Emerson Enhancement Effect in Chloroplast Reactions [1, 2]

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The discovery of the Emerson enhancement effect in photosynthesis (3–6, 8) led to the suggestion that there are 2 photochemical reactions necessary for complete photosynthesis. A likely hypothesis is that the 2 reactions are carried on by different forms of chlorophyll a (10, 11). The enhancement effect in photosynthesis has been studied extensively in several laboratories (19) and it has been shown (12, 14, 15) that the effect also occurs in O_2 evolution in the Hill reaction using quinone as the electron acceptor. Govindjee et al. (13) have recently reported the existence of the Emerson enhancement effect in NADP photoreduction by spinach chloroplasts using white (fluorescent) supplementary light. This paper deals with observations on the enhancement of NADP photoreduction and O_2 evolution in chloroplast preparations, using both white and monochromatic lights.

Materials and Methods

Preparation of Chloroplasts. Chloroplasts from spinach leaves were prepared by a method similar to that of Hill and Walker (16). Fresh spinach leaves were first cooled in ice cold water, deribbed, and then chopped into small pieces. The chopped spinach leaves were ground with white sand using a minimum quantity of a solution containing 0.01 M NaCl, 0.4 M sucrose, and 0.05 M Tris, pH 7.5. The resulting suspension was filtered through 4 layers of cheese cloth and then centrifuged for 1 minute at 200 g. The cell debris was discarded and the supernatant fluid centrifuged for 10 minutes at 1000 to 1500 × *g*. This supernatant material was discarded and the pellet was suspended in buffer and recentrifuged at the same speed for 10 minutes. The supernatant solution was again discarded and the chloroplasts made up in a known volume of the buffer. Chlorophyll concentration was estimated spectroscopically (1).

Reaction Mixture. The reaction mixture used in the experiments had the following constituents in μmoles per 2 ml of total volume: KH_2PO_4, 100 (pH 7.3); $MgCl_2$, 15; ADP, 2; NADP, 1; chloroplasts containing 100 μg of chlorophyll, and an optimal amount of photosynthetic phosphopyridine nucleotide reductase (PPNR).

PPNR was prepared according to the method of San Pietro and Lang (20) and was partially purified through the Dowex-Bentonite step.

Optical System. One beam of light was obtained from a 750 w tungsten lamp controlled by a variac. The light beam was made parallel, passed through a 30 cm water filter and appropriate interference and colored glass filters and was then focussed on a water-cooled Beckman cuvette that contained the reaction mixture. The maxima of the Bausch and Lomb second order intererfence filters (35 % peak transmission, half-band width 10 mμ) and the numbers of their associated colored glasses are: 678 mμ (2-61 Corning), 693 mμ (RG-5 Schott and Gen.), 700 mμ (RG-5 Schott and Gen.), 714 mμ (RG-8 Schott and Gen.), 721 mμ (RG-8 Schott and Gen.), 730 mμ (RG-8 Schott and Gen.), and 740 mμ (RG-8 Schott and Gen.).

A second beam of light, the supplementary white light, was obtained from a 40 w white fluorescent tube. This light did not contain any far-red wavelengths. It was chosen so as to get a mixture of wavelengths that would excite both chlorophyll b and the short wave form of chlorophyll a (chlorophyll a 670). Supplementary red light was obtained from a 300 w slide projector by interposing a 650 mμ interference filter and OG-2 (Schott and Gen.) colored glass in the light path. A 15 cm water cuvette served as a heat filter. This beam was projected on the Beckman cuvette (containing the reaction mixture) from the side opposite to the one which received the first beam of light (678 mμ to 740 mμ).

The optical system in the case of the determination of O_2 evolution and that used in the difference spectrometer are described at appropriate places.

Exposures of the Samples to Light. Each of the 6 cuvettes containing the reaction mixture was exposed for 10 minutes to a different wavelength of far red light (693 mμ to 740 mμ). An identical set was exposed to supplementary light (either white or 650 mμ) and a third set to the combined far-red and supplementary lights. In the combined lights experiment, the beams from the opposite sides hit the same suspension at the same time. A dark control was always maintained. The water bath was regulated at 20°.

