# DELAYED LIGHT EMISSION IN DCMU-TREATED CHLORELLA: TEMPERATURE EFFECTS

P. JURSINIC AND GOVINDJEE

## Introduction

   MAR & GOVINDJEE (1971) observed that preilluminated algae and isola-
ted spinach chloroplasts could be stimulated to give renewed delayed
light emission (TDLE) by a quick temperature jump of about 15°C. We
felt that the most promising approach for gaining further understanding
of the TDLE phenomenon was in finding out how delayed light emission
(DLE), corrected for changes in the quantum yield ($\Phi$) of fluorescence
(LAVOREL 1969) was affected by temperature. LAVOREL (1969) has measured
the decay of the quantity J (= DLE/$\Phi$) 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 (1967) but only at one tempera-
ture. We report here measurements of the decay of DLE and of fluores-
cence yield in the time range of 1 to 10 sec at different temperatures
from 0 to 50°C. From the decay-rate constants of J, one is able to ex-
plain not only the magnitude of the TDLE peak, but also its decay rate.
Using the decay data and a kinetic analysis we are able to predict and
explain a number of properties of TDLE (JURSINIC & GOVINDJEE in press).
(For references to earlier litterature on delayed light emission and
thermoluminescence see JURSINIC & GOVINDJEE (1972), STACY et al. (1971).)
Details of our techniques, materials and methods, as well as most of the
experimental data presented here, are described by JURSINIC & GOVINDJEE
(1972).

