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ELECTROPHYSIOLOGICAL STUDIES ON ULMUS AMERICANA

JILL STANLEY KATHLEEN SHIPMAN

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ELECTROPHYSIOLOGICAL STUDIES ON <u>ULMUS</u> <u>AMERICANA</u>

BY

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DISSERTATION

Submitted to the University of New Hampshire
in Partial Fulfillment of
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in
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Electrophysiology
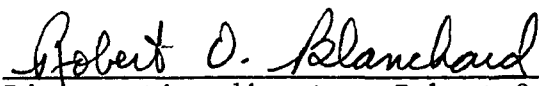
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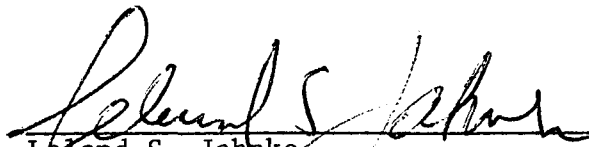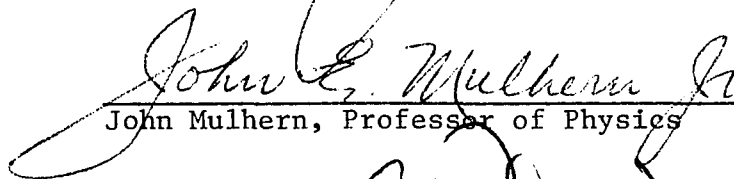
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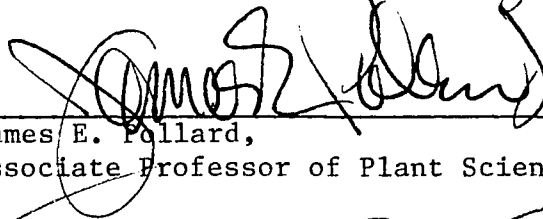
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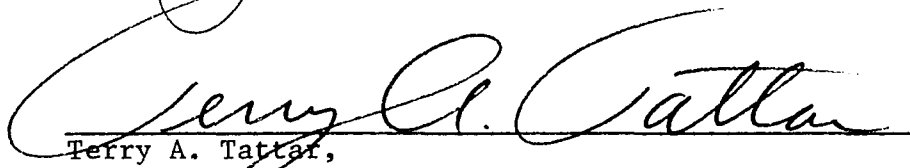
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ABSTRACT

ELECTROPHYSIOLOGICAL STUDIES ON
ULMUS AMERICANA

by

JILL STANLEY KATHLEEN SHIPMAN

University of New Hampshire, May 1982

Although membrane potentials have been measured in algal cells and
in large cells of some higher plants, this investigation is the first to
include such measurements in mesophyll cells of elm (Ulmus americana)
leaves. The inquiry entailed the development of the technique for
measuring elm leaf cell membrane potentials, the determination of
membrane potentials expected of healthy, greenhouse elms, and the
effects of various physical and chemical factors on the membrane poten-
tials of elm leaf cells.

The membrane potential of a specific cell, was measured by ampli-
fying the potential difference between a microelectrode inserted in the
cell and a reference electrode located in the solution bathing the cell.
The potential difference was then visible on an oscilloscope screen and
on a strip chart recorder. Changes in the potential difference with
time or in response to a stimulus were recorded by the strip chart
recorder and could then be compared to measurements taken from other
cells. Random electrical radiations were shielded from the measurement
system by a Faraday cage. Chemical test factors, such as pH, were

tested by flushing the test solution through a perfusion chamber in which the cell being measured was located. Physical factors such as light and temperature were tested with the aid of filters and solutions of various temperature, respectively.

The studies provided an electrophysiological picture of the elm inclusive of healthy and stimulus related effects on membrane potential. Also, an equation was developed which combines several criteria from membrane potential traces to facilitate the comparison of the electrical nature of various cells. The equation also can serve as an internal standard for a particular cell, while simultaneously making a statement about the electrical stability of a cell. Although the equation was only tested for elm, it may be useful in interpreting the electrophysiological nature of other species.

This comprehensive investigation on the electrophysiology of elm has provided elm leaf membrane potential expected for healthy elm and for effects on elm of various physicochemical stimuli. The results suggest that electrophysiological methods have potential for use in pathology and breeding programs for elm.

INTRODUCTION

Historical interest in bioelectric systems can be traced to Aristotle's observations of torpedo fish (Anonymous, 1975). More recently in the 18th and 19th centuries, several investigators claimed beneficial effects of electricity on plant growth (see review by Tattar and Blanchard, 1976), and Warner (1892, 1893), Kinney (1897), and Stone (1909) led the way for future studies in the use of electricity in agriculture. Recent studies have been more physiological and biochemical in nature. For example, reversible movements and touch responses in plants have been shown to be regulated by changes in internal electrical potentials (Simons, 1981). Light and gravity induce positive electrostatic charges in cell membranes of plants and these charges are thought to be involved in growth reactions controlling various physiological activities (USDA, 1979). Electricity also affects development. Examples of its effect on growth and development from the animal, plant, and fungal kingdoms include studies on cardioacceleration (Joseph and Engel, 1981), nerve endings (Borgens, et al., 1981), pollen tubes (Jaffe and Nuccitelli, 1977; Mulcahy, 1974), and hyphal tips and root tips (Heller, 1959; Scott, 1975).

At the cellular level, bioelectrical phenomena are receiving considerable attention. Electrophysiological techniques are being used in both plant and animal cells to better understand metabolic processes and how they influence or are influenced by electrical responses.

Cell electrophysiology is ultimately a study of cell membranes. All living cells are surrounded by membranes that separate components of

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metabolic processes from the external environment. In this sense cells have identity, and the ability of the cell to control fluxes of molecules is dependent upon the integrity of the cell membrane (Jain and Wagner, 1980). Almost every living biological membrane maintains an electrical potential difference between two adjacent solutions (Heinz, 1981). The origin of this potential difference has been summarized by Heinz (1981) and involves potentials from three sources: 1) Equilibrium Potential, which is established when a nonpermeable ion is unequally distributed between two solutions separated by a selectively permeable membrane, 2) Membrane Diffusion Potential, which is established when differences in ion mobility cause unequal concentrations of ions on either side of a membrane, and 3) Electrogenic Pump Potential, which is established when ions are forcibly transported across a membrane, causing an unequal redistribution of ions. Consequently, any changes in cell metabolism or membrane integrity that influence one or more of the three defined potentials will affect the electrical potential difference across the membrane.

Around 1900, W. Nernst, a physical chemist, developed a formula (Nernst Equation) which in essence was a statement showing how the internal and external activities of an ionic species are related to the electrical potential difference across a membrane (Nobel, 1974). However, it was not until 1939 and 1940 that micropipettes were used to record the electrical activity of a single cell and thereby substantiate the Nernst Equation in living cells. By inserting a glass micro-electrode into the lumen of a freshly dissected squid giant axon, Hodgkin and Huxley (1939) in England and Curtis and Cole (1940) in the

United States were able to measure the electrical potential of resting nerve as well as the alteration of this voltage during the propagation of an action potential.

An inherent problem in the use of microelectrodes described by the earlier researchers was that tip diameters ranged from 10-100 u. Since squid giant axon cells are on the order of 500 u, such microelectrodes posed little problem. However, before membrane potentials of smaller cells could be accurately measured, smaller electrodes were required. In response to this need, Ling and Gerard (1949) described a method for preparing glass micropipettes with tip diameters less than 1 um. Since that time, technology has vastly improved microelectrodes and the instrumentation for amplifying and recording membrane potentials in both animal and plant tissues. Subsequent studies have led to an expanding body of knowledge of metabolic processes associated with membrane integrity.

The biophysical aspects of ion transport in plants have developed from studies of electrochemical potentials and ion fluxes across membranes of large algal cells (MacRobbie and Dainty, 1958a, 1958b; Hope and Walker, 1960, 1961). Methods were used which had been developed for animal cells. These methods were subsequently applied to higher plants by Higinbotham and co-workers who established the existence of electropotentials in cells of higher plants and determined that the plasma membrane was the chief electrical barrier (see reviews by Dainty, 1962; Higinbotham, 1973a; MacRobbie, 1970).

Changes in the physical structure of a cell membrane or in the environment around the membrane, may have direct and indirect effects on membrane pathobiology. For example, Chia, et al. (1981) showed that at

physiological temperature, membrane phospholipids undergo a progressive transition to a gel phase and that this transition is correlated with loss of membrane function during cell senescence. They further showed that senescence was related to environmental stresses that affect cell biochemistry. Marx (1974) cited free radicals as a possible source of membrane damage associated with senescence. The herbicide, paraquat (1, 1' - dimethyl - 4, 4' - bipyridinium dichloride), for example, induces formation of the highly-reactive radical, superoxide (O_2^-), and among other effects, disrupts cell membranes (Birchem, et al., 1979). Some pathogens have been shown to change the membrane potentials in higher plants (Novacky, et al., 1976; Novacky and Karr, 1977), which may be related to membrane recognition of toxins. Strobel (1974, 1975) suggested that sugarcane susceptible to the leaf eyespot disease pathogen, Helminthosporium sacchari, bound the toxin helminthosporoside with a membrane protein, while resistant sugarcane did not. He theorized an interaction phenomenon in which the toxin interfered with the membrane's ability to regulate the passage of ions. Strobel (1975) cited membrane potential experiments by Novacky, Jones, and Dropkin of the University of Missouri, where exposure of sugar cane cells to helminthosporoside caused an immediate drop in the electric potential across the membrane. Novacky and Hanchey (1974) also showed depolarization of membrane potentials in oat roots treated with the toxin, victorin.

Although electrophysiological techniques have been used for animal cells since before World War II, only in the last two decades have such techniques been used extensively to elucidate metabolic phenomena in plant cells. Further, even less time has been spent in elucidating the

aspects of membrane involvement in self-recognition and failure to recognize pathogens and/or their metabolites. Goals in plant pathological research should include an understanding of the mechanisms of host-parasite interactions at the cellular level so that target sites for control can be identified. Electrophysiological techniques should aid in this understanding.

Before abnormal aspects of cellular function are elucidated, an understanding of the normal aspects must be addressed. The research described in this dissertation is a first attempt in addressing the membrane potentials of "normal" elm leaf cells. American elm (Ulmus americana L.) was chosen specifically because of the extensive body of knowledge about this species and the diseases it suffers (see Stipes and Campana, 1981). Understanding first what the normal membrane potentials are and how these potentials are affected by various tissue preparations and external stimuli, could hopefully lead to further studies using electrophysiological techniques in elucidating host-parasite interactions in elm diseases.

CHAPTER I

THE HEALTHY ELM

Introduction

The plant cell wall encloses the cell and may be thought of as a continuum of the cell's external environment (Wallach, 1972). The plasmalemma, however, represents a barrier to the environment. Similarly, the many other membranes found within the cell are responsible for the compartmentation of cellular contents. The various cell membranes keep metabolic activities grouped so that the cell can continue to function. A physical barrier, such as the plasmamembrane, can separate charge. The difference in charge on either side of a membrane can be measured and is an indication of cell metabolic activities. The plasmalemma selectively controls the entrance and exit of molecules and ions into the cell. The tonoplast exhibits a similar permeability function. Ions in and around a plant cell, then, account for its electrical properties. The ions change in response to cell metabolic activities and to changes in the cell's ambient milieu. In addition the membrane itself is not stagnant. Components of the membrane change in response to cell metabolism and environmental fluctuations. The potential difference between the two solutions separated by the changing membrane mirrors the chemical activity or inactivity of the cell.

Another possible source of variation in membrane potentials is endogenous current. For example, Black, _et al._ (1971) found that endogenous currents in tomato plant tissues was attributed to either an ion pump or to a redistribution of plant growth regulating substances.

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In a study on pollen tubes, Jaffe and Nuccitelli (1977) found growth was associated with electrical changes. Similarly, Heller (1959) and Scott (1975) found expanding root tips to have electrical properties. Finally, Higinbotham (1974) and Spanswick (1981) have reviewed the works of several investigators illustrating that ion distribution contributes to changes in membrane potentials.

The preceding clearly demonstrate that membrane potentials can be affected by one or a combination of factors. Thus, these membrane potentials are a reflection of the physiology of a cell.

Elm is a majestic tree, subject to many diseases. It provided an excellent focus for plant electrophysiology for three reasons:

1. elm is a woody plant that has been thoroughly studied because of Dutch elm disease,

2. there are numerous other diseases of elm, and

3. the electrical studies of elm leaves may lead to a better understanding of disease mechanisms.

The major objective of this study was to measure the membrane potentials of a large number of healthy elm cells and to compare these measurements of a woody plant with those of non-woody plants. Measurements included maxima and minima membrane potentials and longevity of penetrated cells.

Materials and Methods

The Elms

Elm (Ulmus americana) seeds were collected in Durham, New Hampshire and were stored at $10^{\circ}C$ until planted. Seeds were germinated in flats in a Jiffy mix: soil: perlite mixture (1 :5:1), then were transplanted to and maintained at $21^{\circ}C$ in 8 inch standard pots in a

greenhouse with 16 h light/day. Plants were watered daily and sprayed

once a week with Resmethrin to control white flies. Experiments were

not done on recently sprayed trees. Trees (ca. 1 m in height) were

moved from the greenhouse to the lab at least two days before

measurements were taken. Trees were exposed to ambient laboratory light

including fluorescent and daylight components. Temperature of the

laboratory was $24 \overset{+}{-} 1^{\circ}C$.

The electrophysiology set-up

The electrophysiology set-up (Figure 1) was composed of three

separate parts: the light path, the fluid path and the electrical

circuit.

The Light Path. Light was transmitted from a light source (AO

starlite illuminator, with 6v, 20-watt Tungsten-Halogen lamp). It

traveled either filtered or unfiltered through one side of the perfusion

chamber, through the specimen, through the other side of the perfusion

chamber and then through the microscope to the eye.

The Fluid Path. Either Ringer's solution or a test solution was

gravity fed through Tygon tubing from a shelf 85 cm above the sample.

The flow rate was regulated by a valve on the Tygon tube. The fluid

flowed through the perfusion chamber and then again through Tygon tubing

to a flask (70 cm below) which collected the waste.

The Electrical Circuit. Differences in charge across the cell

membrane and tonoplast were amplified and either recorded on a strip

chart recorder, or visualized on an oscilloscope or both. The measuring

recording instruments were interconnected to the cell via two electrodes

which were connected to the amplifier (Nobel, 1974). Both were

Figure 1. The electrophysiology set-up. The reference electrode (a) and the microelectrode (b) are connected to an amplifier and recording devices. Ringer's solution or a test solution (c) is fluxed through the perfusion chamber (d). The micromanipulator (e) was used to lower the electrode into the cell. The microscope (f) was used for viewing the elm leaf cells. The light source was behind the perfusion chamber (d).
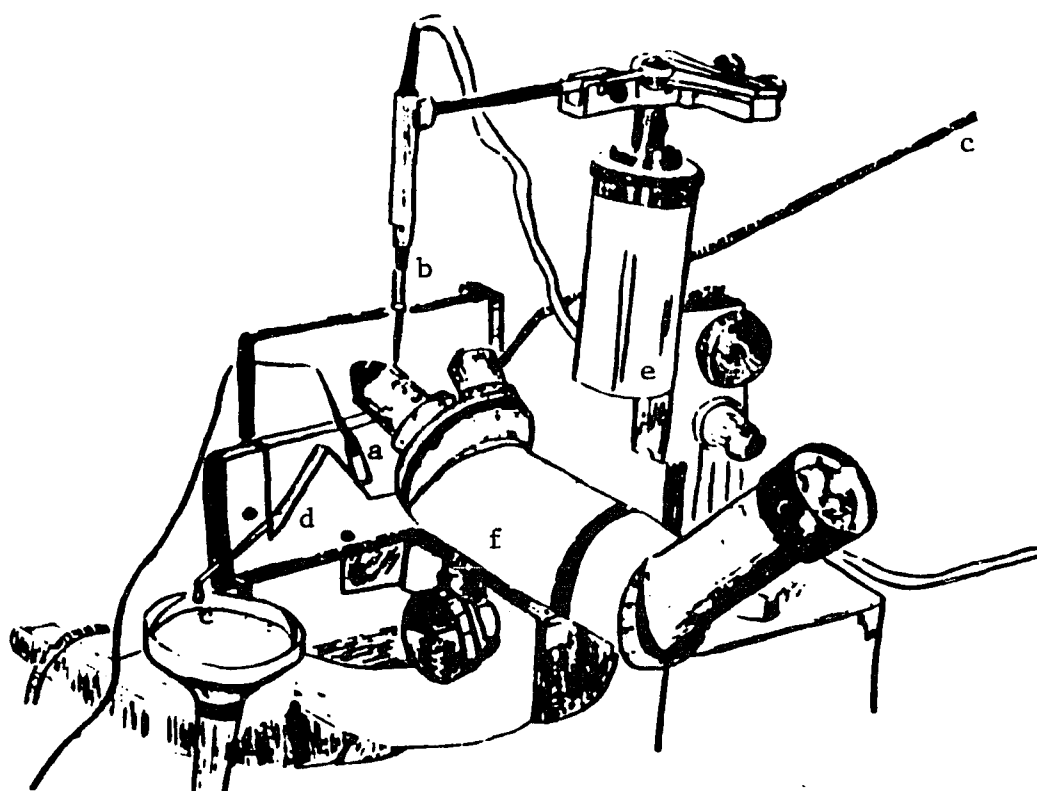
Table 1. Solutions used to study the Electrophysiology of Elm.

