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AN ANALYSIS OF A TRIPLET EXCITON MODEL FOR THE DELAYED LIGHT IN *CHLORELLA**

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Abstract—The time dependence of the delayed light in the green alga *Chlorella pyrenoidosa* has been examined quantitatively in the 1 to 12 msec range after excitation with light pulses ($\lambda = 6328$ Å) of 100 μsec and 4·5 msec duration. We have confirmed the data of Tollin, Ruby, and Bertsch *et al.*, on the time course of the delayed light in the msec range. New experiments, with 100 μsec flash excitation, on the time dependence of the delayed light emitted by *Chlorella* treated with 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DMU), hydroxylamine, methyl violgen, and various combinations of these chemicals are presented. Also, data on the dependence of the delayed light intensity on the intensity of the excitation light in the 1·5 and 5·0 msec range are reported. The square law dependence, reported by Jones, in the 140 and 250 msec range is confirmed in the 1·5 and 5·0 msec range at very low light levels.

The experimental data on delayed light has been analyzed in terms of a model which incorporates triplet exciton fusion. The following major points result from this analysis: (1) A triplet exciton kinetic model can explain both the time dependence and the excitation intensity dependence of the delayed light emitted by *Chlorella*. (2) The density of triplet excitons predicted by the model from the observed delayed light intensity is much less than that which can be detected by flash photolysis measurements. Therefore, the failure of such measurements to detect triplet states *in vivo* does not disprove the model. (3) The possibility of changes in the rate of electron transfer reactions of photosynthesis is included in the kinetic model. The predictions from the model are compared with the effects of chemical additives on the time dependence of the delayed light decay. (4) The proposed triplet exciton model predicts that the delayed light intensity may, under certain specific conditions, be affected by a magnetic field. The negative result of an attempt to observe this effect is reported and discussed. (5) It is concluded that the proposed triplet 'fusion' model is a valid alternative to the electron-hole recombination model.

A. INTRODUCTION

THE CHLOROPHYLL molecules in green plants emit delayed light[1]. Since its discovery by Strehler and Arnold in 1951[2], many workers have attributed this emission to an electron-hole recombination process in the chloroplast[3–8]. Others have suggested that triplet states of chlorophyll may be involved[9–12]. In a process analogous to electron-hole recombination two triplet excitons might annihilate (triplet exciton fusion) to produce an excited singlet state which could decay with the emission of a photon. This mechanism has been well established for a number of organic solids and solutions[13]. The resulting light would have the same spectral characteristics as the normal prompt fluorescence but a slower decay time due either to the longer lifetime of the triplets relative to that of the singlets, or to a slow rate of triplet formation.

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Triplet state mechanisms, however, have not been well received. The strongest argument against the importance of triplet states in the chloroplast is based on flash photolysis measurements which have failed to show any triplet–triplet absorption [14–16]. (In such an experiment the absorption spectrum is monitored immediately after the system has been exposed to an intense light flash, and triplet states are detected as small transient changes in the optical density.) Moreover, phosphorescence from chlorophyll *a in vivo* has not been reported [17]. On the basis of these negative results, it has been stated that, in the msec region, there are too few triplets present in the photosynthetic apparatus to account for the intensity of the delayed light [8].

The present paper reexamines the importance of triplet states in terms of the time dependence of the delayed light and a kinetic model based on triplet exciton fusion. It is found that the kinetic model can describe the time decay of the delayed light in the msec time region. The appropriate parameters of the model are used to estimate the triplet exciton density during this time range and, contrary to the above objection, it is found that a very low triplet density can account for the intensity of the delayed light.

The effect of a magnetic field on the intensity of the delayed light was measured for field strengths up to 18 kG. This measurement was prompted by recent studies of crystalline anthracene and tetracene which indicate that, under certain conditions, the delayed fluorescence produced by triplet exciton fusion is sensitive to a magnetic field [18–21]. Such a magnetic field effect was not observed here within the limits of the experimental accuracy. An analysis of this negative result in terms of the original theory by Merrifield [21] and the appropriate parameters taken from the time decay measurements suggests that this experimental accuracy was not sufficient to confirm or deny the existence of triplet states.

1. *Delayed light emission in green plants*

The basic characteristics of the delayed light emitted by plants are its slow decay, which may persist for many minutes after the exciting light has been turned off [2], and its emission spectrum which is similar to that of the normal fluorescence of chlorophyll *a in vivo* [9, 22–24]. The time decay is complex and does not follow first order kinetics [1, 9, 25, 26]. However, in a qualitative fashion, one can distinguish two major components in the decay, a fast decaying component of a few msec (or faster) duration, and a slowly decaying component of several hundred msec duration. (Here we use the word 'component' qualitatively and do not suggest that the decay can be represented as a sum of two first order decays.)

The intensity of the delayed light relative to that of the normal fluorescence is difficult to define experimentally because the two cannot be measured simultaneously since both have the same emission spectra [cf. Ref. 9]. One millisecond after the excitation light has been turned off the delayed light intensity is between 10^{-3} to 10^{-5} of the steady state fluorescence intensity [1]. Attempts to measure the quantum yield of the delayed light after excitation with blue light have produced values from 10^{-4} to 10^{-6} [22–24, 26].

Delayed light in plants is dependent on the electron transfer reaction of photosynthesis. Chemical poisons, which act on the initial reactions of photosynthesis, have a significant effect on both the intensity and the time dependence of the delayed light [9, 27–29]. In addition, it has been shown in a photosynthetic bacterium *Rhodopseudomonas spheroides* that functional reaction centers must be present in order for delayed

light to be emitted[30]. In green plants, photoreaction II (for a discussion of photoreactions I and II, see Ref. [31–33]) is directly associated with the delayed light[9, 34–36]. Mutants of green algae (*Chlamydomonas* and *Scenedesmus*) that lack system II have negligible delayed light, but those lacking system I have a full complement of it.

2. *Description of the model and the experiment*

In order to test the triplet fusion hypothesis for delayed light in plants, we shall adopt a working model based on the Hill–Bendall scheme of electron transport in photosynthesis[31–33]. Our main assumption is that triplet excitons are produced most efficiently at the trapping molecule of photosystem II (see Section D of this paper). From there they are free to diffuse into the bulk pigment system and eventually decay or annihilate with other triplets produced in a similar manner. The triplets escape the trapping molecule because it is only effective in trapping singlet excitons. This sort of behavior is to be expected if the trap is due to 'environmental effects' similar to those in crystalline anthracene which slightly lower the energy of the first excited singlet although it still remains higher than that of the triplet[35].

The overall process resulting in delayed light can then be described as follows. Following light absorption by a bulk pigment molecule, the energy can be emitted as normal fluorescence or transferred to the trap molecule. At the trap molecule the excitation energy has three choices; it can drive the electron transport chain (chemistry), it can decay by fluorescence or internal conversion, or it can populate a triplet state by intersystem crossing. The triplet excitons can then either decay, probably by a radiationless transition, or undergo fusion, and produce an excited singlet in the bulk.

We also assume that triplets can be produced at the trapping site by chemical back reactions as suggested by Lavorel[9]. The production of singlets in this manner is possibly diminished by the usual energy degradation accompanying the electron transfer reactions[9]. Lavorel[9] has pointed out that only 1·0 eV may be available from the back reaction of system II, but 1·8 eV is needed to form the singlet excited state of chlorophyll *a*. Thus, there is not enough energy for the direct formation of singlets.

Under certain conditions, the delayed light predicted by this model (see Appendix) should be sensitive to a magnetic field. In particular, if $\gamma n_T \ll \beta$ (where γ is the fusion rate constant, n_T is the triplet exciton density, and β is the triplet reciprocal lifetime), then the delayed light intensity is proportional to γ which is dependent on the magnetic field strength. The sensitivity of γ to the magnetic field depends on the magnitude of the annihilation probability (the probability that a particular encounter between two triplet excitons will result in a singlet exciton).

