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THE ELECTROPHOTOLUMINESCENCE OF CHLOROPLASTS AND ITS RELATION TO PHOTOSYNTHETIC MECHANISMS

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THE ELECTROPHOTOLUMINESCENCE OF CHLOROPLASTS AND ITS RELATION TO PHOTOSYNTHETIC MECHANISMS

JAMES L. ELLENSON

DEDICATION

To my parents, whose years of love and concern will not be forgotten.

To the proposition that honesty is still the best policy.

To peace.

If you can look into the seeds of time,
And say which grain will grow and which will not,
Speak then to me . . .

Macbeth

ABSTRACT

One of the more intriguing problems facing biophysical chemists today is the manner in which plant systems are able to convert light energy into chemical energy. The study of delayed light emission from preilluminated photosynthetic systems has received considerable attention as a phenomenon whose understanding promises insight into this problem. The stimulation of delayed light emission by a number of perturbations - (salt, pH and temperature gradients, for instance) have been reported. The work presented in this thesis deals with stimulation of delayed light emission caused by an electric field. This stimulation, or electrophotoluminescence (EPL) was studied as a function of electric field, viscosity, photosynthetic inhibitors and membrane integrity.

EPL emission is observed to appear within several microseconds after the rise of an external electric field. There is an induction period of about 50 μsec lasting much longer than the rise time of the field (<6 usec) leading to a maximum emission in times on the order of 100 μsec. The maximum EPL emission, which is observed after about 100 μsec, depends on E^3 for fields below about 1200 $V\text{-}cm^{-1}$, while an exponential character is observed for higher field intensities. Two components appear to comprise the emission, one which decays faster (370 μsec half life at 1600 $V\text{-}cm^{-1}$) than the other (half decay 10msec). Removal of the field results in a decay in about 38 μsec. Immediate reversal of the field direction results in an emission that lacks the fast component. However, a zero field pause between initial and reversal pulses results in a return of the fast component when the reversed field is applied. The recovery of the fast component takes

about 5 to 10 msec at room temperature.

Retardation of onset kinetics and reduction of EPL intensity is observed when glycerol or sucrose is added to the medium. Chloroplasts suspended in STN buffer show a much reduced EPL emission and little fast component emission.

Detergent treatment, sonication and heat treatment all serve to annihilate EPL emission. The ionophore CCCP serves to abolish emission while gramicidin inhibits the fast component to a greater extent than the slow component. Valinomycin does not appear to have any noticeable effects on emission kinetics or intensities.

The emission capacity for EPL does not decay at the same rate as normal delayed light emission does, and an EPL emission experiment has no noticeable effect on subsequent normal delayed light emission.

A model is proposed to account for the experimental observations, and several field-related chemical phenomena are discussed.

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I. INTRODUCTION

A. PHENOMENOLOGY OF DELAYED LIGHT EMISSION

Sometimes it is possible to elucidate the nature of chemical reactions by observing phenomena which are the result of the reversal of forward reactions of interest. Such is the case in point for the study of delayed light emission associated with photosynthetic organisms, a phenomenon originally reported by Strehler and Arnold (Strehler and Arnold, 1951). The phenomenology is the following: if *any* wild-type photosynethtic organism, be it a primitive photosynthetic bacterium or a green plant cell, is observed in total darkness shortly after illumination, a low level decaying emission of light can be detected. This light is quenched in the presence of photosynthetic inhibitors. In green plants the emission has the spectrum of chlorophyll *a* fluorescence, while in photosynthetic bacteria the emission spectrum is characteristic of bacteriochlorophyll fluorescence. Since the emission spectra match the fluorescence spectra of the pigments, the emission is a result of a chlorophyll singlet to ground state radiative transition.

The turnover time of the photosynthetic electron transport chain is about 10^{-2} sec (Emerson and Arnold, 1932), much longer than the *in vivo* lifetime of the chlorophyll singlet state, about 10^{-9} sec (Clayton, 1965). Since energy cannot be stored as chlorophyll singlet excitation for longer than its lifetime, there has to be some mechanism by which energy is stored in metastable species before the appearance of photosynthetic products. The nature of these species

has remained a persistent and intriguing problem, and the study of delayed light emission has been pursued in the quest for further understanding of these intermediates.

In order for delayed light emission to occur at times longer than the chlorophyll (Chl) excited singlet state, ground to singlet state excitation has to occur at a later time by a non-photonic process. The energy for this re-excitation has to come from sources originally formed in the light, as no delayed light emission from photosynthetic systems is observed without preillumination. The quantum yield of delayed light emission, or the ratio of the number of luminescent quanta to the number of photons absorbed by the organism has been measured to be in the range of 10^{-6} (Tollin et al., 1958) to 10^{-4} (Arnold, 1972). The emission level in the dark decays quickly, but can be visually observed to last several minutes. No single theoretical description has been able to properly describe the dark decay kinetics of the emission over all time periods, but in the range between milliseconds and seconds, the emission has been consistently reported to be second order (Mayne, 1968; Malkin and Hardt, 1971; Ruby, 1971). That the production of delayed light is closely associated with primary photochemical events was early suggested by Tollin, Fujimori, and Calvin (Tollin et al., 1958), who showed that delayed light is emitted from cells which had been frozen and illuminated near liquid nitrogen temperatures.

In order to fit emission of delayed light within the context of the overall scheme of photosynthesis, it is necessary to construct a framework based upon current views of primary photosynthetic reactions. (By primary photochemical reactions I refer to those photoinduced reactions

which are the immediate result of photons impinging upon the molecular apparatus of the photosynthetic machinery and which occur in a time less than about 10^{-9} sec.)

B. THE TWO LIGHT REACTION SCHEME OF PHOTOSYNTHESIS

Nature has provided plant systems with a rather unique molecular architecture which uses many different pigment molecules to harvest light energy, but only a few specialized molecules which are capable, probably by nature of their unique location and/or environment, to make use of radiant energy to do chemical work. The pigments which gather light energy are referred to as "antenna" pigments, and are capable of transferring energy via exciton interactions to the specialized molecule or molecules termed the reaction center. It is at the reaction center that the first separation of chemical oxidizing and reducing potential occurs, initiating photosynthetic chemistry.

In green plants there are apparently two separate types of antenna-reaction center complexes which initiate two different chains of reactions. These two chains are linked together in series and account for the electron transport properties of the light reaction of photosynthesis. The two systems have been named Photosystem I (PS I) and Photosystem II (PS II) and occur in a sequential relation as shown in Fig. 1. A single photochemical event in PS II results in a transfer of an electron from water to an unidentified electron acceptor A. PS II and PS I are connected via an electron transport chain. An electron from reduced A loses its reducing potential in a series of reactions, at least one of which is a site of ATP synthesis (Avron and Neumann, 1968). The flow of

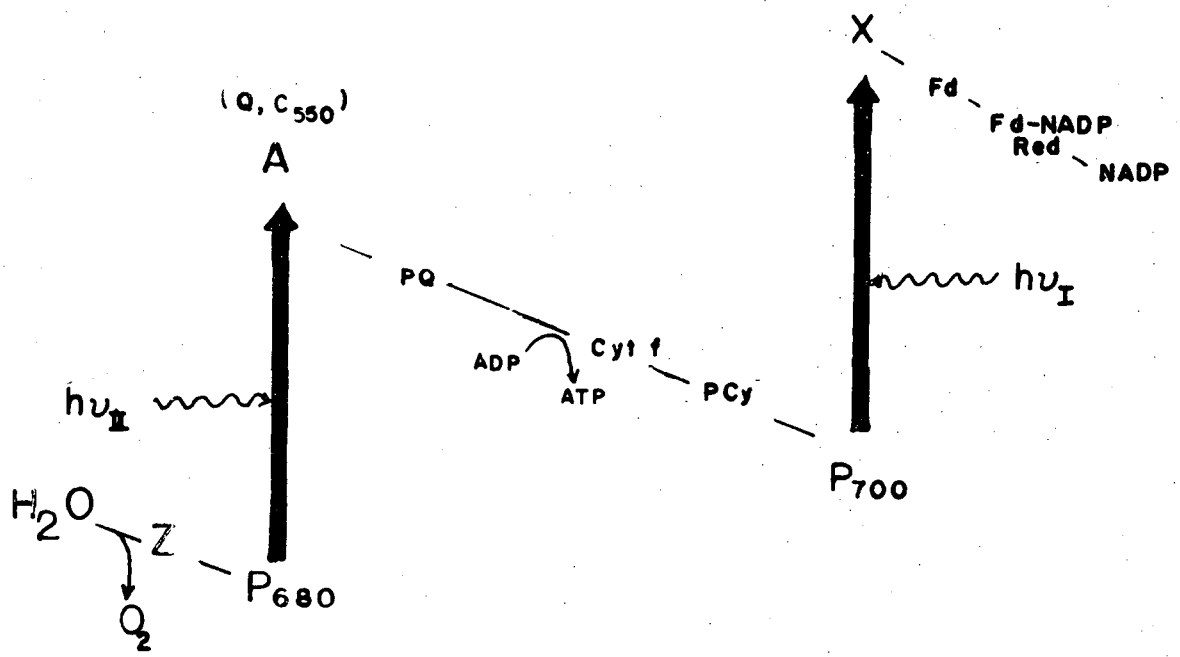

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Fig. 1. The basic elements of the "Z-scheme," two light reaction hypothesis for green plant photosynthesis. Vertical arrows refer to changes in relative reducing potential. Abbreviations: Z, the primary donor to PS II; A, the primary acceptor for PS II; Q, the PS II fluorescence quencher; C_{550}, the spectral component identified with A; PQ, plastoquinone; Cyt f, cytochrome f; PCy, plastocyanine; P_{700}, the reaction center for PS I; X, the primary acceptor to PS I; Fd and Fd-NADP Red., ferredoxin and ferredoxin-NADP reductase.

electrons continues beyond PS I when a photoreaction at that location forms reduced NADP, leaving behind an oxidized center which is in turn reduced by electrons from the donor species created near PS II. Since the oxidation of water to oxygen requires four electrons, four quanta have to be absorbed by each reaction center, or a total of eight, for each molecule of oxygen gas produced.

Each photosystem has its own set of primary acceptors, donors, and reaction center pigments, and various spectroscopic techniques have been employed to try to deduce their nature, as well as the nature of the electron transport species connecting the two photosystems. The identity of the pigments involved in the primary chemistry has been especially difficult to ascertain. However, the reaction center for PS I has been deduced from low temperature photoinduced absorption measurements and chemical titration data (Beinert and Kok, 1964). This reaction center contains one or more chlorophyll a (Chl a) molecules exhibiting a photobleaching maximum at 700 nm, and is called P_{700}. Recent spectral evidence suggests that a pigment absorbing at 680 nm, P_{680}, is a likely candidate for the PS II reaction center (Floyd, et al., 1971). Since delayed light emission is confined to PS II (see below), our concern will lie primarily with the constitution of PS II, the oxygen evolution photoreaction.

It is possible to select certain electron transport inhibitors which block electron flow at particular locations. Sites of inhibition can be determined by a combination of spectral, electrochemical, and biological (e.g., mutant strains) means. For some electron transport inhibitors the addition of exogenous electron acceptors or donors can

reactivate electron flow by uncoupling the normal flow of electrons past the inhibition point. Hill reaction oxygen evolution is a case in point. Oxygen evolution inhibited by an interrupted flow of electrons past PS II can be restored by the addition of various electron acceptors. The selective use of these factors aids in the characterization of electron transfer along the electron transport chain.

Bertsch et al., (1963, 1967) and Mayne (1967) showed that many different photosynthetic poisons, electron transport inhibitors and photophosphorylation uncouplers affected delayed light emission in a manner which demonstrated that delayed light emission is solely a PS II phenomenon. The absence of delayed light in algae lacking PS II activity (Bertsch et al., 1967; Arnold and Azzi, 1968) confirmed these results, and comparison of flash excited oxygen and delayed light emission gives additional correlation.

The study of oxygen evolution resulting from a series of ultrashort flashes (10^{-5} or less sec) allowed investigators to watch oxygen evolution as a result of single photon events. If chloroplasts are left in the dark for a sufficiently long time, a single saturating microsecond flash does not produce any oxygen, and very little is produced on the second flash. A third flash produces a maximum yield, however. If continued flashes are given, damped oscillatory production of oxygen results, with every fourth flash producing a maximum. The oxygen yield per flash eventually reaches a steady state which is about one half the amount produced on the third flash. A model has been proposed by Kok et al., (1970) to account for these observations:

In order for oxygen to be evolved, a series of four oxidizing

equivalents, S_1, S_2, S_3, and S_4 are produced by four successive photoreactions:

$$S_0 \xrightarrow{h\nu} S_1 \xrightarrow{h\nu} S_2 \xrightarrow{h\nu} S_3 \xrightarrow{h\nu} S_4 \longrightarrow O_2$$

The formation of S_4 spontaneously generates an oxygen molecule and regenerates S_0. It was further proposed that both the states S_0 and S_1 are stable in the dark, accounting for the high yield of oxygen on the third flash. The chemical identity of the S states has not been determined.

The appearance of delayed light emission following a series of flashes has also been investigated. Barbieri et al., (1970) and Joliot et al., (1971) reported that delayed emission also shows an oscillatory nature with a period of 4, with maxima on flashes 2 and 6. The entire cycle was advanced one flash compared with oxygen evolution. Zankel (1971), found the same periodicity, but reported maxima on flashes 3 and 7. Joliot et al., (1971) referring to their own scheme, concluded that states S_2 and S_3 were involved in delayed light emission. Little further progress has been made concerning the actual chemical identity of these states.

Properties of the PS II primary acceptor have been deduced from light induced fluorescence changes. A rise in fluorescence yield is observed as one continuously illuminates a suspension of chloroplasts. The "live" fluorescence change has been attributed to the primary electron acceptor of PS II (Clayton, 1969). In the oxidized state, the acceptor quenches fluorescence. As the acceptor becomes reduced, fluorescence rises. Because of the fluorescence quenching properties of the acceptor, it has been named Q.

Bennoun (1970) found that the decay of delayed light corresponds to the reoxidation of Q as measured by fluorescence changes when the experiments were conducted in the presence of DCMU. DCMU is considered to block electron flow from the primary acceptor of PS II, and is a very potent inhibitor of oxygen evolution.

Butler (1973) disagrees that Q is the primary electron acceptor of PS II. In this paper he discussed the evidence that a pigment which shows an apparent primary spectral shift near 550 nm, but does not always parallel fluorescence changes, is the primary acceptor. It is named C_{550}. The actual distinction between C_{550} and Q may be more in their functional definition than in their chemistry (Butler, 1973).

Thus, although the actual species which participate in oxygen evolution and the metastable species responsible for delayed light emission are still unknown, certain characteristic properties have been deduced. Before we further pursue the factors which influence the level of delayed light emission, it is appropriate to discuss two general types of mechanisms that have been proposed in order to account for the luminescent act itself.

C. TWO MECHANISMS FOR EMISSION

1. The Electron-Hole Model

Arnold and Sherwood (1957, 1959) early reported that if photosynthetic organisms are frozen at low temperatures, illuminated and reheated, fluorescent emission is observed to occur at different temperature ranges. This phenomenon is termed thermoluminescence, and the resultant luminescence *vs*. time curve is called a glow curve.

Classical solid state theory concerning thermoluminescence is based upon an electron-hole concept of charge carriers. If an electron can be separated in space from an atom or molecule and trapped at another site, a so-called electron-hole pair has been established, the "hole" being a positively charged center into which an electron can fall. If the electron loses its energy radiatively while falling into the hole, luminescense can be observed. Thermoluminescence occurs upon recombination of electrons and holes, one or both of which were released by thermal excitation. The similarities to solid state phenomena led Arnold and Azzi (1968) to propose an electron-hole model for photosysthesis.

Arnold and Azzi's model involves two separate Photosystem II trapping centers as indicated in equations (1) through (3):

$$\text{TRAP A:}\quad \text{Chl } \underline{a} + h\nu \rightarrow \text{Chl } \underline{a}_M + e^-_T \tag{1}$$

$$\text{TRAP B:}\quad \text{Chl } \underline{b} + h\nu \rightarrow \text{Chl } \underline{b}_M + \oplus_T \tag{2}$$

$$\text{Chl } \underline{a}_M^+ + \text{Chl } b_M^- \rightarrow \text{Chl } \underline{a} + \text{Chl } \underline{b} + h\nu_{DL} \tag{3}$$

Trap A excitation produces a trapped reductive species (e^-_T) and a mobile oxidized chl $\underline{a}_M^+$ molecule which is in contact with other chl $\underline{a}$ molecules, allowing for migration of the hole, Chl a_M^+. At the second trap excitation causes the formation of a trapped oxidative species ($\oplus_T$) and a Chl b^- molecule whose negative charge can also migrate to other chl $\underline{b}$ molecules. Encounter of an electron and hole at the chl $\underline{b}$, chl $\underline{a}$ interface results in the production of a photon of delayed light, $h\nu_{DL}$.

The inclusion of Chl $\underline{b}$ in the reaction scheme resulted in part

from consideration of Nelson's (1968) careful measure of chl a and Chl b oxidized and reduced potentials, and to account for an observation that two photons were necessary for delayed light emission (Jones, 1967).

If the mobile species themselves become temporarily trapped, the rate of emission then becomes proportional to the rate at which electrons or holes can escape from the traps and return to the chlorophyll levels. Arnold and Azzi proposed that, if the traps of photosynthetic systems obey the properties of solid state trapping phenomena, then the rate of detrapping and luminescent intensity should follow the relation (after Randall and Wilkins (1945)):

$$I_{DLE} = - dN/dt = NFexp(-E/kT), \tag{4}$$

where N is the number of trapped species, F is a frequency factor, E is the activation energy of the trap, T is the absolute temperature, and k is Boltzmann's constant. Arnold and Azzi applied their trapping model to an explanation of the thermoluminescence glow curves obtained from frozen chloroplasts. Of three maxima of emission relevant to photosynthetic reactions, two were related to the untrapping of holes, one the detrapping of an electron (Arnold and Azzi, 1968).

Bertsch et al., (1971) proposed a similar electron-hole model, but invoked two separate sets of Cho a molecules, one set responsible for production of a trapped hole and mobile electron, Eq. (5), and another set responsible for a trapped electron and mobile hole, Eq. (6).

$$\text{Chl } \underline{a} + h\nu \rightarrow \text{Chl } \underline{a}^{S} \text{ T Chl } \underline{a} + d^{-}_{M} + \oplus_{T} \tag{5}$$

$$\text{Chl } \underline{a}' + h\nu \rightarrow \text{Chl } \underline{a}'^{S} \rightarrow \text{Chl } \underline{a}' + \oplus_{M} + e^{-}_{T} \tag{6}$$

$$e^{-}_{M} + \oplus_{M} \rightarrow h\nu_{DL} \tag{7}$$

Again, encounter of the mobile electron and hole produces delayed light emission, Eq. (7). The best evidence for the electron-hole mechanisms lies with thermoluminescence data and the fact that oxidized Chl a can be reduced in vivo to give Chl a fluorescence (Goedheer, et al., (1962), Linschitz (1961)).

Variations in the basic electron hole theory and the intensity equation, Eq. (4), have developed as further knowledge has been gathered concerning delayed light emission and the factors which affect it. We will return to these developments after a brief discussion of the triplet state model.

2. The Triplet State Model

A second hypothesis is the triplet state model proposed by Stacy et al., (1971). Instead of electrons and holes migrating within the photosynthetic structure they propose that the triplet state of chlorophyll is involved. The elements of the model are the primary formation of a charge separation, Eq. (8), the formation of a triplet state chlorophyll, Eq. (9), the migration

$$ZChlQ + h\nu \rightarrow Z^{+}ChlQ^{-} \quad (8)$$

$$Z^{+}ChlQ^{-} \rightarrow ZChl^{T}Q \quad (9)$$

$$ZChl^{T}Q + Chl_{ant} \rightarrow ZChlQ + Chl^{T}_{ant} \quad (10)$$

$$2\ Chl^{T}_{ant} \rightarrow Chl^{S}_{ant} + Chl^{0}_{ant} \quad (11)$$

$$Chl^{S}_{ant} \rightarrow Chl^{0}_{ant} + h\nu_{DL} \quad (12)$$

of the triplet away from the reaction center, Eq. (10), into the antenna pigment array, and the subsequent encounter with another triplet from a different reaction center, Eq. (11). The two triplet

states interact to form one Chl ground state molecule, Chl^0 and an excited singlet state, Chl^S, which can radiate while returning to the ground state, Eq. (12), producing delayed light emission. The radiation of light from the antenna array is consistent with the observation that the emission spectrum of delayed light emission is indistinguishable from the fluorescence spectrum of bulk *in vivo* chlorophyll. Radiation from a reaction center would be expected to deviate from ordinary *in vivo* fluorescence because of the assumed different optical properties of a reaction center.

Recent evidence has shown that the triplet state can be observed at low temperatures in chemically reduced reaction center preparations of photosynthetic bacteria (Dutton *et al.*, 1973). However, the triplet state has never been observed spectroscopically under normal *in vivo* conditions. Stacy *et al.*, (1971) claim that undetectable levels of triplet chlorophyll could account for delayed light emission if they are present only as transient intermediates between the trapped species and the excited singlet Chl.

There is not yet enough evidence to discount either of these mechanisms, and the possibility remains that either, or a combination of the two, may account for the emission mechanism. Whatever the actual mechanism of emission, the environmental context in which the delayed light system works has been further elucidated by the discovery that electric fields can play an important role in biological mechanisms.

D. MEMBRANE FIELDS IN PHOTOSYNTHESIS

In 1961 Mitchell published his chemiosmotic mechanism for phosphorylation (Mitchell, 1961). Mitchell proposed that the components of the electron transport chains are arranged across the thylakoid in such a way that electron carriers and hydrogen carriers alternate across the membrane. Electron flow results in a net transfer of protons into the thylakoid, producing a hydrogen ion chemical potential difference. The flow of these ions back through a coupling factor provides the necessary energy for phosphorylation. In addition to the chemical potential provided by the concentration gradient of hydrogen ion, an electrical potential results as a consequence of the separation of charges. Experimentally, a light induced hydrogen ion gradient is readily detected with a glass electrode (Neumann and Jagendorf, 1964), and ATP formation is observed in the dark in response to artificial pH gradients (Izawa and Hind, 1967). A direct measure of the electric field is not possible. However, a particular light induced absorption change has been correlated with the formation of an electric field. The description and evidence is the following:

1. Flash illumination produces an absorption change in green plant systems which has maxima at about 478, 515, and 648 nm, henceforth known as the A_{515} change. The half-time of the absorption rise is less than 2×10^{-8} sec (Wolff *et al.*, 1967; Witt, 1967).

2. The decay of the absorption change is sensitive to factors which would discharge an electric field. Gramicidin D, an ionophore which is known to effect membrane permeability to univalent cationic species (Bangham *et al.*, 1965), increases the rate of decay of the absorption

change (Junge et al., 1969).

3. Nearly identical electrochromic spectral shifts can be observed when monolayers of photosynthetic pigments are subject to electric field strengths on the order of 10^5 V-cm^{-1} (Schmidt et al., 1968; Junge and Witt, 1968).

Junge et al., (1969) and Junge and Witt (1968) interpreted the A_{515} change as due mostly to the electrochromism of Chl b. However, Hildreth (1970) reported that A_{515} changes could be observed in a Chl b-less mutant of barley, and Strichartz and Chance (1972) showed that the A_{515} change could be reconstituted from heptane extraction of chloroplasts by addition of β-carotene. Hildreth (1970) also found a correlation between A_{515} and carotene content. Currently, the absorption change is mostly attributed to a carotenoid electrochromic band shift.

An experimental test of the relation between A_{515} and membrane potentials was performed by Strichartz and Change (1972). They induced potential gradients across dark adapted thylakoid membranes by subjecting them to sudden increases in ionic strength. Absorption changes nearly identical to light induced A_{515} changes were observed, even when electron transport was inhibited with DCMU.

