DIFFERENT FORMS OF CHLOROPHYLL *A* IN VIVO AND THEIR PHOTOCHEMICAL FUNCTION

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INTRODUCTION

From the study of the action spectra of chlorophyll fluorescence in algae containing different pigment complements, it has been concluded that quanta absorbed by pigments other than chlorophyll *a* are transferred by resonance to the latter pigment, with an efficiency between $< 20\%$ (for some carotenoids) and $> 90\%$ (for chlorophyll *b* and phycocyanin). Comparison with the action spectra of photosynthesis suggested that these quanta contribute to photosynthesis in proportion to the efficiency of their transfer—in other words, that ultimately photosynthesis is sensitized only by excited molecules of chlorophyll *a*, whether their excitation be due to direct absorption or to resonance transfer. This simple and eminently plausible picture is, however, unable to explain two phenomena: (I) The quantum yield of photosynthesis declines, in green algae (*Chlorella*) above 680 mμ (12, 13) (Fig. 1), and in red algae (*Porphyridium cruentum*) above 650 mμ (1) (Fig. 2)—i.e., in the region where light is absorbed *only* by chlorophyll *a*. The action spectrum of chlorophyll *a* fluorescence shows a similar "red drop," and it seems that it, too, occurs at considerably shorter wavelengths in red algae than in green algae (8). (II) The quantum yield of photosynthesis in the "red drop" region can be brought up, often to the normal level of about 10 quanta per molecule O_2, by simultaneous absorption of light of shorter wavelengths (9-11, 14). The action spectrum of this so-called "second Emerson effect" follows the absorption curves of the main accessory pigments (chlorophyll *b* in green algae, fucoxanthol in brown algae, the phycobilins in red and blue-green algae); or, more precisely, the curves showing the proportion of incident light absorbed by these pigments. This suggests that excitation of chlorophyll *a*, far from being the only mechanism of sensitization of photosynthesis,
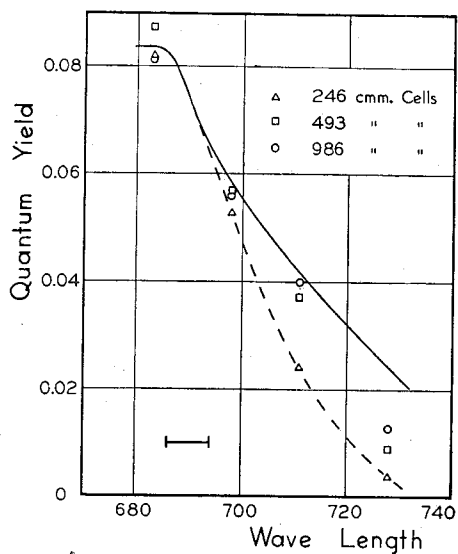
Fig. 1. Decline of quantum yield, ϕ, in the far red in *Chlorella*. Dashed curve, incomplete absorption; solid curve, approach to complete absorption (showing that red drop is not simply the consequence of incomplete absorption). (After Emerson and Lewis, 13).
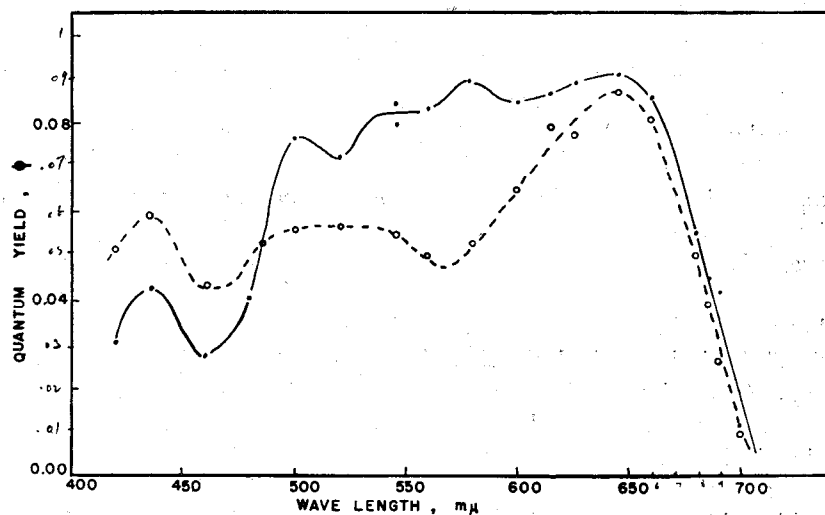