A. $\underline{3M\ KCl}$

$\qquad$ 223.68g/l

B. $\underline{3M\ KCl\ agar}$

$\qquad$ 16g bacto agar

$\qquad$ 500ml 3M KCl

C. $\underline{Stock\ Solutions\ Used\ for\ Ringer's\ Solution}$

compound.	g/l
$MgSO_4$	3.01
KCl	7.456
$Ca(NO_3)\cdot 4H_2O$	23.615
$NaH_2PO_4\cdot H_2O$	13.799
$Na_2HPO_4\cdot 7H_2O$	2.681

D. $\underline{Ringer's\ Solution}$

compound	ml
$MgSO_4$	10
KCl	10
$Ca(NO_3)_2\cdot 4H_2O$	10
$NaH_2PO_4\cdot H_2O$	9
$Na_2HPO_4\cdot 7H_2O$	5
H_2O	956

adjust pH to 5.6

E. $\underline{1mM\ Sodium\ Cyanide\ in\ Ringer's\ Solution}$

$\qquad$ add 0.05g to 1000ml of Ringer's solution

$\qquad$ adjust pH to 5.6

had a tip diameter of about 1μm and was inserted, with a
micromanipulator, directly into the cell to be measured. The complete
circuit then included the amplifier, the microelectrode, the cell, the
bathing medium and the reference electrode. Charged ions carried
current through the liquid phase of the circuit. Electrons carried
current through the metallic phase. The transition between the liquid
and solid or metallic phases of the circuit was accomplished by the
silver/silver chloride half-cells of the electrodes.

Biological membranes have high electrical resistance as do the
micropipette tips (Nobel, 1974). Because of these high resistances, the
amplifier used to measure membrane potentials must have a high input
impedance (Luttge and Higinbotham, 1979). The instrument used was a WPI
high input impedance amplifier (60 Fitch Street, New Haven, Connecticut,
06515). Leaf structure in trees involves a protective cuticle which is
stronger than the tip of the electrode used to measure membrane
potential. Therefore, electrodes were not inserted through the cuticle.
Instead, the cut leaf sections were held vertically in a sponge holder
and the electrode was inserted through the cut edge of the leaf sections
studied. The leaf holder was oriented in the perfusion chamber so the
upper leaf surface faced the researcher. The electrodes were inserted
in the palisade cells for membrane potential measurements. Palisade
cells in elm measure ca. 10 x 25 um.

Tissue Preparation

Fully expanded leaves were removed from the third or fourth node
from the tip of the elm using a sharp razor blade to cut through the
petiole. The excised leaves were inserted into a petri dish containing
Ringer's solution at either $10^{o}C$ or $24^{o}C$ and were cut into 1 cm^{2}

sections. The cut tissues were then aged at room temperature for either

1, 2, 3, 5, 15 or 24 h before membrane potentials were measured.

The membrane potential was measured by the microelectrode -

reference electrode method as described in the section entitled, "The

Electrical Circuit." The cell was illuminated with a microscope

tungsten-halogen lamp. Measurements were done at room temperature

(25°C). Resulting mv readings were statistically compared for the

different treatments.

The sub programs ANOVA, one way analysis of variance with Duncan

range test of means, FREQUENCY, frequencies of several variables of the

membrane traces, and MANOVA, multiple analysis of variance, were used to

compare several variables of the membrane potential traces of the elm

leaf cells.

Results

The major objective of this study was to describe the membrane

potentials of healthy elm leaf cells. There are many characteristics of

a membrane potential trace (Figure 2). Before the microelectrode was

inserted into a cell, the zero was defined on the chart recorder and the

electrode was tested. If the electrode test gave a tip potential

between 15 and 25 megohms, the electrode was inserted into the elm cell.

The membrane potential generally hyperpolarized to a base value. The

time to plateau from the initial measurement to the base value ranged

from one to two minutes. Cells lived for a time period, labeled "cell

longevity," which was often five to seven hours in elm. In some cases,

a trace may have fluctuated during the entire measurement period;

however, even without rapid changes in membrane potential, the trace had

a maximum and minimum apart from initial insertion or cell death. Cell

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Figure 2. Characteristics of a membrane potential trace.
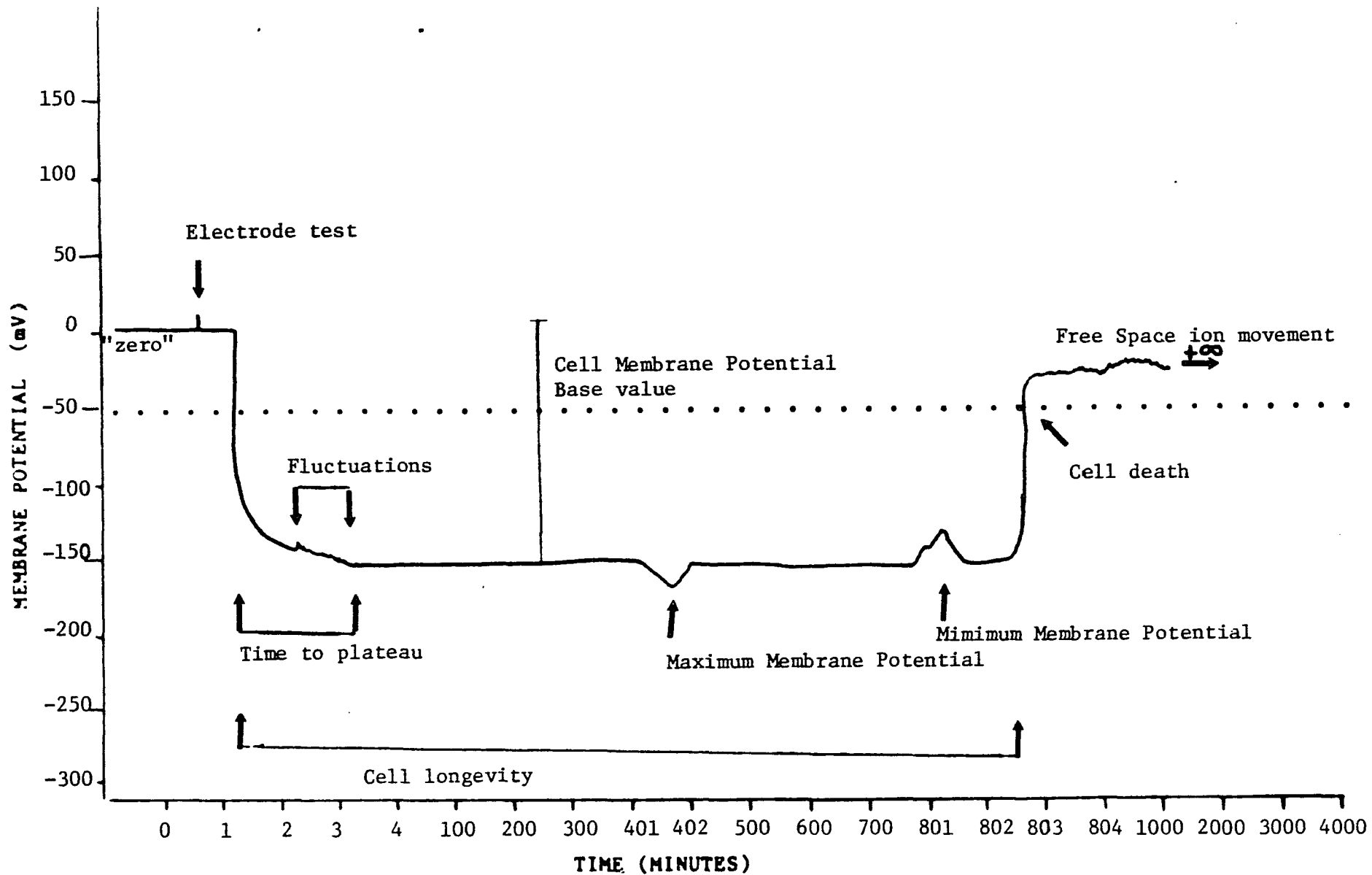
death was defined to occur at the time when the membrane potential depolarized to a voltage less than -50 mV and did not hyperpolarize again. When the electrode was inserted in a dead cell, only ion movement in the free space of the leaf tissue was detected.

Maximum and minimum membrane potentials and cell longevity were compared using subroutines of SPSS including ANOVA, MANOVA, and Duncan's range test of means and frequency.

The study on cell longevity during membrane potential measurements indicated that most cells either die within the first five minutes or they live longer than twenty minutes (Table 2).

Apparently a high proportion of the cells are injured during the wounding by the electrode as shown by the fact that 48% of the cells penetrated died within five minutes. Thirty-three percent of cells penetrated lived longer than twenty minutes. Cells that live past the initial shock of electrode insertion normally live much greater than twenty minutes indicating that they survive the wounding caused by the electrode. The effect of injury on the longevity of the cells was visible at both temperatures and each of the six tissue preparation times.

Frequently the maxima and minima of cell membrane potential traces are used to make comparisons among cells (Spanswick, 1972; Zeiger, Moody, Hepler and Varela, 1977). Because these factors are standard in electrophysiology experiments, they were compared for healthy elm cells subjected to the various tissue preparation time and temperature regimes. The electrical maxima and minima, greatest and least absolute values respectively, of the membrane potential measurements for elm leaf cells at the various preparation times and

Table 2. Effect of treatment time and temperature on elm leaf cell longevity during membrane potential measurements. Numbers represent total cells dying in the longevity interval indicated.

Cell longevity (minutes)	Treatment time (h)											
	1		2		3		5		15		24	
	10°C	24°C	10°C	24°C	10°C	24°C	10°C	24°C	10°C	24°C	10°C	24°C
0-5	0[a]	3	2	5	1	0	3	2	4	1	4	4
6-10	0	0	0	0	1	2	1	0	0	1	0	0
11-15	0	1	1	0	0	0	0	0	1	1	0	1
16-20	0	0	1	0	0	0	0	0	0	0	0	0
over 20	5	1	1	0	3	3	1	3	0	2	1	0

[a]Each column includes five cells.

temperatures are shown in Table 3. The mean membrane potential for the entire population of cells tested was -117 mv. The mean membrane potential for those cells prepared at $10^O C$ were -103 mv and -128 mv for minima and maxima, respectively and for those cells prepared at 24° were -102 mv and -133 mv for minima and maxima, respectively.

In the comparison of membrane potentials in elm leaf cells by priori contrast (Table 4), it can be seen that tissue preparation times of 1, 2, 3, 5, and 15 hours showed no significant difference in electrical minima. There are significant differences when comparing 1 and 24 hour, 5 and 24 hour, and, 15 and 24 hour tissue preparation times. Similarly for electrical maxima of elm leaf cell membrane potential, there are no significant differences found among the measured tissues which were prepared for 1, 2, 3, 5 or 15 hours (Table 5). There were significant differences, however, when 1 and 24 hour and 5 and 24 hour tissue preparation times were compared. The actual membrane potentials can be seen in Table 3.

Discussion

Chance for successful disease control increases with our understanding of how a host plant interacts with both the environment and the pathogen. Before various chemical and physical factors could be manipulated and their effects on the membrane potential of elm leaves studied, "normal" or "baseline" membrane potentials were required. Prior to experimentation, no data were available on elm membrane potential.

Cells that are penetrated with an electrode are injured. The location of the penetration point, the physical state of the cell, the

Table 3. Effect of treatment time and temperature on the minimum and maximum membrane potentials of elm leaf cells.

| Treatment time (h) | Membrane potential (mv)[a] | | | |
| | Minima | | Maxima | |
	10°C	24°C	10°C	24°C
1	-89	-85	-119	-116
2	-100	-123	-126	-132
3	-111	-118	-123	-145
5	-104	-72	-116	-114
15	-83	-92	-119	-149
24	-135	-125	-163	-139
Column $\bar{X}$	-104	-102	-128	-133
Column S.D.	31.8	41.4	29.3	32.4
Overall $\bar{X}$ = -117				
Overall S.D. = 33.7				

[a]Membrane potentials are means of five replicates

Table 4. Priori contrast between different treatment times for minimum membrane potential in elm leaf cells.

Treatment Time (h)	1	2	3	5	15	24
1	-	-	-	-	-	$+$[a]
2	-	-	-	-	-	-
3	-	-	-	-	-	-
5	-	-	-	-	-	+
15	-	-	-	-	-	+
24	+	-	-	+	+	-

[a]+ indicates a significant difference at 95% confidence (Duncan's Range Test of Means).

Table 5. Priori contrast between different treatment times for maximum membrane potential in elm leaf cells.

Treatment Time (h)	1	2	3	5	15	24
1	−	−	−	−	−	+[a]
2	−	−	−	−	−	−
3	−	−	−	−	−	−
5	−	−	−	−	−	+
15	−	−	−	−	−	−
24	+	−	−	+	−	−

[a] + indicates a significant difference at 95% confidence (Duncan's Range Test of Means).

condition of the electrode, and the extent of introduced vibrations on the electrode by the researcher can individually or collectively determine the ability of the cell to respond to the injury. Several authors have reported increases ("a steady, progressive hyperpolarization") in membrane potential with time following wounding (Macklon and Higinbotham, 1968; Koopowitz, Dhyse and Fosket, 1975). Successful recovery is necessary for further experimentation on individual cells, i.e., cells must have an acceptable longevity time. Of the cells penetrated in this study, 48% died within the first five minutes (Table 2). Sixty-five percent of those that survived for at least five minutes, lived for more than 20 minutes. From these values it can be seen that, using the apparatus as described in materials and methods, the researcher would know by five minutes whether a penetrated cell could be useful for experimentation in terms of its survivability. Equilibration times (treatment) of one and three hours yielded the most usable cells in terms of longevity (Table 2) but other factors (dealt with in CHAPTER II) afforded more accurate predictions. Although cell walls (sic, membranes) often seal tightly around microelectrodes, and electrical activity of penetrated cells are maintained (Fein, 1977), there are reports of "jumps" in potential following electrode insertion (Koopowitz, Dhyse and Fasket, 1975). It is well-known that wounding induces developmental and physiological changes in higher plant tissues (Koopowitz, Dhyse and Fosket, 1975). Changes in membrane potential following wounding have also been reported (Koopowitz, Dhyse and Fosket, 1975). Since I looked at membrane potential in sections of elm leaves, the studied tissues were wounded once during sectioning of the leaf tissue and again during electrode insertion. None the less, results of

studies on elm membrane potential do not conflict with similar studies on large-celled algae which are injured only during electrode insertion.

In elm cells, as in cells of other higher plants, the total electrical potential between the vacuole and the solution outside the cell is negative. Although, positive membrane potentials of isolated protoplasts and vacuoles have been found, suggesting severe changes in membrane permeability, most membrane potentials in plants range between -100 and -200 mv (Luttge and Higinbotham, 1979). Membrane potentials in elm cells vary within individual cells, i.e., there are maxima and minima potentials, and between cells (Table 3). The mean membrane potential for all elm cells measured was -117 mv with a standard deviation of 33.7. Variation within and between cells is affected by the temperature at which leaf tissues are cut and by the time allowed for tissues to equilibrate in Ringer's solution before insertion of electrodes (Tables 3, 4, 5).

Maximal and minimal values have been used by previous researchers to describe membrane potential traces and to characterize differences between experimental and control groups. (Spanswick, 1972; Zeiger, Moody, Hepler and Varela, 1977).

Early plant electrophysiology was facilitated by studying giant celled algae, such as Nitella, Chara and Halicystis (Luttge and Higinbotham, 1979; Spanswick, 1981). Since the cells of these plants are large compared to other plants, conclusions based on work from these plants need to be tested before they can be considered general electrical characteristics However, mean maxima and minima of membrane potentials in elm are comparable with those found in algae as well as those found in other higher plants.

Characteristics which describe a "normal" elm leaf cell have been condensed in Figure 2. It is clear from the data obtained that general patterns are repeatable, but specific patterns of an individual cell are unique. Because variations in membrane potentials occur during "normal" cell activity, experimental studies should not be limited to elm cells lacking these variations.

CHAPTER II

THE CELL STABILITY INDEX

Introduction

Electrophysiology of plants began with studies on large algal cells
and has since involved work on tissues as varied as oat coleoptile and
carrot and oat root cells (Novacky and Hanchey, 1974; Etherton, 1963;
Rubinstein, Maher, and Tattar, 1977). Presently, membrane potentials of
leaf cells are being used to detect relative resistance of plants to
specific pathogens and to elucidate mechanisms of host-parasite
interactions (Novacky, Karr and Van Sambeek, 1976). A prerequisite to
applying electrophysiology in the study of plant disease is knowledge of
the electrical nature of the healthy plant. A healthy plant can carry
out its physiological functions as expressed in its genetic make-up.
Deviation from health interferes with these physiological functions and
can thusly interrupt the balance of ions between a cell and its
environment. A change in ion distribution from the norm surfaces in
measurements of membrane potential. However, the healthy status of a
plant does not in and of itself indicate that membrane potential must
remain constant. Some changes in ion balance are expected in a healthy
plant as a part of its "normal" metabolism (Luttge and Higinbotham,
1979).

Frequently the only points considered on a membrane potential
versus time trace are the maximum and minimum membrane potential values
(Higinbotham 1973a). Some consideration is given to the baseline value,
or approximate millivolt level of the entire curve. In elm, the

baseline membrane potential is sometimes variable (CHAPTER I). A method was needed to distinguish between the normal variability associated with a membrane potential trace of a healthy elm (Fig. 2) and changes in membrane potential caused by an experimental treatment. The scientific method involves a flow process from known facts, to hypothesis, to experimentation which furnishes more facts that will cancel, strengthen or alter the hypothesis (Little and Hills, 1978). The facts that membrane potentials in elm exhibit variability and have differing maxima and minima, and that the cells last for various lengths of time (CHAPTER I) led to the hypothesis that the stability of the membrane potential could be determined by a formula. This formula could in turn be used to predict cell longevity or could make a statement about the combined metabolic effects contributing to membrane potential. Experimentation to determine healthy elm membrane potential under various treatments of tissue preparation and temperature (CHAPTER I) have shown that variations in membrane potential are attributable in some cases to manipulation of the treatment factors, or to natural variability.