Under the proper experimental conditions it is possible to calculate the magnitude of induced potential using the Goldman equation (Goldman, 1943):

$$E_{o-i} = \frac{RT}{F} \ln \frac{\sum_C P_c[C]_i + \sum_A P_a[A]_o}{\sum_C P_c[C]_o + \sum_A P_a[A]_i} \qquad (13)$$

where P_c and P_a are permeability coefficients for univalent cations C

and anions A. Subscripts i and o refer to concentrations inside and outside, respectively. This equation is valid for passive independent ion flow and for a linear potential gradient (Goldman, 1943). If one of the species is made much more permeant than the others, as when potassium salts are added in the presence of the ionophore valinomycin (Moore and Pressman, 1964), Eq. (13) reduces to Eq. (14):

$$E_{o-i} = \frac{RT}{F} \ln \frac{[C]_o}{[C]_i} \tag{14}$$

If the observed change in absorbance is directly proportional to the magnitude of the field applied, then Eq. (14) and the measured A_{515} produced as a result of an ion concentration jump can be combined to calibrate the membrane potential based on absorption changes. For a given inner concentration of K^+, there should be a direct relation between the measured absorption change and the logarithm of K^+ concentration. This relation was confirmed by Jackson and Crofts (1971) and by Strichartz and Chance (1972). The magnitude of the membrane fields calculated from absorption changes is in the range of 10^5 V-cm^{-1} (Barber, 1972; Schmidt _et al_., 1968).

Preilluminated chlorplasts are also observed to emit a stimulated signal of delayed light emission when a salt concentration jump experiment is performed in the dark. Barber and Kraan (1970) applied the membrane potential model to the production of this stimulation. By allowing for a change in trap activation energy by the size of the potential given by Eq. (14), Barber and Kraan rewrote Eq. (4) as:

$$I_{DLE} = \Phi NF\exp[-(E-(\frac{RT}{F} \ln \frac{[C]_o}{[C]_i}))/kT] \tag{15}$$

where Φ is a yield factor. This equation may in turn be rewritten as:

$$I = \frac{A[C]_o}{[C]_i} \tag{16}$$

where $A = \Phi NF\exp(-E/kT)$ (Crofts et al., 1971). Barber and Kraan were able to show that the amplitude of stimulated emission was approximately linear in the concentration of externallyy added K^+ (Barber and Kraan, 1970).

Tributsch (1971, 1972) has derived a similar expression, but based more formally upon the precepts of electrochemistry. His expression, written as a time dependent equation, predicts that the observed delayed light intensity is given by:

$$I_{DLE} = A \cdot \exp\{[B \cdot \exp(-k_1 t)] - k_2 t\} \tag{17}$$

where A, B, k_1 and k_2 are constants and t the time. This equation fits fairly closely a curve of delayed light emission published by Lavorel (1971) if it is assumed that $k_2 << k_1$.

E. ELECTROPHOTOLUMINESCENCE

Besides salt induced luminescence, emission is also stimulated by fast acid-base transitions (Miles and Jagendorf, 1969), acid addition only (Miles and Jagendorf, 1969, Hardt and Malkin, 1971), temperature jumps (Mar and Govindjee, 1971), and the injection of organic solvents (Hardt and Malkin, 1972). Recently, the stimulation of delayed light emission upon the direct application of an external electric field to preilluminated chloroplasts was reported by Arnold and Azzi (1972). This experiment provided the first unambiguous electric field effect on delayed light emission.

There are solid state analogies to this electrical phenomenon. As early as 1920 Gudden and Pohl reported an enhancement of phosphorescence from zinc sulfide crystals when an electric field was applied (Gudden and Pohl, 1920). Similar phenomena became known generically as "Gudden-Pohl" effects. Ivey (1957), in a review of various types of solid state emission phenomena, categorized such phenomena as electrophotoluminescence, as opposed to electroluminescence, which is field caused light emission not requiring preillumination. Light emitting diodes owe their properties to electroluminescence. To avoid introducing yet another term for a biological variation of a solid state phenomenon, the electrical stimulation of delayed light will also be called electrophotoluminescence, or simply EPL. It is the characterization of this phenomenon that is the subject of this thesis.

The discovery that an electric field produces perturbations in emission intensity opens a whole new time domain for observation. Electric fields can be applied and removed in microseconds, much faster than any of the previously used techniques, all of which require mixing times of at least milliseconds. A distinct advantage of the technique is the fact that the perturbing influence (the field) can be completely removed, unlike the other perturbations. Such advantages promise to reveal kinetic processes which cannot easily be monitored using the slower perturbation techniques based on mixing.

In what ways electrical perturbations disturb the concentration of metastable intermediates, and whether this disturbance is equivalent to the pH, ionic, solvent and temperature perturbations are fundamental questions which immediately arise. What follows is a report of the

pursuit of answers to these questions and others, the results which were obtained, and the implications which have been derived therefrom.

II. MATERIALS AND METHODS

A. CHLOROPLAST SAMPLES

Broken chloroplasts were prepared from growth chamber grown (10 hrs. light, 14 hrs. dark) spinach plants, which were 6 to 8 weeks old. A typical preparation consisted of grinding about 10 g of deveined spinach leaves in about 75 ml of a buffer adjusted to pH 7.8 consisting of 0.4 M sucrose, 0.05 M tricine, and 0.01 M NaCl (STN buffer). The resulting suspension was filtered through either several layers of cheesecloth, a layer of glass wool, or a filter made out of Miracloth, of product of Chicopee Mills, Inc., New York City. The latter proved the most expedient. The filtrate was then centrifuged at 1000 × g for about 5 min. The supernatant was discarded, leaving a pellet of broken chloroplasts. "Aqueous" chloroplasts were prepared from the broken chloroplasts by resuspending them in distilled water and recentrifuging for 10 min. at 1000 × g. The resulting pellet was resuspended in about 5 ml of distilled water. Aliquots of that stock solution were diluted in 5 or 10 ml of distilled water, or distilled water plus added reagents.

Tris-washed chloroplasts were prepared by suspending broken chloroplasts for 30 to 60 min. in about 20 ml of 0.8 M tris buffer, pH 8.0, at 3°C. The suspension was centrifuged from the tris buffer and recentrifuged in distilled water. The resulting pellet was resuspended in a medium which depended on the particular experiment being performed.

Heat treatment consisted of suspending a test tube containing a chloroplast solution in 55°-60°C water for about 3 min.

Chlorophyll concentrations were measured by the standard technique

of diluting 0.1 ml of stock chloroplast preparation in 20 ml of 80% acetone, 20% water mixture, and measuring the absorption of the filtered solution at 652 nm. The milligrams of chlorophyll in the original solution is obtained by multiplying the absorbance at 652 nm by 5.8 (Sun, 1972). Typical Chl concentration of 10 to 100 Mg of Chl per ml were used. Absorption measurements were made on a Cary 14 or Aminco DW-2 spectrophotometer.

B. ELECTRO-MECHANICAL APPARATUS

The apparatus for producing and detecting EPL (see Fig. 2) consists of a light source (L), two stepping motor shutters (S_L and S_D), a multiplier phototube (MPT), and two platinum electrodes (E). The experiment is controlled by logic circuitry (LC) whose sequential pulses direct shutter positions, voltage pulses, and recording devices. MD is the motor drive unit for the stepping motors (Phillips PD-22), and PG is the pulse generating network. The shutters opened or closed in about 15 msec. The regimen of the experiment is to preilluminate for a time t_p (by opening shutter S_L with S_D still closed) a sample of chloroplasts which fills the space between the two electrodes, close shutter S_L, and open shutter S_D. After a time t_d a voltage pulse of length t_f is triggered across the electrodes, producing electrophotoluminescence. Starting with the triggering of the voltage pulse the signal detected by the photomultiplier is digitally recorded in the 128 channel memory of a Biomation 610 transient recorded (B10 610), which was triggered simultaneously with the voltage pulse. The 128 channels can record an event 12.8 μsec to 6.4 sec total duration. The stored signal is then

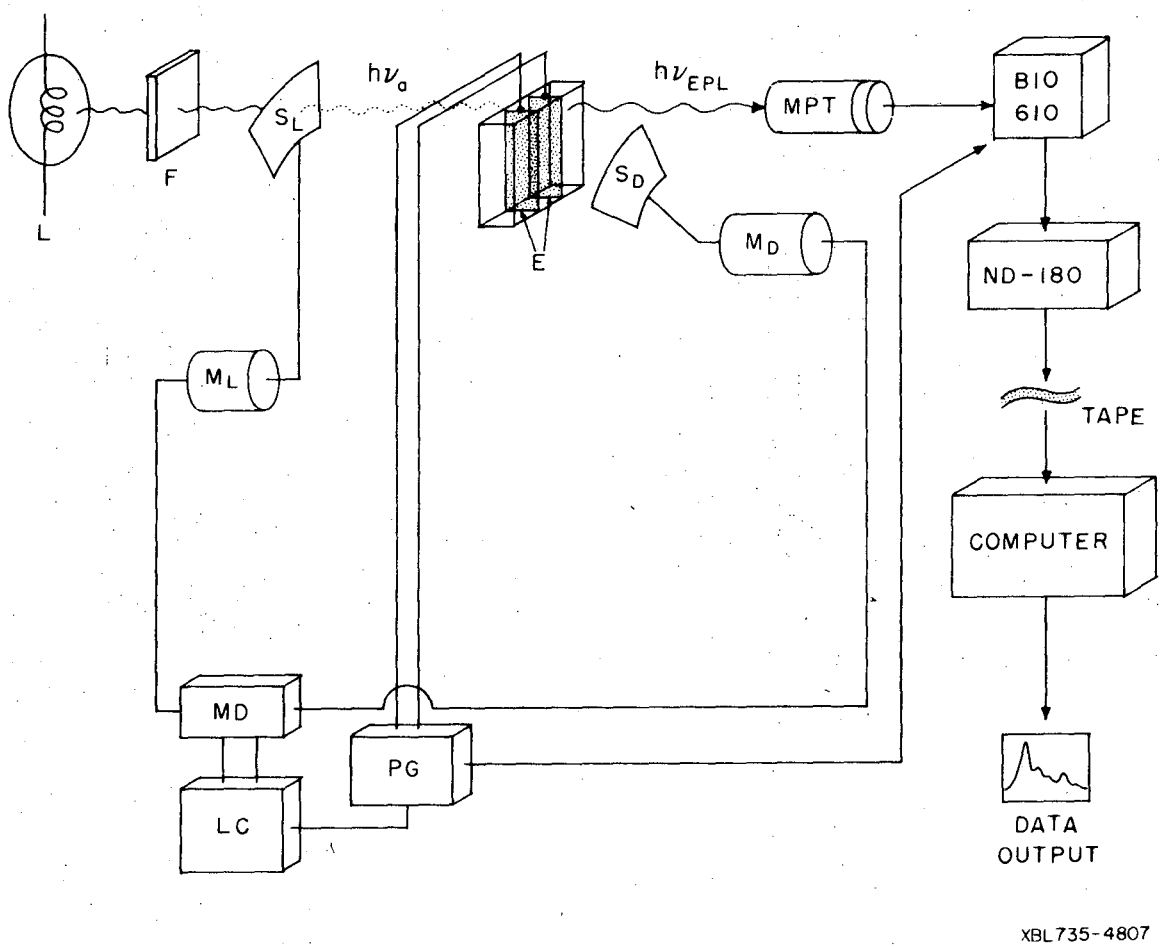

Fig. 2. Schematic diagram of experimental apparatus. See text for description of components.

transmitted to a Nuclear Data Enhancetron or Nuclear Data ND-180M memory unit (ND-180). Both systems provide output on paper tape for computer interpretation and graphing, or the results can be directly plotted in analog form on X-Y recorders.

A schematic diagram of the electrode and detecting apparatus is shown in Fig. 3(a and b). The electrode spacing in Fig. 3(a) is 0.25 cm. The electric field is applied transverse to the optical path. It was found that a lucite light guide (LG) and a magnetic focusing lens (MFA) improved signal to noise. Fig. 3(c) and (d) show two other electrodes employed in the course of the experiments. The advantages of the electrodes in Fig. 3(a) was that it could be filled and emptied without altering the electrode position, whereas the electrodes in Fig. 3(c) and (d) had to be raised out and lowered into a cuvette, so that electrode repositioning was not exact.

The light source usually used was a 500-watt tungsten lamp operated from a regulated DC power source. The exciting light passed through 10 cm of water and suitable optical filters to give a broad band (400-500 nm) excitation. A 1/4-meter Bausch and Lomb monochromator was used in place of the filters whenever any monochromatic light was needed. The stimulated emission passed through a Corning 2-64 filter (nearly transparent to light with $\lambda > 660$ nm) before researching the photomultiplier.

Short light flashes (10 μsec half-width) were produced by a xenon flash lamp assembly designed by ILC Inc. of Sunnyvale, California.

The "Bipolar energy switch", which is designated as PG in Fig. 2, was designed to produce a bipolar rectangular pulse having a duration of 1 to 10 msec. The rise time of the pulse was less than 6 μsec. The

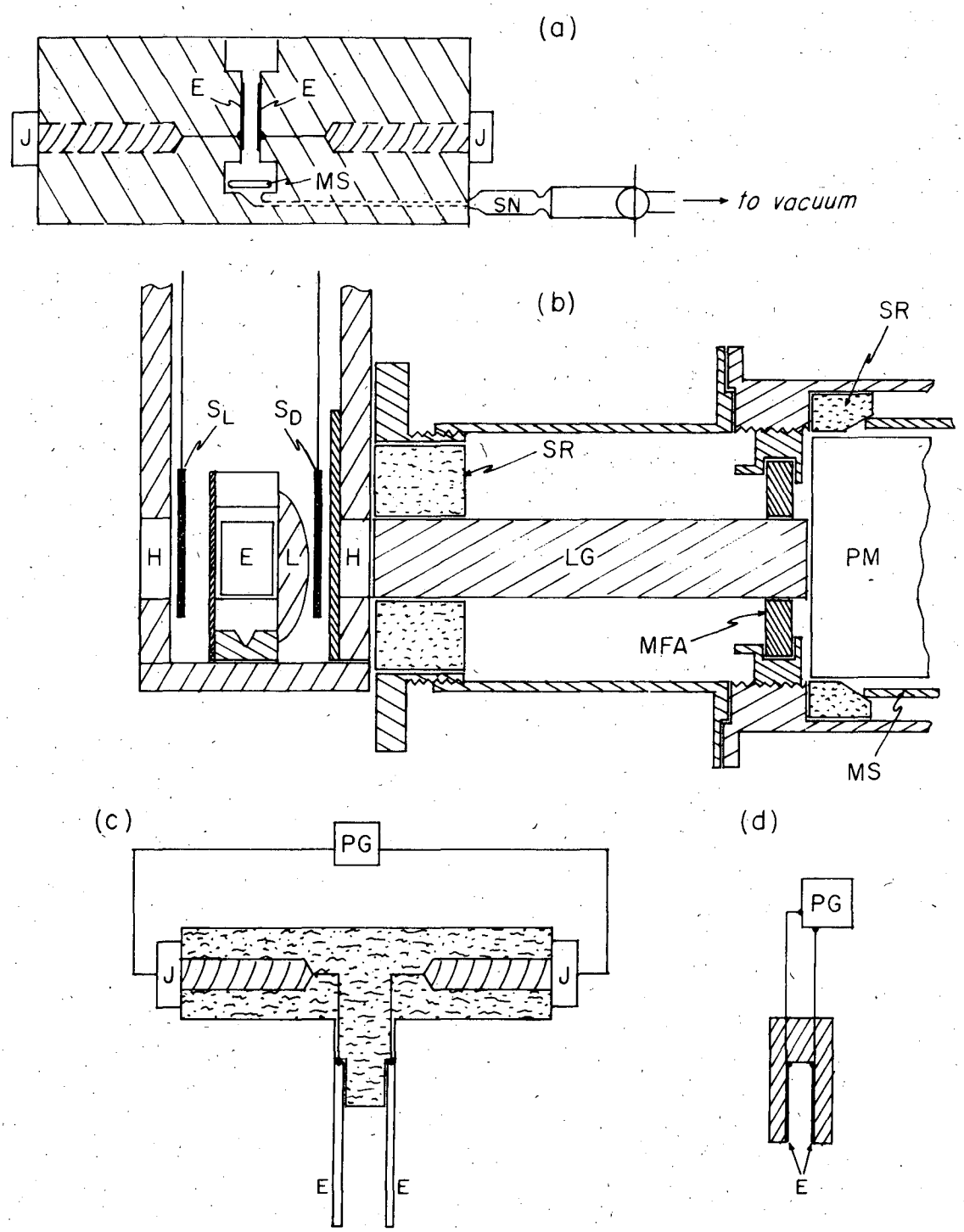

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Fig. 3. (a) Self-contained electrode and cuvette components. Elements: J, electrical jack; E, platinum electrodes; MS, magnetic stirrer; SN, syringe needle. (b) Cross-section of electrode housing and detector assembly. Elements: H, light port; S_L and S_D, paddle shutters; E, platinum electrodes; L, converging lens; SR, lucite support ring; LG, lucite light guide; MFA, magnetic focusing assembly; PM, photomultiplier; MS, magnetic shield. (c) and (d): Immersible electrode assemblies with gold electrodes; PG, pulse generator.

unit could be modified to produce a single unipolar pulse, two separated unipolar pulses, or two separated bipolar pulses. These wave forms are shown in Fig. 4. The device is limited to a maximum total voltage of 400 volts applied through an impedance of about 100 ohms. The magnitude of the field across the electrodes depended upon the interelectrode distance and the applied voltage (field = voltage applied/distance applied). The electronic schematics for the apparatus are assembled in LCB electronics Schematic #16 X 484.

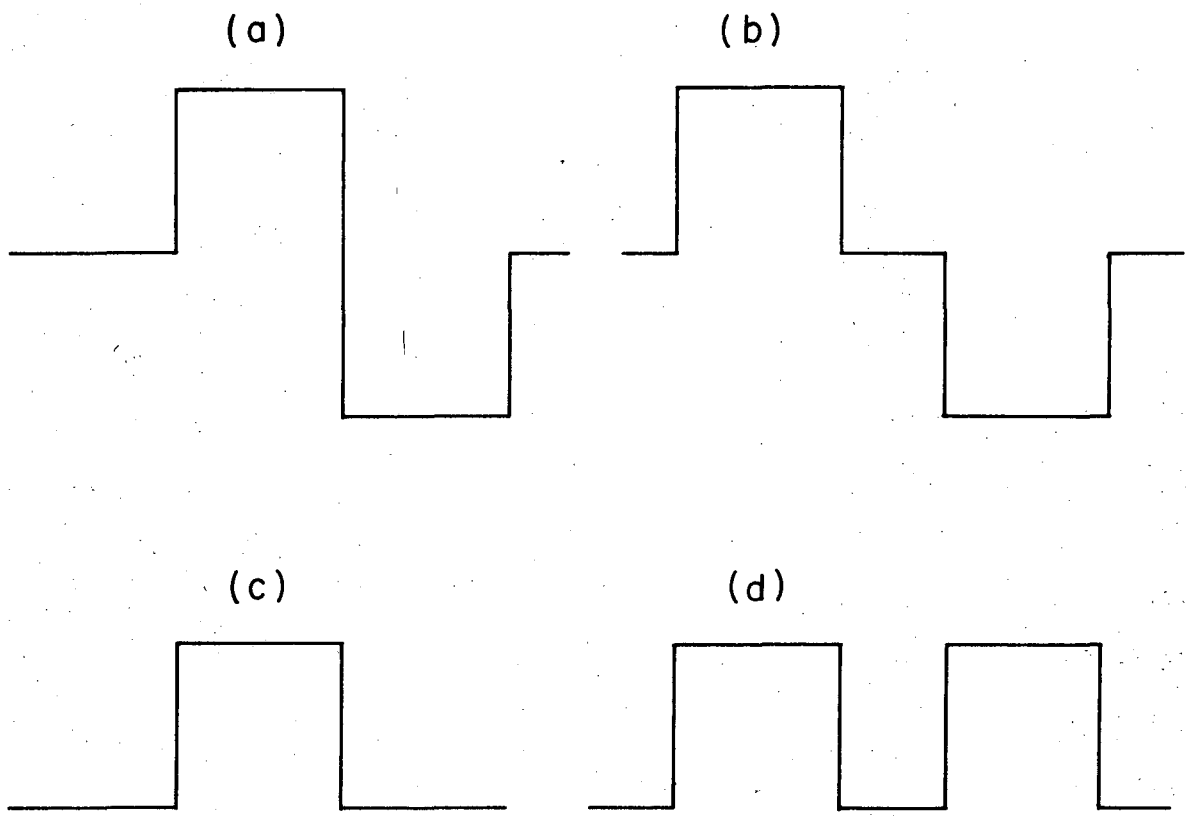

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Fig. 4. Pulse shapes available from pulse generating network: (a) bipolar; (b) sequential bipolar; (c) unipolar; (d) sequential unipolar. The two pulses in (a), (b), and (d) may or may not be of equal length, depending on the choice of conditions.

III. RESULTS

A. GENERAL NATURE OF EPL EMISSION

The general nature of the photosynthetic electrophotoluminescent phenomenon is a large enhancement of delayed light emission which occurs when an electric field is applied to preilluminated chloroplasts. The emission spectrum is characteristic of Chl fluorescence. The enhancement appears within about 10 μsec of the onset of the field and disappears in a time less than 100 μsec from the time the field is removed. An unusual facet of this perturbation is that it produces no noticeable reduction in the amount of delayed light emission from that which would occur in the absence of a perturbation. A representation of the delayed light emission and the stimulated response from chloroplasts is shown in Fig. 5. Figure 6 reveals an expanded time scale of a characteristic profile of emission from aqueous chloroplasts perturbed by a rectangular voltage pulse producing a field of about 1600 $V\text{-}cm^{-1}$. The time scale begins with the field application.

In order to gather some insight into the nature of the emission, the kinetic form of the luminescence during the field-on time t_f was studied. The bulk of the experiments used to characterize the emission were obtained using aqueous chloroplasts. These chloroplasts had concentration of NaCl less than 0.1 mM and a tricine concentration less than 0.5 mM. Aqueous samples were chosen to minimize current requirements for the electronic circuitry and to minimize joule heating effects of the sample. Aqueous samples were found to reveal a greater complexity of emission character than chloroplasts suspended in STN, while at the

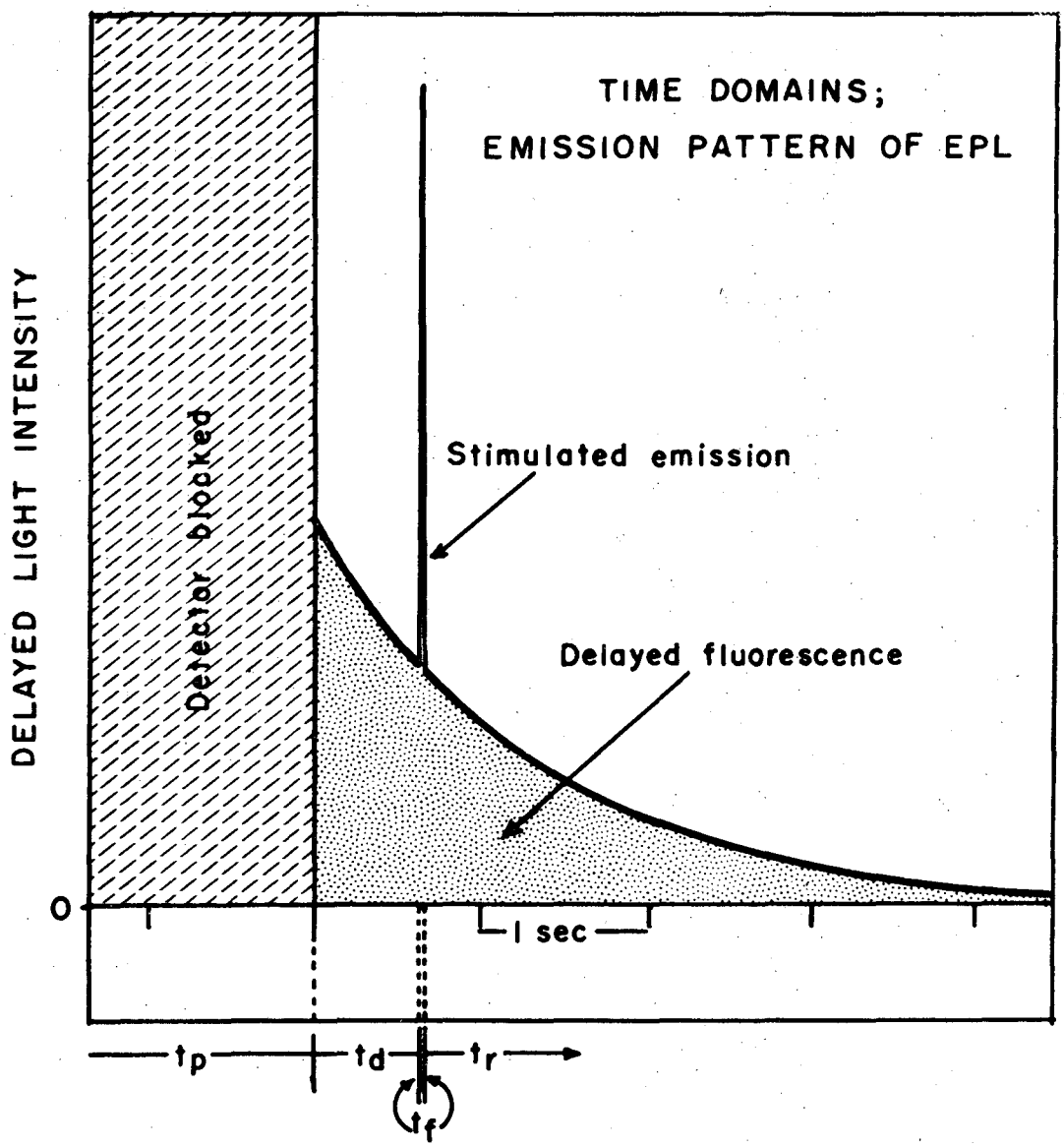

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Fig. 5. Overall time domains and emission pattern for EPL experimental regime. The sample is illuminated for time t_p (typically 3-5 sec) during which the detector is blocked. Actinic light is then blocked, and a shutter opens to allow the detector to observe subsequent delayed emission in the dark during time t_d. The electric field is applied for the time t_f. The time after the electric field pulse is designated t_r. Field pulses generally were not shorter than 500 μsec nor longer than 10 msec.

