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THERMOLUMINESCENCE AND TEMPERATURE EFFECTS ON DELAYED LIGHT EMISSION (CORRECTED FOR CHANGES IN QUANTUM YIELD OF FLUORESCENCE) IN DCMU-TREATED ALGAE*

P. JURSINIC† and GOVINDJEE‡
Departments of Botany, Physiology and Biophysics, University of Illinois, Urbana, Illinois 61801, U.S.A.

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Abstract—(1) The simultaneous measurements of delayed light emission (DLE) and chlorophyll (Chl) fluorescence yield in DCMU§ treated *Chlorella* were made in the time range of 1 to 10 sec at various temperatures from 0 to 50°C. Similar measurements were made for DCMU treated thermophilic strain of *Synechococcus* in the temperature range of 0 to 75°C.

(2) Using the basic assumption that DLE is produced by the back reaction of primary photoproducts of system II, and that two such reactions are required for it, a linear relationship between $J^{-1/2}$ (where J is energy per unit time available for DLE) and time after illumination was derived. This second-order relationship was confirmed experimentally at several temperatures (2°, 5°, 10° and 15°C). From these analyses, reaction rate decay constants, at specific temperatures, were calculated.

(3) An Arrhenius plot was made for these calculated rate constants. Its slope (8–10 kcal/mole) agreed well with previous reports; however, it had a region of zero slope which occurred at the physiological temperature of the organisms used.

(4) Thermoluminescence or temperature jump delayed light emission (TDLE) was measured using various temperature conditions and it was found that not only the magnitude of the temperature jump (ΔT), but the initial and final temperatures of the sample were important. For example, a temperature jump of 8°C from 2 to 10°C gave much higher TDLE than from 12 to 20°C.

(5) Many properties e.g., magnitude, temperature dependence and time independence of TDLE could be explained by the DLE decay data (corrected for changes in fluorescence yield) and the kinetic analysis.

(6) It is suggested that, in addition to the back reaction of Z^+ (the primary oxidized photoproduct of system II) with Q^- (the primary reduced photoproduct of system II), a reducing entity, beyond the sites of DCMU and antimycin *a* action, is somehow involved in the production of slow DLE.

INTRODUCTION

ONE OF the major goals of photosynthesis research is to understand how the electronic excitation energy, absorbed by chlorophyll (Chl) molecules, is converted into chemical energy. After the absorption of light the energy appears in various physical forms. The most immediate form is fluorescence which has a lifetime of 10^{-9} sec[1–5]. The earliest evidence of a chemical form occurs in 10^{-2} sec[6], the turnover time of the entire electron transport chain. The magnitude of these time differences implies the necessity

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‡Reprint requests should be addressed to Govindjee, Department of Botany, 297 Morrill Hall, University of Illinois, Urbana, Illinois 61801, U.S.A.

§Abbreviations: Chl, chlorophyll; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DLE, delayed light emission; TDLE, temperature jump delayed light emission; Q, primary acceptor of electrons of system II; Z, primary donor of electrons of system II.

for some type of metastable energy storage. It is believed that delayed light emission (DLE), discovered by Strehler and Arnold[7], reflects the state of this metastable energy. This light emission is of low intensity, is observed immediately after the illumination is terminated and continues for many minutes. The emission spectrum of DLE is identical to that of normal fluorescence of Chl *a in vivo*[8, 9]. Also, the action spectra of DLE match the action spectra of photosynthesis in the organisms studied [10]. DLE data from system II lacking mutants[9, 11] show that it is associated mainly with a functional photoreaction center II, the oxygen evolving photoreaction in the Hill and Bendall scheme[12].

The decay of this low intensity DLE[13, 14] has been found to be very complex [14–16]. The two hypotheses that explain the DLE phenomena most satisfactorily are based (1) on an electron-hole model[17–20] and (2) a charge transfer redox model [9, 14, 21]. In the former theory energy is made available for DLE by the recombination of electrons and holes in a Chl crystal structure, while in the latter theory DLE is produced by energy provided by a back reaction of system II ($Z^+\text{Chl}a_2Q^- \rightarrow Z\text{Chl}a_2Q$ + energy). The effects of the chemical poisons hydroxylamine and DCMU are explained as follows. Hydroxylamine has been shown[22–26] to take the place of water in feeding electrons to an oxidized form of Z, the initial electron donor at reaction center II. Thus, at long times there is no Z^+, and thus no delayed light. DCMU treatment blocks the electron flow between the two photosystems[27]. Q^- can return to the oxidized form Q, mainly by a back reaction of Z^+ and Q^-; this leads to DLE.

One of the most important concepts in the present area of research is the intimate relationship of DLE and fluorescence[9]. DLE, just like fluorescence, is due to the decay of an excited Chl molecule. The only difference is in the manner by which the excited state is created. In fluorescence the singlet excited state is populated directly by absorbed light quanta, while in DLE the singlet state is populated mainly via chemical back reaction. Nevertheless, the singlet to ground state transition is still fundamental to both processes. With this in mind, the general relation $F = \phi I$ has now been extended to DLE by Lavorel[9] into the following relation:

$$L = \phi J.$$

In these formulae, F is the number of quanta emitted per unit time as fluorescence, L is the number of quanta emitted per unit time as DLE, ϕ is the fluorescence yield, I is the number of quanta absorbed per unit time, and J is the amount of energy made available per unit time for emission as DLE.

Until recently few investigators realized the direct effect of the fluorescence yield on DLE [also see Clayton, 28]; it was never taken into account and as a result much of the previous work dealing with DLE may have to be repeated and corrected for this effect. Recent research has dealt with how DLE is perturbed by a quick discontinuous change in the experimental environment: acid–base transition[29–31] and salt treatment[32]. One of the most important experiments in stimulated DLE was performed by Kraan *et al.*[33]. They simultaneously measured the fluorescence yield and DLE induced by acid–base shifts and sudden increases in ionic strength upon the addition of salts. In this manner Lavorel's equation $L = \phi J$ could be used and the primary DLE parameter J could actually be measured, instead of just the DLE intensity (L) as previous investigators had done. It was found that the fluorescence yield varied slowly and

was relatively insensitive to acid–base shifts and ionic strength changes. However, the importance of measuring fluorescence yields and not merely the DLE intensity was re-emphasized. Many of the previous results were, therefore, put on much more rigorous grounds.