Fig. 2. Decline of quantum yield, ϕ, in the red in *Porphyridium*. Solid curve, cells adapted to green light; dotted curve, cells adapted to blue light. (After M. Brody and R. Emerson, 1.)

is in itself insufficient to produce photosynthesis, and needs supplementation by the absorption of quanta by one of the accessory pigments.

This picture, too, seems consistent and plausible—but it fails to explain the above-mentioned parallelism of the action spectra of photosynthesis and of chlorophyll *a* fluorescence. Why should excitation of chlorophyll *a* need supplementation by excitation of an accessory pigment, if the latter excitation is transferred practically quantitatively to chlorophyll *a?*

Prior to Emerson's experiments on the red drop, Duysens (8) suggested, as explanation of the apparent low photochemical efficiency of chlorophyll *a* in red algae, the presence in these organisms of two forms of chlorophyll *a,* one of which is preferentially coupled to the accessory pigments. This form is fluorescent and photochemically active, while the rest of chlorophyll *a* is non-fluorescent and photochemically inactive. To account for the "red drop," it could be further assumed that the two forms of chlorophyll have somewhat displaced absorption bands. Specifically, it could be suggested that the main red bands of both *Chlorella* and *Porphyridium* consist of two superimposed component bands—one with a peak at about 670 mμ ("Chl *a* 670"), which belongs to the photochemically active form of chlorophyll *a,* and another with a peak at 690-700 mμ ("Chl *a* 690"), which belongs to the photochemically inactive form of the same pigment. To explain the action spectrum of chlorophyll fluorescence, one would naturally suggest that the Chl *a* 670 form is fluorescent and the Chl *a* 690 form, non-fluorescent. (However, there is evidence (2) of a fluorescent band at 710-715 mμ probably associated with the 695 mμ absorption band.) It was suggested by Brody (2, 3) that the difference in the location of the red drop in *Chlorella* (at 680 mμ) and in *Porphyridium* (at 650 mμ) can be due simply to a much larger proportion of the inactive form, Chl *a* 690, in the latter. However, this interpretation probably is insufficient. The shape of the band is, in every case, suggestive of the presence of a third, and perhaps most abundant component, with a peak near 680 mμ—"Chl *a* 680." This is particularly clear in French's "derivative spectra" (4, 16). (He assigns positions at 673, 683, and 695 mμ to the three component bands in *Chlorella*). The question of the nature, the photochemical role, and the fluorescence of Chl *a* 680 naturally poses itself and the answer to it is far from clear. We will return to this subject in discussing the new experimental results presented in the following section.

NEW OBSERVATIONS

Action Spectra of the Emerson Effect

The hypothesis that two (or more) forms of chlorophyll *a*, with different photochemical properties, are present in vivo was confirmed by new and more precise measurements of the action spectrum of the Emerson effect (Figs. 3 A, B). Fig. 3A applies to the green alga, *Chlorella*. It shows the extent to which the quantum yield of photosynthesis produced by the absorption of light in a "far red" band (685-700 mμ) is enhanced by monochromatic supplementary light of different wavelengths. The curve has two sharp maxima. The one at 650 mμ (already observed by Emerson) must belong to chlorophyll *b*; the other, at 670 mμ, not noted by Emerson, indicates the existence of a chlorophyll *a* form having an absorption peak in this region—a form whose excitation can supplement that in the far-red band as effectively as does the excitation of chlorophyll *b*.