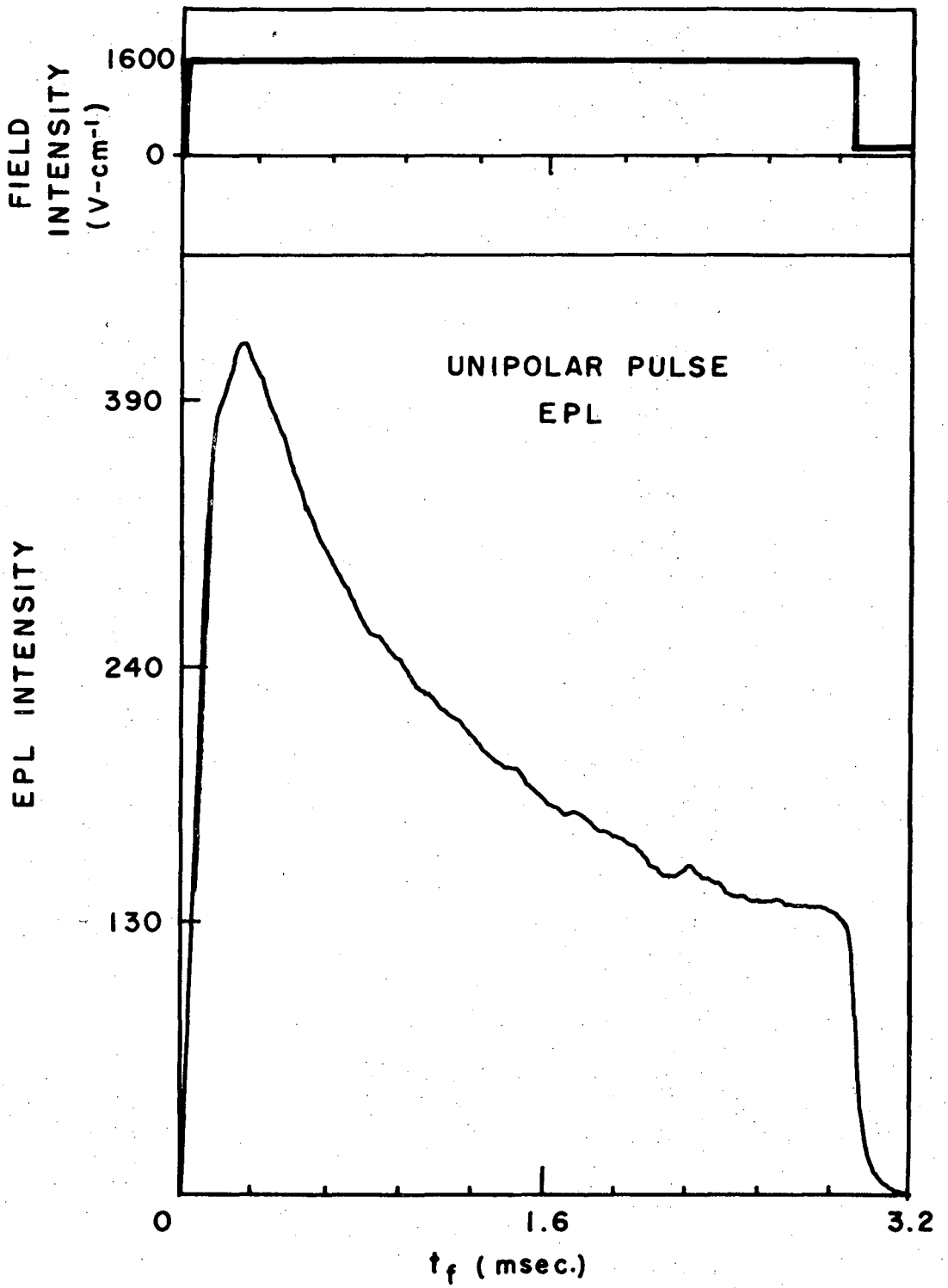

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Fig. 6. Stimulated emission from aqueous chloroplasts subjected to a 1600 V-cm^{-1} field pulse. t_p = 4 sec, t_d = .1 sec. The curve is an average of 10 scans.

same time the magnitude of the observed signal was enhanced. These details will be discussed below.

The instantaneous emission intensity may be written as

$$I_{EPL} = \Phi_{EPL} \cdot [Chl^*]$$

where Φ_{EPL} is the emission efficiency and [Chl*] is the instantaneous concentration of excited singlet states. The time dependent change in emission which is observed during a pulse may be a field alteration of either or both of the terms on the right.

B. FIELD DEPENDENT PRODUCTION AND DECAY KINETICS

During the time t_f the emission is characterized by a fast rise to a maximum (Fig. 6) followed by a slower decay. Removing the field results in a final decay of the emission to a non-perturbed level. For this particular sample the level of unperturbed delayed light emission intensity was approximately 1% of the maximum EPL emission which occurred during the time t_f. The rise to maximum emission is sigmoid in nature, as the greater t_f time expansion in Fig. 7 shows. The various characteristics of the emission pattern were studied as a function of variables such as light intensity, electric field, various inhibitors and different suspension media.

1. Onset Kinetics

If the reciprocal of the time required to reach one-half the maximum EPL intensity, $(t_{1/2})_f^{-1}$, is plotted as a function of applied field strength, a linear relation is observed (curve A of Fig. 8). The resultant curve is an indication of the change in rate of emission as a function of field strength. Two other samples were chosen

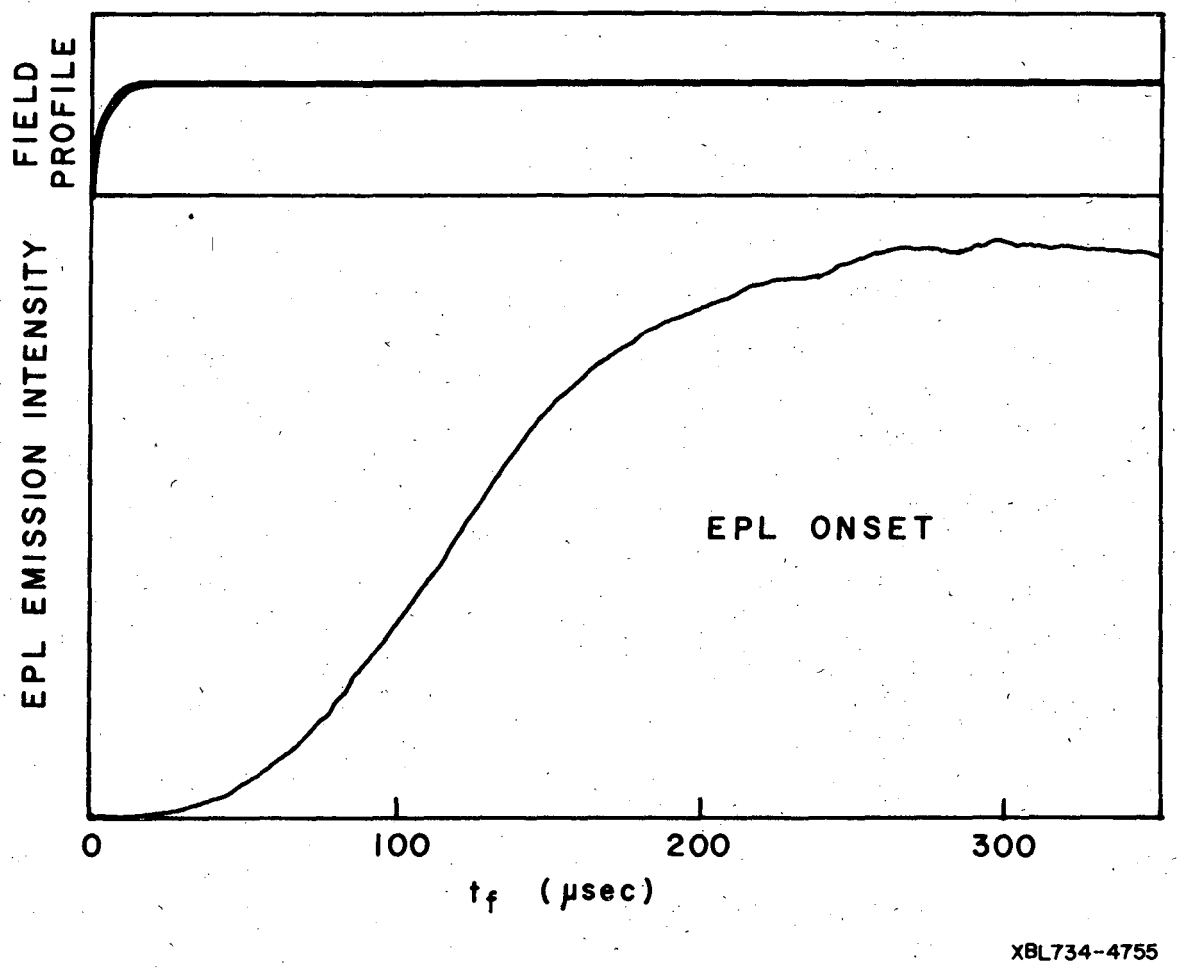

Fig. 7. Expanded time scale starting with field application showing the sigmoid nature of the EPL onset kinetics. This particular curve was obtained from aqueous chloroplasts subjected to a 1200 V-cm^{-1} field pulse and at a temperature of 3°C.

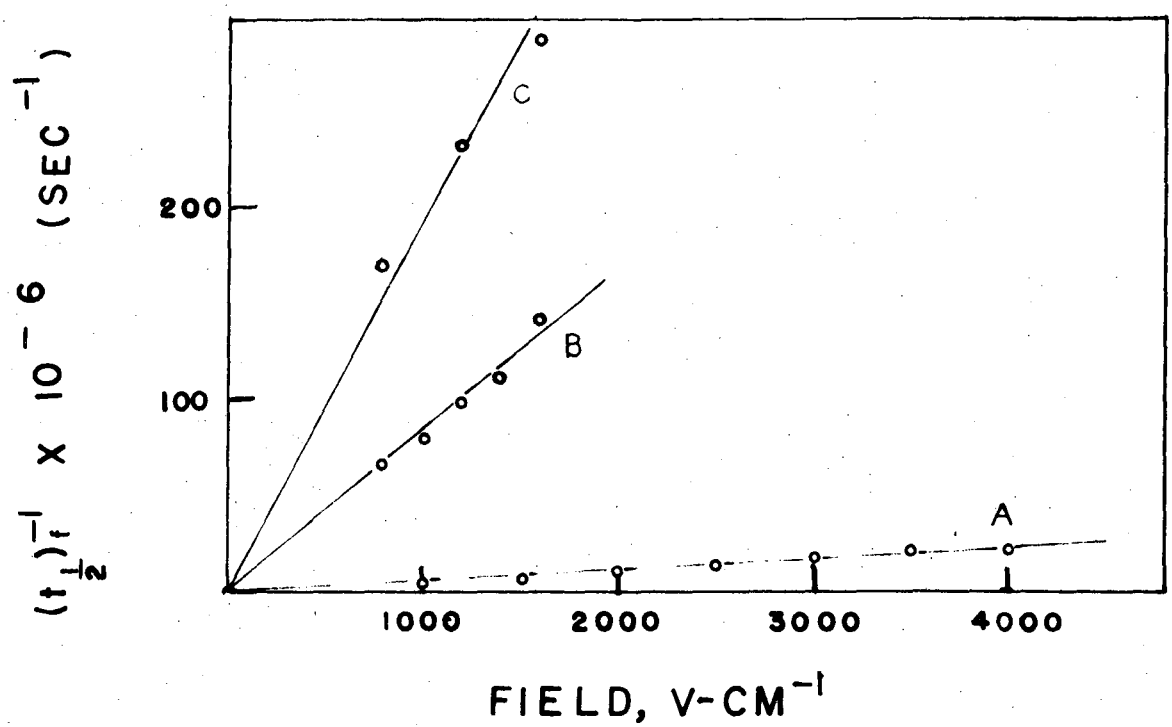

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Fig. 8. Variation of reciprocal of time to reach one-half maximum EPL emission, $(t_{1/2})_f^{-1}$, as a function of applied electric field. Curves are for chloroplasts suspended in water with no treatment (A), tris-washed aqueous chloroplasts (B), and tris-washed chloroplasts with 10^{-5} M o-phenanthroline added (C).

which had different levels of electron transport integrity compared to the untreated aqueous chloroplasts. These were aqueous tris-washed chloroplasts (curve B, Fig. 8) and aqueous tris-washed chloroplasts to which had been added o-phenanthroline (curve C, Fig. 8). Tris-washed chloroplasts have a disrupted flow of electron transport between water and PS II (Yamashita and Butler, 1968), while o-phenanthroline is believed to block electron flow from the primary acceptor of PS II (Bennoun, 1970). All three curves in Fig. 8 are linear over the field values which were applied.

As the temperature is lowered, the $(t_{1/2})_f$ times increase. Table 1 summarizes the changes in the $(t_{1/2})_f$ parameter for the same preparations used for Fig. 8. Activation energies were calculated for the three different preparations and are noted in Table 1. There was an apparent discontinuity in the $(t_{1/2})_f$ parameter for tris-washed chloroplasts at 30°C, which manifested itself as an almost total inhibition of emission from tris-washed chloroplasts having 10^{-4} M o-phenanthroline added at that temperature. Anomalies in rates of delayed light emission (Kindergan, 1972) and the intensity of T-jump stimulated emission (Hardt and Malkin, 1973) have been previously noted in this temperature range.

The $t_{1/2}$ parameter was found to be extremely sensitive to addition of non-electrolytic solutes such as glycerol or sucrose. Table 2 shows the variation in $(t_{1/2})_f$ with several low concentrations of sucrose. Addition of similar concentrations of electrolytic solutes such as NaCl or KCl did not change the overall appearance of the emission curve, but resulted in lower emission intensities. These lower intensities are thought to reflect electrode-electrolyte resistance phenomena, and

TABLE I

VARIATIONS OF HALF-MAXIMAL RISE TIMES WITH TEMPERATURE AND TREATMENT

Samples used were aqueous (H_2O), Tris-washed (TW), and Tris-washed with 10^{-5} M o-phenanthroline added.

T°C	$(t_{1/2})_f^{-1}$		
	H_2O	TW	OP + TW
0	0.0165	0.0643	0.165
10	0.0281	0.0694	0.188
20	0.0446	0.0735	0.234
30	0.0608	0.141	-----
E_a	5 ± 2	1 ± 0.5*	3 ± 1

*Calculated from 0, 10, and 20°C data only

TABLE II

VARIATION OF $(t_{1/2})_f$ WITH ADDITION OF SUCROSE AT 0°C.

% Sucrose added	$(t_{1/2})_f$, μsec
0	60.0
1	284.0
2	640
5	1070

inter-electrode potential drop measurements using a vacuum tube voltmeter were consistent with this idea. More than 90% of the potential drop occurs across the bulk of the solution in the case of aqueous samples, whereas less than 50% of the potential drop occurs across the bulk of the solution for salt concentrations higher than 10 mM. The rest of the potential drop occurs at the electrode-electrolyte interface.

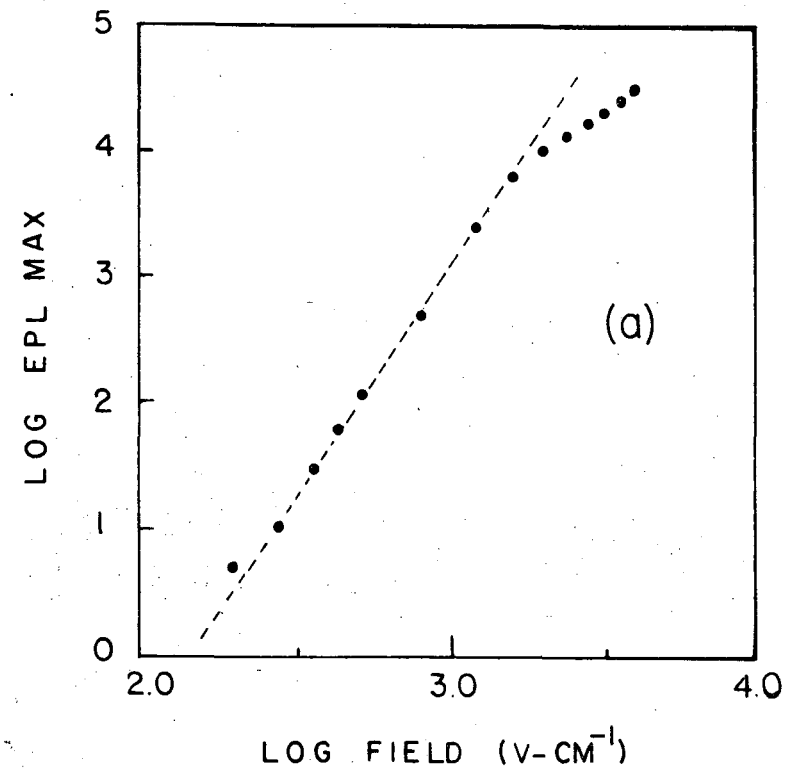
2. Field-off Decay of EPL

When the field is removed, EPL emission relaxes during the time t_r back to a non-perturbed level. The decay is characterized by a single exponential component having a half-life of 38 ± 3 μsec at 20°C, and about 50 μsec at 3°C. The decay rate was measured after a 500 μsec unipolar pulse and also after the reversed field of a bipolar pulse. Both results were identical to within experimental error. The decay appeared to be independent of field strength.

3. Field Dependent EPL Maxima

A superlinear relation between emission intensity and applied field was observed under all circumstances. For aqueous chloroplasts, the maximum emission intensity varied approximately as the cube of the applied field for field values less than 1600 $V\text{-}cm^{-1}$. The intensity tended towards an exponential behavior up to the maximum field magnitudes employed, about 4000 $V\text{-}cm^{-1}$. See Fig. 9(a) and (b). Inter-electrode arcing phenomena occurring at higher field values prohibited obtaining any further information.

The possibility that the field-on decay of EPL emission is composed of more than one component was shown when sequential bipolar field pulses were applied to aqueous and STN chloroplasts.



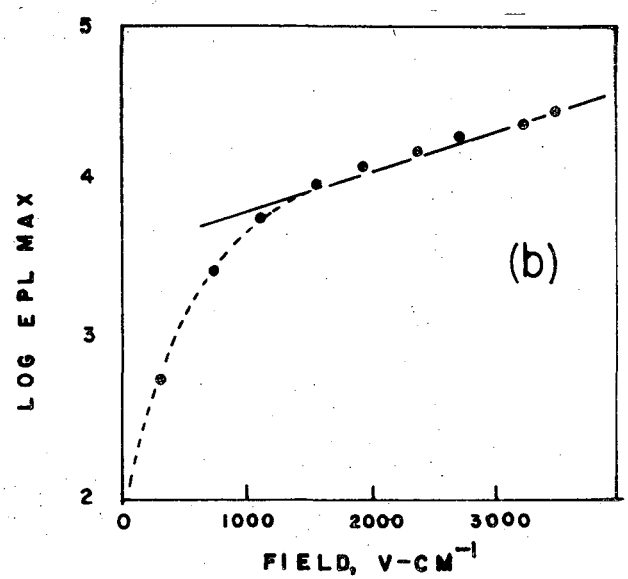


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Fig. 9. Log-log, (a), and semi-log, (b), plots of maximum EPL emission measured during a 1 msec, 1600 V-cm^{-1} field pulse as a function of applied field.

C. BIPOLAR EPL EMISSION

An electric field pulse of equal magnitude but opposite sign was applied to aqueous chloroplasts directly after the initial pulse, resulting in the emission shown in Fig. 10. In the case of aqueous chloroplasts the time dependence of the emission resulting from the reversed pulse is clearly different from that obtained from the initial pulse. The total emission intensity is lower and has both a longer rise time and slower decay during the field pulse. The same result obtains whether the first pulse is negative or positive with respect to a given electrode. If, instead of a reversed pulse, a sequential unipolar pulse is applied, the observed EPL emission is affected only when the interpulse spacing is long enough compared to the 38 μsec field-off decay to allow for a reasonable reduction in intensity, as shown in Fig. 11(a)-(c). These results are to be compared to the bipolar EPL emission shown in Fig. 11(d). Thus it is the change in sign which is the significant factor. It should also be noted that although the rise time for the second unipolar pulse appears to be as rapid as for the initial pulse, the peak intensity is never as great as the first pulse; a depletion effect is indicated.

