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A PHYSIOLOGICAL BASIS FOR ELECTROPHYTOGRAMS

by

Alan H. Goldstein

A Dissertation Submitted to the Faculty of the

DEPARTMENT OF PLANT SCIENCES

In Partial Fulfillment of the Requirements
For the Degree of

DOCTOR OF PHILOSOPHY
WITH A MAJOR IN AGRONOMY AND PLANT GENETICS

In the Graduate College

THE UNIVERSITY OF ARIZONA

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THE UNIVERSITY OF ARIZONA
GRADUATE COLLEGE

As members of the Final Examination Committee, we certify that we have read
the dissertation prepared by Alan H. Goldstein
entitled A PHYSIOLOGICAL BASIS FOR ELECTROPHYTOGRAMS

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Final approval and acceptance of this dissertation is contingent upon the
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Alan H. Goldstein

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ABSTRACT

A passive system that may be useful for measuring electrochemical changes occurring in in vivo, extracellular plant tissue has been developed within the past few years. The technique involves the placement of a small (250 μm diameter) noble metal probe into the anatomical region of interest. The electrode potential of this probe is then established relative to a reference electrode. The time variation of this potential (up to 100 mV or more per day) is termed an electrophytogram. The changes are coherent and reproducible. In this dissertation theoretical physiological bases for the voltage fluctuations have been developed. One theory involves modeling the electrophytogram as a chemical thermodynamic system passing through a series of "frozen" equilibria. The electron is considered as the chemical species of interest in this system and changes in the redox potential are interpreted in terms of an electron electrochemical potential ($\tilde{\mu}_e$). The relationship between changes in the electrochemical nature of the solution contiguous with the probe and the $\tilde{\mu}_e$ intensity variable is then represented for several limiting cases. In the case of a redox reaction accompanied by a proton exchange, the limiting equation shows that pH changes well within the physiological range for extracellular plant fluid can account for the observed voltage fluctuations. An alternative representation of the electrophytogram as an electrostatic field phenomenon is more difficult to analyze due to a lack of

information about the three dimensional structure of the probe/tissue interface. However, a cylindrical capacitor model shows that fluxes in the range of a few hundred monovalent ions per μm^3 of extracellular volume could easily explain the measured voltage changes. The third model involves the polyelectrolyte gel nature of the plant cell wall. The electrophytogram voltage signal is considered to result from surface interactions between the metal probe and the cell wall. This interaction is analyzed by using the theory of interacting electrochemical double layers. Output from a computer simulation shows that if these two surfaces approach within two Debye lengths, a voltage signal will be generated at the electrophytogram probe. Furthermore, slight fluctuations in the surface to surface distance result in voltage fluctuations of a magnitude equal to those observed in vivo. Physiological processes known to occur in plants are discussed with respect to the generation of electrochemical potential gradients and other physical conditions necessary to "drive" the various models. I conclude that the electrophytogram is most likely the result of surface interactions between the probe and the polyelectrolyte gel components of the cell wall.

Elucidation of the physiological basis for electrophytograms must also involve an accurate anatomical interpretation of the position of the probe relative to the plant tissue. Therefore the results of a freeze-fracture electron microscopic (FFSEM) examination of the probe/tissue interface after wound healing are included. Electron micrographs show cell wall material appressed directly against the probe, indicating that the electrophytogram provides a method for monitoring the

electrochemical status of the cell wall space. Since cell wall material is hygroscopic, it is reasonable to assume that the smallest probe/tissue separation distance observed in the FFSEM's represents a maximum in vivo value. Since this distance is less than 10nm, these data support the hypothesis that the metal surface is within two Debye lengths of the cell wall surface in vivo. Insertion of the probe into mature, fully elongated tissue appears to cause minimal damage to nonxylem tissue beyond the adjacent cell layers and virtually no damage to the xylem region.

INTRODUCTION

Overview

Variations in bioelectric potential and other related electrochemical phenomena have been shown to play a significant role in several aspects of plant growth and metabolism (Galston and Slayman 1979). Measurements carried out by plant electrophysiologists usually involve either intracellular glass microcapillary electrodes or pairs of saline-bathed wick or similar type electrodes attached to the plant surface. Unfortunately, physical limitations preclude the use of either of these systems for long-term monitoring of a plant under normal in situ environmental conditions. As an alternative to these techniques, a passive monitoring system has been developed that involves the use of a penetrating Palladium probe. The Palladium wire (250 μ m in diameter) is inserted 2 to 4 millimeters into the anatomical region of interest. After wound healing the probe remains firmly in place. The electrode potential of this probe is then established relative to a reference electrode and the time variation of the potential is recorded. This total phenomenon has been termed an electrophytogram (EPG), and observed changes in the electrical signal, as a function of time, are coherent and reproducible (Gensler 1974, 1978a, 1978b). These time dependent changes presumably reflect electrochemical changes in plant tissue adjacent to the probe. The instrumentation involved is detailed elsewhere (Gensler 1974).