Fig. 3B refers to the diatom *Navicula*. The action spectrum of the Emerson effect in this diatom shows three peaks, at 535 mμ (attributable to fucoxanthol—this is not the peak of the fucoxanthol band, but the region of maximum participation of fucoxanthol in total absorp-
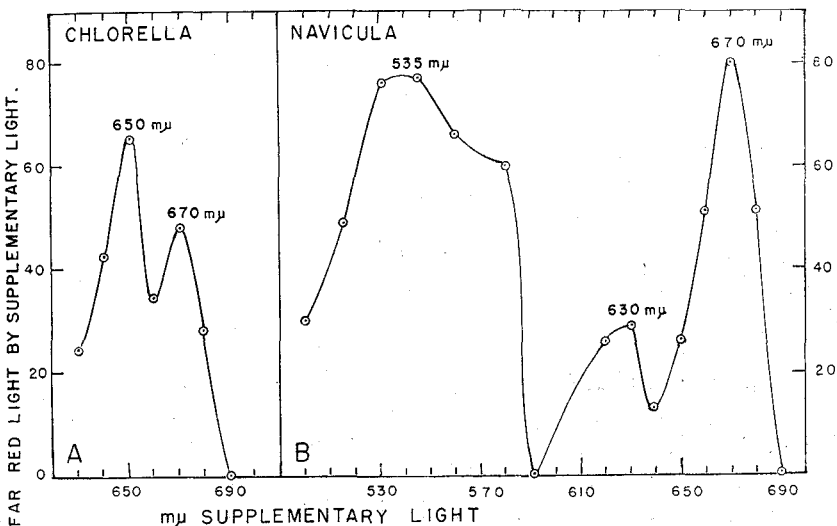

Fig. 3. A, Action spectrum of Emerson effect in *Chlorella* (after Govindjee and Rabinowitch, 17). B, Action spectrum of Emerson effect in *Navicula* (after Govindjee and Rabinowitch, 17).
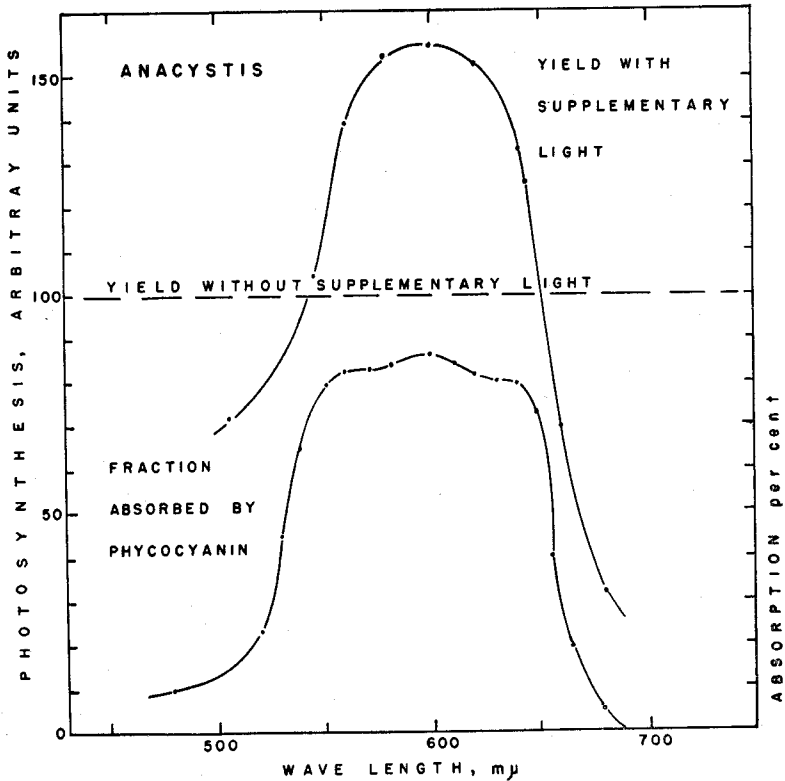
Fig. 4. Upper curve, action spectrum of Emerson effect in *Anacystis*. Lower curve, fraction of absorbed light absorbed by phycocyanin. (After Emerson and Rabino-witch, 14).

tion, cf. Tanada, 19), at 630 mμ (chlorophyll c), and at 670 mμ. There is no chlorophyll b in these algae and therefore no peak at 650 mμ.