At low light levels, when $\gamma n_T \ll \beta$, the intensity of the delayed light is proportional to the square of the excitation intensity because two triplets requiring the absorption of two quanta are needed (see Appendix). At higher light levels, when $\gamma n_T \ll \beta$, the quadratic dependence becomes linear. This type of dependence on the intensity of the exciting light has been reported by Jones[38] in a study of the luminescence intensity at low light levels emitted by *Chlorella*. Jones[38] has shown that, in the time range of 140 to 250 msec after the excitation flash, the integrated signal is proportional to the square of the excitation intensity in 'dark-adapted' algae.

The experimental work reported here may be divided into two parts. The first part is a study of the delayed light intensity as a function of the exciting light intensity and of a magnetic field. At low light levels, when the delayed light intensity is proportional

to the square of the exciting light intensity, the presence of a magnetic field of several kG might be expected to produce a slight decrease in the delayed light intensity.

Our measurement of the excitation light dependence differs from the work by Jones [38] primarily in the region of the time studied. Jones used a flow system in which the algae were exposed to 1·7 msec light flashes at one point in space and observed by a photomultiplier at another. In this way he measured the delayed light intensity between 140 and 250 msec after the exciting flash. In the present work the delayed light is detected between 1·5 and 5 msec after the exciting flash.

The second part of this research is a reinvestigation of the time decay of the delayed light. By solving the appropriate rate equations suggested by the triplet-fusion model, one can predict the time decay and compare it with the experimental results. Three types of experimental parameters can be varied: the excitation pulse shape (important when the decay is non-exponential), an external magnetic field, and the rate of electron transport by the addition of chemicals.

The chemical additives used in this work, hydroxylamine, methyl viologen, and DCMU (3-(3,4-dichlorophenyl)-1, 1-dimethylurea), were selected because they interfere in the normal electron transport in photosynthesis at different 'points'. DCMU blocks [39, 40] the electron transport between Q (the primary electron acceptor of light reaction II) and the system I. Hydroxylamine blocks the electron transport from water to Z (the primary electron donor of light reaction II); it also feeds electrons at the level of Z [41–43]. The third compound, methyl viologen, accepts electrons from reduced X (X being the primary electron acceptor of light reaction I) [44, 45]. The qualitative effects of DCMU and hydroxylamine on the decay characteristics of the delayed light have been previously reported [9, 27, 28]. (See Ref. [29] for the effects of ferricyanide, DCMU and uncouplers of phosphorylation on spinach chloroplasts.) In particular, DCMU increases and hydroxylamine decreases the relative intensity of the slow time component mentioned above. The purpose of the present work was to measure and compare these effects quantitatively with the above model.

B. EXPERIMENTAL METHODS AND APPARATUS

1. *Low light level measurements*

The experimental arrangement used to monitor delayed light intensity at low light levels was that of a modified phosphoroscope. A helium neon gas laser (Spectra-Physics Model 115 or Model 130) provided an excitation beam at 6328 Å. After passing through a mechanical chopper the light beam was incident on the sample, which was positioned between the pole faces of a 4 in. water cooled electromagnet (Varian Model V-4004).

The emission was detected through a 4 ft. quartz light pipe (0·5 in. diameter) by an RCA 7265 photomultiplier. Two Corning CS2-64 'sharp cut-off' filters were employed to reduce stray 6328 Å light. The output of the light pipe was chopped by the same light chopper. The spatial position of the laser beam was adjusted so that the overall effect was that of a phosphoroscope. The light pipe was blocked when the laser beam was incident on the algae, and *vice versa*.

A lock-in amplifier (PAR HR-8) amplified the photomultiplier signal and the final output was displayed on a chart recorder. A second optical system consisting of a gas laser, a photodiode, and a small mirror fastened to the chopping wheel, provided the necessary reference signal for the lock-in amplifier.

The intensity of the excitation beam was varied over four orders of magnitude (10^{15} to 10^{11} photons/cm^2 sec) by the use of calibrated neutral density filters and two Glan-Thompson polarizing prisms. The chopping rate was such that the sample was illuminated by a square light pulse of 3·5 msec duration at a repetition rate of 50 Hz. A calibrated photodetector (Yellow Springs Inst.) measured the intensity of the excitation beam.

The sensitivity of the photomultiplier tube to stray magnetic fields necessitated the use of the long quartz light pipe. The photomultipler housing was also heavily shielded with 'mu' metal. With this arrangement, the magnetic field perturbation on the photomultiplier signal was kept below 2% at all light levels.

2. *Time decay measurements*

As in the low light level measurements, the excitation source for the time decay measurements was a helium neon gas laser operating at 6328 Å. The light pulse duration and repetition rate was determined by a mechanical chopper.

In place of a phosphoroscope arrangement, the photomultiplier was electronically switched on after the excitation light pulse[46]. This enabled the photomultiplier to detect the delayed fluorescence within 0·1 msec after the exciting pulse and still avoid saturation by the much brighter prompt fluorescence. The minimum dead time of the mechanical phosphoroscope was 1·5 msec.

The photomultiplier signal was amplified by an oscilloscope preamplifier and then fed into a computer of average transients (Mnemetron Division of Technical Measurements Corp. CAT Model 400C). By continually adding successive chopping cycles (400 storage units in a 31·25 msec sweep), the CAT effectively averages out transient and non-coherent noise. The accumulated contents of the memory units were recorded with a printer.

Two different excitation pulse durations were used in these experiments. The first, flash excitation, consisted of a spike with a width of 100 μsec. The second was a square wave pulse with a 4·5 msec duration and rise and fall times of about 50 μsec each. The time dependence of the delayed light was markedly different for these two cases and they will be discussed separately in the Experimental Results section.

Pulse repetition rates of 1/15, 1, 25, and 50 Hz were used. The two slower rates were obtained by placing a mechanical shutter behind the chopping wheel. The shutter speed was adjusted so that only one pulse from the chopping wheel was allowed through when the shutter was triggered. The appropriate repetition rate of the shutter was provided by a timing system connected to a triggering electric solenoid.

3. *Sample preparation*

The unicellular green alga *Chlorella pyrenoidosa* (Emerson's strain No. 3) was used in most of the experiments. In some experiments, we used the blue-green alga *Anacystis nidulans*. The conditions for growth of these cells are described in Refs. [47–49]. After three days of growth, the cells were removed from the growth medium and placed in a buffer solution which allowed the cells to photosynthesize but halted further multiplication. The buffer (Warburg's buffer No. 9) consisted of 15% of 0·1 *M* $KHCO_3$ and 85% of 0·1 *M* $NaCO_3$. The concentration of the algae suspension was adjusted so that the absorbence of 6750 Å in a 1 cm cuvette was about 0·5. All data on normal (without additives) algae was taken within 30 min from the time the algae were removed from the

growth medium. The suspension was contained in a thin-walled plastic cuvette to avoid a long-lived decay emitted by most Pyrex containers.

The additives, DCMU, hydroxylamine, and methyl viologen were added in concentrations of 10^{-5}, 10^{-3}, and 10^{-3} *M* respectively. The effectiveness of these concentrations was checked each time by recording the initial fluorescence transients. This method has been described elsewhere [48, 49].

C. EXPERIMENTAL RESULTS

1. *Low light level measurements*

Using the experimental setup described in Section B.1., the intensity of the delayed light was measured as a function of the intensity of the excitation light. A logarithmic plot of this data for *Chlorella* is shown in Fig. 1. The actual signal plotted here is the luminescence intensity integrated between 1·5 and 5·0 msec after the excitation pulse. The excitation intensity referred to is the amplitude of the 3·5 msec rectangular excitation pulse.