The response of STN chloroplasts to a bipolar pulse (Fig. 12) is much more symmetric than bipolar EPL response from aqueous chloroplasts, apparently from a lack of the initial fast-rising, high intensity emission exhibited by aqueous chloroplasts. These results, plus others to be discussed below, suggest that EPL emission from aqueous chloroplasts consists of the sum of two different components: a fast rising component having a high maximum intensity and a slower rising component having a lower maximum. The reversal of field stimulates only the

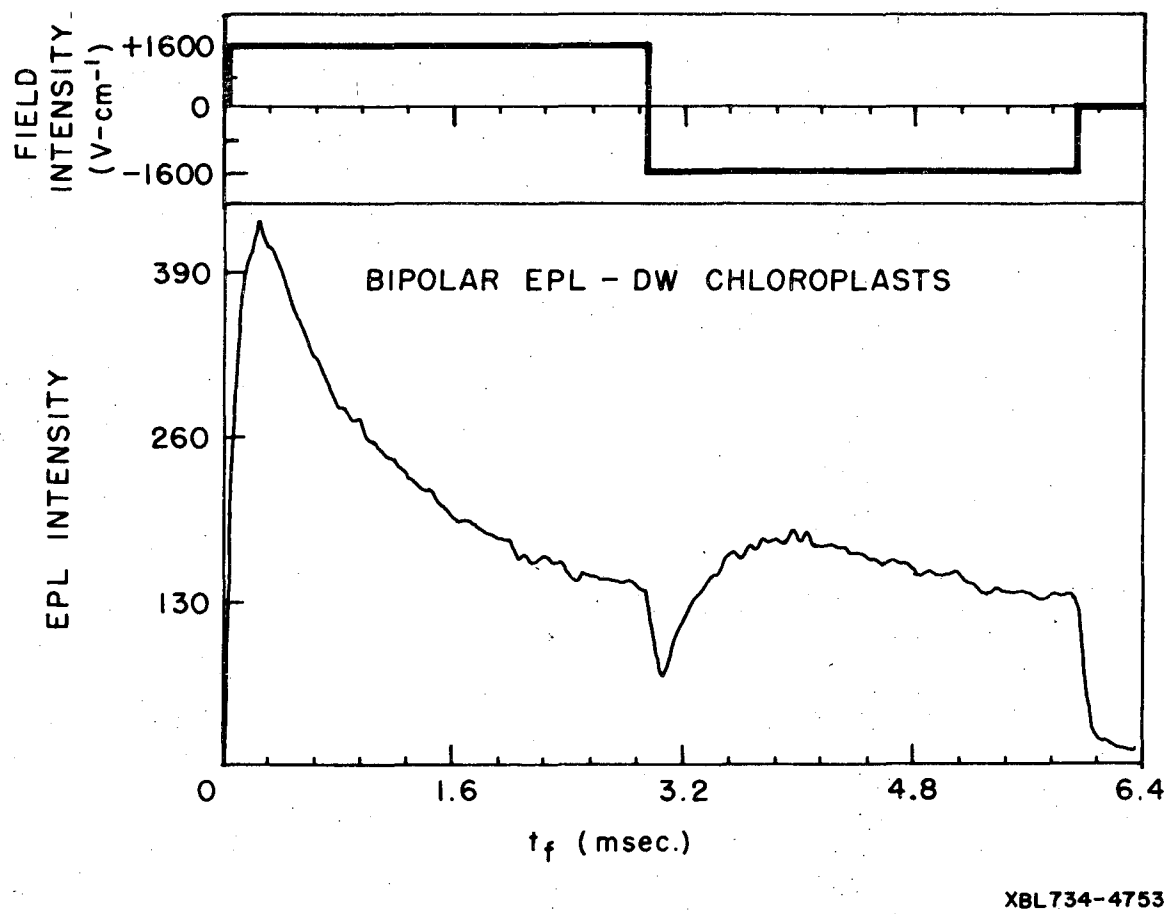

Fig. 10. EPL emission from aqueous chloroplasts resulting from a 1600 V-cm^{-1} bipolar electric field pulse (top). Other conditions the same as in Fig. 6.

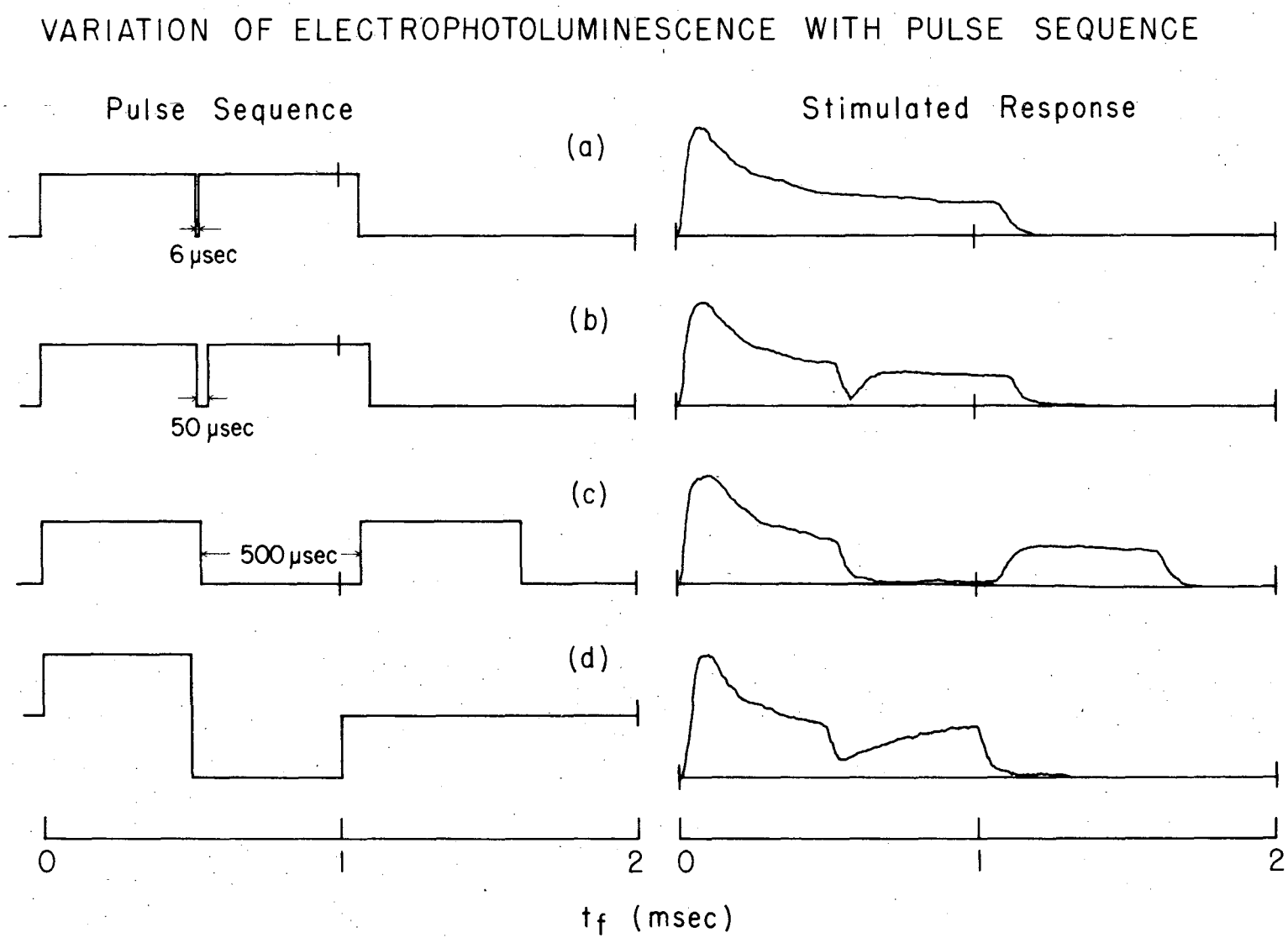

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Fig. 11. Variation of EPL with delayed unipolar pulse sequences, left, (a)-(c), compared to bipolar emission shown in (d).

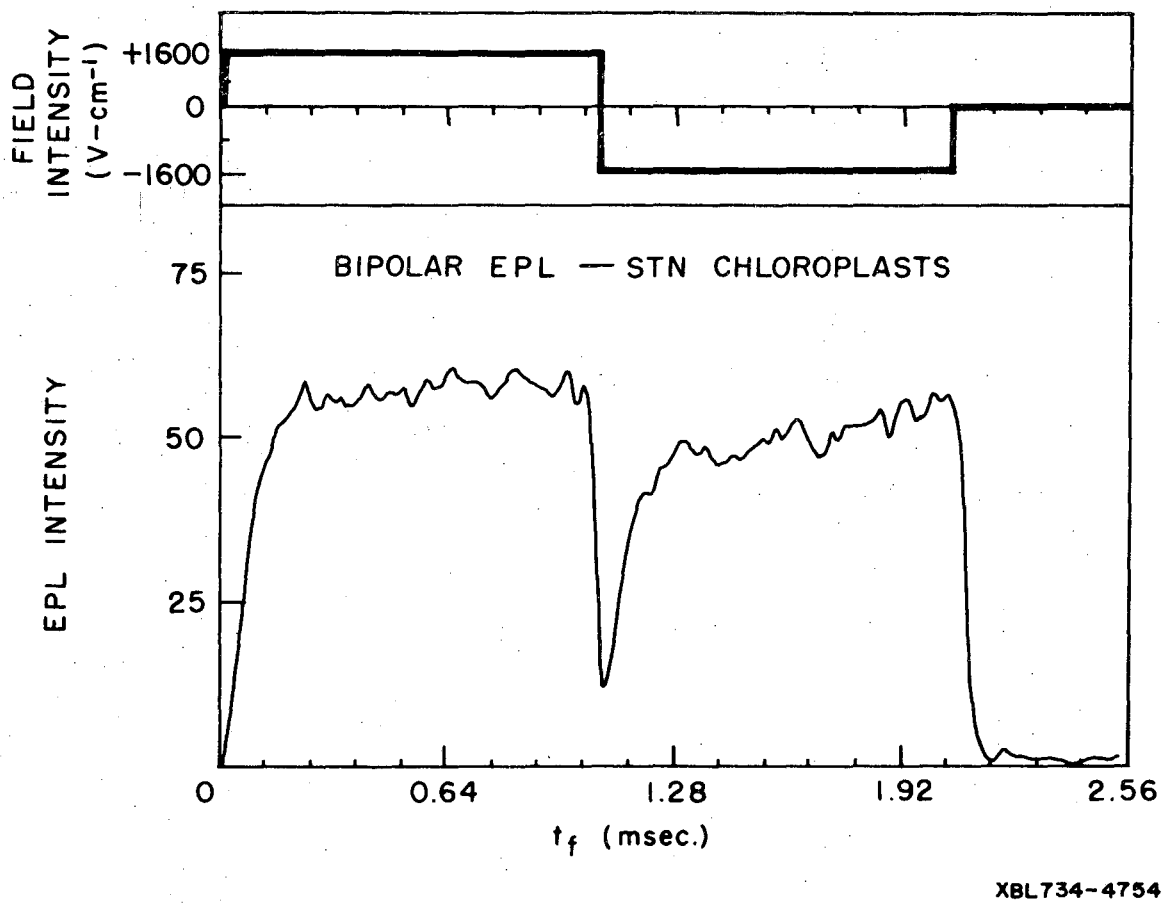

Fig. 12. Bipolar EPL emission from chloroplasts suspended in STN buffer. t_d and t_p times similar to Fig. 6.

slower of these two components. The emission from STN chloroplasts further suggests that the contribution from the slower component is the same regardless of field direction. Subtraction of the slower reversed component (curve B, Fig. 13) from the total initial pulse EPL (curve A, Fig. 13) results in a point by point difference, which, when plotted on a semilog scale, exhibits a single expotential decay (Fig. 13 (b)). These data support the hypothesis that the first emission pulse from chloroplasts has at least two separate components, and that selection of proper conditions should allow for their separation. That there is indeed a separate fast component is further verified by interposing a zero field spacing between the initial and reversed pulse, as shown in Fig. 17. After a 1.7 msec zero field spacing, the emission from the reversed pulse is observed to contain a larger fast component emission compared to a reversed pulse occurring only about 100 μsec after a short initial pulse.

Various treatments were also used to try to separate the two components. Tris-washed chloroplasts show one of the more dramatic effects. Fig. 14(a) shows typical unipolar EPL response from tris-washed aqueous chloroplasts. The response consists of what appears to be a large initial transient with a rapid decay superimposed upon a lesser component with a retarded decay. Treatment of these tris-washed chloroplasts with 10^{-7} M gramicidin D results in the abolition of the fast decaying transient, Fig. 14(b), but appeared to have little effect on the lesser component. The latter is observed to have a rapid rise.

A parallel effect is observed with untreated (i.e., no tris-washing)

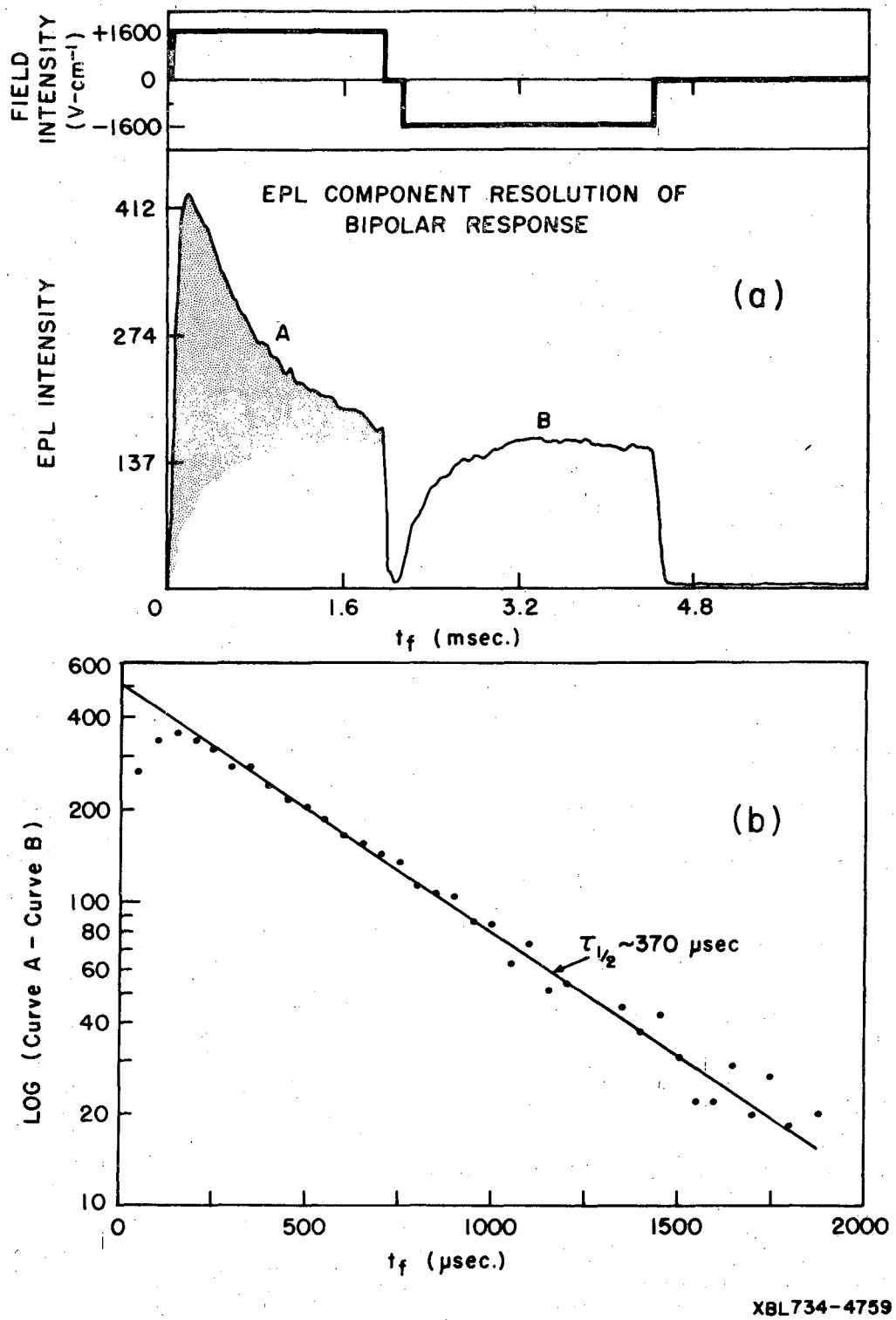

Fig. 13. Delayed bipolar emission (a) resolved into two components by subtracting Curve B from Curve A. The shaded portion of A is the residual. (b) Point by point residuals of Curve A minus Curve B plotted semilogarithmically against time.

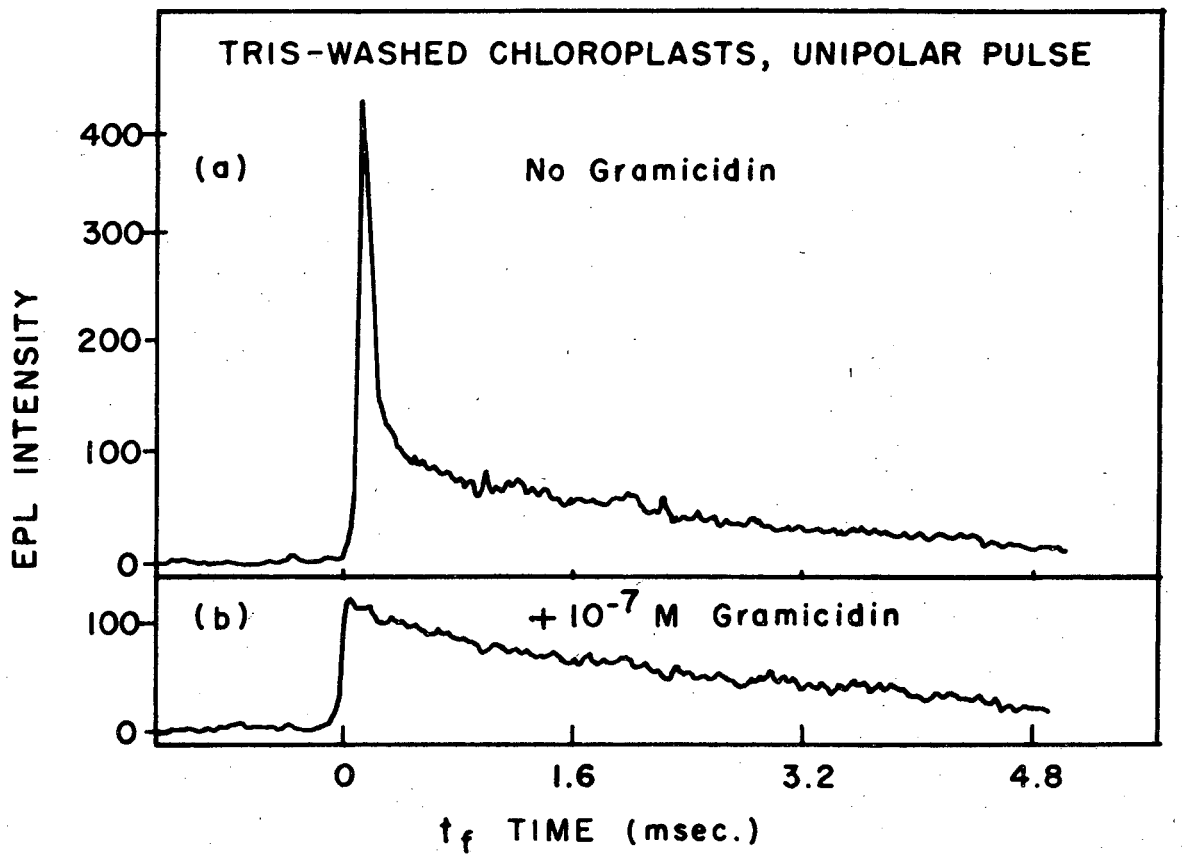

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Fig. 14. EPL emission from tris-washed aqueous chloroplasts which have no additions, (a), and to which has been added 10^{-7} M gramicidin, (b). The field pulse was a 5 msec unipolar DC transient. Chlorophyll concentration was ~100 μg Chl/ml.

aqueous chloroplasts. A series of bipolar EPL emissions from aqueous chloroplasts treated with varying amounts of gramicidin D is shown in Fig. 15. Even though the intensity as a whole is decreased, it is clear that the relative size of the fast rise component is more strongly inhibited.

A comparison of EPL emissions (Fig. 16) shows that an increase in viscosity resulting from addition of 30% glycerol suppresses the fast transient for both aqueous and tris-washed chloroplasts. In the case of the tris-washed chloroplasts, where little emission is seen upon field reversal, an increase in viscosity appears to restore the emission kinetics to a nature akin to that of untreated aqueous chloroplasts.

D. PARALLEL EFFECTS ON A_{515} and EPL

The electric field related nature of EPL and A_{515} (see INTRODUCTION) suggest that, if the origins of the two phenomena are related, conditions which are known to affect A_{515} should have parallel effects on EPL. For instance, the A_{515} phenomenon is highly dependent upon the integrity of the membrane. Depending on whether intact chloroplasts (which still retain their outer membrane (Larkum and Bonner, 1972)), broken chloroplasts (Schliephake *et al.*, 1968) or sub-chloroplasts preparations (Neumann *et al.*, 1970) are used, different A_{515} characteristics are observed. The integrity and condition of thylakoid membranes is also found to be a critical factor in determining the nature of EPL emission which can be obtained from a particular preparation. Sonication, for instance, totally annihilates EPL emission, even though delayed light emission from these same chloroplasts is clearly evident.

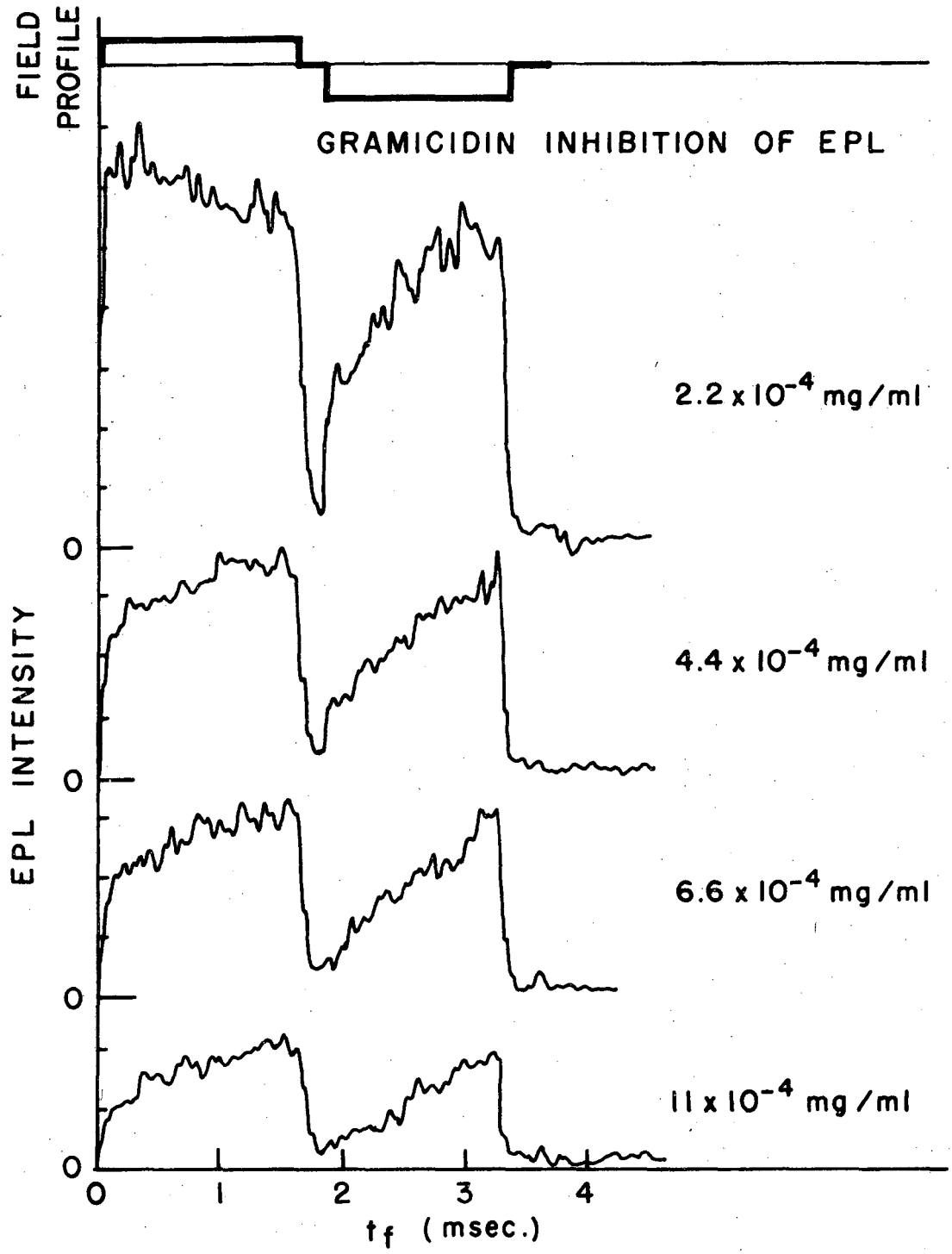

Fig. 15. Inhibition of EPL emission from aqueous chloroplasts using varying amounts of gramicidin noted in the figure. Chlorophyll concentration was ~ 50μg Chl/ml.