## Temperature-Jump Delayed Light

   Figure 1 shows the renewed delayed light in DCMU-treated *Chlorella*
when a temperature jump of 8°C (from 2° to 10°C) was given. TDLE is
much more clearly observed in this experiment than in our preliminary
work (MAR & GOVINDJEE 1971) when a jump of 12°C (from 26° to 38°C) was
given. TDLE depends not only on the magnitude of the jump but also on
the initial and final temperatures. For example, a jump of 8°C (from
12°C to 20°C) hardly gives any TDLE (Fig. 2). Table I summarizes our
data at various initial and final temperatures. A comparison of the
ratios of the slopes of the $J^{-1/2}$ versus time curves at the initial
(Ti) and final (Tf) temperatures with the ratios of DLE shows that
they are approximately equal for different temperature jumps. We also
found that the ratio of DLE at the final to that at the initial temp-
erature was independent of time. For example, a temperature jump of
2° to 10°C gave a ratio of 1.7 no matter what time after illumination
the temperature jump was made. Furthermore, the rate of DLE decay after
the temperature jump was characteristic of the sample's final tempera-
ture.
   TDLE with DCMU treated cells were several fold smaller than with
untreated cells. For example, a temperature jump of 8°C (from 2° to
10°C) gave a value of 1.75 for DLE at Tf/DLE at Ti in DCMU-treated
cells in contrast to a value of 3.3 in untreated cells.
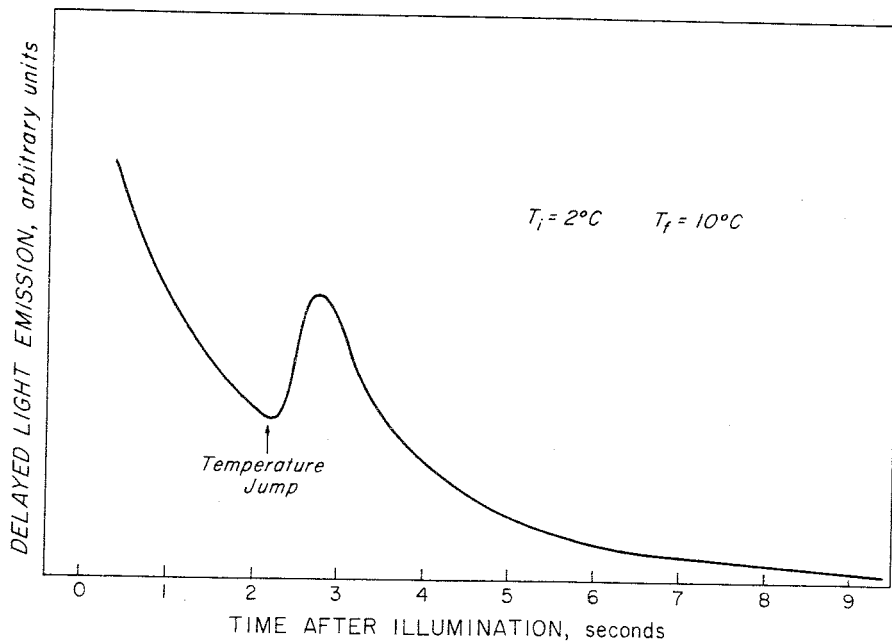
Fig. 1: Delayed Light Emission in DCMU-treated *Chlorella*; a temperature jump from 2° to 10°C was given at about 3 sec after the illumination ($2 \times 10^4$ ergs cm$^{-2}$ sec$^{-1}$ blue light) ended.
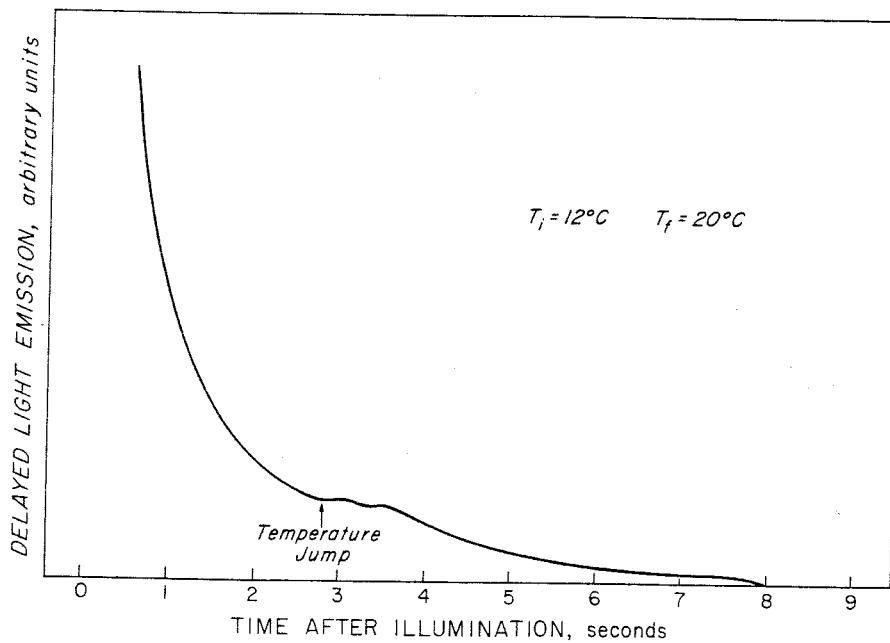


Fig. 2: Delayed Light Emission in DCMU-treated *Chlorella*; a temperature jump from 12° to 20°C was given at about 3 sec after the cessation of illumination ($2 \times 10^4$ ergs cm$^{-2}$ sec$^{-1}$ blue light).

Table I. Temperature-Jump Induced Delayed Light Emission.

These 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 $J^{-1/2}$ versus time curves at temperature $T_f$ and $T_i$ respectively.

| $T_i$ | $T_f$ | DLE at $T_i$ | DLE at $T_f$ | Ratio: $\dfrac{\text{DLE }(T_f)}{\text{DLE }(T_i)}$ | $\dfrac{\text{Slope }(T_f)}{\text{Slope }(T_i)}$ | $\dfrac{\text{Ratio}}{T}$ | Activation energy* |
|---|---|---|---|---|---|---|---|
| 2°C | 5°C | 2.5 | 3.5 | 1.40 | 1.30 | .467/°C | 13.4 |
| 2°C | 10°C | 2.5 | 4.3 | 1.72 | 1.73 | .215/°C | 10.6 |
| 2°C | 15°C | 2.5 | 5.5 | 2.20 | 1.73 | .169/°C | 6.8 |
| 2°C | 20°C | 2.5 | 7.5 | 3.00 | 1.82 | .167/°C | 5.3 |
| 12°C | 20°C | 2.3 | 2.4 | 1.04 | 1.05 | .130/°C | 1.0 |
| 24°C | 30°C | 2.5 | 3.0 | 1.20 | 1.20 | .200/°C | 5.4 |
| 24°C | 35°C | 2.5 | 4.5 | 1.80 | 2.00 | .164/°C | 7.8 |

