BIOCHEMICAL STUDIES OF THE DELAYED LIGHT EMISSION FROM ISOLATED CHLOROPLASTS

Thesis for the Degree of M. S.
MICHIGAN STATE UNIVERSITY
PETER FELKER
1973

ABSTRACT

BIOCHEMICAL STUDIES OF THE DELAYED LIGHT EMISSION FROM ISOLATED CHLOROPLASTS

By

Peter Felker

The purpose of the research reported in this thesis was to attempt to correlate delayed light emission (DLE) with the much better understood effects of uncouplers, electron transport inhibitors, and ion movements on isolated chloroplast biochemistry.

Electron transport inhibitors appear to behave in two classes with respect to delayed light emission. It has long been known that electron transport inhibitors, which act between plastoquinone and photosystem II, always drastically inhibit DLE. New evidence is presented which shows that inhibitors, which act between plastoquinone and photosystem I, actually stimulate the DLE under some conditions.

All uncouplers of photophosphorylation except NH_4Cl markedly inhibit the DLE. Under proper conditions NH_4Cl can markedly stimulate the DLE. The conditions for this stimulation of DLE by NH_4Cl were very similar to the conditions for light-activated NH_4^+ uptake by

chloroplasts. Also, methylamine which uncouples phosphorylation in chloroplasts particles, abolishes the DLE in chloroplasts, but not in subchloroplast particles.

Several nucleotides have also been found to stimulate delayed light emission. ATP and ADP gave the greatest stimulation of the DLE from chloroplasts. This new observation of DLE stimulation by ATP and ADP appears to be well correlated with the previously known stimulation of the H^+ uptake and inhibition of electron transport by ADP and ATP.

A scheme is presented to explain these phenomenon in which a cation uptake creates a membrane potential which in turn stimulates a back-reaction of holes and electrons in the membrane to give an excited chlorophyll molecule and thus yield DLE.

BIOCHEMICAL STUDIES
OF THE DELAYED LIGHT EMISSION
FROM ISOLATED CHLOROPLASTS

By

Peter Felker

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Botany and Plant Pathology

1973

ACKNOWLEDGEMENTS

I sincerely wish to thank my guidance committee for advice and support through the course of this study. I especially wish to thank Dr. Norman E. Good, my major professor, for his advice and critical suggestions. I wish to thank Dr. Seikichi Izawa for his excellent day by day counseling on chloroplast biochemistry and for construction of the NH_4^+ selective electrodes. I wish to thank Dr. Alfred Haug for so generously making his phosphoroscope available to me and for his help with controls and elctronics. Finally I wish to thank Dr. Robert Bandurski for his encouragement and support.

This work was supported in part by a teaching assistantship granted by the Department of Botany and Plant Pathology, and by the United States Veterans Administration.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

ADP	adenosine-5'-diphosphate
5' AMP	adenosine-5' -monophosphate
ATP	adenosine-5'-triphosphate
Chl	chlorophyll
Cyt	cytochrome
DBMIB	3, 6-dibromo-2-methyl-5-isopropylbenzoquinone
DCMU	3-(3, 4-dichlorophenyl)-1, 1-dimethylurea (diuron)
DLE	delayed light emission
E. T.	electron transport
FeCy	potassium ferricyanide
Fluor.	fluorescence
GDP	guanosine-5'-diphosphate
HEPES	N-hydroxymethylpiperazine-N'-ethanesulfonic acid
ITP	inosine-5'-triphosphate
K_m	Michaelis constant
MES	N-morpholinoethanesulphonic acid
mM	millimolar
msec	millisecond
MV	methylviologen
PC	plastocyanin
Pi	orthophosphate
PS I, II	photosystem I, II
P.M.	photomultiplier

PQ	plastoquinone
Q^-	reduced primary electron acceptor for photosystem II
S	substrate concentration
TAPS	N-tris (hydroxymethyl) methyl-3-aminopropane-sulfonic acid
Tricine	tris (hydroxymethyl) methylglycine
uM	micromolar
UTP	uridine-5'-triphosphate
v	velocity of an enzyme reaction
V_m	maximal velocity of an enzyme reaction
Z^+	oxidized primary electron donor for photosystem II

INTRODUCTION

It has long been known that photosynthetic organisms emit light for a short time after illumination has ceased. The emitted light is very weak and some of it continues for minutes. However, the highest intensity is immediately after the light is turned off, with over half of the luminescence occuring in a small fraction of a second. The luminescence which can be observed in green plants, in photosynthetic bacteria, and in isolated chloroplasts, has the emission spectrum characteristic of the type of chlorophyll present.

This phenomenon was first observed by Strehler and Arnold (1951) while they were using the firefly luminescence assay in an attempt to detect ATP in chloroplast preparations. They determined that the action spectrum for the weak red luminescence of the chloroplasts was the same as the action spectrum for photosynthesis. In living algae there was a sharp temperature optimum at 37°C, an inhibition by ultraviolet light, and a stimulation by CO_2, all of which strikingly parallel the effects of these conditions on photosynthesis. These workers postulated that the luminescence (now called delayed light emission or DLE) involved at least one reversible enzyme reaction.

Many other workers subsequently studied the phenomenon. Frank and Brugger (1958) studied the DLE from a variety of plant material under N_2, O_2, and CO_2 atmospheres and with a variety of poisons and potential electron acceptors. Tollin et al. (1958) found the DLE measured 0.1 sec or longer after illumination was maximum at 25°C, but at shorter times the temperature optimum was -50°C. They also found the quantum yield to be 10^{-6} for Chlorella and 10^{-7} for spinach chloroplasts. Bertsch and Azzie (1964) also found that the DLE, measured 0.5 sec after illumination, peaked fairly sharply between 15°C and 35°C, depending on the color of the exciting light. They found the decay kinetics of the luminescence to be affected by the color of the exciting light. When the exciting light was 600 nm, the decay curve was monotonic. However, when far red light was added to the 600 nm exciting light, a shoulder appeared in the decay curve. The decay curves after illumination at different wavelengths actually crossed each other.

Goedheer (1964) studied the action spectrum of DLE. He found that 660 nm stimulated DLE but longer wavelengths decreased the DLE. The most effective wavelenghts for decreasing DLE was 700 nm in both Chlorella and spinach chloroplasts. He also showed (1962) that addition of reductants such as dithionite, ascorbate, or cysteine caused a stimulation of the DLE in chloroplasts. The addition of oxidents such as ferricyanide caused a drop in DLE until the oxidant was reduced.

Chloroplasts also emit a weak red luminescence when they undergo a rapid acid to base transition (Mayne and Clayton, 1966). This luminescence, which requires pre-illumination and is therefore a special case of DLE (Mayne, 1968), is abolished by uncouplers of phosphorylation, such as ammonia, methylamine, carbonylcyanide-3-chlorophenyl-hydrazone (CCCP) and by inhibitors of electron transport such as DCMU. Since Uribe and Jagendorf (1966) had shown that similar acid-base transitions support an uncoupler-sensitive phosphorylation reaction, it was natural to suggest that the same ATP-generating potential was required for both DLE and phosphorylation. Electron transport inhibitors such as DCMU, however, inhibit only the DLE associated with acid-base transitions, not the phosphorylation. Therefore, it was reasonable to postulate that DLE required both the "high energy state" responsible for phosphorylation AND at least part of the electron transport pathway. Consistent with this picture is the fact that not only uncouplers but also ADP and phosphate, which use up the ATP generating potential, decrease the DLE measured at 4 msec after illumination (Mayne, 1967). Furthermore phlorizin, which blocks the ATP generating reaction between ADP and phosphate, restores the full DLE.

Miles and Jagendorf (1969) repeated the acid-base transition luminescence studies and extended them to show that a transition from low salt conditions to high salt

conditions also caused luminescence, even though these salt changes did not support phosphorylation. As in the acid-base transition and in ordinary DLE studies, the presence of DCMU abolished the phenomenon. The nature of the temporary trans-membrane ion gradients contributing to DLE is not clear. Barber and Kraan (1970) reported that a low-salt to high-salt transition with sodium salts gave an increased luminescence if gramicidin was present. Since gramicidin is known to increase membrance permeability to a number of monovalent cations including sodium, this implies that a sodium gradient aids DLE. On the other hand valinomycin, which increases permeability to potassium ions, gave increased DLE when potassium salts were present. Therefore it seems that a potassium gradient can also aid DLE. Barber and Kraan (1970) therefore suggested that DLE may result from an electrical membrane potential generated by the diffusion of any ion. Fleischman (1971) showed that a jump in K^+ concentration (a transition from a choline chloride medium to a KCl medium) gave luminescence in bacterial chromatophores, but only if the potassium-carrying antibiotic valinomycin was present.

Fleischman (1971) and Crofts, Wraight, and Fleischman (1971) have suggested a model for DLE in chloroplasts. This model was suggested by Jackson and Croft's demonstration (1969) of a linear correlation between the magnitude of the artificially induced membrane potential

in Rhodopseudomonas spheroides chromatophores and the extent of the carotenoid spectral shift, which is in turn well correlated with the 4 msec DLE.

In their model of chloroplast DLE, they place the primary electron acceptor of photosystem II,Q, on the outside of the thylakoid, in equilibrium with the plastoquinone pool. They place the primary electron donor of photosystem II, Z, on the inside of the membrane, in equilibrium with the water oxidation site. Since the reduction of plastoquinone to give plastohydroquinone would consume H^+ on the outside of the membrane and the oxidation of water would produce H^+ on the inside of the membrane, electron transport via photosystem II should involve a H^+ "pump". This "transport" of H^+ across the membrane, if unaccompanied by a counter ion, would generate a membrane potential with the inside being positive and the outside negative. The forward reaction of electron transport in photosystem II can therefore be depicted as

$$Z + Q + h\nu \xrightarrow{\text{chlorophyll}} Z^+ + Q^-$$

and the back reaction which yields the DLE can be depicted as

$$Z^+ + Q^- \xrightarrow{\text{chlorophyll}} Z + Q + h\nu$$

However this back reaction is almost impossible unless activation energy is added from another source. Now the

above mentioned membrane potential is just such an additional energy source.

Evans and Crofts (1973) have also implicated the trans-membrane H^+ gradient as a factor in causing DLE because of the similarity of the rise and decay kinetics of the DLE and H^+ gradients.

There has been much discussion in recent years concerning the relationship between DLE and fluorescence. Since DLE has the same emission spectrum as fluorescence, it's reasonable to assume that the same chlorophyll is involved in both processes. The intensity of prompt fluorescence is obviously a measure of the efficiency of light emission from chlorophyll which has been excited by light (either by direct absorption or by energy transfer). On the other hand, DLE is a measure of the efficiency of fluorescence of chlorophyll which has been excited by some unspecified chemical reaction. Knowing the efficiency of prompt fluorescence, we are therefore in a position to apply this to the DLE in order to arrive at conclusions regarding this chemical generation of excited chlorophyll. See Lavorel (1968), Clayton (1969), and Wraight (1972). Since our main interest has centered on those back-reactions of photosynthesis which generate excited chlorophyll, we have routinely measured the prompt fluorescence and the DLE simultaneously and we have attempted to separate effects on prompt fluorescence from effects on the chemical mechanism responsible for DLE.

With the advent of mutant strains of Scendesmus obliquus it has been almost conclusively shown that the DLE originates from PSII by Bertsch et al. (1967), and by Haug et al. (1972).

Haug's group (1972) measured the entire time course of the DLE and showed that the apparent prompt fluorescence contained less than 1% of a DLE componenet.

There has been some recent work correlating electron transport and DLE. Bertsch et al. (1971) confirmed that DCMU markedly inhibited DLE when chloroplasts were reducing ferricyanide. Bertsch and Lurie (1971) showed that Tris poisoning near PS II inhibited DLE but this could be restored by addition of artificial electron donors to PS II. Itoh et al. (1971) also studied the effect of donors of electrons to PS II on DLE and fluorescence. Butler (1971) reported that destruction of C-550 with pancreatic lipase markedly inhibited the DLE (C-550 is an unidentified component detected as a light-induced absorbance change). Kessler et al. (1957) showed that a severely Mn-deficient Ankistrodesmus culture gave only one-fourth as much DLE as the control. Arnold (1972) has recently reported that an alternating electric field of several hundred volts can stimulate the DLE up to 50 fold. Zankel (1971) found that by using single flashes of light the DLE could be made to oscillate in a period of four in the same way as does oxygen production (Joliot, 1969). Mar and Govindjee

(1971) have reported that chloroplasts subjected to a quick temperature jump of about 15°C emit light.

It appears that DLE is primarily a function of two major processes: first, an enzymatic catalyzed reversal of electron flow, as originally suggested by Strehler and Arnold (1951), and second, the involvement of a "high energy state" and/or membrane potential, as suggested by Mayne (1968), Fleischman (1971), and Wraight and Crofts (1971).

Electron flow therefore can be expected to affect DLE in two ways, by generating the high energy state-membrane potential or by influencing the size of the pools of back-reacting endogenous electron donors and electron acceptors.

Keeping these mechanisms in mind, we studied the effects of various electron transport inhibitors on DLE. In particular we investigated some new inhibitors, specifically dibromomethylisopropylbenzoquinone (DBMIB) a plastoquinone antagonist (Trebst el al. (1970) and KCN, a plastocyanin inhibitor (Quitrakul and Izawa, 1973). We also investigated correlations between DLE and membrane gradients using various nucleotides, ions, and phosphorylation uncouplers.

METHODS

Chloroplasts were usually prepared by the method of Saha et al. (1971) from spinach (Spinacia oleracea L.) obtained at a local market. The leaves were ground for 10 seconds in a Waring Blendor in a medium containing 0.3M NaCl, 2mM $MgCl_2$, and 0.04 M N-2-hydroxyethylpiperazine-N'-ethanesulfonic acid-NaOH (pH 7.6). The homogenate was squeezed through several layers of cheesecloth and centrifuged at about 3000 x g for 4 min. The pellet was resuspended in a medium containing 0.2 M sucrose, 2mM $MgCl_2$, and 0.01 M N-2-hydroxylethylpiperazine-N'-2-ethanesulfonic acid- NaOH (pH 7.6). The suspension was centrifuged at about 1200 x g for about 20 seconds to remove debris, and then centrifuged again at 3000 x g for 4 min. The washed chloroplasts were finally suspended in a small volume of the same sucrose-N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid -$MgCl_2$ medium. All operations were carried out at as near to O C as possible. The chlorophyll content of the chloroplast suspension was measured by the method of Arnon (1949).

Crucial experiments were confirmed using chloroplasts prepared by the same methods from the spinach variety "Bloomsdale Longstanding", grown in a growth chamber. This spinach was a gift from Dr. N. E. Tolbert.

Under some conditions (in the presence of NH_4Cl) chloroplast preparations gave more DLE if the leaves of commercial spinach used were illuminated overnight in a moist chamber with their petioles in water and if the chloroplasts stock solution was held in 0.1% bovine serum albumin (BSA) during the experiments. These procedures were therefore used routinely for all later experiments, including all those reporting the effects of ammonia.

Oxygen evolution was measured using a Clark-type oxygen electrode. The signal from the oxygen electrode was passed to a preamplifier constructed of standard design, consisting principally of a zener diode regulated voltage supply and a Zeltex Zel-1 operational amplifier. The signal was recorded on a sargent recorder.

Electrodes for measuring NH_4^+ were constructed by Dr. S. Izawa by using two concentric pieces of pyrex glass tubing sealed at the lower end with 10 mu filter held by epoxy glue. See Figure 1. The central glass tube held a solution of 0.01 M KCl in which was inserted an Ag/AgCl electrode. The outer tube held Orion K^+ liquid Ion Exchanger. For reference, another Ag/AgCl electrode was used. It was immersed in a LiCl (0.03M) solution and connected to the experimental vessel with a LiCl (0.03M) 4% agar bridge.

The potential difference between the two silver electrodes, which is a function of the NH_4^+ concentration,

Figure 1.--Diagram of the electrode and the apparatus used to measure the uptake and release of NH_4^+ by chloroplast suspension. A 4 ml reaction volume was placed in a transparent thermostated water jacket at 20 C. The reference electrode was connected to the reaction vessel by means of a LiCl (30 mM), 4% agar bridge in plastic surgical tubing. A copper ground wire was connected to the power supply ground and to the reaction vessel solution. Orion K^+ "Liquid Ion Exchanger" was placed in the outer jacket of the electrode. The 10 mu millipore filter was sealed onto the glass with epoxy glue.