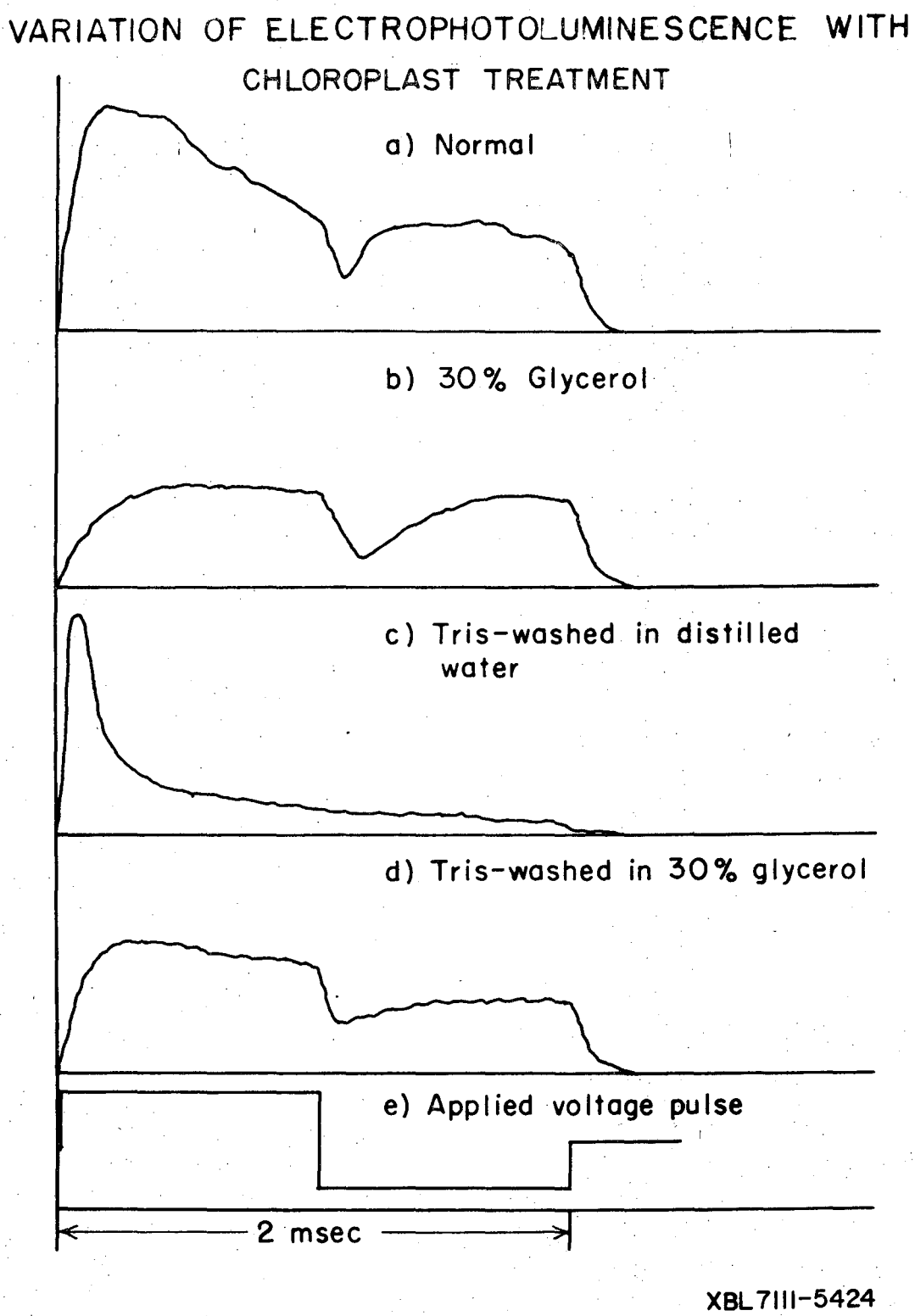

Fig. 16. Variation of EPL emission from aqueous chloroplasts treated as noted in the figure. Chlorophyll concentration was ~ 50 μg/ml.

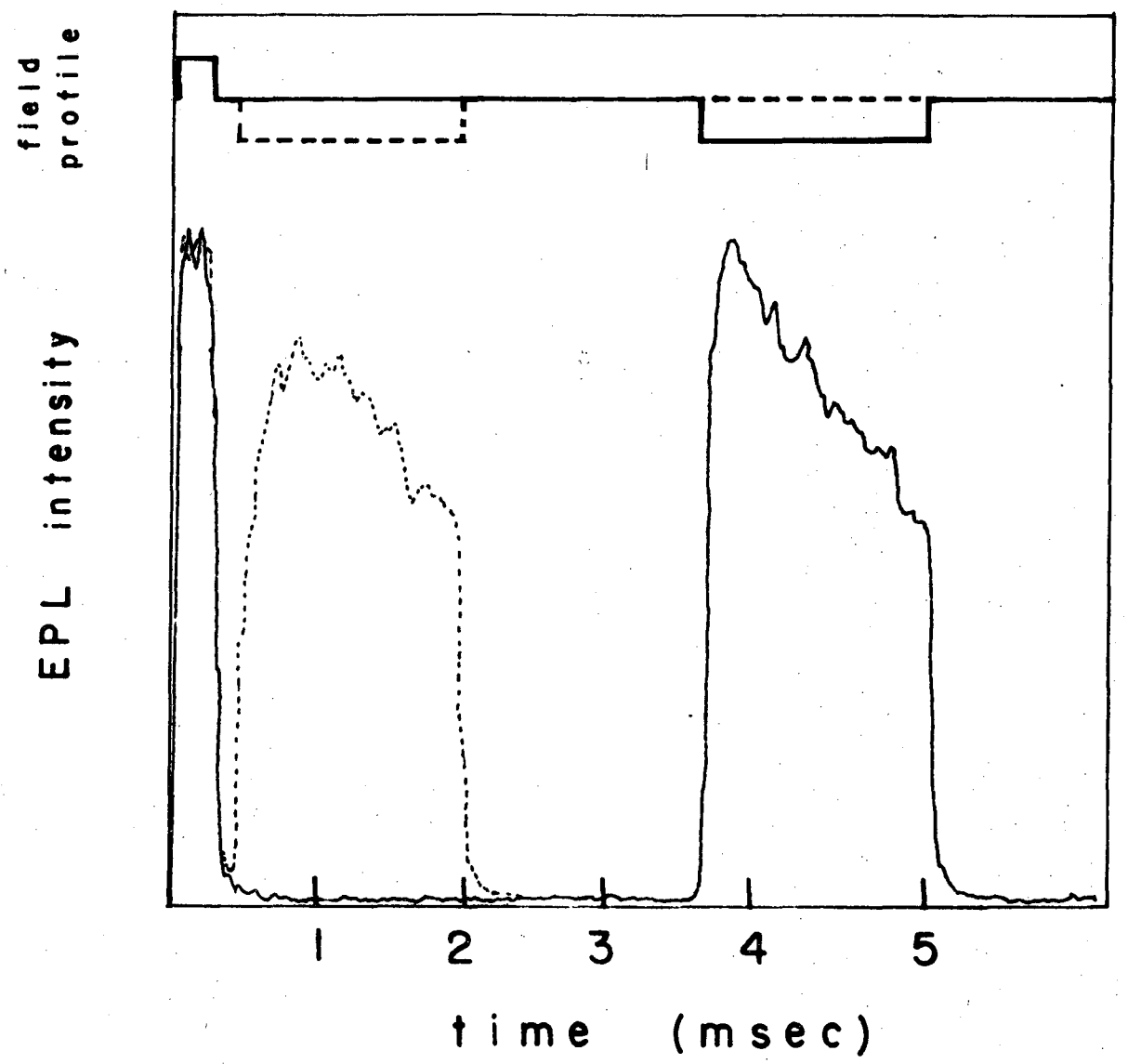

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Fig. 17. Asymmetric delayed bipolar EPL responses resulting from a zero field spacing of 100 μsec (dashed curve) and 3.2 msec (solid curve), showing recovery of fast component emission after an initial field pulse.

Solubilization of the membrane by detergent treatment is a potent annihilator of both A_{515} changes and EPL. Kraan et al., (1970) reported that low (0.01%) concentrations of Triton X-100 effectively abolish steady-state A_{515} changes. A similar concentration of Triton X-100 totally abolishes EPL emission. Normal delayed light emission is not seriously affected at these low detergent concentrations (Barber and Kraan, 1970).

Junge and Witt (1968) have shown that suspension of chloroplasts in a hypo- or hyperosmotic medium resulted in a flash-induced absorbance change which was dominated by a fast decaying expotential component with a $t_{1/2}$ = 50 msec component that dominated the decay. A comparison between the EPL emission from aqueous and STN chloroplasts suggests that osmolarity could be an important factor. However, it has already been shown that a viscosity effect may dominate in this situation. At low osmotic strength, it would appear that viscosity is a dominant factor, since glycerol or sucrose causes marked kinetic changes in EPL emission, whereas NcCl or KCl at similar concentrations have little or no effect on the kinetics of EPL emission.

A great deal of the A_{515} literature is based upon the effects of various ionophores upon the absorbance characteristics. Ionophores such as gramicidin and valinomycin affect the membrane functionality by making the membrane permeable to certain ions. Gramicidin is one of the most potent substances known to affect A_{515}. Concentrations as low as 10^{-10} M have been claimed to be effective in altering the characteristics of this spectral change (Junge and Witt, 1968). The basic effect of the ionophore is to hasten the decay of the light-induced absorption change, presumably

by rendering the membrane permeable to all common univalent ions (Junge and Witt, 1968). This compares to the suppression of the fast component emission noted in Fig. 14(b) and Fig. 15.

Valinomycin is selective for potassium ion transport, and when added to preparations also containing only endogenous potassium (Strichartz and Chance, 1972) valinomycin can effectively inhibit A_{515}. Presumably, since valinomycin would allow unimpeded potassium flow, a photoinduced electric field would be countered by a flow of potassium ions in response to that field. However, valinomycin (10^{-7} M to 10^{-5} M) was found to produce no noticeable effect on EPL emission either when it was added alone or in the presence of added potassium ion up to 10 mM.

CCCP (carbonyl cyanide-m-chlorophyenylhydrazone) affects membrane permeabilities and is thought to be a hydrogen ion ionophore (Vredenburg, 1969; Henderson et al., 1969). It also uncouples photophosphorylation (Avron and Neumann, 1968; Mitchell, 1966), inhibits the light-induced pH gradients formed across photosynthetic membranes (Avron and Neumann, 1968), and is known to accelerate the decay of A_{515} (Neumann et al., 1970; Fleischman and Clayton, 1968). Concentrations of CCCP which have noticeable effects on these responses are in the 10^{-8} to 10×10^{-6} M range. CCCP added in the range of 10^{-7} M to preparations of aqueous chloroplasts begins to inhibit EPL; at concentrations of 10^{-5} M all EPL emission is absent. Delayed light emission was also observed to be strongly inhibited at these concentrations of CCCP.

Evidence was cited in the INTRODUCTION which indicates that the A_{515} shift is an electrochromic response of carotenoids to an electric field. No evidence has been presented, however, that the pigment is

actually responsible for the formation of the field, but just the indication of it. EPL emission does not appear to depend upon the carotenoid pigments, since chloroplasts which had been extracted according to Strichartz' method still show what appears to be an unaffected EPL signal.

E. LIGHT DEPENDENCE OF EPL EMISSION

The preillumination dependence of the emission intensity after a given t_d and for a given field intensity demonstrates a saturation behavior shown in Fig. 18. The points on the lower part of the curve are proportional to the square root of the excitation intensity, as shown in Fig. 19. Similar light dependence has been reported for the 1 msec (Clayton, 1968) and 5 msec (Ruby, 1971) delayed light emission, and HCl-included emission (Hardt and Malkin, 1973).

The entire kinetic character of EPL was found to be independent of light intensity. Curves obtained under identical conditions except for a difference of nearly two orders of magnitude in preilluminating light intensity were found to be superimposable after normalization with respect to intensity.

F. DARK DECAY OF EPL CAPACITY

1. Normal Decay

Chloroplasts lose their capacity to respond to electric fields with increasing time, t_d, in the dark following illumination. As was already discussed in the INTRODUCTION, delayed and stimulated delayed light emission have been reported to decay via second order kinetics. Figure 20 shows that EPL emission also follows this trend: if the

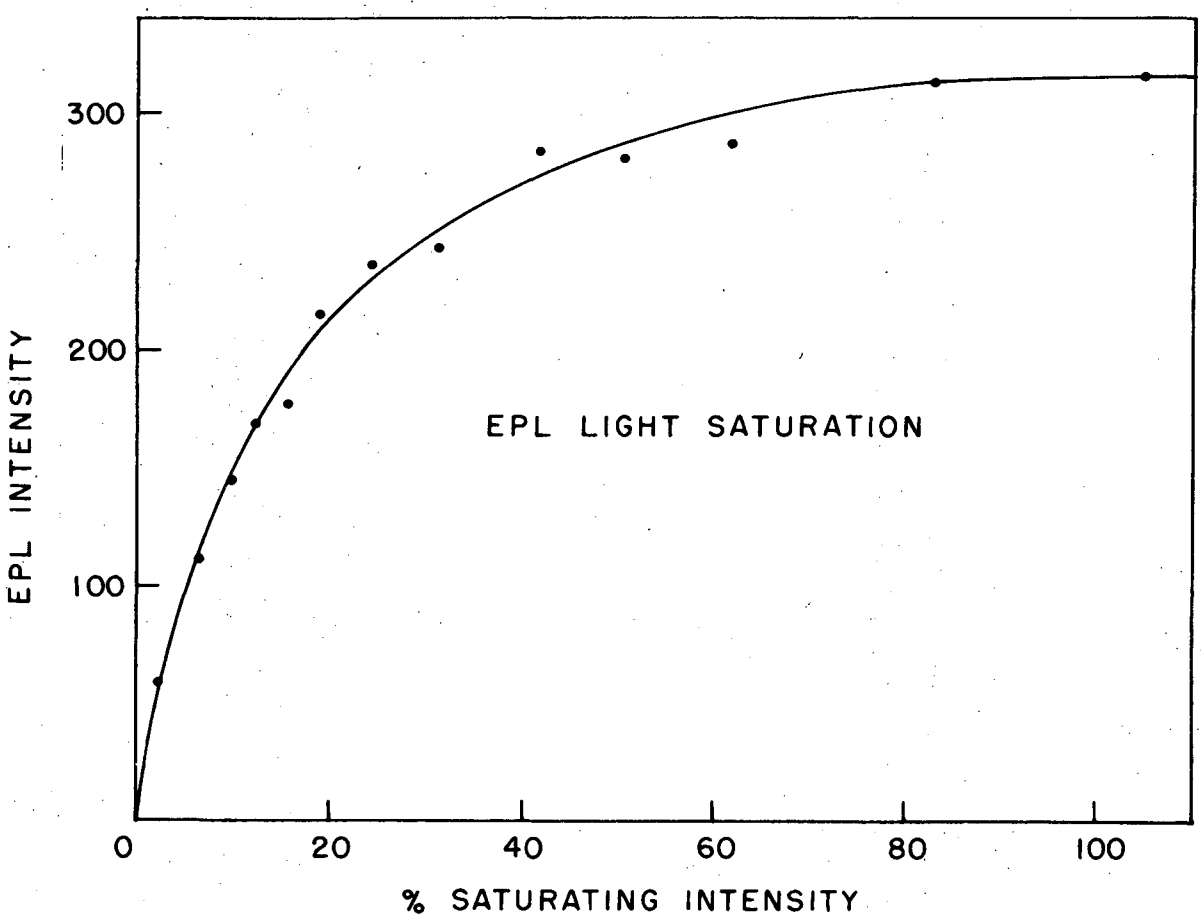

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Fig. 18. EPL emission maxima measured for varying intensities of broad band blue light. Maximum intensity used was about 1 mWatt-cm^{-2}-sec^{-1}. Preillumination time was about 4 sec. Chlorophyll concentration was about 10 μg/ml.

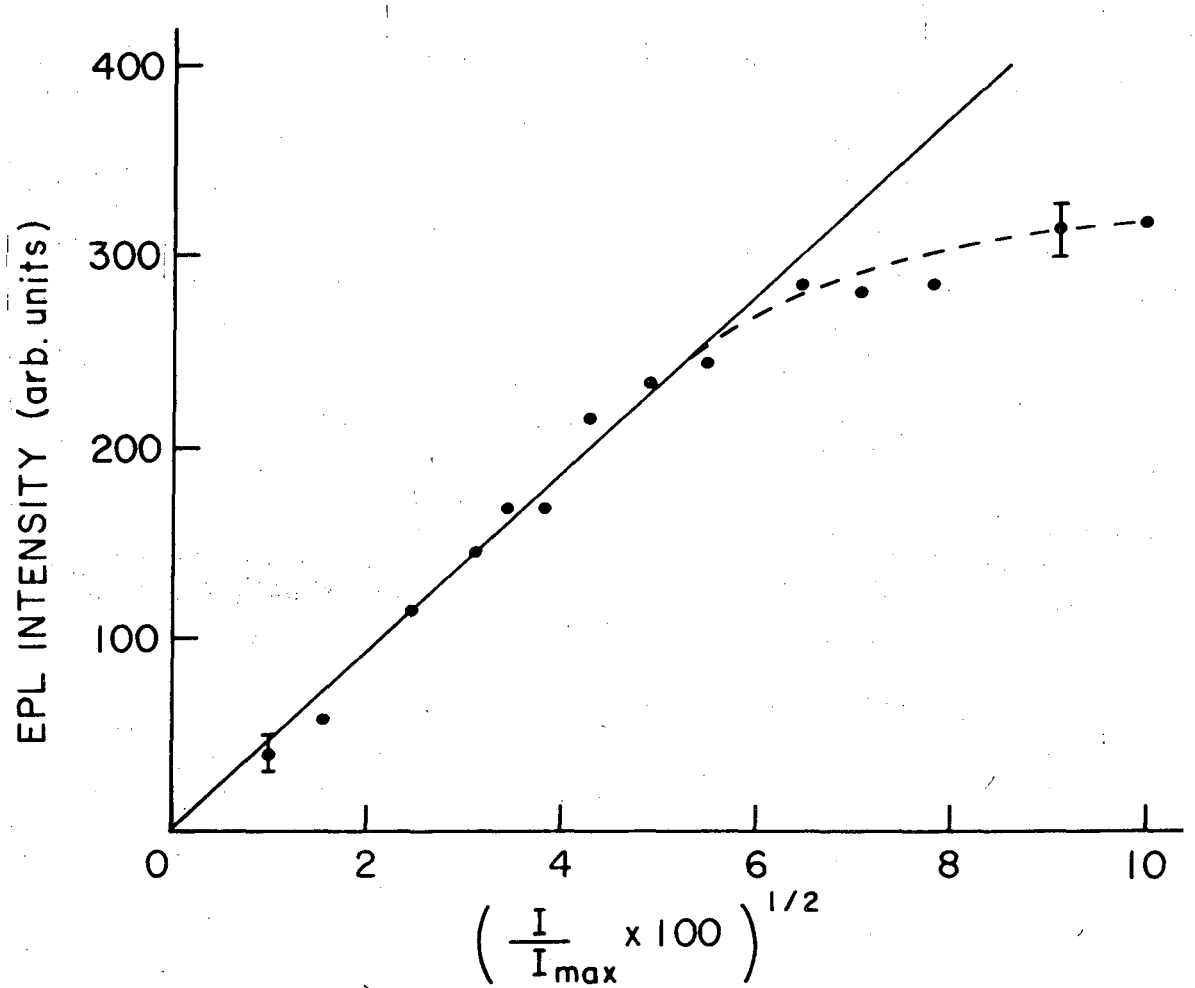

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Fig. 19, Data of Fig. 18 plotted as EPL intensity vs. the square root of preillumination intensity.

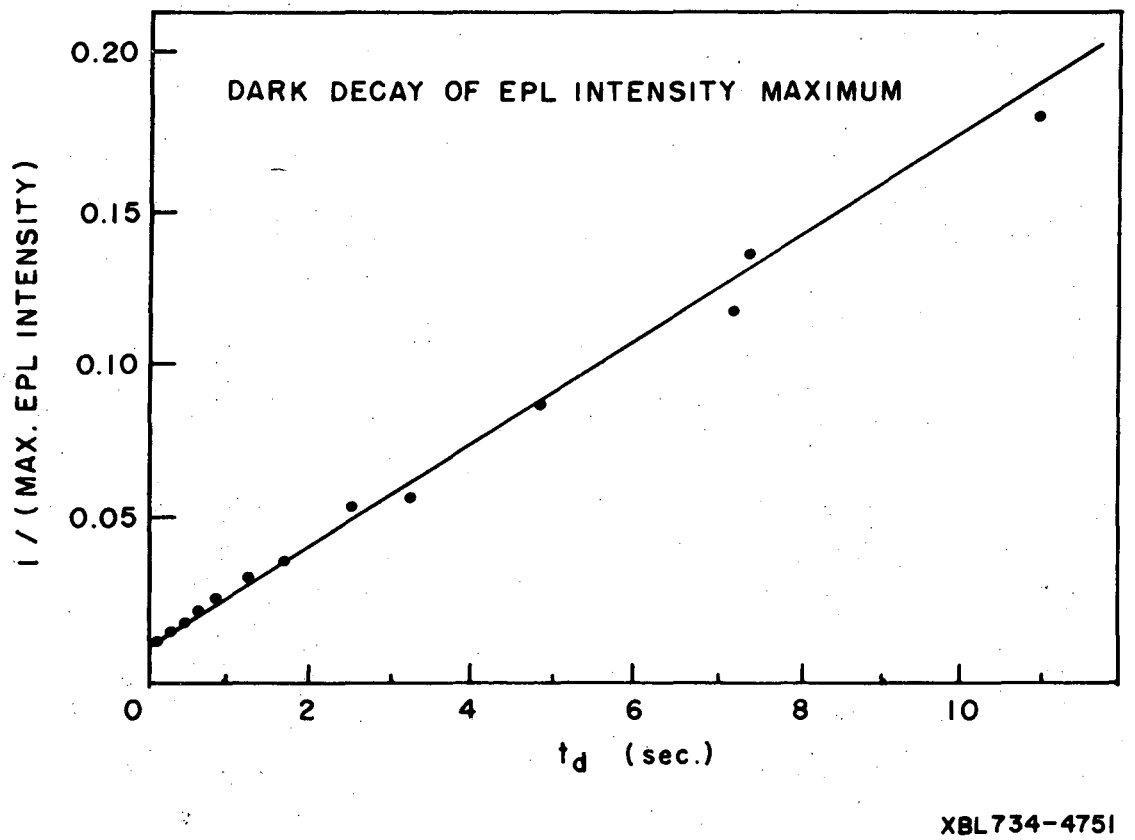

Fig. 20. Second order plot of the maximum EPL emission measured after a dark time t_d after preillumination.

time t_d is varied, with all other experimental parameters fixed, the reciprocal of the maximum emission level during t_f plotted against dark time t_d yields a linear relation.

The use of DCMU to selectively block flow of electrons from Q^- to the rest of the electron transport chain between PS II and PS I has been a classic criterion for determining whether a particular phenomenon is closely associated with the reducing side of PS II. It is consistent with the tenet that delayed light emission is a result of reverse electron flow through the ZChlQ complex that DCMU has been found to have various effects on delayed light emission, depending on the organisms and time period studied. Ruby (1971) lists literature reports concerning the effect of DCMU on various components of delayed light emission in chloroplasts and algae. The effect of 10^{-5} M DCMU upon delayed light emission from aqueous and STN chloroplasts is shown in Fig. 21. The effect of DCMU on STN chloroplasts, a general flattening of the emission decay curve and increase in intensity for t_d times > 0.2 sec, is similar to what has been reported in the literature (Clayton, 1969). However, the effect of DCMU on aqueous chloroplasts is different, in that a small decrease in intensity is observed, but the shape of the decay curve is not so obviously affected.