* Calculated as $EA = \log \dfrac{k'}{k''} \dfrac{(2.303 \times R)\ T_1 \cdot T_2}{T_2 - T_1}$ , where $k'$ and $k''$ were read off from figures, R was 1.987 cal/mole °K, and T in °K was taken from columns (1) and (2) of the above table.

## Decay of Fluorescence Yield as a Function of Time at Different Temperatures

As discussed by LAVOREL (1969), DLE should be corrected for changes in the quantum yield of fluorescence. We measured the changes in the quantum yield of fluorescence as follows. A sample was placed in the measuring chamber with only the analytic beam on. The intensity of this beam was then adjusted to give a fluorescence signal about 40% of the usual DLE signal; this insured that the intensity was low enough so as no not 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 seconds by both the actinic (saturating) and analytic beams, then a shutter was closed blocking both light sources, and the DLE signal alone was recorded. To measure the DLE plus fluorescence signal the same procedure was followed except, after illumination, only the actinic beam was blocked leaving the analytic beam on. Therefore, the recorded signal was DLE plus fluorescence. The fluorescence signal alone was obtained by taking the difference of the two measured signals. The fluorescence signals, normalized to the value of 1.0 at long times, are shown in Fig. 3. It is clear that the decay of fluorescence yield varies with temperature. These yield changes continue upto 10 secs
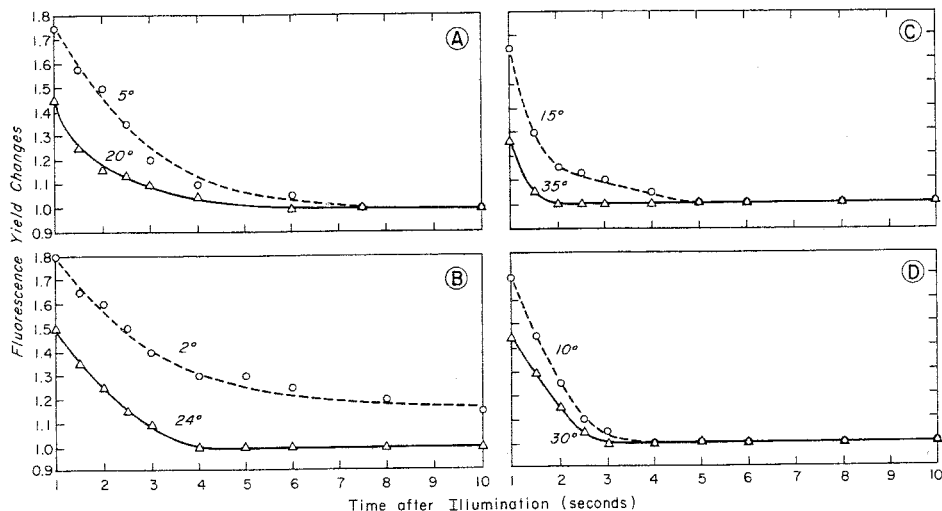
Fig. 3: Fluorescence yield changes in DCMU-treated *Chlorella* after the cessation of illumination (2 x $10^4$ ergs $cm^{-2}$ $sec^{-1}$ blue light). (See text for details).

(or more) after the cessation of illumination at 2°C, upto 8 secs at 5°C, 4 secs at 10°C, 5 secs at 15°C and at 20°C. 4 secs at 24°C, 3 secs ar 30°C, and 2.0 secs at 25°C. At times shorter than mentioned above, corrections for changes in the quantum yield of fluorescence are needed for all DLE data, but at longer times, no such corrections appear to be essential.