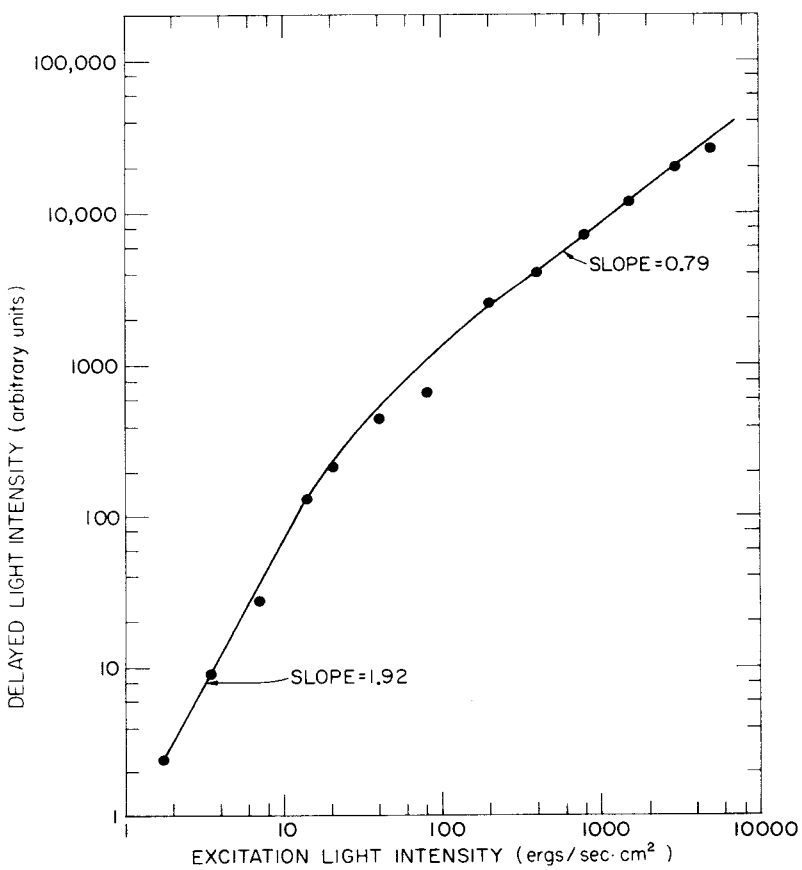


Fig. 1. Logarithmic plot of the intensity of delayed light (1·5 to 5 msec) vs. the intensity of an exciting light pulse of 3·5 msec duration in *Chlorella pyrenoidosa*. Instrumental accuracy is approximated by the dot size.

The slope of 1·92 at low excitation levels clearly suggests that, in this range, the delayed light varies as the square of the excitation intensity. This is expected from the triplet fusion model (see Section D) because two triplets are involved, and one needs therefore two quanta to create them. At higher intensities, the dependence becomes more linear. At excitation levels around 10^3 ergs/cm^2 sec, the exponent is approximately 0·8.

The quadratic intensity dependence was not observed with *Anacystis*. The delayed light yield of *Anacystis*, however, was found to be about an order of magnitude less than that of *Chlorella*. It is possible that the necessary light level for a quadratic intensity dependence in *Anacystis* was below the limits of the apparatus.

We monitored the delayed light intensity at all excitation levels accessible in the presence of magnetic fields up to 19 kG. No magnetic field effect was observed at any excitation intensity. The experimental accuracy was such that, in the region of quadratic intensity dependence, any magnetic field effect had to be less than 2% of the measured signal to remain unobserved.

2. *Time decay measurements*

As mentioned above, a high signal-to-noise ratio in the time decay measurements was obtained by repetitively exposing the sample to excitation pulses and adding the resulting decay curves to get an average time decay. The repetition rate of the excitation flashes was limited by the slow time component of the delayed light. It was found that a detectable contribution to the signal baseline appeared during the averaging time when the repetition rate was 1 Hz or greater. The time constant of this 'build-up' was approximately 1 sec.

The main disadvantage of such low repetition rates is the resulting long averaging times. The necessary averaging times for repetition rates of 1 Hz and 1/15 Hz were 15 min and at least 35 min respectively. These times were inconvenient because the shape of the decay curve for normal algae changed appreciably within one hour after the algae were removed from the growth medium. With flash excitation, the decay curve eventually resembled that of poisoned algae (DCMU) in which the electron flow was blocked. However, with square-wave excitation, there was negligible change with time in the decay curve of the normal algae. In order to obtain comparative data for three chemical additives and the four combinations on the same batch of algae, faster repetition rates were necessary.

The compromise solution adopted was to use a flash repetition rate of 25 Hz and subtract the measured 'build-up' signal from the data. This slower lifetime effect was easily measured with an oscilloscope set at a slow sweep rate. At 25 Hz the build-up for normal *Chlorella* was approximately 10% of the maximum signal recorded. A comparison of the normal algae decay curves obtained in this way with those obtained with slower flashing rates showed no noticeable difference.

Typical decay curves for *Chlorella* exposed to flash excitation are shown in Figs. 2, 3, and 4. All of the data for these three figures was taken on the same batch of algae; the excitation pulse had a width of 100 μsec and a rise time of 50 μsec. The plots are labeled 'intrinsic delayed light' to indicate that any measured build-up in the signal baseline—measured as discussed above—was subtracted. The solid curves in these figures represent the calculated data from the kinetic model discussed in the next section. The units of the delayed light intensity are photons/sec per cm^2 of emitting

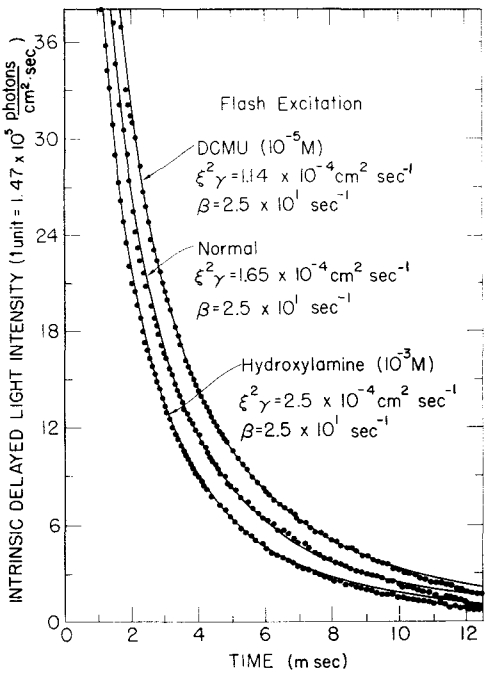


Fig. 2. Flash-excited delayed light intensity vs. time (1–12 msec) for normal (without additives) *Chlorella*, and for those treated with DCMU and hydroxylamine. Full scale $\sim 5{\cdot}3 \times 10^6$ photons/cm^2 sec; flash repetition rate, 25 Hz; excition pulse, 100 μsec wide, with 50 μsec rise time; solid dots: experimental points; solid lines: plots from the kinetic model of triplet-fusion discussed in the text.

lamellae. The manner in which this area term was calculated is discussed in Section D.2.

The apparent effect of the additives was to increase or decrease the luminescence decay rate as suggested by the shapes of the curves. Hydroxylamine increases the decay rate and completely eliminates the slow time component from the luminescence. The baseline signal did not build-up whenever hydroxylamine was added to the cell suspension. DCMU always reduced the rate of decay of delayed light while the addition of methyl viologen had no major effect on the decay rate. The combination of DCMU and hydroxylamine produced a curve shape very similar to that of the normal algae while the combination of DCMU and methyl viologen resulted in a decay rate similar to that of DCMU. The combination of all the three additives yielded a decay rate very similar to that of hydroxylamine alone. These effects are discussed quantitatively in the next section.

An attempt was also made with this experimental setup to determine the effect of a magnetic field on the decay curve for flash excitation. The luminescence decay of *normal Chlorella* in a magnetic field was monitored for field strengths up to 18 kG. Again, no magnetic field effect was observed.