The delayed light emission from untreated and DCMU-treated STN and aqueous chloroplasts was measured and plotted on a second order scale shown in Fig. 22. In Fig. 22 the curves have all been normalized by dividing the actual time dependent emission curve by the maximum emission at the first measured time. The first point always has a value of 1. The only curve which does not plot linearly in the time range

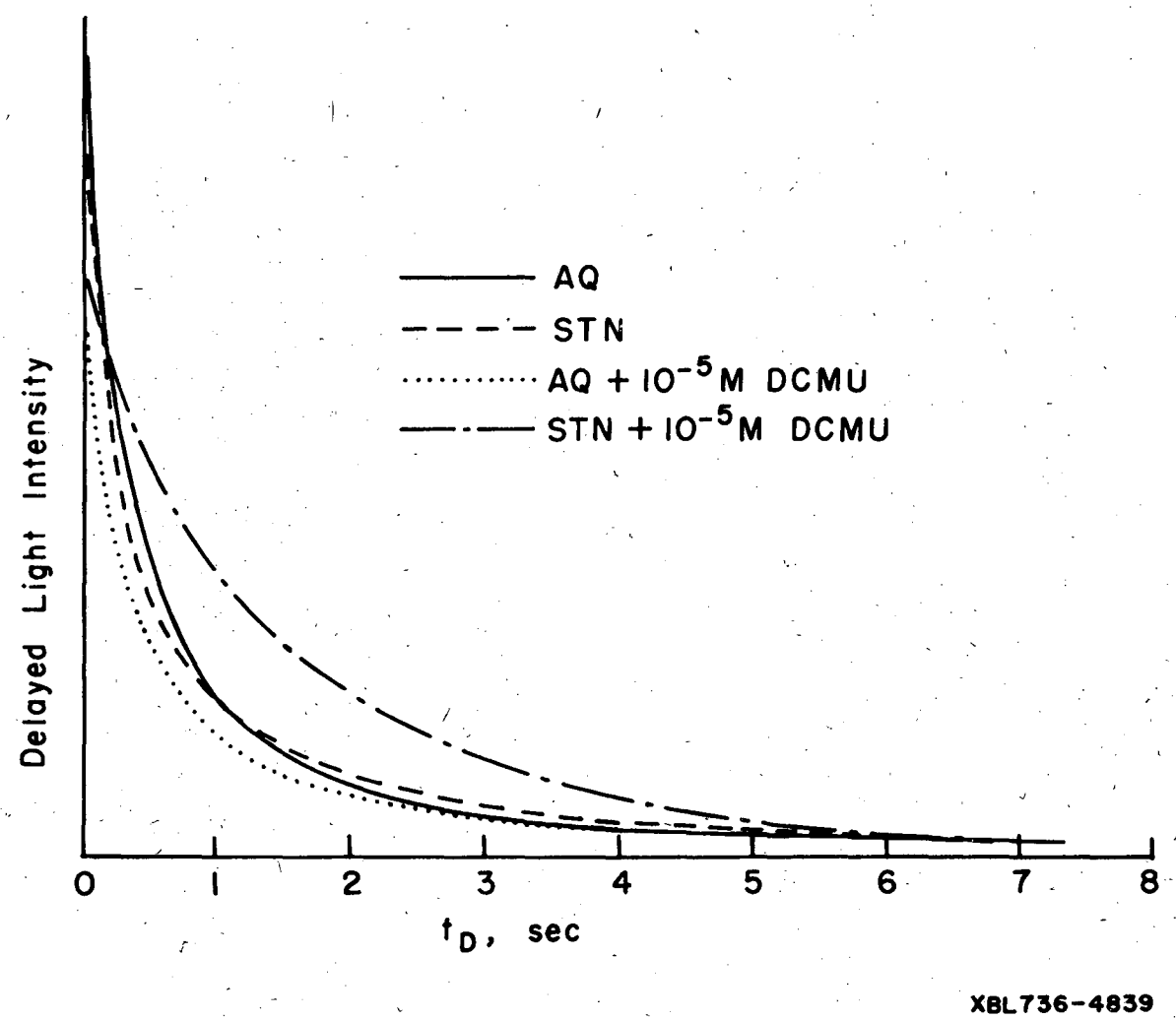

Fig. 21. Time decay of normal delayed light emission for chloroplasts suspended in water without (———) and with (·····) 10^{-5} M DCMU and chloroplasts suspended in STN buffer without (------) and with (- — - — -) 10^{-5} M DCMU. Chlorophyll concentration was ~ 50 μg/ml.

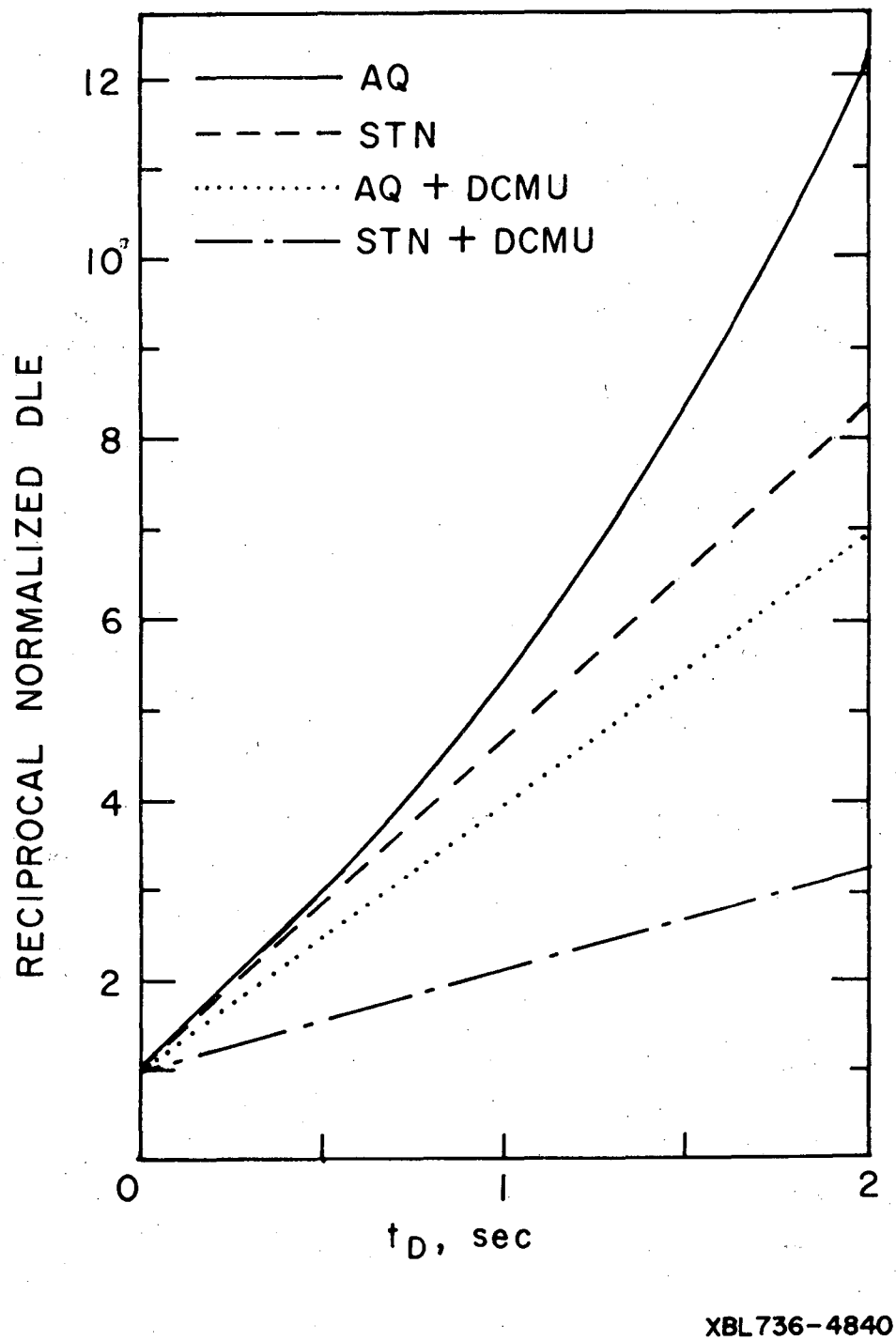

Fig. 22. Second order plots for emission curves in Fig. 21.

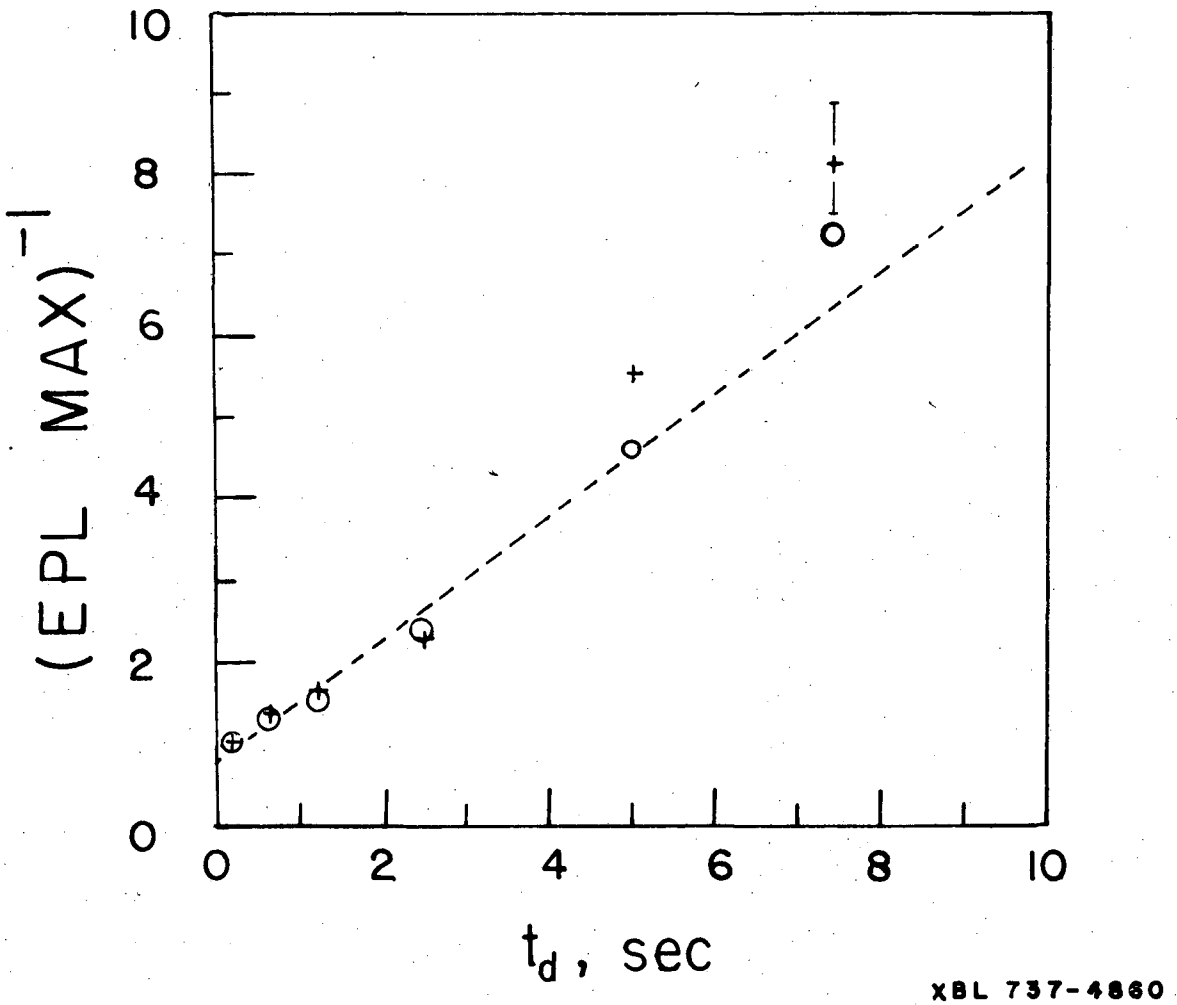

Fig. 23. Normalized (see Text) second order decay plots for EPL emission maxima measured after time t_d after preillumination. Open circles refer to untreated aqueous chloroplasts, crosses are data points for chloroplasts with 10^{-5} M DCMU added.

employed in Fig. 22 is the curve for the non-DCMU-treated aqueous chloroplasts. It also does not fit a single exponential decay.

2. Comparison to Delayed Light Emission

To compare EPL emission decay rate on the same scale as delayed light emission, the same normalization procedure was used. If the emission levels of EPL and delayed light differ only by a yield factor, that is, if

$$I_{EPL} = AI_{DLE}$$

where A is some proportionality constant, I_{EPL} is the EPL emission intensity and I_{DLE} is the delayed light intensity, then the two normalized curves should be superimposable. This would be the case if both delayed light emission and EPL were direct indicators for the same pool of metastable species.

EPL emission from control aqueous and DCMU treated aqueous chloroplasts was measured, normalized, and plotted on a second order scale shown in Fig. 23. Within experimental error the two curves coincide.

An inspection of Fig. 22 and Fig. 23 shows that none of the delayed light emission curves will superimpose on the EPL emission curve. The STN DCMU-treated sample has a decay of delayed emission which is closer to the EPL decay curve than those of the other two samples.

A control experiment was performed to make certain that the DCMU used was functioning as a photosynthetic inhibitor. Ferricyanide supported Hill reaction oxygen evolution from the same aqueous chloroplasts was monitored in the absence and presence of 10^{-5} M DCMU. Oxygen evolution was effectively abolished when the DCMU was added.

3. Single Flash Effects

A single saturating 10 μsec flash does not produce as much EPL emission as when EPL emission is observed after two or more saturating flashes which are spaced within several milliseconds of each other. However, if more than about 0.5 sec lapses between sequential saturating pulses, the EPL intensity declines so that the intensity observed after the last flash is no greater than that following a single saturating flash.

G. FURTHER INHIBITOR EFFECTS

If 10^{-5} M o-phenanthroline was added instead of DCMU, a striking change in emission character was noted, as shown in Fig. 24. The EPL rise time was accelerated, as was already noted in Fig. 8, and the peak intensity increased, but the field reversal component was similar to the untreated case. From the emission record it is apparent that either the fast component has been magnified and accelerated, or else a third component has appeared.

Dibromothymoquinone (DBMIB), is a relatively new inhibitor which has been shown to be Photosystem II selective (Gimmler and Avron, 1972) and is thought to be a plastoquinone antagonist (Bohme *et al.*, 1971). Addition of DBMIB resulted in complete inhibition of EPL emission; 3×10^{-5} M DBMIB reduced EPL intensity by 75%. All components of emission seemed to be equivalently reduced in magnitude.

Also consistent with the hypothesis that EPL is related to Photosystem II activity is the reduction in EPL intensity caused by addition of 10^{-4} M ferricyanide, which tends to cause flow of electrons away

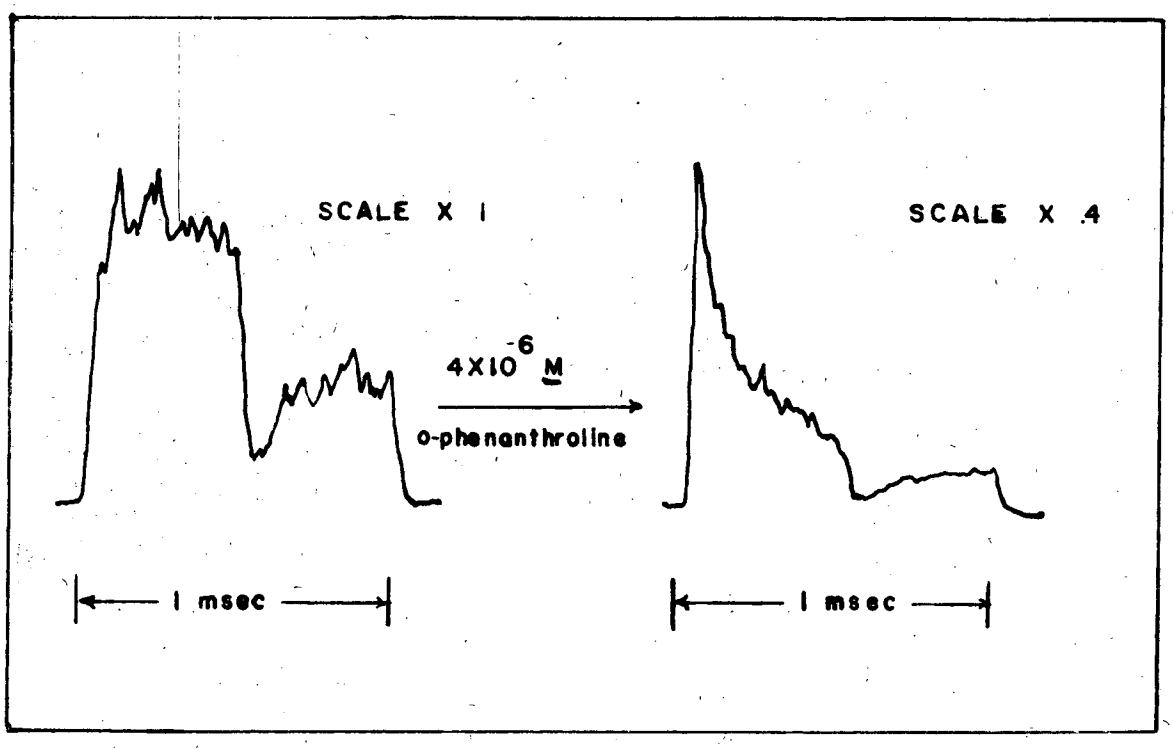

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Fig. 24. Comparison of bipolar emission for untreated chloroplasts, left, and the same preparation to which had been added 4×10^{-6} M o-phenanthroline. For comparison, the vertical coordinate of the figure on the right should be multiplied by 2.5.

away from Photosystem II by acting as an oxidant, and total inhibition of EPL emission by 10^{-4} M hydroxylamine, which is thought to quickly reduce any oxidized species created by Photosystem II reaction centers (Bennoun, 1971).

The only other organisms which were studied to determine whether they yield EPL emission were chloroplasts from leaves of both the normal and a Chl b-less mutant leaves of barley and the alga Chlorella pyrenoidosa. Both types of barley chloroplasts (aqueous) yielded EPL comparable with that from spinach chloroplasts. Chlorella did not yield any detectable emission. This result is in agreement with Arnold and Azzi (1971); they attributed the lack of emission from Chlorella to the insulating nature of the cell wall.

IV. DISCUSSION

A. EPL AND RELATED PHENOMENA

Electrophotoluminescence, like other reported forms of stimulated delayed light emission, is the result of an increased rate of conversion of stored chemical energy into light. In spite of the plenitude of observations reported in the literature, little light has been shed upon possible kinetic models which could explain the production of stimulated emission. The EPL experiments reported here, however, provide information in time domains which do allow speculations concerning possible mechanisms leading to electrically stimulated emission. By comparing the nature of the EPL phenomenon with stimulated emissions of other varieties, it may be possible to come to meaningful conclusions as to the pathways by which emission is stimulated.

We should then be able to clarify whether current photosynthetic models such as the "Z-scheme" can properly encompass such conclusions.

In trying to understand the EPL phenomenon, we want to fit the experimental observations within a reasonable scheme. We have shown that EPL emission depends upon:

1. the electric field. EPL emission is observed to appear within several microseconds after the rise of an external electric field. There is an induction period of about 50 μsec lasting much longer than the rise time of the field (<6 usec) leading to a maximum emission in times on the order of 100 μsec. The maximum EPL emission, which is observed after about 100 μsec, depends on E^3 for fields below about 1200 $V\text{-}cm^{-1}$, while an exponential character

is observed for higher field intensities. Two components appear to comprise the emission, one which decays faster (387 μsec half life at 1600 V-cm^{-1}) than the other (half decay ∿ 10msec). Removal of the field results in a decay in about 38 μsec. Immediate reversal of the field direction results in an emission that lacks the fast component. However, a zero field pause between initial and reversal pulses results in a return of the fast component when the reversed field is applied. The recovery of the fast component takes about 5 to 10 msec at room temperature.

2. viscosity. Retardation of onset kinetics and reduction of EPL intensity is observed when glycerol or sucrose is added to the medium. Chloroplasts suspended in STN buffer show a much reduced EPL emission and little fast component emission.
3. photosynthetic inhibitors. DCMU lowers EPL emission intensities but does not alter the kinetics. O-phenanthroline increases the maximum emission intensity and accelerates emission rise times. O-phenanthroline does not appear to affect the slower component. Hydroxylamine serves to annihilate emission completely.
4. membrane integrity. Detergent treatment, sonication and heat treatment all serve to annihilate EPL emission. The ionophore CCCP serves to abolish emission while gramicidin inhibits the fast component to a greater extent than the slow component. Valinomycin does not appear to have any noticeable effects on emission kinetics or intensities.

The emission capacity for EPL does not decay at the same rate that normal delayed light emission does, and an EPL emission experiment has

no noticeable effect on subsequent normal delayed light emission.

In order to interpret EPL in the context of other delayed emission phenomena, some of the published experimental observations concerning these emissions will be presented and compared to the characteristics of EPL.

1. Time Dependent Decay of Emission Capacity

The second order decay rate which was observed for the dark decay of EPL capacity is similar to the decay rate reported by Mayne (1968) for acid-base stimulated emission and by Barber and Kraan (1970) for salt-induced emission. Malkin and Hardt (1971) also measured the emission capacity for acid, acid-base, salt and T-jump perturbations. However, of these, the acid, acid-base, and salt emission capacity convincingly plotted on an exponential scale, while the reciprocal square root of thermally stimulated emission was found to be linear in time.

It is not clear why the acid and salt-induced emission data of Malkin and Hardt should plot according to one scale while that of Mayne and of Miles and Jagendorf should plot on a different scale, although Hardt and Malkin's experiments were performed on lettuce chloroplasts; the other experimenters used spinach chloroplasts. All of the literature data above are for decay times up to 35 sec.

By comparison, the decay of delayed light emission in the time range between 1 msec and 1 sec occurs via second order kinetics (Hardt and Malkin, 1972; Clayton, 1968; Mayne, 1968; Ruby, 1971). Relative maxima have been observed in delayed light emission measured for times greater than several seconds (Bertsch and Azzi, 1968). The only evidence that indicates that any stimulated emission is directly proportional to

to the level of delayed light emission is Arnold and Azzi's (1971b) report that the ratio of electrically stimulated emission to delayed light was nearly constant in time from 1 msec to 30 minutes. Unfortunately, few details were given about the conditions of the experiment. This is contrary to the results which were obtained by this author for aqueous chloroplasts (Cf. Fig. 22 and 23).

It is not exactly clear why Arnold and Azzi (1971a,b) found a direct relation between the intensity of EPL emission and delayed light emission, whereas we did not. They also found EPL saturation occurring at electric fields $\sim$ 600 V-cm^{-1}. No saturation was observed up to fields $\sim$ 4000 V-cm^{-1} in our work. One possibility certainly lies in the fact that Arnold and Azzi used continuous alternating current; continuous fields may produce other depletion effects which have not been observed with our pulse technique. In particular, the fast and slow components of EPL emission would not behave similarly to one another in response to an AC field.

2. Light Activation

The $I^{1/2}$ dependence of $I_{EPL}(t_d)$ is also paralleled by similar dependencies in the literature. Clayton (1969) reported an $I^{1/2}$ dependence for the 1 msec component of delayed light, and Ruby (1971) found similar relation for the 5 msec component of delayed light. Hardt and Malkin (1973) also present data for the intensity of HCl-induced emission as a function of the intensity of a single flash. The lower intensity data plotted on a $I^{1/2}$ vs. t_d scale also gives a reasonably linear relation. Mayne (1968) reported a light dependence for acid-base stimulated emission which he plotted according to the function

$L = L_{max}\,[1 - \exp(\alpha J)]$ with α a constant, J proportional to light intensity, and L the observed emission for a given J. His data also plot reasonably well on an L vs. $J^{1/2}$ scale. Mayne's data were from .5 sec flash activation experiments.

3. Effect of Inhibitors

In general, the effect of various inhibitors on stimulated emissions is less well documented than the effect of inhibitors on delayed light emission. However, many pertinent results have been recorded. Table III is a summary of the data discussed below. Table III contains information relevant to stimulation of emission resulting from a single preillumination and subsequent perturbation and also includes results regarding normal delayed light emission.

