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July 1958

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DELAYED LIGHT EMISSION IN GREEN PLANT MATERIALS: TEMPERATURE-DEPENDENCE AND QUANTUM YIELD*

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INTRODUCTION

The discovery of the delayed light emission of plant materials by Strehler and Arnold in 1951[1] has stimulated a good deal of interest in this rather remarkable property. The emitted light has been shown to be due to an electronic transition between the first excited singlet state of chlorophyll and the ground state.[2,3] At room temperature, a luminescence is observable from about 0.01 second[4] to several minutes[5] after excitation. Thus, the electronic transition cannot be rate-determining and the process represents neither normal fluorescence nor normal phosphorescence. Indeed, there is some evidence[4,6] that the decay curve of the luminescence is the resultant of more than one rate-limiting process. Strehler and co-workers[4,7]

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* The work described in this paper was sponsored in part by the United States Atomic Energy Commission and in part by the Department of Chemistry, University of California, Berkeley, California.

have been able to demonstrate the existence of many relationships between delayed light emission and photosynthesis and thus have been led to interpret the luminescence phenomena as a consequence of the reversibility of some of the enzymatic photosynthetic reactions. However, Tollin and Calvin[6] have shown that the faster decaying components of the delayed light are present to as low a temperature as -100°C, suggesting that the early processes following light-absorption are non-enzymatic in nature. These latter observations, in conjunction with several other types of experimental and theoretical information,[8-15] have suggested an interpretation of the physical processes leading to delayed light emission, and, by analogy, to photosynthesis, in terms of semiconductor theory.[16,17,18]

The earlier investigations in this laboratory[3,6] have been limited to the study of the light emitted approximately 0.1 second after excitation by a flash discharge. The recent reports of luminescences at still shorter times after excitation[4,19] have prompted the construction of a device capable of continuously observing the light emission of a sample of plant material from 0.0015 second to about 30 seconds after the onset of flash excitation. The present work describes a series of experiments carried out with this apparatus.

MATERIALS AND METHODS

The chloroplast material was prepared as outlined previously.[10] Chlorella and Scenedesmus were grown in continuous culture in our laboratory and samples were prepared for the luminescence measurements by centrifugation of a suspension of the algae to obtain a relatively thick paste. In general, measurements were begun within 10 minutes of harvesting.

A block diagram of the apparatus used in the experiments is shown in

Figure 1. Conceptually, it is quite similar to the apparatus described previously[6] with the exception that the mechanical shuttering system is replaced by an electronic device. This latter involves a "gating" of the photomultiplier (Dumont K-1292) during the exciting light flash through the application of a voltage pulse to the first two dynodes and the shield which results in a reversal of their polarity with respect to the photocathode. This serves to prevent most of the photoelectrons liberated at the cathode from being multiplied in the tube. The duration of this gating pulse is continuously variable from a few microseconds to about 5 milliseconds and thus may be matched to the duration of the flash. The gate is also used to start the sweep of a Tetronix 514-D cathode-ray oscilloscope. A delay of about 500 microseconds is introduced between the beginning of the gating process and the "triggering" of the flash to allow the gate to become fully operative before the light commences. Thus, this arrangement permits the onset of the observation of the luminescence to begin in a time limited only by the duration of the flash excitation. The flashtube used in the experiments is the General Electric FT-230 which has the advantage of combining a relatively short duration (about 1.5 milliseconds under the conditions of luminescence measurement) and a small source size (spherical with a diameter of about 4 mm.) with a reasonably high light intensity in a suitable wavelength range. The flashtube is operated at 2000 volts with a 32 microfarad load capacitor. The triggering system is standard. All electrical leads were kept as short as possible to achieve optimum pulse-transmission characteristics.

In summary, then, the sequence of events in a measurement is as follows. A push button activates the photomultiplier gate and simultaneously triggers the oscilloscope sweep. Five hundred microseconds later the flash is fired

and 1.5 milliseconds after this occurs the gate turns off and the photomultiplier is then ready to observe light from the sample.

The exciting light is focused onto the sample through a filter system consisting of a Corning No. 5113, a Corning No. 4303 and a Corning special infrared filter. This combination passes a band of light of wavelengths between 3700 Å and 4700 Å having a maximum at about 4150 Å. A Corning No. 2030 and another Corning special infrared filter is placed between the sample and the photomultiplier. This latter filter system allows only those wavelengths between 6500 Å and about 9500 Å to reach the photomultiplier. Such an arrangement of filters minimizes the light from the flash discharge which is incident upon the photomultiplier during excitation, although it by no means completely eliminates the scattering problem. However, no transient phenomena or saturation effects in the photomultiplier response are observed. The geometrical arrangement of exciting source, sample and photomultiplier is essentially the same as that reported previously.[6]

The photomultiplier anode is supplied with a 2 megohm load resistor and the voltage developed across this resistance is fed directly into a Sanborn stabilized D.C. preamplifier (gain = 1000). The preamplifier in turn feeds the oscilloscope, a Sanborn Model 151 recorder and a Leeds and Northrup "Speedomax" recorder. The photomultiplier is normally operated at 1300 volts.

For the measurement of the quantum yield of the luminescence the photomultiplier was calibrated using a National Bureau of Standards lamp and a bolometer. The photomultiplier then was used to measure both the light incident on the sample from the flash and the light emitted from the sample. It was assumed that all of the light reaching the sample was absorbed. Such

an assumption is probably quite satisfactory inasmuch as the samples used were almost black in appearance. Appropriate corrections were applied for the filters, for the geometry of the system and for self-absorption in the sample. The values obtained for the quantum yield of the light emitted between 0.0015 second and 30 seconds after excitation are probably accurate only to within a factor of 2 or 3. Furthermore, the yield is too low in terms of total light emitted inasmuch as we are unable to detect emissions of duration longer than 30 seconds or shorter than 0.0015 second. It is unlikely, however, that inclusion of this energy would raise the quantum yield value by more than an order of magnitude inasmuch as extremely sensitive quantum counting devices are needed to observe the longer term emissions[5] and the present results indicate a steadily decreasing quantum yield as one goes to shorter times after excitation (see below).