## Delayed Light Emission as a Function of Time at Various Temperatures

DLE decay curves at 2° and 35°C, normalized to the arbitrary value of 100 at one second after illumination, are shown in fig. 4. At the higher temperature, the decay rate of DLE is much greater. DLE decay was measured at various temperatures from 2° to 50°C. It was found that samples kept at 45°C for three or more minutes lost their ability for DLE.

We calculated the values for J (= DLE/$\Phi$) by combining the DLE and fluorescence yield ($\Phi$) data. In Figs. 5-7, the quantity $J^{-1/2}$ versus time is plotted for 2°, 5°, 10°, 15°, 30°, and 35°C. Similar curves for 20° and 24° gave intermediate values. 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 involved. At lower temperatures (2°-15°C) the $J^{-1/2}$ versus time graphs are linear. At 20°C, slight deviations from linearity are seen at times above 6 seconds. At 24°C and above, the deviations occur progressively earlier in time. For temperatures
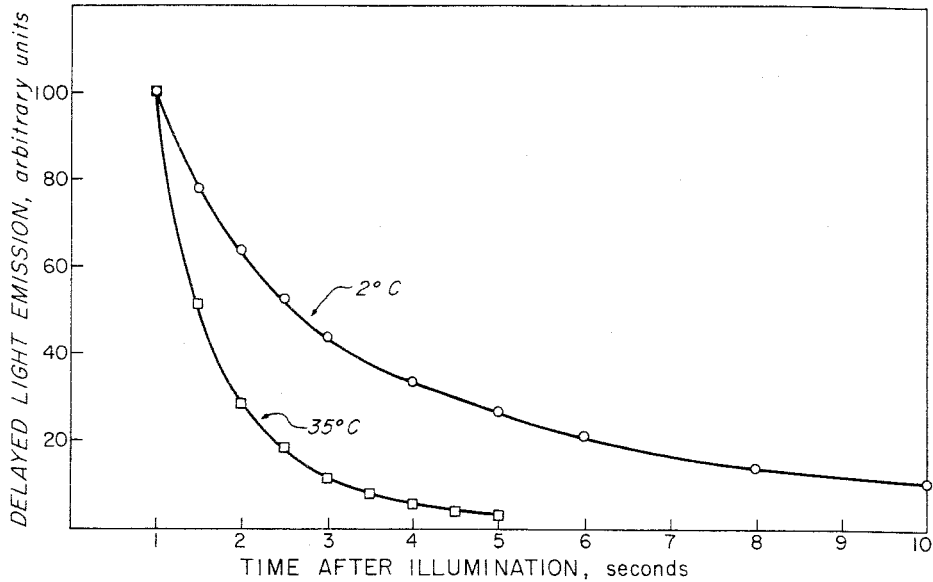
Fig. 4: Delayed Light Emission decay for DCMU-treated *Chlorella* at 2° and 35°C; the rate of decay is sensitive to temperature.
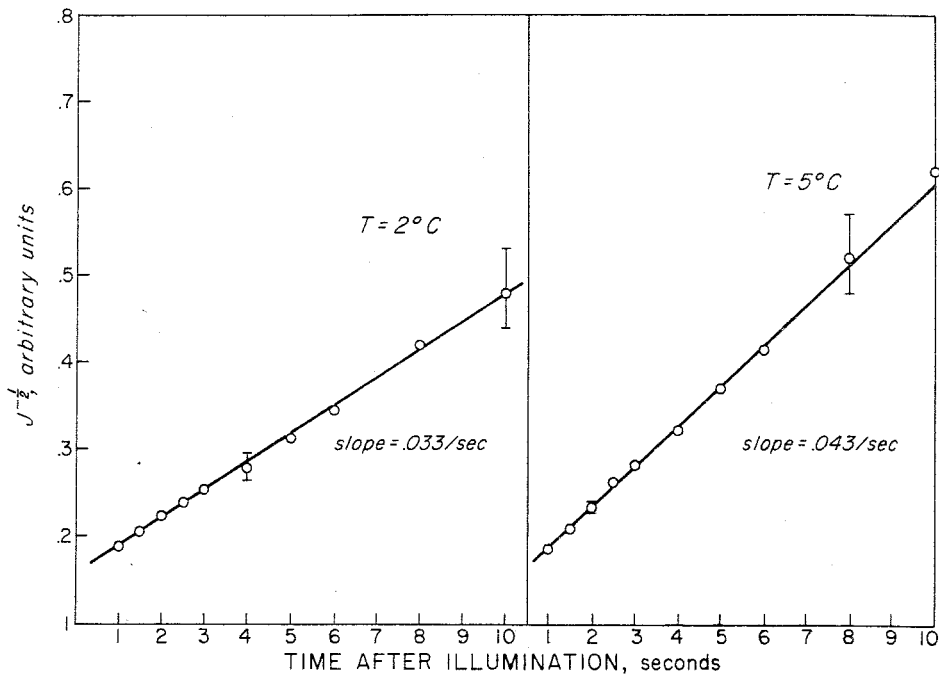