This dissertation will focus on developing theoretical physiological bases for the voltage fluctuations measured in electrophytogram. To reach this objective, three biophysical models are developed. In the first model the EPG is analyzed as a manifestation of a chemical thermodynamic system passing through a series of 'frozen' equilibria (see following section). Taking a somewhat novel approach (but see Clark 1960, or Dutton and Wilson 1974), the electron is considered to be the chemical species of interest and the changes in redox potential are interpreted in terms of an electron electrochemical potential, $\tilde{\mu}_e$. Finally, the variation in $\tilde{\mu}_e$ is related to the types and concentrations of chemical components in the volume surrounding the probe. This approach is taken in order to determine the effect of changes in the electrochemical nature of the solution contiguous with the probe on the $\tilde{\mu}_e$ intensity variable. The relative concentrations of various compounds are related to physiological processes occurring within the plant.

A second model is developed which treats the EPG as a pure electrostatic phenomenon. This model visualizes the volume around the probe (the cell wall space) as being capable of accumulating a net coulombic charge and that the amount of charge present changes as a function of time.

The third model takes into account the likely spatial configuration of the probe/tissue interface in vivo. If these two materials are close enough to one another, surface mediated electrochemical interactions may provide a basis for the measured EPG voltage fluctuations. In particular, since the cell wall contains a high percentage of polyelectrolyte gel material, the cell wall surface will have a modified

Debye atmosphere. This will occur because each charged group on the cell wall surface will have a Debye atmosphere. If the concentration of such groups is high, the entire surface may be considered to have an associated Debye atmosphere for the purposes of an electrochemical analysis. As a result, the cell wall surface can be visualized as an electrical double layer region and this double layer may then interact with the metal surface to produce the observed EPG voltage fluctuations. This phenomenon is analyzed quantitatively using the classical treatment of interacting electrochemical double layers developed in the colloid stability theory.

In addition to the theoretical analysis, the results of an electron microscopic study are reported. These results represent an attempt to elucidate the structure of the probe/tissue interface and to gain insight into the anatomical implications of probe insertion. It is necessary to understand exactly what is occurring at the point of contact between the metal and the plant. Gensler (1978b) has pointed out the paucity of literature on the subject of plant wound healing around a penetrating, chemically inactive object. It might be expected that, if an object is truly inert, normal repair processes would occur. The biochemistry and anatomy of these mechanisms have been examined by several workers (Wyllie 1930a, 1930b, 1931; Bloch 1941, 1952; Lipetz 1970) and the time course of wound healing has also been characterized (Tucker 1975). The results presented here are concerned with the anatomical structure observed at the probe/tissue interface after a probe has been inserted into mature plant tissue and allowed to heal for at

least 14 days. These are the conditions under which EPG data are gathered.

The primary objective of this anatomical study was to determine if wound healing that occurred in response to the insertion of probes into cotton (Gossypium hirsutum L.) petioles and peduncles resulted in a complete closure of the wound. Complete closure at any point along the wound is defined for this experimental system as contiguity between the metal surface and the plant apoplast as seen in the electron microscope. The use of freeze-fracture scanning electron microscopy (FFSEM) allowed the observation of the probe/tissue interface to a resolution of ≥ 10 nanometers.

A secondary goal of this study was to obtain an estimate of the degree of disruption of tissue organization in the region adjacent to the probe. An understanding of the anatomy of the tissue/probe interaction zone after healing is crucial to the proper evaluation of voltage fluctuations measured with the electrophytogram technique. Interpretation of future, more sophisticated techniques such as ion-specific electrode implants must also involve an analysis of the plant's wound healing response to a penetrating object.

In order to evaluate the feasibility of the physiological models, a critical discussion of the present state of our knowledge of several aspects of biophysical plant physiology is presented in the form of a literature review. This review, when combined with the results of the FFSEM study, provides a framework within which the various models may be examined, and judged in terms of their respective abilities to

stimulate metabolic processes known to occur in plants. This type of analysis is presented in the discussion section.

Electrode Potential and the Electrophytogram

Figure 1a shows a schematic diagram of the electrophytogram system with the various sources of electrical potential difference indicated. A fundamental theoretical electrochemical limitation is the inability to measure the absolute value of any of these potential differences. The only variable that can be measured is the galvanic cell potential difference, E_m , and variations of this difference, ΔE_m . The usefulness of the technique lies in the interpretation of E_m in terms of potential changes along the galvanic circuit path. Broadly speaking, there are several relatively definitive locations for potential difference. The interface between the measuring electrode and the plant tissue electrolyte yields an electrode potential. The path from this interface down through the plant stem and trunk may contain unneutralized charges which could give rise to an electrical potential. The root-soil interface is the source of another possible potential difference. The galvanic path would continue through the soil itself and reach the soil reference electrode interface. Potential differences are present at each interface. Further potential differences can arise from less well defined interfaces; however, it is very probable that the circuit path is apoplastic and any other potential differences arise from unneutralized charge concentration.

Multiple probes with part of the circuit paths in common permit one to partially localize the source of potential variation (Gensler 1974). Procedures of this type have given evidence that many, but not

Figure 1. The electrophytogram system.