Arnold and Sherwood[17, 34] observed thermoluminescence (glow curves) when preilluminated *dried* chloroplast films were *slowly* heated (13°C/min). Involvement of five different activation energies (0·69 to 0·93 eV) was suggested. Arnold[35] later showed that *Chlorella* cells, first frozen at −10° to −200°C, then illuminated in cold, and finally heated rapidly in dark to 95°C, gave off light. (Heating to 95°C may cause irreversible changes in the cells and may not be desirable.) Rough calculations gave activation energies of about 0·5 eV. Arnold and Azzi[20] reported four thermoluminescence peaks (glow curves) when *Chlorella* cells (at −10° to −196°C) were illuminated and then heated in darkness at 3°C/sec. These peaks were at −155°C, −6°, +30° and +52°C which they arbitrarily labelled Z, A, B and C. The Z peak was *not* related to the operation of photosynthesis as it was excited only by blue light, and was present in plants heated to 100°C for 5 min. However, A, B, and C peaks disappeared if samples were heated to 55°C for 5 min. Addition of DCMU lead to the disappearance of A and C peaks—the B peak, however, remained. Upon heating (20°C/min) preilluminated frozen *Chlorella* cells, Rubin and Venediktov[36] observed several "glow peaks" (at −10°, +10°, +25° and +50°C). The +10° and the +50°C peaks were observed even in the presence of $10^{-5}M$ DCMU. These authors concluded that storage of electrons in the presence of DCMU occurs in smaller traps, *i.e.*, only carriers located before the site of action of DCMU are involved. Shuvalov and Litvin[37] have described, in detail, five major components of DLE and have attempted to relate them to the various thermoluminescence peaks.

Mar and Govindjee[38] observed that preilluminated algae and isolated spinach chloroplasts (at 10–20°C) could be stimulated to give renewed DLE by a quick temperature jump of about 15°C. They suggested that the thermoluminescence or temperature-jump delayed light emission (TDLE), perhaps, had the same underlying mechanism as the emitted light due to pH or ionic strength changes. However, results with added DCMU and hydroxylamine were explained in terms of the back reaction of Z^+ with Q^-. We felt that the most promising approach for gaining further understanding of the TDLE phenomenon was in answering the fundamental question of how DLE (especially J) was affected by temperature. A number of investigators[7, 15, 18, 39] had already observed how DLE decay was dependent upon temperature; however, none of this work took the simultaneous fluorescence yield changes into consideration. More recently Lavorel[9] has measured the decay of J at two different temperatures, but only at times less than 150 msec. The decay of J in the time range of seconds has been measured by Clayton[28], but *only* at one temperature. In the time and temperature range we were interested in, these measurements had never been made. Therefore, we measured the DLE decay and fluorescence yield simultaneously in the time range of 1 to 10 sec at different temperatures from 0 to 45°C. Also, we calculated the back reaction decay rate constant of Z^+ and Q^- at specific temperatures. From these decay rates alone, we were able to not only explain the magnitude of the TDLE peak but also its decay rate. Our measurements also allowed us to make an Arrhenius plot for the back reaction rate constant fundamental to DLE using data which had been corrected for fluorescence yield. We found that this Arrhenius plot has a segment with zero slope

at the physiological temperatures of the organisms used. A non-linear Arrhenius plot of this type has never been reported before for the DLE back reaction. However, Sweetser *et al.* [39, (Table 3)] show some data which in fact do deviate from a linear Arrhenius plot, but these data were not emphasized in that paper. Our analysis is supported by the DLE decay data which has been corrected for fluorescence yield changes. Then, using the decay data and our kinetic analysis we are able to predict and explain a number of properties of TDLE.

MATERIALS AND METHODS

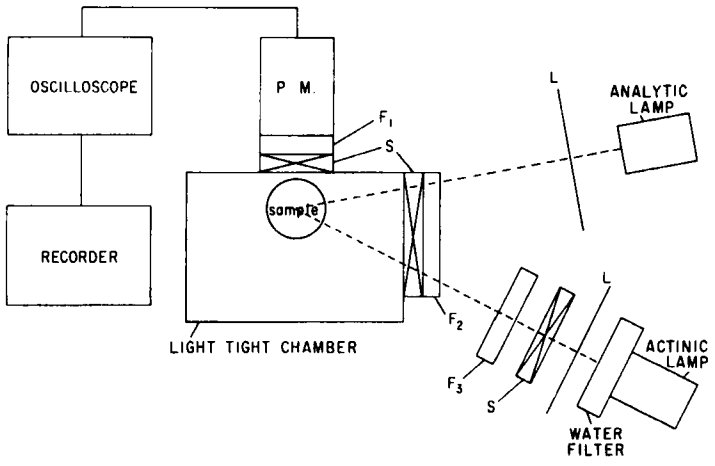
In most experiments the unicellular green alga *Chlorella pyrenoidosa* was used. The techniques for growing this alga have been described previously [40]. In a few experiments we also used the blue-green alga *Anacystis nidulans* and a high temperature strain of the blue-green alga *Synechococcus lividus*, grown as described in Ref. [41] and [42], respectively.

Measurements of the DLE of 2, 3, 4, 6 and 8 day old samples of *Chlorella* showed that the decay and level of DLE appeared approximately the same for the 2, 3, and 4 day old samples, whereas the 6 and 8 day samples gave more DLE which decayed with a lower rate. Therefore, to keep the sample variability as small as possible, 3 day old samples were used in all experiments. Cells were removed by centrifugation from the growth medium and resuspended in a buffer solution (15% of 0·1 *M* $KHCO_3$ and 85% of 0·1 *M* $NaCO_3$). The algae concentration was adjusted to an absorbance of 0·3, in a 1 cm cuvette, at 675 nm (Chl concentration, 40 μg/ml).

A block diagram of the instrument used for DLE measurements is shown in Fig. 1. The sample (1 ml of suspension) was contained in a thin walled transparent plastic cuvette. The filter F_1 allowed the Chl emission to reach the photomultiplier (EMI 9558 B) while blocking the blue excitation light transmitted by F_2. The shutters allowed flexibility in protocol for measuring DLE and fluorescence. The photomultiplier signal was displayed on an oscilloscope and recorded with a Midwestern instruments model 801 oscillograph, or with an Esterline Angus Recorder (model E11015). The temperature of the sample was measured with a TRI-R electronic thermometer; no special means was used to hold the sample temperature constant when it differed from room temperature. A typical measurement took a total of 20 sec and in this time span the sample temperature varied less than two degrees; this variation does not significantly affect our results, and conclusions. A quick temperature jump was effected by injection with a syringe of 1 ml of buffer heated to various temperatures.