RESULTS

Some typical decay curve data obtained from Chlorella at 21°C are shown in Figure 2. All of the results presented in this section have been obtained from data of this type by an evaluation of the luminescence intensity at various times after excitation. It is apparent that the signal-to-noise ratio is generally quite good and thus fairly accurate decay curves may be calculated.

The results of the quantum yield measurements for whole spinach chloroplasts, Scenedesmus and Chlorella are shown in Table 1. The total light emission from different samples of the same organism shows some variation in intensity, presumably due to differences in the physiological state of the material. However, this rarely amounts to more than a factor of two. Strehler and Arnold[1] have reported a figure of 10^{-6} for the ratio of the

quanta emitted by Chlorella suspensions near the beginning of the observed decay curve to the quanta absorbed. The quantum yields reported in the present work are consistent with this number.

A comparison of the room temperature emissions of spinach chloroplasts, Scenedesmus and Chlorella is given in Table 2. The choice of time ranges is somewhat arbitrary. It is seen that the algae give a much higher absolute yield of luminescence than do the chloroplasts (see also Table 1) and that this higher yield is mainly due to a disproportionately greater quantity of slowly decaying light obtained from the former as compared with the latter. The decay curves for Chlorella and for Scenedesmus are, in fact, quite similar, essentially differing only by a scale factor.

In Figures 3, 4 and 5 and Tables 3 and 4 are given the results of experiments in which the luminescence of spinach chloroplasts and of Chlorella are studied as a function of the temperature. It is apparent that the temperature-dependence of the light emission in both types of material is quite complex. Furthermore, at no temperature can the decay curve be represented by a simple kinetic expression (either unimolecular or bimolecular). The most striking change upon cooling in both materials is the fairly rapid changeover from a decay curve in which most of the light is emitted in the longer times (greater than 0.1 second) to a decay curve in which essentially all of the emission decays rapidly (see Tables 3 and 4, Column 4). Another change of interest is the general (although not quite monotonic) increase in the absolute intensity present in the faster components as one cools to intermediate temperatures, followed by a decrease in this intensity as one cools still further (see Figure 5). This effect is particularly large in spinach chloroplasts and results in an almost threefold increase in the

total integrated intensity obtained at -36°C as compared with that obtained at 21°C (see Table 3, Column 5). In Chlorella, it appears that what would otherwise be a peak in total integrated intensity at intermediate temperatures is masked by the rapid decrease in the intensity of the slowly decaying light (which represents a much larger percentage of the total intensity in Chlorella than it does in chloroplasts). This results in a monotonic decrease in the total integrated intensity with the suggestion of a possible maximum appearing as a leveling-off at intermediate temperatures (see Table 4, Column 5). It is of interest that the maximum of the fastest component comes at a higher temperature in Chlorella than it does in chloroplasts.

Experiments involving the use of various filters between the sample and the photomultiplier demonstrate that the emission in the first few milliseconds after excitation has the same wavelength distribution as do the slower components[3] (i.e., emission is occurring from the first excited singlet state of chlorophyll) at both 21°C and at -170°C. This is a further indication of the failure of the triplet state of chlorophyll to emit,[3] although it may be involved in the sequence of events leading to luminescence.[18]

In Figure 6 the decay curves for spinach chloroplasts and for Chlorella at 21°C and at -165°C are plotted as log intensity vs. time. The low temperature curves for both materials are quite similar in shape. It is possible to interpret these curves as being the resultant of two unimolecular luminescent processes having half-lives of about 1-2 milliseconds and about 30 milliseconds, respectively. The validity of such an interpretation is, of course, open to some question. However, it is of interest that as one approaches these low temperatures the changes in the luminescence for a given temperature increment become progressively smaller and, in fact, the decay

curve for spinach chloroplasts does not undergo any measurable changes between -128°C and -168°C. This would suggest a corresponding simplification in mechanism.

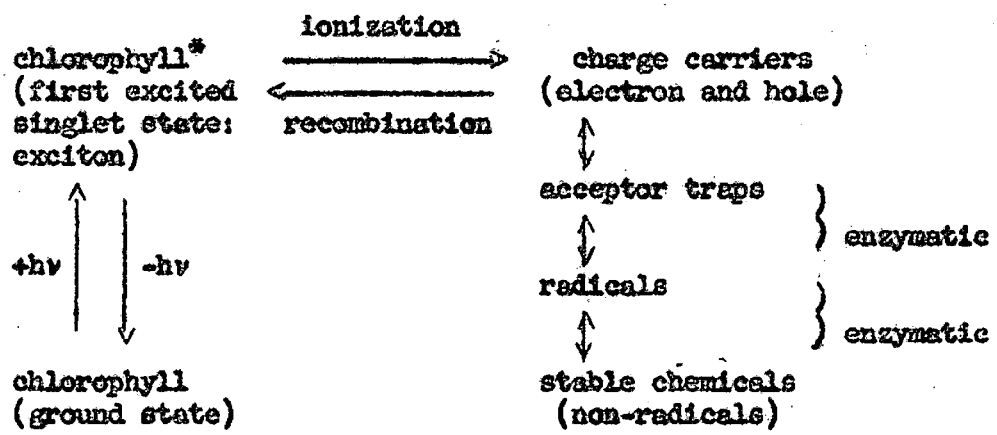
Some interesting effects are obtained if one allows the samples to age on the sample holder in the dark at room temperature. A typical result is shown in Table 5. A marked decrease in the total integrated light intensity is observed after 20 hours of aging with the slower components having decreased to a much greater extent than the faster ones. The material is quite dry and hard at this time. If one rewets the sample with a drop of water one can achieve some reactivation. However, the percent of the original intensity present after wetting is much greater for the faster components than for the slower ones. Even after as much as ten days of aging, the fast decays are still present and some reactivation of the slow decays is possible.