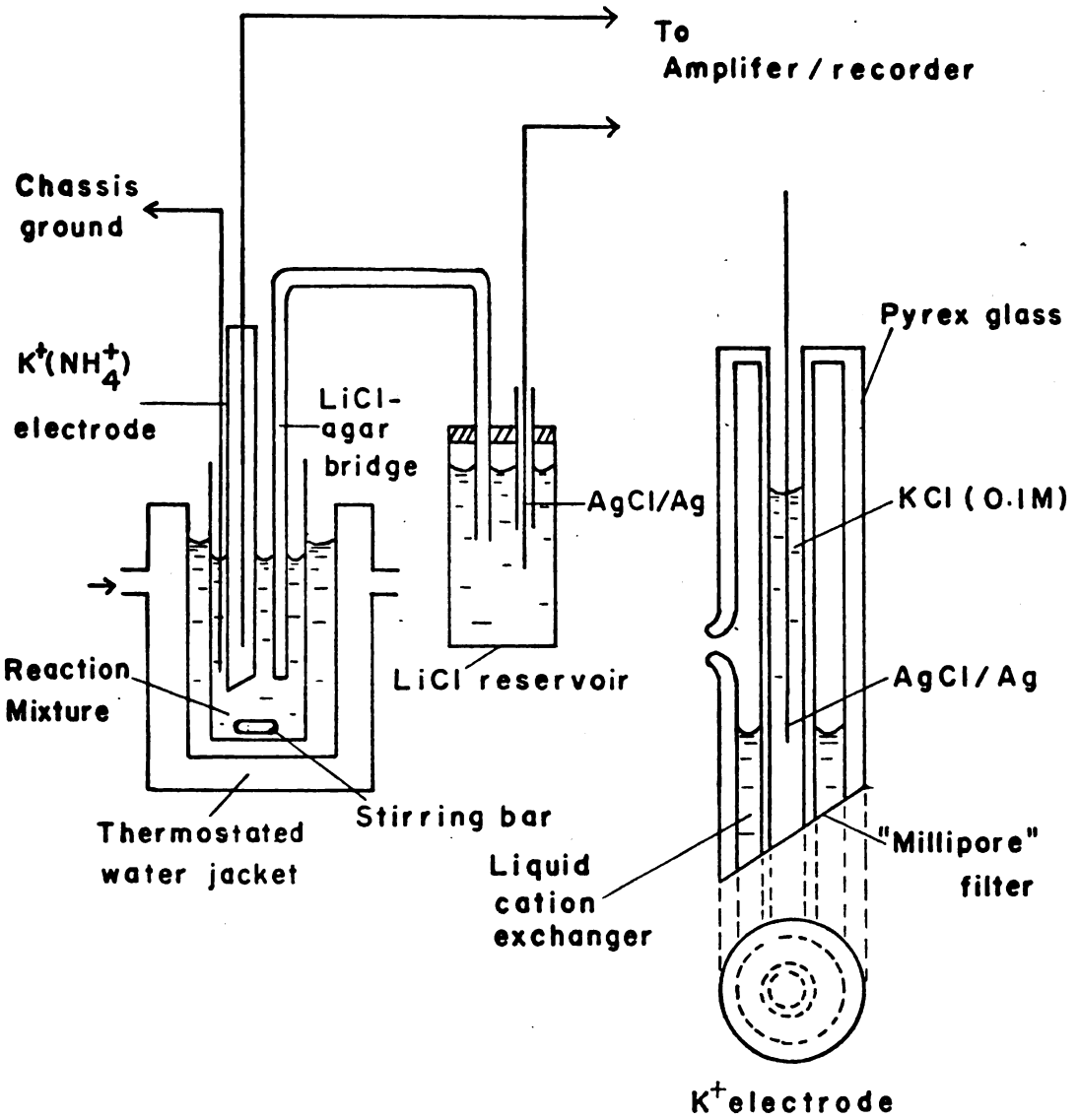
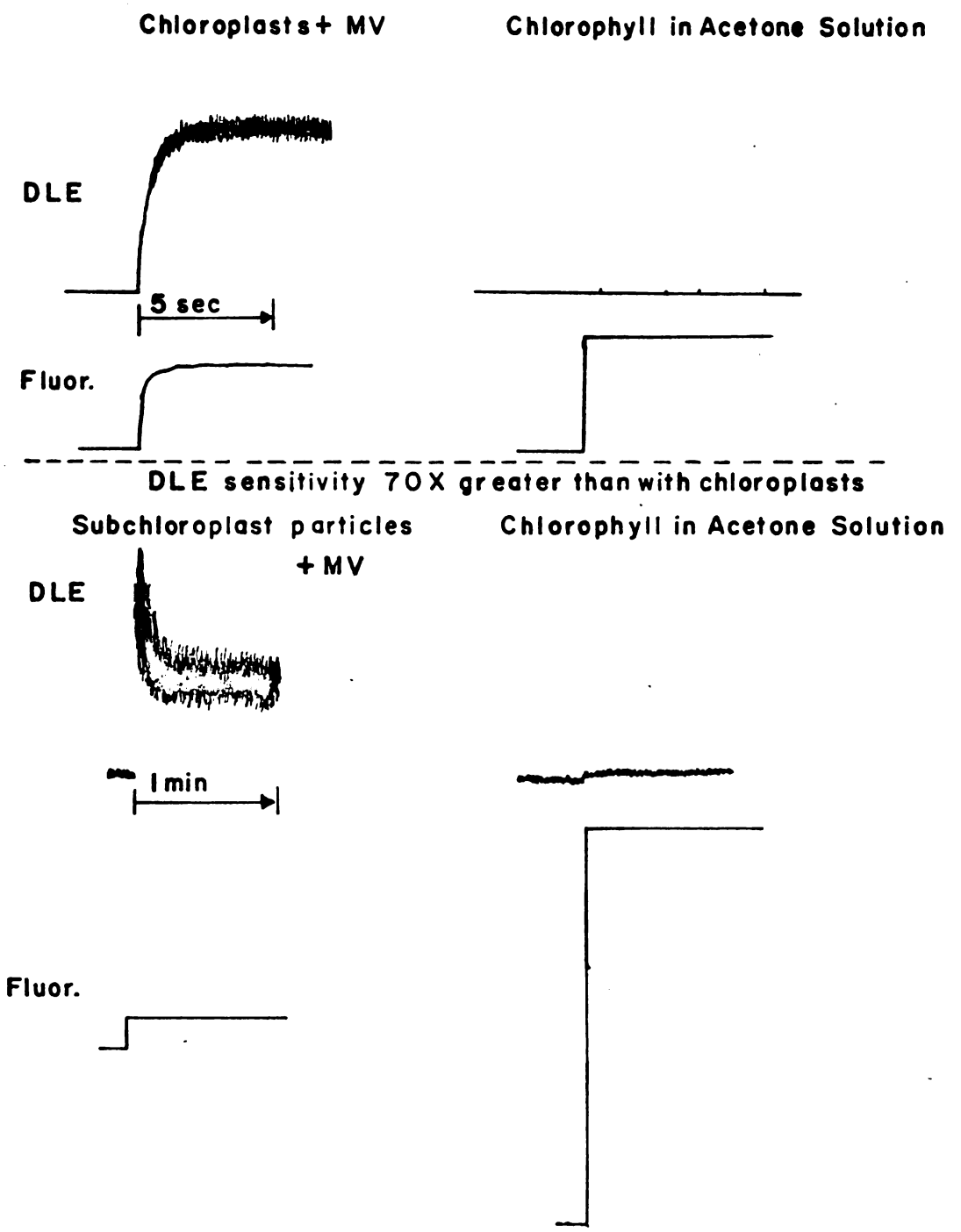

To
Amplifer / recorder
Chassis
ground
$K^+(NH_4^+)$
electrode
LiCl-
agar
bridge
AgCl/Ag
Pyrex glass
KCl (0.1M)
Reaction
Mixture
LiCl reservoir
AgCl/Ag
Thermostated
water jacket
Stirring bar
Liquid
cation
exchanger
"Millipore"
filter
K^+electrode

Figure 2.-- Separation of the DLE and prompt fluorescenceby the instrument described. Curves showing the DLE and prompt fluorescence from a chloroplast suspension are above the dotted line on the left. Similar curves from a solution of chlorophyll in acetone are given on the right. Below the dotted line, curves for DLE and prompt fluorescence in subchloroplast particles and chlorophyll solution are shown. Note that the sensitivities of the instrument were different above and below the dotted line; below the dotted line, the response to DLE was about 70 times as sensitive while the response to prompt fluorescence was actually lowered.


Chloroplasts + MV
Chlorophyll in Acetone Solution
DLE
5 sec
Fluor.
DLE sensitivity 70X greater than with chloroplasts
Subchloroplast particles
+MV
Chlorophyll in Acetone Solution
DLE
1 min
Fluor.

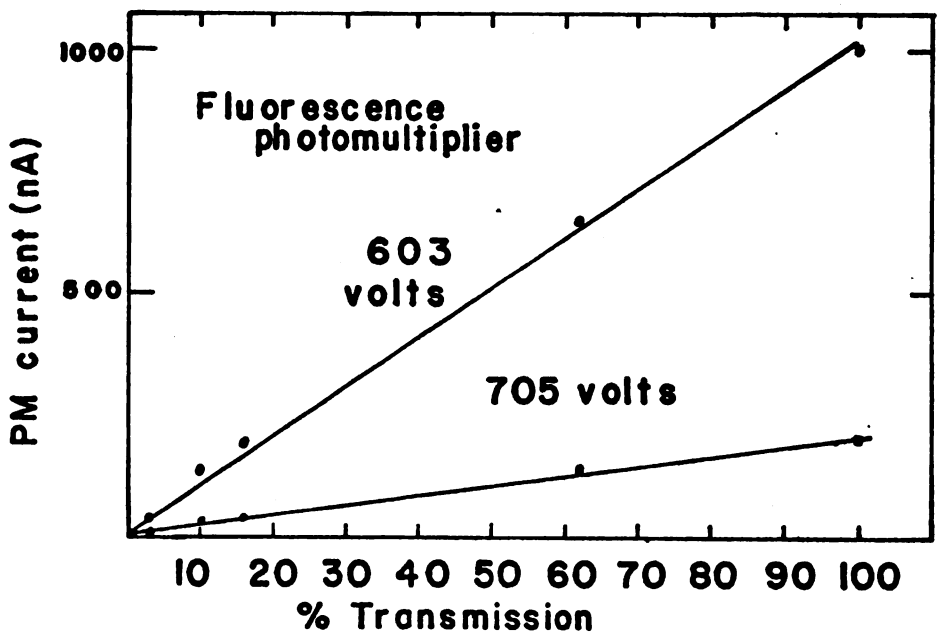
was measured with a solid state amplifier constructed according to the method of Brand and Rechnitz (1970). The amplifier parts were generously loaned by Dr. Clarence Suelter. The amplifier had very high impedance inputs from both electrodes and consequently the reaction solution had to be grounded. A DC power for the amplifier was constructed with voltage regulation provided by a Silicon General S. G. 1501 integrated circuit. A flashlight battery was used to construct a DC offset and the output of the amplifier was recorded on a Sargent recorder.

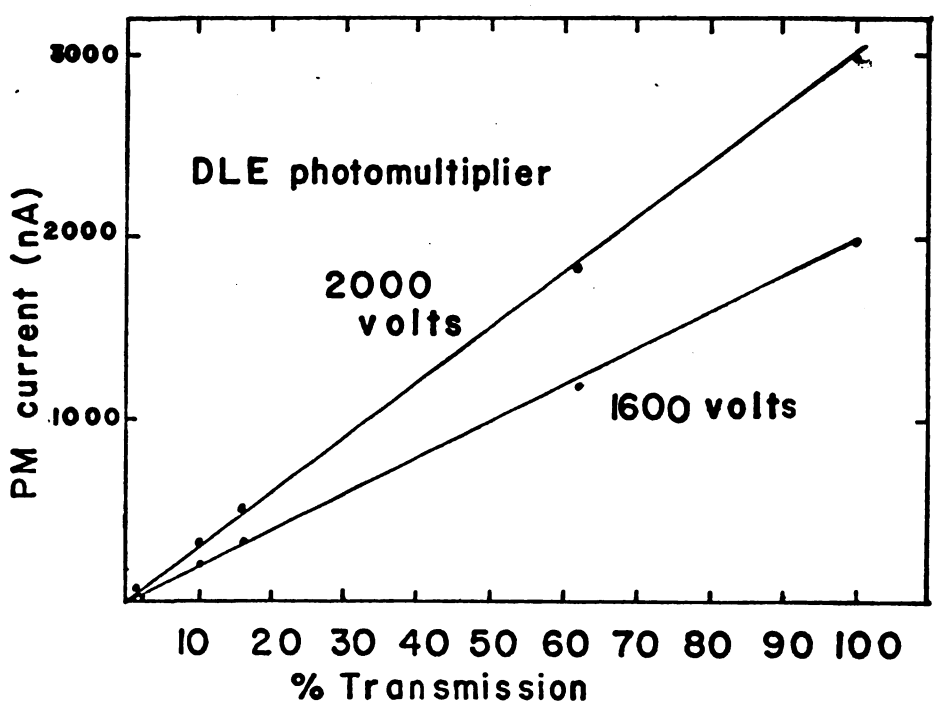
The phosphoroscope apparatus used to measure DLE was a modification of one previously described by Priestly and Haug (1968). It consisted of a 10 inch diameter rotating bucket, with a half inch slot subtending not quite 180 degrees cut out of it. The exciting light and the DLE detection system were placed exactly opposite each other so that the exciting light could never shine on the DLE detection system. The fluorescence detection system was at right angles to the sample but was prevented from seeing the exciting light by a system of complementary filters. The exciting light from the mercury-xenon lamp was filtered through 6 cm of saturated $CuSO_4$ solution, the filter being cooled with a fan. Corning 2030 red transmission filters were used in front of the DLE and fluorescence photomultiplier tubes to prevent scattered exciting light from reaching them. An example of the

separation of fluorescence and DLE achieved by this instrument is given in Figure 2 which compares chloroplasts with an acetone solution of chlorophyll, since the latter obviously produces no DLE. An RCA 31000E photomultiplier was used for DLE measurements and for best results (reduction of noise) was cooled for 36 hours with dry ice, and typically operated at 1800 volts. An EMI 9558B photomultiplier used for fluorescence measurements was typically operated at 650 volts. Both photomultipliers were powered simultaneously by a Keithley 246 high voltage supply via a voltage divider circuit. The linearity of both photomultipliers is shown in Figure 3. The output from the fluorescence detecting photomultiplier went directly to one channel of a Hewlett Packard 7702B recorder. The output from the DLE detecting photomultiplier went to a Keithley 417 high speed picoammeter for amplification and then to a Princeton Applied Research Boxcar Integrator cw-1 for processing, and then to the other channel of the Hewlett Packard recorder for recording. In addition a trigger amplifier was constructed to raise the photocell trigger pulse to operate the Boxcar Integrator. The system's faithfulness in reproduction is shown in Figure 4 in which the signal integrated over 5 minutes (bottom curve) is compared with the amplified photomultiplier output (middle curve) from voltage pulses on a photodiode (upper curve).

A small plexiglass box was constructed to hold sample cuvettes and about 50 mls of water to buffer

Figure 3.--Tests of the linearity of the photomultipliers used for measuring DLE and fluorescence. A 2 watt light bulb connected to a rheostat was placed in the sample position in the DLE apparatus with the amplifier and Boxcar Integrator at normal sensitivity. The voltage on the light bulb and the iris in front of the photomultiplier were adjusted until the signal gave a full scale deflection at the desired photomultiplier voltage. To be on the safe side, voltages were chosen both considerably above and considerably below normal working voltages and the linearity was tested at these voltage extremes. Light neutral density filters, calibrated on a Cary 15 spectrophotometer, were placed between the light-source and the photomultiplier. The resulting recorder heights were then plotted versus the percent transmission of the filter combination used.


1000
800
PM current (nA)
Fluorescence
photomultiplier
603
volts
705 volts
10 20 30 40 50 60 70 80 90 100
% Transmission


3000
2000
1000
PM current (nA)
DLE photomultiplier
2000
volts
1600 volts
10 20 30 40 50 60 70 80 90 100
% Transmission

temperature changes. After all pipettings were completed the cuvette was swirled in a beaker of water at room temperature for 30 seconds and then placed in the plexiglass container for 30 seconds before measurements were begun. We found that Beckman pyrex cuvettes gave almost as much "DLE" from a chlorophyll in acetone solution as did a chloroplast suspension. We were unable to measure any "DLE" when the same chlorophyll in acetone solution was transferred to quartz cuvettes. Probably our pyrex cuvettes contained phosphorescent impurities which were excited either by excited chlorophyll or by the fluorescence given off by excited chlorophyll.