The intensity of the actinic illumination, measured at the sample by a thermopile, was 2×10^4 ergs cm^{-2} sec^{-1}; this saturated DLE. The intensity of analytic illumination was adjusted so that the fluorescence signal it caused was about 40 per cent of the DLE signal observed 1 sec after illumination. Thus the intensity of the analytic light was in the range used by Lavorel [9] and Kraan *et al.* [33].

We observed DLE and TDLE after the addition of 10^{-5}, 10^{-6} and 10^{-7} *M* DCMU to algal suspensions. All the concentrations used gave similar effect on DLE and TDLE. Consequently 10^{-6} *M* DCMU was used in all experiments. As was explained in the introduction, DCMU blocks the electron flow between the two photosystems [27]; therefore, the decay of Q^- is simplified by removing the competition by the electron flow from Q^- through the electron transport chain to photosystem I. In other words,



F_1, red cut off filter, C.S. 2-64
F_2, blue filter, C.S. 4-96
F_3, neutral density filter
L, lens
P.M., photomultiplier
S, shutters

Fig. 1. Diagram of the experimental apparatus for measuring delayed light emission (DLE), temperature-jump delayed light emission (TDLE) and chlorophyll fluorescence yield.

DCMU assures that Q^- will return to the oxidized form mainly by the DLE back reaction.

EXPERIMENTAL METHODS

In saturating light intensity (2×10^4 ergs cm^{-2} sec^{-1}), the magnitude of the DLE was insensitive to the duration of illumination in a time range of 1 sec to many minutes. In all DLE measurements an illumination time of 5 or 10 sec was used.

The temperature jump experiments were conducted in a variety of temperature conditions. Different initial sample temperatures were tried and various magnitudes of temperature jumps were conducted at each initial temperature. A continuous recording was made of the DLE beginning with the end of illumination. The injection of heated buffer caused a fast mixing and thus a temperature jump to occur within 0·5 sec.

The measurement of the fluorescence signal was carried out in the following manner. A sample was placed in the measuring chamber, and the analytic beam was turned on. The intensity of the analytic beam was then adjusted to give a fluorescence signal about 40 per cent of the usual DLE signal; this insured that the intensity was low enough so as not to cause a significant change in the reduction of Q to Q^-, but was capable of measuring the level of Q^-, indicated by the fluorescence yield. Next the DLE signal alone was measured. The sample was illuminated for 10 sec by both the actinic and analytic lamps, then a shutter was closed blocking both light sources and the DLE signal was recorded. To measure the DLE plus fluorescence signal the same procedure was followed except after activation only the actinic beam was blocked with a shutter

so that only the analytic beam remained on. Therefore, the signal recorded was DLE plus fluorescence. The fluorescence signal (F) alone was obtained by taking the difference of the two measured signals: $F = (\text{DLE}+F)-(\text{DLE})$. The fluorescence signal was normalized to the value of 1·0 at long times. Thus, we were able to measure the delayed light and fluorescence yield at identical times after the illumination was terminated.

RESULTS

Illumination intensity. The light curve for DLE was established by Strehler and Arnold[7] when they discovered DLE. Somewhat later Goedheer[42] measured the light curves of spinach chloroplasts for both DLE and Hill reaction in the same sample as he wanted to learn about the relation of these two processes.

A light curve for TDLE has never been measured. Having this curve would be quite useful since it would enable us to choose an illumination intensity which would be in the saturation region of the TDLE light curve. Such a choice is desirable since the TDLE would then be independent of small fluctuations in the illumination intensity. It was also advantageous to measure the DLE light curve since it could be compared to the TDLE curve. The light curves for DLE and TDLE are shown in Fig. 2. (The intensity of exciting light was measured at the sample.)

The protocol was as follows. The sample was illuminated for 5 sec and at 1 sec after illumination the DLE intensity was measured. For the TDLE the temperature jump was carried out at 1 sec after illumination and the peak of the induced luminescence was measured. In the TDLE measurements, reported in Fig. 2, the initial sample temperature was 24°C and the final temperature was 45°C.

Light curves were also made for DLE and TDLE at times other than 1 sec. In all cases the light curves obtained were similar to those in Fig. 2, with saturation occuring at about 1×10^4 ergs cm^{-2} sec^{-1}. On the basis of these experiments we chose an illumination intensity of 2×10^4 ergs cm^{-2} sec^{-1} for all the experiments to assure that the samples would be in the saturation region of the curves.

Decay of J in chlorella at various temperatures. The importance of measuring J for

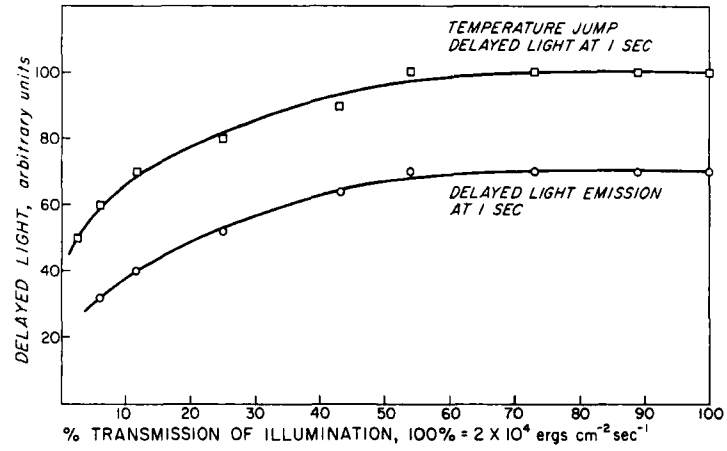


Fig. 2. Light curves for both DLE and TDLE. Saturation occurs for both at about $1\cdot1\times10^4$ ergs cm^{-2} sec^{-1}.

the understanding of DLE has been pointed out in the introduction. Since J represents the energy flux which is available for DLE we felt that learning about its temperature dependence behavior would be useful in understanding TDLE.

As pointed out in the introduction, the decay of J has been previously measured. However, this has not been done in the time range and in the extensive temperature range used in the present study. To determine the decay of J both the DLE and fluorescence yield decays were measured and then J was calculated using Lavorel's[9] formulation: $J = L/\phi$.

DLE or L decay curves at 2 and 35°C, normalized to the arbitrary value of 100 at 1 sec after illumination, are shown in Fig. 3. At the higher temperature the decay rate of DLE was much greater. However, if the sample was kept at 45°C for 3 or more min, it lost its capability for DLE.

The values obtained for the stimulated Chl *a* fluorescence yield at several temperatures and at various times after illumination are shown in Table 1. These, however, are relative yield values obtained by assigning the value of 1·00 to the fluorescence yield at long times after the actinic illumination was terminated. Table 1 shows that the fluorescence-yields change significantly at short times after illumination; it is clear that the fluorescence yield decay rate, after the actinic illumination is terminated, is sensitive to temperature and is larger at higher temperatures.