Figures 5 and 6 show the decay curves for *Chlorella* exposed to a rectangular excitation pulse. One reason for using such a pulse in addition to the flash excitation data is to test the kinetic model. Because the time dependence of the delayed light emission is not a simple exponential decay, it might be expected to depend on the duration of the

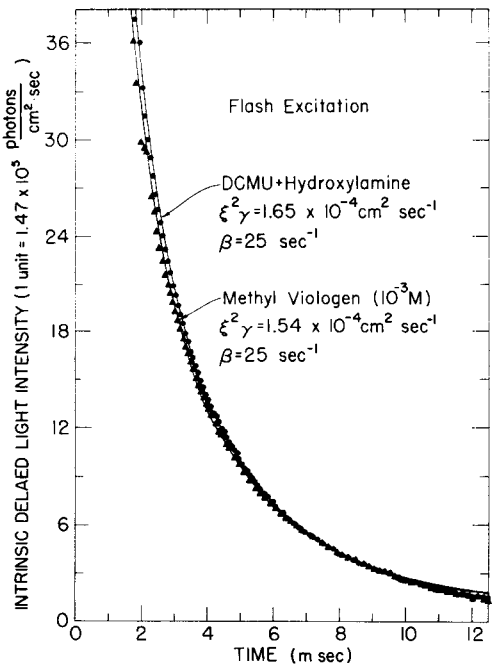


Fig. 3. Flash-excited delayed light intensity vs. time for *Chlorella* treated with methyl viologen and the combination of DCMU and hydroxylamine. (For details see legend of Fig. 2).

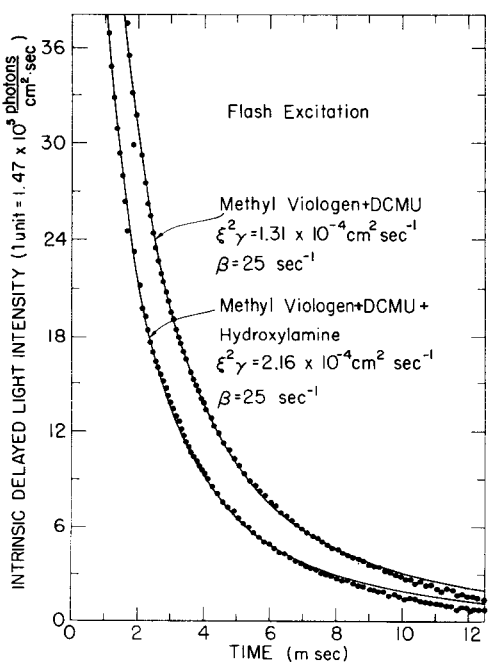


Fig. 4. Flash-excited delayed light intensity vs. time for *Chlorella* treated with the combinations of methyl viologen + DCMU and methyl viologen + DCMU + Hydroxylamine. (For details see legend of Fig. 2).

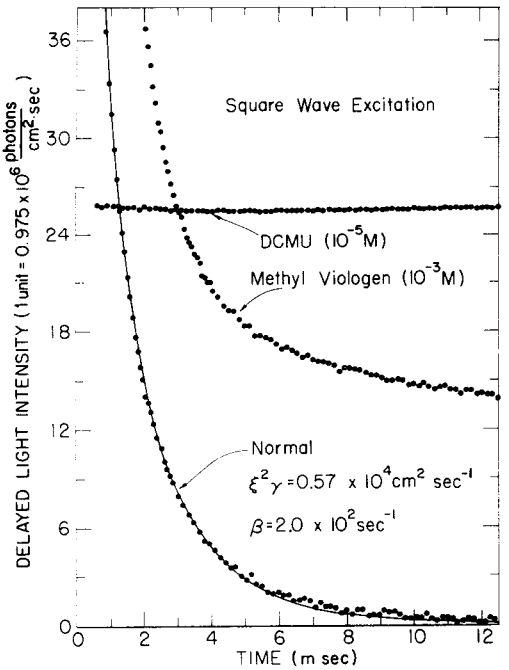


Fig. 5. Square wave excited delayed light intensity vs. time for *Chlorella*: normal (without additives), with DCMU and with methyl viologen. Full scale ~ $3{\cdot}7 \times 10^7$ photons/cm^2 sec: flash repetition rate, 25 Hz; excitation pulse, 4·5 msec with a rise time of 50 μsec. Solid dots: experimental points; solid line: plot from the kinetic model of triplet-fusion discussed in the text.

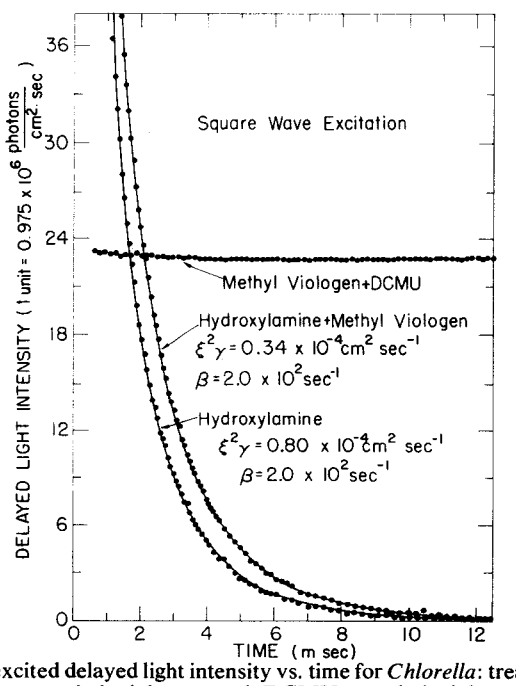


Fig. 6. Square wave excited delayed light intensity vs. time for *Chlorella*: treated with hydroxylamine, hydroxylamine + methyl viologen, and DCMU + methyl viologen. (For details, see legend of Fig. 5.)

excitation pulse. The kinetic model should be capable of predicting such a dependence. A second reason for employing a longer excitation pulse is to allow a comparison of the present data with those from other laboratories. Much of the work on the effects of various chemical additives on the delayed light has been obtained from phosphoroscopes with excitation pulses of a few msec duration [34].

The baseline build-up (see above) in this data was subtracted only from the decay curve for normal *Chlorella*. As in the flash excitation case, all cells treated with hydroxylamine exhibited no build-up in the signal baseline. The decay curves for cells treated with DCMU, DCMU and methyl viologen, or methyl viologen, had very large build-up components which could not be separated from the remainder of the signal. In fact the decay curves for DCMU or the combination of DCMU and methyl viologen consisted of only a very slowly decaying function and had no fast components.

The combination of hydroxylamine and DCMU thoroughly quenched the delayed light when excited by the 4·5 msec excitation pulse. Both this combination and the mixture of all three additives yielded a negligible signal on the scale of Figs. 5 and 6.

It has been reported [27] that the addition of hydroxylamine to *Chlorella* results in nearly first order decay of the delayed fluorescence. This has been suggested as supporting evidence that the normal decay curve may be resolved as a sum of exponential decays [26]. In the present work all samples of *Chlorella* treated with hydroxylamine yielded decay curves which were clearly nonexponential. Figure 7 is

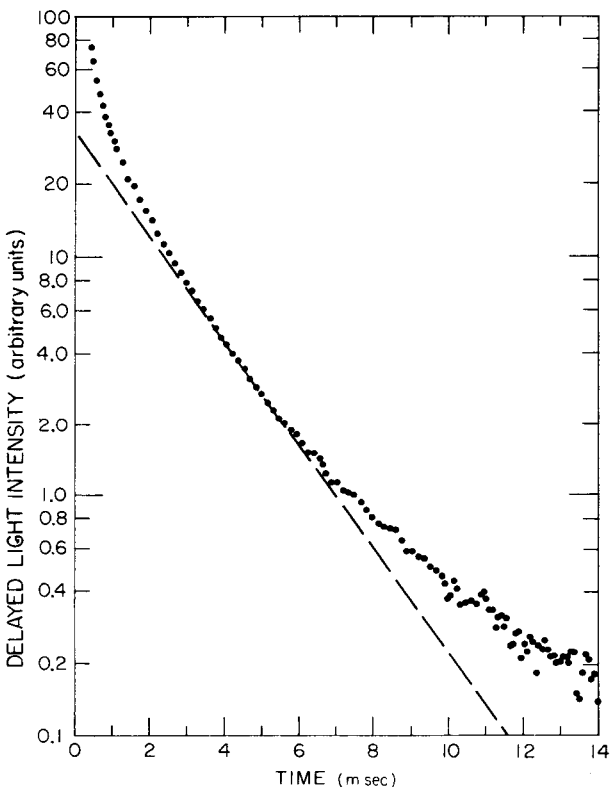


Fig. 7. Similogarithmic plot of the delayed light intensity vs. time for square-wave excited *Chlorella* treated with 10^{-3} *M* hydroxylamine.

a semilogarithmic plot of typical hydroxylamine data for *Chlorella* exposed to a 4·5 msec excitation pulse.