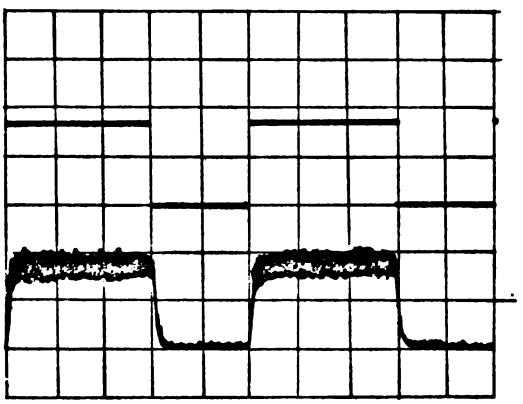
Cyanide treated chloroplasts were prepared by incubating the chloroplasts at 0°C for 90 minutes in a 30 mM KCN solution buffered at pH 7.8 as described by Ouitrakul and Izawa (1973). Controls were incubated in a similar medium in which KOH replaced KCN.

Thymoquinone was prepared from thymol by the method of Gilman and Blatt (1961). Dibromothymoquinone was prepared from thymoquinone as outlined by Carstanjen (1871). This method consisted of adding slightly more than two equivalents of liquid bromine to a container containing the thymoquinone and water. The mono and dibromo derivatives were separated using the fact that monobromo compound is much more soluble in -20°C ethanol than is the dibromo compound.

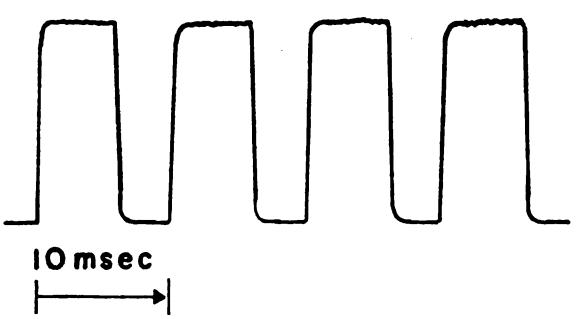
Figure 4.--Waveform distortion test of the DLE apparatus. A 100 hz, 5 volt pulse from the rear of a Hewlett-Packard counter was placed on one channel on a dual beam oscilloscope and across a Monsanto MV-4 light emitting diode (red), which was placed in the sample chamber facing the photomultiplier. The output from the photomultiplier and amplifier combination was connected to the other channel of the dual beam oscilloscope and to the input of the Boxcar Integrator. It can be seen that the diode-photomultiplier-amplifier combination damps the original signal to give a rise time of approximately 0.5 msec. The output from the Boxcar Integrator recorded on the chart paper, if closely examined, can also be seen to have a rise time of 0.5 msec, with a much reduced signal to noise ratio.

Voltage pulse to
light emitting diode

Output from P. M.
and amplifier



Output from Boxcar
Integrator recorded
at chart speed of
1 inch/5 minute.



Subchloroplast particles were prepared from chloroplasts isolated in the usual manner (see above). Five mls of chloroplast suspension having a chlorophyll concentration of 600 ugm/ml were diluted to 20 mls with a medium consisting of sucrose 0.2M and HEPES buffer 0.01 M at pH 7.4 (no $MgCl_2$) to give a final chlorophyll concentration of 150 ugm/ml. One ml of a 2% solution of digitonin in ethylene glycol was added. The preparation was shaken and incubated at 4°C for 15 minutes. It was then centrifuged for 10 minutes at 6,000 rpm in a Sorvall RC-2. The pellet was discarded and the supernatant was centrifuged for 20 minutes at 16,000 rpm in the RC-2. The pellet was resuspended in sucrose 0.2 M, HEPES 0.01 M, and $MgCl_2$ 2mM, and was washed once and was finally resuspended in the same medium.

RESULTS

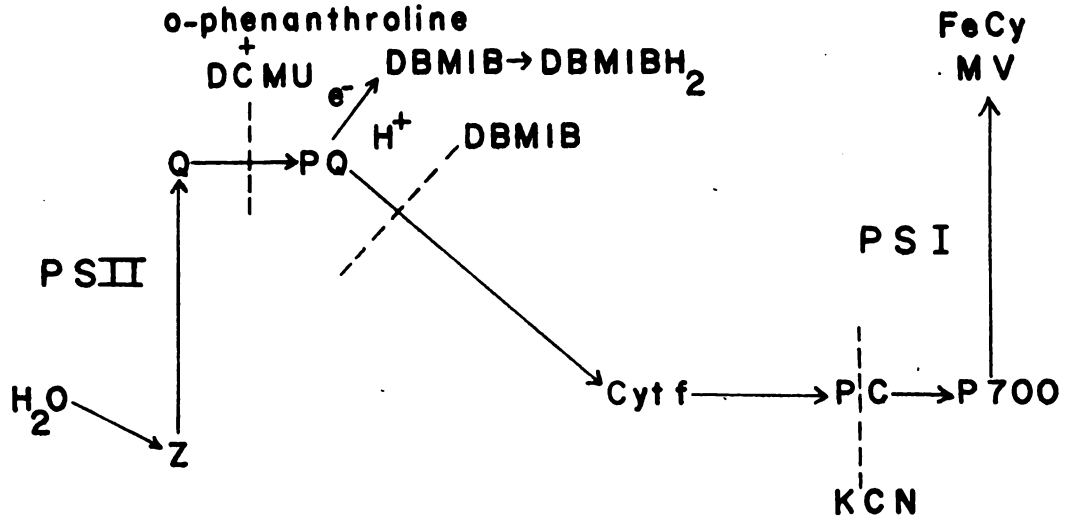
I. Electron Transport Inhibitors On Delayed Light Emission

As was pointed out in the introduction, DLE seems to depend on having the primary electron acceptor of PS II in the reduced form, the primary electron donor of PS II in the oxidized form and the thylakoid membrane in an "energized state". Electron transport inhibitors obviously affect the oxidation levels of all of the electron acceptors and electron donors in the transport chain. In this way they presumably affect DLE. However, electron transport also generates the "energized state" of the membrane, whatever that may be. In an attempt to sort out these complicated interactions we investigated the effects of a number of very dissimilar inhibitors on DLE.

The inhibitors used were orthophenanthroline, (3, 4-dichlorophenyl) -1, 1-dimethylurea (DCMU), dibromo-methylisopropylbenzoquinone (DBMIB) and KCN treatment. At this point it is worthwhile reviewing the site of inhibition of these inhibitors.

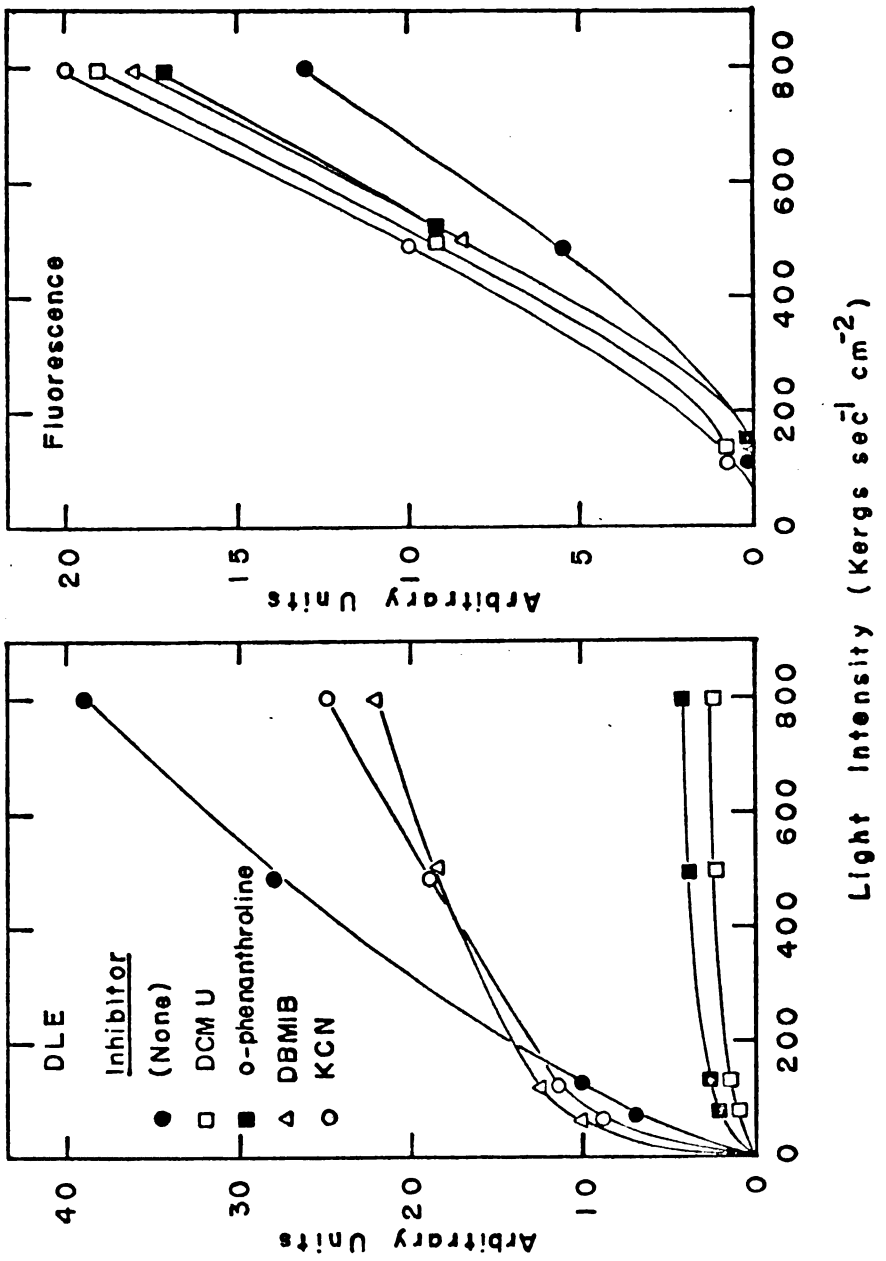
DCMU is believed to inhibit between the primary electron acceptor of PS II, Q, and plastoquinone (Duysens and Sweers, 1963). Orthophenanthroline seems to inhibit at the same place (Nishmura, 1967). The cyanide treatment

has been shown to inhibit by reacting with plastocyanin (Quitrakul and Izawa, 1973). The newest inhibitor, DBMIB, was first found to inhibit at the level of plastoquinone (Trebst et al. 1970) and later it was postulated that DBMIB inhibits on the PS I side of the plastoquinone pool by Gould and Izawa (1973). These sites of inhibition are shown in the preceding scheme.



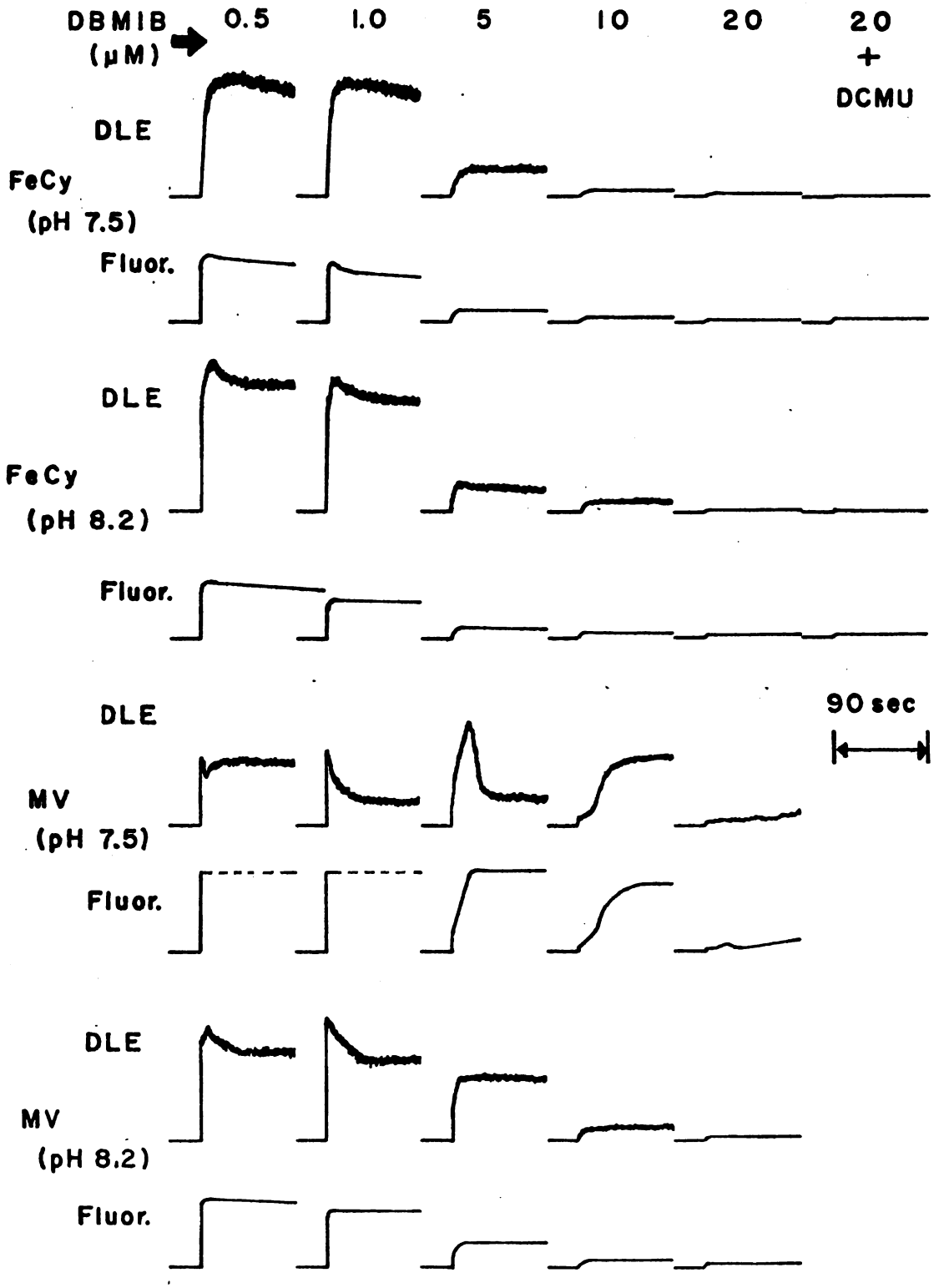
It can be seen that these four inhibitors belong to two different classes on the basis of their effects on DLE. See Figure 5. Orthophenanthroline and DCMU markedly inhibit the DLE at all light intensities. In contrast DBMIB and KCN treatment only inhibit DLE slightly, and this inhibition was only apparent at very high light intensities. At low and moderate light intensities these inhibitors actually stimulated DLE.

Figure 5.--Effects of various electron inhibitors on the DLE and prompt fluorescence. The reaction mixture contained the following compounds: sucrose, 0.1M; Tricine-NaOH(pH 8.3), 0.04M; and $MgCl_2$, 0.002 M. It also included chloroplasts containing 30 ugm chlorophyll in 2 mls. KCN-treated chloroplasts were prepared as described in methods. Final concentrations of other inhibitors were: DBMIB, 5×10^{-7}M; o-phenanthroline, 1×10^{-4}M; and DCMU, 1×10^{-6}M. The electron acceptor methylviologen was 5×10^{-5}M and was present under all conditions. Light intensity was measured, with the light-chopping bucket stopped, after the light passed through 6 cm. of saturated $CuSO_4$. The intensity of DLE 3.5-4.0 msec after the termination of each light period was measured after the chloroplasts had been subjected to one minute of periodic illumination. Light intensities were varied with neutral density filters. The arbitrary units used in measuring DLE were unrelated to and much smaller than the fluorescence units.


DLE
Inhibitor
(None)
DCMU
o-phenanthroline
DBMIB
KCN
Arbitrary Units
0
10
20
30
40
0
200
400
600
800
Fluorescence
Arbitrary Units
0
5
10
15
20
0
200
400
600
800
Light Intensity (Kergs sec^{-1} cm^{-2})

It was noticed that at concentrations of DBMIB forty times greater than those needed for complete inhibition of methylviologen reduction DLE was greatly decreased. However it was noticed that these high concentrations of DBMIB also completely inhibited the fluorescence. Since Lavorel (1968) postulated that both the DLE and fluorescence shared common factors affecting the emmission yield, an experiment was carried out to look at the relationship between fluorescence and DLE as a function of DBMIB concentration. Figure 6 presents the results of that experiment. It can be immediately seen that, in three out of four conditions, high concentrations of DBMIB inhibited both the DLE and the fluorescence. Izawa et al. (1973) have pointed out that these high concentrations of DBMIB support electron transport from PS II; DBMIB itself acts as an electron acceptor. It could be argued that transport of electrons to DBMIB quenches the fluorescence. This argument is invalid since it is shown in Figure 6 that DCMU which inhibits all electron transport does not relieve the strong quenching of fluorescence by high DBMIB. It seems that the quenching is due to some kind of direct effect of DBMIB on the chlorophyll itself. This is borne out by the fact that there is a concomitant decrease in the quantum efficiency of electron transport, and the fact that DBMIB at similar concentrations quenches the fluorescence of chlorophyll solutions.