The objective of this study was to test whether a hypothesized formula included all terms necessary to describe elm membrane potentials and to determine if the formula could be used as a predictor of cell stability for further membrane potential experimentation.

Materials and Methods

Because electrophysiology experiments in plants have used minimum and maximum membrane potential as well as cell longevity as descriptors of membrane potential and because preliminary experiments on elm leaf

cells showed that their membrane potentials exhibited variability, a quantitative representation for the relationship among these variables was postulated:

$$Q = \frac{|\Delta x|}{200} + \frac{10}{y} + z.$$

Where Q = the cell stability index

Δx = the change in membrane potential,

y = a set time chosen for observation by the researcher and,

z = the number of positive fluctuations in membrane potential.

The data from CHAPTER I were further analyzed to see if additional terms should have been included in the formula or if any of those included should have been discarded. The formula was weighted in an attempt to balance the effect of the terms. Since the change in membrane potential could have had a value such as -190 mV this term was weighted with 200 which would bring the term close to 1. Similarly the length of time sufficient for an experiment was twenty minutes and to bring this term close to one, the time was divided into 10. The number of fluctuations was generally under 10 so this was left as a units term.

The membrane potentials of elm cells were measured in healthy tissue sections cut at either 10 or $24^{o}C$ and allowed to equilibrate at one of six tissue preparation times: 1, 2, 3, 5, 15 and 24 h. The experiment was therefore designed as a two by six with a total of twelve blocks. Within each block, five replicates were made and several

characteristics of the membrane potential traces were compared. These

characteristics were

1.   the minima and

2.   maxima of membrane potential,

3.   the change in potential from maximum to minimum,

4.   the number of fluctuations during given time intervals and,

5.   the duration of the fluctuations during the same time

intervals.

The sub programs ANOVA, one way analysis of variance with Duncan

range test of means, FREQUENCY, frequencies of variables of the membrane

traces, and MANOVA, multiple analysis of variance, were used to compare

the variables of the membrane potential traces of the elm leaf cells.

Results

Multiple analysis of variance showed the effects of

1.   temperature at which the elm leaf cell was cut,

2.   treatment time between cutting the leaf tissue and electrode insertion, and

3)   the interaction of the temperature and treatment time, on the membrane potentials of healthy elm leaf cells.

The mean maximum potential for the total population was −121 mv (Table 6). The mean maximum potentials for cells of leaf tissues prepared at $10^{\circ}C$ and $24^{\circ}C$ were −128 mv and −133 mv, respectively. Based on treatment time, membrane potentials of cells measured at 1, 2, 3, 5, 15 and 24 h ranged from a mean of −116 mv at 5 h to a mean of −152 mv at 24 h. Although at 5 h the mean was lower than the mean maximum potentials at the other treatment times, the mean maximum membrane potentials at 1, 2, 3, 15 and 24 h were greater with increasing treatment time. The interaction between temperature and treatment time showed mean membrane potentials that ranged from −114 mv at 5 h, $24^{\circ}C$ to −163 mv at 24h, $10^{\circ}C$.

The mean minimum membrane potential of healthy elm leaf cells was −103 mv (Table 7). The mean minimum potentials for cells of leaf tissues prepared at $10^{\circ}C$ and $24^{\circ}C$ were −104 mv and −103 mv, respectively. The means of cells measured at 1, 2, 3, 5, 15 and 24 h

Table 6. Mean maximum membrane potentials of elm leaf cells showing the effects of temperature at which the leaf was cut, the effects of treatment time between cutting and electrode insertion, and the effects of the interaction between cutting temperature and tissue preparation time.

MULTIPLE ANALYSIS OF VARIANCE		
	MAXP	Maximum Membrane Potential per Elm Leaf Cell Measured (mV)
BY	TEMP	Temperature of Ringers Solution During Leaf Cut (^{o}C)
	TRT	Treatment Time (Hours)

TOTAL POPULATION

-131 (60)[a]

TEMP					
10	24				
-128 (30)	-133 (30)				

TRT					
1	2	3	5	15	24
-118 (10)	-130 (10)	-134 (10)	-116 (10)	-134 (10)	-152 (10)

TEMP	TRT					
	1	2	3	5	15	24
10	-120 (5)	-126 (5)	-123 (5)	-117 (5)	-119 (5)	-163 (5)
24	-116 (5)	-133 (5)	-145 (5)	-114 (5)	-149 (5)	-137 (5)

[a]Number in parentheses indicate replications.

Table 7. Mean minimum membrane potentials of elm leaf cells showing the effects of temperature at which the leaf was cut, the effects of treatment time between cutting and electrode insertion, and the effects of the interaction between cutting temperature and tissue preparation time.

			MULTIPLE ANALYSIS OF VARIANCE			
		MINP	Minimum Membrane Potential per Elm Leaf Cell Measured (mV)			
	BY	TEMP	Temperature of Ringers Solution During Leaf Cut (^{o}C)			
		TRT	Treatment Time (Hours)			

TOTAL POPULATION

-103 (60)

TEMP

10	24				
-104 (30)	-103 (30)				

TRT (hours)

1	2	3	5	15	24
-87 (10)	-112 (10)	-115 (10)	-88 (10)	-88 (10)	-130 (10)

	TRT					
	1	2	3	5	15	24
TEMP						
10	-89 (5)	-101 (5)	-111 (5)	-104 (5)	-83 (5)	-135 (5)
24	-85 (5)	-123 (5)	-118 (5)	-72 (5)	-93 (5)	-126 (5)

[a]Numbers in parentheses indicate replications.

ranged from -87 mv at 1 h to -130 mv at 24 h. The means of minimum membrane potentials for five replicates in each temperature, treatment time block ranged from -72 mv at 5 h, 24°C to -135 mv at 24 h, 10°C.

Multiple analysis of variance of the effects of temperature, treatment, and the interactions between temperature and treatment on membrane potentials showed that treatment (tissue preparation time) had a significant effect on both maximum, and minimum membrane potentials (95% confidence level, Duncan's Range test of means).

The effect of treatment time and temperature on the change in membrane potential (maximum minus minimum) showed that at both tissue preparation temperatures, 10°C and 24°C, the mean differences between maximum and minimum were greatest at treatment times of 15 h (Table 8). They were least at either 3 h for the 10°C group or 2 h for the 24°C group.

The mean number of fluctuations in membrane potentials of the total population of elm leaf cells was 1.1 (Table 9). The mean number of fluctuations in membrane potentials for cells of leaf tissues prepared at 10°C and 24°C were 1.3 and 0.9, respectively. The means of fluctuations in cells measured at 1, 2, 3, 5, 15 and 24 h ranged from 0.6 at 24 h to 1.4 at 15 h. When the interaction between temperature and treatment time was tested, the lowest mean number of fluctuations in membrane potentials was found at 3 h, 10°C. The greatest mean number of fluctuations was found at 15 h, 10°C.

The mean time of fluctuations in membrane potential for the elm population studied was 2.9 minutes (Table 10). The mean time of fluctuations in membrane potentials for cells of leaf tissues prepared at 10°C and 24°C was 4.6 and 1.1 minutes, respectively. Separation of

Table 8. Effect of treatment time and temperature at which leaves were cut on the change in membrane potential (maximum minus minimum) of elm leaf cells.

Treatment time (h)	Mean change in membrane potential (mv)[a]	
	at $10^{O}C$	at $24^{O}C$
1	30.8	31.0
2	25.6	9.4
3	11.6	27.4
5	12.6	42.0
15	36.0	56.8
24	28.4	14.0

[a]Numbers are means of five replicates.

Table 9. Mean number of fluctuations in membrane potentials of elm leaf cells showing the effects of temperature at which the leaf was cut, the effects of treatment time between cutting and electrode insertion, and the effects of the interaction between temperature and tissue preparation time.

MULTIPLE ANALYSIS OF VARIANCE					
NODIPS Number of Fluctuations in Membrane Potential					
BY TEMP Temperature of Ringers Solution During Leaf Cut ($^\circ$C)					
TRT Treatment Time (Hours)					

TOTAL POPULATION

1.1 (60)

TEMP

10	24
1.3 (30)	0.9 (30)

TRT (hours)

1	2	3	5	15	24
1.1 (10)	1.3 (10)	0.8 (10)	1.3 (10)	1.4 (10)	0.6 (10)

TRT (hours)

	1	2	3	5	15	24
TEMP						
10	1.6 (5)	1.6 (5)	0.2 (5)	1.6 (5)	1.8 (5)	0.8 (5)
24	0.6 (5)	1.0 (5)	1.4 (5)	1.0 (5)	1.0 (5)	0.4 (5)

[a]Numbers in parentheses indicate replications.

Table 10. Mean times of fluctuations in membrane potentials of elm leaf cells showing the effects of temperature at which the leaf was cut, the effects of treatment time between cutting and insertion, the effects of the interaction between temperature and tissue preparation time.

			MULTIPLE ANALYSIS OF VARIANCE				
		DIPTIME	Total Time of Fluctuations in Elm Membrane Potential (Minutes)				
	BY	TEMP	Temperature of Ringers Solution During Treatment ($^{\circ}$C)				
		TRT	Treatment Time (Hours)				

TOTAL POPULATION

2.9 (60)

TEMP

10	24
4.6 (30)	1.1 (30)

TRT (hours)

1	2	3	5	15	24
15.0 (10)	0.3 (10)	0.5 (10)	0.8 (10)	0.5 (10)	0.2 (10)

TRT (hours)

TEMP	1	2	3	5	15	24
10	26.1 (5)	0.4 (5)	0.1 (5)	0.3 (5)	0.6 (5)	0.3 (5)
24	3.9 (5)	0.2 (5)	0.8 (5)	1.2 (5)	0.4 (5)	0.1 (5)

[a]Numbers in parentheses indicate replications.

the measurements by tissue preparation time showed a maximum mean of 15 minutes fluctuation time for the 1 h group and a minimum mean of 0.2 minutes fluctuation time for the 24 h group. Interaction between temperature and treatment groupings showed a range of mean fluctuation time from 0.1 minutes at both 3 h, $10^{o}C$ and 24 h, $24^{o}C$ to 26.1 minutes at 1 h, $10^{o}C$.

Priori contrast between different treatment times for duration of fluctuations in membrane potential demonstrated a significant difference between 1 h and all other tissue preparation times tested (Table 11).

Table 11. Priori contrast between different treatment times for durations of fluctuation in membrane potential in elm leaf cells.

Treatment Time (h)	1	2	3	5	15	24
1	−	+[a]	+	+	+	+
2	+	−	−	−	−	−
3	+	−	−	−	−	−
5	+	−	−	−	−	−
15	+	−	−	−	−	−
24	+	−	−	−	−	−

[a] + indicates a significant difference at 95% confidence (Duncan's Range Test of Means).

Discussion

The hypothesized formula

$$Q = \frac{|\Delta x|}{200} + \frac{10}{y} + z$$

was expected to describe an individual cell and index its usability for
further experimentation. A cell which lived indefinitely with no
changes in membrane potential would have a low index. The lower Q (the
cell stability index), the more stable the cell. If, however y was an
interval chosen by the researcher, a cell not lasting the selected time
interval could not be used with the index. The formula was expected to
describe the characteristics of the elm membrane potential and to
predict cell health based on a stable membrane potential. Comparisons
of means of several characteristics of membrane potential traces, show
that the formula does describe elm leaf cell membrane potential.
Membrane potential characteristics vary for individual cells and are
reflective of both environmental fluctuations and internal metabolic
fluctuations. Two external manipulations (temperature at which tissues
were cut and time allowed for equilibration before electrode insertion)
did interact to significantly affect membrane potential.

Maximal and minimal membrane potentials do differ depending upon
tissue preparation (Tables 6, 7, 8), therefore x is a factor which
contributes to the validity of the index. Since, in elm, the change in
constant, 200, in the denominator of the first term of the equation
could be lowered or even eliminated. The y factor, if set by the
researcher, contributes the same amount to the index of each cell

studied. If instead y is used to express longevity of the cell the formula can not be used as a predictor because the cell would have expired before y was determined. However, the longevity term is necessary in developing a predictive model. The formula can be refined so that the cell index is determined at a set time after electrode insertion. From the data in Chapter 1, it is clear that the time interval selected should be at least 5 minutes and need not be more than 20 minutes. The z factor (number of fluctuations in the membrane potential contributes to the validity of the formula because such fluctuations are typical in measurements of healthy elm leaf cells (Table 9). It might be assumed that fluctuations would interfere with results from experimental stimuli. However, disregarding data from cells in a control group which have fluctuations while not eliminating them from the experimental group would bias the data. Use of the cell index gives an indication of natural variability of cells in both control and experimental groups before a stimulus is applied. Because of this, the number of fluctuations is an especially important factor in the formula. Analyses of experimental results show that cells with the lowest Q, and therefore those described as the most stable, were those measured in tissues cut at $10^{o}C$ and allowed to equilibrate 3 h before electrode insertion.

In the original formula the number of membrane potential fluctuations was considered, but the time duration of these fluctuations was not. Duration of fluctuations was significant only between treatment equilibration time of 1 h and all other treatment times (Table 11). However, when treatment times were coupled with temperature at which tissues were cut (Table 10), substantial differences were shown.

These results suggest that fluctuation time would be a reasonable factor to add to the cell stability formula.

Results show that the membrane potential "fingerprint" of individual cells is unique and is affected by methods of tissue preparation. However, variations should not be used empirically to label a cell as usable or unusable for further experimentation. The cell stability index aids in quantifying and describing the membrane potential of an individual elm cell and comparing it to potentials of a population of elms. The hypothesized index is by no means complete, but it is useful as a descriptior in its present form. Based on the results of the multiple analysis of variance, the formula can be refined to include Δx and z at a selected time interval between 5 and 20 minutes. As data is accumulated over the years, addition of other factors, such as fluctuation time, will undoubtedly yield a formula that in addition to being descriptive is sufficiently refined so that it is also highly predictive.

CHAPTER III

LIGHT AND HEALTHY ELM MEMBRANE POTENTIAL

Introduction

Exposing green algal cells to light causes hyperpolarization (see Glossary) and enhanced ion transport (Higinbotham, 1973). Green cells of higher plants are also hyperpolarized by light. Hydrogen ion movement, pH gradient and electrogenic pumps have been suggested explanations (Higinbotham, 1973). In light membrane potential is approximately 50 mv more negative than in the dark. This difference in potential has been attributed to an electrogenic pump (Higinbotham, 1973). Light at 660 nm triggers hyperpolarization and at 730 nm depolarization (Galston and Satter, 1976). These changes are perhaps caused by structural changes in the pigment phytochome (Galston and Satter, 1976).

There are two photosystems thought to operate in photosynthesis, photosystem II and photosystem I. Both photosystems are activated by red or blue radiation. There is a light driven proton gradient formed by the thylakoid membrane (Luttge and Higinbotham, 1979; Jagendorf, 1977; Junge, 1977). Photosystems II and I both contribute about equally to the electric potential difference after excitation of chloroplasts with a short flash of light (Junge, 1977). In the light, the inner thylakoid membrane drops in pH indicating increased concentration of protons (Jagendorf, 1977).

Light does affect disease development, sometimes in conjunction with temperature effects. In light – grown bean hypocotyls, for example, anthracnose symptom development was temperature dependent

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(Bailey, 1974). It has been suggested that fungi have the ability to metabolize the phytoalexins which counter the disease at lower temperatures (Bailey, 1974).

In higher plants, as in other photosynthetic species, many enzymes are light-modulated (Anderson, Ashton, Mohamed and Scheibe, 1982). Both activation and inactivation of enzymes can be light-modulated (Anderson, Ashton, Mohamed and Scheibe, 1982). In the case of the light-modulated enzyme chloroplast fructose – 1, 6-bisphosphatase, for example, modulation involves conformational change (Anderson, Chin and Gupta, 1979a). Some light-modulated enzyme changes shift pH dependency (Anderson, Chin and Gupta, 1979a), or affinity for substrate (Anderson, Hansen and Anderson 1979b).

Inhibition of light-modulated enzymes is probably responsible for part of the toxic effect of SO_2 on plants (Anderson and Duggan, 1977). Novacky and Karr (1976) have indicated a light-dependent component of membrane function that is vulnerable to leaf-damaging pathogens. Such light-dependent susceptibility of the membrane illustrates the importance of fully understanding light effects on membrane potential.

The purpose of this experiment was to test the effect of presence or absence of light of various wave lengths on the membrane potential of healthy elm leaf tissue.

Materials and Methods

Leaves of 1 year old, greenhouse-grown, elm trees were submerged in Ringer's solution at $10^{\circ}C$ and were cut into 1 cm^2 sections. The leaf sections were then allowed to age for three hours. A leaf section was then positioned in front of a horizontal microscope in a perfusion chamber. Ringer's solution was fluxed through the perfusion chamber.

Using the standard electrophysiological set-up (Figure 1), membrane

potentials were measured for 5 minutes with the source light on, then

the source light was shut off for five minutes. After 5 minutes, the

source light was turned on again. There frequently was ambient light in

the room. The experiments to test effects of presence or absence of

light were repeated with the ambient laboratory light blocked out so

that when the source light was out, no light was present. Flux

densities of light used to study elm membrane potential, measured with a

Quantum/Radiometer/Photometer (Li Cor, Lincoln, Nebraska [Lambda

Instrument Corp.] Sr. No.:ZRPA 306-781), ranged between 17 and 32

microeinsteins m^{-2} sec^{-1} for white light. The red filter passed

wavelengths longer than 580 nm. The flux densities for red light ranged

between 5.3 to 10 microeinsteins m^{-2} sec^{-1}. The blue filter passed

wavelengths from 360 nm to 500 nm. The flux densities for blue light

ranged between 0.6 to 11.5 microeinsteins m^{-2} sec^{-1}.