- a. The electrophytogram system represented as an electrochemical cell, indicating the possible sources of electrical potential.
- b. An enlarged view of the probe/tissue interface, considered to be the source of the measured changes in electrode potential. This diagram represents the EPG as a two phase, closed thermodynamic system.

Figure 1. The electrophytogram system.

all of the electropotential variations arise at the measuring probe-tissue interface. Use of a specialized and extensively tested reference electrode design has resulted in a very high probability that the changes in electrical potential do not arise from changes in the reference electrode interface (Gensler 1974, 1980). Placement of the reference electrode in bulk soil yields the same range of values of E_m as encountered in measurements employing hydroponic nutrient solutions as a root environment. Although soil is a very rich redox potential medium (van Olphen 1977), field tests in bulk soil indicate the value of E_m is independent of the location of the reference electrode. Faradaic voltage differences in the soil and plant itself are reduced to insignificance with the use of high impedance amplifiers to measure E_m . These results indicate that a logical initial analysis of the electrical potential variations in the EPG should focus on the measuring probe-tissue interface.

The Galvani potential difference at the measuring probe-tissue interface is a potential difference from the bulk metal $E^{(m)}$ to the bulk extracellular electrolyte $E^{(s)}$, where the superscripts indicate the metal and solution phases respectively. It is equal to the difference in the work functions of the metal and the solution. Although the absolute value of this Galvani potential is inaccessible, changes in the potential can be equated to changes in the measured potential, ΔE_m , assuming all other potential changes in the circuit path are zero, that is:

$$\Delta (E^{(m)} - E^{(s)}) = \Delta E_m \quad (1)$$

Equation (1) provides the transition of a measured electrical potential

to a spatially and temporally defined electrical potential. The problem at this point is to relate the electrical variables to electrochemical variables and thereby be able to obtain information about changes in the chemical environment surrounding the probe.

Although the physical picture of the probe/tissue interface is likely to be complex in nature, there are essentially only two types of interactions that may occur between the noble metal probe and the adjacent plant electrolyte volume. In the first case, electrons are exchanged between the probe and chemical species in solution. This situation leads to the analysis of the measured electrical potential as an oxidation-reduction potential. The solution adjacent to the probe is considered to contain a mixture of reduced and oxidized species that equilibrate with the inert electrode; the electromotive force (emf) of this electrode relative to the reference electrode yields a voltage measurement commonly known as an electrode potential (Ives and Janz 1961). Changes in this electrode potential over time would then be considered to reflect changes in the types or relative concentrations of various redox couples in the solution phase.

Alternatively, if chemical compounds capable of exchanging electrons with the probe are not present, charged species may still interact with the probe at the level of their associated electric fields. Changes in the types and numbers of charged species near the probe can alter the strength of the electric field sensed by the mobile electrons in the probe and therefore affect the measured voltage. Finally, the possibility exists that both electric field effects and the concentration of redox couples may be acting to produce the observed voltage.

In the next two sections I develop models that will describe the behavior of the EPG system either as the reflection of a changing redox potential or an electric field phenomenon.

The Electrophytogram System

In order to extract physiological information from the electrophytogram measurements a relationship must be established between the observed variation in voltage and changes in the concentration of one or more chemical species within the volume immediately adjacent to the probe. This extension of the voltage information may be accomplished via a chemical thermodynamic analysis in which the voltage changes are related to changes in the chemical potential (and electrical potential if the species is charged) of one or more compounds. A derivation of the desired relationship will be presented in the next section.

Figure 1 shows the representation of the EPG as a thermodynamic system.

Figure 1b also represents the EPG as a two-phase closed thermodynamic system. This system is composed of the probe (phase M) and surrounding volume of plant tissue (phase S). The dimensions of phase S are dictated by: (a) the effective distance (r_H) from which electrons may be exchanged or electric field effects occur between the probe and the surrounding media; and (b) the part of the probe that is actually engaged in electron exchanges or interacting electrostatically with the media, i.e., the effective length (l). The actual volume of phase S must ultimately be varied as part of a sensitivity analysis of the EPG model. For the purposes of a thermodynamic analysis it is only necessary for the volume to be fixed at some constant value. Phase M is

considered to be the volume of the metal probe delimited by the plant surface.

LITERATURE REVIEW

Modern Aspects of Plant Electrophysiology

Electrophysiology is defined as the study of electrical phenomena associated with physiological processes. Physiological processes, in turn, encompass the entire realm of functions and activities of living matter. This being the case, the electrophysiologist is constantly working at the interfaces between disciplines as diverse as quantum mechanics and botany. The initial result of mixing two or more previously separate bodies of knowledge together is often confusion and frustration. However, the potential for generating new and useful insights into the nature of biological processes at these interfaces is great. Just as molecular biology revolutionized the field of genetics, modern approaches to bioelectrochemistry and the related fields of colloid and surface chemistry may radically alter (and in some cases create) mechanistic theories for such physiological events as photosynthesis and respiration. As is often the case in an emerging discipline, theoreticians advance hypotheses faster than experimentalists can test them. This review will be restricted to those regions of plant electrophysiology where theory and experiment may be usefully compared.