Combining the DLE and fluorescence yield data, values for J were calculated. In Figs. 4–7 the quantity $J^{-1/2}$ vs. time is shown for several different temperatures. Each point in these graphs represents the average of values obtained from as many as eight separate experiments. Error bars representing one standard deviation unit are shown at various points on these graphs to give an indication of the experimental variability. At low temperatures the graphs are linear for times longer than 10 sec, the slopes of these lines are shown on the figures. At 20°C slight deviations from linearity are seen at long times. At 24°C and above the deviations occur progressively earlier in time. For temperatures where deviations occur, the early linearity is projected with a broken line and the slope is indicated. As can be seen from Figs. 4–7 these slopes are dependent upon the temperature of the samples.

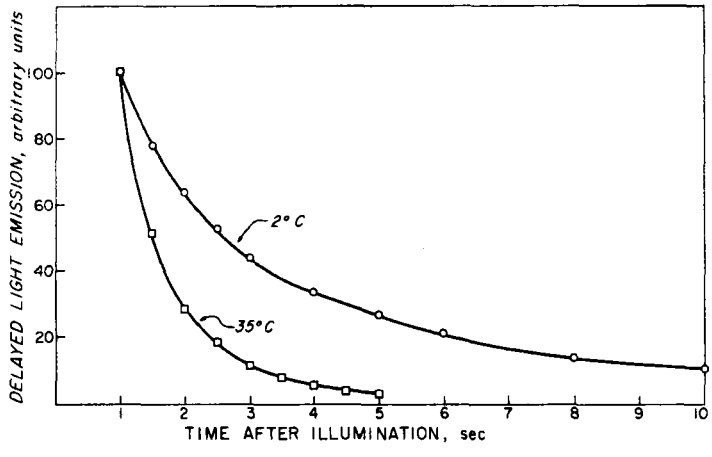


Fig. 3. DLE decay for *Chlorella* at two different temperatures. The rate of decay is seen to be sensitive to temperature.

Decay of J in synechococcus. Since we were interested in the effects of temperature upon the decay of J, testing the generality of the *Chlorella* results with *Synechococcus*, which grows at a different temperature (65°–70°C), was desirable. We measured the DLE of these algae from 0 to 75°C. The DLE at 0°C was barely distinguishable above the signal noise. Easily measureable intensities of DLE were not observed until sample temperatures were greater than 15°C. Even at 20°C the DLE was low in intensity and had very slow decay kinetics. At all temperatures the DLE decay rates of these high temperature algae were smaller than those for *Chlorella*. But these algae had normal DLE at temperatures above 45°C where *Chlorella* could not function. Keeping *Synechococcus* at 75°C for 3 min, however, did destroy the capability for DLE.