Results

Following a light-dark change, the membrane potential

hyperpolarizes about 10 mv, then depolarizes slightly before returning

to its beginning level. The membrane potential depolarizes about 20 mv

following a dark-light change, then hyperpolarizes to return to near its

previous value (Figure 3).

Similarly, with ambient laboratory light present (less than 0.3

microeinsteins $m^{-2}S^{-1}$), a white-blue light change resulted in a

hyperpolarization followed by a return to the starting level and a

blue-white light change caused a depolarization in

the membrane potential followed by a return to near its previous level

(Figure 4).

Figure 3. The effect of light-dark and dark-light changes on membrane potential in elm leaf cells. The trace is typical of more than 100 traces. White light was shut off at the point labled dark and turned on again five minutes later at the point labled white light.

Figure 4. The effect of white-blue and blue-white light changes on membrane potential in elm leaf cells. The trace is typical of the reaction in more than 25 cells. Ambient laboratory light was present during the blue measurement.
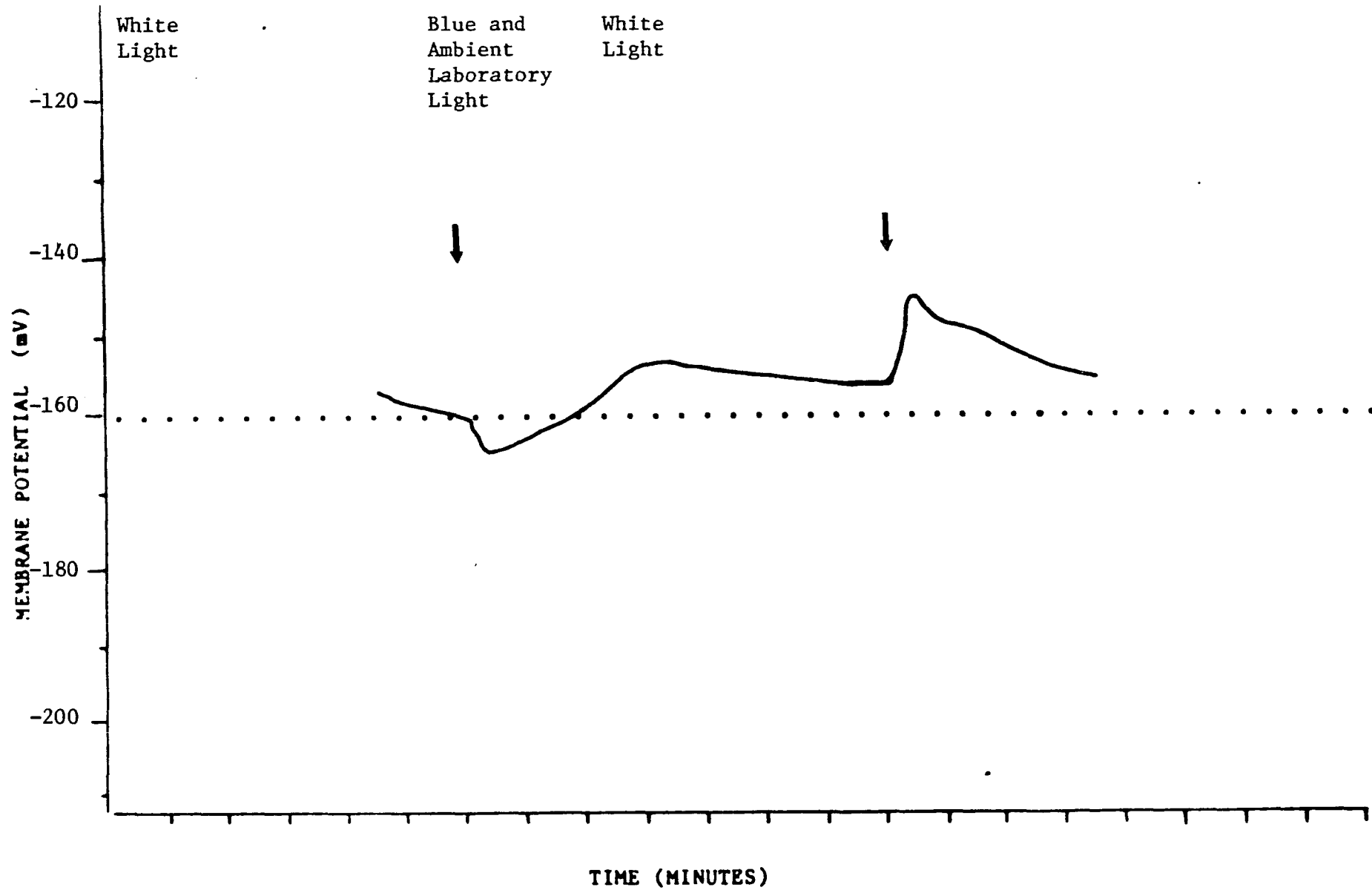
However, when no ambient light was present except the source light, a white-blue light change resulted in a depolarization followed by a slight repolarization and then a gradual depolarization about 20 mv. When a blue-white change was made, there was a rapid depolarization of about 20 mv followed by a hyperpolarization to near its previous level (Figure 5).

A white-red light change caused a hyperpolarization about 10 mv followed by a gradual depolarization. A red-white light change caused a depolarization, about 10 mv, followed by a gradual hyperpolarization. With ambient laboratory light, the white-red, red-white light change caused a similar hyperpolarization-depolarization, depolarization-hyperpolarization membrane potential pattern (Figure 6).

Discussion

Transport systems in the membrane of Chara corallina which are light stimulated include Cl^- influx and OH^- efflux. Lichtner, Lucas and Spanswick (1981), suggested that if these systems represent major conductance pathways in the membrane, the regulation of transport by light might account for the rise of the membrane resistance in the dark.

Membrane potential of green cells is generally hyperpolarized on illumination. It has been assumed that light activates the putative H^+ pump which acts electrogenically. (Kawamura, Shimmen and Tazawa, 1980).

Transient potential changes observed from switching from dark to light, or vice versa are probably a result of ionic diffusion. For example, in light, H^+ is taken up by chloroplasts Atriplex spongiosa and K^+ is extruded (Higinbotham, 1973). Possibly K^+ efflux from chloroplasts is involved in Nitella flexilis since pH in the vacuole shifts downward during depolarization (Higinbotham, 1973).

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Figure 5. The effect of white-blue and blue-white light changes on
membrane potential in elm leaf cells. The trace is typical of the
reaction in more than 25 cells. No ambient laboratory light was present
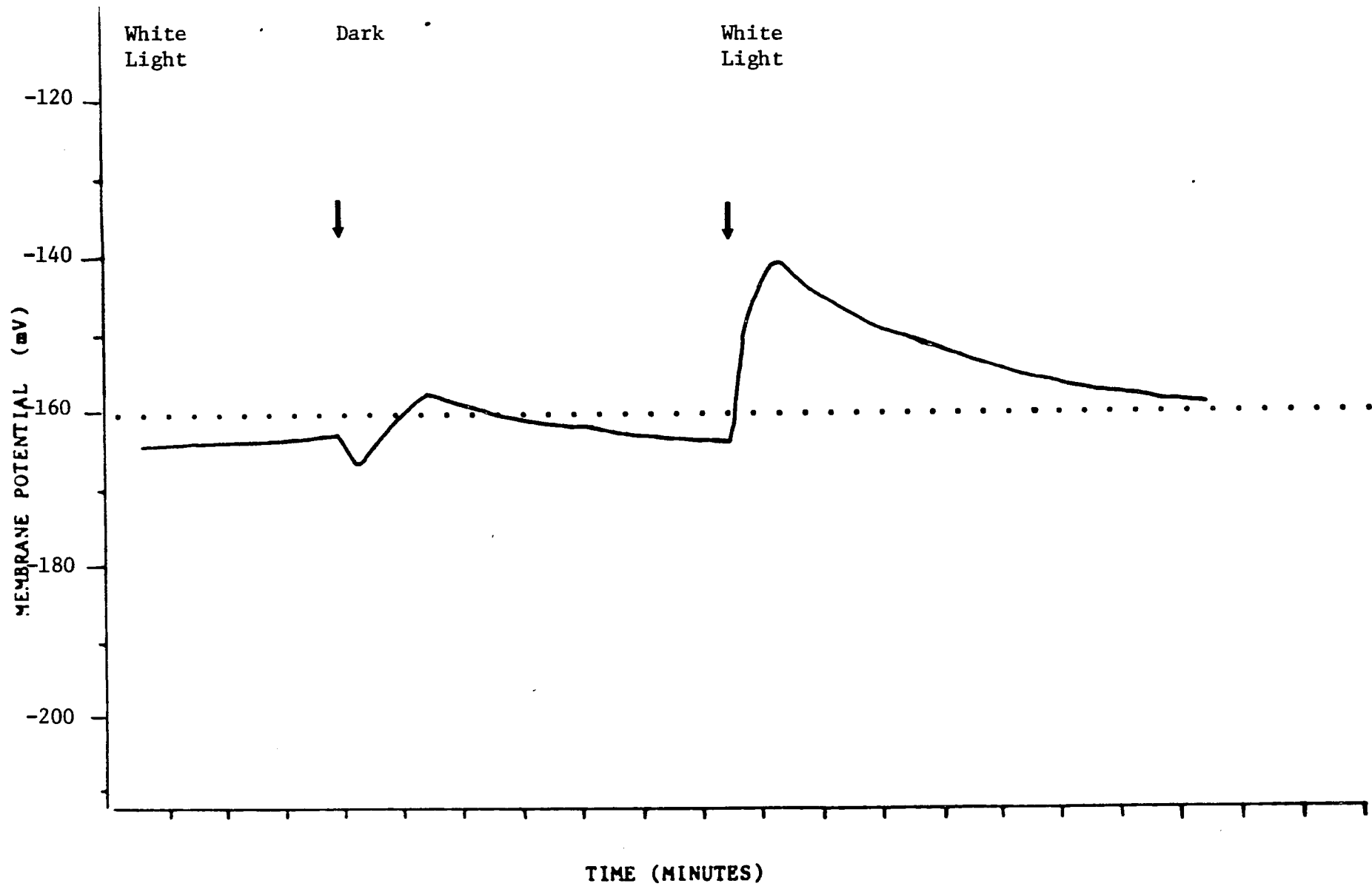during the measurements.
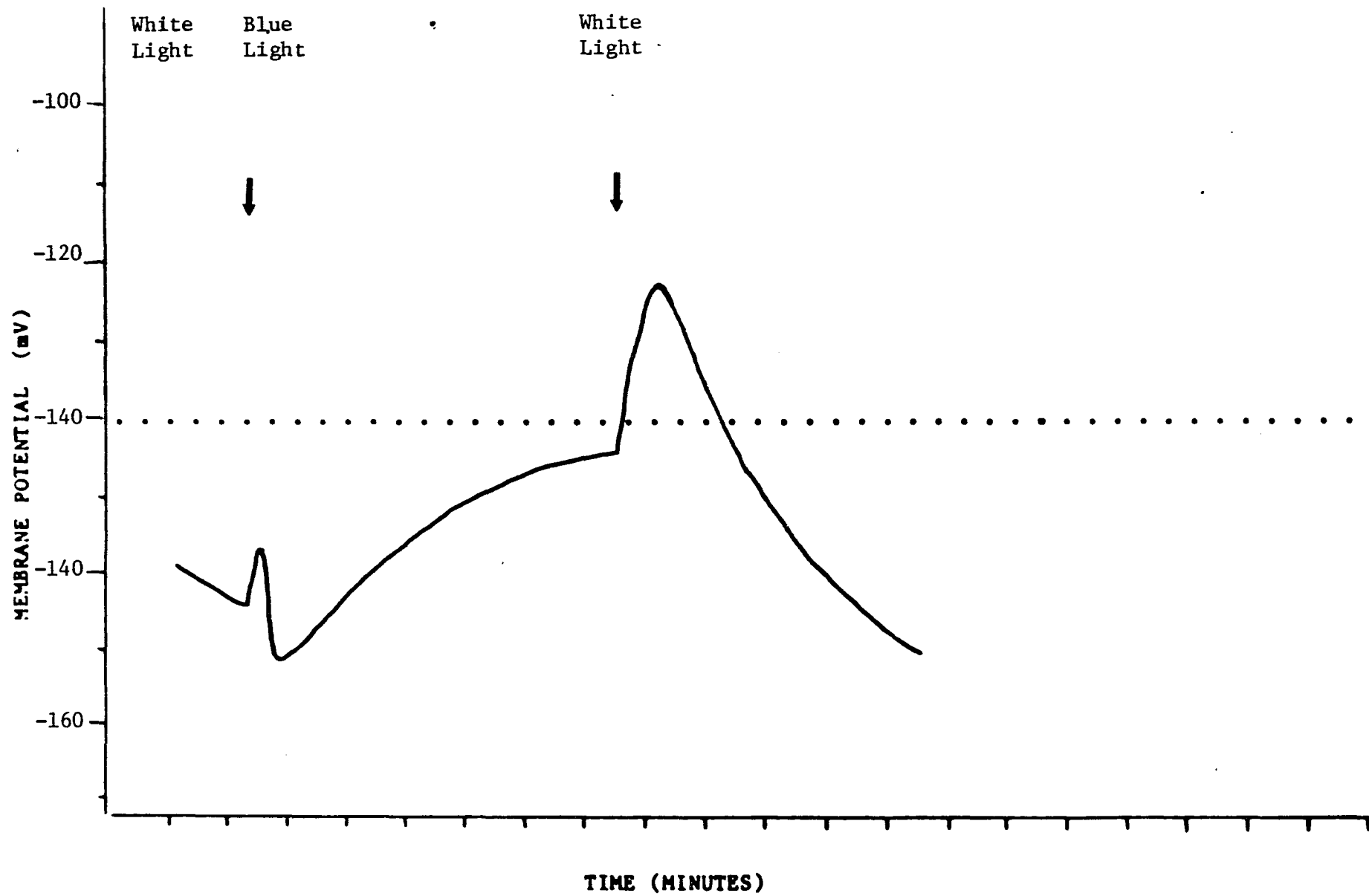
Figure 6. The effect of white-red and red-white light changes on membrane potential in healthy elm leaf cells. The trace is typical of the reaction in more than 25 cells. No ambient laboratory light was present during measurements.

White
Light

Red
Light

White
Light

-80

-100

-120

-140

-160

MEMBRANE POTENTIAL (mV)

TIME (MINUTES)
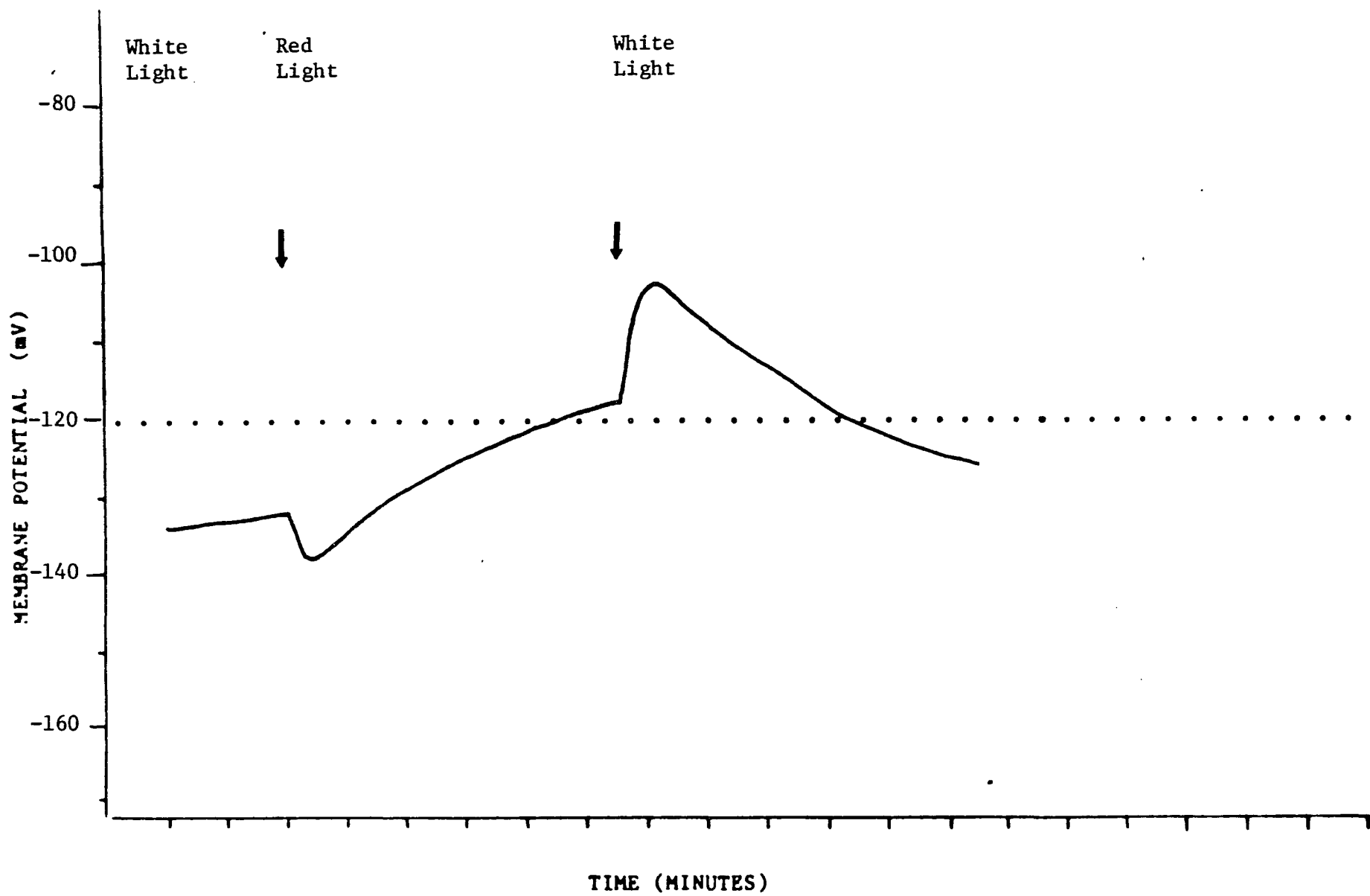
Photosynthetically active light has both depolarization and hyper-polarization effects on cell membrane potentials (Bentrup, 1974b; Felle and Bentrup, 1974a,b; Jeschke, 1970a,b). Electrogenic pumps are assumed to be involved in the light-dependent changes in membrane potential (see Review: Bentrup 1974). Transient membrane potential phenomena associated with the presence or absence of light may last longer than 1 h (Luttge and Higinbotham, 1979). Resting potential (baseline membrane potential) is often re-established after light-dark or dark-light induced oscillations, however.