Figure 6.--Effects of increasing DBMIB concentrations on the DLE and prompt fluorescence. The reaction mixture was identical to that of figure (5) except that HEPES buffer replaced the Tricine buffer at pH 7.5. The light intensity was 400 Kergs/sec/cm^2 of blue light. The ferricyanide when used was 0.4 mM and the DCMU was 1.0 uM. Fluorescence measurements with methylviologen at pH 7.5 and with DBMIB at 0.5 uM and 1.0 uM went off scale at the level indicated by the dashed line. As in figure 5 the data presented are recordings of the 3.5-4.0 msec DLE. The noise level could be reduced but not without yielding "overdamped" rise curves.


DBMIB (μM)
0.5
1.0
5
10
20
20 + DCMU
DLE
FeCy (pH 7.5)
Fluor.
DLE
FeCy (pH 8.2)
Fluor.
DLE
90 sec
MV (pH 7.5)
Fluor.
DLE
MV (pH 8.2)
Fluor.

Two pH's and two electron acceptors were used in this experiment for reasons based on the work of Gould and Izawa (1973). Under certain conditions DBMIB can be reoxidized by oxygen or by ferricyanide. DBMIB can be reoxidized at either pH 8.2 or pH 7.5, but the DBMIB can only be reoxidized by oxygen at pH 8.2. Thus, as shown in Figure 3, the DLE and fluorescence is very markedly quenched when the DBMIB is oxidized i.e., at pH 7.5 and 8.2 with ferricyanide, and at pH 8.2 with oxygen. At pH 7.5 with methylviologen and O_2 and at DBMIB concentrations of 5 and 10 uM, the DLE and fluorescence are initially low. However, during the early stages of illumination the DBMIB becomes reduced and cannot become reoxidized. The chlorophyll-DBMIB interaction therefore ceases and the DLE and fluorescence rise together.

As Gould and Izawa (1973) pointed out, since DBMIB is in the quinone form, its reduction to the hydroquinone form must involve the uptake of 2 H^+ ions as well as 2 electrons. This is how they accounted for the one pH rise and fall in the light, which on subsequent illuminations gave no more pH changes. This one and only one pH rise, they attributed to the consumption of H^+ ions by the reduction of DBMIB. It is felt that the initial pH 7.5 DLE and fluorescence rises are the result of DBMIB reduction which removes the chemical quenching of the

chlorophyll fluorescence. The high subsequent fluorescence is presumed due to the remaining block of electron transport at plastoquinone which keeps Q reduced. It is felt that the subsequent decay of the DLE is the result of gradual loss of the pH gradient as reported by Gould and Izawa (1973).

II. Effects of Ammonia and Organic Amines on Delayed Light Emission

It has long been dogma that the presence of uncouplers decreased the DLE from chloroplasts (Mayne, 1967). However this is not always the case. Ammonium ion, at concentrations which totally abolish phosphorylation actually increase DLE. See Figure 7. The DLE data are presented as decay curves from 3 msec to 30 msec after light extinction. (Note that the initial steep rise and the final steep fall are purely instrumental, representing the mechanical opening and closing of slits and are without biological signifance). It can be seen that the presence of ammonia increases the DLE at 3 msec but that the decay of the delayed light is much faster under these conditions. Thus at 30 msec the DLE with ammonia is decreased. Valinomycin, which makes membranes permeable to NH_4^+ (as well as K^+), has little effect by itself but it abolishes most of the DLE when NH_4^+ is present. The uncouplers gramicidin and atebrin also abolish most of the DLE. It is important to note that while the DLE is

Figure 7.--Effects of NH_4Cl and other uncouplers on the DLE decay curves with methylviologen as electron acceptor. Reaction mixture ingredients were as in figure 5. Concentrations of the added substances were, when used: NH_4Cl, 10 mM; valinomycin, 5×10^{-7} M; gramicidin D, 4 ugm/ml; and atebrin, 1.2×10^{-5}M. Data presented are decay curves from 3.0-30.0 msec after light extinction. The leading and trailing edges of the traces result from the slit opening and closing and contains no biological information. F_{max} values are the values of the maximum prompt fluorescence observed during the same experiment.

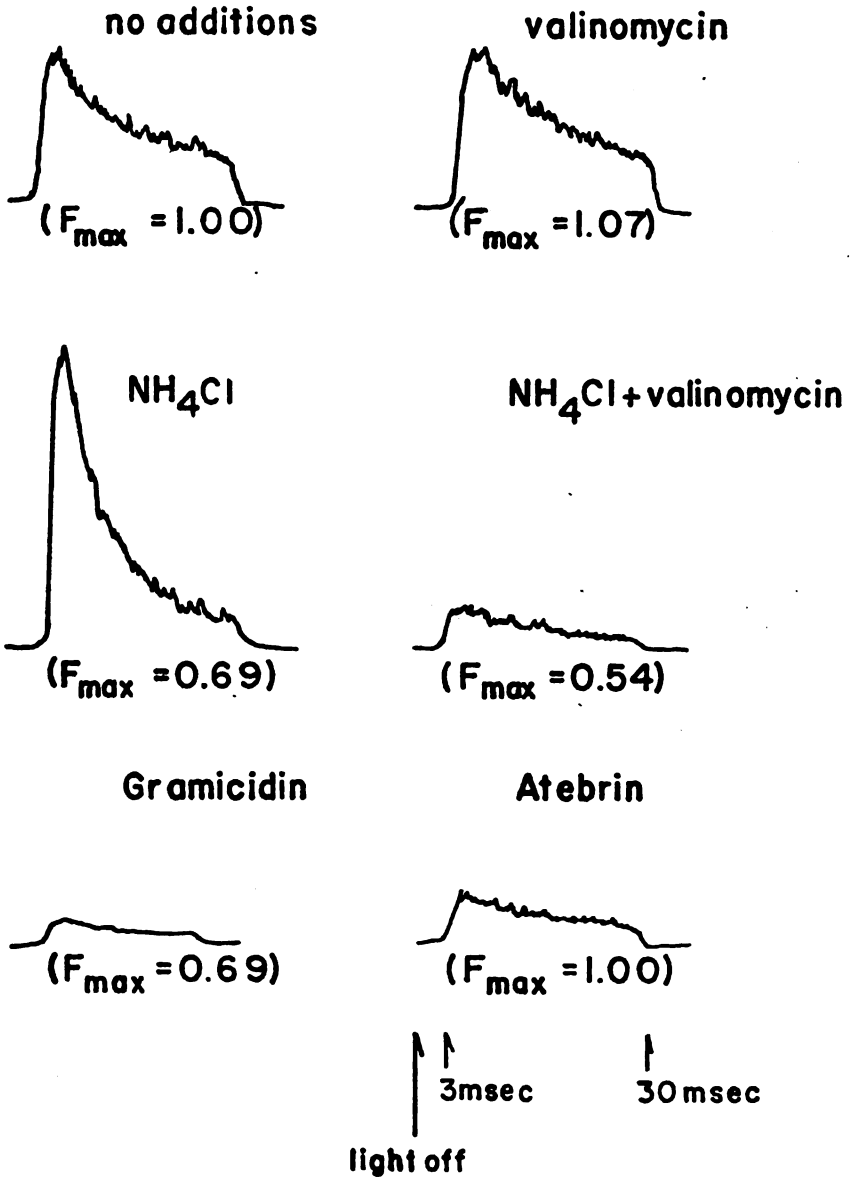
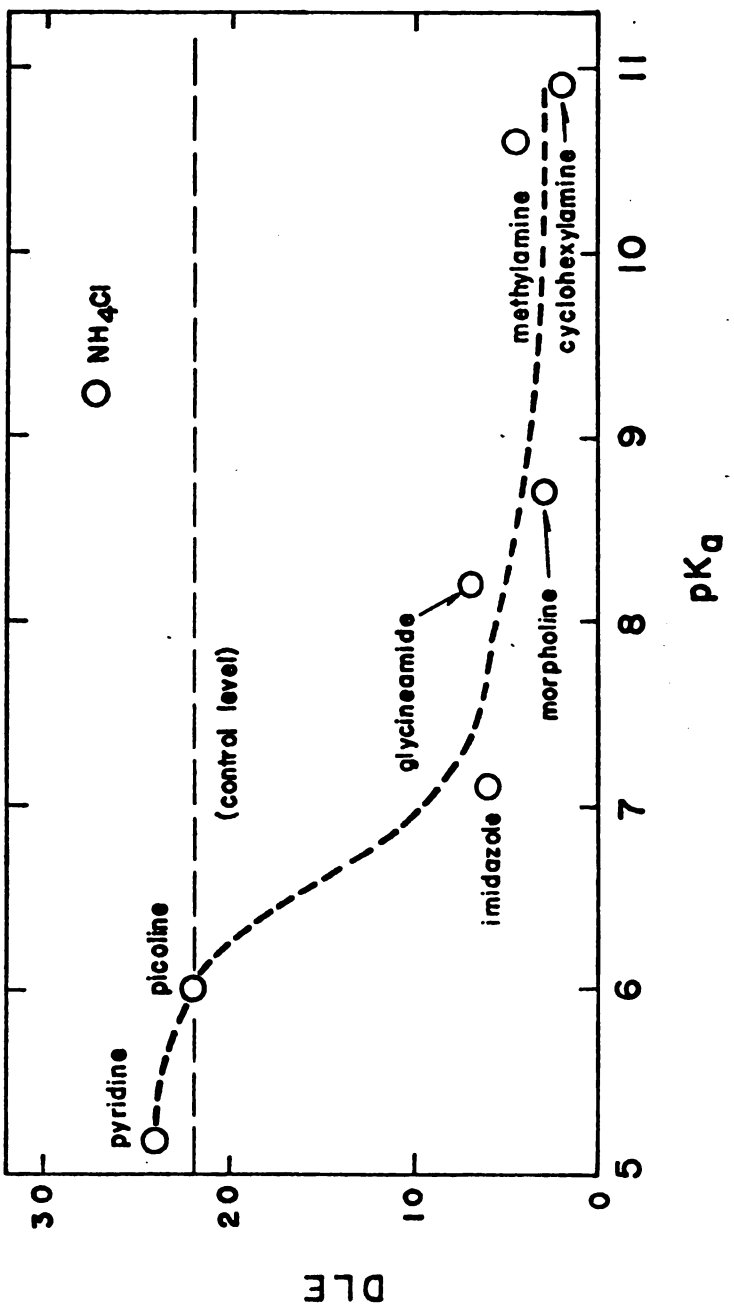

no additions
(F_{max} =1.00)
valinomycin
(F_{max} =1.07)
NH_4Cl
(F_{max} =0.69)
NH_4Cl+valinomycin
(F_{max} =0.54)
Gramicidin
(F_{max} =0.69)
Atebrin
(F_{max} =1.00)
3msec
30msec
light off

Figure 8.--Effects of amines of various pK_a's on the 3.0-3.5 msec DLE. The DLE was measured one minute after the beginning of chopped illumination. The amines were prepared in aqueous solution and adjusted to about pH 8.0 before they were added to the reaction mixture. Final concentration of all amines were 10 mM. The reaction mixture was identical to figure 5 except that the pH was 7.9.

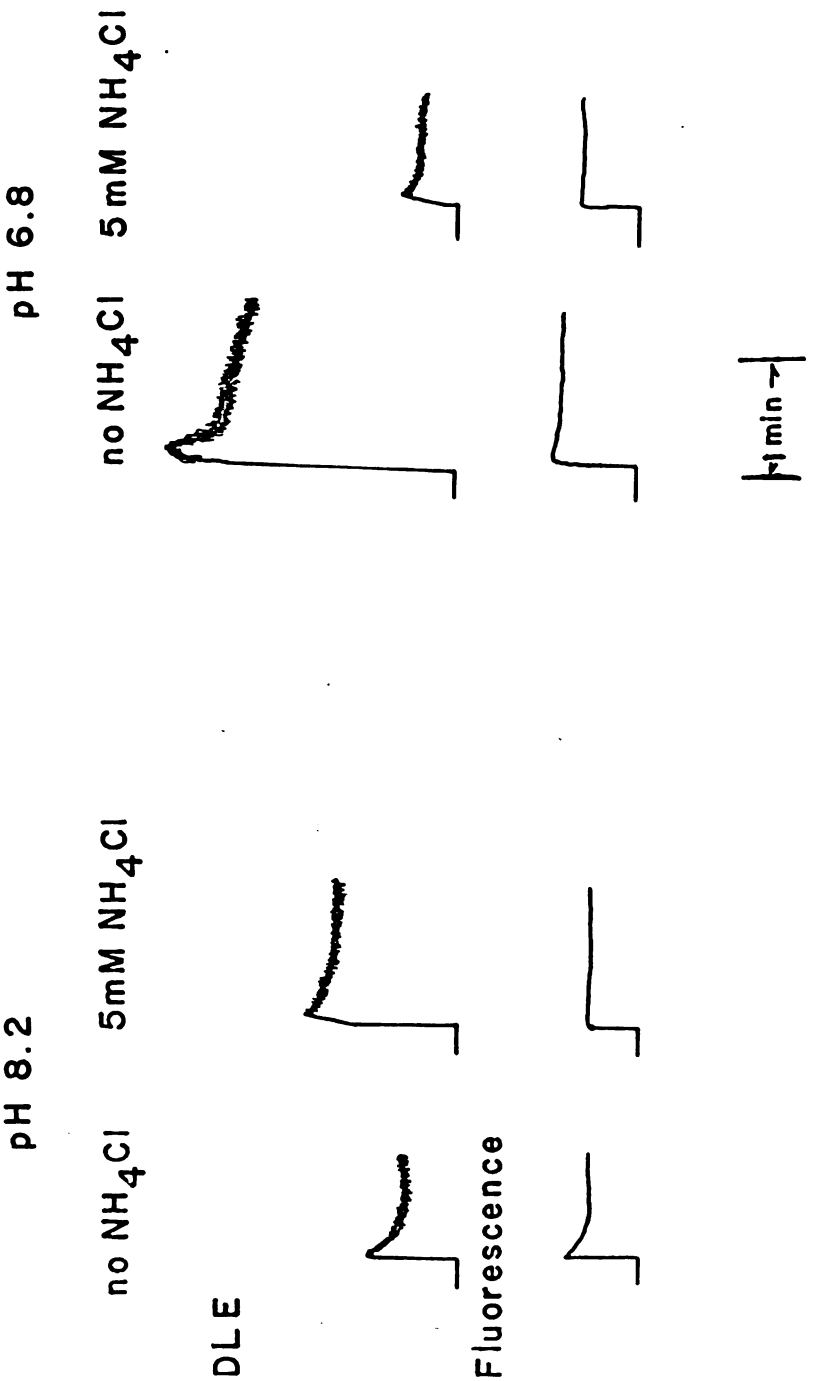

DLE
30
20
10
0
5
6
7
8
9
10
11
pKa
O NH4Cl
(control level)
pyridine
picoline
glycineamide
imidazole
morpholine
methylamine
cyclohexylamine

stimulated by NH_4Cl, the prompt fluorescence is somewhat inhibited. Therefore the stimulation of DLE by NH_4Cl cannot be due to an increase in the fluorescence yield of the chemically excited chlorophyll.

Other amines, which are equally effective uncouplers of phosphorylation, do not have this effect of increasing DLE. On the contrary they, like all other uncouplers studied, inhibit DLE. Thus ammonia seems to be the exception rather than the rule. See Figure 8. It is not clear why ammonia is unique. When we first noted that ammonia increased DLE while methylamine decreased DLE, we considered that the marked difference in pK_a might be responsible. At pH's used in these studies (about 8.0) an appreciable amount of ammonia is present in the uncharged, unprotonated, membrane permeating form, whereas very little of the methylamine is similarly uncharge. However as Figure 8 clearly shows there is no correlation between ammonia's pK_a and its effect on DLE.

Early in the course of these experiments it became apparent that the reaction mixture pH was very critical in determing the stimulation of the DLE by NH_4Cl. At pH 8 and higher the stimulation was always present whereas at lower pH's the stimulation was replaced by an inhibition. See Figure 9. At the lower pH's, for example 6.8, no concentration of NH_4Cl up to 85 mM gave any stimulation and indeed, increasing concentrations of

Figure 9.--Stimulation of DLE by NH_4Cl at high pH and inhibition of DLE by NH_4Cl at low pH. DLE was again measured at 3.0-3.5 msec after each cycle of illumination. Reaction mixture conditions were as described for figure 5 except that 40 mM MES was used at pH 6.8.