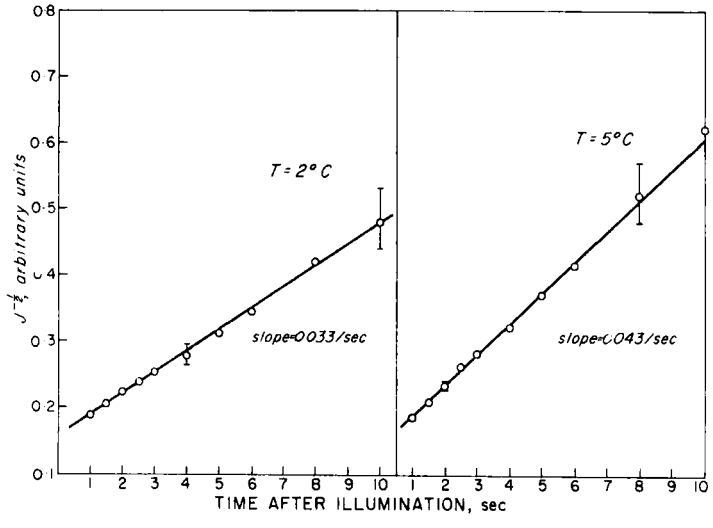


Fig. 4

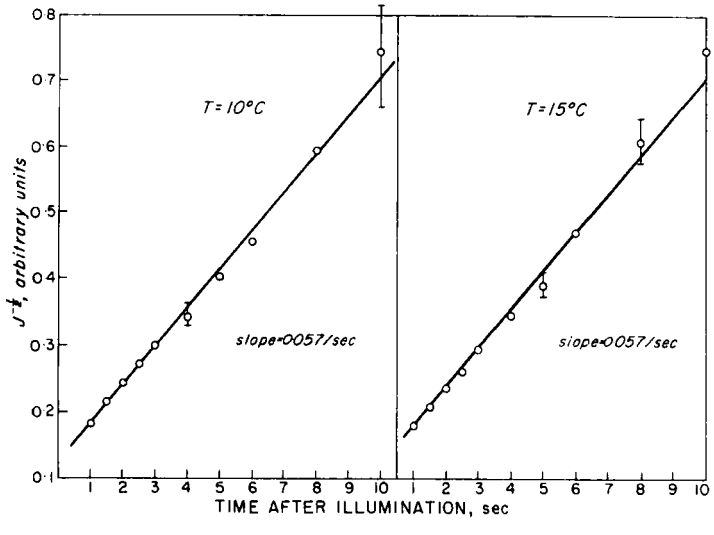


Fig. 5

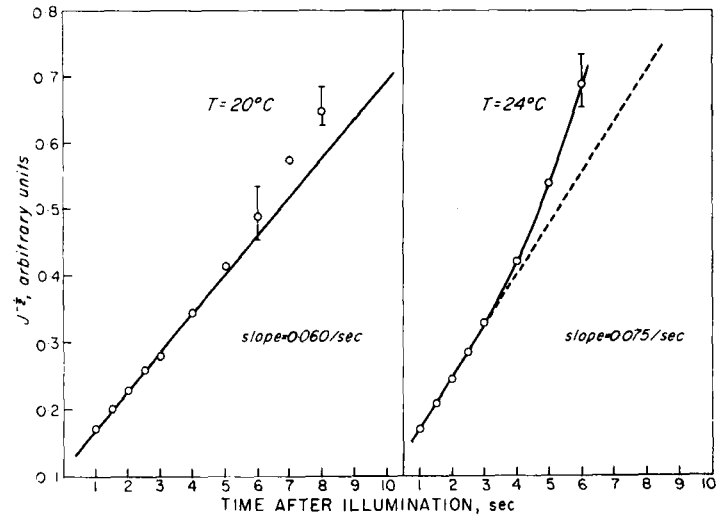


Fig. 6

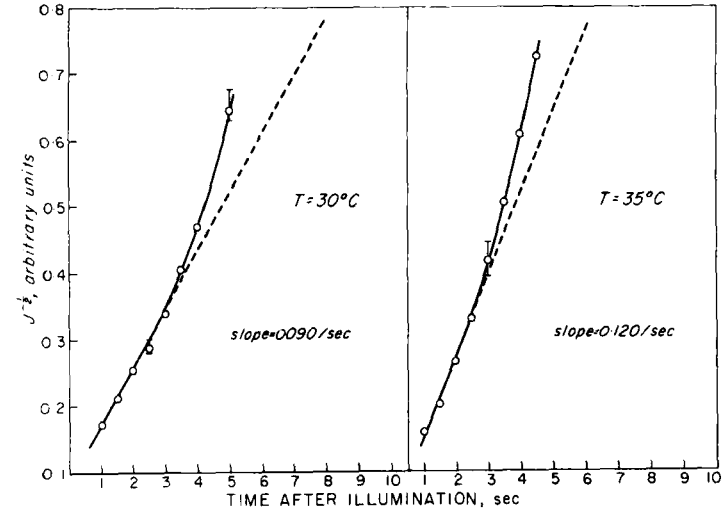


Fig. 7

Figs. 4–7. Decay curves of $J^{-1/2}$ for DCMU treated *Chlorella* at temperatures from 2 to 35°C. The curves start to deviate from linearity at 15°C and above. Projected linearity is shown by a broken line.

Figure 8 shows the $J^{-1/2}$ plot for *Synechococcus* at two different temperatures. At 35°C linearity is still quite good in contrast to *Chlorella* at this temperature (cf. Fig. 7). However, at 45°C deviations from linearity are noticeable. In fact, the J decay for *Synechococcus* is quite similar to that of *Chlorella* except the temperature has been shifted by 30°C. Note that the slope of the $J^{-1/2}$ vs. time curve at 5°C for *Chlorella* is the same as that for *Synechococcus* at 35°C.

Temperature jump delayed light in chlorella. In the temperature jump experiments, *Chlorella* was used as in Ref. [38]. However, we extended the experimental procedure

Table 1. Fluorescence yield values during decay of delayed light. This table shows values of the fluorescence yield from 1 to 10 sec after illumination and at different sample temperatures. These data are for DCMU treated *Chlorella*.

Time after Illumination	Temperature of sample (°C)							
	2	5	10	15	20	24	30	35
1·0	1·80	1·75	1·70	1·65	1·45	1·50	1·45	1·27
1·5	1·65	1·58	1·45	1·30	1·25	1·35	1·30	1·05
2·0	1·60	1·50	1·25	1·15	1·16	1·25	1·15	1·00
2·5	1·50	1·35	1·10	1·13	1·14	1·15	1·05	1·00
3·0	1·40	1·20	1·05	1·10	1·10	1·10	1·00	1·00
4·0	1·30	1·15	1·00	1·05	1·05	1·00	1·00	1·00
5·0	1·30	1·10	1·00	1·00	1·00	1·00	1·00	1·00
6·0	1·25	1·05	1·00	1·00	1·00	1·00	1·00	1·00
8·0	1·20	1·00	1·00	1·00	1·00	1·00	1·00	1·00
10·0	1·15	1·00	1·00	1·00	1·00	1·00	1·00	1·00

by using various initial and final temperatures and varying the time after illumination at which the temperature jump was given. We calculated the ratio of the DLE values immediately before and after the temperature jump, i.e., DLE(T_i) and DLE(T_f) respectively (Table 2). As can be seen, not only the magnitude of the temperature jump, but the initial and final temperatures are also important. This fact is most clearly seen by comparing Fig. 9 with Fig. 10. In both cases a net temperature jump of 8°C took place; however, the 2° to 10°C jump stimulated a much greater DLE than the 12 to 20°C jump. The temperature jump delayed light emission is much more clearly observed in our work here than in the preliminary work of Mar and Govindjee[38]. It was also found that these DLE ratios were independent of time. For example, a temperature jump of 2 to 10°C gave a ratio of about 1·7 no matter what time after the illumination the temperature jump was made. However, at times longer than about 10 sec (after illumination) the DLE intensity usually was so small that the calculated ratio was not very reliable.

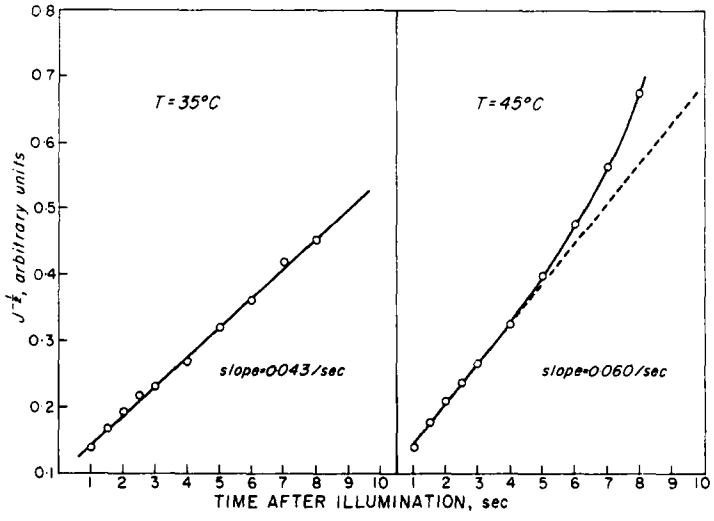


Fig. 8. Decay curves of $J^{-1/2}$ for DCMU treated *Synechococcus* at 35 and 45°C. The decay is still linear at 35°C but deviates from linearity at temperatures of 45°C and higher.

Table 2. Temperature-jump induced delayed light emission
Data are for various temperature jumps conducted on DCMU treated *Chlorella* samples. T_i and T_f are the temperatures before and after the temperature jump. The DLE at T_i and DLE at T_f are the DLE immediately before and after the temperature jump. The Slope (T_f) and Slope (T_i) are the slopes of the $J^{-1/2}$ vs. time curves at temperatures T_f and T_i respectively.