Because membrane potentials alter with wavelengths of photosynthetic light, hydrogen ion changes have been studied (Luttge and Higinbotham, 1979). Work with intracellular pH-microelectrodes (Davis, 1974) supports the following sequence of events:

1. presence of light (after a dark period) causes photosynthetic ion transfer and hydrogen ions are taken up by the thylakoids,

2. the stroma of the chloroplast becomes more alkaline,

3. the membrane potential depolarizes (the cytoplasm could lose hydrogen ions which, according to the Nernst and Goldman equations, would depolarize the membrane potential), and

4. hydrogen ions are taken up into the cell (Luttge and Higinbotham, 1979).

The reverse process seems to occur following a change from light to darkness (Luttge and Higinbotham, 1979). Photosynthetic CO_2 assimilation has also been proposed to explain the changes in membrane potential, assuming that the pumps transporting HCO_3^- and OH^- operate with different kinetics (Denny and Weeks, 1970). Although, Neumann and Levine (1971) suggest that hydrogen ion movements at the thylakoids are

not reflected at the chloroplast envelope or at the plasmalemma, work with the uncoupler F-CCP and the electron acceptor p-benzoquinone on Elodea (Hope et al., 1972) implies that part of oscillations in membrane potential may be coupled to photosynthetic electron flow. With intact cells of Phaeoceros laevis transient pH changes do occur following alterations of light and dark (Davis, 1974). Vredenburg and Tonk (1973) observed a rapid light-triggered reaction which caused a decrease in membrane resistance of Nitella translucens cells and postulated that transport of a reaction product, an intermediate or an ion across the tonoplast might be involved. A slower light-dependent change in membrane potential also occured in Nitella (Vredenburg and Tonk, 1976). Bentrup (1974) suggests that the effect of small, light-dependent, cytoplasmic pH changes may be magnified by protonation at the plasmalemma.

Wright and Fisher (1981) observed an effect of light on Salix sieve tube membrane potential. A light-dark change induced a hyperpolarization of 10 to 15 mV that reached a maximum in 5 min., followed by a slower depolarization to the original potential (Wright and Fisher, 1981). A dark-light change caused a rapid depolarization followed by a slow repolarization in the sieve tube membrane potential. They did not feel the changes were caused by electrode effects or temperature but did not speculate as to their origin.

According to Chemiosmotic theory of Mitchell developed for mitochondria and chloroplasts (Mitchell, 1967), ATP synthesis results from an electrogenic separation of H^+ and OH^- ions across the membrane. The H^+ is driven outward in mitochondria and inward in chloroplasts; the process is reversible in the presence of suitable pH gradients (Higinbotham, 1973).

Rapid electrical changes in onion guard cell membrane potentials are among the fastest known stomatal responses (Zeiger, Moody, Hepler and Varela, 1977.)

Researchers look for photo receptor systems through which plants sense light conditions which require mass synthesis of protective pigments (Drumm-Herrell and Mohr, 1981), for example. Phytochrome is often implicated in this type of sensory system (Drumm-Herrell and Mohr, 1981).

Conclusion

The effects of the presence or absence of light on the membrane potential of healthy elm leaf tissue were tested. Elm leaf sections were cut in $10^{o}C$ Ringer's solution and aged for three hours at room temperature, $24^{o}C$. In addition, the effects of red and blue filtered light were studied. Photosynthesis was inhibited with DCMU and potentials were measured however, results were inconclusive and will not be discussed further.

The results of this study indicated that:

1. A light-dark, dark-light change caused a hyperpolarization - depolarization, depolarization - hyperpolarization in elm membrane potential.

2. A white-blue, blue-white light change caused a depolarization - repolarization - depolarization, depolarization - hyperpolarization pattern in elm membrane potential (Figure 4).

3. The white-blue, blue-white light induced changes in elm membrane potential were masked by the presence of ambient laboratory light (Figure 5).

4. A white-red, red-white light change caused a hyperpolarization
    - depolarization, depolarization - hyperpolarization pattern
    in elm membrane potential (Figure 6). This pattern was not
    affected by the presence of ambient laboratory light.

5. Light-dark and dark-light induced changes in membrane
    potential were similar to patterns found for other species.

The work on elm showed light-dark induced membrane potential
oscillations similar to those found with other plants. However, white
light to red or to blue light caused opposite initial effects on the
membrane potentials of elm leaf cells. The fact that red and blue light
both allow photosynthesis, leads to the expectation that the membrane
potentials would both follow the same pattern if the oscillations in
membrane potential were entirely related to photosynthesis. Perhaps
another mechanism is involved. Measurements of elm membrane potential
when the tissue is subjected to narrower bands of light could lead to
further speculation and perhaps suggest the involvement of a phytochrome
receptor.

Additional wavelength studies, particularly in the UV range, would
strengthen the study on light effects on elm membrane potential.
None-the-less, this study on the effects of light on membrane potential
has contributed to our basic understanding of elm electrophysiology.
Specifically, induced patterns of membrane potential activity in
response to various light changes were determined.

This work is a significant contribution electrophysiology is to be
applied to plant pathology since light effects are often involved in
plant growth and development, cold hardiness and resistance to disease.

CHAPTER IV

EFFECTS OF INHIBITORS

Introduction

The microelectrode and it's associated electrical apparatus allow

the study of the effects of metabolic inhibitors on intact or whole

cells rather than on subcellular organelles.

Membrane potential in several species is thought to contain both

diffusion and energy-dependent components (Kawamura, Shimmen, and

Tazawa, 1980). Dependence of the Membrane Potential of Chara Cells on

External pH in Planta 149 213-218. The Presence or Absence of Internal

Adenosinetriphosphate).

Cyanide inhibits photosynthesis and respiration. The reduction

of plastoquinone involves both electrons and protons (Avron, 1981).

Plastoquinone is a proton carrier (Avron, 1981). DCMU acts after Q at

plastoquinone (Avron, 1981).

Materials and Methods

Plant Material

Elm leaf tissue was prepared as described in CHAPTER I.

Preparation of Solutions

Cyanide was added to the Ringer's solution which was fluxed through

the perfusion chamber. Composition of the cyanide solution is defined

in Table 2.

Electrical Measurements

The membrane potential differences between the external solution
and the cell vacuoles was measured as described in Chapter 1.
Microelectrodes containing 3M KCL were connected, using silver- silver
choloride electrodes, to both the cell interior and a singal amplifier.
The amplified signals were output on both a chart recorder and an
oscilloscope.

Experimental Procedure

The treatment time was 3h and the temperature of the Ringer's
solution during tissue preparation was $10^{o}C$.

During electrical measurement, leaf tissue was oriented in a
split-sponge tissue holder (Appendix C) in the perfusion chamber. The
upper leaf surface was facing the ocular of the microscope. Either
Ringer's solution or the cyanide test solution (Table 2) was fluxed
through the chamber. The tissue was illuminated with a microscope lamp
(see CHAPTER III). Cyanide solution was in one bottle and Ringer's
solution in another. These were connected to the perfusion chamber with
Tygon tubing. A valve allowed selection of one or the other solution.
Cell membrane potentials were measured for 15 minutes before the cyanide
was perfused through the chamber. Cyanide solution exposure was five
minutes, then, Ringer's solution without cyanide was again fluxed
through the chamber.

Results

Cyanide fluxed through the electrophysiological system does affect
the membrane potential readings in elm. The general trend of the
cyanide effect is a depolarization of the membrane potential. Shortly

after applying the cyanide solution, the electropotential decreases

approximately 40% (Figure 7). When the cyanide is removed from the

perfusion chamber by flushing it with fresh Ringer's solution, the

membrane potential repolarizes (Figure 7).

Discussion

An increase in membrane permeability means a decrease in R

(resistance) or, following Ohms law, a corresponding increase in current

if the voltage stays the same. Similarly, a blockage of ion transport

leads to a blockage of current.

Cyanide at 10^{-5} to 10^{-3}M, within about 5 minutes, reduces the cell

electropotential by as much as 50%; recovery requires approximately 30

min (Anderson, Hendrix and Higinbotham, 1974). DNP and Azide also cause

depolarizations in membrane potentials (Higinbotham, 1973).

The swift depolarization caused by respiratory poisons is probably

the most critical evidence for the presence of electrogenic ion pumps at

membranes (Anderson, Hendrix and Higinbotham, 1974).

An electrogenic pump occurs when active transport, in which net

charge is transferred across a membrane at the expense of metabolic

energy, creates a potential difference (Higinbotham, 1973). Because a

respiratory inhibitor blocks the source of metabolic energy, the

potential difference changes causing rapid depolarization in membrane

potential (Higinbotham, 1973).

In this section, the effects of CN^- poisoning on membrane

potentials in elm leaf cells are described. These results are compared

with similar results of other higher plant cells.

Figure 7. The effect of flushing the perfusion chamber with 1mM sodium cyanide on membrane potential in healthy elm leaf cells. The trace is typical of the reaction in more than 25 cells.

Return to Ringer's Solution
Cyanide ·
Flashing started
5 minutes before
this point
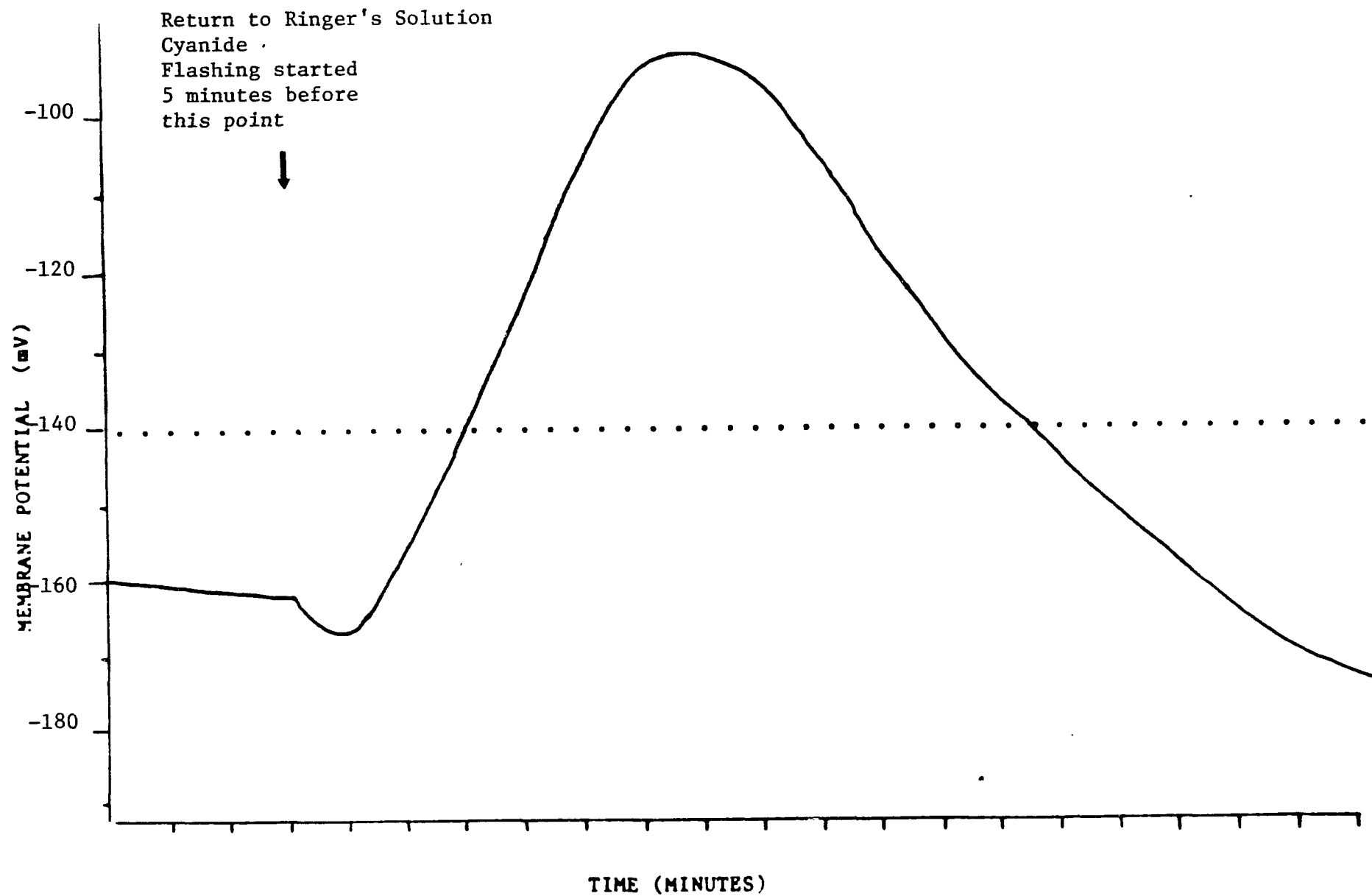
Cyanide poisoning causes a very large membrane depolarization in all plants previously studied and also increases their membrane resistances (Anderson, Hendrix, and Higinbotham, 1974). The studies on elm membrane potential support the findings on other plants that membrane potential depolarizes by as much as forty to fifty percent. Hyperpolarization induced by light was found to be inhibited by Azide, CN, CCCP, and DCMU as expected with an electrogenic pump (see Higinbotham, 1973). Cyanide is a complex inhibitor, in addition to halting cytochrome oxidase, its effects on membrane potential may involve inhibition of iron-containing compounds, for example (Anderson, Hendrix, and Higinbotham, 1974).

CHAPTER V

THE EFFECTS OF pH

Introduction

There have been tremendous increases in sulfur dioxide (SO_2) from

the incineration of fossil fuels and other air pollutants through the

last century (Bringi, Seliga and Dochinger, 1981). Ozone and SO_2

pollution are widespread and have a large impact on the forest.

For example, pines, ash, larch, oak, aspen birch and elm are all

susceptible to these two pollutants (Bringi, et. al, 1981), and are

showing various degrees of injury.

The fallout of dilute solutions of sulfuric and nitric acid from

SO_2 and NO_2 pollution is the major cause of acid precipitation (Manion,

1981). Unpolluted rain generally has a pH value of 5.6 (Likens, Wright,

Galloway, and, Butler, 1979). Dissolved carbonic acid caused by

dissolving of CO_2 from the atmoshere, in unpolluted rain, accounts for

its slight natural acidity. The ratios of the various components in

acid precipitation vary from time to time and place to place (Cowling

and Linthurst, 1981).

Rain with pH as low as 2.4 has been recorded as a result of

industrial pollution (Manion, 1981). Significant effects of acid

precipitation on terrestrial plant ecosystems are being documented

(Harper and Jones, 1982).

Terrestrial effects of acid precipitation are felt directly through

erosion of plant leaf cuticles and indirectly through the leaching of

essential elements from soils. In a forest, precipitation is

intercepted by vegetation where dissolved substances can induce various

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physiological changes before reaching the soil, including erosion of leaf surface waxes and leaching of essential elements and various metabolites from foliar organs (Cowling and Linthurst, 1981). It is possible that an additional indirect effect is that plants are predisposed to disease and insect injury by a weakening of the plant caused by the effects of acid precipitation.

Air pollution injury on forest trees has traditionally been reflected by changes in leaves, changes in volume of wood and in changes in weight of wood (Patton, 1981). Direct and indirect damage to crops and forest trees have been reported in various field, greenhouse and laboratory investigations of the effects of synthetic rain, equivalent in chemical composition and rate of deposition to natural rains (Cowling and Linthurst, 1981). Decomposition of litter on the forest floor is also affected by acid precipitation, thus, in turn affecting nutrients available for absorpiton by the trees (Cowling and Linthurst, 1981). A study on nutritive effect of low doses of SO_2 showed growth enhancement in some tree species (Ziegler, 1979 and Maugh 1979; both as cited by Patton, 1981). The nutrient elements essential for growth of plants can be taken up readily through foliar organs in addition to absorption by roots from the soil solution (Wittwer and Bukovac, 1969).

Soil acidity governs the availability of nutrients to the tree (Pirone, 1978). As acid rain seeps into the soil, it leaches away valuable mineral nutrients but makes toxic metals more soluble. Plants can then absorb the poisons (Angyal, 1980). Adding lime may increase the toxicity of metals released by the acid (Angyal, 1980). Young trees are particularly sensitive to the detriments of acid rain. Whether the pH toxicity or poisoning by heavy metals is responsible, seed

germination is inhibited, seedling growth is stunted, and bud formation is limited (Angyal, 1980). Acid precipitation has also caused nutritional problems, related to aspects of fertilization (Abrahamsen, 1980). The most favorable soil pH range for Ulmus spp. is 6.5 to 7.5 (Pirone, 1978).

The purpose of this experiment was to determine the effect of pH on membrane potential of elm leaf cells and to relate results to acid rain.