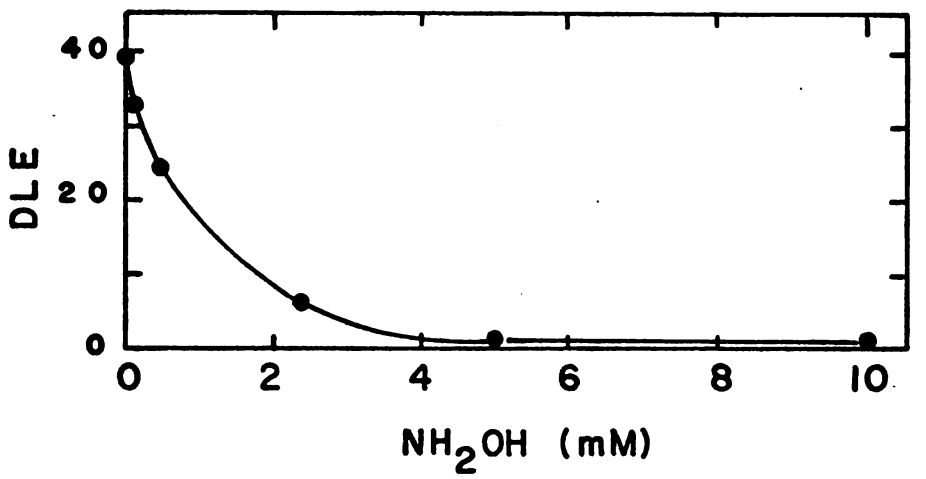

pH 8.2
pH 6.8
no NH_4Cl
5mM NH_4Cl
no NH_4Cl
5 mM NH_4Cl
DLE
Fluorescence
1 min

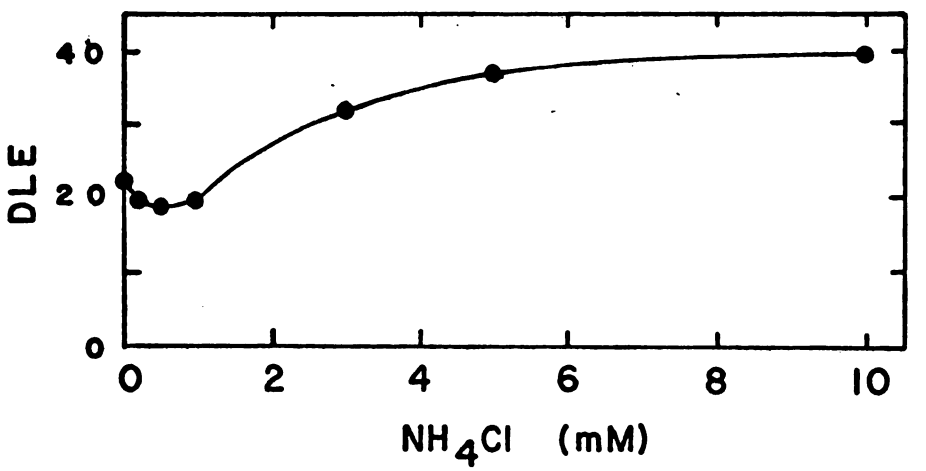
NH_4Cl simply gave lower levels of DLE. It should be emphasized that in this Figure, in contrast to Figure 7, the curves presented are not DLE decay curves. Rather they represent repeated samplings of the DLE 3.0-3.5 msec after light extinction. In other words the curves in this Figure represent changes with time in the peak heights depicted in Figure 7. Again the differences cannot be correlated with fluorescence efficiencies since these did not change very much.

Since the stimulation of the DLE by NH_4Cl was maximal at high pH, and since ammonia and other amines are known to inhibit water oxidation at high pH, it was suspected that perhaps the stimulation of DLE by NH_4Cl might somehow be a result of electron transport inhibition. If this were true then the electron transport inhibitor hydroxylamine, which inhibits water oxidation much as does high pH and NH_4Cl, should also stimulate DLE. Figure 10 gives concentration curves for the effects of hydroxylamine and NH_4Cl on the DLE. It is readily apparent that there is little similarity between the effects of these two compounds on the DLE.

The DLE in the presence of NH_4Cl and the uptake of ammonium ion by chloroplasts show remarkably similar pH optima. Furthermore, these optima are similar to the optima for basal electron transport and for noncyclic phosphorylation. See Figure 11. The pH optima for DLE

Figure 10.--A comparison of the effects of NH_2OH and NH_4Cl on the DLE. Reaction mixture conditions were as in figure 5 except that the pH was 8.5 for the NH_2OH treatment.

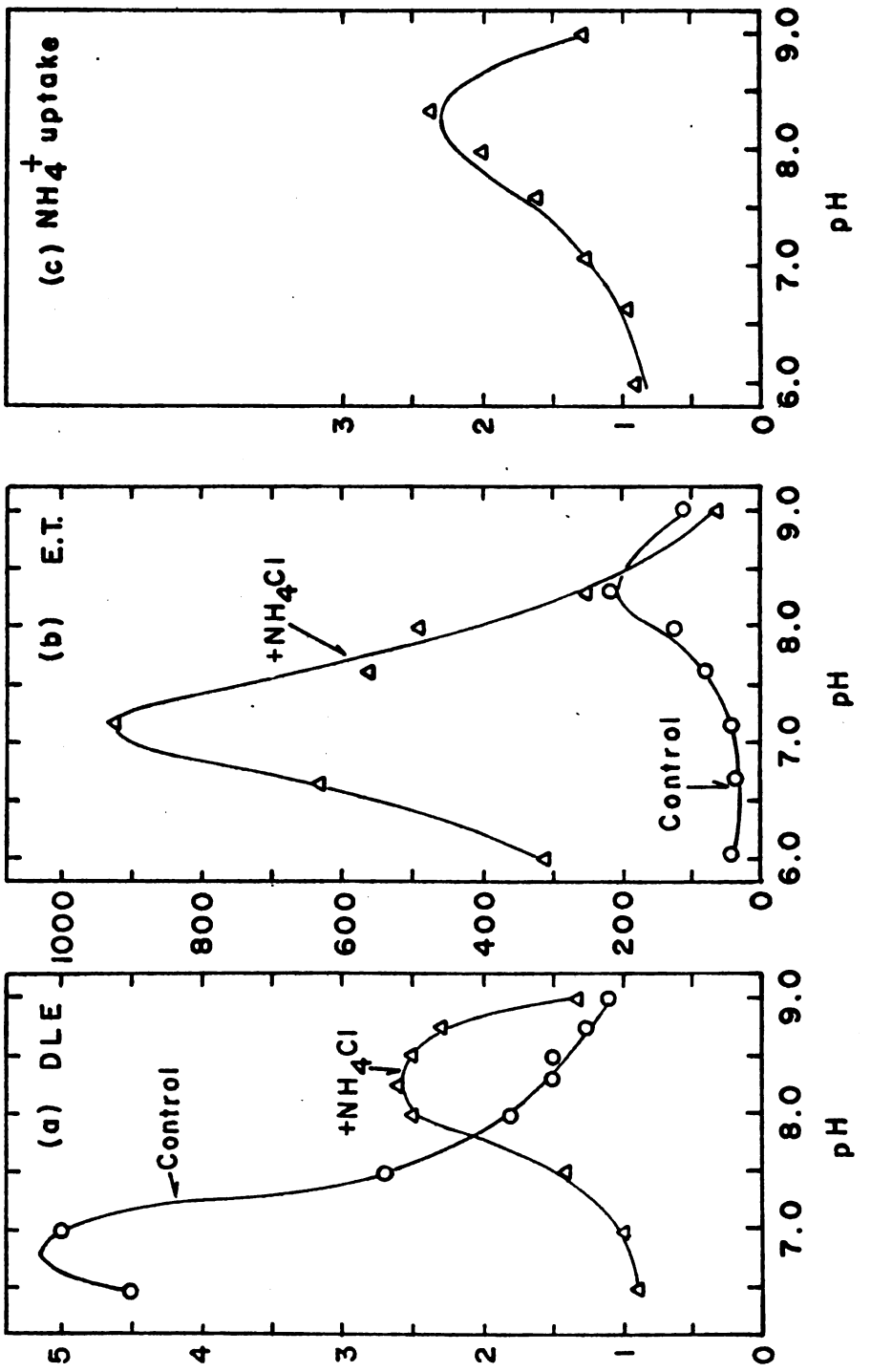

40
20
0
DLE
0
2
4
6
8
10
NH2OH (mM)


40
20
0
DLE
0
2
4
6
8
10
NH4Cl (mM)

in the absence of NH_4^+ and the pH optima for electron transport in the presence of NH_4^+ are quite different.

The DLE and ammonium ion uptake were compared at various pH's in the presence and absence of the salt LiCl (50 mM). See Figure 12. LiCl was used instead of NaCl or KCL since it does not interfere with the electrode responses used to measure NH_4^+. It is important to note that the DLE intensities reported in this Figure represent the luminescence 3.0-3.5 msec after light extinction in each 70 msec cycle of the intermittent illumination. Thus the insert in the upper right hand corner does not represent the decay of luminescence with time but rather represents changes in the 3 msec DLE with overall time of illumination. Clearly the DLE shown in this inset Figure is biphasic with a fast initial spike and a subsequent lower steady state level. The pH curve for the steady state component of the DLE is very similar to the pH curve for the extent of the uptake of NH_4^+ in the absence of LiCl. It should be noted particularly, however, that the extent of the uptake of NH_4^+ in the presence of LiCl showed a quite different pH optimum. Thus the extra NH_4^+ uptake in the presence of the chloride salt did not seem to be correlated with the DLE at all. It should also be noted that the spike of DLE was not correlated with the NH_4^+ uptake either. This spike was in any case, very variable occuring mostly at high intensities of actinic light (greater than 400 Kergs sec^{-1} cm^{-2}).

Figure 11.--A comparison of DLE, electron transport and NH_4^+ uptake as functions of pH. MES-Li buffer was used below pH 7.0, HEPES-Li buffer was used at pH 7.2 and 7.6, Tricine -Li buffer was used at pH 8.0 and 8.3, and TAPS-Li buffer was used at pH 8.5, 8.7, and 9.0. All buffer concentrations were 40 mM. The reaction mixtures used in the measurement of DLE and electron transport were as described in figure 5 except for the buffer substitutions noted. The reaction mixtures used for measuring NH_4^+ uptake consisted of sucrose, 0.1 M; buffer, 0.04 M; $MgCl_2$, 0.002 M; and chloroplasts containing 250 ugm of chlorophyll, all in 4 ml. When NH_4Cl was used it was 5 mM.


(a) DLE
Control
+NH4Cl
(b) E.T.
+NH4Cl
Control
(c) NH4+ uptake
pH
pH
pH

A study of the effects of other salts and of mannitol on DLE was also undertaken. While the nature of the anion present did affect the intensity of the DLE somewhat, these effects were not striking. The same is true of all concentrations of mannitol employed up to 0.8M.

Since methylamine and NH_4Cl do not uncouple phosphorylation in digitonin particles of chloroplasts (Nelson et al. 1970), DLE from these particles was investigated. Figure 13 shows that DLE in the particles was stimulated at pH 8.3 by NH_4^+ as in intact chloroplasts. However DLE in the particles was also stimulated by methylamine, even though methylamine strongly inhibits the DLE in intact chloroplasts. Moreover, NH_4Cl, which inhibits DLE at pH 6.5 in intact chloroplasts, did not inhibit DLE in the particles. As expected the DLE was obliterated by DCMU and by the combination of NH_4Cl and valinomycin.

III. Effects of Nucleotides on Delayed Light Emission

As has already been pointed out in the introduction, DLE in chloroplasts is inhibited by the presence of ADP and Pi (Mayne 1967). Table I confirms this observation but adds the new information that ADP and ATP actually increase DLE if phosphorylation is prevented either by omitting phosphate or by adding deoxyphlorizin. Deoxyphlorizin was included in this experiment because Winget et al. (1960) and Izawa et al. (1966) have shown that

Figure 12.--A comparison of the initial rate and extent of NH_4^+ uptake with the spike and "steady state level" of the 3.0 msec DLE, with and without high salt (50 mM LiCl). Reaction mixtures for DLE measurements were as in figure 5, while reaction mixtures for NH_4^+ uptake measurements were as described in figure 11. For the high salt condition LiCl was added since NaCl or KCl would have interfered with NH_4^+ determinations.

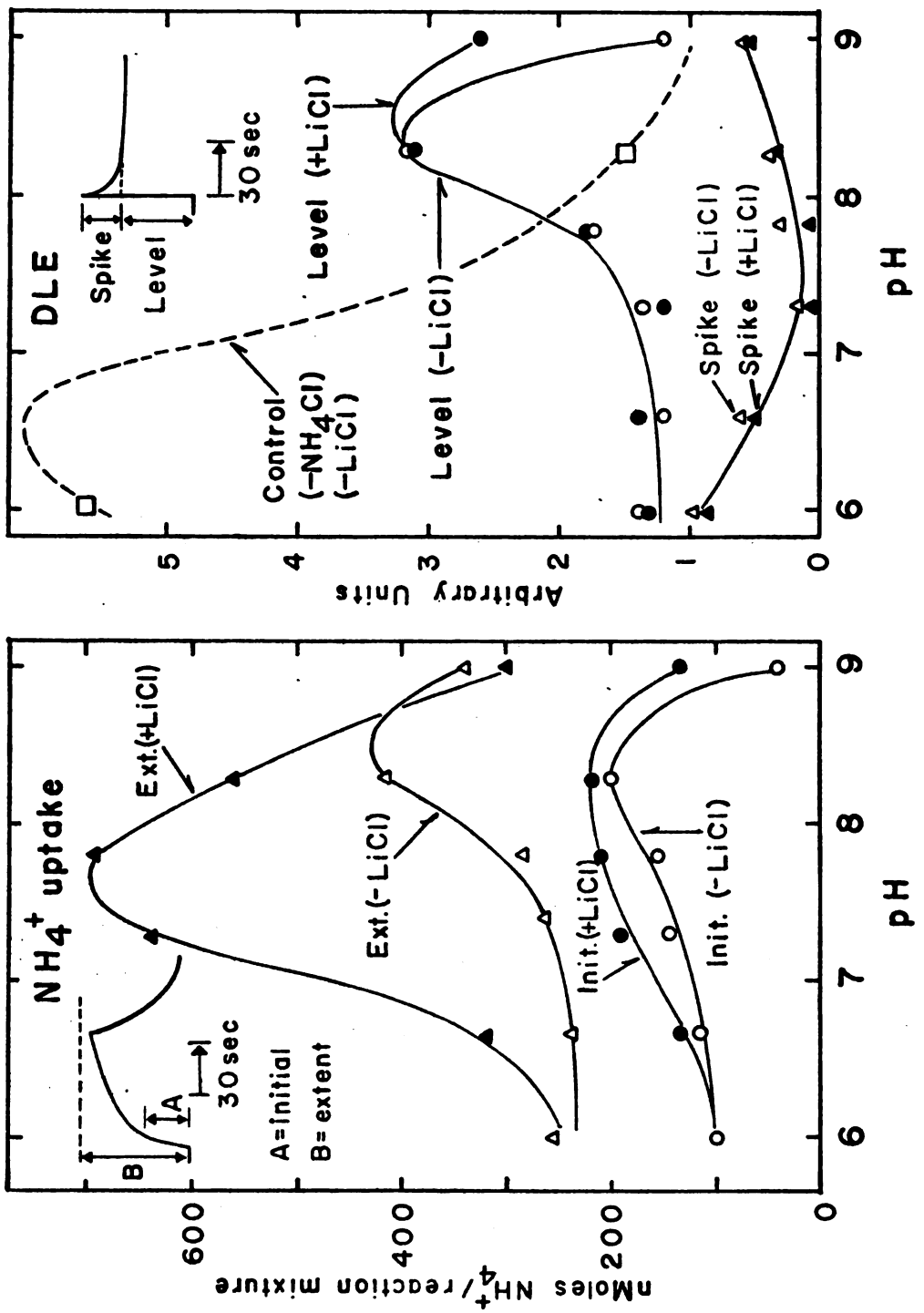
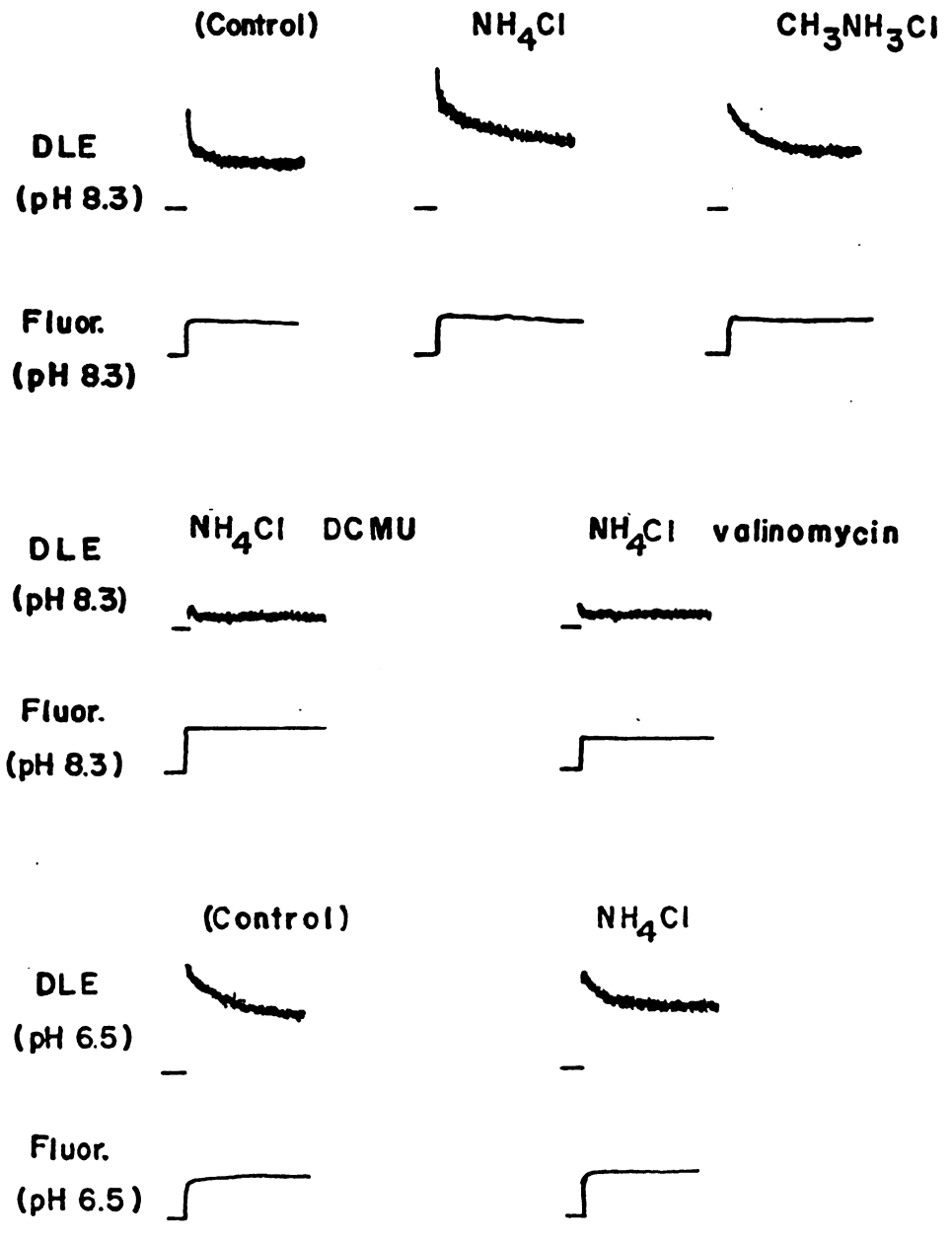