DISCUSSION

It is highly unlikely that a temperature-dependence as complex as that observed in the present experiments for delayed light emission can be a reflection of an underlying mechanistic simplicity. Indeed, the general features of the changes in the luminescence decay curves upon cooling may readily be accounted for qualitatively by postulating a series of either parallel or sequential rate-limiting processes as constituting the basic kinetic pattern of the luminescence phenomenon. Such a complicated sequence of events leading to an electronically excited chlorophyll molecule is consistent with what is known about the photosynthetic pathway and about metabolic processes in general.

In terms of the above picture, one can explain the increase in the intensity of the faster light emissions at intermediate temperatures as being due either to the undetected presence of still faster decay processes of high yield whose rates are decreased upon cooling or to a "freezing-out" of the slower mechanisms with a corresponding increase in the amount of energy being emitted in the faster processes. It is not possible to say which of these points of view is applicable to the present situation. The rationale for the subsequent decrease in intensity upon further cooling is obvious.

The most significant result of the present study is the observation of a substantial luminescence decay at temperatures as low as -170°C. This strongly suggests that the basic mechanisms of the early processes following light absorption do not involve the migration of atomic nuclei and thus are purely physical. This is, of course, quite consistent with the general features of the semiconductor mechanism proposed earlier.[16,17,18] According to this hypothesis, the pathway of the light quantum may be depicted in terms of the following scheme (for simplicity, the triplet state of chlorophyll has not been included in the sequence):



If one grants the validity of separating the -170°C decay into two components, one could identify the faster of these decays with a charge carrier lifetime and the slower decay with the emptying of a shallow trapping level. Both of these processes should be relatively temperature-independent over the range studied. It is striking that the time constant of the slow decay ($\sim$ 0.03 second) is of the same order of magnitude as those observed for: (a) the minimum dark time for photosynthesis by Chlorella in flashing light by Emerson and Arnold,[20] (b) the corresponding dark time for the Hill reaction in Chlorella by Clendenning and Ehrmantraut,[21] (c) the decay time in Chlorella for absorption spectra changes at 5150 Å by Witt[22] and (d) the minimum dark time for oxygen production in the Hill reaction in Scenedesmus in the presence of thioctic acid by Bradley and Calvin.[17] Such a correlation would argue that the rate-limiting step in these processes is physical rather than enzymatic.

The fact that algae yield a much greater light intensity in the slow decays than do chloroplasts is probably a consequence of the partial removal of enzymes and smaller molecules in the preparation procedure. This suggests chemical transformations as the rate-limiting steps of the slower components. An explanation in these terms would also be consistent with the large temperature coefficient of these components as observed in the cooling experiments (Tables 3 and 4), with the aging experiments (Table 5), and with the results of Strehler and co-workers.[4,7]

The extremely low quantum yields observed in the present experiments are in accordance with the interpretation of delayed light emission as an indication of the reversibility of at least part of the photosynthetic pathway. These low values are probably a reflection of the high efficiency with which the absorbed quanta pass over into the chemical processes involved in

photosynthesis. It is interesting to note that the much higher quantum yield of fluorescence[13] ($\sim 10^{-2}$) suggests a considerably lower order of efficiency of quantum conversion for the earliest physical stages.

SUMMARY

A device has been constructed which is capable of recording the decay curve of green plant luminescence from 0.0015 second to approximately 30 seconds after excitation by a flash discharge. Absolute quantum yield measurements of the emitted light give values of the order of 10^{-6} for Chlorella and Scenedesmus and 10^{-7} for spinach chloroplasts. Such low yields are in accord with the interpretation of delayed light emission as a reversal of photosynthesis. The luminescence has been found to exhibit an extremely complex temperature-dependence which is suggestive of a multiprocess mechanism. A substantial luminescence decay is measurable at temperatures as low as -170°C. This is interpreted as demonstrating that the early processes following light absorption are physical rather than enzymatic. Evidence is presented to support the contention that the later stages of emission are of an enzymatic nature. At all of the temperatures investigated, the luminescence originates in the first excited singlet state of chlorophyll.

The authors wish to express their appreciation to Mr. Fred Vogelsberg of the Radiation Laboratory for his invaluable assistance in setting up the apparatus used in this work.

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Table 1

ABSOLUTE QUANTUM YIELDS OF LUMINESCENCE OF VARIOUS GREEN PLANT MATERIALS

Material	Φ (Quanta Emitted/Quanta Absorbed)
Spinach Chloroplasts	4×10^{-7}
Scenedesmus	3×10^{-6}
Chlorella	4×10^{-6}

Table 2

ROOM-TEMPERATURE LUMINESCENCE OF VARIOUS GREEN PLANT MATERIALS

Material	Integrated light intensities in various time ranges after excitation has ceased (normalized to 0.001-0.01 sec. range of spinach chloroplasts)				Total integrated light intensity (normalized to spinach chloroplasts)
	0.001-0.01 sec.	0.01-0.1 sec.	0.1-1.0 sec.	1.0-10.0 sec.	
Spinach Chloroplasts	1.0	1.5	2.6	4.1	1.0
Chlorella	3.2	6.2	24.1	53.5	9.4
Scenedesmus	2.0	4.1	17.3	36.0	6.4