Materials and Methods

Sulphuric acid was added to Ringer's solution (normal pH = 5.5) to produce pH levels typical for acid rain: 3.5 and 4.5. Elm tissues were prepared by cutting leaf sections in $10^{o}C$ Ringer's solution and allowing them to equilibrate at room temperature for 3 h. Leaf sections were then placed in the holding chamber and bathed in fluxing Ringer's solution (see CHAPTER I for details of this procedure and the subsequent electrode insertion). Individual cells were penetrated with a microelectrode and allowed to equilibrate for five minutes. Membrane potential traces were monitored on a strip chart recorder. Then, Ringer's solution at one of the prepared pH levels was fluxed through the holding chamber. Flushing was allowed to continue for five minute sand was then replaced with normal Ringer's solution. Several cells were penetrated and treated in this manner for both pH levels.

Results

A typical membrane potential trace of elm leaf cells exposed to pH values of 3.5 and 4.5 is shown in Figure 8. Membrane potentials of penetrated cells were allowed to equilibrate for about five minutes, and

Figure 8. The effect of acid pH ranges on membrane potential in healthy
elm leaf cells. The trace is representative of measurements from 3
cells at pH 3.5 and 3 cells at pH 4.5.
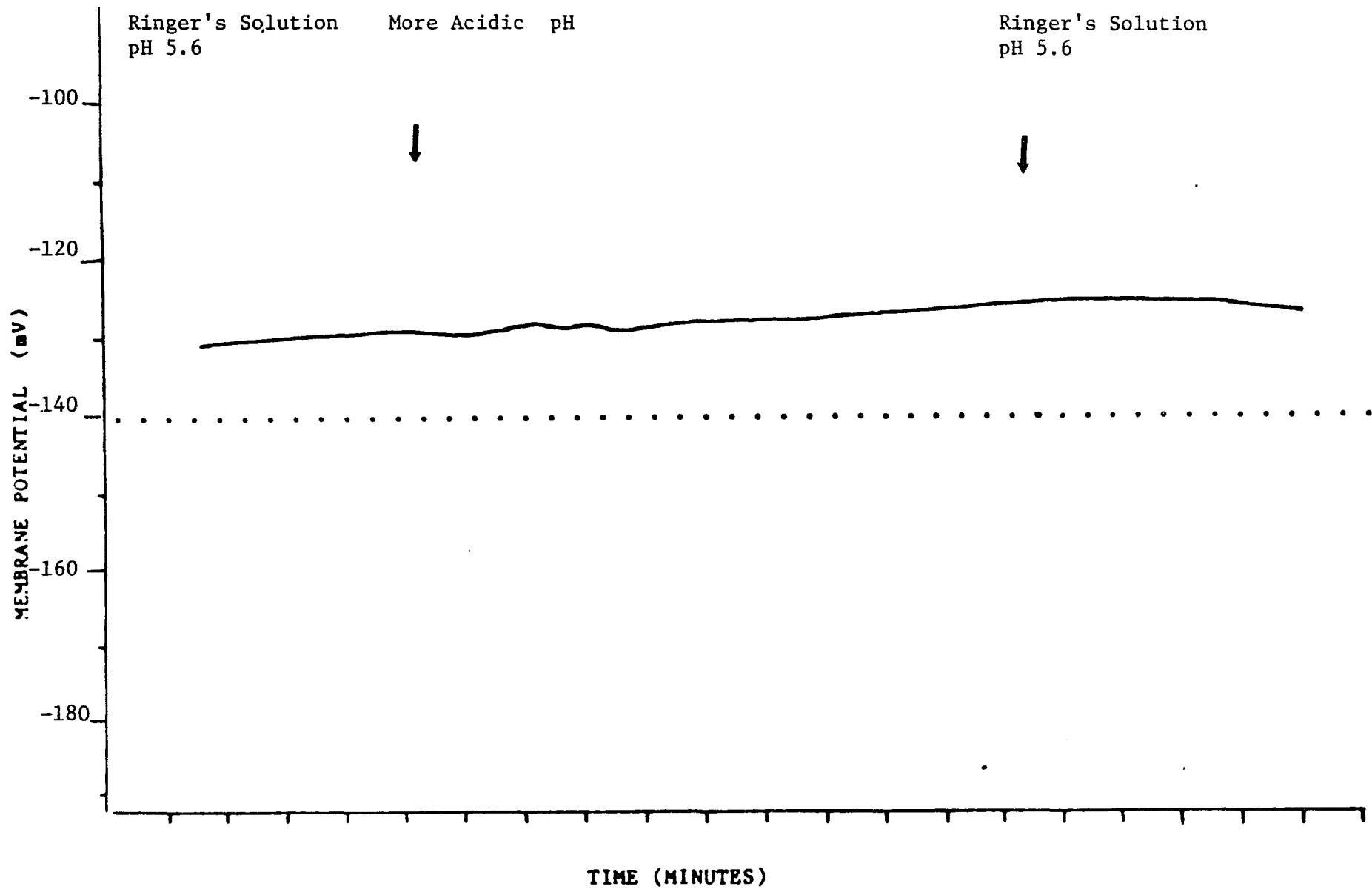
most levelled off between -120 and -130 mv. The flushing Ringer's

bathing solution, with a pH of 5.5, was shut off at the arrow (Figure 8)

and was replaced with flushing Ringer's at either pH 3.5 or 4.5. Within

three minutes, the membrane potential began to hyperpolarize and

continued to do so gradually for five to eight minutes. The membrane

potential then levelled out at 25-30 mv more negative than the original

potential. When the altered pH Ringer's was replaced with normal

Ringer's, the membrane potential gradually depolarized to the original

level. This same pattern occurred for all cells tested and at both pH

levels.

Discussion

In plant cells, membrane potential is considered strongly dependent

on external pH (pH_o). This dependency is decreased when cell metabolism

is blocked by inhibitors or low temperature (Saito and Senda, 1973,

Bentrup et al. 1973 and Richards and Hope 1974 as cited by Kawamura,

Shimmen and Tazawa, 1980). Lichtner et al. (1981) showed that changing

external pH from 8 to 6 initially displaces the membrane potential

toward a more positive value in Chara corallina. Also in Chara Kamura,

et al. (1980) found that the membrane potential became more positive at

a rapid rate as pH changed from 4.4 to 7.4. As pH changed from 7.4 to

8.4, the membrane potential again became more positive but at a

decreased rate.

In this study on elm leaf cells, only two levels of pH were

studied. As with studies on Chara, membrane potentials did initially

become more positive, but then resumed initial potentials. Both pH

levels produced membrane potential traces similar to those produced by

light-dark changes (Figure 8). Although initial studies here showed

little impact of lowered pH on elm cells, it should be understood that pH changes were of short duration. Under prolonged stress, as would be expected with acid rain, cells might exhibit significant impact of air pollutants that could be studied electrophysiologically.

Before further inferences concerning various tree species can be made, more tests with other varieties and ages of trees, at various pollutant levels and at different time intervals are needed. Acid precipitation parallels increases in the emission of sulfur and nitrogen oxides from the combustion of fossil fuels (Likens, Wright, Galloway, and Butler, 1979). It would be worthwhile to study the effect of these emission increases on trees such as elm by acid treatments of trees which parallel these increases and to utilize the results for future electrophysiological studies (Shipman, 1981).

CHAPTER VI

SUMMARY AND FUTURE OUTLOOK

Discussion

Dutch elm disease, caused by Ceratocystis ulmi (Buisman, C.

Moreau), and aided by the vectors Scolytus multistriatus and

Hylurgopinus rufipes has had devastating effects on the elm populations

of two continents. Because of the widespread incidence of the disease,

much research has been done on its pathology. The abundance of studies

on the elm provide a solid background on which to base

electrophysiological studies. They include pesticide research, genetics

and breeding, fungal strains, resistant and susceptible varieties.

While electrophysiological studies of elm are still in elementary

stages, they have vast potential to contribute to our understanding of

wilt diseases, to breeding programs, to pesticide testing and to our

basic knowledge.

The interaction of various physical factors on elm tree health is

complex enough to make separation of single factors very difficult. The

electrophysiology set-up allows the physical factors to be singled out

and tested on one aspect of tree health, the nature of the leaf cell

membrane potential. Leaf cell membrane potential, however, is

indicative of the interaction of many cell metabolic activities. While

it is unclear as to what metabolic activity or what combination of

metabolic activities are causing changes in the membrane potential, the

measurement of membrane potential in itself indicates rapid biochemical

changes within the cell. The rapid detection of biochemical activity

indicated by this technique is of value since current biochemical

methods cannot uncover the reactions which occur within the first few seconds after a stimulus is provided. In fact, during that time, often the plant tissue is still in the stage of being ground-up.

Refinement of the detection of metabolic activity observed with electrophysiological methods is perhaps possible as the techniques are improving rapidly (Appendix C). Specific ion electrodes and beveled electrodes for example will provide more clues to the reasons for the electrical changes in the membrane potential. Combinations of membrane potential measurements with other techniques such as membrane resistance measurements, labeling, antibody work and electron microscopy will help unroll the riddles of the electrical results of metabolism. The direct and indirect effects of physical factors, chemicals and pathogens on membrane potential will begin to be resolved.

Interactions of factors seem to affect plant membrane potentials differently. For example:

1.   Ambient light can mask the effects of blue light (CHAPTER III).

2.   Temperature and treatment interaction affect membrane potential (CHAPTER II).

3.   Membrane potentials of certain cells are affected by the interaction of sucrose and pH (Racusen and Galston, 1977).

In nature it is probable that the combination of physical and chemical stimuli interact in a manner similar to those of the interactions visible in laboratory electrophysiological studies. The electrical activities within and surrounding the plant may be involved in both:

1. plant resistance or susceptibility to disease, and,

2. plant response to phytotoxic elements of the environment. I believe that mechanisms of resistance and susceptibility to the effects of plant disease, phytotoxins and environmental stress can be determined at least in part by using electrophysiological methods.

Philip Abelson (1982) indicated that analysis of presentations at a conference on "Biomass Substitutes for Liquid Fuels" held in February 1982 in Brazil showed that, "a combination of factors will guarantee the increasing importance of the culture of trees and the applications of forest products." The factors discussed included:

1. the need to develop renewable alternatives to oil;

2. the growing world requirements for food and energy;

3. the need to decrease soil erosion.

Although we are in the early phases of improvement in biomass yield from trees, farmers currently obtain larger economic return from other crop plants than trees and profit yields of wood from natural forests are small (Abelson, 1982).

Traditional plant-breeding techniques, high-energy inputs, and vast chemical inputs led to great gains in agricultural productivity during the Green Revolution (Krogmann and Key, 1981). It is true that plant breeders and plant pathologists together have greatly improved the yield of food plants by selecting varieties that are resistant to disease, that yield well and that are adapted to specific climates, soils, and farming methods (Strobel, 1975). However, since selection through breeding is a slow process especially when maturity of the plant species takes years, as in trees, and since dissemination of a single new variety can itself be a hazard, electrophysiological techniques offer

new promise. Though the methods need to be further developed and tested, they show potential for rapid screening of young plants for characteristics such as disease resistance and cold hardiness.

Krogmann and Key (1981) pointed out the concern that more research be directed toward solving future agricultural gains. More fundamental knowledge about plants appears essential to solving practical agricultural problems (Krogmann and Key, 1981).

Griesbach, Koivuniemi, and Carlson (1981) stated that, "current genetic engineering technology is not very well developed for use in plant improvement. Some of the problems in applying somatic hybridization and in vitro mutagenesis to plant breeding have demonstrated a need to re-examine classical agronomic and horticultural traits and to develop new technology specifically designed for plant improvement." Cell, tissue and organ culture of selected genotypes of tree species offers considerable potential for rapid, economical propagation (Brown, 1976 and Durzan and Campbell, 1974; as cited by Karnosky, 1981). Indeed, the improvement of tree species, as with all horticultural and agronomic plant species, depends upon selecting superior varieties and increasing genetic variability to broaden the genetic base from which to select new types with characteristics that make them in-demand. It is now difficult to make major genetic improvements in tree species because of the lengthy maturation time. Consequently, many breeders work with economic plant species with shorter maturation times. Using clonal rather than seed propagules to establish a stand of trees, none-the-less, offers considerable savings in time and money (Karnosky, 1981). With this technique, however, a single variety can constitute a large proportion of a number of

plantings. When a large fraction of any crop is one variety, the entire crop can be lost to a previously unknown or unimportant disease (Strobel, 1975). It is possible that electrophysiological methods, used in conjunction with both traditional plant breeding and tissue culture techniques, could reduce this threat by providing a rapid way for several potentially-resistant varieties to be developed simultaneously.

Although it is possible to produce haploids and regenerate whole plants from cultured cells, or even from protoplasts for some species, there are difficulties with the new breeding methods. One of the difficulties limiting the new techniques is selecting hybrid cells from the parent cell population (Griesbach, Koivuniemi, and Carlson. 1981). New types of genetic manipulations such as chromosome-mediated transformation and in situ selection help to overcome this and other limiting factors in plant breeding (Griesbach, Koivuniemi, and Carlson. 1981). Since most agronomically important traits are expressed in only one of several tissues in a plant (Griesbach, Koivuniemi, and Carlson. 1981) a technique which would allow the researcher to identify characteristics of the whole plant or key desired traits when they are not phenotypically visible would broaden the selection. In situ selection has been used to produce herbicide tolerant tobacco plants, for example (Radin and Carlson, 1978; as cited by Griesbach, Koivuniemi, and Carlson. 1981). In situ rescue or selection combines classical genetic methods with tissue culture to recover whole plants with specific, desired traits. Cell electrophysiology could also be used in conjunction with these and other methods to enhance current breeding programs. My studies show that for plant diseases and stress situations which affect plant membranes, the electrophysiology set-up offers

promise as a rapid scan of parent material in the selection processes of plant breeding. Particularly in trees, such a technique could speed-up breeding programs.

Our understanding of the basic electrical natures of plant cells is quite fragmentary. While emphasis in this dissertation is on the elm eletrophysiology, it has frequently been necessary to call attention to conclusions drawn from work with micro-organisms, other animals and plants. Numerous problems of plant electrophysiology could be illuminated by the closer application of the information and the techniques which have been developed with elms and with other organisms. For example, halophyte characteristics cannot be ignored in attempts to breed crop plants for salt tolerance (Jefferies, 1981). These characteristics include 1) a high internal ionic concentration, 2) a strong asymmetry in ion distribution within cells, 3) a reservoir of soluble organic compounds located mainly in the protoplast, and, 4) an ability to divert energy from other cell activities to maintain solute asymmetrics and to encourage growth and reproduction despite low osmotic potentials (Jefferies, 1981). Since many of these characteristics are related to the electrical nature of the cell, it is highly probable that electrophysiological studies will contribute to the determination of such questions as whether or not halophytic genera are suitable for agricultural purposes in saline environments, and, whether a non-halophytic crop-plant has enough halophytic characteristics to be useful in breeding for salt tolerance. Accelerated optimal growth of trees under protective culture requires regulation of all environmental factors including light, temperature, minerals, water, carbon dioxide,

growing media, competition, mycorrhizae, and pests (Hanover, Young, Lemmien and Van Slooten, 1976).

The common occurrence of chemical compounds is often indicative of a close phylogenetic relationship (Crawford and Giannasi, 1982). Perhaps these compounds can be detected electrochemically with microelectrodes. Until researchers look for electrical differences between species or cultivars or races, the use of electrophysiology in plant breeding will be limited. However, as information is accumulated for various higher plants, the technique will take its place among traditional and tissue cultural plant breeding.

Electrophysiology as a plant research technique offers integration of basic and applied science. This technique allows observation on the interaction of several factors. It's immediate success perhaps cannot be measured in terms of increased agricultural yield per acre. However, both scientific progress in terms of understanding underlying processes of plant growth and development, and, agricultural yield in terms of applicable disease-resistance, or hardiness, screening techniques for breeding, and toxicity tests, are possible in the foreseeable future using the electrophysiology set-up.

Both physical and chemical stress on plants can be measured. The results can be applied locally and nationally for the improvement of crops.

These studies on the electrophysiology of elm have given me cause to believe that electrophysiology as a tool in plant research may yield important information on:

1) plant disease mechanisms;

2) plant disease resistance;

3)   plant breeding programs, especially for trees;

4)   chemical stress studies (water, minerals);

5)   physical stress studies (temperature, light);

6)   toxicity studies; and

7)   pure and applied tissue culture research.

With these basic data, we can consider more realistically the subsequent fate of the tree with respect to the animate and inanimate entities - spores, viruses, pollutants, fungicides and herbicides. More exhaustive surveys of membrane potentials in higher plants followed by computer assisted statistical analysis might reveal previously unrecognized trends and correlations between both chemical and physical stimuli, and, electrophysiological effects on plants. Digital storage oscilloscopes with computer interfaces and computers allow data to be gathered more rapidly and with considerably less effort than tracing the plots produced by analog chart recorders and either manually picking off values for "important" points or using a digitizer to trace the anolog plots. Similarly, recorders with both digital to analog and analog to digital capabilities, and, computer interfaces are available on the market today. Electrophysiology researchers should take advantage of the available instruments which will facilitate their studies particularly as the equipment becomes more affordable.

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APPENDICES

APPENDIX A

Membrane Models and Biomembranes

Cell electrophysiology

Cell electrophysiology is ultimately a study of cell membranes. Transport of molecules back and forth across the membrane, whether as a result of metabolism or as a result of cell leakiness, is an indicator of membrane integrity. Cell membrane integrity, on the other hand, is often related to health, stress, or disease. The electropotential of a cell is dependent on cell metabolism, cell reaction to environment, recognition of self and of other, and on membrane maintenance. Pumping mechanisms also contribute to cell membrane potential.

There are many membrane models today. The most widely accepted of these is the fluid-mosaic model (Singer, 1971). Like earlier models, this model accounts for lipids and proteins, but does not account for the incorporation of water and ions into the membrane. Since water and ions are responsible for the electrical characteristics of membranes, they should be included in the models. The nature of a membrane depends on its thickness, its molecular structure and its fluidity. These characteristics affect the passage of particles, such as ions. Since transport of charged particles is affected by membrane properties, the electropotential of the membrane is also affected.