Decay curves for *Anacystis* excited by a square wave pulse are shown in Fig. 8. A build-up in the signal baseline was observed only from the normal algae and those treated with methyl viologen. The magnitude of the build-up in these two cases, how-

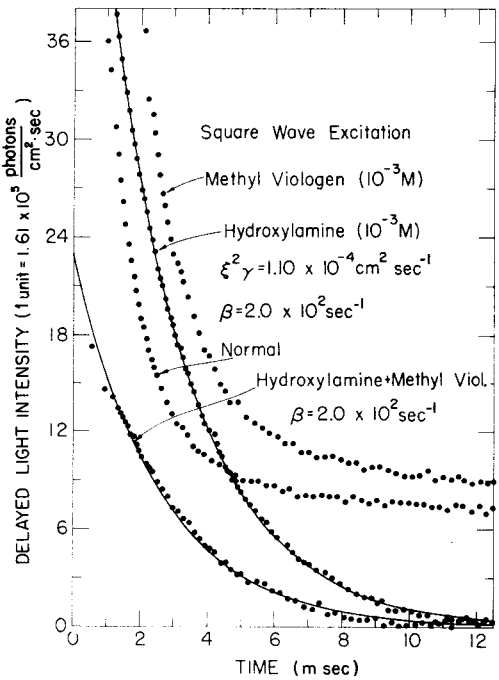


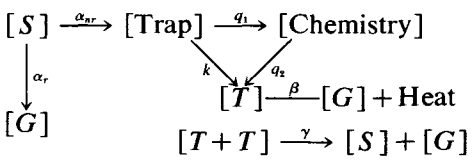
Fig. 8. Square wave excited delayed light intensity vs. time for *Anacystis nidulans*: normal, with hydroxylamine, methyl viologen and hydroxylamine + methyl viologen. Full scale ~ 5·8 × 10^6 photons/cm² sec. (For other details, see legned of Fig. 5).

ever, was large and could not be unambiguously separated from the faster components of the decay. Treatment with hydroxylamine again eliminated all slow components in the decay but the effect of DCMU on the delayed light from *Anacystis* was strikingly different from the corresponding effect in *Chlorella*. In contrast to the slowly decaying emission in *Chlorella*, the delayed light intensity from *Anacystis* was greatly reduced by the addition of DCMU. By itself or in combination with either of the other two chemical additives, DCMU yielded a negligible signal on the scale of Fig. 8. The combination of methyl viologen and hydroxylamine also reduced the delayed light intensity but not to the extent of DCMU.

D. DISCUSSION: THE KINETIC MODEL

1. *The rate equations*

The model which we wish to investigate may be represented by the following schematic of Photosystem II:



Here S represents a singlet exciton in the bulk pigment system which can decay with the radiative constant α_r to leave a ground state molecule G and a photon or can transfer its energy to the trap (reaction center) with the non-radiative rate constant α_{nr}. The excited trap molecule can be deactivated chemically with rate q_1 or can result in a triplet exciton with intersystem crossing rate k. One may question whether k/q_1 in the scheme can be significantly different from zero. If it is not zero, as suggested here, the traps would not be 100% efficient – that they are only 70% efficient in mature cells of algae, used in this work, has been calculated from measurements of the lifetime of excited state with and without DCMU added [59]. For our present purposes, other modes of trap decay such as fluorescence or internal conversion may be grouped into q_1. The rate of triplet production from chemical back reactions is represented by q_2. The delayed light results from the fusion of two triplet excitons which happen to collide in the bulk pigment system. The triplets may also decay with the monomolecular rate constant β. We denote by γ the bimolecular fusion rate constant.

At low light intensities, and at short times (milliseconds) after light flashes we assume that the chemical back reactions are negligible (i.e., $q_2 = 0$). With this assumption, we can write the following three rate equations:

$$\frac{dn_s}{dt} = -(\alpha_r + \alpha_{nr})n_s + \tfrac{1}{2}\gamma n_T^2 + G'(t) \tag{1}$$

$$\frac{dx}{dt} = \alpha_{nr} n_s - (q_1 + k)x; \alpha_{nr} = C(N - x)^* \tag{2}$$

$$\frac{dn_T}{dt} = kx - \beta n_T - \gamma n_T^2 \tag{3}$$

where $G'(t)$ = rate of singlet generation; it should not be confused with G (for ground state) above
n_s = singlet exciton population density
n_T = triplet exciton population density
x = density of excited trap molecules
N = density of trap molecules
C = constant

In the above formulation, the system is assumed to be homogeneous. This is a valid assumption based on the experimental evidence that the fluorescence lifetime increases linearly with increase in the quantum yield of fluorescence (Tumerman and Sorokin [50], and Briantais *et al.* [51]).

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*α_{nr} may not be such a simple function of density of unexcited trap molecules. However, it should not effect too much our results (Lavorel, personal communication).

The three coupled differential equations cannot be solved in closed form. Solutions can be obtained, however, for limited time intervals. In particular we wish to solve for the delayed light emission after the system has been excited by a brief flash of light. For times t long on the normal emission time scale ($t^{-1} \ll \alpha_r + \alpha_{nr}, k + q_1$), the faster response times of n_s and x compared to that of n_T allow us to make the following approximations [52].

$$\frac{dn_s}{dt} = 0 \tag{4}$$

$$\frac{dx}{dt} = 0 \tag{5}$$

$$G'(t) = n_s(0)\delta(t) \tag{6}$$

where the zero of time is set at the instant the excitation light is shut off. Under conditions such that $N \gg x$, equation (3) becomes

$$\frac{dn_T}{dt} = -\xi\gamma n_T^2 - \beta n_T \tag{7}$$

where

$$\xi = 1 - \tfrac{1}{2}(1+\phi)\left(\frac{k}{k+q_1}\right) \tag{8}$$

in terms of the total fluorescence efficiency $\phi = \alpha_r/\alpha_r + \alpha_{nr}$. Equation (7) can be solved with the initial condition, $n_T(0) =$ constant. The solution is

$$n_T(t) = \frac{\beta n_{T0}}{(\xi\gamma n_{T0} + \beta)\exp(\beta t) - \xi\gamma n_{T0}} \tag{9}$$

where $n_{T0} = n_T(0)$. The delayed light produced by triplet exciton fusion is

$$L(t) = \tfrac{1}{2}\gamma\phi n_T^2(t) \tag{10}$$

Combining equations (9) and (10), and assuming ϕ independent of time,* we get

$$L(t) = \frac{\beta^2 L_0}{\{[(2\xi^2\gamma L_0/\phi)^{1/2} + \beta]\exp(\beta t) - (2\xi^2\gamma L_0/\phi)^{1/2}\}^2} \tag{11}$$

where $L_0 = L(0)$. An important characteristic of equation (11) is that, at low light levels such that $(2\xi^2\gamma L_0/\phi)^{1/2} \ll \beta$, it is a simple exponential decay with a reciprocal lifetime equal to 2β.