Fig. 5: $J^{-1/2}$ versus time for DCMU-treated *Chlorella* at 2° and 5°C ($J = DLE/\Phi$).

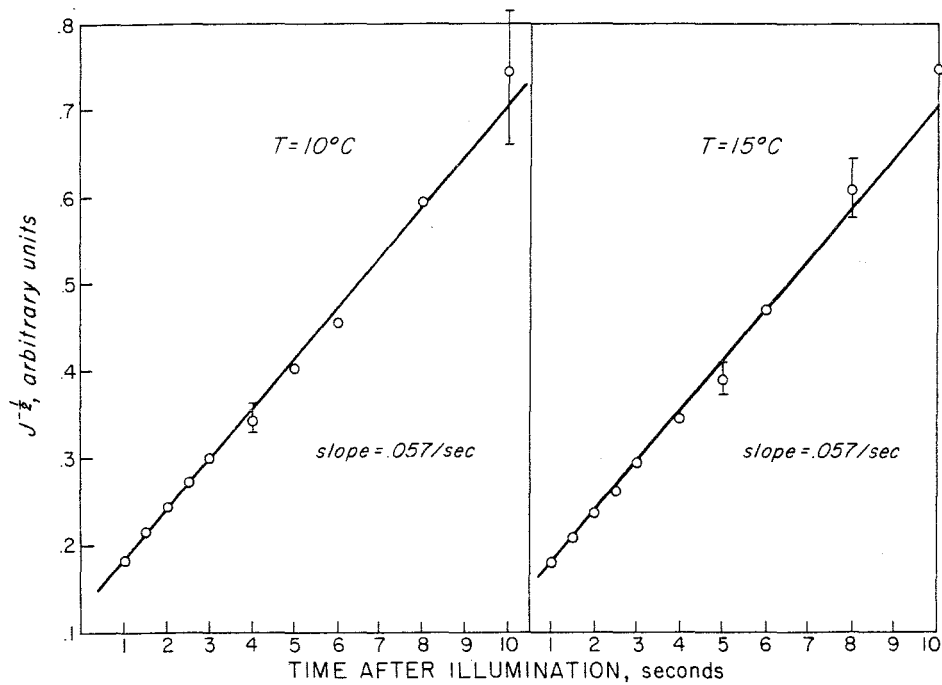Fig. 6: $J^{-1/2}$ versus time for DCMU-treated *Chlorella* at 10° and 15°C.