Determination of the Amount of NADP Reduced. After exposure, the chloroplasts were centrifuged out of the reaction mixture. The optical density of

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the clear supernatant solution was measured at 340 mμ in a Cary recording spectrophotometer, model 14.

Measurement of O_2 Evolution. O_2 evolution was measured polarographically by the use of a Clark electrode. The light from a 500 w tungsten lamp was made parallel and passed through a 15 cm water bath. The light was divided into 2 beams which were passed through 2 different filters and then reconverged on the same surface of the reaction vessel, which was submerged in a water bath maintained at 10°. The signal from the electrode was amplified and recorded.

Measurement of Absorption and Energy. The per cent absorption by the chloroplast suspension (50 μg chlorophyll/ml) at different wavelengths was determined in an integrating sphere. The energy of the 2 incident beams was measured by a photocell. The galvanometer readings were converted into absolute units by previously determined calibration values.

Results

The initial experiments were to determine (1) whether the rate of reduction of NADP was linear with time, (2) that the rate of reoxidation of reduced NADP was not significant, and (3) that we were working in the linear portion of the light curve (rate of NADP reduction versus intensity of light).

Rate of NADP Photoreduction as a Function of Time and Rate of Reoxidation of Reduced NADP in the Dark. In order to obtain a continuous plot of the NADP concentration as a function of time, we used a difference spectrophotometer. The 650 mμ light was isolated from a 500 w tungsten lamp by means of an interference filter-colored glass combination. The measuring monochromator of the difference spectrophotometer was set at 340 mμ and a Corning glass 7-60 was used to eliminate the second order overlap. The rate of NADP reduction was found to be linear up to 10 minutes at the intensity (5 μeinsteins/10 minutes) and with the chlorophyll concentration (50 μg/ml) used. 5 μg/ml and 10 μg/ml concentrations of chlorophyll also gave linear curves. This ensured that neither NADP nor the enzyme was a limiting factor in our experiments.

The rate of dark reoxidation of NADP (after the exposure to light) was determined both in the difference spectrophotometer and in the Cary spectrophotometer. This rate did not exceed 0.01 OD/100 seconds.

Rate of Photoreduction of NADP versus Intensity of Light. The rate of photoreduction of NADP as a function of light intensity was measured at 714 mμ and 650 mμ. The results are presented in figure 1. The rate of NADP reduction increases linearly with increase in incident light of 714 mμ. The rate versus light intensity curve of 650 mμ is linear up to the incident intensity of 0.2 μeinsteins/minute. The slope of this curve gets progressively smaller with further increase in incident intensity. This will tend to mask the enhancement effect even when it is really present at these intensities.

Red Drop in NADP Photoreduction. Emerson and Lewis (7) had discovered the existence of a decline in the quantum yield of photosynthesis in the far-red end of the spectrum. We undertook the measurement of the quantum yield of the photoreduction of NADP in the 678 mμ to 740 mμ region. The quantum yield was calculated as the number of μmoles of NADP reduced per minute divided by the number of μeinsteins of light absorbed per minute.

Figure 2 shows the quantum yield of NADP versus the wavelength of light. The decline in the yield begins at 690 mμ and the yield is half at 707 mμ. This is the region where the longwave form of chlorophyll a becomes the prime absorber of light energy.

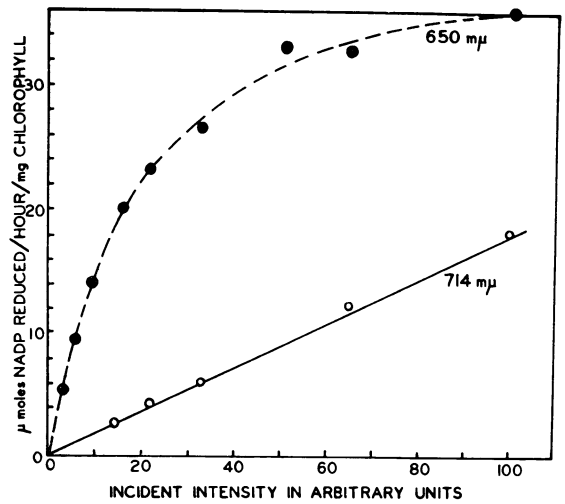
Emerson Enhancement Effect with White Fluorescent Light. The middle curve of figure 2 shows the quantum yield of NADP reduction as a function of wavelength in the presence of supplementary white light. The Emerson enhancement represented by the equation below:

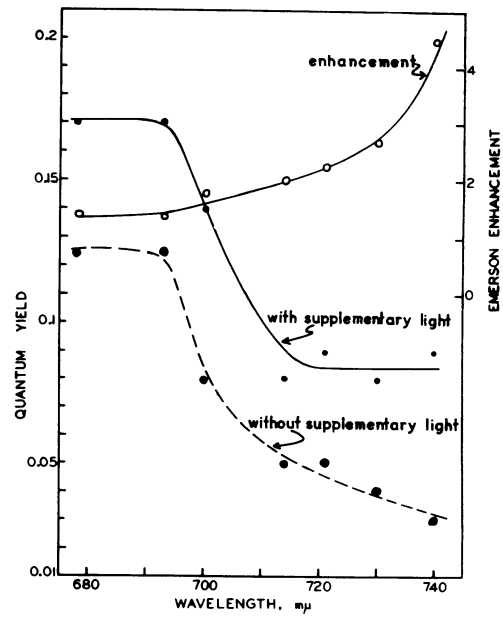
$$\frac{R_{(\text{far red + supplementary lights})} - R_{(\text{supplementary light})}}{R_{(\text{far red light})}}$$

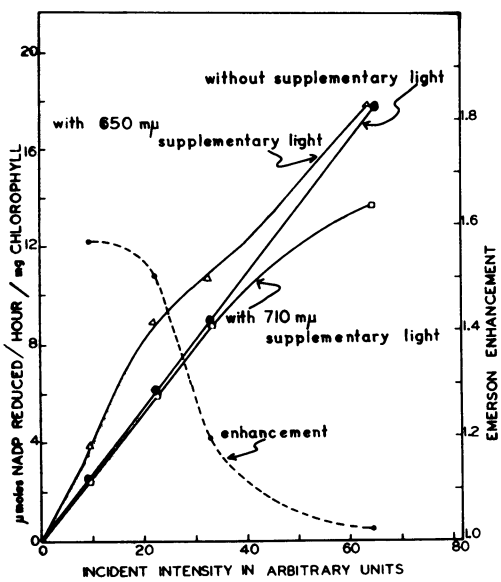
where R represents the rate of the photochemical reaction is shown by the upper curve.

Since some enhancement was also observed with 678 mμ, the following questions can be raised: if Emerson's assumption that the yield in the short-wave light is maximum, is correct; and if the enhancement is also possible on the short-wave light by the long-wave light. We have not attempted to answer these questions here.

Emerson Enhancement Effect with Monochromatic Light. The rate of NADP reduction as a function of light intensity (quantum yield) was determined at 714 mμ singly and in combination with a background of 650 mμ light. Figure 3 shows the results. The yield at 714 mμ is greater when determined on a background of 650 mμ than it is in 714 mμ light alone. An enhancement effect is clearly seen. The Emerson enhancement decreases with the increase in the intensity of 714 mμ light. In other words, it increases with an increase in the ratio of far red to supplementary lights. The point at the highest intensity of 714 mμ apparently shows no enhancement. The light intensity of the combined beams is in the non-linear (sloping) part of the light-curve. A spurious enhancement can be obtained by sigmoid light curves at far red wavelength, addition of any light would then give an apparent enhancement. This point was checked by determining the light curve for NADP reduction at 714 mμ on a background of 710 mμ. No enhancement is seen with these 2 wavelengths as no change in the 714 mμ light curve is observed upon adding the 710 mμ background except for a falloff of rate at the highest intensity, doubtless due to saturation.