Table 3

TEMPERATURE-DEPENDENCE OF SPINACH CHLOROPLAST LUMINESCENCE

Temp. °C	Time after excitation has ceased (sec)	Integrated light intensities (normalized to 0.001-0.01 range 21°C)	% of total integrated intensity	Total integrated light intensity (normalized to 21°C)
21	0.001-0.01	1.0	10.8%	1.0
	0.01-0.1	1.5	16.3	
	0.1-1.0	2.6	28.3	
	1.0-5.0	4.1	44.6	
11	0.001-0.01	2.3	53.5	0.42
	0.01-0.1	0.84	19.5	
	0.1-1.0	0.66	15.3	
	1.0-5.0	0.50	11.7	
5	0.001-0.01	3.1	40.5	0.80
	0.01-0.1	1.1	14.4	
	0.1-1.0	1.2	15.7	
	1.0-5.0	1.95	25.4	
0	0.001-0.01	3.5	68.4	0.56
	0.01-0.1	1.6	31.3	
	0.1-1.0	0.02	0.3	
	1.0-5.0	0	0	
-4	0.001-0.01	3.8	73.1	0.55
	0.01-0.1	1.4	26.9	
	0.1-1.0	0	0	
	1.0-5.0	0	0	
-18	0.001-0.01	5.9	73.8	0.86
	0.01-0.1	2.1	26.2	
	0.1-1.0	0	0	
	1.0-5.0	0	0	
-36	0.001-0.01	13.1	53.9	2.6
	0.01-0.1	9.1	37.4	
	0.1-1.0	2.1	8.7	
	1.0-5.0	0	0	
-62	0.001-0.01	7.5	49.3	1.7
	0.01-0.1	5.2	34.2	
	0.1-1.0	2.7	16.5	
	1.0-5.0	0	0	
-95	0.001-0.01	3.7	80.8	0.50
	0.01-0.1	0.78	17.0	
	0.1-1.0	0.10	2.2	
	1.0-5.0	0	0	
-128	0.001-0.01	1.7	77.3	0.24
	0.01-0.1	0.5	22.7	
	0.1-1.0	0	0	
	1.0-5.0	0	0	
-168	0.001-0.01	1.7	77.3	0.24
	0.01-0.1	0.5	.7	
	0.1-1.0	0	0	
	1.0-5.0	0	0	

Table 4

TEMPERATURE-DEPENDENCE OF CHLORELLA LUMINESCENCE

Temp. °C	Time after excitation has ceased (sec)	Integrated light intensities (normalized to 0.001-0.01 range 21°C)	% of total integrated intensity	Total integrated light intensity (normalized to 21°C)
21	0.001-0.01	1.0	3.7%	1.0
	0.01-0.1	1.9	7.1	
	0.1-1.0	7.5	27.7	
	1.0-10.0	16.6	61.5	
13	0.001-0.01	1.01	4.6	0.80
	0.01-0.1	1.4	6.3	
	0.1-1.0	5.0	23.0	
	1.0-10.0	14.3	66.1	
5	0.001-0.01	1.7	13.5	0.46
	0.01-0.1	0.9	7.0	
	0.1-1.0	2.5	20.0	
	1.0-10.0	7.4	59.5	
0	0.001-0.01	1.9	28.7	0.24
	0.01-0.1	0.6	9.4	
	0.1-1.0	1.3	19.9	
	1.0-10.0	2.7	42.0	
-5	0.001-0.01	2.0	38.1	0.19
	0.01-0.1	0.65	12.5	
	0.1-1.0	0.9	17.0	
	1.0-10.0	1.7	32.4	
-13	0.001-0.01	2.1	43.3	0.18
	0.01-0.1	0.85	17.3	
	0.1-1.0	0.5	10.3	
	1.0-10.0	1.4	29.1	
-38	0.001-0.01	0.3	40.1	0.03
	0.01-0.1	0.5	59.8	
	0.1-1.0	0	0	
	1.0-10.0	0	0	
-65	0.001-0.01	0.23	65.2	0.013
	0.01-0.1	0.17	34.8	
	0.1-1.0	0	0	
	1.0-10.0	0	0	
-110	0.001-0.01	0.25	59.6	0.015
	0.01-0.1	0.17	40.4	
	0.1-1.0	0	0	
	1.0-10.0	0	0	
-170	0.001-0.01	0.29	55.1	0.020
	0.01-0.1	0.24	44.9	
	0.1-1.0	0	0	
	1.0-10.0	0	0	

Table 5

EFFECT OF AGING AND REWETTING ON ROOM-TEMPERATURE SCINEDESMUS LUMINESCENCE

Material	Integrated light intensities in various time ranges after excitation has ceased (normalized to 0.001-0.01 sec. range of fresh material)				Total integrated light intensity (normalized to fresh material)
	0.001-0.01 sec	0.01-0.1 sec	0.1-1.0 sec	1.0-10.0 sec	
Fresh	1.0	2.05	8.7	18.0	1.0
Aged 20 hours	0.20	0.23	0.41	0.12	0.03
Rewet after aging	0.63	0.95	2.3	2.25	0.21
% of original intensity recovered	63%	46%	26%	12%	21%