T_i (°C)	T_f (°C)	DLE At T_i	DLE At T_f	$\frac{DLE(T_f)}{DLE(T_i)}$	$\frac{Slope\ (T_f)}{Slope\ (T_i)}$	Activation energy* (kcal mole^{-1})
2	5	2·5	3·5	1·40	1·30	13·4
2	10	2·5	4·3	1·72	1·73	10·6
2	15	2·5	5·5	2·20	1·73	6·8
2	20	2·5	7·5	3·00	1·82	5·3
12	20	2·3	2·4	1·04	1·05	1·0
24	30	2·5	3·0	1·20	1·20	5·4
24	35	2·5	4·5	1·80	2·00	7·8

*Activation energy (E_a) was calculated from the equation: $E_a = \log (k_i/k_f)\ (2{\cdot}303)\ (R)\ (T_f.T_i/T_f - T_i)$, where k_i and k_f are the rate constants (read off from Figs. 4–7) at temperatures T_i and T_f, R is the gas constant (1·987) in cal mole^{-1} °K^{-1}, and the T_i and T_f are the initial and final temperatures in °K (instead of in °C as given in the above table).

Also shown in Table 2 are the ratios of the slopes of the $J^{-1/2}$ curves of Figs. 4–7 for the appropriate initial and final temperatures of the temperature jump experiments. (Slope (T_i) is for the initial and (T_f) is for the final temperature.) As can be seen, the DLE ratio and the slope ratio are approximately equal to each other for the different temperature jumps shown. Both the time independence of the DLE ratios and the equality of the DLE and the slope ratios—noted above—had not been reported before but are predicted by our analysis (see Discussion). Also it was anticipated that the rate of DLE decay after the temperature jump occurred would be characteristic of the

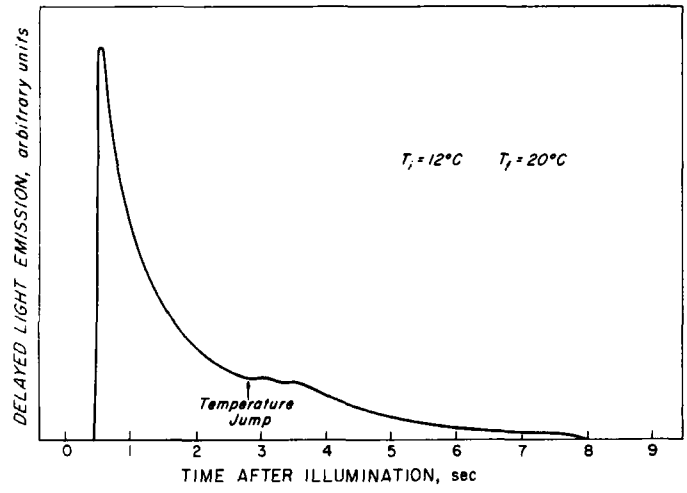


Fig. 9. Chart trace of DLE and TDLE: A temperature jump from 12 to 20°C given at about 3 sec after the illumination ended, for a sample of DCMU treated *Chlorella*. In this and the next figure, the initial trace (between 0–1 sec) is due to the movement of the recorder pen when the DLE measurements begin, and it should be ignored.

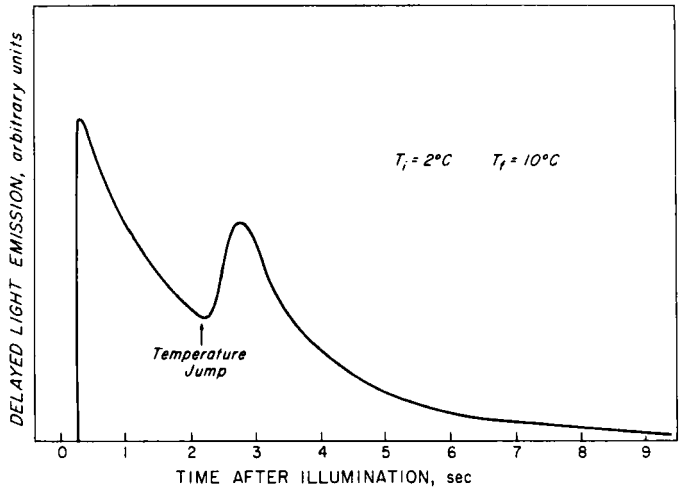


Fig. 10. Chart trace of DLE and TDLE: A temperature jump from 2 to 10°C; other details as in Fig. 9.

sample's final temperature. For example in Fig. 10 the DLE decay after the temperature jump had the decay rate of a 10°C sample. This effect was observed for all temperature jumps.

The last column in Table 2 lists the calculated activation energies for various temperature jumps.

Temperature jump experiments were also conducted using *Anacystis* and *Synechococcus* giving results similar to those of *Chlorella*. These experiments were carried out in order to test the generality of the TDLE phenomenon; the results are not shown here. However, one interesting fact was that, as in *Chlorella*, there was a temperature region in *Anacystis* where the TDLE decreased even though the Δt was the same as in other temperature regions.

DLE in untreated (normal) chlorella. Measurements of DLE and TDLE were also made with *Chlorella* that had not been treated with DCMU. The DLE decay in these untreated cells was different in two respects. First, the rate of DLE decay was slower and second, the decay was complicated and could no longer be described by a second order kinetic analysis. DLE decays for samples with or without DCMU at 10°C, arbitrarily adjusted at 1 sec, are shown in Fig. 11. The intensity of DLE, at 1 sec, in untreated samples, was 1·5 to 2·0 times that in DCMU-treated samples.

The TDLE measurements also showed changes when DCMU was not present. For a temperature jump of 2 to 10°C the TDLE ratio in untreated cells was 3·3, whereas in DCMU treated cells this ratio was 1·75.

One possible explanation for this is that DCMU not only blocks the electron flow from Q^- through the electron transport chain but that it also blocks the back-reaction of Z^+ with some reducing entity (in addition to Q^-) which is further up the electron transport chain than the DCMU block. To test this idea we conducted an experiment using cells treated with 10^{-5} *M* Antimycin A, which is believed to cause a block further down the electron transport chain than DCMU. It was found that these samples had slightly faster DLE decay and smaller TDLE ratios compared to DCMU treated samples. From this result it seems that this additional back reaction entity may be

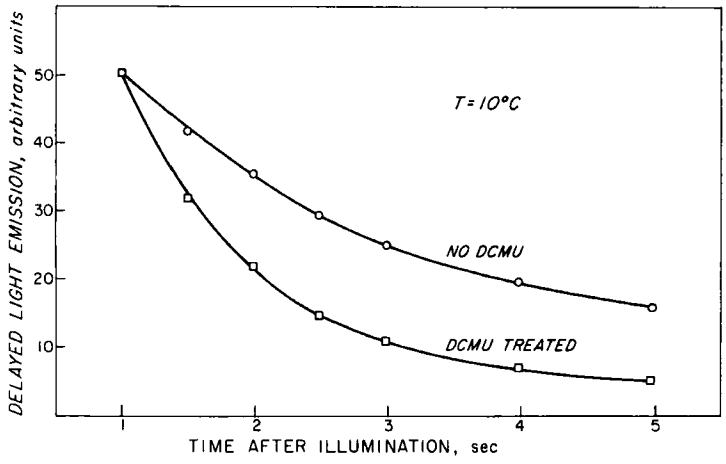


Fig. 11. DLE at 10°C for samples with and without DCMU. The DLE intensities were normalized to the arbitrary value of 50 at 1 sec.

further down the electron transport chain beyond the site where Antimycin A blocks. Further experiments of this type are needed to show the nature of the proposed entity other than Q^-.