The time dependence of the delayed light excited by a rectangular pulse can be obtained by integration. Denoting the pulse length by T and setting the zero of time at

*The kinetics of the delayed light have also been discussed in terms of the time dependence of the fluorescence efficiency [29, 53].

the instant the excitation pulse shuts off, we have

$$n_T(t) = \frac{1}{T}\int_{-T}^{0} \frac{\beta n_{T0}\,du}{(\xi\gamma n_{T0}+\beta)\exp(\beta t-\beta u)-\xi\gamma n_{T0}} \tag{12}$$

Combining equation (12) with equation (10) yields

$$L(t) = \frac{L_0}{(\lambda T)^2}\left[-\beta T + \ln\frac{(\lambda+\beta)\exp(\beta t+\beta T)-\lambda}{(\lambda+\beta)\exp(\beta t)-\lambda}\right]^2 \tag{13}$$

where $\lambda^2 = 2\xi^2\gamma L_0/\phi$.

Equations (11) and (13) are difficult to compare analytically. A numerical study of the two expressions indicates that, as one might expect, the effect of a longer excitation pulse is to decrease the luminescence decay rate. This is a small effect, however, and appears to be negligible in the range of parameters studied here.

2. *Comparison with experiments*

The solid curves in Figs. 2–6 and 8 represent either equation (11) or (13). The technique for fitting these expressions to the appropriate data involved the manipulation of two adjustable parameters, $\xi^2\gamma$ and β. L_0 was always adjusted for a given combination of $\xi^2\gamma$ and β such that the theoretical curve intersected the data at approximately 1·0 msec. The values for $\xi^2\gamma$ and β were then varied by trial and error to obtain the best fit to the data. This method resulted in unique values for $\xi^2\gamma$ and β over the range tested (several orders of magnitude for both). That is, large changes of 10% or more in one parameter could not be compensated by changes in the other parameter.

It was found that, for a given batch of algae, the changes in the decay shape due to chemical additives could be accounted for by corresponding changes in $\xi^2\gamma$ while β was held fixed. The values of $\xi^2\gamma$ and β for algae treated with the same additives but from different growth batches were found to vary. While the values for $\xi^2\gamma$ varied by a factor of about 2, the values of β^{-1} ranged from 5 msec to 40 msec for different batches of algae. It is difficult to understand why β, a physical constant, should vary. However, β may depend on the state of the cells.

The fitted values of $\xi^2\gamma$ for the flash-excited *Chlorella* of Figs. 2, 3, and 4 are listed in Table 1. The units of $\xi^2\gamma$ are reciprocal density times reciprocal time. The definition of the density term depends on the geometry of the system in which the triplet excitons move. In line with the lamellar membrane picture of the photosynthetic apparatus

Table 1. Decay parameters for flash-excited *Chlorella*

Algae treatment	$\xi^2\gamma$ (cm²/sec)	ξ/ξ^{normal}
Normal	$1{\cdot}65\times10^{-4}$	1·00
Hydroxylamine (Hydrox.)	$2{\cdot}50\times10^{-4}$	1·23
DCMU	$1{\cdot}14\times10^{-4}$	0·83
Methyl viologen (MV)	$1{\cdot}54\times10^{-4}$	0·97
Hydrox. + DCMU	$1{\cdot}65\times10^{-4}$	1·00
MV + DCMU	$1{\cdot}31\times10^{-4}$	0·89
Hydrox. + MV + DCMU	$2{\cdot}16\times10^{-4}$	1·15

[31–33], we have assumed a two dimensional structure. Thus n_T and $\xi^2\gamma$ have units of cm^{-2} and cm^2/sec respectively.

The fitted values of $\xi^2\gamma$ in the proper units are easily obtained by noting that the product $(\xi^2\gamma L_0)^{1/2}$ has the units of reciprocal seconds. Thus, the evaluation of $\xi^2\gamma$ requires an absolute measurement of the intensity of delayed light and the total area of emitting lamallae. Such a measurement is difficult to obtain to any accuracy, particularly the measurement of the total volume of emitting cells. The number of cells per volume of suspension was estimated from the optical density at the red absorption maximum (10^8 cells/cm^3 at O.D. $\simeq 0{\cdot}1$). The area of absorbing lamellae per cell was set equal to the cross-sectional area of one cell ($\sim 10^{-7}\,cm^2$). The resulting uncertainty in $\xi^2\gamma$ is estimated to be about one order of magnitude.

Because the algae concentrations were the same for a given batch, a study of the relative values of $\xi^2\gamma$ avoids the error in an absolute measurement. The ratio $\xi\gamma^{1/2}/(\xi\gamma^{1/2})_{normal}$ is listed in the third column of Table 1 and, since only ξ is expected to vary with the addition of a chemical additive, the $\gamma^{1/2}$ term has been factored out.

Of particular interest are the ξ/ξ_{normal} values for hydroxylamine and DCMU. Hydroxylamine increases ξ over that of a normal *Chlorella* while DCMU causes a decrease in ξ. From equation (8) it can be seen that an increase in ξ implies an increase in q_1 and *vice versa* assuming that ϕ and k remain constant. Thus, the data may indicate that DCMU decreases q_1, the rate at which the trap molecule is chemically deactivated, while the addition of hydroxylamine has the opposite effect.

As mentioned in the introduction, treatment with DCMU blocks the electron flow between the two pigment systems [39, 40] and, it is suggested here that a slight reduction in q_1 takes place. It is generally assumed that DCMU does not affect q_1 but only the reoxidation of Q^- to Q. Hydroxylamine has been shown to take the place of water [41–43] in feeding electrons to an oxidized form of Z, the initial electron donor at reaction center II. Whether this should increase or decrease q_1 requires some speculation as to the identity of the rate-limiting component of q_1 and the efficiency with which hydroxylamine reduces Z. The present data indicates that hydroxylamine increases q_1 in *Chlorella*. Similar statements can be made about the addition of methyl viologen. Methyl viologen is a strong electron acceptor just above the level of ferredoxin [44, 45] and whether its presence *should* increase or decrease q_1 is also open to question. The present data suggests that q_1 is not greatly affected by methyl viologen in *Chlorella*.

The *Chlorella* square wave data of Figs. 5 and 6 could be fitted with equation (13) in only three cases: normal, hydroxylamine, and the combination of methyl and viologen and hydroxylamine. In these three cases the slow build-up component of the decay curve was either negligible or so small that it could be unambiguously subtracted. The decay parameters for this data are listed in Table 2. As in the flash-excited data, the apparent effect of hydroxylamine was to increase q_1.

Table 2. Decay parameters for square-wave-excited *Chlorella*

Algae treatment	$\xi^2\gamma(cm^2/sec)$	ξ/ξ^{normal}
Normal	$0{\cdot}57\times10^{-4}$	1·00
Hydroxylamine (Hydrox.)	$0{\cdot}80\times10^{-4}$	1·19
Hydrox. + Methyl Viologen	$0{\cdot}34\times10^{-4}$	0·77

The failure of equation (13) to fit the data for methyl viologen, DCMU, and the combination of methyl viologen and DCMU may be due to the presence of the slow build-up component in the decay curves. This effect was explicitly left out of equations (1)–(3) by setting q_2 equal to zero. Certainly the methyl viologen decay curve could be explained by including the back reaction represented by q_2 and its corresponding first order rate equation.

The two DCMU curves (DCMU and DCMU + methyl viologen) require additional comment. The problem here is to explain the total absence of a fast component in the decay curve; the entire signal consists of the slow build-up component. As explanation of this effect can be given in terms of the reaction center complex of photosystem II. In terms of the initial electron donor Z, the initial electron acceptor Q, and the excitation trapping molecule (Trap), the production of triplet excitons at the reaction center can be represented by

$$Z(\mathrm{Chl})Q + h\nu \rightarrow Z(\mathrm{Chl})^S Q \begin{cases} \xrightarrow{q_1} Z^+(\mathrm{Chl})Q^- \xrightarrow{q_2} Z(\mathrm{Chl})^T Q \\ \xrightarrow{k} \end{cases}$$

Because DCMU blocks the oxidation of Q^-, a gradual build-up of $Z^+(\mathrm{Chl})Q^-$ must occur after the excitation light is turned on. In this state the excitation of the trap molecule to an excited singlet is blocked and triplets can be produced only by

$$Z^+(\mathrm{Chl})Q^- \xrightarrow{q_2} Z(\mathrm{Chl})^T Q$$

Thus, the delayed light for *Chlorella* treated with DCMU is rate-limited by the q_2 back reaction. The presence of a fast component in the DCMU flash excited data suggests that a sufficient concentration of $Z^+(\mathrm{Chl})Q^-$ could not build up during the excitation pulse to block the intersystem crossing transition.