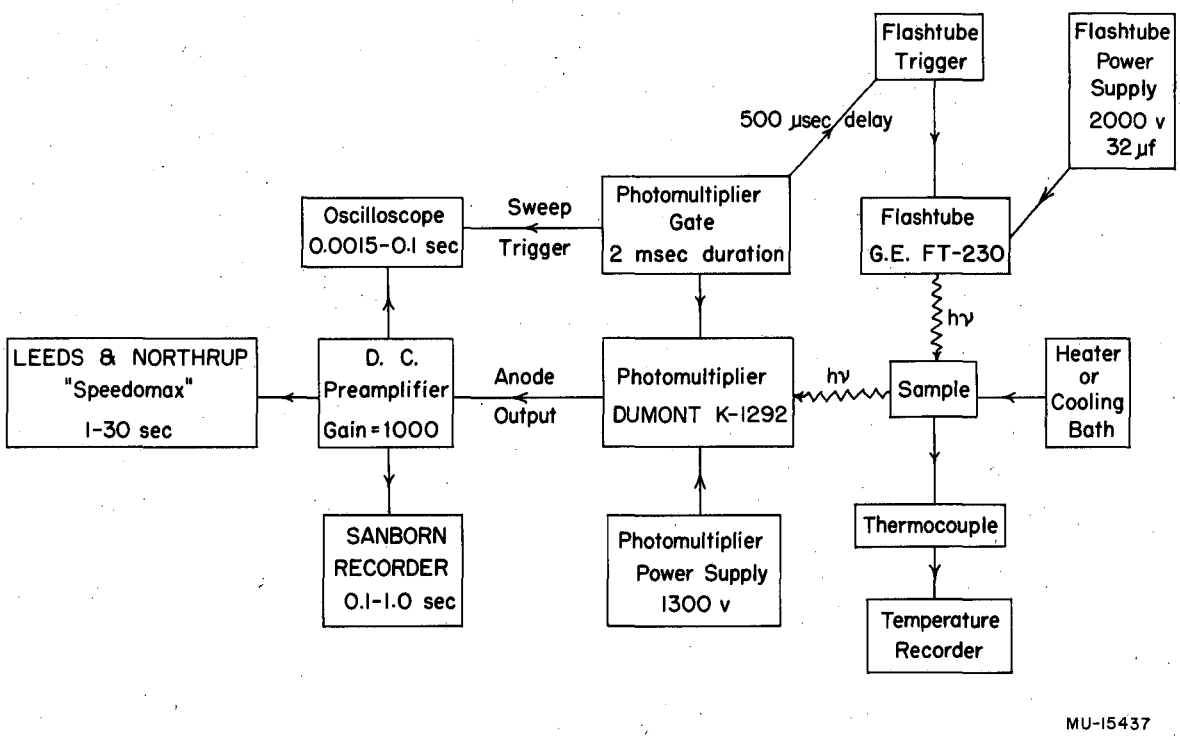


Fig. 1. Block diagram of luminescence apparatus.

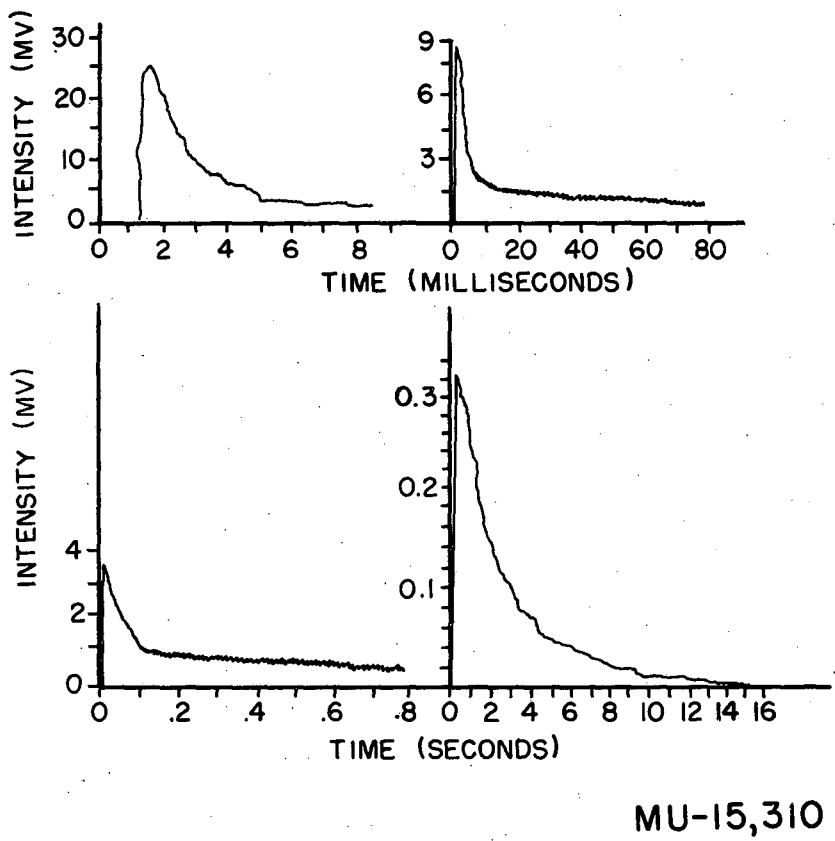


Fig. 2. Tracings of decay curve data obtained from Chlorella at 21°C. The three longer-term curves were obtained with a single flash excitation. The fastest curve (0.0015-0.009 second) was obtained independently.