DISCUSSION

The preliminary experiments of Mar and Govindjee[38] showed that preillumination was necessary for TDLE, and that TDLE occurred in DCMU treated cells, but was absent when hydroxylamine was present. They explained their results in terms of the back reaction of the primary photoproducts Z^+ and Q^- of photosystem II. One of the first experiments we performed established the light curves for DLE and TDLE (Fig. 2). Both processes reached saturation at the same light intensity; thus it appeared that DLE and TDLE might originate from the same fundamental process. Also earlier work[9, 14] explained DLE in terms of the back reaction of Z^+ and Q^-. If this back reaction is fundamental to both DLE and TDLE, learning how the back reaction is affected by temperature should help in the understanding of TDLE.

In order to describe the effects of temperature on the back reaction of Z^+ and Q^-, a relation between their decays and the measured DLE must be established. Using DCMU, the competition for Q^- by the electron transport chain is eliminated.

The basic reaction scheme is assumed to be as follows[9, 14]:

$$ZChlQ \xrightarrow{h\nu} Z^+ChlQ^- \xrightarrow[DCMU]{} \text{electron transport chain} \quad (1)$$

$$Z^+ChlQ^- \xrightarrow{k_1} ZChl_tQ \quad (2)$$

$$2ZChl_tQ \xrightarrow{k_2} Z\,Chl_sQ + ZChlQ \quad (3)$$

$$ZChl_sQ \longrightarrow ZChlQ + h\nu' \text{ (delayed light)} \quad (4)$$

Where Chl_t is a chlorophyll triplet, Chl_s is a chlorophyll (excited) singlet, k_1 is the back

reaction constant of Z^+ and Q^-, and k_2 is the bimolecular fusion rate constant; k_1 is assumed to be rate limiting.

It is clear from the above scheme that the emission of a quantum of delayed light requires the cooperation of two reactions. Two quanta of light must be absorbed to give two triplets, and their fusion gives delayed light. This scheme leads to a second order kinetics. Second order decay had been earlier suggested by Clayton[21], Mayne[30], and Barber *et al.*[31] when they referred to either J or DLE.

To provide for the high photochemical efficiency of photosynthesis, the primary donor and acceptor should be in a complex, and therefore its decay should be first order. However, delayed light that requires the cooperation of two such complexes would be a second order.

The rate at which the singlets are formed is:

$$\frac{ds^*}{dt} = k_1(Z^+Q^-)^2 \tag{5}$$

Or simply,

$$\frac{ds^*}{dt} = k_1(Q^-)^2 \tag{5a}$$

as ZQ is one complex.

Since J in Lavorel's[9] formulation is the energy per unit time available for DLE, then it should be proportional to the rate at which the singlets are developed:

$$J\alpha\frac{ds^*}{dt} = k_1[Q^-]^2 \tag{6}$$

If DLE follows second order kinetics then plotting $J^{-1/2}$ vs. time should give straight lines. The slope of these lines being the back reaction constant for Z^+ and Q^- at that particular temperature.

Figures 4–7 show $J^{-1/2}$ plots for DCMU treated algae at various temperatures. Linear plots do occur at temperatures below 20°C for *Chlorella* and below 45°C for *Synechococcus*, confirming the second order relationship. Deviations above these temperatures are presently unexplained, but they may be due to a conformational change implying thereby that at higher temperatures a "substrate", upon which the back reaction of Z^+ and Q^- takes place, changes its form. In this manner the effective rate constant of the back reaction may change.

Taking the slopes from Figs. 4–7 an Arrhenius plot of the back reaction constant in *Chlorella* can be made. This is shown in Fig. 12. The slope of this plot represents an activation barrier for the back reaction. Previously reported values were 14 kcal/mole [21] and 10 kcal/mole[39]. The section of zero slope in the Arrhenius plot, which occurs in the physiological temperature range of *Chlorella*, is a new finding. In Clayton's work[21] only two temperatures were used so a non-linear Arrhenius plot could not be detected. And as previously stated Sweetser *et al.*[39] did not emphasize the point which deviated from linearity in their data.

This region of zero slope may be significant in relation to the idea of a conformational change mentioned above. First, the deviations from linearity of the $J^{-1/2}$ vs. time

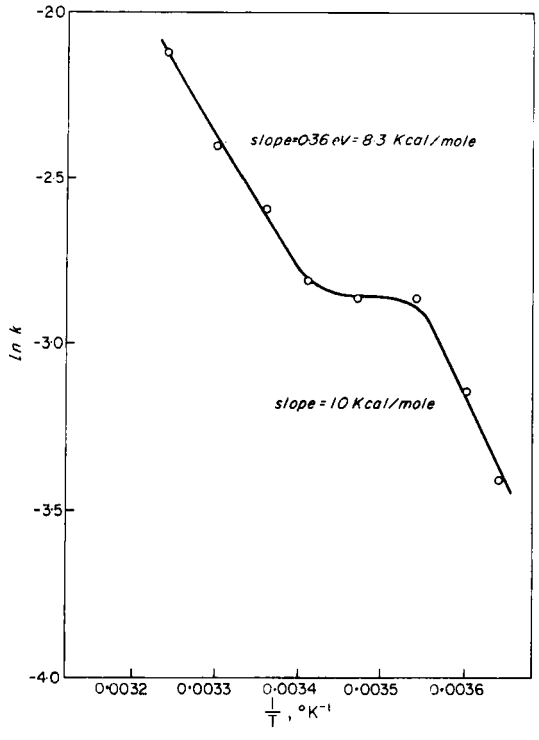


Fig. 12. Arrhenius plot of the back reaction constant of Z^+ and Q^- in *Chlorella*. The section of zero slope occurs in the (physiological) temperature range of 10°C → 20°C. The slopes of the curves differ slightly for temperatures above and below the zero slope temperature range.

curves begin at the same temperature, between 10°C and 15°C, as the zero slope section of the Arrhenius plot. Second, the activation barrier for the back reaction is different in the temperature regions above and below the zero slope section of the Arrhenius plot [cf. Table 2]. Perhaps these events are indicative of the suggested conformational change. The zero slope section of the Arrhenius plot may be a temperature region where a transition between two conformation states exists.