The smallest biological unit upon which electrophysiological measurements may be made is the intact cell. There is a great deal of literature available on the role of electrical phenomena in the function of individual molecules (e.g., protein conformation, Tanford 1961) and

molecular aggregates (e.g., macromolecular complexes, Katchalsky 1954) but this information has not been integrated into existing physiological structure/function models. For example, electrostatic attraction plays a significant role in DNA:Histone interactions, yet the nature and magnitude of the forces involved remain to be determined. A major problem is that intermolecular forces depend on the three dimensional structure of the molecules in vivo. Progress in this area must occur, to a large degree, via the methods of spectroscopy and crystallography. On the other hand, many cell types are large enough to allow the use of microscopic instruments to make direct measurements of electrical properties. The starting point in any study of cellular electrophysiology is the measurement of an electrical potential difference (P.D.) between two phases or between two points in one phase (Findlay and Hope 1976). Resistance and capacitance measurements may also be made.

The electrical properties of plant cells are controlled to a great extent by the plasmalemma and compartments within the cells (Hope 1971). Electrode systems used to make voltage measurements between cells or on an individual cell are reviewed by several authors (Findlay and Hope 1976; Nobel 1974; Carter and Silver 1961). However, it is worth noting that the often used term, microelectrode, is a misnomer since the interior of the cell is connected to the measuring system via a micro-salt-bridge (usually a Pyrex glass tube drawn out at one end to a fine point and filled with 3M KCl) while the electrode itself is macroscopic in size. An artificial situation may be created when a micropipette (tip diameter between 0.5 and 10 μm) filled with concentrated KCl is shoved through the cell wall, plasmalemma and into the

cytoplasm of a cell. Gushing of sap into the micro-salt-bridge tip caused by the cell's turgor pressure may decrease the cell volume sufficiently to alter the turgor pressure (Findlay and Hope 1976). In addition there may be leakage around and/or plugging of the micro-salt-bridge (Poole 1966; Findlay 1962). Finally, there is the fundamental question of whether the micro-salt-bridge has truly eliminated any liquid junction potential between the electrode and the measured phase. Overbeek (1956) has pointed out that a small diffusion potential between saturated KCl and a colloidal solution is by no means a certainty. In particular, in the tail of the concentration gradient of KCl, where the equivalent concentration of KCl is of the same order as that of the colloidal ions, the mobilities of K^+ and Cl^- may be considerably different in the high electric fields around the particles. Since these mobility changes would presumably be in opposite directions, a large diffusion potential could develop.

Assuming that the micro-salt-bridge method of making electrical contact with the interior of the cell was electrochemically valid, workers proceeded to make several types of measurements on plant cells and tissues. Voltage differences across the plasmalemma (membrane potentials) have been studied intensively. This is especially true in the case of large algal cells (Walker 1955), where electrical coupling with other cells is eliminated. Electrical resistances of membranes have been estimated from the steady changes in P.D.s produced by the passage of an electric current through the membranes (Findlay and Hope 1976). The capacitative component of cell membranes has become apparent from the response of the membrane to a flow of sinusoidal alternating

current. An equivalent electrical circuit for a given cell must be postulated to estimate the actual membrane capacitance. The instrumentation used to make resistive and capacitative measurements as well as more sophisticated techniques such as voltage clamping are discussed by Findlay and Hope (1976).

It is generally accepted that plant cells exhibit a transmembrane P.D. such that the cytoplasm is electrically negative with respect to (w.r.t.) the external medium. There will also be a P.D. across the tonoplast between the vacuole and the cytoplasm, although its value is usually quite small. Transmembrane P.D.s are considered to result from three mechanisms (Luttge and Higinbotham 1979): (1) passive (or facilitated) diffusion of ions; (2) Donnan systems; (3) active ion transport (also called electrogenic pumping). While the first two mechanisms may be treated by the methods of equilibrium thermodynamics (including the usual assumptions of electroneutrality and electrochemical equilibrium), mechanism three has been most successfully analyzed by nonequilibrium methods. Since the specific equations developed to calculate the membrane P.D. from ion fluxes or concentrations (or vice versa) are reviewed in all of the recent texts on transport in plants (Walker and Pitman 1976; Luttge and Higinbotham 1979; Nobel 1974), they will not be rederived here. Nernst and Donnan-type potentials are discussed with respect to their applicability to the electrophytogram measurement in other chapters of this text (see pp. 31 and 46). Part of the transmembrane P.D. may occur across the cell wall, which is really a second membrane that is often physically inseparable from the plasmalemma (Preston 1976). It now seems clear that, in cells of higher plants,

the plasmalemma is the major electrical barrier (Luttge and Higinbotham 1979). Measurements of tonoplast P.D.s show only a small voltage difference between the vacuolar sap and the cytoplasm. Positive plasmalemma P.D.s reported for isolated wall-less protoplasts (Heller 1977; Rona, Cornel and Heller 1977) and vacuoles (Racusen, Kinnersley and Galston 1977) probably reflect disruption of the membrane during isolation.