DLE
Spike
Level
30 sec
Control
(-NH4Cl)
(-LiCl)
Level (+LiCl)
Level (-LiCl)
Spike (-LiCl)
Spike (+LiCl)
Arbitrary Units
pH
NH4+ uptake
Ext.(+LiCl)
Ext.(-LiCl)
Init.(+LiCl)
Init. (-LiCl)
A
B
30 sec
A=Initial
B= extent
nMoles NH4+/reaction mixture
pH

Figure 13.--Presence of DLE with CH_3NH_3Cl in sub-chloroplast particles. Particles were prepared as described in the methods. NH_4Cl and CH_3NH_3Cl concentrations were 10 mM. The DCMU was 1×10^{-6}M, and the valinomycin was 5×10^{-7}M. MES-Na buffer was used at pH 6.5. All other conditions were as in figure 5.


(Control)
NH_4Cl
CH_3NH_3Cl
DLE
(pH 8.3)
Fluor.
(pH 8.3)
NH_4Cl DCMU
NH_4Cl valinomycin
DLE
(pH 8.3)
Fluor.
(pH 8.3)
(Control)
NH_4Cl
DLE
(pH 6.5)
Fluor.
(pH 6.5)

phlorizin and its analogs inhibit ATP synthesis and the latent chloroplast ATPase. It seems, therefore, that the stimulation of DLE by added ATP cannot be attributed to any reaction which involves energy from the hydrolysis of ATP.

This stimulation of DLE by nucleotides seems to be specific for ATP, ADP, and ITP, with ITP showing a considerably smaller effect. This specificity is very similar to the nucleotide specificity of the phosphorylation related activities of chloroplasts and coupling factor (Avron, 1960). See Table II.

The actual recorder tracings which document some of these contentions are presented in Figure 14. It can be seen that the fluorescence change with nucleotides present or absent are negligible in comparison with the large DLE changes. As expected ADP and Pi inhibited DLE, while ATP or ADP without phosphate stimulated DLE, and Pi alone was without effect. The last tracing in Figure 14 shows the results obtained when phosphate and a limited amount of ADP were added. This amount of ADP should have been almost completely phosphorylated within a minute or two. As is shown initially the ADP and Pi inhibited the DLE, but gradually as the ADP was converted to ATP the DLE rose in a curious two step reaction which is not understood.

The increased DLE in the presence of the nucleotides ADP and ATP seems to be related to the increased H^+

gradient which is observed under the same conditions (McCarty et al. 1971). These conditions also inhibit the basal non-phosphorylating electron transport (Izawa and Good, 1969). Figure 15 shows these phenomenon as functions of the ATP or ADP concentration in the same chloroplast preparation. It is evident that the stimulations and inhibitions all plateau and become maximal at about 15 uM. The DLE data of Figure 15 shows that the ATP stimulated the DLE more than ADP at the same concentration. We always found this to be true at or below 50 uM, ADP or ATP, but we also found that the difference between ADP and ATP stimulations varied considerably between chloroplast preparations. Differences between the ADP and ATP stimulated H^+ uptake at the same concentration are not considered to be experimentally significant. Nevertheless, the stimulation over the control is quite significant and does plateau at about 15 uM. Differences between electron transport inhibition by ATP and by ADP are considered to be experimentally significant with ATP inhibiting slightly more than ADP. The correlations among these three phenomenon, are not believed to be purely coincidental as will be explained in the discussion section.

TABLE I.--Stimulation of Delayed Light Emission by ADP and ATP.*

Additions	Delayed Light (arbitrary units)	
	-deoxyphlorizin	+deoxyphlorizin
Ferricyanide alone	100	95
Ferricyanide plus ADP	153	166
Ferricyanide plus Pi	113	100
Ferricyanide plus ADP and Pi	60	107
Ferricyanide plus ATP	166	166

*The reaction cuvette contained 2 ml. of reaction mixture consisting of 40 mM tricine-NaOH buffer (pH 8.2), 0.1 M sucrose, 2 mM $MgCl_2$, and potassium ferricyanide 0.4 mM. Other concentrations when used were 0.5 mM ADP, 0.5 mM ATP, 5 mM Pi, and 0.5 mM deoxyphlorizin. The amount of chloroplasts suspended in the 2.0 ml reaction mixture was equivalent to 30 ugm chlorophyll. Values were for steady state delayed light taken one minute after starting the chopped illumination. Measurements were taken on the segment of the decay curve from 2.5 to 3.0 msec after light extinction.

TABLE II.--Stimulation of Delayed Light by Various Nucleotides.*

Nucleotide	Concentration (uM)	DLE (arbitrary units)
None	---	100
ATP	50	157
ADP	50	136
5'AMP	50	100
Adenosine	500	95
GDP	50	104
UTP	50	104
ITP	50	123

*50 uM methylviologen was used as electron acceptor. Other conditions were as in Table I.

Figure 14.--Effects of ADP, Pi, and ATP on the DLE and fluorescence. The reaction mixture was as in figure 5. The Pi in all experiments was 5 mM. The ATP was 150 uM as was the ADP concentration when the ADP was in excess. When the ADP was limited it was 45 uM. The intensity of the blue actinic light was 130 Kergs/sec/cm^2. The DLE was measured 3.0-3.5 msec after the illumination cycle ended.

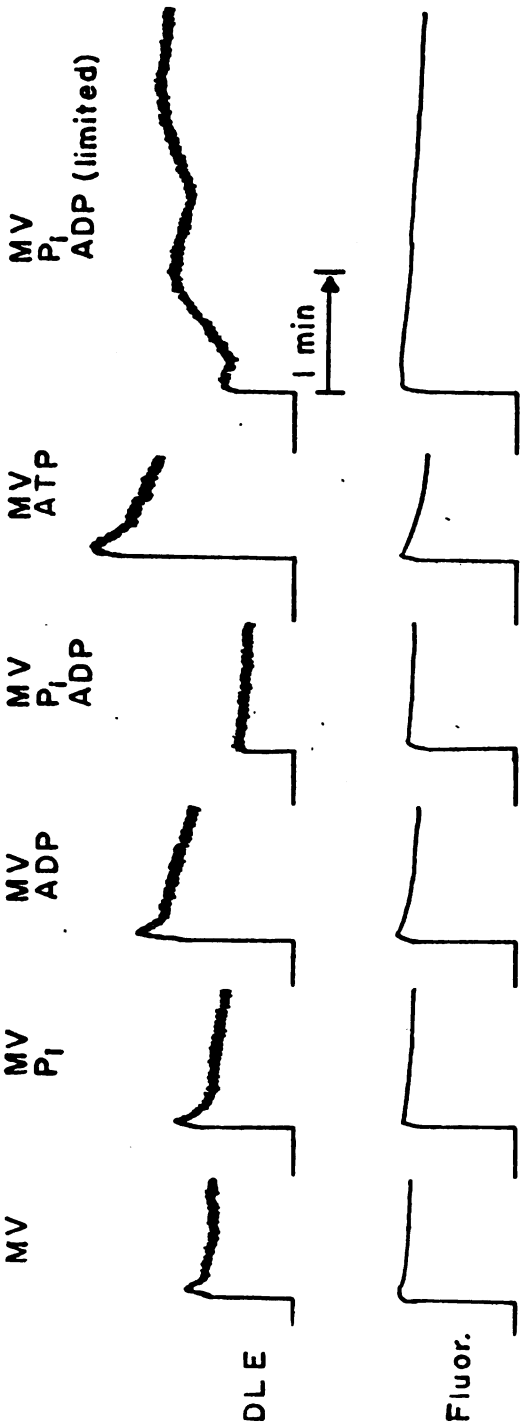
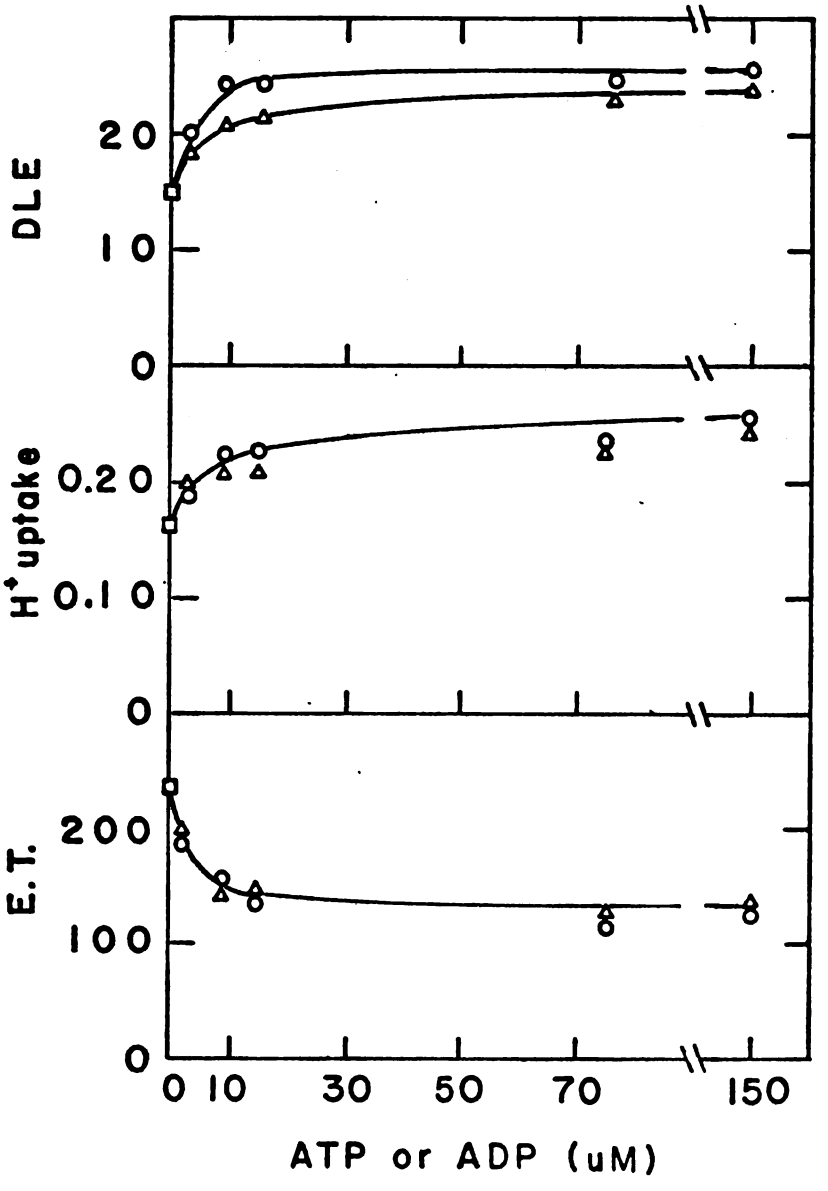

MV
MV
P_i
MV
ADP
MV
P_i
ADP
MV
ATP
MV
P_i
ADP (limited)
1 min
DLE
Fluor.

Figure 15.--The effects of ADP and ATP on the DLE, H^+- uptake and electron transport. The DLE experiment and the H^+ uptake were carried out on the same day with the same chloroplast suspension. Units for the DLE are arbitrary, units for electron transport are microequivalents/hr/mg chlorophyll, and units for H^+ uptake are micromoles/mg chlorophyll. The reaction mixture for the DLE measurements and the H^+ uptake measurements consisted of methylviologen, 5×10^{-5}M; sucrose, 0.1 M; $MgCl_2$, 0.005 M; and HEPES buffer 0.001 M at pH 7.8. The DLE experiments used chloroplasts containing 30 ugm chlorophyll while the H^+ uptake experiments used chloroplasts containing 100 ugm chlorophyll. The reaction mixtures in both cases were 2.0 mls. Electron transport was measured as O_2 consumption using an oxygen electrode. Conditions for electron transport measurements were as in figure 5. Circles represent ATP concentrations, triangles represent ADP concentrations, and squares represent no additions.


DLE
20
10
0
H+uptake
0.20
0.10
0
E.T.
200
100
0
0 10 30 50 70 150
ATP or ADP (uM)

DISCUSSION

The mechanism of DLE remains obscure. Strehler and Arnold (1951) postulated that a reversible enzyme reaction was involved. However it would have to be a very exergonic enzyme reaction since almost 40,000 calories or 1.8eV is released in the formation of an einstein of red light. Mayne's (1967) observation that uncouplers greatly inhibit DLE suggests that the a "high energy state" of the chloroplast membrane somehow contributes to DLE. Further, his observation (Mayne and Clayton, 1966) that an acid-base transition which is capable of causing phosphorylation also elicits DLE from preilluminated chloroplasts adds support to this concept. However, on considering the energetics of the situation, it becomes clear that six or seven ATP's or ATP precursors (squiggles) operating in a concerted fashion reaction would be required to produce an einstein of red light. Similarly it would take a pH difference across the membrane of 30 units at 60 mv/unit, to produce the energy for an einstein of red light! These considerations of energy requirements and the demonstrated requirement for preillumination in the acid-base transition studies make it seem very probable

that DLE somehow also involves a back-reaction of products of the initial forward photoreactions.

There have been several proposals concerning the ways in which these back-reactions might occur. Lavorel (1969) postulates that DLE is the result of the recombination of the oxidized donor of electrons to PS II (Z^+) and the reduced acceptor of electrons from PS II (Q^-).