These two curves seem to suggest that the form Chl a 680 does not supplement effectively the photochemical action of Chl a 690.

So far, so good; but now we are up for trouble. Fig. 4 was obtained by Emerson with the blue-green alga *Anacystis*. It shows only one peak, at 600 mμ, clearly attributable to phycocyanin; but when we move to longer waves, not only is there no clear peak at 670 mμ, but there is a *negative* region. This means that combination of far-red light (685-700 mμ) with monochromatic light in the region 670-700 mμ produces, in *Anacystis, less* photosynthesis than the two light beams separately! Emerson's measurements cf. 14 showed negative

effects not only in the action spectrum of *Anacystis,* but also in those of *Navicula* and *Porphyridium.*

The negative effect in the region 670-700 mμ in *Anacystis* suggests that this alga contains relatively little, if any, of the "activating" form, Chl *a* 670, and that the main chlorophyll *a* component in it, Chl *a* 680, not only does not complement the photochemical action of Chl *a* 690, but counteracts it! (The existence of such photochemical countereffects is well known in various photobiological phenomena.)

Another approach to the interpretation of the negative effects is through the study of light saturation in monochromatic light. So far, it has been presumed, on theoretical grounds—and confirmed by observations in filtered colored light—that light saturation of photosynthesis is independent of the spectral nature of the absorbed light, being imposed by the limited amount of some enzyme, and a consequent "ceiling" on the overall rate of photosynthesis. Some preliminary observations suggest, however, that the saturation rate may be much lower at 690 and 700 mμ than at the shorter wavelengths. If this is true, then the superposition of two beams, both belonging to the far red region, may produce a negative effect simply by bringing the process into the light-saturation range, even though one would not expect this to happen from the known extension of the "linear range" in white light. Such low saturation level may be itself conceivably due to some kind of a "photochemical counter effect."

Difference Spectra

The different fates of several chlorophyll *a* components in photosynthesis are suggested also by the shape of the "difference spectrum" of *Chlorella* (cf. Fig. 5, based on observations by Coleman in our laboratory; 5). The reversible bleaching of chlorophyll in strongly illuminated *Chlorella* cells is shown by this curve to affect Chl *a* 680 more strongly than Chl *a* 670 or Chl *a* 690; in fact, it seems possible that a reversible transformation of Chl *a* 680 into Chl *a* 670 and Chl *a* 690 takes place in light. Since, however, the whole difference spectrum remains negative above 600 mμ, this conversion must be superimposed on the transformation of Chl *a* into a compound with no red absorption band at all. (This compound may absorb in the 520 mμ band, and may perhaps be identical with Krasnovsky's "pink" reduced chlorophyll, for which the name "eosinophyll" was suggested (14).)