The absence of a delayed light for the two hydroxylamine + DCMU combinations (hydroxylamine + DCMU and hydroxylamine + DCMU + methyl viologen) for square-wave-excited *Chlorella* can be explained in a similar manner. The effect of hydroxylamine is to reduce the Z^+ and thus transform $Z^+(\mathrm{Chl})Q^-$ to $Z(\mathrm{Chl})Q^-$. In this state the q_2 recombination transition is blocked and no triplets are formed. Again, the finite signal for flash excitation suggests that a sufficient concentration of $Z(\mathrm{Chl})Q^-$ could not build up during the exciting pulse.

Due to a build-up in the signal baseline similar to that of *Chlorella*, the square wave excitation data for *Anacystis* yielded only two decay curves which could be fitted by equation (13); these are curves for hydroxylamine and the combination of hydroxylamine and methyl viologen. For hydroxylamine, a value for $\xi^2\gamma$ of $1{\cdot}1 \times 10^{-4}$ cm^2/sec was obtained with the assumption that the exposed lamellar area of *Anacystis* is the same as that of *Chlorella*. This is in good agreement with the corresponding values obtained for *Chlorella*. The decay curve produced by the hydroxylamine + methyl viologen combination is interesting in that it closely approximates an exponential decay and could be fitted by equation (13) independent of the choice of $\xi^2\gamma$ (for values of $\xi^2\gamma$ comparable to that for hydroxylamine). This can be explained by the low value of L_0

and the statement following equation (11). The absence of delayed light when DCMU was added to the cell suspension suggests that, in *Anacystis*, DCMU retards q_2. Such an effect might occur if the chain of back reactions responsible for q_2 extends beyond the point at which DCMU acts in the Hill-Bendall scheme.

3. *The absence of a magnetic field effect*

According to the discussion in the Appendix, the magnitude of the low-light-level magnetic field effect depends on the magnitudes of the molecular zero field splitting energy and the branching ratio k_2/k_{-1}. The zero field splitting for chlorophyll *a* is not known. In the cases of anthracene and tetracene the zero field splitting is such that the magnetic field effect on the delayed fluorescence saturates at about 3 kG [18–20]. Thus, it would seem that 18 kG is sufficiently large to enable the Zeeman splitting to dominate in chlorophyll *a* and lead to a magnetic field effect.

According to equations (A.6) and (A.10), the fractional decrease in the delayed light intensity can be related to the branching ratio, $k_2/k_{-1} = \epsilon$ in the following manner:

$$\frac{\Delta L}{L} = \frac{2}{3}\left(\frac{\epsilon}{3+2\epsilon}\right) \tag{14}$$

Thus, an experimental accuracy of 2% implies that the branching ratio must satisfy

$$\epsilon < 0{\cdot}1 \tag{15}$$

The physical significance of this parameter is contained in equation (A.8). Thus,

$$\gamma < 0{\cdot}01 k_1 \tag{16}$$

where k_1 is the collision rate between two triplet excitons. In other words, at zero field less than one collision in 100 leads to annihilation. This ratio in crystalline anthracene is about one collision in 25 [21].

The collision rate for a two dimensional system can be expressed in terms of the triplet exciton diffusion constant and lifetime as [54]

$$k_1 = -4\pi D/\{\ln\left[\tfrac{1}{2}(\beta/D)^{1/2}R\right] + 0{\cdot}577\} \tag{17}$$

where D is the diffusion constant, R is the interaction radius, and it is assumed that $(D/\beta)^{1/2} \gg R$. Setting $\gamma = 10^{-4}$ cm^2/sec, $\beta \simeq 100$ sec^{-1}, and $R \sim 10$A, we obtain from equations (16) and (17)

$$D > 8 \times 10^{-4} \text{ cm}^2/\text{sec}. \tag{18}$$

This value is certainly reasonable in an order of magnitude sense. The corresponding value for anthracene is $1{\cdot}8 \times 10^{-4}$ cm^2/sec for motion in the *ab* plane [55].

Thus the absence of a magnetic field effect to the present accuracy does not imply an unreasonable value for the triplet diffusion constant. A lower limit on the size of the magnetic field effect can be estimated from the value of γ obtained in the luminescence decay measurements and the assumption that chlorophyll triplets *in vivo* have about the same diffusion rate as triplets in organic crystals such as anthracene. Assum-

ing that the diffusion constant is no greater than $\sim 10^{-1}$ cm^2/sec implies that the annihilation probability cannot be less than 10^{-4}. The branching ratio ϵ must be greater than $\sim 10^{-3}$ and, therefore, the minimum magnetic field effect should be $\sim 2 \times 10^{-4}$. A convincing check of the triplet fusion model should improve the 2% accuracy of the present magnetic field measurements by about two orders of magnitude.

4. *The triplet exciton density*

The triplet exciton density can be estimated with equation (10). At 1 msec after the excitation light was turned off a typical emission rate (L) for *Chlorella* was $\sim 10^8$ photons/cm^2 sec (see Figs. 5 and 6). With $\gamma \sim 10^{-4}$ cm^2/sec and $\phi \sim 2 \times 10^{-2}$, the triplet density at 1 msec is $n_T = (2L/\gamma\phi)^{1/2} \sim 10^7$ cm^{-2}. If the lamellar area of chlorophyll molecule is 2×10^{-14} cm^2[56], then the proportion of molecules in the triplet state is approximately 2×10^{-7}. Thus, the expected change in the absorption spectrum corresponding to the above triplet density is less than 10^{-6}. To our knowledge, the most sensitive search for chlorophyll triplets in the chloroplast has been a flash photolysis measurement conducted by Porter and Strauss with an instrument capable of resolving O.D. changes down to only 0·5%[16].

5. *Difference between the electron-hole model and the triplet fusion model*

An important difference between the model discussed here and the electron-hole recombination model for delayed light is the required number of trapping sites in photosystem II. The electron-hole model requires two trapping centers, one for electrons and one for holes[7, 8], while the triplet fusion model requires only one. As a result of this difference, the electron-hole model suggests that the delayed light and the chemistry of photosynthesis operate energetically in parallel while the triplet fusion model suggests that they are in competition.

Evidence for this aspect of the electron-hole model has been the work of Bertsch, Hill, and West[57], who have shown that the addition of ferricyanide to isolated chloroplasts increases the intensity of the delayed light at 1 msec. It is then concluded that the electron transport of photosynthesis and the delayed light must not be in competition for the energy provided by the initial act of light absorption.

We wish to point out that the triplet fusion model provides an alternate explanation of the results of Bertsch *et al.*[57]. In this case, the slow decay of the delayed light emitted by isolated chloroplasts without any added oxidant is determined by the low value of q_2 as in the case of algae treated with DCMU (see previous discussion). The addition of ferricyanide to the isolated chloroplasts results in the oxidation of Q^- and triplets can then be formed at the faster rate k and the relative values of the initial delayed light intensity result from the different rates of triplet formation q_2 and k. An analogous result arises when one compares the decay curves for normal *Chlorella* and those treated with DCMU under square wave excitation.

E. CONCLUSIONS

The results reported in this paper indicate that the delayed light emitted by plants can be explained by a triplet fusion model in which the production of triplets is mediated by the reaction center of photosystem II. This conclusion is based on an extensive study of the time dependence of the luminescence decay and the observation that, at low light levels, the delayed light intensity is proportional to the square of the excitation intensity

(also, see Jones[38]). Direct proof in the form of a magnetic field effect on the delayed light was not obtained.