This suggestion receives a good deal of support from biochemical studies. The hydroxylamine - induced inhibition of the oxidation of water by Z^+ (Joliot, 1966, Izawa et al. 1969, Cheniae and Martin, 1971) results in an inhibition of DLE (Lavorel, 1969). Furthermore, Barbieri et al. (1970) have provided evidence that intermediates in water oxidation (Z_o to Z_4 in the formalism of Joliot et al. 1969) are involved in DLE. Similarly, inhibition of electron transport by DCMU, which is reputed to prevent the flow of electrons between Q and plastoquinone (Duysens, 1964), also abolishes the DLE (Sweetser et al. 1961). These observations, taken together imply not only that the back-reaction between Z^+ and Q^- is required for DLE but also that the limiting factors in determining the amount of DLE are, on the one hand, the back-reaction of plastoquinone with Q and on the other hand the back-reaction of some intermediate in the water oxidation reaction with Z.

Lavorel's proposal should be considered from the standpoint of energetics. Although Z^+ must be more than +0.8 volts to oxidize water and Q^- is reputed to be somewhere between +0.2 volts (Kok et al. 1967) and -0.2 volts (Cramer and Butler, 1969) it still does not seem that the recombination could provide quite all of the requisite energy. Nor does it explain how the "high energy state" of the membrane is involved. Nevertheless the potentials quoted are so uncertain that one must apply such energy computations with caution.

Arnold (1968) rationalizes the fact that there is apparently not enough energy in the reaction of Z^+ with Q^- to give DLE by abandoning the "Z scheme" of Hill and Bandall (1960) to use Z^+ with an electron from ferredoxin. The combination of these two might then have sufficient energy to support DLE. However, Goedheer (1962) had already shown that PS II light stimulates DLE while PS I light markedly decreases DLE. This suggests that the removal of electrons from the plastoquinone pool decreases DLE, which in turn suggests that the electrons used in DLE come from plastoquinone and not from ferredoxin or anything on the reducing side of PS I.

Lavorel (1969) approached the problem of the energy required for DLE in a different manner. He postulated that the product of the reaction of Z^+ and Q^- produced one triplet exciton. Then two of these triplet excitions

"collide" to form the chlorophyll singlet which decays in a normal manner to give the DLE. The major drawback to this theory is that while Livingston (1960) has presented extensive data on triplet activity in chlorophyll solutions, the triplet state has never been detected in chloroplasts. Further doubt is cast upon the triplet model by the finding of Lumpkin and Hillel (1973) that a pulsed 30 kgauss magnetic field does not affect the DLE.

Fleishman (1971) and Crofts, Wraight, and Fleischman (1971) have suggested a mechanism by which the deficiency in energy of the reaction between Q^- and Z^+ might be overcome while at the same time accomodating the fact that the high energy state of the membrane is required for DLE. They propose that the additional energy needed comes from an electrochemical potential which is formed across the membrane. They interpret Mayne and Clayton's (1966) observation that acid-base transitions contribute to the DLE, and Mayne's (1967) subsequent observation that uncouplers inhibit the DLE in terms of this model, with the acid-base transition producing the electrochemical (H^+) gradient across the membrane and uncouplers abolishing this gradient, by making the membranes permeable to H^+.

Other studies have implicated trans-membrane electrochemical gradients. Miles and Jagendorf (1969) showed that a transition from a low salt medium to a high

salt medium also contributed to DLE. Several different anions and cations were employed. It is important to note that such gradients, unlike H^+ gradients, do not support significant phosphorylation. Subsequently, there were other reports of cation gradients which stimulated DLE but did not support phosphorylation. For example, Barber and Kraan (1970) showed that a low sodium chloride to high sodium chloride transition in the presence of gramicidin (which makes membranes permeable to Na^+ but utterly abolishes phosphorylation) gave DLE. Similarly, a low K^+ to high K^+ transition gave DLE in the presence of valinomycin (which makes membranes permeable to K^+). Thus DLE does indeed seem to require a membrane potential, but this potential does not need to be of a kind which supports phosphorylation. In fact DLE occurs under conditions where no phosphorylation would be possible (in the presence of gramicidin as reported above and as in the ammonia experiments reported herein).

These ammonia experiments show a stimulation of DLE by NH_4Cl and, under low salt conditions, the stimulation seems to be correlated with NH_4^+ uptake. At least both NH_4^+ uptake and DLE under these conditions show the same pH optimum. See Figure 11. This correlation disappears under high salt conditions. However Izawa and Good (1966) have shown that the chloroplast volume increases drastically with NH_4^+ and high salt and this swelling

probably destroys any systematic relationship between NH_4^+ uptake and the NH_4^+ concentration gradient which might be determining the membrane potential associated with DLE. The reason why this effect of NH_4^+ on DLE is unique among amines is unclear. However, it is probably relevant that methylamine also stimulates DLE in subchloroplast particles instead of inhibiting the DLE as it does in intact chloroplast lamellae. Perhaps this is somehow related to the fact that ammonia and aimes do not uncouple phosphorylation in subchloroplast particles.

It is important to remind the reader that the energy from an acid-base transition or a low salt to high salt transition is not alone sufficient to create DLE, since a recent preillumination has been shown to be required by Mayne (1968). Thus there seems to be a requirement for a membrane potential AND a Z^+ and Q^-. Fleischman (1971) and Crofts and co-workers (1971) visualize the membrane potential as somehow adding to the energy of the reaction between Z^+ and Q^- in such a way as to effectively lower the activation energy of this reaction. Even if the combined membrane potential and the combined redox potentials of the Z^+ and Q^- do not provide the 1.8 eV necessary for the DLE, there must be a few "individuals" in the Boltzman distribution that can reach the 1.8 eV. Furthermore the greater the membrane

potential due to, for instance, a H^+ gradient, the greater should be the number of "individuals" with enough energy to make DLE, and the faster should be the rate of back-reaction between Q^- and Z^+ i.e., the decay of the DLE. See Figure 7.

The amount of DLE is very small. It has been determined by Tollin et al. (1958) to have a quantum yield of 10^{-6}. Thus one would expect the activation energy to be rather large. Indeed, this is fortunate since it is the function of the chloroplast to get excitation energy away from the excited chlorophyll, into the hands of the enzymatic machinery to make ATP and NADPH, and not DLE.

Fleischman (1971) and Crofts et al. (1971) do not limit themselves to a discussion of energetics, but also postulate certain topographical features in the chloroplast membrane. We would like to develop their concepts with new evidence. Basic features of their model are that water splitting, and its consequent H^+ production, occurs on the inside of the thylakoid membrane " in equilibrium" with the Z/Z^+ couple, while the plastoquinone pool occurs on the outside of the membrane in equilibrium with the Q/Q^- couple. Then, since the H^+ pump creates an electric field, positive inside and negative outside of the membrane, the resulting membrane potential would act to oppose the electron from Q^-, going

to the inside of the membrane to back-react with Z^+. Thus energy would have been added to the reaction between Z^+ and Q^-.

However, there is good evidence that the pool of electrons used in the back reaction of DLE actually resides in plastoquinone and not in the primary electron acceptor, Q. DCMU and o-phenanthroline (Nishimura, 1967) which probably blocks the transport of electrons from Q^- to plastoquinone abolish DLE. However, inhibitors which block electron transport on the PS I side plastoquinone actually increase the quantum efficiency of DLE, as shown in Figure 5. Thus KCN treatment which inhibits at the level of plastocyanin (Quitrakul and Izawa, 1973) and DBMIB which inhibits by preventing the reoxidation of reduced plastoquinone (Trebse et al. 1970) cause more, rather than less DLE at all but very high light intensities.

Furthermore, there is evidence that the back-reaction of Q^- with Z^+ does not require much activation energy since it is said to occur at liquid nitrogren temperatures (Tollin et al. 1958). Thus the "activation energy" supplied by the membrane potential probably somehow contributes to the transport of electrons from the storage form in plastoquinone to Q, and perhaps to the transport of electrons from Z to the intermediates in the water oxidation process.

With these considerations in mind we would like to modify the Fleischman-Crofts model by suggesting that plastoquinone and the water oxidation machinery are on the outside and inside respectively of the membrane and that the membrane potential "accelerates" reverse electron flow on the way from plastoquinone to the water oxidation site. We therefore suggest that the primary photochemical charge separation may occur across some sort of chlorophyll containing layer, which occupies much less than the 70-90°A width of the membrane (Kirk, 1971) and that subsequent electron transport events (Q^- to plastoquinone and water oxidation to Z^+) take place across the rest of the membrane. Such a model is outlined in Figure 16.

This model would explain our data in the following manner. First, the DBMIB and KCN blocks stimulate DLE at low light intensities by backing up the electrons in the plastoquinone pool which can back react to reduce the Q. The Q reduction is very unfavorable but under conditions where the electric field from ion gradients make it more favroable, it can go to a small degree. This reduction of Q by plastoquinone can be blocked by DCMU or o-phenanthroline which then abolishes the DLE. If Q is reduced by plastoquinone it can react with Z^+ to give an excited chlorophyll molecule which then gives DLE. The stimulation by NH_4Cl is due to the fact that ammonium ion uptake establishes an electrical gradient, as

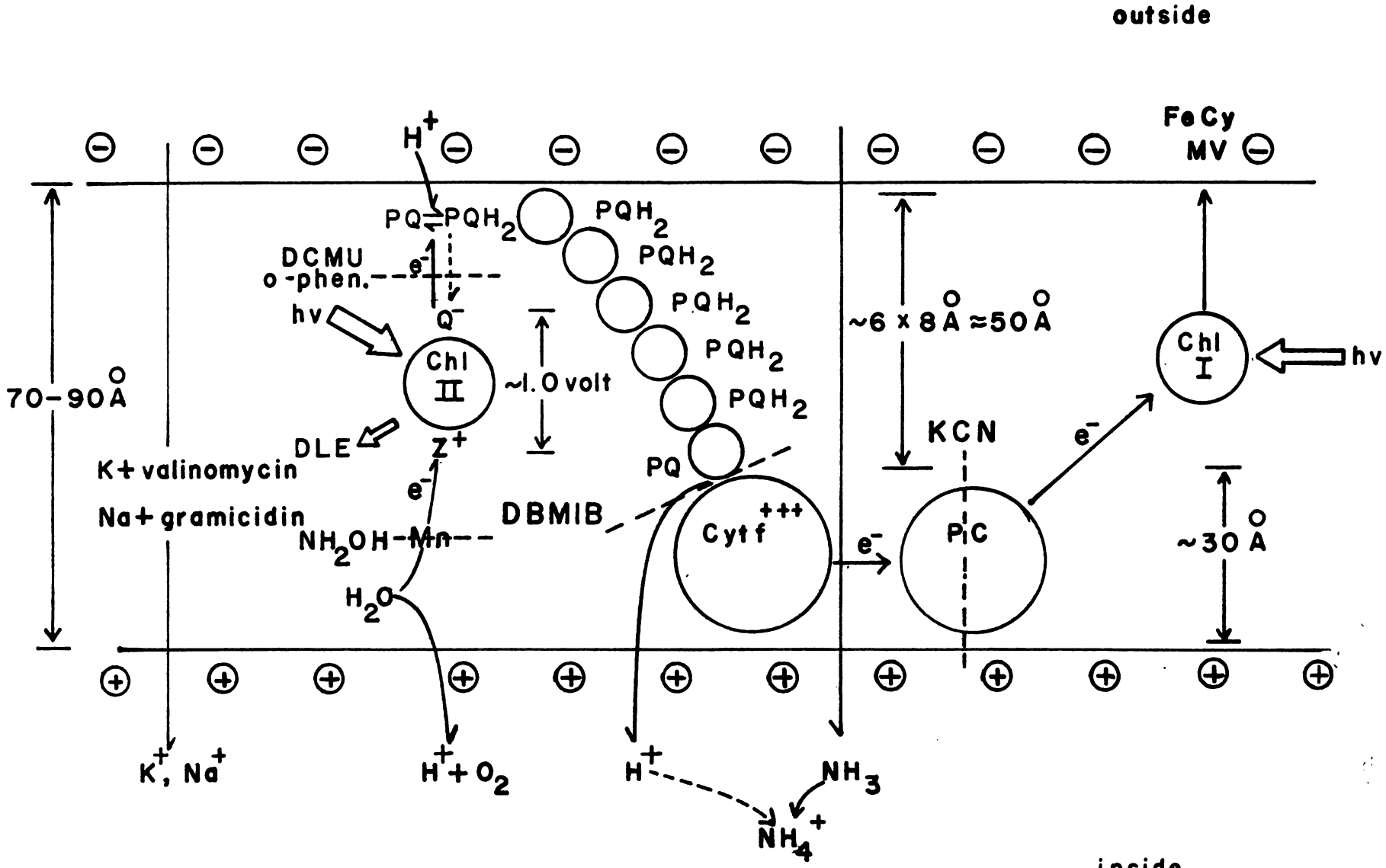
did the H^+ gradient, and this electrical field caused by the ammonium ion gradient partially counteracts the thermodynamically unfavorable Q reduction by plastoquinone.

It should be noted that according to our model and to the model of Fleischman and Crofts, uncouplers only abolish DLE if they abolish all membrane potentials. Obviously some kinds of ion gradients and membrane potentials which are incapable of supporting phosphorylation are capable of contributing to DLE.

Probably ADP and ATP stimulate the DLE because they stimulate the H^+ uptake, presumably by interaction with the coupling factor, and the resulting membrane potential once again stimulates the rate of Q reduction by plastoquinone. The fact that ADP and Pi together inhibit the DLE is also easily explained since they consume H^+ to make ATP. This consumption of H^+ reduces the membrane potential and makes it more difficult for electrons to go from plastoquinone to Q.

The corollary to this model is that in decreasing the activation energy for the reduction of Q with electrons from plastoquinone by applying an electric field favorable for the reverse electron movement, one simultaneously increases the activation energy for the forward reaction. That is if one stimulates the DLE, all other conditions being equal, one should inhibit electron transport. Figure 15 shows precisely that. ATP and ADP stimulate the DLE

Figure 16.--Postulated general configuration of the thylakoid membrane discussed in this thesis. In this model, the membrane normally becomes charged because of the transport of H^+ ions unaccompanied by counter ions. It can also become charged due to passive diffusion of Na^+ or K^+ along a concentration gradient. The membrane potential and/or H^+ gradient add to the energy available for the back reaction which produces DLE i.e. the transfer of electrons from PQH_2 to water oxidizing center designated Mn (to imply the involvement of manganese). The H^+ "pump" is represented as a consumption of H^+ in the reduction of plastoquinone occuring on the outside of the membrane, the oxidation of the PQH_2 by the electron carrier cytochrome f with concomitant H^+ production on the inside, and the oxidation of water to H^+ and O_2 also on the inside. The sizes of the components of the model are indicated in a very rough way in this figure. Probably these components could be accomodated within the membrane. Note that DCMU and NH_2OH, (which interfere with the back-reaction) inhibit DLE. In contrast DBMIB and KCN, which inhibit electron transport to the same degree, but at subsequent sites more remote from the photochemistry, do not inhibit DLE.


outside
FeCy
MV
H+
PQ⇌PQH2
PQH2
PQH2
PQH2
PQH2
PQH2
DCMU
o-phen.
e-
Q-
hv
Chl
II
~1.0 volt
70-90 Å
DLE
Z+
K+ valinomycin
Na+ gramicidin
e-
NH2OH-Mn
H2O
DBMIB
PQ
Cyt f+++
~6 x 8 Å ≈50 Å
KCN
e-
Chl
I
hv
e-
PC
~30 Å
K+, Na+
H+ + O2
H+
NH3
NH4+
inside

by increasing the H^+ uptake while at the same time also decreasing electron transport. When ADP and Pi were added, H^+ ions were consumed, decreasing the electrical gradient opposing the forward reaction and thus allowing electron transport to proceed at a faster rate. Similarly uncouplers, by decreasing the H^+ gradient, would decrease the electrical gradient opposing the forward reaction, and thus allow electron transport to proceed at a much faster rate.