The sensitivity of the back reaction constant, k_1, to certain temperatures is seen in Fig. 12. One question of interest is: would a change in k_1 alone be able to explain the TDLE observed? This is expected if TDLE is only caused by the back reaction of Z^+ and Q^-. A temperature jump from T_i to T_f at time t is represented as:

$$\text{at } T_i,\ \text{DLE}(T_i, t) = k_1(T_i)[Q^-(t)]^2, \tag{7}$$

and

$$\text{at } T_f,\ \text{DLE}(T_f, t) = k_1(T_f)[Q^-(t)]^2. \tag{8}$$

Therefore

$$\frac{\text{DLE}(T_f)}{\text{DLE}(T_i)} = \frac{k_1(T_f)}{k_1(T_i)}, \tag{9}$$

where, $DLE(T_i, t)$ is the DLE at time t and temperature T_i, $DLE(T_f, t)$ is the DLE at time t and temperature T_f, $K_1(T_i)$ is the back reaction rate constant (slope of the $J^{-1/2}$ vs. time curve) at temperature T_i, $k_1(T_f)$, the same at temperature T_f, and $[Q^-(t)]$ is the concentration of Q^- at time t.

From this relationship it is seen that the magnitude of the TDLE, or the "DLE ratio", should be equal to the slope or rate constant ratio [equation (9)]. Comparing columns five and six in Table 2, it can be seen that the above prediction is true except for the 2 to 15°C and 2 to 20°C temperature jumps. From Fig. 12, a temperature jump in the 10 to 20°C range would be expected to cause very little TDLE since the back reaction rate constant is insensitive to those temperatures. This is confirmed by the negligible TDLE shown in Fig. 9.

Another prediction from [equation (9)] is that the "DLE ratio" of a temperature jump should be independent of the time after illumination at which the temperature jump occurs. This, in fact, was observed in all the experiments that tested for it.

In conclusion, it seems that a second order decay for J is correct in some temperature ranges, but not for high temperatures. The DLE back reaction appears to have an activation barrier of zero in a specific temperature region. Furthermore, many aspects of the TDLE phenomenon may be explained by the change in the back reaction rate constant of Z^+ and Q^- caused by a temperature change. Further experiments with faster temperature jumps might be a fruitful approach for better understanding of the primary reactions of photosynthesis. It is tempting to speculate on the relationship of DLE and TDLE observed in this work with those observed by Arnold and coworkers[17, 20, 34, 35], by Rubin and Venediktov[36] and by Shuvalov and Litvin [37]. Our experiments are different from the thermoluminescence experiments of Arnold and Sherwood[17, 34]: We change the temperature quickly (within fractions of seconds) and these changes are small (3 to 15°C), whereas the rate of the temperature change was 14°C/min in Arnold and Sherwood's experiments. Also, we use fresh *Chlorella* suspensions and they used *dried* materials. Thus, it is difficult to compare our results with those of Arnold and Sherwood. However, it is possible to make some comparisons with the experiments of Arhold[35], Arnold and Azzi[20], Rubin and Venediktov[36] and Shuvalov and Litvin[37]. As mentioned in the introduction, *Chlorella* cells were first frozen (to -10 to -200°C) and then heated either slowly (20°C/min)[20, 35–37] or rapidly (3°C/sec)[35]. Upon slow heating, four (or five) thermoluminescence peaks [at -155°C (Z peak), at -6 to -15°C (A peak), at $+10$°C or $+20$–30°C (B peak), and at 52°C (C peak)] were observed. It is tempting to assume that, in the present work, we are looking at the component responsible for Arnold's B peak or Rubin and Venediktov's $+10$°C peak because these are the peaks that are insensitive to DCMU. Arnold and Azzi[20] suggested that the B peak is due to "electron untrapping", and calculated the activation energy to be about $-0{\cdot}42$ eV (9·7 kcal/mole). Our value of activation energy is in qualitative agreement with it but our data show a more complicated situation (Fig. 12). In both Arnold and Azzi's and our pictures, TDLE occurs in pigment system II, although the explanations for TDLE are different in the two cases.

It is difficult to compare our component(s) with the 5 components described by Shuvalov and Litvin[37]; only their component IV appears to have some common features with the DLE observed here: The duration of component IV is in seconds ($\tau = 1{\cdot}59$ sec), and the thermoluminescence maximum is in the temperature range of

20°C. However, it is *intensified* under the action of DCMU, is insensitive to O_2, an energy trap of 0·9 eV (= 21 kcal/mole) is assumed to be responsible for it, and it is assumed to be filled from the singlet level of Chl during excitation of pigment system I. It remains to be seen, however, whether pigment system I or II is involved. We have assumed—as noted above—that system II is involved in our component(s) in agreement with Arnold and Azzi, and with the earlier data that suggest that most of DLE originates in System II. Our component is not due to component *V* ($\tau \simeq 10$–15 sec) of Shuvalov and Litvin[37] because the latter is completely suppressed by DCMU. The components I ($\tau = 5 \cdot 10^{-3}$ sec) and III ($\tau = 1 \cdot 7$ sec) of Shuvalov and Litvin[37] are suggested to be due to Chl of System II involving directly (I) or indirectly (III) triplet levels of chlorophyll in agreement with other recent suggestions for DLE [9, 14, 38]. However, our component does not correspond to these components I and III as the duration of "I" is too short (msec), and "III" is severely depressed by DCMU. Lastly, the component II ($\tau \simeq 0 \cdot 1$ sec)—that may be equivalent to the Z peak of Arnold and Azzi[20]—is excited only by 400–500 nm light[20, 37], is present in plants heated to 100°C for 5 min[20], is responsible for a thermoluminescence peak at −160°C, and has an emission peak at 740 nm[37]. We are certainly not looking at this peak as we can observe DLE and TDLE with red light (unpublished data), and heating to 45°C for 3 or more min destroys all DLE in our experiments. Thus, further work is needed to correlate DLE observed here with the various components reported by Shuvalov and Litvin[37].

[Finally, the reader is referred to two recent papers on delayed light emission that came to our attention during the preparation of this manuscript; the first one by Ruby [43] deals with the preillumination effects on DLE in *Chlorella*, and the second one by Björn[44] deals with the effects of various chemicals on DLE in min region. Neither of these papers contains information similar to that presented here].

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