The low conductance ($0.5 - 20 \Omega^{-1} \text{ m}^{-2}$) of plant cell membranes may reflect the limited ability of the cytoplasmic membranes to conduct ionic current (Findlay and Hope 1976). Rectification properties (Hope and Walker 1961; Fujita 1962) demonstrated by plant membranes may be the result of the operation of unidirectional ion carriers. Conductance measurements in intact tissues are complicated by the intercellular connections resulting from the presence of plasmodesmata. Although the plasmodesmata are narrow (40-100 nm diameter) cytoplasmic strands, they may occupy anywhere from 0.08 to 0.002 times the area in common between adjacent cells (Tyree 1970). Electrical measurements on tissues show the existence of a high conductivity pathway for ionic current flow (Goodwin 1976) and are interpreted as evidence for a 'symplastic pathway' for large scale intercellular ion fluxes (Spanswick 1976). Plant membranes also exhibit capacitance values ranging from $0.01 - 0.03 \text{ Fm}^{-2}$ (Goldsmith, Fernandez and Goldsmith 1972).

It has long been known that the cells of many charophyte plants (e.g., Chara sp., Nitella sp.) exhibit true action potentials (Cole and Curtis 1939; Harvey 1942). Recent work in this area (reviewed by Findley and Hope 1976) has shown that different types of macroscopic stimuli, ranging from a flash of UV light to mechanical injury, may

cause the initiation of an action potential. At the molecular level, it appears that depolarization of the plasmalemma P.D. below a threshold level triggers the action potential response (Kishimoto 1968). The ionic basis of the action potential in nerve cells was established by Hodgkin and Huxley (1952) and it is likely that a similar mechanism operates in plant cells. However, the specific types of ions affected by permeability changes in the membrane have yet to be determined with certainty (compare Gaffey and Mullins 1958 with Findlay 1962). Well defined, propagated action potentials are also known to occur in certain types of higher plants exhibiting rapid movement (e.g., Mimosa) but transient plasmalemma P.D. fluctuations in a variety of 'normal' plant cells are often highly variable (Pickard 1973). Therefore any generalized electrophysiological role for this phenomenon in seed plants remains speculative.

Electrophysiological measurements at the tissue level are of several types. Cell to cell electrical coupling has been investigated using a multiple micro-salt-bridge experimental set-up and current injection. The results of these experiments strongly suggest symplastic continuity (see Spanswick 1976 or Goodwin 1976 for reviews) via the plasmodesmata as previously discussed. In addition to these intercellular pathways it has been shown that tissues are capable of driving ion currents through the external medium with the tissue as the return circuit. This phenomenon results in extracellular P.D.s around roots and other types of tissue. Electrogenic ion pumping systems could provide the driving emf. Although these types of effects have been studied most intensively in animal systems (Jaffe, Robinson and Nuccitelli 1974),

ionic currents are postulated to play a role in a developmental control system involving such plant physiological trigger devices as the membrane potential, the osmotic potential and the ionic permeability of the membrane (Scott 1957, 1962). External electric fields have also been measured in animal systems (Jaffe, Robinson and Nuccitelli 1974) however, a unified 'bioelectric field theory' that would integrate the electrical phenomena with other known biochemical mechanisms involved in growth and differentiation (e.g., gene transcription, translation, etc.) has not yet been developed.

The transition from tissue to organ generally requires the electrophysiologist to shift from microscopic to macroscopic experimental techniques. The majority of measurements at this level involve P.D.s between different regions of the plant. The experimental set-up usually involves pairs of saline-bathed wick, wire or capillary-suction electrodes (Galston and Slayman 1979). One member of the pair is placed at an 'interesting spot' on the organ and the other at a distant, 'neutral' location. While simpler in implementation than the micro-salt-bridge insertion technique, the surface electrical measurement is more prone to artifacts since it depends on several variable properties of the whole recording circuit: the quality of the electrode/tissue contact, the size of the electrodes relative to the size of individual cells, the presence of insulating 'dead' spaces (e.g., the waxy cuticle), the constancy of the potential near the 'neutral' electrode and the relative resistance between cells vs around cells. As a result of these circuit variables, extracellular recorded potentials range from a few microvolts to hundreds of volts (Galston and Slayman 1979) whereas

membrane potentials measured via the micro-salt-bridge vary only over a range of about 300 mV.