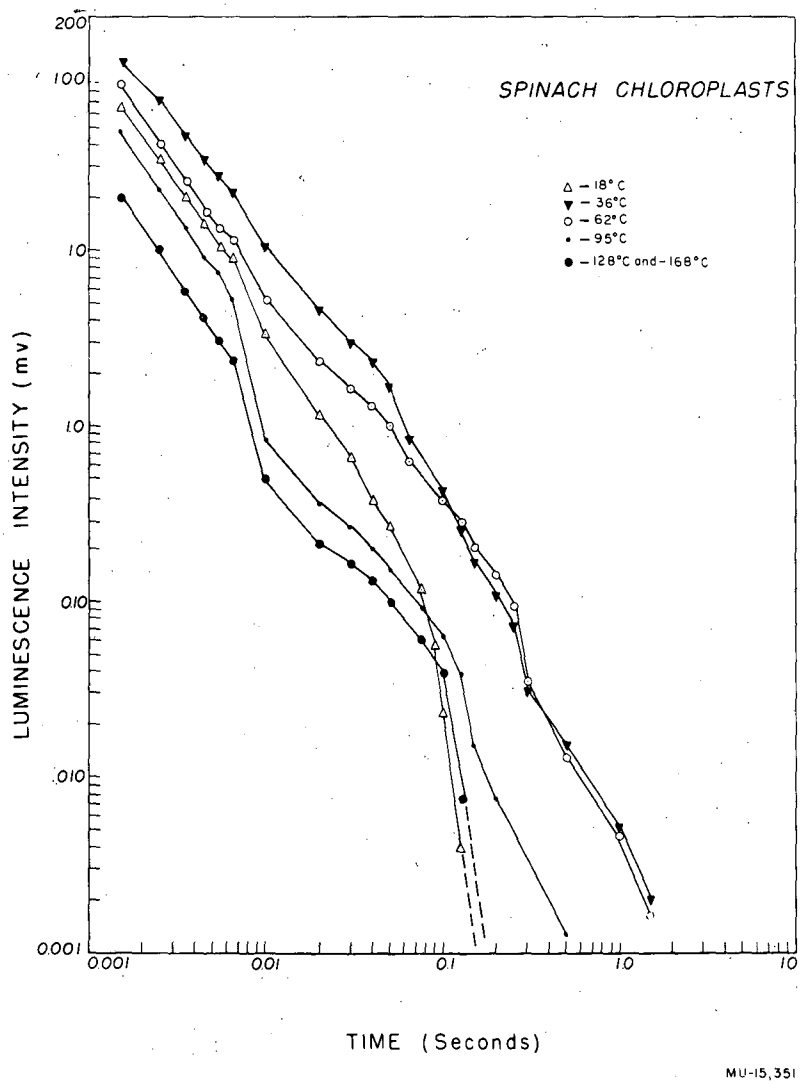
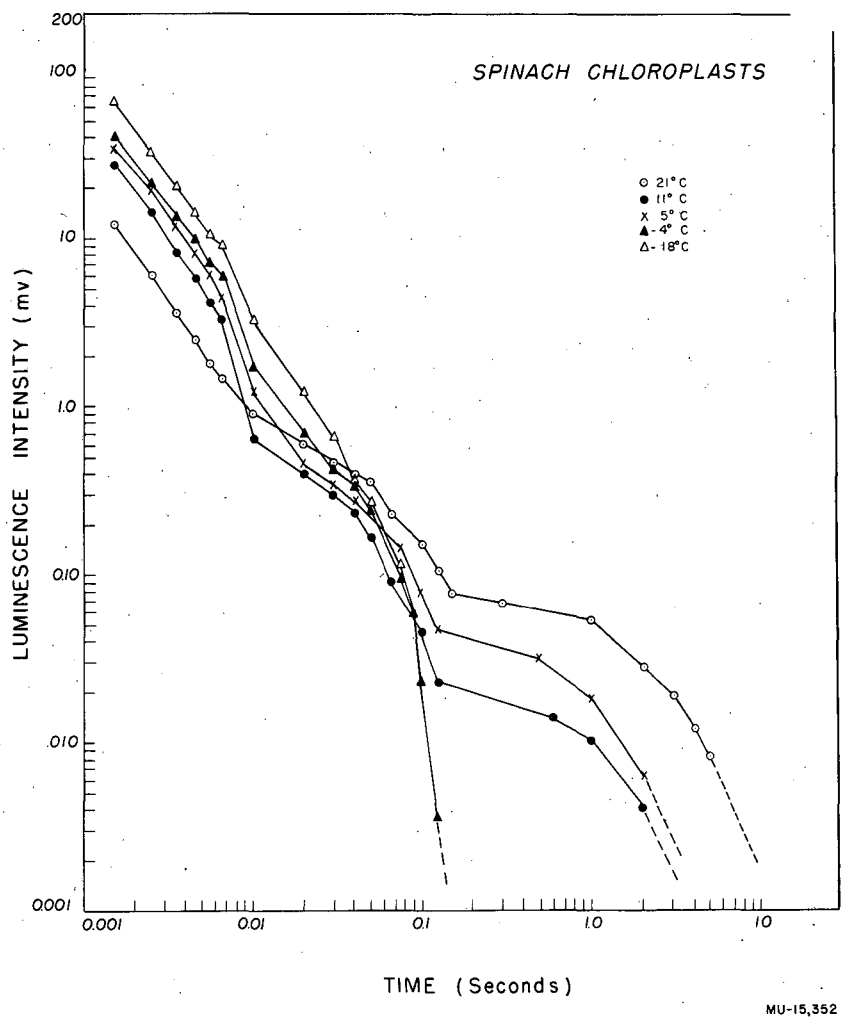


Fig. 3. Temperature-dependence of spinach chloroplast luminescence.