Krasnovsky (18, 20, 21) has observed preferential bleaching of Chl *a* 670 in light; but this was not confirmed by Brown and French (4),
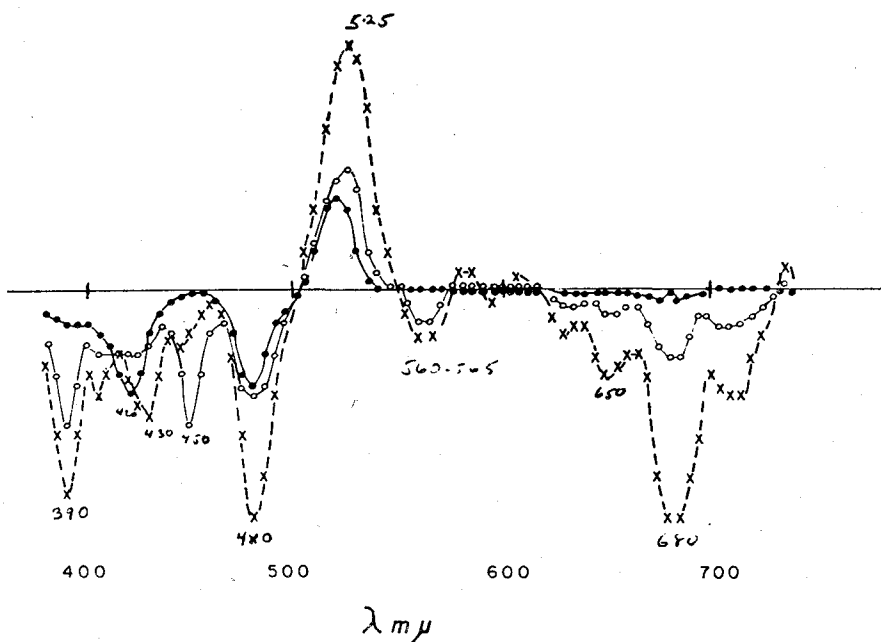
Fig. 5. Difference spectrum of *Chlorella* (after Coleman and Rabinowitch, 5). The three curves correspond to three light intensities (3.4, 12.0, and 31.4 $\times$ 10^{14} $h\nu/$ (sec $\times$ cm²). Reversible bleaching in the red chlorophyll *a* band is observed only at the higher intensities. It is strongest at 680 mμ, weaker at 670 mμ and 690 mμ. Maximum effects are of the order of 0.2%.

who found that the bleaching affects most strongly the 690 mμ form, next the 680 mμ form and least of all the 670 mμ form. In any case, these observations applied to more extensive and not immediately reversible bleaching, rather than to the small ($\sim$0.1%) instantaneous and fully reversible effect shown in Fig. 5.

CONCLUSIONS

All these results suggest that, in addition to the "one-dimensional" action spectra of photosynthesis, one should investigate systematically also "two-dimensional action spectra," i.e., the effect on the yield of photosynthesis of combinations of two monochromatic light beams of systematically varied wavelengths. That certain combinations of light colors have specific effects on photosynthesis has been asserted in the past, e.g., by Danilov (6, 7); but conclusions were unconvincing because of the primitive techniques used. It seems that these

studies are worth being repeated using more precise photochemical methods.

The enhancement of the quantum yield in the far red by auxiliary light suggests that the "inactive" form, Chl *a* 690, is not entirely inactive, but requires a balanced co-excitation of an "active" form to contribute fully to photosynthesis. It seems natural to think in this connection of the possibility that the primary photochemical process in photosynthesis might consist of two steps. Excited Chl *a* 690 may be able to bring about only one of these steps, while excited Chl *a* 670 may be able to sensitize both of them. More specifically, Franck (15) suggested that the Chl *a* 690 excitation leads practically entirely to the triplet state (because it occurs in chlorophyll associated only with lipides, in which the singlet-triplet conversion is accelerated by the existence of an intermediate n,π state) ; while the Chl *a* 670 excitation leads to a singlet excited state long-lived enough to emit fluorescence (because it occurs in chlorophyll *a* associated with water, in which the n,π state lies above the singlet excited π,π^* state). Why the cooperation of a chlorophyll *a* molecule, in an excited singlet state, with a chlorophyll *a* molecule in the triplet state is needed to bring about photosynthesis, is a separate question.

Franck's hypothesis is, of course, not the only possible explanation of the different photochemical capacities of Chl *a* 670 and Chl *a* 690 and of their cooperation in photosynthesis. Another alternative would be, for example, to postulate a limited photochemical capacity of a dimeric or polymeric form of chlorophyll *a*, and more versatile capacities of the monomeric form.