Although Miles and Jagendorf (1969) find that DCMU inhibits acid-base, acid, and high salt induced emissions, Malkin and Hardt (1973) claim that DCMU does not inhibit HCl- or methanol-induced emissions. This discrepancy may be due to Hardt and Malkin's interpretation of the word "inhibit." Hardt and Malkin's 1973 paper mentions a result reported by Jursinic and Govindjee (1972). Jursinic and Govindjee showed that DCMU reduces the magnitude, but does not totally annihilate stimulated delayed emission resulting from a temperature jump. Hardt and Malkin interpreted these results as meaning that DEMU does not "inhibit" T-jump stimulated emission. To clarify this point, any further use of the word "inhibit" will infer the common understanding of the term, which is to lessen or reduce a given response. Inhibition does not imply total abolition. In another report, Mayne and Clayton (1966) found DCMU to "abolish" acid-base induced emission.

TABLE III. Comparison of various emission types and factors which affect them.

EMISSION TYPE	ADDED FACTOR				
	DCMU	CCCP	HYDROXYLAMINE	GRAMICIDIN	VALINOMYCIN
Normal delayed light					
1-100 msec	I[i]	I[i]	K[k]	I[i]	I[f]
long term	S[j]	I[i]	I[k]		
Stimulated emissions				S[f]	
Salt	I[a,f]	I[a], S[f]			
Acid		I[a,f], N[c]			
Acid-base	I[a,h], A[b], N[c]	I[f], A[b]	I[a]	I[f,h]	S[f]
temperature-jump	I[c]	I[g]			
Organic solvent	N[c]	A[d]	K[c]	N[d]	N[d]
EPL	Slight I	A	A	I, K	N

I = inhibited, N = not inhibited, S = stimulated, A = abolished, K = altered kinetics

a. Miles and Jagendorf (1969)
b. Mayne and Clayton (1966)
c. Hardt and Malkin (1973)
d. Hardt and Malkin (1972)
e. Jursinic and Govindjee (1971)
f. Barber and Kraan (1970)
g. Mar and Govindjee (1971)
h. Mayne (1968)
i. Mayne (1967)
j. Clayton (1969)
k. Bertsch *et al.* (1969)

Miles and Jagendorf (1969) and Barber and Kraan (1970) found opposite results using CCCP. Miles and Jagendorf found 1.0 μM CCCP to reduce salt- and acid-induced luminescences, while Barber and Kraan found .1 μm CCCP to inhibit acid-base induce luminescence, but to stimulate salt-induced emission. Barber and Kraan also found gramicidin at .1 mg/l to have similar effects to those they found for CCCP. Mayne and Clayton (1966) reported abolition of acid-base stimulated emission using 2.7 μM CCCP.

Depending on the type of stimulated emission observed, different results have also been obtained for addition of hydroxylamine. Whereas Mar and Govindjee (1971) found hydroxylamine strongly to inhibit T-jump induced emission, Hardt and Malkin (1972) found that hydroxylamine or DCMU added alone did not inhibit organic solvent in induced emission, but added together, the two reagents abolished the stimulated emission. However, Hardt and Malkin (1973) found hydroxylamine alone to affect the nature of oscillations of short flash stimulated emission intensity as a function of flash number for acid- and methanol-induced emission.

Not listed in Table III are results obtained from salt perturbation of continuously observed 1 msec delayed light emission reported by Barber (1972). Those experiments differ in that the sample is continuously illuminated through a rotating sector wheel. Delayed light emission is observed while a sector momentarily blocks the actinic light source. Addition of concentrations of gramicidin greater than 3.3×10^{-7} M caused a complete inhibition in stimulation caused by KCl or NaCl addition. Addition of valinomycin also tended to inhibit emission, but had different magnitudes of effect for KCl and NaCl.

4. Comparison of Light Activation Dependencies

The question of whether multiple reaction types are involved in different emission types has been considered by Hardt and Malkin (1971, 1972). Their evidence indicated that delayed light emission and stimulated emissions occur via different pathways and reflected different precursors. Their conclusions were based on the following results:

The emission observed from acid-base, acid, or salt-induced perturbations rose to a maximum and then declined to lower values as the length of time of preillumination increased. Delayed light emission measured at 22 msec after the preillumination rose to a stable maximum and did not decrease as the length of preillumination rose to a stable maximum and did not decrease as the length of preillumination increased. While the emission intensity vs. time of preillumination curves for acid-base, acid, and salt-induced luminescences were pretty much superimposable, the curve for normal delayed light emission lagged the three other curves, and reached the maximum level with nearly an order of magnitude longer preillumination time ($\sim$ 1 sec as opposed to $\sim$ .1 sec). Variation in different light intensities and preillumination times emphasized the differences for delayed light emission and stimulated emissions. Although the yield of induced emission due to long flashes goes through a maximum as preillumination time increases, Malkin and Hardt found intensity saturation for HCl-induced emission following single microsecond flashes (Malkin and Hardt, 1973). They concluded from the above results that some induced emissions may result from secondary species formed as a result of long illumination times. These secondary species may interact with species which are produced as a

result of a single light act. Delayed light emission may reflect those species which have not reacted to form secondary precursors.

5. Sequential Perturbations

Reports concerning sequential application of different types of perturbation techniques also lead to the conclusion that different precursors and/or emission pathways exist. Miles and Jagendorf (1969) found that if an acid-base induced experiment is followed by a salt-induced experiment, or *vice versa*, the emission observed at the time of the second perturbation is always less than the emission which results if the other perturbation does not precede it. A competition between the two processes is indicated. In contrast however, Malkin and Hardt (1971) found that if either a salt or acid-induced luminescence precedes a temperature induced emission, almost no change in emission compared to the control occurs. In summarizing the conclusions from their data and that of Miles and Jagendorf, Malkin and Hardt concluded that four separate emission types must be involved to explain the various observations. These were acid-base or salt, acid, T-jump, and delayed light at 22 msec. The first two were distinguished by competition criteria, and the second two by differences in kinetics (Malkin and Hardt, 1971, 1973).

In view of the conflict in the literature as to what the decays of emission capacity for various luminescences really are, it is difficult to identify EPL emission as being directly related to any particular emission type. It is reasonable to suggest that the decay of EPL emission corresponds to a component of normal delayed light emissions. However, it is clear that EPL emission capacity does not decay with the same kinetics as does the *overall* delayed light emission.

Barber and Kraan (1970) have also shown that acid-base stimulated emission is more sensitive to detergent treatment than either salt stimulated emission or normal delayed light emission. They also found KCl induced emission to be more sensitive to heat treatment than either normal, acid or acid-base stimulated emission (Barber and Kraan, 1970).

Barber and Kraan also showed evidence that the back reaction of metastable species was apparently not due to a reversal of electron flow. If a direct reversal were responsible for emission, the yield of variable fluorescence should increase as reversed electron flow is stimulated, increasing the concentration of Q^- (see INTRODUCTION). However, in measuring the stimulated emission from chloroplasts subjected to acid-base or salt treatments, a concomitant increase in fluorescence was not noted (Barber and Kraan, 1970).

The factors mentioned above lead to the conclusion that different mechanisms of stimulation exist and that different emission types depend upon membrane integrity to differing extents. However, Malkin and Hardt's conclusions that separate precursors account for differences in light activation are valid only if light does not affect the separate decay pathways independently from the perturbation. For instance, the amount of emission generated by a pH jump could depend not only upon the concentration of a metastable energy species, but also upon the concentration of a species transported in the light. The emission intensity would then assume a behavior dependent upon the product of two separate concentrations. Thus the relative amount of pH (or salt, etc.) induced emission would not necessarily show a light dependence similar to that of normal delayed light emission, even though the same initial metastable state

was involved.

No clear pattern emerges from the accumulated data in Table III. However, no data have been found that indicate that stimulated emissions ever occur when normal delayed light emission does not. Nearly every investigator cited in Table III used different conditions of chloroplast preillumination and chloroplast preparation, and a proper comparison of all the data can only be made for similar experimental conditions. For these reasons one must be cautious in drawing conclusions only from similarities or dissimilarities of inhibition affects.

6. Absorption Changes - A_{515}

Because the two phenomena of EPL and the A_{515} changes reportedly arise from the presence of an electric field, it is profitable to explore the similarity of A_{515} and EPL experiments.

Strichartz and Change (1972) showed that addition of alkali cation salts to fragmented chloroplasts in the dark resulted in an absorption change very similar to the light-induced A_{515} change. DCMU did not inhibit the salt induced absorbance change at a concentration that inhibited 90% of the light induced change. Larkum and Bonner (1972) also found light-induced A_{515} for intact chloroplasts to be inhibited by DCMU and Valinomycin, while CCCP completely abolished the change. Valinomycin was found to stimulate salt-induced A_{515} changes at concentrations similar to those that inhibited A_{515} activated by continuous illumination (Strichartz and Chance, 1972), a probable result of enhanced decay rates noticed for laser flash-induced changes (Neuman *et al.*, 1970). Sonication diminished both light and salt induced changes (Strichartz and Change, 1972).

Junge and Witt (1968) observed that gramicidin transformed flash-induced A_{515} changes from slow component decay to fast component decay.

It is a formidable task to try to build a single framework on which to structure all of the data which have been presented. However, it is possible to ask whether present models are sufficient to account for EPL emission phenomena without contradicting other pertinent published data, and without invoking an entirely independent EPL mechanism disconnected from documented electron transport pathways.

B. A WORKING HYPOTHESIS

The outline of a working hypothesis in which we will try to incorporate the results which have been obtained is presented below. The overall scheme is shown in Figure 26. The elements of the hypothesis are:

1. Metastable energy is trapped in a state or species which we denote as M_1^*. The effect of the electric field is to produce a secondary species E_1' from a light activated form E_1. E_1' and M_1^* interact in a time less than 38 μsec in a second order field independent process with rate constant K_{12} to produce metastable species M_2^*. It is the formation of E_1' and subsequent reaction with M_1^* which is responsible for the induction (onset delay) upon turning on the field. Approximately 100 μsec elapse from the time when the field is turned on until the maximum concentration of E_1' is achieved. The interaction of E_1' with M_1^* is postulated to be viscosity dependent, which explains why viscosity decreases the EPL onset kinetics and reduced EPL intensity (Figurure *16* and Table II).

2. It is further postulated that the field-stimulated emission has

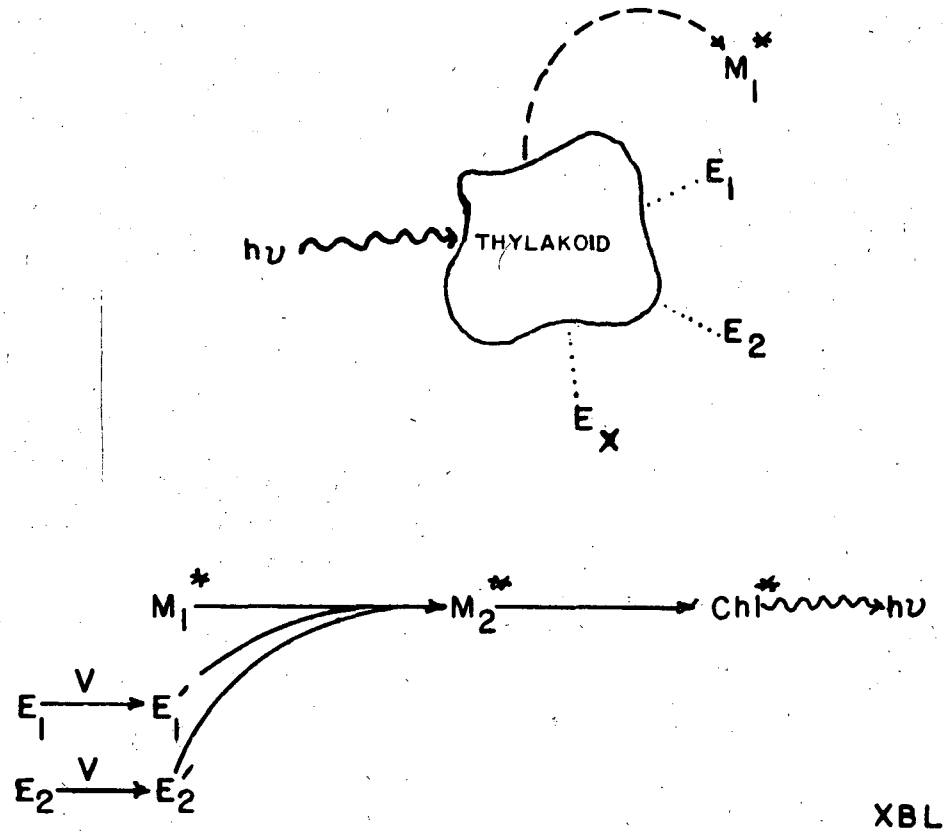

Fig. 25. Model system in which light produces a metastable energetic species M_1^* while at the same time light activated species E_1, E_2 and E_x are generated. E_1, E_2, and E_x could be light activated pH gradients, ion concentration gradients, etc. The production of stimulated emission is postulated to occur via the sequence at the bottom, where a field dependent change in E_1 and E_2 is followed by an interaction with M_1^* to produce M_2^*, which spontaneously produces an excited state chlorophyll molecule, Chl^*, which yields the EPL photon.

little effect on the total concentration of M_1^* ([E] total << $[M_1^*]$ total). As a consequence the field-on decay expression for the fast component, $K_{12}[M_1^*][E_1']$, is pseudo-first order in $[E_1']$, and the decay of the fast component appears as a first order process (Figure *13*). For the experimental conditions of Figure *13* the half-life of the decay of E_1' is about 370 μsec. This postulate is consistent with the observation that the application of an electric field pulse does not disturb the intensity of normal delayed light emission subsequent to the field pulse (figure 5).

3. M_2^* is a state through which *all* delayed light emission energy must pass and is a state that leads directly to the Chl singlet state. The conversion of M_2^* into Chl* is a first order, field independent process with a half-life of ∿38 μsec. The postulation of the M_2^* species is based in part upon the similarity of field-off emission decay after a short (500 μsec) unipolar pulse, in which the fast component emission dominates, and after a reversed pulse which is composed of slow component emission only (see RESULTS). These results suggest that the decay of a single species is responsible for the field-off emission decay.
4. The interaction of M_1^* with E_1' is a specific reaction which is responsible for fast component EPL emission. Slow component emission results from the conversion of a second inactive species E_2 to E_2' which can also interact with M_1^* to form M_2^*. The conversion of E_2 to E_2' is slower than the field mediated conversion of E_1 to E_1'; a maximum in E_2' concentration does not occur until .5

to 1.0 msec after field application (curve B in Figure *13*). Other emission types (salt, pH, etc.) are accounted for by the inclusion of different E species, E_x, (see Figure 25), each of which is converted to an E' species by the appropriate perturbation.

5. Normal delayed light emission occurs in part via conversion of M_1^* to M_2^* along a pathway independent of the E species entering into the perturbation reactions.

Figure 26 shows a suggested interrelation between the species M_1^*, M_2^*, E_1, and E_1' and the resultant EPL.

The inclusion of different E species (e.g., E_1 and E_2) produces stoichiometric limitations to EPL field-on decay processes. Since the two species E_1 and E_2 are independent, the field-on decay of emission produced by the E_1', M_1^* interaction is independent of the E_2', M_1^* interaction. This aspect of the model accounts for the independent field-on onset and decay rates which are observed for the fast and slow EPL emission components.

This scheme also predicts that the dark decay of emission capacity for different emission types may differ, depending on the dark decay of the E species involved in each reaction type. It is therefore reasonable that EPL emission capacity does not decay at a similar rate as the decay of normal delayed light emission (Figure *22* and *25*).

The differences in light activation for various emission types observed by Hardt and Malkin (1971) can also be attributed to the independence of separate E species for different triggered luminescences. On the other hand, similarities in light activation and the decay of emission capacity in the dark may reflect a similar E species for different

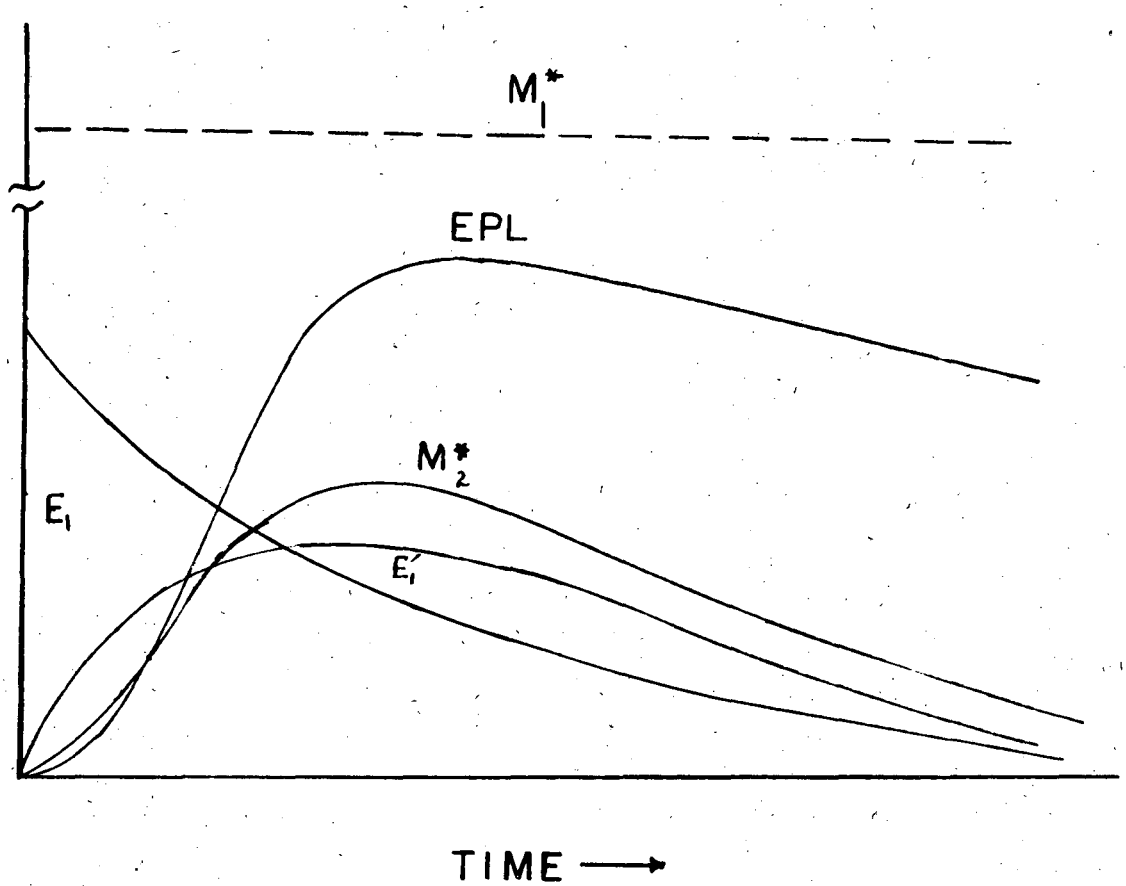

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Fig. 26. Population levels of interacting species showing an onset delay (induction) phenomenon.

emission types. For instance, the data of Malkin and Hardt (1971) and Miles and Jagendorf (1971) suggest that E species for acid-base and salt induced emissions may be the same, while those for acid and T-jump emission are different.

Since the removal of the field abruptly stops production of E_1' or E_2', the model also predicts that the field-off decay of emission should reflect a single process, which is the decay of M_2^* to give Chl*. The 38 μsec field-off decay reflects the field independent decay of the M_2^* species. Since it has been postulated that M_2^* is a species common to EPL and normal delayed light emission, one might expect to find a similar emission component in the published literature on delayed light emission. Such a parallel does indeed exist.

Zankel (1971) has observed three sub-millisecond first order components of microsecond flash-induced delayed light emission that have half-lives of 10, 35 and 200 μsec. DCMU treatment of the chloroplast samples left only the 35 μsec component intact. He attributed this decay to either a reaction preceding the reduction of Q, a reaction on the donor side of PS II, or a decay in a side reaction. It is significant that this component of delayed light emission should show both a decay time and an insensitivity to DCMU similar to that observed with EPL emission. These similar experimental results suggest a confluence of EPL and delayed light emission at the M_2^* species.

It is possible to assign the interaction of various inhibitors within the EPL scheme that has been introduced and to relate our scheme to the overall Z-scheme of photosynthesis.

The interaction of ionophores can be considered to be either an

inhibition of the formation of either the E species or M_1^*, or a rapid dissipation of the E species. Gramicidin may interact preferentially with the light activated E_1 species and to a lesser extent on the E_2 component (Figure *15*). Since CCCP inhibits the production of delayed light emission as well as electron transport (Yamashita and Butler, 1968), it may inhibit the formation of M_1^*. The effect of tris-washing appears to be an acceleration of the M_1^*, E_1' interaction. Although tris-washing appears to lower the total slow component emission intensity, it may accelerate the onset kinetics (Figure *14*). It should also be noted that an apparent increase in viscosity tends to retard the tris-washing acceleration noted above (Figure *16*). The above results strongly suggest that the transport of ionic species is essential to the EPL process.

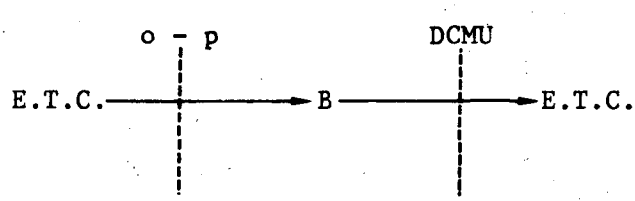
C. EPL AND THE Z-SCHEME

The best evidence which localizes EPL as being related to PS II chemistry is the effect that various inhibitors and treatments have on the emission. The inhibitors CCCP and hydroxylamine have both been implicated in affecting electron transport between water and PS II. Evidence indicates that CCP must act somewhere between the primary donor for PS II and water (Kimimura *et al.*, 1971), while Katoh *et al.*, (1970) and Bennoun (1970) suggest that hydroxylamine reacts specifically with the primary electron donor of PS II. Tris-washing treatment has been partially localized as interrupting electron flow from water to PS II (Yamashita and Butler, 1966a); the effect of heat treatment has also been located between water and PS II (Yamashita and Butler, 1968b). All of these treatments have an effect on EPL consistent with the interpretation that a donor species between water and PS II is required for EPL

emission. These results suggest that the M_1^* species is related to the donor side of PS II. It is the dissimilarity in effects caused by o-phenanthroline and DCMU which is difficult to relate to the Z-scheme.

O-phenanthroline and DCMU are both Photosystem II specific inhibitors. Both compounds block oxygen evolution but do not interfere with cyclic photophosporylation, characteristic of PS I activity (Forti and Zanetti, 1969; Arnon, 1969). Both compounds also have similar or identical effects on fluorescence induction phenomena (Papagerogiou and Govindjee, 1969; Bennoun, 1970; Delosme, 1967). Both Bennoun (1970) and Delosme (1967) found that o-phenanthroline or DCMU produced identical results on the nature of the fluorescence induction kinetics. Thus, the nature of interaction of o-phenanthroline and DCMU indicated that the site of electron transfer inhibition was very close, if not the same. The EPL results indicate that, at least for this phenomenon, o-phenanthroline and DCMU do not appear to act similarly. The question is whether these results are in conflict with the currently accepted Z-scheme model, or whether there is a feasible resolution to the problem.

The Z-scheme presents electron flow as a linear process. If o-phenanthroline and DCMU do not act on the same species, then, according to the Z-scheme, they must act upon nearby members of the same chain. The differences in action between o-phenanthroline and DCMU on EPL can be made consistent with the Z-scheme in two different manners. If o-phenanthroline and DCMU are postulated to affect two different locations very close to each other along the electron transport chain:



where E.T.C. is the electron transport chain and B is a member of it, under continuous lighting conditions electrons would be effectively blocked near PS II whether o-phenanthroline or DCMU was used. If electron blockage at these two nearby sites effectively poise Q in the same condition, similar fluorescence induction and oxygen evolution inhibition effects should be observed for both inhibitors. A second alternative is that o-phenanthroline affects two different sites, one of which is identical to the DCMU site. The interaction at the second site produces a change in the kinetic dependence of the E_1', M_1^* reaction.

The first alternative could best be examined using short flashes while watching oxidation or reduction of various electron transport chain components. A difference in transients caused by o-phenanthroline and DCMU treatment would indicate that electrons had been advanced to different points before blockage occurred. No difference in observations would suggest that the second alternative is viable, and one should pursue an investigation to find a separate o-phenanthroline effect on such phenomena as hydrogen ion transport. Whichever alternative proves more reasonable, the differences in EPL emission caused by o-phenanthroline and DCMU are the first results that definitely show that o-phenanthroline and DCMU do not have identical effects on all photosynthetic reactions.