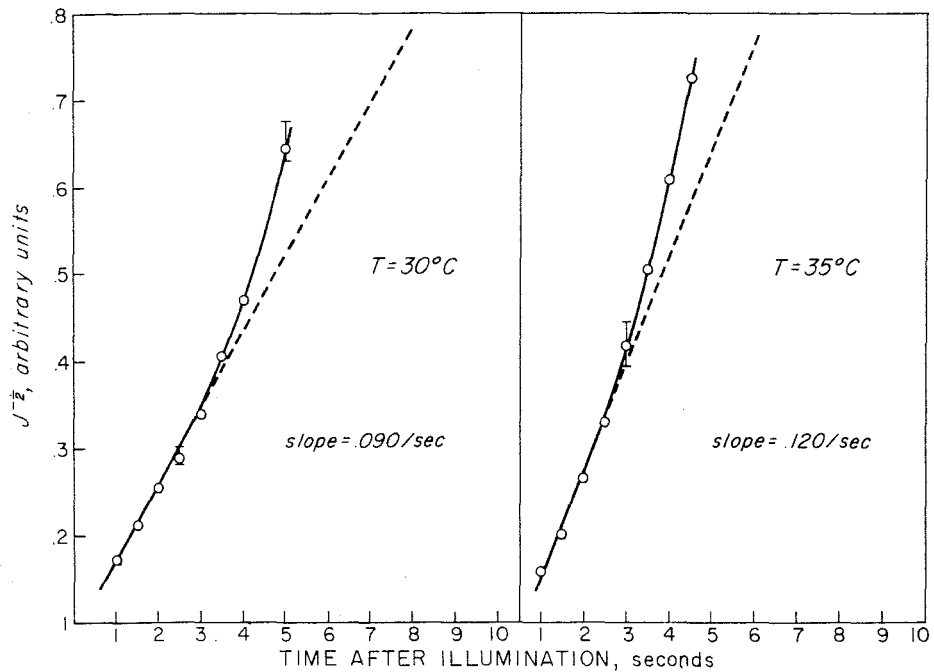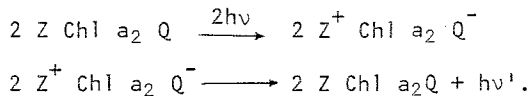Fig. 7: $J^{-1/2}$ versus time for DCMU-treated *Chlorella* at 30° and 35°C.

where deviations occur, the early linearity is projected with a broken line and the slope is indicated. The slopes of $J^{-1/2}$ versus time curves are dependent on temperature. They are: 0.033/sec at 2°, 0.043/sec at 5°, 0.057 at 10° and 15°, 0.060/sec at 20°, 0.075 at 24°, 0.090 at 30° and 0.120 at 35°C.

The second order relationship of the quantity J with time is clearly established at lower temperatures (2-20°C). Since an exciton for delayed light must originate following two photochemical reactions (see LAVOREL 1969; STACY et al. 1971), this second order relationship is easily understood. For example, we can imagine the following sequence of events:

$$2 \text{ Z Chl } a_2 \text{ Q} \xrightarrow{\;2h\nu\;} 2 \text{ Z}^+ \text{ Chl } a_2 \text{ Q}^-$$

$$2 \text{ Z}^+ \text{ Chl } a_2 \text{ Q}^- \longrightarrow 2 \text{ Z Chl } a_2\text{Q} + h\nu'.$$

## Arrhenius Plot

Taking the slopes from all the $J^{-1/2}$ versus time curves, an Arrhenius plot of the back reaction constant in *Chlorella* was made (Fig. 8). The slope of this plot ($\ell n$ K versus 1/T) represents an activation barrier for the back reaction. Previously reported values were 14 Kcal/mole (CLAYTON 1967) and 10 Kcal/mole (SWEETSER et al. 1961). The section of zero slope in the Arrhenius plot (Fig. 8), which occurs in the physiological temperature range of *Chlorella* is a new finding. In CLAYTON's work (1967) only two temperatures were used, and therefore a non-linear plot could not be detected. SWEETSER et al. (1961) did not emphasize one point in their data which deviated from linearity.

The region of the zero slope may be significant in relation to the idea of a possible confirmational change that might affect the back reaction of $Z^+$ with $Q^-$. The deviations from the linearity of the $J^{-1/2}$ curves begin at the same temperature, between 10°C and 15°C, as the zero slope section of the Arrhenius plot. Also the activation barrier for the back reaction appears to be slightly different in the temperature regions above and below the zero slope section of the Arrhenius plot. One may speculate: 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 conformation states exist.