REFERENCES

1. Brody, M., and Emerson, R., *J. Gen. Physiol.,* 43, 251 (1959).
2. Brody, S. S., *Science,* 128, 838 (1958).
3. Brody, S. S., pers. commun.
4. Brown, J. S., and French, C. S., *Plant Physiol.,* 34, 305 (1959).
5. Coleman, J. W., and Rabinowitch, E., *J. Phys. Chem.,* 63, 30 (1959).
6. Danilov, A. N., *Sovet. Botan.,* No. 4, 3 (1935).
7. Danilov, A. N., *Eksp. Botanika,* 2, 5 (1936).
8. Duysens, L. N. M., Ph.D. Thesis, Univ. of Utrecht (1952).
9. Emerson, R., *Science,* 125, 746 (1957).
10. Emerson, R., *Science,* 127, 1059 (1958).
11. Emerson, R., and Chalmers, R. V., *Phycol. Soc. Am. News Bull.,* 11, 51 (1958).
12. Emerson, R., Chalmers, R. V., and Cederstrand, C., *Proc. Natl. Acad. Sci., U. S.,* 43, 133 (1957).
13. Emerson, R., and Lewis, C. M., *Am. J. Bot.,* 30, 165 (1943).
14. Emerson, R., and Rabinowitch, E., *Plant Physiol.,* 35, 477 (1960).
15. Franck, J., *Proc. Nat. Acad. Sci. U. S.,* 44, 941 (1958).
16. French, C. S., *Brookhaven Symposia in Biol.,* 11, 65 (1959).

17. Govindjee, and Rabinowitch, E., *Science,* **132,** 355 (1960).
18. Krasnovsky, A. A., and Kosobutskaya, L. M., *Doklady Akad. Nauk. SSSR,* **104,** 440 (1955).
19. Tanada, T., *Am. J. Botany,* **38,** 276 (1951).
20. Vorobyova, L. M., and Krasnovsky, A. A., *Biokhimiya,* **21,** 126 (1956).
21. Vorobyova, L. M., and Krasnovsky, A. A., *Biokhimiya,* **23,** 760 (1958).

DISCUSSION

DR. FRANCK: I am grateful for the opportunity to compare the basic differences of Dr. Calvin's concept of the photochemical steps of photosynthesis with mine. That I accept his biochemical results on the pathway of the carbon in photosynthesis, is self-evident. Our main difference is that Dr. Calvin, as practically all biochemists, takes it for granted that the reduction of the final photosynthetic oxidant proceeds by dark reactions with a photochemically produced reducing agent. On the other hand, according to my view, physical evidence indicates direct photochemical reduction acts with direct contact between the oxidants and the excited chlorophyll molecules. In the last years Dr. Calvin emphasized the hypothesis that the only light reaction of photosynthesis consists in a photoionization of chlorophyll which by migration of electrons and positive holes, produces a charge of a 'solar battery' sufficient for electrolysis of water. The original model of this battery is, according to my opinion, based on very improbable assumptions; the new one presented today avoids some difficulties, but others remain. I enumerate the main ones briefly:

1) I still have grave doubts whether the quantum yield of photoionization can be high enough to match the high yield of photosynthesis. That a limited photoionization occurs, is shown by the behavior of the afterglow of chlorophyll, found by Strehler and Arnold, and explained as recombination luminosity of positive chlorophyll ions with electrons, by Arnold. That this hypothesis fits all observations of afterglow, has been discussed in a paper of Brugger and Franck.

2) The assumption that light energy is fully utilized for photoionization has failed to explain the very characteristic relations between chlorophyll fluorescence intensity and utilization of light energy for photosynthesis. All factors which lower the photosynthetic utilization of excitation energy, as low temperature, absence of CO_2, induction phenomena, addition of enzymatic poisons, etc., enhance the fluorescence, but not more than by a factor of ca. 2, under conditions where photosynthetic rates are zero during illumination. A detailed up-to-date description of such observations and their interpretation based on the concept of direct contact between oxidants and excited chlorophyll, will be found in an article on chlorophyll afterglow in the *Handbuch für Pflanzenbiologie,* Vol. V. (The article was originally written several years ago and is finally due to appear this spring).

3) Another point not considered so far in Dr. Calvin's concept is an