Using the whole-tissue recording technique Lund (1947) found the growing tip of the seedling leaf sheath of oat plants to be about 100 mV negative to the base of the organ. Longitudinal electrical gradients have also been observed along other plant organs such as stems and roots (Briggs and Hope 1958). These gradients may be altered by several environmental factors including temperature and light (reviewed by Lund 1947). Transverse electrical gradients of 100 mV or more across stems and other organs have been observed in response to light or gravitational fields. This polarization correlates with the redistribution of auxin within the organ and subsequent growth curvature (Shrank 1957). These same types of phenomena have been associated with light-induced phytochrome-mediated effects (Newman and Briggs 1972). With respect to interorgan (long distance) transport phenomena, an electroosmotic flow mechanism has been proposed (Fensom 1957; Spanner 1975). This hypothesis depends on the plant's ability to generate potential gradients across the pores of the phloem sieve plates. This scenario appeared to be possible based on early electron micrographs showing the pores clogged with 'P-protein' to a degree where only diffusional movement of solutes would have occurred. However, new fixation methods have allowed plant anatomists to demonstrate that the sieve pores are probably open in vivo (Esau 1960), allowing for hydraulic flow and eliminating a crucial element of the electroosmotic flow model.

As an alternative to these techniques, Gensler (1974, 1978a, 1978b, 1979, 1980) has developed the electrophytogram measuring system.

The anatomical consequences of the placement of a small (250 μm diameter) noble metal probe into the tissue and the possible biophysical and biochemical bases for the observed P.D.s are discussed at length in this dissertation.

Transport in the Apoplast Space

The physiological models developed in this dissertation must provide mechanisms whereby rather large (hundreds of millivolts) fluctuations in the electropotential of a noble metal probe embedded in the apoplast can occur. If the measured EPG voltage is a redox potential (see p. 40), then any change in voltage would presumably be the result of a change in the type and/or concentration of redox couples (or pH) in the region adjacent to the probe surface. Alternatively, the EPG voltage may be the result of an electrostatic field effect produced by electrogenic pumping in the region of the probe (see p. 57). Here again, some type of change in the chemical composition of this region is implied. Finally, there is a model based on the electrochemical double-layer at the surface of the cell wall (see p. 69). The biophysical properties of the cell wall, a polyelectrolyte gel, are a function of both the pH and the ionic concentration of the bathing fluid. It can be seen that all three models invoke, to some degree, dynamic transport phenomena in the apoplast transport space. In this section, modern theories for apoplast transport will be discussed in relation to the various EPG model systems.

While great progress has been made in analyzing and defining the individual processes from which the plant makes its metabolic living, real insight into the physiology of plant growth and development must

involve an analysis of these processes at the level of a dynamical, interactive whole system. The central role of transport phenomena in coordinating the numerous metabolic activities that are occurring simultaneously in the living plant has been recognized by plant scientists. A large amount of diverse and highly detailed information has been accumulated in recent years relating to the various types of transport phenomena that can occur in plants. This interest in transport physiology has sparked a dramatic increase in the number of publications on this subject. There are now books that deal to some degree with every aspect of the logistics of moving matter into, through and out of a plant. It is becoming clear that, in addition to the more obvious inter-cellular systems for solute movement such as the xylem, phloem and plasmodesmata, the cell wall mediates many transport processes (Lauchli 1976). In some cases, the cell wall may simply be acting as a passive conduit for fluid flow (Briggs, Hope and Robertson 1961). However, there is now a great deal of evidence to support the concept of the non-xylem apoplastic space as a complex multiphase system capable of interacting, through its electrochemical properties, with the symplasm and/or the external environment (Preston 1976; Lauchli 1976).

Preston (1976) considered the cell wall to be a rigid or semi-rigid deposition upon the outer surface of the plasmalemma. The wall is chemically distinct from the plasmalemma insofar as the structurally important components are polysaccharides, and their carboxylic acid derivatives. Non-polysaccharide components are also present in many types of modified cell walls (Albersheim 1976). In a plant tissue, the cell wall of each cell merges with the walls of adjacent cells giving

the tissue physical coherence and strength (Esau 1960). The term 'wall' is in itself somewhat misleading since the physical properties of this material appear to be more closely related to those associated with a membrane (specific properties are discussed later in this section). The term apoplast was first applied by Munch (Gunning 1976) to the non-living part of the plant. The lumen of dead xylem elements is often included in this definition. However, the mechanisms of solute movement in the cell wall space differ from those operating during long distance xylem transport.

To aid in the conceptual separation of these two regions of the apoplast, the concept of the free space (F.S.) was introduced by Briggs and Robertson in 1957. Further, an apparent free space (A.F.S.) was operationally defined in order to extract information about the free space from data obtained using ion accumulation techniques (Briggs, Hope and Robertson 1961). The free space was defined as, "that part of the plant into and through which the solute and the solvent from the external solution move readily." The idea of the free space (often called the diffusion free space) has proven to be heuristically useful in the analysis of salt uptake studies employing a cell or piece of tissue transferred between two solutions of differing ionic concentration. It was recognized that the cell wall would consist of at least two physically distinct phases present in most tissues, i.e., the cell wall material itself and the intercellular spaces. Therefore, the model was further refined to allow for a water free space (W.F.S.; anatomically, the intercellular spaces) which contains only water and diffusible ions and a Donnan free space (D.F.S.; the cell wall space proper) which

contains indiffusible macroions. These macroions were shown to modify the concentration of diffusible ions in the D.F.S. relative to the external solution and/or the W.F.S. (Briggs, Hope and Robertson 1961). Briggs et al. (1961) point out that even in root tissue, the intercellular spaces in vivo are not normally injected with a solution so that the W.F.S. is really an artificial concept introduced to account for an in vitro artifact inherent in ion exchange experiments carried out in a liquid media. It appears reasonable to ignore the W.F.S. in this preliminary treatment of non-xylem apoplast transport. It is important to point out that the W.F.S. undoubtedly serves as an 'air-free space' in vivo to facilitate gas exchange processes.