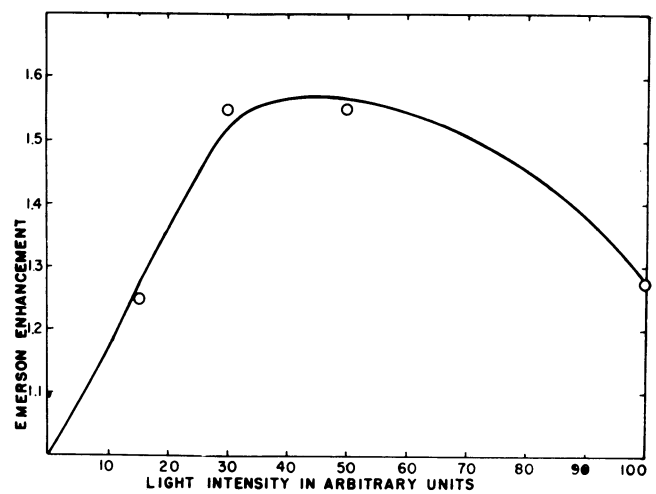


FIG. 1 (*upper left*). The rate of photoreduction of NADP versus the incident intensity of 650 mμ (dashed curve with solid dots) and 714 mμ (solid line with open circles) lights. Temperature is 20° and the chlorophyll content is 50 μg/ml reaction mixture; per cent absorption at 650 mμ is 86 and at 710 mμ is 18; 100 % for the incident intensity of 650 mμ is 2.12 μeinsteins/minute and it is 4.06 μeinsteins/minute for 710 mμ.

FIG. 2 (*upper right*). The quantum yield of NADP photoreduction at different wavelengths of light (dashed curve with solid dots). The yield is half at 707 mμ. Temperature, 22° and time, 10 minutes; chlorophyll content, 20 μg/2 ml reaction mixture; absorbed intensity, 0.06 μeinsteins/minute (energy adjusted so as to get equal number of absorbed quanta at all the wavelengths). Solid line with solid points, the quantum yield of NADP reduction in the presence of constant intensity of white (fluorescent) light. Solid lines with open circles, Emerson enhancement.

FIG. 3 (*lower left*). μmoles of NADP reduced as a function of the intensity of 714 mμ light with and without background of 650 mμ and 710 mμ lights. The amount of NADP reduced by different intensities of 714 mμ is shown by solid points, that by combined (710 mμ + 714 mμ) minus 710 mμ light by open squares and that by the combined (650 mμ + 714 mμ) minus 650 mμ by open triangles. NADP reduced/mg/hr chlorophyll by 650 mμ alone equals 5.4 μmoles and that by 710 mμ equals 4.3 μmoles. 65 on the intensity scale is equivalent to 2.9 μeinsteins/minute incident 714 mμ light; temperature is 22° and the chlorophyll content is 50 μg/ml reaction mixture. The dashed curve with solid points shows the Emerson enhancement obtained at various far red wavelengths with 650 mμ supplementary light.

FIG. 4 (*lower right*). Emerson enhancement versus intensity of 650 mμ light. Temperature, 20°; Time, 10 minutes; chlorophyll content is 50 μg/ml reaction mixture. Incident intensity of 714 mμ is 0.98 μeinsteins/minute; 100 % of 650 mμ intensity is 5.15 μeinsteins incident/minute.

An experiment was made in which 714 mμ light intensity was kept constant and that of 650 mμ was varied. The Emerson enhancement calculated from this experiment increases with increasing intensity of 650 mμ light up to a point and then saturates. However, the highest intensity used showed a lesser enhancement since the intensity of the combined beams reached the sloping part of the light curve. Figure 4 is a plot of the Emerson enhancement as a function of the intensity of the 650 mμ light. The intensity relationship shown here is the same as that described in literature on enhancement phenomenon (3, 19).

Unpublished results of L. Yang and Govindjee show clearly a peak at 650 mμ (due to chlorophyll b) and another one around 675 mμ (due to chlorophyll a) in the action spectrum of the Emerson effect in the Hill reaction using NADP as the electron acceptor. These results also find their parallel in the experiments on photosynthesis (10, 11).