It should be stressed that the present results do not exclude the electron-hole model. The importance of chlorophyll *b* suggested by the detailed model of Arnold and Azzi[7] seems unlikely since, in the present work, *Anacystis* produced results similar to those of *Chlorella*. Yet, a simpler scheme of photosystem II such as that proposed by Bertsch [8] could be represented by a kinetic model analogous to that presented here. In an electron-hole picture, γ would become the recombination rate and β the charge carrier reciprocal lifetime. In this case, $\gamma \sim 4\pi D$ and the charge carrier diffusion constant is $\sim 10^{-5}$ cm^2/sec. This value is in good agreement with the measurements of Nelson[58] on solid chlorophyllide.

It is shown in this paper, however, that the triplet fusion model presented here is equally valid for the delayed light *in vivo*. At 1 msec, after the excitation light was turned off, the proportion of molecules in the triplet state was estimated to be only 2×10^{-7}. Thus, experiments which have not reported the existence of triplets *in vivo* have not ruled out the triplet fusion model.

We recognize that more work is necessary to determine the mechanism of delayed light in plants. A confirmation or rejection of the triplet fusion model awaits a more sensitive search for a magnetic field dependence of the delayed light coupled, perhaps, with an ultra-sensitive flash absorption measurement.

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APPENDIX A

Magnetic field effect on delayed light

The intensity of the delayed light can be derived by first solving equations (1), (2), and (3) in the steady state limit. This yields the following equation for the triplet exciton density:

$$\xi\gamma n_T^2 + \beta n_T = 2(1-\xi)\mu I \tag{A.1}$$

where $\xi = 1 - \frac{1}{2}(1-\phi)(k/k+q_1)$, μ is the absorption coefficient for the exciting light and I is the excitation flux. Instead of solving (A.1) exactly we consider the limit $\xi\gamma n_T \gg \beta$. In this case,

$$\xi\gamma n_T^2 = 2(1-\xi)\mu I \qquad \text{(A.2)} \tag{A.2}$$

and the delayed light (L) is

$$L = \frac{1-\xi}{\xi}\phi\mu I, \quad \xi\gamma n_T \gg \beta \tag{A.3}$$

Thus at high light intensities such that the above inequality is satisfied, the delayed light intensity is proportional to the excitation intensity and is independent of the fusion rate constant γ.

In the other limit $\xi\gamma n_T \ll \beta$,

$$\beta n_T = 2(1-\xi)\mu I \tag{A.4}$$

and the delayed light is

$$L = 2\gamma\phi(1-\xi)^2\mu^2 I^2/\beta^2, \quad \xi\gamma n_T \gg \beta \tag{A.5}$$

At low intensities the delayed light is proportional to the square of I and is directly proportional to γ. Because

the magnetic field dependence is contained entirely in γ, the low intensity region is the one which we wish to investigate.

Although equation (A.1) was obtained in the steady state limit, the experimental chopped light conditions can be accounted for with a minor adjustment. In the low light level region $\beta \gg \gamma\xi n_T$ and thus the decay is a simple exponential. The experimentally observed luminescence is then

$$L(t) = \frac{2\gamma\phi(1-\xi)^2\mu^2 I^2 \exp(-2\beta t)}{\beta^2} \tag{A.6}$$

In order to obtain the room temperature magnetic field dependence of γ we follow the original treatment by Merrifield[21]. The relevant parameters are defined by the following schematic of the annihilation of two triplet excitons.

$$T + T \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} (TT) \xrightarrow{k_2'} S$$

Two free triplets diffuse together at a rate represented by k_1. When sufficiently close they can either scatter with rate constant k_{-1} or annihilate to produce a singlet exciton at a rate equal to k_2'. The two triplets in close proximity (represented by (TT) form a manifold of nine possible pair states ψ_i. If spin is conserved in the fusion process, then k_2' for a given pair state is proportional to S_i^2 where $S_i = |\langle S|\psi_i\rangle|$ is the amplitude of the singlet component for this state. Letting $k_2' = k_2 S_i^2$, the probability of fusion from the i^{th} pair state is $k_2 S_i^2/(k_{-1} + k_2 S_i^2)$. The fusion arate constant γ is then the product of the collision rate constant and the total fusion probability.

$$\gamma = \frac{1}{0}\sum_{i=1}^{9} \frac{k_1 k_2 S_i^2}{k_{-1} + k_2 S_i^2} \tag{A.7}$$

The magnetic field dependence of this expression can be shown by considering two cases, the zero field case and the high field case.

Zero field case

In the absence of an external magnetic field the dominant term in the spin Hamiltonian for a triplet exciton is the dipolar interaction

$$\mathscr{H} = D(S_z^2 - \tfrac{1}{3}S^2) + E(S_x^2 - S_y^2)$$

where D and E are constants (zero field splitting parameters) and S is the total spin operator. The eigenfunctions are $|x\rangle$, $|y\rangle$, $|z\rangle$ which have their spin quantized along the corresponding principal axes of the dipolar tensor. If the triplet–triplet interaction energy is sufficiently small then the zero field pair states can be written as $|xx\rangle$, $|xy\rangle$, $|xz\rangle$, $|yy\rangle$ etc. The singlet pair state can then be written as

$$|S\rangle = \frac{1}{3^{1/2}}[|xx\rangle + |yy\rangle + |zz\rangle].$$

Thus, only three pairs states have singlet character, $\langle S|xx\rangle| = (\frac{1}{3})^{1/2}$. $|\langle S|yy\rangle| = (\frac{1}{3})^{1/2}$. $|\langle S|zz\rangle| = (\frac{1}{3})^{1/2}$. The fusion rate constant is

$$\gamma = \frac{k_2}{9}\left(\frac{\epsilon}{1+\epsilon/3}\right) \tag{A.8}$$

where

$$\epsilon = k_2/k_{-1}.$$

High field case

At high fields such that the Zeeman splitting is large compared to the zero field splitting, the spin states are quantized along the field and the pair state eigenfunctions can be written in terms of $|00\rangle$, $|0+\rangle$, $|0-\rangle$, $|++\rangle$, etc. The three pair functions with singlet character are $|00\rangle$, $|+-\rangle$, and $|-+\rangle$ but the last two are degenerate and must be replaced by the symmetric and antisymmetric combinations. Thus only two of the nine possible pair eigenstates have singlet character. The singlet pair state is

$$|S\rangle = (\tfrac{1}{3})^{1/2}[(00\rangle - |+-\rangle - |-+\rangle]$$

and the two pair eigenstates with singlet character are

$$\psi_1 = |00\rangle, S_i = (\tfrac{1}{3})^{1/2}; \psi_2 = (\tfrac{1}{2})^{1/2}[|+-\rangle + |-+\rangle], S_2 = (\tfrac{2}{3})^{1/2}.$$

The fusion rate constant in the high field case is

$$\gamma_H = \frac{k_1}{9}\left[\frac{\epsilon/3}{1+\epsilon/3}+\frac{2\epsilon/3}{1+2\epsilon/3}\right] \tag{A.9}$$

A comparison of (A.8) and (A.9) shows that the effect of the magnetic field is to decrease the fusion rate. The relative magnitude of this effect is

$$\frac{\Delta\gamma}{\gamma} = \frac{\gamma-\gamma_H}{\gamma} = \frac{2}{3}\left(\frac{\epsilon}{3+2\epsilon}\right) \tag{A.10}$$

If $\epsilon = 0{\cdot}5$ then $\Delta\gamma/\gamma = 0{\cdot}08$. A typical zero field splitting energy for an organic molecule such as anthracene is approximately $0{\cdot}03\ \mathrm{cm}^{-1}$ [18–20]. This corresponds to the Zeeman energy of an electron in a magnetic field of about 0·3 kG.

In summary, then, at low light intensities, such that the delayed light is proportional to the square of the excitation intensity, the presence of a magnetic field may decrease the delayed light intensity. The magnitude of the effect is dependent on both the zero field splitting energy of the molecule and the ratio k_2/k_{-1}.