Membrane asymmetry

Membrane asymmetry is a fairly well-established concept today (Wallach, 2977). There are many types of asymmetry in biomembranes including those of kinetics, transfer capacity and morphology. There can also be asymmetry of the membrane environment. Membrane asymmetry means that 1) the kinetics of the components of a membrane varies in different sections of the membrane, 2) there is directional transfer capacity through the membrane. and, 3) the structure of the membrane itself is not bisymmetrical. In addition, the fluid environment on either side of a membrane can differ. Both membrane asymmetry and asymmetry of the membrane environment contribute to the electrical nature of a plant cell. Electrical asymmetry of the membrane contributes to membrane potential.

Kinetic asymmetry affects both the speed of ion movement through a membrane and the lateral and transverse movements of the lipid and protein components of the membrane.

Transfer asymmetry, affects differences in ion concentration maintained by pumps (Appendix B). For example, a rapid and large influx of Ca^{+2} through Ca^{+2} specific ion channels in the membranes of paramecia results from depolarization (Eckert, Naitah and Machemer, 1976 as cited by Simons, 1981). Another example is found in Hodgkin and Huxley's report (1952) that in cerebellum tissue accumulation of extracellular potassium could lead to membrane depolarization, which, if large enough, could inactivate the sodium channel and possibly block conduction (Hodgkin and Huxley, 1952, as cited by Malenka, Kocsis, Ransom and Waxman, 1981).

Morphological asymmetry contributes to electrical asymmetry because different lipids and protein molecules possess different charges on their parts. Since the molecules are spatially arranged, these charges are held to a particular side of the membrane, or are tucked inside, protected by some other portion of the molecule. A series of negatively charged lipids, for example, could yield a negative portion of a membrane. Similarly, ions may interact with charges in the cell membrane or plant cell wall affecting either membrane asymmetry or the environment on opposing sides of a membrane (Dainty, 1963).

A biomembrane is a lipoprotein structure that isolates cells or parts of cells into structural and functional compartments. It may be described as a tri-laminar sandwich of lipid and protein that is impermeable to water soluble materials,and which serves as a diffusion barrier between various compartments. Eukaryotic membranes may compose 80% of the dry weight of a cell. For example, the rat liver cell is basically a synthetic cell and has predominantly synthetic membranes:

Rat liver cell

        plasma membranes 3%
        nuclear 0.7%
        outer mitochondrial membranes 1.2
        inner mitochondrial (no data)
        rough endoplasmic reticulum (RER) - 60%
        smooth endoplasmic reticulum (SER) - 35%   95%

There are six major functions of membranes. (Stoeckenius and Engelmann 1969).

General Functions of Biomembranes

    1. continuous barrier
    2. selective transport systems
    3. support and orientation of enzymes and transport carriers

Specialized Membranes

    4. energy transducers
    5. impulse conduction
    6. electrical insulator

Most all membranes perform functions 1, 3, and 4. The other functions are common only to specific types of membranes. Unit membranes, or tripartate membranes, reform closed structures when broken. Bio-membranes form compartments within cells.

Lipids are polar molecules which provide functions 1,2, 3 and 6, that is:
    1. continuous barrier
    2. selective transport
    3. support and orientation
    6. electrical insulation

While proteins are responsible for:

>antigenic recognition
>cell-cell communication
>shape and architecture (support and orientation
>receptors and transmitters (impulse conduction)
>enzymes in compartments

Definition of a biomembrane

A biomembrane is:

1. lipoprotein structure that isolates cells or parts of cells into structural and functional compartments,
2. tri-laminar, made of lipid and protein. impermeable to water soluble materials, and
3. a diffusion barrier between various compartments of a cell or tissue.

Components of a biomembrane

The components of biomembranes include proteins, lipids, carbohydrates, water, ions.

The components of membranes included in membrane models.			
Membrane Components	Most models	Some models	Few or No models
Proteins	X	X	X
Lipids	X	X	X
Carbohydrates		X	X
Water			X
Ions			X

When referring to membranes, "functional" approximately equals "enzyme associated."

Structures

When considering structures of membranes, ask:

1. How the various lipids and proteins are arranged in membranes
2. How such architecture affects and regulates specific membrane functions, and
3. What mechanisms are responsible for the individual molecules which comprise membranes synthesis.

Approaches to membrane models

1. Detailed model to closely approximate the specific membrane under study: Bracker (ultrastructure, biochemistry, etc.)


2. General System based on evolution of cell structure. (Provides an approximation for a wide variety of membranes
   a. Davison-Danielli
   b. Fluid-Mosaic

Carbohydrate, ions, and water are nearly always omitted from membrane models.

Three General Types of Membrane Models

Models of membranes are considered in three general categories. These include 1) the bi-layer or unit type models, 2) the globular subunit models, and, 3) compromise models. Each type of model will be discussed in turn.
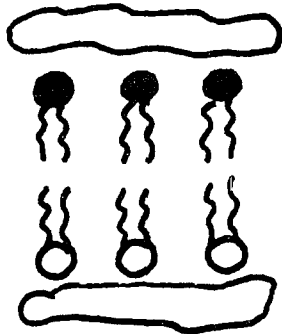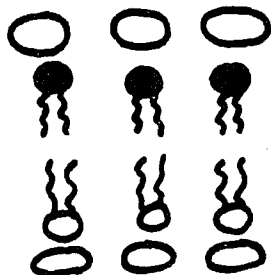
1.Bi-layer or unit membrane type models
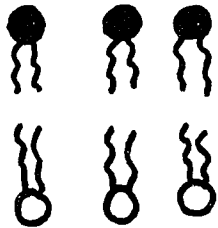
Asymmetric

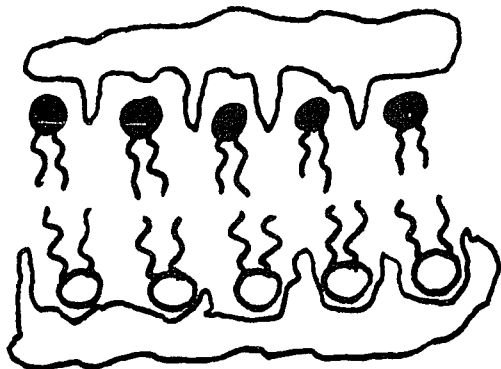1925 – Gorter and Grendel.
Red blood cell membrane

1934 – Davison and Danielli
   recognized that protein was associated with this bilayer.

1937 – realized sheet of protein didn't explain things very well so they changed it to globs of protein

1955 - Davison and Danielli proposed penetration of protein into the lipid bilayer

1955 - J.D. Robinson proposed the trilaminar or unit structure
1970 - Deamer proposed lipid bridge models: dark, light, semi-dark, light, dark bands of lipid and protein known as protein lipid protein sandwich models. Lateral movement of lipids is restricted.
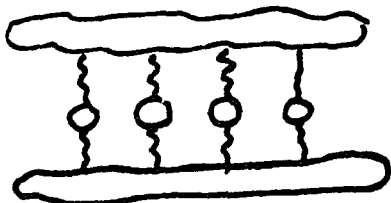
final evolution of unit or lipid bilayer model.

lipid composition
electrical
permeability
surf tension
physical structure

metabolic activity
enzymic sequences
cellular control of membrane
  synthesis and function
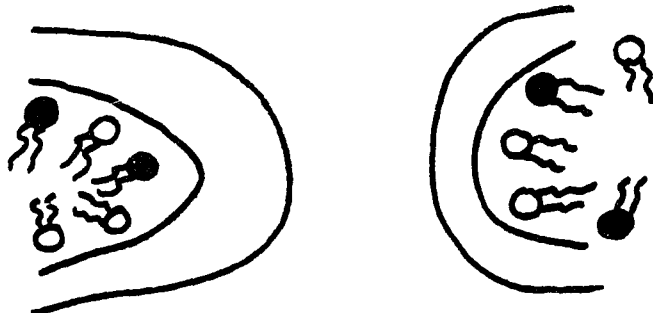
2. <u>Globular subunit</u> (actually are specific models originally thought
    to be general)
    (in Plants) - Sjostrand 1963

Globules of a lipid core surrounded by proteins associated into layers
of mitochondrial membrane.
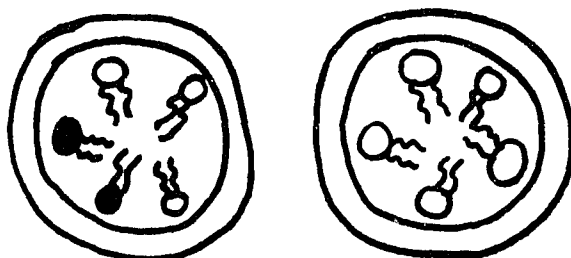
Green - 1966
Sjostrand - 1967 - globules - lipo protein micelles
Green - 1969 - inner mitochondria membrane or chloroplast membrane
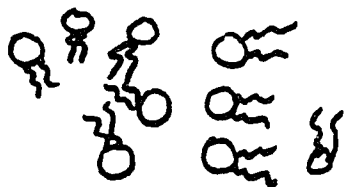   1970 - protein crystal model -
                Explains 1) high protein content
                         2) explains unit appearance after removal of
                            lipids

3. <u>Compromise Models</u>

Lipid bilayer with areas of irregularity
1964 - Lucey

Discontinuity in the lipid layer
1966-69 Wallach et al.

𝛼 Helical proteins dispersed in the lipid bilayer

1971-72 Singer   library research

fluid mosaic model
protein icebergs in a sea of lipids.
randomly arranged
globular or leaflets exposed
𝛼 helical submerged
peripheral or extrinsic
integral or intrinsic
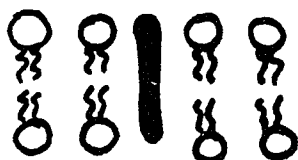
The fluid mosaic model is consistent today in terms of thermodynamics
however it is specific for red blood cells (RBC).
Nature of the proteins

Freeze Fracture Electron
Microscopy

Can explain lateral movement

For many types of membranes the fluid mosaic model is not consistent with chemical composition.

Consider these points:

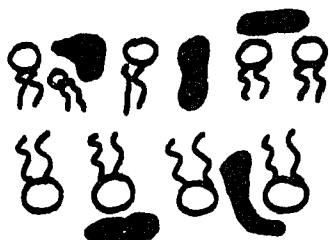1.   Ca associates with charges on polar lipid head groups.
EDTA - which ties up calcium.
Calcium ion electrostatic bulges.

2.   Carbohydrate (CHO) is thought associated with protein on the environmental side of the membrane.

3.   Hydrocarbon chains of fatty acids and lipid anuli?   What is the role of water in the membrane?

4.   Membranes look alike under electron microscope -- possibly oversimplification caused by artifact.

Degree of membrane structure

There are various levels of organization in membranes.   These levels are defined as degrees of membrane structure.

$1°$ = molecular composition
$2°$ = confirmation of the various molecules
         hydrophilic
         hydrophobic
$3°$ = organization of molecular components and forces
$4°$ = interaction between various membranes
     cell-cell interaction
     fusion of cell membranes
$3°$ organization will be discussed

Singer understood that:
1.   largest fraction of membrane proteins is water insoluble
2.   amino acid composition was not particularly different from amino acid composition of water soluble proteins
3.   most membrane proteins are α-helical

Mosaic structure was consistent with hydrophobic and hydrophilic forces.
It accounted for:
1. Ionic attraction and repulsion
2. Vanderwaals forces
3. Cohesion
4. Polarity

molecular packing effect
(lipid annulus)
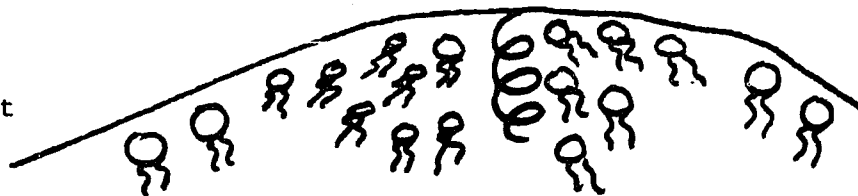
deformation and non-homogeneity
of lipid bilayer

peripheral or extrinsic – salt or EDTA
integral – strong detergents or intrinsic

97

Types of intrinsic proteins

1. Outside cell - functional properties - only enough of the protein in the lipid layers to anchor it.

2. The major part of functional activity is inside the cell.

The lipid composition in two bilayers is asymmetric. For example, in some cases intermediate hydrocarbon zones <u>only</u> contain sphingomyelin and cholesterol.

Short range vs. long range order.

1. Short range
       Localized units of more or less repeating structure.
2. Long range
       Whole membrane ordered or rather it is patchy with short range orders distributed randomly.

    Short range order is common. Long range is rare except in some like inner mitochondrial of proteins and lipids.

fluid
dynamic (not static)
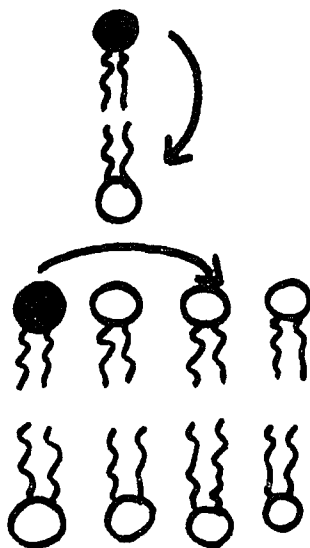
Transverse or flip-flop exchange doesn't occur very often.
Lateral movement is more frequent.

For both lipids and proteins, it takes seconds or minutes for lateral movement, however, hours or days for flip flop exchange.

Membrane Proteins

Membrane proteins are a special class of proteins distinct from the general secretory proteins of a cell. An α-helical region is perhaps responsible for the difference.

Additional Biomembranes References

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Membrane Components

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Carbohydrates

Weissman and Claiborne, Chapter 6, p. 55

Hughes, Essays in Biochem <u>11</u>, p. 1

Hughes, <u>Membrane Glycoproteins</u>

Jarnefelt et al., TIBS (May 1978), 110

Yamakawa and Nagai, TIBS (June 1978), 128

The plasma membrane has been described as, "a lipoprotein layer [that] acts as an electrical insulator to ion frequency currents," (Tattar and Blanchard, 1976).

APPENDIX B

PUMPS IN THE LIVING CELL

Differences in concentration are maintained by pumps, driven by the metabolism of the cell, that push ions through the membrane one direction or the other (Solomon, 1962). Neutral pumps transport zero net charge across the membrane and therefore have no direct effect on membrane potential (Spanswick, 1981). Electrogenic pumps contribute to membrane potential (Higinbotham, 1974).

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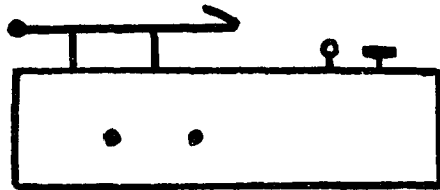
APPENDIX C

ELECTROPHYSIOLOGICAL TECHNIQUES

GETTING READY TO PULL ELECTRODES

Wear Goggles

1. Turn on the electrode puller.
2. Hold switch (magnetic switch) to the right.
this switch does not look like a switch.
3. While holding switch, push start.
holding switch for 2 minutes: The coil will
heat up. Release switch.
4. Then you can pull electrodes.

Electrode puller

PULLING ELECTRODES

Wear Goggles

(Read section on getting ready to pull electrodes).

1. Open clamp, insert prepared micropipette, close clamp.
2. Move switch to right. Open lever so that the switch "grabs" the end of the pipe, release lever.
3. Push start button.

> DON'T LET ANYONE STAND IN LINE WITH THE MACHINE OR PUT A FACE OR ARM THERE. SOMETIMES THE GLASS SHATTERS.

4. Electrodes may need "trimming" at the shank end. Use a three-corner file. Refer to Figure 2.
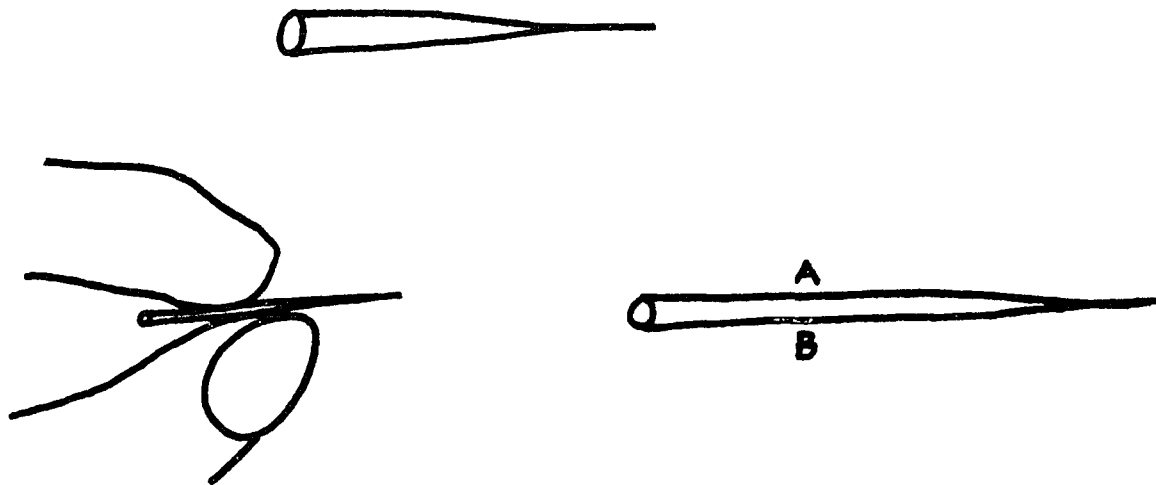
(Run file across points a and B....A, then, roll the pipette, then repeat.)

