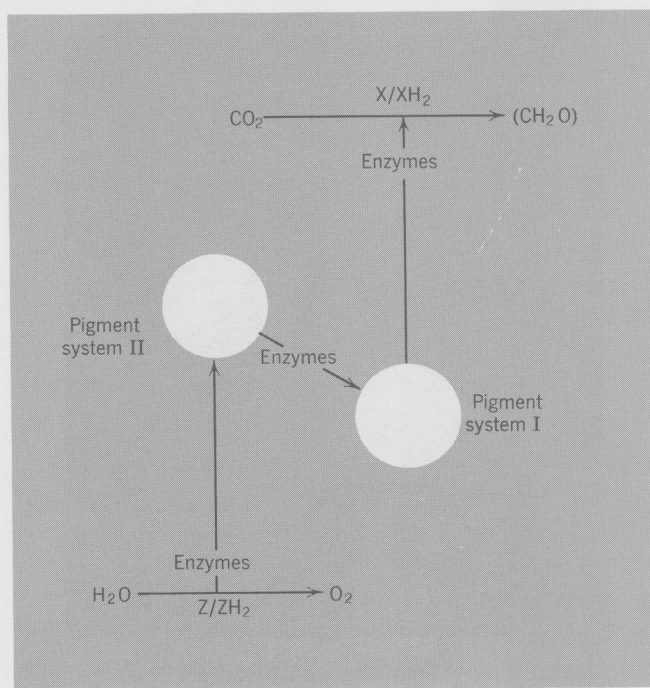
FIG. 13.9 Scheme of photosynthesis with two photochemical steps (compare with Fig. 5.4) sensitized by two pigment systems (I and II).

to proceed with the highest quantum yield? Two different hypotheses have been suggested. In one of them, called the "spill-over" hypothesis by J. Myers (University of Texas in Austin), all excess light energy absorbed in pigment system II is assumed to "spill over" into system I—but not vice versa, because quanta absorbed in PSI are drained into the long-wave component Chl a 695, from which their return to Chl a 680 and Chl a 670 is obstructed by an energy barrier. The spill-over leads to balanced excitation of the two systems throughout the part of the spectrum where more light is absorbed by system II than by system I, and explains the drop-off of the yield in the far-red (beyond 680 nm) where light is absorbed (in green cells) mainly by Chl a 695 in system I. In phycobilin-containing algae, where most Chl a is in system I, the drop begins at 650 nm, where absorption in phycocyanin yields to absorption by chlorophyll a.

This hypothesis explains the high quantum yield of photosynthesis in green cells at wavelengths <680 nm, and the "red drop" in the action spectrum of photosynthesis beyond 680 nm (in higher plants and green algae) and beyond 650 nm in red algae.

In the second hypothesis, called by J. Myers *"separate package"* hypothesis, an approximately balanced excitation of both systems is achieved, without any "intersystem transfer," through the presence in both systems of the same pigments, even if in somewhat different proportions. According to this hypothesis, the action spectrum of photosynthesis must show minima wherever one of the two systems absorbs more than the other. This must cause a fine structure in the action spectrum. Some such structure is present in the action spectrum of photosynthesis as well as that of fluorescence; for example, the dip in the action spectrum of photosynthesis in *Chlorella* at about 660 nm, first noted by Emerson, was clearly demonstrated as real by recent careful measurements of Rajni Govindjee in our laboratory.

Altogether, the "separate package" theory, first proposed by L. N. M. Duysens, seems at present to be more plausible than the spill-over hypothesis. The spatial separation of PSI from PSII, indicated by fractionation experiments (to be described in Chapter 16) can well explain the absence (or at least, relative weakness) of "intersystem" transfer. Furthermore, one has to take into account that the potential barrier opposing the return of energy quantum from Chl a 695 to Chl a 680 (and thus also from PSI to PSII) should not be prohibitive at 300°K.

THE HILL REACTION AND THE BACTERIAL
PHOTOSYNTHESIS: TWO SYSTEMS OR ONE?

In the Hill reaction (as observed with NADP$^+$ as oxidant), the quantum requirement of 8 (per O_2 evolved), together with the occurrence of red drop and of an Emerson effect (R. Govindjee and also M. Avron, and coworkers) suggests the operation of both photochemical systems. (However, with ferricyanide as oxidant, at low light intensities, no Emerson effect could be detected in the Hill reaction.) Hill oxidants such as quinone and ferricyanide, with E_0-values of 0.0 volt (or higher) could conceivably enter the reaction sequence of Fig. 13.9 in the middle (instead of on top), so that PSII alone would be put to work, while PSI would idle. This would not decrease the quantum requirement, since one half of the light energy absorbed in the unoperative pigment system I would go to waste; and a "red drop" would still be present.

It seems that whenever the oxidation-reduction potential of the Hill oxidant is more negative than 0.0 volt, both pigment systems are needed; this is clearly the case in the Hill reaction with NADP$^+$ as oxidant. But if E_0 of the Hill oxidant is positive, one pigment system may suffice, at least under certain conditions (such as low light intensity, and sufficiently high concentration of the oxidant).

The energy requirement of bacterial photosynthesis could be easily satisfied by one light reaction; nevertheless, the quantum requirement is of the order of 8 quanta per reduced molecule of CO_2. No decline in the quantum yield at the far red end of the absorption band and no Emerson enhancement effect have been observed in bacteria. However, this does not preclude the existence of two photosystems; their absorption spectra may be simply more closely identical in bacteria than in the higher plants. Recently, Sigehiro Morita in Japan, M. A. Cussanovich, R. G. Bartsch, and Martin Kamen in La Jolla, California, and Christiaan Sybesma in our laboratory, all found evidence of the involvement of two photosystems in the photosynthesis of bacteria.