A unifying factor further relating stimulated emissions to oxygen evolution is the recent discovery that HCl-, methanol-, sodium benzoate- and T-jump-induced emissions following preillumination flashes oscillate with a period of 4 flashes (Hardt and Malkin, 1973). As with the flash stimulated delayed light emission, maxima occurred on the second and sixth flash. This result relates these emission types directly to

Kok's S states (see Introduction). The definitive experiment to determine whether EPL emission will also display this oscillatory nature has yet to be done. If it is determined that EPL is also an oscillatory phenomenon, one will have an additional perturbation technique which can help indicate what types of species are involved in oxygen evolution.

The effect caused by electric field reversal is best considered after a discussion of why the physical structure of the thylakoid is important to EPL emission and how that structure can be responsible for an electrostatic mechanism that generates very high transmembrane electric fields.

D. ROLE OF THE MEMBRANE IN EPL PRODUCTION

1. Membrane Integrity

The evidence that the integrity of the membrane is an important factor in EPL emission is the following:

1. Physical disruption of the membrane inhibits EPL emission. This includes sonication, detergent treatment, and heat treatment.
2. Factors which affect the ionic permeability of the membrane affect EPL. Included here are effects caused by CCCP and gramicidin.
3. Hypo-osmotic and isotonic conditions produce different EPL characteristics.

Parallel effects have been observed for the A_{515} change (see Sect. IV-A-(6). In order to observe EPL and A_{515} effects, it is apparent that the membrane must define an inner space that is electrically insulated from the surrounding medium. It is also apparent that EPL and the A_{515} change are related to electrical phenomena. We may now pursue these similarities and ask what magnitude of field characterizes the A_{515} shift and compare that to the fields which have been applied in the EPL experiments.

2. Effective Membrane Fields

Schliephake *et al.*, working from their deduction that two protons are translocated across a thylakoid membrane per photon absorbed, and assuming certain physical properties of the membrane, calculated that a 50 mV potential could be induced across a 30 Å lipid layer, corresponding to an electric field of about 1.7×10^5 V-cm^{-1} (Schliephake *et al.*, 1968). Barber also estimated that a field of about 10^5 V-cm^{-1} could be light induced across a membrane, but deduced this value using salt-induced millisecond delayed light emission as an indicator of the potential (see Introduction; Barber, 1972). If we compare these magnitudes with the magnitude of free space fields that have been used in the EPL experiments, an apparent anomaly exists. The free space field, equivalent to the magnitude of the applied voltage divided by the distance through which it is applied, varied from about 3×10^2 to 4×10^3 V-cm^{-1}, and yet produced quite large enhancements of emission. However, when one investigates the electrical properties of an enclosed low permeability membrane, a plausible resolution is found.

An intact chlorplast is composed of an external membrane which encloses the stacked membrane structures of the thylakoids. Hypo-osmotic conditions burst the outer membrane but leave the thylakoids as swollen, membrane enclosed structures (Izawa and Good, 1968). Under these conditions we may idealize the theylakoid membrane as a spherical shell with inner radius a and outer radius b, as shown in Figure *27*. The thickness of the membrane relative to its diameter has been magnified to help visualize the problem. We wish to consider what kind of electrical potentials will be felt across such a spherical shell if it is placed in an initially uniform field E_o. This problem has been considered by Maxwell (1892).

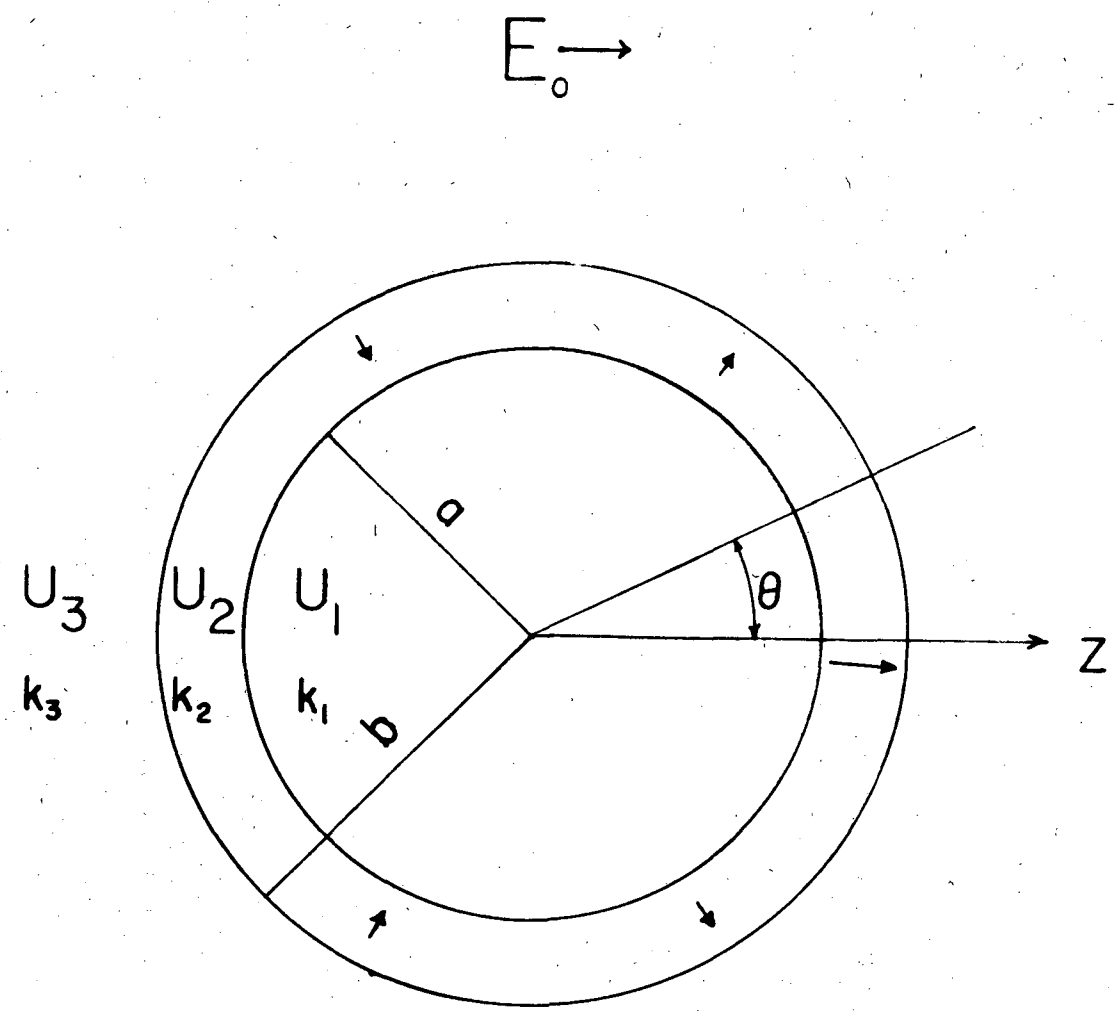


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Fig. 27. Spherical shell model discussed in text. U_1, U_2, U_3 are the potentials in Regions 1, 2, and 3, while k_1, k_2, and k_3 are the resistivities for these regions. E_o is the external applied electric field in the Z direction. The quantitative description of the variance of the field and potential in the three regions predicts that the radial electric field across region II (correponding to a membrane) is of equal magnitude and has the same Z component for θ and $-\theta$ (note the arrows referring to field directions and magnitudes).

There are three different regions of space to consider: the internal volume, the shell itself, and the suspending medium. We will call these regions 1, 2 and 3, respectively. Each region has a specific resistance k_1, k_2, or k_3. We define a spherical coordinate system as follows: The direction of the initially uniform electric field defines the polar z direction. The origin of the coordinate system coincides with the center of the sphere. The two angular coordinates are the azimuthal angle ϕ and the polar angle Θ. For each region of space there will be a potential defined as $U(r,\Theta,\phi)$. However, due to the axial symmetry of the model, the potential will be independent of ϕ. Then $U = U(r,\Theta)$. The potential functions for the three different regions will be U_1, U_2, and U_3.

Electrostatic theory (see e.g., Reitz and Milford, 1967) requires certain boundary and limit conditions to be met. These are:

1. The potential must be continuous everywhere. Specifically, at the boundaries,

$$U_1(a,\Theta) = U_2(a,\Theta) \tag{18}$$

$$U_2(b,\Theta) = U_3(b,\Theta) \tag{19}$$

2. The component of current passing normal to the interface of two boundaries must be equal in the two contacting regions. This requires that

$$\frac{1}{k_1}\frac{\delta U_1(a,\Theta)}{\delta r} = \frac{1}{k_2}\frac{\delta U_2(a,\Theta)}{\delta r} \tag{20}$$

$$\frac{1}{k_2}\frac{\delta U_2(b,\Theta)}{\delta r} = \frac{1}{k_3}\frac{\delta U_3(b,\Theta)}{\delta r} \tag{21}$$

3. The potentials must obey Laplace's equation ($\nabla^2 U(r_1\Theta) = 0$). Since the potentials are functions of r and Θ only, the solutions

to each of the potentials can be expressed as Legendre Polynomials having two terms (Reitz and Milford, 1967):

$$U_1 = A_1 r\cos\Theta + \frac{B_1}{r^2}\cos\Theta \tag{22}$$

$$U_2 = A_2 r\cos\Theta + \frac{B_2}{r^2}\cos\Theta \tag{23}$$

$$U_3 = A_3 r\cos\Theta + \frac{B_3}{r^2}\cos\Theta \tag{24}$$

where the A's and B's are constants. These equations can be solved using the relations (18)--(21) and two other conditions which must hold. In Region 3 at great distances from the center of the sphere the electric field in the z direction must be E_o. Since the field is given by $E_z = -\frac{\delta U}{\delta z}$, then

$$\frac{\delta U_3}{\delta z} = \frac{\delta \delta U_3}{\delta r\cos\Theta} = A_3$$

and

$$A_3 = -E_o.$$

At the center of the sphere the potential must be finite, so that B_1 must be zero.

Thus there are six coefficients to obtain, two of which are already known. Equations (18) - (21) are the four other relations which define a solution of the other coefficients. The four simultaneous linear equations can be solved (straightforward but tediously); the results for the coefficients A_2 and B_2 are:

$$A_2 = \frac{9k_1k_2}{(2k_1 + k_2)(2k_2 + k_3) + 2(k_1 - k_2)(k_2 - k_3)(a/b)^3}$$

$$-\frac{1}{a^3}\left\{1 + \frac{1}{b^3} \cdot \frac{(k_2 - k_3)(2k_1 + k_2)b^3 + (k_1 - k_2)(k_2 + 2k_3)a^3 - 9k_1k_2b^3)}{(2k_1 + k_2)(2k_2 + k_3) + 2(k_1 - k_2)(k_2 - k_3)(a/b)^3 \{1/b^3 - a^{1/3}\}^{-1}}\right\}.$$

$$B_2 = 1 + 1/b^3 \frac{(k_2 - k_3)(2k_1 + k_2)b^3 + (k_1 - k_2)(k_2 + 2k_3)a^3 - 9k_1k_2b^3}{(2k_1 + k_2)(2k_2 + k_3) + 2(k_1 - k_2)(k_2 - k_3)a^3/b^3 \{1/b^3 - 1/a^3\}^{-1}}.$$

These rather formidable looking terms can be simplified when we make some physically relevant approximations. If we compare the specific resistivity of the membrane with either the suspending medium or the internal volume, we can with reasonable justification surmise that $k_2 >> k_1 \sim k_3$. The justification is the following:

Junge and Schmidt (1971) calculated ion currents that flowed through the thylakoid membrane in response to light activated transmembrane electric fields. The decay of the A_{515} shift was used to monitor the decay of electric field. Typical ion currents of about 7×10^6 amp-cm^{-2} across the thylakoid resulted from transmembrane potentials of about 100 mV. The resistance of a 1 cm^2 patch of membrane is thus (from Ohm's Law) $\sim 2 \times 10^4$ ohms. The specific resistance, ρ, of a substance is found from the relation $\rho = RA/L$, where R is the resistance, A the cross-sectional area of the conductor, and L is its length. For a 70 70 Å membrane, $\rho = k_2 \sim 2 \times 10^{10}$ ohm-cm. A resistance of about 1500 ohms was measured for a 1 cm^2 × .25 cm volume of aqueous medium. This results in a specific resistance k_3 of about 6000 ohm-cm.

It is reasonable to assume that the internal specific resistance of a thylakoid will be no lower than the specific resistance of the suspend-

ing medium. Hence we are justified in saying $k_2 >> k_3 \sim k_1$. With this approximation A_2 and B_2 become:

$$A_2 = -\ 3/2\ (1/\ (1 - (a/b)^3)\ )\ E_o$$

$$B_2 = \ \ 3/2\ (a^3/\ ((a/b)^3 - 1)\ )\ E_o$$

The electric field vector in the radial direction for Region 2 is given as

$$[E_a(r)]\cdot\hat{r} = |-\delta U_2/\delta r\cdot\hat{r} = (A_2 \cos\theta - 2B_2\ r^{-3} \cos\theta)\ \cdot\ \hat{r}$$

or

$$[E_2(r)]\cdot\hat{r} = \{3/2\ [1/(1-(a/b)^3)\}E_o\cos\theta + 3[a^3/(1-(a/b)^3)]E_o 1/r^3\cos\theta\}\hat{r} \quad (25)$$

For r = a,

$$E_2(r) = [9/2\ \{1/(1-(a/b)^3)\}\ E_o\cos\theta]\ \cdot\ \hat{r} \quad (26)$$

where $\hat{r}$ is the unit vector in the r direction. Notice that for $|\theta| > \Pi/2$, the unit vector is negative. The z component of the radial field everywhere has the same sign as the external field.

From this relation it can be seen that the magnitude of the radial field is dependent upon:

1. The magnitude of the polar angle, θ. The radial field is maximum at the poles ($\cos\theta = \pm 1$) and is zero at the equator ($\cos\theta = 0$). The field is axially symmetric, and for any given radius depends only on the magnitude of θ.
2. The relative size of the shell thickness to its radius. When *a* approaches *b*, the magnitude of the radial field increases rapidly.

It should also be noted that the induced membrane field, although in the same direction on both sides of the membrane relative to the applied field, is directed inward on one side of the membrane and outward on the other side. For a 5000 Å thylakoid (see e.g., Izawa and

Good, 1968) with a 70 Å membrane, the radial field at the poles is $\sim$ 100 times the externally applied field. This means that for free space fields on the order of 10^2 to 10^3 V -cm^{-1}, effective membrane fields on the order of 10^4 to 10^5 V - cm^{-1} may be obtained.

Without finer detail to our knowledge concerning the exact electrical properties of thylakoid membranes, it is difficult to pursue any further electrodynamic or electrostatic calculations. The problem has been treated in a DC manner, and for ways in which surface conductivities and AC fields can enter into the picture, see Miles and Robertson (1932) and O'Konski (1960). This simplified model has provided a rationale for understanding why relatively low free space fields can cause locally high fields across membrane surfaces.

We may now return to our model to incorporate an additional element:

6. A field pulse converts E_1 to E_1' only when the external field is of the proper sense relative to the transmembrane direction. If the field is of the opposite sense, it diverts E_1 via a secondary pathway to E_1''. E_1'' cannot react with M_1^* to form M_2^* in the presence of an electric field.

This element of the hypothesis accounts for the absence of the fast component upon field reversal. The relative slow ($\sim$5 msec) reversion of E_1'' to E_1 or replacement of E_1'' with fresh E_1 after the removal of the field accounts for the reactivation of the fast component after a zero field spacing interrupts the initial pulse from a reversed pulse.

E. POSSIBLE ELECTRIC FIELD EFFECTS

The actual identity of the M and E species involved in the scheme and the effect of electric fields on them is not known. However, there

are several clues which are offered by the EPL emission character which suggest directions in which to pursue their identity. From the discussion up to this point it is evident that the membrane plays an important role in stimulated or triggered emissions, and we will focus on possible testable electric field related phenomena that could change the properties of membrane processes.

The effect of ionophores draws our attention to ways in which an electric field can bring about changes in ion activities. High electric fields are known to cause changes in electrolyte conductivity and weak electrolyte dissociation constants. These changes are usually referred to as Wien effects, and can be explained as follows: The increase in conductivity can be explained by the inability of a counter ion charge cloud to keep up with the velocity of a central ion accelerated in a high field. The central ions then become independent and have a greater mobility. Weak electrolytes in a strong field experience a strong pull which tends to keep the component ions apart, with a consequent increase in dissociation constant. Such an effect can result in a perturbation in any reaction which depends on the dissociation constant of a weak electrolyte; pH dependent processes are susceptible to the Wien effect.

Wien type effects have been observed for biologically interesting cases. Neumann and Katchalsky attributed spectroscopically observed long-lived conformational changes in polyelectrolytes to a field related shift in the charge cloud surrounding the molecules. The shift in counter ions apparently results in a large induced dipole which in turn causes a repulsion of strands in the helically entwined molecules of the polynucleotides studied. Although not specifically attributed to the Wien

explored using dichroism or birefringence techniques, or possibly an optical absorption technique such as used by Neumann and Katchalsky (1972) in their work discussed above. Any of these techniques should be capable of detecting changes which occur in sub-millisecond time ranges. The possibility of two separate conformational changes, one which occurs as a part of the emissive M_1^*, E' interaction sequence of our model, and the other which reflects the non-emissive E' → E" conversion, may show up in a spectroscopic measurement as two separate resolvable kinetic components.

It is possible to use radioactive tracer techniques to explore the possibility that field effects result in a transport of ions across the membrane. Because such a mechanism is likely to be time and light independent, comparisons of internal and external ion concentrations for thylakoid preparations which had and had not been subjected to electric fields can be made over time periods long enough to prove whether field caused transmembrane ion conduction can be an important factor.

Whether membrane surface ion conductivities plan an essential role in the EPL mechanism(s) can be tested in a number of ways. The charge density which resides on the membrane surface is a property of the nature of the ionic groups exposed to the exterior such as zwitterionic protein residues and phosphate groups on membrane phospholipids. This charge density, and hence surface conductivity can be changed as a function of pH and ionic strength. Isoelectric zwitterionic buffers can serve to separate pH effects from ionic strength effects, since low counterion concentrations accompany zwitterionic buffers under isoelectric conditions. The recovery of the fast component emission intensity can

effect by the authors, the resultant shift in ions has the characteristics of a Wien effect.

Other electrically induced conformations have been observed in calf thymus DNA (O'Konski and Stellwagen, 1965, and aggregation of poly-Y-benzyl-L-glutamate caused by electric fields has been studied (Gregson, *et al.*, 1970).

Recent consideration has been given to the Wien effect as a possible electric field related mechanism of nervous conduction (Bass and Moore, 1968). The applicability of the effect in this case centered around small pH changes which can cause critical depolarizations across nerve membranes. Bass and Moore elegantly demonstrated how a reduction in the Wien effect could account for the critical depolarizations required to initiate action potentials.

There have also been reports that some membranes may be susceptible to disorganization at high electric field strengths. The investigations of Ohki (1972) showed that the stability of homogeneous and heterogeneous lipid bilayer membranes depends on the degree of asymmetry in environments on different sides of the membrane. Asymmetric distributions in pH, ionic strength, and electric field decreased membrane stability to the point where breakdown occurred, detected by a sudden increase in membrane conductivity.

The Wien effect suggests that a search for a change in conformation, a change in transmembrane ion conductivities, or a change in membrane surface ion mobilities might be reasonable courses to pursue to relate EPL emission to specific membrane transients.

The possibility of a field induced conformational change can be

also give clues to what changes the electric field brought about.

In face of the fact that a recovery does take place, three different alternatives can be suggested to account for the reactivation. Either the depleted species is itself electronically recharged, it is effectively replaced by an unaffected species, or a given pulse only selects certain specific M states which have the correct (time dependent) "sense" to the effect of the external electric field.

In principle, one could distinguish the former case from the latter two by the use of an appropriate electron transport inhibitor which would disallow any such transfer of metastable energy. The second alternative, the replacement scheme, would have to involve some type of diffusion process. Replacement mechanisms could include the rotary diffusion of the whole thylakoid, resulting in a new surface being presented in the directions most affected by the field, the diffusion of species along the surface of the membrane, or the lateral diffusion of species within the membrane itself.

The rotary diffusion of the entire membrane can be discounted on the basis of a computation of the rotational diffusion relaxation time, τ_{rot}, which for spheres is

$$\tau_{rot} = \frac{4\Pi\eta r^3}{kT}$$

Plugging in values for the radius r = 5000 Å, the viscosity, η, (of water) = 1 centipoise, and T = 300° K, the rotational diffusion time is about 400 msec. Thus, on the average, a thylakoid does not rotate fast enough to account for the reappearance of the fast component which occurs in 3 to 10 msec.

The ways for testing for surface ion diffusion are the same as those discussed on page 50, but now one would look for kinetic effects on EPL emission during the time t_f.

Lateral diffusion of species within the plane of the membrane is another alternative. Lateral diffusion rates of phospholipids in biological membranes have been measured as high as 5×10^5 Å-sec^{-1}, or 500 Å-msec (Scandella *et al.*, 1972). Surprisingly, the time and distance factors are suitable for this type of mechanism to account for a reactivation of the fast component over a period of a few milliseconds if one assumes that a species has to migrate on the order of 1000 Å during that time. One possible way to test this alternative would be to apply the same type of spin labeling techniques used by Scandella *et al.*, in their phospholipid study. The problems of labeling functional biological membranes is not trivial, but methods used by Scandella *et al.*, (sonication of membranes with medium containing appropriate labels to incorporate the labels in the membrane) are encouraging.

In principle, it should be possible to discover which process is involved in both the production of the fast component emission and its subsequent reappearance. The slower component, less well characterized than the fast, can be similarly explored. The implications of a final resolution of the mechanism(s) involved are far-reaching. If it turns out that the recovery of the fast component reflects such basic membrane phenomena such as ion transport or molecular diffusion, the emission itself can be used as a direct, real time *in vivo* measure of these activities, which are to a large extent not directly detectable with other present techniques.

F. SUMMARY

The information which has been obtained in the course of work reported above leads this author to believe that EPL is the result of a sequence of reactions which are at least partially independent of the molecular processes which result in normal delayed light emission.

A model has been presented to account for the EPL observations noted by this author and which is compatible with stimulated emissions produced by other (pH, salt, etc.) perturbations. Emission is pictured to occur as a result of a field dependent interaction of a metastable energy species M_1^* with a light activated species (E_1, E_2) to produce a second metastable species M_2^* which is directly accessible to the Chl singlet state. Other stimulated emissions are presented as originating from the same M_1^* state, but involve different light activated species (different E species).

The actual mechanism which results in EPL emission may very well be the result of the Wien effect; however, several alternative mechanisms have been presented.

Fields on the order of 10^5 V-cm^{-1} are generated locally across the thylakoid membrane as a result of the electrical conductivity properties of biological membranes. The transient emission which results when an electric field is applied to preilluminated aqueous chloroplasts consists of at least two components. One, decaying faster than the other, has a half-maximum rise time of about 50 μsec and a half-time decay of about 370 μsec with an applied field of 1600 V-cm^{-1}. This fast component shows irreversible depletion upon field reversal. This is thought to be the result of induced emission occurring from only one side of the

membrane while the field is on, with concomitant inhibition of emission processes occurring in the other direction. Field reversal cannot produce emission from those sites affected in an inhibitory nature by the first pulse.

A second component having a half maximum rise time of about 1 msec and a much longer decay time not only lasts longer, but does not show the depletion character upon field reversal and may be due to a different type of interaction. The fast component appears to be especially sensitive to treatments which influence membrane permeability. The slow component has not been so characterized.

EPL emission most likely reflects the population level of one or more components of the electron transport scheme described as the Z-scheme hypothesis. Further work is necessary to determine exactly what the relation of the metastable source(s) of EPL emission is to Kok's S states.

It has also been suggested that the further exploration of the electrophotoluminescence phenomenon may provide valuable insight into the nature of membrane chemistry and its relation to photosynthesis.

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