5. Place the electrodes in 3 M KCl bath. Let sit Keep the 3 M KCl bath covered except when placing or removing electrodes.

KCl should be filtered through filter paper in a funnel.

BACKFILLING THE ELECTRODES

1. This stage called "Backfilling" since the tips should be full from sitting in the bath.
2. Fill the syringe with 3 M KCl.
3. Insert the syringe in the back or shank end of the electrode.
4. Fill, forcing out bubbles from the electrode as you go.
5. Place electrode in 3 M KCl bath or use immediately. (Preferably use just after filling.
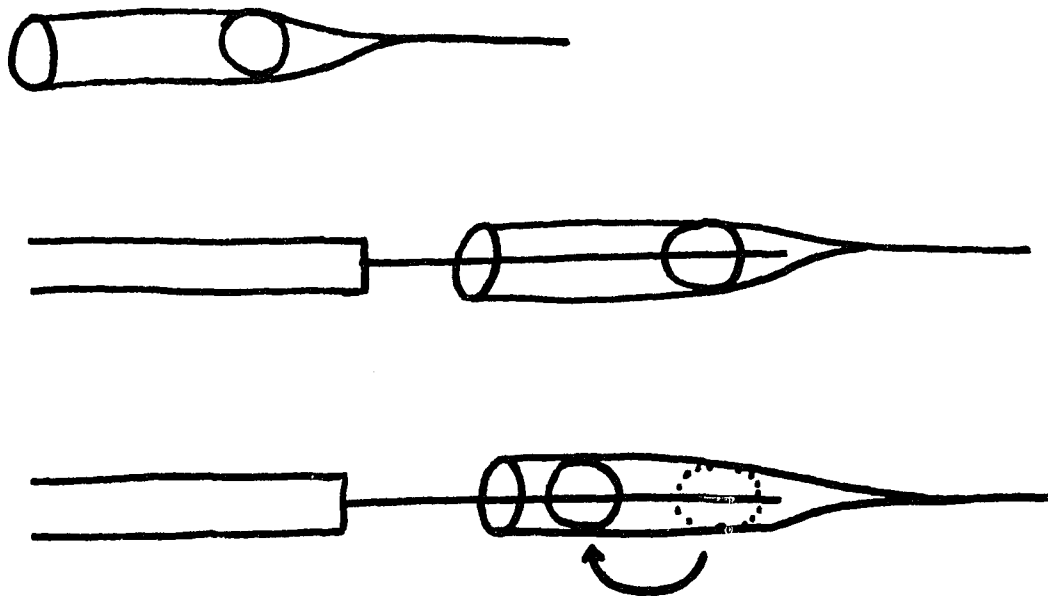
Backfilling the electrode, removing air bubbles.

MOUNTING AN ELECTRODE IN THE ELECTRODE HOLDER

The electrode holder

1. Fill the electrode holder with 3 M KCl using a syringe. Leave a drop on the top of the rubber part (Figure 5).
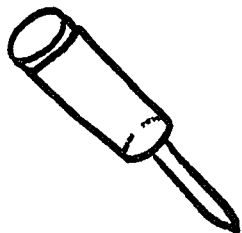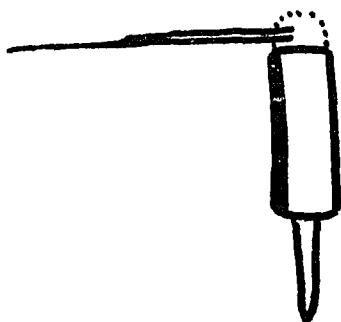
2. Bring the electrode in sideways. Put the back into the drop, then, tip it vertically into the hole. Gently slide the electrode into the electrode holder. Check for air bubbles.

3. Carefully place the electrode holder in the brass fitting. Measurements may then be taken with the electrode. Test the electrode using the electrode "test" button on the amplifier.

The electrode holder shown in relation to the drop of 3 M KCl and the electrode.

REDUCING TIP POTENTIAL

The microelectrode tip potential can be reduced if the pH of the 3 M KCl used to fill the electrodes is adjusted to 2 (Lichtner, Lucas and Spanswick, 1981).

OVERCOMING HIGH INPUT RESISTANCE

Because the tip of a micropipette is small, it has a high electrical resistance. To overcome this high resistance, the instrument measuring the cell membrane potential must be capable of measuring a small voltage

in the presence of a large resistance (Nobel, 1974). In effect, the measurement of cell membrane potential is like interconnecting a volt meter resistance in parallel with the membrane resistance. The total resistance (R_t) measured is therefore determined by the formula for the resistance of a parallel circuit:

$$\frac{1}{R_t} = \frac{1}{R_v} + \frac{1}{R_m}$$

where R_v is the resistance of the voltmeter and R_m is the resistance of the membrane. By making R_v large, the R_t measured approaches R_m in value. This figure is used in measuring membrane potential.

TISSUE HOLDER

A sponge with a slit in it served well as a holder for leaf tissue. The tissue studied protruded above the sponge several millimeters so that light and fluids could readily get to the tissue involved. The sponge did not crush and injure the tissue. A further advantage to this technique is that the same holder can support slices of tubers or tissue culture callus for electrophysiological study.

ELECTRODES TO MEASURE INTRACELLULAR pH

A good reference on this topic is: Marx, Jean L. 1981. Investigators focus on intracellular pH. Science 213:4509 pp. 745-747.

CALCIUM ION-SELECTIVE MICROELECTRODE

Refer to: Shafie, Lamir M. 1980. Sodium-calcium exchange in rabbit heart muscle cells: direct measurement of sarcoplasmic Ca^2 activity. Science. 209:4457. pp 699-702.

ANTISTATIC MAT

An antistatic mat helps reduce build up of static electricity. It is a useful addition to a computer assisted laboratory, especially when digital instrumentation is used for cell measurements. (3M carries one.)

FLOW RATE

A flow valve from an intravenous unit works very well to control the rate of influx of Ringer's solution into the perfusion chamber.

REPLACING THE REFERENCE ELECTRODE

Bananna plug
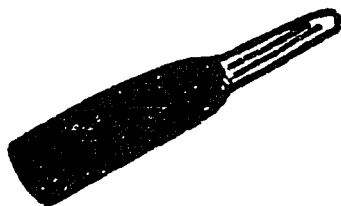
Be sure to put wire through Red plastic part of bananna plug <u>first</u>.

Don't cut wire shot - use about 1/4" of wire.

DECREASING ELECTRICAL NOISE

1. Check all ground connections to ensure that they are properly attached. Can you ground anything else?

2. Use a Faraday Cage.

3. Change the reference electrode. The lead on the reference electrode often breaks. Generally the break is not visible.

4. Use fiber optics.

5. Use rheostat switches.

CHEMICALS AND EQUIPMENT

The chemicals and equipment used in the elm electrophysiology experiments were:

1,2-Dichloroethane
2,4-Dinitrophenol
Amplifiers (2)
Calcium Chloride, Dihydrate, Granul
Calcium Nitrate, Granular
Clamps
Cordycepin=CCCP
Dissecting Microscope
Electrode Holders
Glassware
Goggles
Horizontal Compound Microscope
Magnesium Sulphate, 7 Hydrate, CRYS
Micromanipulator
Micropipettes
Oscilloscope
Pasteur Pipettes
Perfusion Chamber
Petri Plates
Pi-Pumps
Plexiglass
Potassium Chloride
Potassium Chloride, Crystal
Recorder
Recorder Pens
Recorderpaper
Reference Cells
Refrigerator
Silicon Glue
Sodium Cyanide
Sodium Nitrate
Sodium Phosphate Dibasic, Anhydros
Sodium Phosphate Monobasic, Monohyd
Sodium Thiosulphate
Strainer
Syringes
Tubing (ID 3/32; OD 5/32; W 1/32

ELECTROPHYSIOLOGICAL SUPPLY SOURCES

W.P. Instruments
60 Fitch Street
P.O. Box 3110
New Haven, Connecticut 06515

Frederick Haer
P.O. Box 337
Industry Road
Brunswick, Maine 04011, USA
(207) 729-1601

ADDITIONAL SUPPLIES

These additional supplies may be ordered from:
      Industrial Science Associates, Inc.
      63-15 Forest Ave.
      Ridgewood, NY 11227
      (212) 821-0209

Rubber mat for under electrode puller.
Draft shield for micropipette puller.

ADDITIONAL TECHNIQUES

Additional techniques which may supplement electrophysiological studies include:

1.   electron microprobe analysis,

2.   aequorin microluminescence,

3.   chlorotetracyclic microfluorescence, and

4. ^{45}Ca autoradiography (Simons, 1981).

APPENDIX D

Elm

The elm is quite susceptible to highly destructive diseases (Pirone, 1978). A recent review of elm diseases (Stipes and Campana, 1981) is available from the American Phytopathological Society.

Dutch Elm Disease

The most visible symptoms of Dutch Elm disease are the wilting and yellowing of the leaves. As with other vascular wilts, however, it is rather the blockage of water translocation which occurs in the branches and stem that results in the leaf symptoms than the halting of the leaf functions of photosynthesis and transpiration.

Dutch elm disease, which results from infection of the elm tree by Ceratocystis ulmi, led to widespread study of elm. There are several diseases of elm, however: The combined effects of Dutch elm disease, elm phloem necrosis and other elm diseases have discoraged elm plantings. The elm, however, is easily transplanted, and becomes quickly established (Pirone, 1978).

Fertilizing elm trees causes increased vessel size making the tree more susceptible to Dutch elm disease (Pirone, 1978).

As Dutch elm disease makes its mark, scientists race to understand the host-pathogen complex well enough to combat the disease. Because the Dutch elm pathogen is so successful in attacking its host, elm becomes a prime candidate to serve as a model for electropathological research.

A very high molecular weight phytotoxic glycoprotein present in the filtrates of ceratocystis ulmi cultures was isolated by gel filtration (Salemink, Rebel, Kerling and Tchernoff, 1965; Rebel and Salemink, 1968 as cited by Ballio, 1972). Salemink (1965, as cited by Dimond, 1972) demonstrated that the glycopeptide toxin produced by Ceratocystis ulmi appears to enter cells and damages membranes permanently. The history of research on this toxin involves the work of several people including Zentmyer, Dimond, Feldman, Kerling, Beckman and Salemink and is reviewed by Lousberg and Salemink (1972).

Variability in cultural morphology and its relationship to pathogenicity is being studied using a variety of Ceratocystis ulmi isolates. Inoculation trials with American and Siberian elms indicate that cultures reisolated from ioculated trees rarely differ in cultural characteristics from the original isolate. Polyphenol oxidase, catalase and peroxidase were suspected of being associated with darkly pigmented isolates. Peroxidase and catalase activity was detected in spectrophotometric assays of mycelial extracts, but their activity could not be associated with the onset of pigmentation or sporulation. Both pigmented and nonpigmented isolates showed similar enzyme activity (Hindale, 1981).

Ceratocystis ulmi

Nine isolates of this fungus were obtained from Dale Hindale and
have been cultured on potato dextrose agar (PDA). Synnema producing
medium (SPM) and PDA have been used to compare morphological
characteristics of the isolates received with those characteristics
previously described for those starins.

The fungi are being grown in liquid culture so that fungal extracts
can be used for electrophysiological studies on elm. Experiments are
underway to inoculate 2 year old greenhouse elms with the different
strains of Ceratocystis ulmi and to observe changes in membrane
potential with time in infected and control elms.

GLOSSARY

GLOSSARY

Action Potential

Originally this term was applied to electrical events in nerves which cause a biological action, such as muscle contraction. There is little agreement, though, in the literature about the definition of an action potential in plants but Simons (1981) suggests the following criteria of its properties:

(i) transient and rapid change in transmembrane potential difference;

(ii) a typical voltage curve usually features a sharp spike, followed by a more gradual return to the original 'resting' potential;

(iii) can only be triggered after a critical level of excitation has been reached – a phenomenon known as the all-or-nothing response;

(iv) if sub-threshold stimuli are delivered over limited periods, sufficient excitation can be accumulated to trigger an action potential;

(v) during and immediately after the passage of an action potential, it is not possible to elicit another action potential on the same membrane site without a rest (refractory) period;

(vi) can be triggered by electrical stimuli (Simons, 1981).

Amplifier

An electrical circuit for increasing the strength or amplitude of an electric signal (Ackermann, 1972).

Background

In electrical measurements, unwanted or extraneous signals not related to the desired signal. Similar to noise. (Ackermann, 1972)

Bioinstrumentation

The development and use of instruments for recording and transmitting physiological data (Webster's New Collegiate Dictionary).

Chemiosmotic hypothesis

Electron transport (oxidation) and ATP synthesis (phosphorylation(are coupled by a proton gradient rather than by a covalent high-energy intermediate or an activated protein (Mitchell, 1961). Both the respiratory chain and ATP ase are vectorially arranged in the inner mitochondrial membrane (Mitchell, 1961).

Cytochrome

A cytochrome is a protein that contains a heme prosthetic group and that transports electrons. The ferrous (+2) state of cytochrome is reduced while the ferric (+3) state is oxidized (Stryer, 1981).

Depolarization

A decrease in electrical potential. As plant cell interiors are negative with respect to the outside medium, depolarization is generally a change to a more positive potential (Simons, 1981).

Direct current

An electrical current in which the electrons flow uniformly in one direction (Ackermann, 1972).

Electrode Polarization

The deposition of gas on one or both electrodes of an electrolytic cell increasing the resistance and setting up a counter electromotive force (Websters, 1973).

Electrogenic pump

An ion pump that contributes to membrane potential (Higinbotham 1974).

Electron carriers

Electron carriers include flavins, iron-sulfur complexes, quinones, and hemes (Stryer, 1981).

Electron-transfer potential

NADH and $FADH_2$ in oxidative phosphorylation have free energy, called electron-transfer potential, which are converted to the phosphate-transfer potential of ATP. E_0^1, the redox potential is the measure of electron-transfer potential (Stryer, 1981).

Electroosmosis

The flow of an electrolyte solution through a pore when a potential is applied (Higinbotham, 1973).

Facilitation

An increase in membrane potential over limited periods of time with successive stimulation (Simons, 1981).

Feedback

A circuit in which a portion of an amplifier is returned to the input. When the current or voltage fed back is opposite in phase or polarity to the input, negative feedback results, which decreases the overall gain of the amplifier but makes for increased stability (Ackermann, 1972).

Gain

The amount input current or voltage is increased by an amplifier in output (Ackermann, 1972).

Ground

Contact made with the earth or other point considered to be at zero electrical potential (Ackermann, 1972).

Half-cell

The redox potential of a $H^+:H_2$ couple is defined as zero volts.

Hyperpolarization

An increase in electrical potential. As plant cell interiors are negative with respect to the outside medium, hyperpolarization is generally a change to a more negative potential (Simons, 1981).

Impedance

The generalized resistance to the flow of an alternating current (Ackermann, 1972).

Junction Potentials

Potential differences at boundaries, for example, between the electrode and the plant (Simons, 1981).

Noise

Any unwanted or extraneous signals which tend to interfere with a desired signal (Ackermann, 1972).

pH scale

The pH scale is a measure of acidity that is logarithmic. For example, solutions of pH 6, 5, and 4 respectively contain one, ten, and, one hundred microequivalents of acidity (Likens, Wright, Galloway, and, Butler, 1979). To expalin further, "each unit change in pH represents a tenfold change in acidity" (Angyal, 1980).

Pigment

A substance which strongly absorbs visible light. Most absorb only certain regions of the spectrum and transmit all other wavelengths; consequently, they appear colored (Govindjee and Govindjee, 1974).

Phosphate-transfer potential

ATP has phosphate-transfer potential, which is measured as $\_\_ G^{ol}$ for the hydrolysis of the phosphate compound (Stryer, 1981).

Receptor Potentials

Transient changes in membrane potential which converts stimuli into electrical analogues. Receptor potentials always precede, and often trigger, action potentials, but they do not have the all-or-nothing, nor the propagating, properties of action potentials. The voltage curve of receptor potentials is highly variable and reflects specific properties of the stimulus (Simons, 1981).

Redox potential

Rodox potential is a measure of affinity a substance has for electrons. A negative redox potential means a substance has a lower affinity for electrons than H_2 does while a positive redox potential means it has a greater affinity for electrons than does H_2. A strong reducing agent, for example NADH, has a negative redox potential where as a strong oxidizing agent, for example O_2, has a positive redox potential (Stryer, 1981).

Reference Electrode

The reference electrode is silver, coated with silver chloride, and immersed in a concentrated (3M) potassium chloride agar which communicates to the measuring electrode via the Ringer's solution (or other bathing solution) and the cell cytoplasm (Ackermann, 1972).

Resistance

Opposition offered to the passage of an electric current, symbolized by the letter, R, and, measured in ohms (Ackermann, 1972).

Specific ion electrode

An electrode which is used to measure the concentration of a specific ion in a solution. A common example of a specific ion electrode is the glass electrode of the pH meter (Ackermann, 1972).

Streaming Potentials

Streaming potentials are the converse of electroosmoses. The potential developed when an electrolyte solution is forced through a pore (Higinbotham, 1973).

Transducer

Any device for converting one form of energy to another (Ackermann, 1972).

Variation Potentials

First described by Houwink (1935) in the wound-induced changes in membrane potential in Mimosa, these are apparently confined to plants. Variation potentials develop relatively slowly over a period of minutes, rather than seconds (as in action potentials). They do not conform to the all-or-nothing property of action potentials, but are conducted away from the site of stimulation. The shape of the voltage curve is extremely variable (hence the name), often consisting of many small spikes superimposed on a slowly developing hyper- or depolarizing potential (Simons, 1981).

Volt

The unit of electrical potential, symbolized by V and equivalent to the potential difference required to make a current of one ampere flow through a resistance of one ohm (Ackermann, 1972).