## DLE in Untreated (Normal) Chlorella

Figure 9 shows the delayed light emission in *Chlorella* (with and without DCMU) after illumination with an intense blue light ($2 \times 10^4$ ergs $cm^{-2}$ $sec^{-1}$). (The two curves were arbitrarily adjusted to a value of 50 at 1 sec.). The decay of DLE in untreated *Chlorella* is clearly slower than in DCMU-treated *Chlorella*. (However, recent experiments in our laboratory show opposite results in isolated chloroplasts.) Also, in the 1-10 sec time range, there was more DLE in the untreated than in treated *Chlorella* (data not shown). Furthermore, the decay of untreated cells could not be described by a second-order kinetic analysis; it was complex. 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 (other
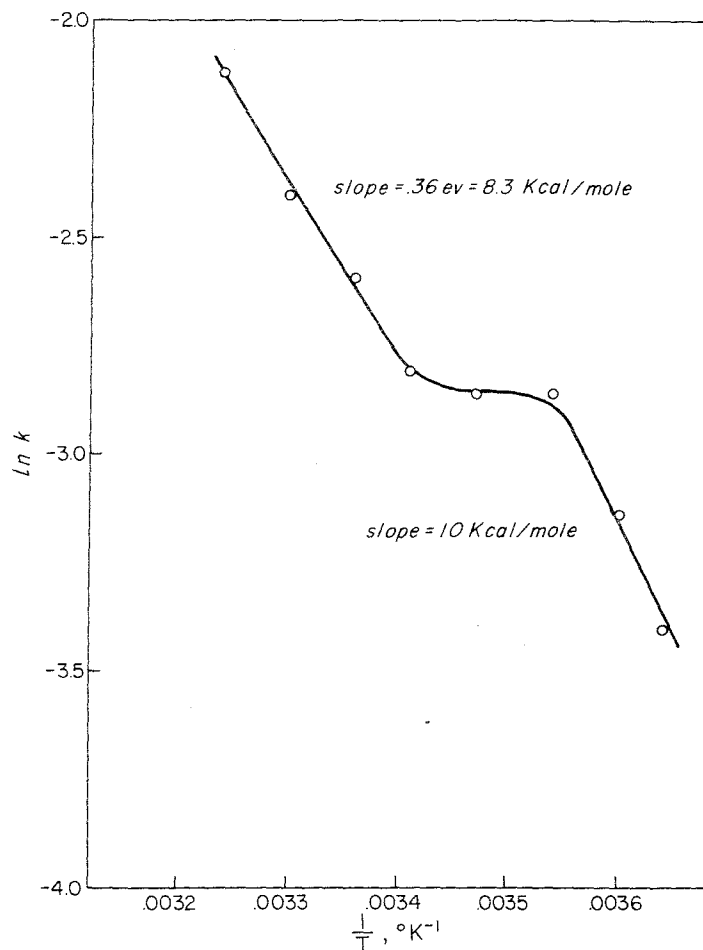
Fig. 8: The Arrhenius plot (ℓn K versus 1/T) for DCMU-treated *Chlorella*; the section of zero slope occurs in the physiological temperature range of 10° to 20°C. The slopes of the curves are 8.3 Kcal/mole and 10 Kcal/mole for higher (20°-35°C) and lower (2°-10°C) temperatures respectively.

