Effect of Combining Far-Red Light with Shorter Wave Light on the Excitation of Fluorescence in Chlorella

Recent experiments on the rate of photosynthesis in far-red light alone and in the presence of supplementary light of shorter wavelengths (1–8), have revealed a previously unsuspected complexity of the light-absorbing pigment system. It appears to contain several forms of chlorophyll *a* with slightly different absorption bands and different photochemical capacities, forms whose excitations can complement each other in bringing about photosynthesis.

Because of the parallelism which Duysens (9) found between the efficiency with which light absorbed by a certain pigment produces photosynthesis and its efficiency in exciting the fluorescence of chlorophyll *a*, it seemed natural to look for similar complications also in the action spectrum of the excitation of chlorophyll *a* fluorescence *in vivo*. It is known that the quantum yield of fluorescence in *Chlorella* drops when excitation is achieved by light above 680 mμ (10). It is an open question whether this drop is due to dimerization, as in chlorophyll solutions (11, 12), or to some other reasons, related to those responsible for the decline of the quantum yield of photosynthesis in the far red. (Alternatively, the latter decline, too, may be ascribed to the dimerization of chlorophyll *in vivo*, as suggested by Brody (13)).

While attempting a systematic study of the intensity and spectrum of chlorophyll *a* fluorescence *in vivo* excited by monochromatic light of different wavelengths, we noted unexpectedly an effect opposite in sign to the "second Emerson effect" in photosynthesis (1–8), namely: combining a far-red beam (700 ±5 mμ) with a beam of shorter wavelength caused a *decline* (rather than an enhancement) of the yield of fluorescence, compared to that produced by the two beams separately. Thirteen experiments of this type are summarized in Table I.

Chlorella pyrenoidosa Strain 3 were used, grown (usually for seven days) in combined light from fluorescent and incandescent lamps, at 20°C. in a "standard nitrate medium" (14). Two light beams ("A" and "B") isolated by two monochromators with 10 mμ wide exit slits, were arranged so as to hit the same spot on the face of the cuvette under 45° to the surface, with an angle of 90° between their directions; fluorescence was measured normally to the surface. When beam "A" consisted of blue light (436 mμ), the slit needed to obtain enough light was as wide as 40 mμ. There was no danger, however, of contaminating fluorescence by scattering, since fluorescence was observed through a sharp-cut off filter RG8 and a third monochromator, set at 685 mμ with a band width of 30 mμ.

The intensity of fluorescence was measured by a RCA 6217 photomultiplier connected to a mirror galvanometer. Separation of fluorescence from scattering was reliable for the short-wave exciting beam "A"; but not for the far-red beam "B" (700 mμ). Consequently, the intensities listed in column B in Table I represent sums of the intensities of fluorescence excited by the 700 mμ beam, and the intensities of scattered light from this beam; the second component may well be the dominant one, because fluorescence excited by the 700 mμ beam must be largely "anti-stokes" fluorescence with a correspondingly low yield.

Since no sudden decrease in the scattering of the 700 mμ beam is likely to be caused by the addition of a 436 mμ or 670 mμ beam, the effect in column E must be due practically entirely to a decrease in the yield of fluorescence. Whether it is the yield of fluorescence excited by beam "A" which is lowered under the influence of beam "B," or vice versa, cannot be answered categorically. However, a large part of the observed decrease in the yield of fluorescence must be due to the first cause, because in some experiments, the decrease was greater than the sum of fluorescence and scattering in 700 mμ light (D > B). Considering that only a small part of the intensities shown in column B is likely to be due to fluorescence, we can surmise that better elimination of scattered light would bring also other ratios 100D/B above 100%, thus suggesting a preponderant (and perhaps exclusive) contribution to the observed effect of the quenching, by the 700 mμ beam, of fluorescence excited by the shorter-wave beam.

To make sure that the change in yield was due to changes in spectral composition, and not in the intensity of exciting light, we made measurements of fluoresence yield at different intensities of incident beam "A" alone, and found strict proportionality.

Perhaps, excitation at 700 mμ generates an

TABLE I

EFFECT OF SIMULTANEOUS EXCITATION BY FAR-RED LIGHT AND SHORTER WAVE LIGHT ON FLUORESCENCE OF CHLOROPHYLL *a in vivo*

1–10 *Chlorella pyrenoidosa* Strain 3; 11–13 *Chlorella* mutant G-10, obtained from Dr. Mary B. Allen; 1–7 and 12–13, 7-day-old cultures; 9–10, 4-day-old cultures; 8 and 11, uncertain age. Short wave light: 436 mμ in experiments 5 thru 13; 670 mμ in 1 thru 4. Observing at λ = 685 mμ.

Exp. No.	Approximate relative intensities of short wave light "A"	Fluorescence due to short wave light "A" A	Fluorescence + scattering due to far-red light "B" (700 mμ) B	Fluorescence + scattering due to far-red light (700 mμ) + short wave light C	A + B − C D	% quenching (calculated by assuming change in fluorescence due to short wave light alone) 100 D/A E
1	10	28.2	32.0	55.1	5.1	18.1
2	5	15.0	13.0	19.4	8.6	57.4
3	10	53.5	39.0	86.2	6.3	11.8
4	5	18.8	29.0	37.9	9.9	52.6
5	5	9.4	12.0	17.5	3.9	41.5
6	1	1.9	1.9	3.4	0.4	21.0
7	1	5.5	1.1	5.3	1.3	23.6
8	5	27.0	35.7	42.5	20.2	75.0
9	1	8.0	14.5	19.5	3.0	37.5
10	1	8.2	3.5	11.0	0.7	8.6
11	—	28.4	29.0	24.4	33.0	116.0
12	—	29.0	0.1	29.0	0.1	0.3
13	—	5.3	6.9	11.8	0.4	7.55

"energy sink;" the quenching of fluorescence may then be due to migration of the excitation supplied by the 670 mμ (or 436 mμ) beam, into this sink. Why the effects on photosynthesis and fluorescence have opposite signs, we do not know. It remains to be seen whether the sign of the effect can be changed by changing the relative intensity of the two beams, as we sometime find it in photosynthesis.

The phenomenon may be associated either with a special form of chlorophyll *a*, or with an unknown pigment.

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Chromatographic Evidence for the Biosynthesis of a "New" Heptulose in Plants

Thus far sedoheptulose is the only seven-carbon sugar which has been assigned a physiological role in plants (1). The possibility that manno-heptulose phosphate might be a common metabolic intermediate was suggested by Nordal and Benson (2) who isolated this sugar phosphate from avocado leaves, but a specific role has not been defined. The free sugar had been isolated much earlier from avocado fruit by LaForge (3).

Studies were undertaken in an attempt to elucidate the physiological origin of mannoheptulose. Since previous studies had revealed that this sugar occurs in alfalfa and we had employed this plant in earlier investigation, we decided to use it for the present experiments. The plants were grown in nutrient cultures using techniques similar to those previously described (4). Heptuloses were detected chromatographically by spotting the expressed juice or alcoholic extracts of the plants on Whatman No. 1 paper, irrigating with ethyl acetate–pyridine–water (8:2:1), and dipping in orcinol reagent (5).