Fig. 3 continued



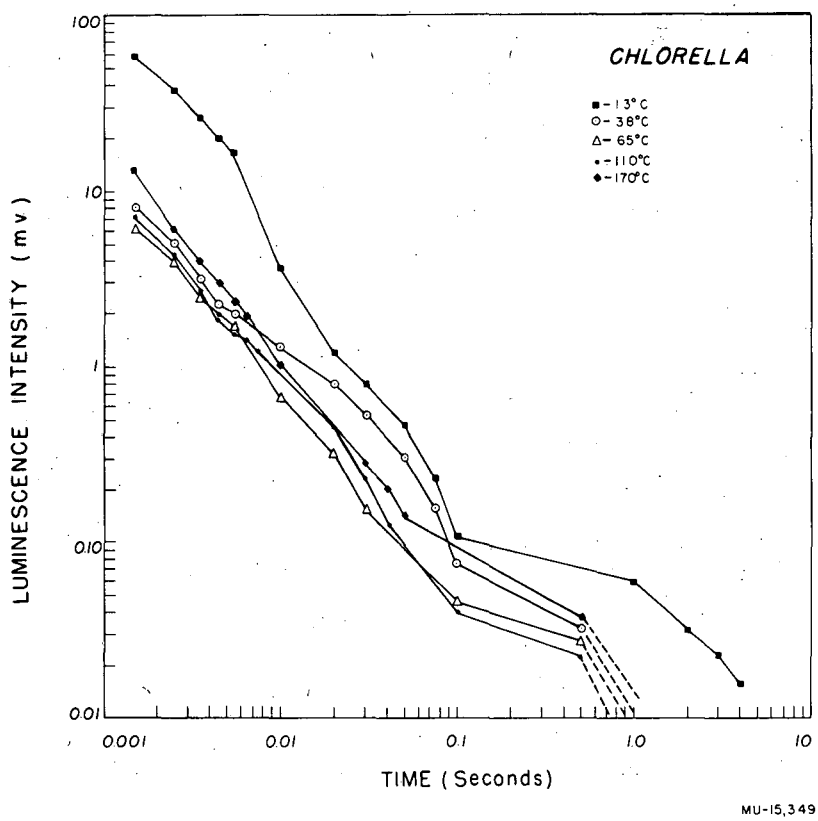
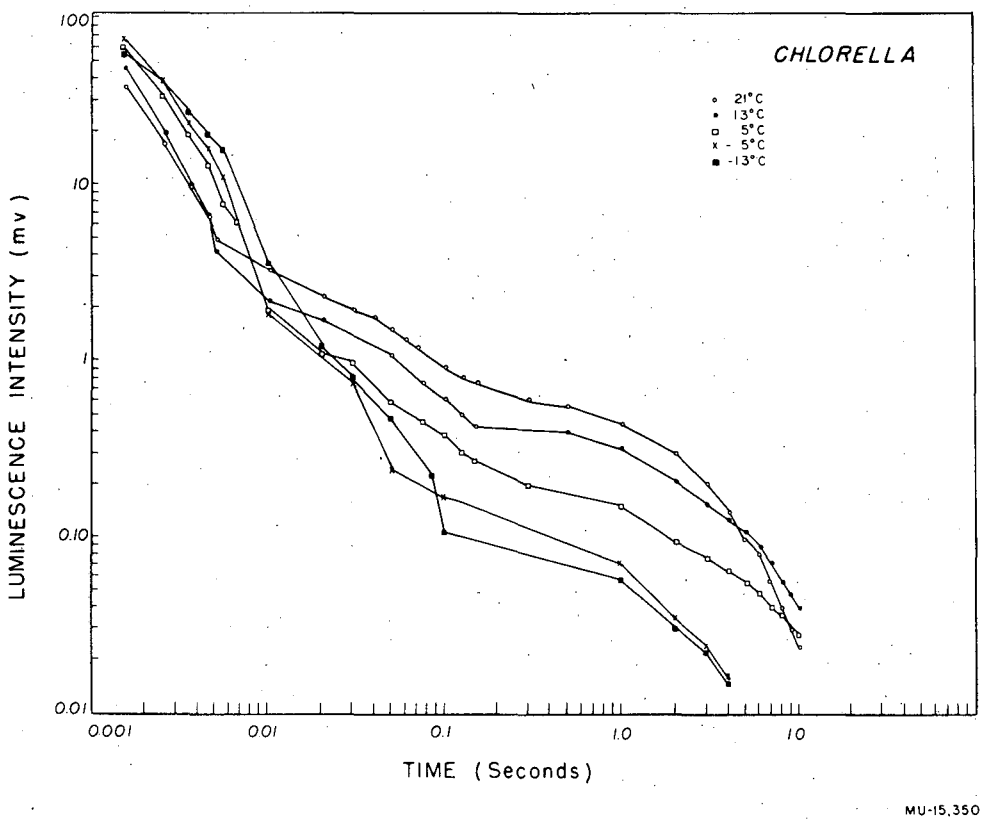


Fig. 4. Temperature-dependence of Chlorella luminescence.