In the course of the nucleotide stimulation of DLE work, an interesting observation was made, one for which we have no good explanation. The raw data for this observation is presented in Figure 14. It can be seen that Pi had little effect on the DLE, that ADP and ATP stimulated the DLE, but that ADP and Pi inhibited the DLE. These results were predictable. The question then arose, if a limited amount of ADP was added, what would happen to the DLE as the ADP was converted to ATP. Would the DLE, which was initially very low, gradually rise as the ADP was consumed to the level characteristic of the ATP and Pi reaction mixture. The last tracing in Figure 14 shows that precisely this did happen. Following a 15 second lag the DLE rises to its maximal height in two steps. Since the fluorescence bore no resemblance to the DLE curve, we assume that this phenomenon has to do with the chemical excitation of chlorophyll. Whether

this could be related to successive bindings or releases of ADP and ATP to a protein such as the coupling factor is purely speculative. Perhaps this phenomenon is in the same related to the two step inhibition of phosphorylation by mercurials (Izawa and Good, 1969)., Bradeen et al. (1973). It is not possible to determine very accurately the phosphorylation rate from this process since the substrate concentration is less than the K_m (which is 1.4×10^{-4}, Izawa et al. 1966) and

$$V = \frac{V_m(S)}{K_m}$$

so that the phosphorylation rate is proportional to the substrate concentration. Consequently it is difficult to relate this curve of rising DLE to the decrease in the rate of phosphorylation which must have been taking place over the same period of time.

In these studies we have measured the intensity of DLE and attempted to deduce therefrom the rate of chemical excitation of the chlorophyll. However, there is a major complicating factor which must be considered, namely the effect of conditions on the fluorescence efficiency of the excited chlorophyll. This factor can be evaluated by observing prompt fluorescence which is, of course, a measure of the fluorescence efficiency of light excited by chemical back-reactions with which we are

primarily concerned. As can be seen in Figure 6 there is striking parallel between DLE and prompt fluorescence when DBMIB is added at various concentrations. This parallel is particularly noticeable when DBMIB is kept oxidized by having ferricyanide present at the same time (or oxygen at the higher pH's). Under these conditions DBMIB acts as an electron acceptor (Gould and Izawa, 1973). However the suppression of DLE and prompt fluorescence by DBMIB is in no way related to electron transport since it also occurs in the presence of DCMU. The quenching of both kinds of fluorescence is very probably due to a direct chemical reaction of this inhibitory quinone with chlorophyll. In the first place the concentrations of DBMIB involved greatly decrease the quantum efficiency of electron transport (Gould and Izawa, 1973) as must happen if there is a direct chlorophyll quenching, and in the second place, DBMIB quenches the fluorescence of chlorophyll solutions (5 mM DBMIB quenches 50% of the fluorescence of a 5 ugm/ml solution of chlorophyll in acetone).

At pH 7.5 with methylviologen as electron acceptor the DBMIB cannot be reoxidized. One can see in Figure 6 that both the DLE and the fluorescence are initially low with 5 uM DBMIB present. However the DBMIB becomes reduced to $DBMIBH_2$ in the light and cannot be reoxidized. Its reduction creates a reducing environment

around plastoquinone and PS II thus raising the prompt fluorescence and the DLE simultaneously. Gould and Izawa, (1973) measured the DBMIB reduction in chloroplasts while looking at pH changes with a pH electrode. They found a H^+ uptake due solely to the $DBMIB_2$ reduction. The H^+ uptake stopped in the light as soon as the DBMIB became reduced and then decayed in the light. The more DBMIB present the longer the pH rise lasted. The kinetics of Gould and Izawa's pH rise (with 5 um DBMIB and methylviologen, pH 7.5) and the DLE presented under the same conditions are practically identical. Thus while the DLE and fluorescence rise identically, after the fluorescence reaches its maximal value, the high level is maintained, but the DLE being partially due to the pH rise the level of DLE declines after reaching a maximal value. At 10 um DBMIB a longer time is required in order to reduce all of the DBMIB while the H^+ uptake is presumably going on for a longer time too. This is undoubtedly the reason that the fluorescence and DLE remain longer at a higher level.

Since there is considerable electron transport and phosphorylation under all of these conditions, we would expect considerable DLE. The absence of DLE in the presence of DBMIB, taken together with the fact that prompt fluorescence is also quenched, argues strongly that DLE shoudl be evaluated on the basis of the prompt fluorescence efficiency as suggested above and as

Lavorel (1969) has proposed. That is to say it seems very probable that prompt fluorescence and the chemiluminescence we have been concerned with here, share the same fluorescence yield characteristics. Perhaps the same chlorophyll molecules are fluorescing. This does not mean that the DLE must rise every time the prompt fluorescence rises, since according to our model, the mechanism of generation of the excited chlorophyll is entirely different.

We conclude that the DLE observed at 4 msec after light extinction under "steady state" conditions, at room temperature, and at fairly high light intensity:

1. Is not an obligatory function of the ability of chloroplasts to phosphorylate, though it is very often associated with this ability.
2. Is an obligatory function of some kind of membrane potential.
3. Absolutely requires both the reduced form of the primary electron acceptor for PS II Q^-, and the oxidized form of the primary electron donor for PS II Z^+.
4. Is dependent upon the reverse flow of electrons, from plastoquinone to Q which is in turn causally related

to the above mentioned cation generated membrane potential.

5. Has a final production step which is dependent on the fluorescence yield.

LITERATURE CITED

LITERATURE CITED

Arnold, W., and J. R. Azzi. 1968. Chlorophyll energy levels and electron flow in photosynthesis. Proc. Natl. Acad. Sci. U. S. A. 61: 29-35.

Arnold, W., and J. R. Azzi. 1971. The mechanism of delayed light production by photosynthetic organisms and a new effect of electric fields on chloroplasts. Photochem. Photobiol. 14: 233-240.

Arnold, W. 1972. The ratio between delayed light and fluorescence emitted by chloroplasts. Biophys. J. 12: 793-796.

Arnon, D. I. 1949. Copper enzymes in isolated chloroplasts: polyphenoloxidase in Beta vulgaris. Plant. Physiol. 24: 1-15.

Avron, M. 1960. Photophosphorylation by swiss-chard chloroplasts. Biochim. Biophys. Acta. 40: 257-272.

Barber, J., and G. P. B. Kraan. 1970. Salt induced light emission from chloroplasts. Biochim. Biophys. Acta. 197: 49-59.

Barbieri, G., R. Delosme, and P. Joliot. 1970. Comparaison entre l'emission d'oxygene et l'emission de luminescence a la suite d'une serie d'eclairs saturants. Photochem. Photobiol. 12: 197-206.

Bertsch, W. F., and J. R. Azzi. 1964. A relative maximum in the decay of long term delayed light emission from the photosynthetic apparatus. Biochim. Biophys. Acta. 94: 15-26.

Bertsch, W., J. R. Azzi, and J. B. Davidson. 1967. Delayed light studies on photosynthetic energy conversion I Identification of the oxygen evolving photoreaction as the delayed light emitter in mutants of Scenedesmus obliquus. Biochim. Biophys. Acta. 143: 129-143.

Bertsch, W., J. West, and R. Hill. 1971. Delayed light studies on photosynthetic energy conversion III Effect of 3- (3, 4-Dichlorophenyl) -1, 1-Dimethyl urea on the millisecond emission from chloroplasts performing

photoreduction of ferricyanide. Photochem. Photobiol. 14: 241-250.

Bertsch, W., and S. Lurie. 1971. Delayed light studies on photosynthetic energy conversion IV Effect of Tris poisoning, and its reversal by hydrogen donors, on the millisecond emission from chloroplasts. Photochem. Photobiol. 14: 251-260.

Bradeen, D. A., J. M. Gould, D. R. Ort, and G. D. Winget. 1973. Site-specific inhibition of photophosphorylation in isolated chloroplasts by mercuric chloride. Submitted Plant. Physiol.

Brand, M. J. D., and G. A. Rechnitz. 1970. Differential potentiometry with ion-selective electrodes. Anal. Chem. 42: 616-622.

Butler, W. L. 1971. The relationship between C-550 and delayed fluorescence. FEBS Letters. 19: 2, 125-127.

Carstanjen. 1871. Ueber chinonartige abkommlinge. J. Fuer. Pract. Chemie. 3: 55-65.

Cheniae, G. M., and I. F. Martin. 1971. Effects of hydroxylamine on photosystem II. Plant. Physiol. 47: 568-575.

Clayton, R. K. 1969. Characteristics of prompt and delayed fluorescence from spinach chloroplasts. Biophys. J. 9: 60-76.

Cramer, W. A. and W. L. Butler. 1969. Potentiometric titration of the fluorescence yield of spinach chloroplasts. Biochim. Biophys. Acta. 172: 503-510.

Crofts, A. R., C. A. Wraight, and D. E. Fleischmann. 1971. Energy conversion in the photochemical reactions of photosynthesis and its relation to delayed fluorescence. FEBS Letters. 15: 2, 89-100.

Duysens, L. N. M. 1964. Photosynthesis. Prog. Biophys. Mol. Biol. 14: 1-31.

Duysens, L. N. M., and H. E. Sweers. 1963. Mechanism of two photochemical reactions in algae as studied by means of fluorescence. In Studies On Microalgae and Photosynthetic Bacteria. University of Tokyo Press, Tokyo. p. 353-372.

Evans, E. H., and A. R. Crofts. 1973. The relationship between delayed fluorescence and the H^+ gradient in choroplasts. Biochim. Biophys. Acta. 292: 130-139.

Fleischmann, D. E. 1971. Luminescence in photosynthetic bacteria. Photochem. Photobiol. 14: 277-286.

Franck, J., and J. E. Brugger. 1958. Experimental and theoretical contributions to studies of the afterglow of chorophyll in plant materials. Arch. Biochem. Biophys. 75: 465-496.

Gilman and Blatt. 1961. Thymoquinone In Organic Synthesis, second edition. John Wiley and Sons. vol. I, p. 511-513.

Goedheer, J. C. 1962. Afterglow of chlorophyll in vivo and photosynthesis. Biochim. Biophys. Acta. 64: 294-308.

Gould, J. M., and S. Izawa. 1973, Studies on the energy coupling sites of photophosphorylation. I Separation of site I and site II by partial reactions of the electron transport chain. In Press. Eur. J. Biochem.

Haug, A., D. D. Jacquet, and H. C. Beall. 1972. Light emission from the Scenedesmus obliquus wild type, mutant II strains, measured under steady-state conditions between 4 nanoseconds and 10 seconds. Biochim. Biophys. Acta. 283: 92-99.

Hill, R., and F. Bendall. 1960. Function of the cytochrome components in chloroplasts: a working hypothesis. Nature. 186: 136-137.

Itoh, S., S. Katoh, and A. Takamiya. 1971. Studies on the delayed light emission in chloroplasts. II Participation of primary electron donor and acceptor of photoreaction II in producing the delayed light emission. Biochim. Biophys. Acta. 245: 121-128.

Jackson, J. B., and A. R. Crofts. 1969. The high energy state in chromatophore- from Rhodopseudomonas spheroides. FEBS Letters. 4: 3, 185-189.

Hess, G. P., and J. A. Rupley. 1971. Structure and function of proteins. Ann. Rev. Biochem. 40: 1,013-1,044.

Izawa, S., and N. Good. 1966. Effects of salts and electron transport on the conformation of isolated chloroplasts. I Light-scattering and volume changes. Plant. Physiol. 41: 3, 533-543.

Izawa, S., T. N. Connolly, G. D. Winget, and N. E. Good. 1966. Inhibition and uncoupling of photophosphorylation in chloroplasts. Brookhaven Symp. Biol. 19: 169-187.

Izawa, S., R. L. Heath, and G. Hind. 1969. The role of chloride in photosynthesis. III The effect of artificial electron donors upon electron transport. Biochim. Biophys. Acta. 180: 388-398.

Izawa, S., and N. Good. 1969. Effect of p-chloromercuribenzoate and mercuric ion on chloroplast photophosphorylation. In: Prog. In Phtosyn. Research vol. III International Union of Biological Sciences. p. 1,288-1,298.

Joliot, A. 1966. Actions du ehloromethyluree et de l'hydroxylamine sur la reaction photochimique d'emission d'oxygene (system II) Biochim. Biophys. Acta. 126: 587-590.

Joliot, P., G. Barbieri, and R. Chabaud. 1969. Un nouveau modele des centres photochimiques du systeme II. Photochem. Photobiol. 10: 309-320.

Kirk, J. T. O. 1971. Chloroplast structure and biogenesis. Ann. Rev. Biochemistry. 40: 161-196.

Lavorel, L. 1969. On a relation between fluorescence and luminescence in photosynthetic systems. In: Prog. in Photosyn. Research, Vol. III International Union of Biological Sciences. p. 883-898.

Kok, B., S. Malkin, O. Owens, and B. Forbush. 1967. Observations on the reducing side of the O_2 evolving act. Brookhaven Symp. Biol. 19: 446-459.

Kressler, E., W. Arthur, and J. E. Brugger. 1957. The influence of manganese and phosphate on delayed light emission, fluorescence, phtoreduction and photosynthesis in algae. Arch. Biochem. Biophys. 71: 326-335.

Livingston, R. 1960. Physciochemical aspects of some recent work on photosynthesis. Quarterly Reviews, 174-198.

Lumpkin, O., and Z. Hillel. 1973. Isolation of a fast decay in submillisecond Chlorella luminescence. Biochim. Biophys. Acta. 305: 281-292.

Mar, T., and Govindjee. 1971. Thermoluminescence in spinach chloroplasts and in Chlorella. Biochim. Biophys. Acta. 226: 200-203.

Mayne, B. C., and R. K. Clayton. 1966. Luminescence of chlorophyll in spinach chloroplasts induced by acid-base transition. Proc. Nat'l. Acad. Sci. U. S. A. 55: 494-497.

Mayne, B. C. 1967. The effect of inhibitors and uncouplers of photosynthetic phosphorylation on the delayed light emission of chloroplasts. Photochem. Photobiol. 6: 187-197.

Mayne, B. C. 1968. The light requirement of acid base transition induced luminescence of chloroplasts. Photochem. Photobiol. 8: 107-113.

McCarty, R. E., J. S. Fuhrman, and Y. Tsuchiya. 1971. Effects of adenine nucleotides on hydrogen-ion transport in chloroplasts. Proc. Nat'l. Acad. Sci. U. S. A. 68: 10, 2,522-2,526.

Miles, C. D., and A. T. Jagendorf. 1969. Ionic and pH transitions triggering chloroplast pot-illumination luminescence. Arch. Biochem. Biophys. 129: 711-719.

Nelson, N., Z. Drechsler, and J. Neumann. 1970. Photophosphorylation in digitonin subchloroplast particles. J. Biol. Chem. 245: 1, 143-151.

Neumann, J., J. Barber, and P. Gregory. 1973. The relation between photophosphorylation and delayed light emission in chloroplasts. Plant. Physiol. 51: 1,069-1,073.

Nishmura, M. 1967. Oxidation-reduction reactions of cytochromes in red algae. Brookhaven Symp. Biol. 19: 132-142.

Quitrakul, R., and S. Izawa. 1973. Electron transport and photophosphorylation in chloroplasts as a function of the electron acceptor II Acceptor-specific inhibition by KCN. Biochim. Biophys. Acta. 305: 105-118.

Priestly, E. B., and A. Haug. 1968. Phosphorescence spectrum of pure crystalline naphthalene. J. Chem. Phys. 49: 622-629.

Saha, S., R. Quitrakul, S. Izawa, and N. E. Good. 1971. Electron transport and photophosphorylation in chloroplasts as a function of electron acceptor. J. Biol. Chem. 246: 10, 3,204-3,209.

Strehler, B. L., and W. Arnold. 1951. Light production by green plants. J. Gen. Physiol. 34: 809-820.

Sweetser, P. B., C. W. Todd, and R. Titersh. 1961. Effect of photosynthesis inhibitors on light re-emission in photosynthesis. Biochim. Biophys. Acta. 51: 509-518.

Tollin, G., E. Fujimori, and M. Calvin. 1958. Delayed light emission in green plant materials: Temperature dependence and quantum yield. Proc. Nat'l. Acad. Sci. U. S. A. 44: 1,035-1,047.

Trebst, A., E. Harth, and W. Draber. 1970. On a new inhibitor of photosynthetic electron transport in isolated chloroplasts. Z. Naturforsch. 25b: 1,157-1,159.

Uribe, E., and A. T. Jagendorf. 1966. ATP formation caused by acid-base transition of spinach chloroplasts. Proc. Nat'l. Acad. Sci. U. S. A. 55: 170-177.

Winget, G. D., S. Izawa, and N. E. Good. 1969. The inhibition of photophosphorylation by phlorizin and closely related compounds. Biochemistry. 8: 2,067-2,073.

Witt, A. T., G. Doring, B. Rumberg, P. Schmidt-Mende, U. Siggel, and H. H. Stiehl. 1966. Electron transport in photosynthesis. Brookhaven Symp. Biol. 19: 161-168.

Wraight, C. A., and A. R. Crofts. 1971. Delayed fluorescence and the high energy state of chloroplasts. Eur. J. Biochem. 19: 386-397.

Zankel, K. L. 1971. Rapid delayed luminescence from chloroplasts: Analysis of components; the relationship to the O_2 evolving system. Biochim. Biophys. Acta. 245: 373-385.