An exact physical description of the ultrastructure of the D.F.S. must await the elucidation of the three-dimensional configuration of plant cell walls. This configuration will obviously vary from plant to plant although it has been postulated (Albersheim 1976) that most higher plant primary cell walls are roughly similar in their infrastructure. It should be possible to develop a preliminary model for movement through cell walls using present knowledge of cell wall structure and existing theories for flow processes through various types of porous media. Preston (1976) has reviewed the results obtained using several analytical methods for cell wall structure determinations. He concludes that plant cell walls, while ultimately a multiphase system, may be viewed as containing only two phases in the first approximation: a crystalline polysaccharide (cellulose in most higher plants) embedded in a matrix consisting of an amorphous mixture of polysaccharide and other compounds (amorphous only in the sense of lacking crystallinity). The

crystals of cellulose, often termed micelle strands (Luttge and Higinbotham 1979) are wound into microfibrils. The tight hydrogen bonding that occurs between cellulose molecules results in the exclusion of solute molecules from the intramicellar space under all but the most extreme conditions (Luttge and Higinbotham 1979). Intermicellar spaces (micelle-spaces) of $\sim 1\text{nm}$ do exist (Lauchli 1976) and may be considered as part of the D.F.S. In addition, intermicrofibrillar spaces (micro-spaces) of $\sim 10\text{nm}$ are known to exist (Lauchli 1976) and will make a large contribution to the volume of the D.F.S. In order to complete the preliminary picture, it is necessary to fill in the micro-spaces with amorphous matrix substances capable of acting as immobile macroions (a more detailed description of the electrochemical properties of the matrix will be presented; see p. 69). This will reduce the average pore size within the micro-spaces. While no definite work has been done in this area, it is reasonable to assign a new average pore size of approximately 1 nm to the macroion filled micro-spaces. The apoplast space may then be viewed as a porous polyelectrolyte gel. The presence of the crystalline cellulose micelles adds only a physical path length tortuosity factor to the total expression for the flow.

An adequate description of the movement of uncharged (i.e., nonionic) molecules through the apoplast may be obtained using current theories of the hydrodynamics of micropores. Gunning (1976) has noted that the huge hydrodynamics literature has been largely neglected by plant transport physiologists. In these hydrodynamic models, much effort has been devoted to characterizing the permeability to water and solutes of natural and artificial membranes in terms of the presence of

equivalent pores. These pores are conceptualized as cylindrical channels piercing the membranes. They are given dimensions that could account for the observed permeabilities and ultrafiltration properties of the membrane. Much of the work in this area has been the result of the need to develop quantitative expressions to describe chromatographic separation phenomena.

Even more closely related to the physical circumstances involved in nonelectrolyte apoplast transport is the process of glomerular filtration, the biophysical basis of the excretory function of the vertebrate kidney (Pappenheimer 1953; Renkin and Gilmore 1973). This process has been subjected to an extensive theoretical analysis by renal physiologists. Also, these workers have accumulated a large amount of experimental data which, in turn, have been used to verify and further refine the derived flux equations. At this time, animal transport physiologists are ahead of their counterparts in the plant sciences and their treatment will be used.

The transport equation of Verniory et al. (1973) presented in this review is the latest in a series of refinements to the so-called "pore theory" that was developed to describe the transport of uncharged solutes across porous membranes. According to this theory, convective flow and net diffusion contribute to solute flow across the membrane, with both processes being impeded by steric hindrance at the entrance of the 'pores' and by frictional forces within the pores. Reproducing the derivation of Verniory et al. (1973) would serve no useful purpose, but it is important to note the significant innovations and limitations of this pore theory equation. Of particular relevance to cell wall flow

is the introduction of a path length tortuosity factor into the expression for solute flow due to diffusion. This was calculated as $(D(C_1 - C_2) A_w / \Delta x) \cdot (A_s / A_w)$ where D is the free diffusion coefficient, C_1 and C_2 , the concentrations of the solute on the two sides of the porous membrane and $A_w / \Delta x$ the pore area freely available to water per unit of length. The term A_s / A_w (A_w is the pore area freely available to solute) describes the restriction to motion and is calculated as $1/K_1 \times S_d$ where $S_d = (1 - A_s / r)^2$. All symbols are defined in Appendix D. Several other modifications were added including; a steric restriction factor in the convective flow expression, 'wall correction factors' (i.e., the frictional resistance to free diffusion or convection relative to that in the pore) and the use of a mean intramembrane solute concentration term in the convective flow expression. With respect to the fixed charges within the plant cell wall space, it is reasonable to suppose that interactions between these charges and nonionic solutes would manifest themselves primarily as van der Waals forces. These forces could, in turn, be incorporated into the effective frictional resistance term.