Fig. 4 continued



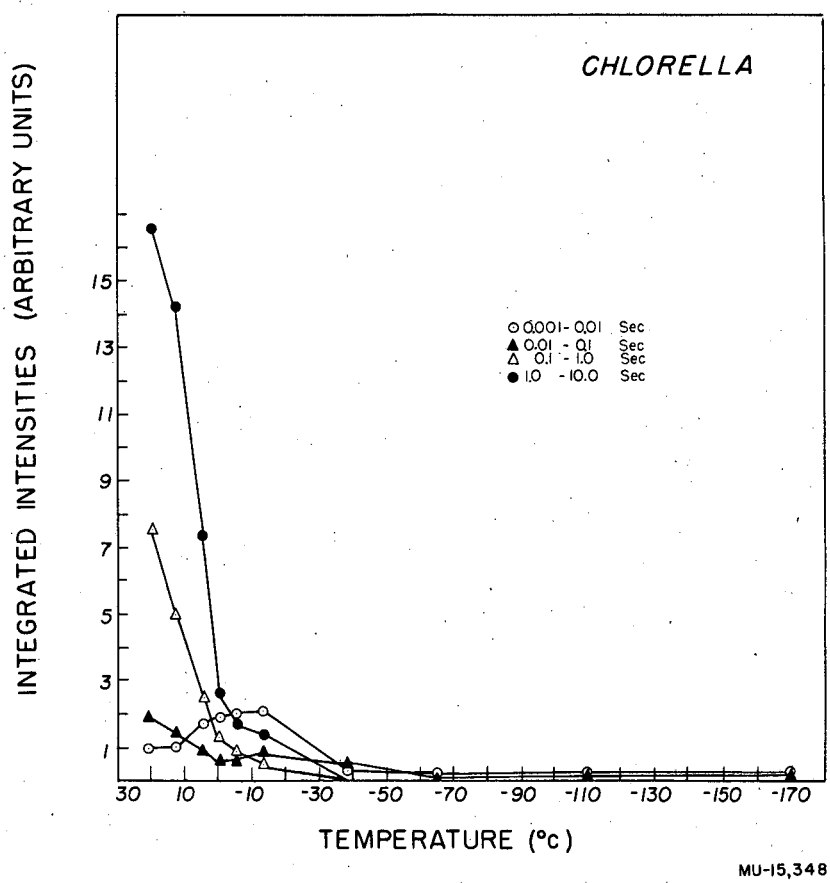
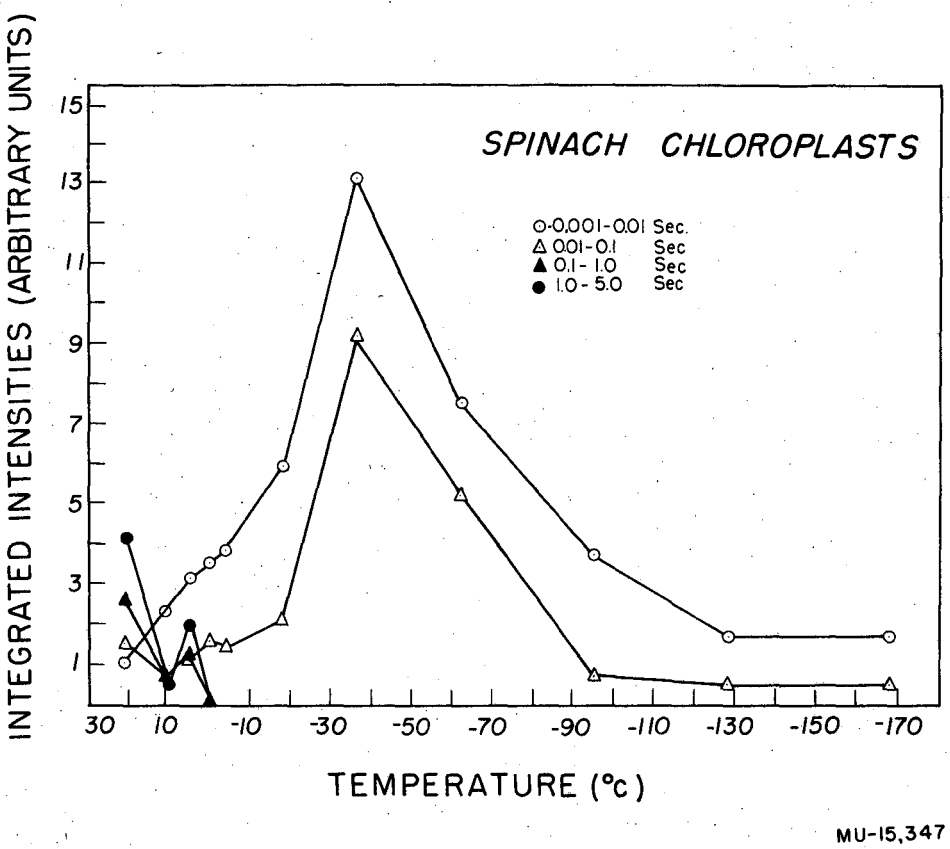


Fig. 5. Temperature-dependence of the integrated light intensities of Chlorella and spinach chloroplasts in various time-ranges. The integrated intensity values have been normalized for each organism to the 0.001-0.01 second range at 21°C.

Fig. 5 continued



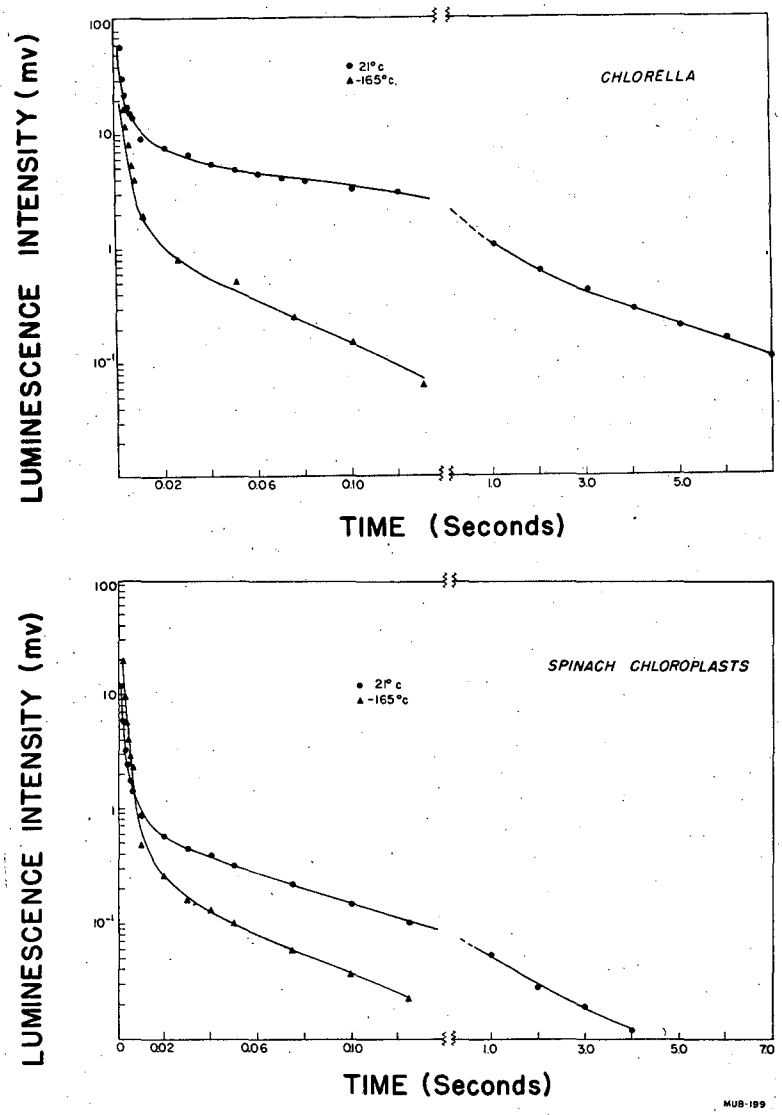


Fig. 6. Luminescence decay curves of Chlorella and spinach chloroplasts at two temperatures.