than Q⁻) which is further down the electron transport chain than the DCMU block. To test this idea we made an experiment using cells treated with $10^{-5}$ M antimycin $a$ which is believed to cause a block further down the electron chain than DCMU. It was found that these samples had slightly faster DLE decay and smaller TDLE ratios compared to DCMU treated samples. It seems that this additional back reaction entity is further down the electron transport chain beyond the sites antimycin $a$ blocks. Further experiments of this type are needed to show the nature of the proposed entity.
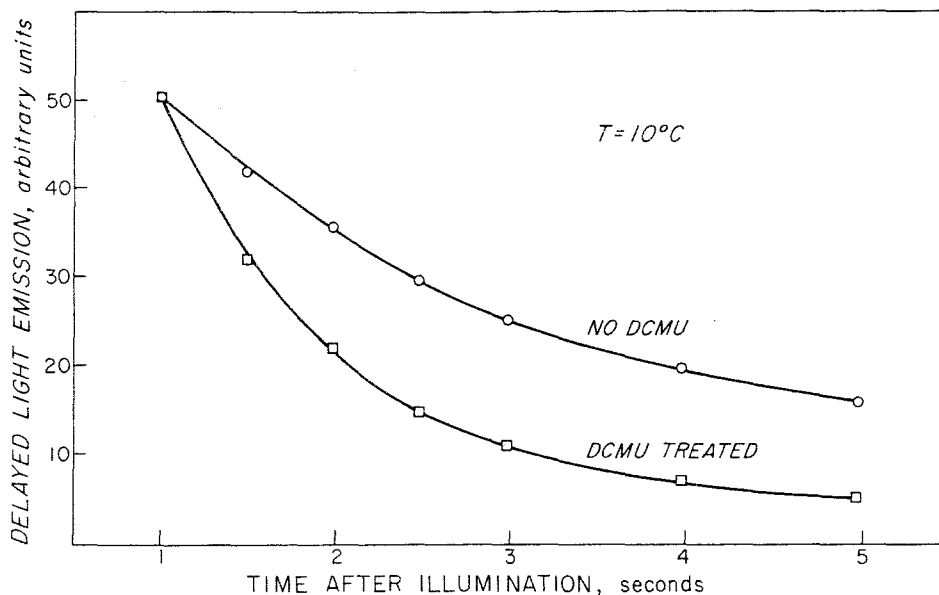
Fig. 9: Delayed Light Emission for *Chlorella* with and without 5 x 10$^{-6}$ M DCMU. The intensities were normalized to the arbitrary value of 50 at 1 sec.

## Summary

Thermoluminescence or temperature-jump induced delayed light emission (TDLE) in DCMU-treated *Chlorella* is described here. It is found that not only the magnitude of the temperature jump ($\Delta T$), but the initial and the final temperatures of the sample are important. For example, a temperature jump of 8°C from 2 to 10°C, than from 12 to 20°C, gives 20 times (or more) TDLE.

From the measurements of both DLE and chlorophyll fluorescence yield ($\Phi$) in DCMU-treated *Chlorella*, the relationship of the quantity $J^{-1/2}$ where J = DLE/$\Phi$) with time (1 to 10 sec after illumination), at various temperatures from 0° to 50°C, is established. The second-order relationship is confirmed at lower temperatures (2 to 15°C).

The Arrhenius plot ($\ell$n K versus 1/T), made from the calculated rate constants, gives a slope of 8.3 Kcal/mole (0 to 10°C), then zero (10 to 25°C), and finally 10 Kcal/mole (25-45°C). The occurrence of a zero slope may indicate the possibility of conformational changes affecting the back reaction of $Z^+$ (Z being the primary donor of electrons of system II) with $Q^-$ (Q being the primary acceptor of electrons of system II) responsible for delayed light.

On the basis of the action of DCMU and of antimycin $a$, it is suggested that in untreated cells, DLE may arise not only from a back reaction of $Z^+$ with $Q^-$, but also with another reducing entity, perhaps, beyond the sites of action of the chemicals used.

## Acknowledgement

## Abbreviation

DCMU = 3-(3,4-dichlorophenyl)-1,1 dimethylurea.

## Bibliography

CLAYTON, R.K. - 1967 - *Arch. für Mikrobiol.*. 59, 49.
*JURSINIC, P. & GOVINDJEE - 1972 - *Photochem. Photobiol.*, in press.
LAVOREL, J. - 1969 - *Progr. Photosynth. Res.*, 2, 883.
MAR, T. & GOVINDJEE - 1971 - *Biochim. Biophys. Acta*, 226, 200.
STACY, W.T., T. MAR, C.E. SWENBERG & GOVINDJEE - 1971 - *Photochem. Photobiol.*, 14, 197.
SWEETSER, P.B., C.W. TODD & R.T. HERSH - 1961 - *Biochim. Biophys. Acta*, 51, 509.

*See this paper for references to literature.

Authors' address:

Department of Botany,
University of Illinois,
Urbana, Illinois 61801,
U.S.A.