Emerson Enhancement Effect in the O_2 Evolution of Chloroplasts. The amount of O_2 evolved by chloroplasts was also measured in both separate and the combined beams by a polarographic method, using a Clark electrode. The reaction mixture was the same as that for the measurement of NADP reduction. Several combinations of far red light (730 mμ, 721 mμ and 713 mμ) with red light (653 mμ, 663 mμ and 668 mμ) were tried and the data are presented in table I. The last column clearly shows the existence of an Emerson enhancement effect in the O_2 evolution by chloroplasts. When the intensity of the far red beam is the same (as in lines 2 and 3), the Emerson effect increases with the increase in the intensity of the 653 mμ supplementary beam. It is also shown that for about the same ratio (1:7–1:8) of far red to supplementary light intensities, the enhancement is seen with 653 mμ, and 668 mμ (14). Lines 1, 4, and 7 show that for approximately the same intensity of 653 mμ, the Emerson enhancement is present on all the 3 wavelengths of the far red light.

Discussion

The results presented here on the red drop and the Emerson effect find close parallelism to the data obtained with studies on photosynthesis (3, 6, 8, 11, 19). This emphasizes that a common or similar mechanism exists both for the Hill reaction (using NADP as electron acceptor) and complete photosynthesis.

It was earlier reported (14, 15) that there exists a red drop and the Emerson effect in the Hill reaction in whole Chlorella cells using quinone as the electron acceptor. Mayne and Brown (18) could not confirm this at the intensities they used. However, the enhancement in the Hill reaction using quinone as the electron acceptor, has further been demonstrated in pokeweed chloroplasts (12). In this paper we have clearly demonstrated the existence of the red drop and the Emerson effect in the Hill reaction (with NADP as electron acceptor). An enhancement in O_2 evolution is shown to occur in the same system. Bishop and Whittingham (3) have recently observed Emerson enhancement in a chloroplast reaction using ferricyanide as the electron acceptor. We interpret these to mean that there are at least 2 photochemical reactions necessary for the complete Hill reaction to take place (17).

Gordon (9) has reported the existence of enhancement in the yield of NADP reduction by far red light, when the algae were preilluminated with 660 mμ. Our experiments reported here have not been done under those conditions and we have not attempted to study the effect of preillumination. However, preliminary experiments of Yang and Govindjee (unpublished) have failed to demonstrate the enhancement effect caused by preillumination in spinach chloroplasts. If the results of Gordon represent enhancment in the same manner as in photo-

Table I

Emerson Enhancement Effect in Oxygen Evolution by Chloroplasts Using NADP as the Electron Acceptor

	Wavelengths*		Rate of O_2 evolution in arbitrary units			Emerson enhancement	Δ C−(B+A)
	Far-red mμ	Supplementary mμ	A Far-red light	B Supplementary light	C Combined lights		
1.	713 (100 %)	653 (57 %)	+15.5	+49.5	+73.5	1.55	8.5
2.	721 (57 %)	653 (15 %)	+ 5.0	+14.0	+21.0	1.40	2.0
3.	721 (57 %)	653 (57 %)	+ 3.0	+36.0	+43.0	2.33	4.0
4.	721 (100 %)	653 (57 %)	+ 6.4	+42.6	+57.1	2.33	8.1
5.	721 (100 %)	663 (57 %)	+ 8.0	+55.8	+69.0	1.65	5.2
6.	721 (100 %)	668 (57 %)	+ 6.5	+53.8	+65.4	1.79	5.1
7.	730 (100 %)	653 (57 %)	+ 2.8	+45.2	+58.4	4.74	10.4

* These wavelengths were obtained by the use of second order Bausch and Lomb interference filters combined with sharp cut-off colored glasses. The figures within brackets indicate the different intensities of light used as per cent of the total available.

synthesis, present schemes for the mechanism of the 2 light reactions (based on cytochrome coupling) must be modified. However, the 2 effects may arise from the same cause. We do not, however, suggest that the mechanism of all the different Hill reactions is one and the same.

Summary

The Emerson enhancement effect has been shown to occur in the photoreduction of nicotinamide adenine dinucleotidephosphate by spinach chloroplasts. Greater than additive rates were obtained where far-red light beams were mixed with supplementary white (fluorescent) or monochromatic 650 mμ light in nicotinamide adenine dinucleotidephosphate reduction, and O_2 evolution. In magnitude, wavelength, and intensity dependence the enhancement effect as measured in the Hill reaction appears analogous to that occurring in complete photosynthesis.

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