Limitations on the pore theory are created by the assumptions of the mathematical model. These assumptions are: (a) the biological membrane is crossed by cylindrical pores with a uniform radius and of length much greater than this radius; (b) solute molecules are simulated by rigid spheres moving slowly inside the pores; (c) the solvent flows according to Poiseuille's law (i.e., it behaves as a 'Newtonian' fluid); (d) the steady state assumption holds; (e) there is no interaction between solute molecules inside the pores; and (f) all gradients are normal to the plane of the membrane. This normal is arbitrarily

labeled as the x axis. In spite of these restrictions, the refined pore theory flow equation provides good agreement with molecular sieving data obtained from experiments with biological membranes (Verniory et al. 1973). This expression (Equation 17 from Verniory et al. 1973) is:

$$J_s = \left(\frac{D_s^o}{K_1} S_d \frac{(C_s^{(2)} - C_s^{(1)})}{\Delta x} \right) + \left(\frac{K_2}{K_1} J_v S_f \bar{C}_s \right) + \left(\frac{1}{K_1} \frac{\bar{V}_s \bar{C}_s}{F_{sw}^o} \frac{\Delta P_{2,1}}{\Delta x} S_d \right) \quad (2)$$

The first term on the right describes solute transport by diffusion. The second term represents solute molecules being dragged along by the fluid (convection) while the third term represents the flow of solute due to a gradient in effective pressure across the membrane (see Appendix D for definitions of all symbols).

A change in the concentration of uncharged solutes in the apoplast space will not directly affect any of the mechanisms of the three models proposed to explain the EPG phenomenon. However, these types of compounds could alter the dielectric constant or water potential of the solution thereby contributing indirectly to the behavior of the electrostatic and double layer models (see p. 57 and p. 69). Since Equation 2 is a steady state flow equation, several approximations would be involved in its application. In particular, initial concentration and pressure gradients across the apoplast would have to be estimated. The value of $\bar{C}_s$ may be calculated using the assumption that $(d/dx) J_s = 0$ and then solving the differential form of Equation 2 (Verniory et al. 1974, Equations 15, 18 and 19). Finally, some type of compartmentation and 'frozen equilibrium' approximation would have to be used. Firstly, the apoplast space containing the EPG probe would have to be considered

to divide two compartments. Various anatomical interpretations of these compartments are possible. Initial values for the pressure and concentration gradients must then be assigned to the two compartments. Transients would have to be ignored and the steady state flow frozen to allow the calculation of $\bar{C}_s$ in the apoplast space adjacent to the probe. New concentration and pressure gradient values would then be assigned and a new $\bar{C}_s$ calculated, etc.

A description of the movement of ions or ionized molecules through the apoplast requires that the effect of electrical forces be represented in the flux equation. The physical situation in the cell wall is approximated quite closely by models developed to describe permeation through ion exchange membranes with narrow pores. Membrane physiologists have long recognized the importance of the effects of bound charges on membrane permeability and have been at the forefront of both theoretical and experimental developments in this area (Teorell 1953). In addition, significant contributions have been made by physical chemists interested in fully understanding the principles that govern the behavior of synthetic ion-exchange membranes and gels used for chromatography. These synthetic materials have been used extensively as experimental systems for testing the various derived flux equations (Meares 1974).

It has been recognized for over forty years that the selective permeabilities of some membranes towards ions of different valencies and/or charge densities were often the result of the attachment, either by chemical bonds or electrochemical adsorption, of ionic groups to the membrane matrix material (Meares 1974). The existence of oriented polar

groups probably results in additional electrical effects within this region. A total elucidation of the interrelationship between all of the forces operating during ion flux across an 'ionic membrane' has not yet been achieved. However, as with glomerular filtration, flux equations have been derived that predict flow rates similar to those observed under a variety of experimental conditions (Woermann 1974). To account for the wide range of possible biophysical situations, two different theoretical approaches to membrane transport problems have been developed. The transition (or activated) state theory was first proposed by Davson and Danielli (1943) and is especially adapted for non-aqueous (hydrophobic) membranes that separate two aqueous phases. Alternatively, the fixed charge theory was developed by Teorell (1953) and Meyer and Sievers (cited by Teorell 1953) for application to aqueous systems. The cellulosic and pectic polymers that form the plant cell wall are among the most hygroscopic biological materials. It appears reasonable to assume that the fixed charge theory (with slight modifications) will provide an accurate description of ion flux phenomena in the apoplast. Using modified fixed charge theory, comparisons of simulated and empirical data have shown close agreement in the case of hydrophilic synthetic ionic membranes (Meares 1974; Woermann 1974).

The original fixed charge theory was basically the result of a synthesis of two theoretical advances made in the field of electrolyte solution chemistry. In particular, the Nernst-Planck flux equation, which describes the flow of a single ionic species in solution under the total driving force of an electrochemical potential gradient (reviewed by Bockris and Reddy 1970), was combined with the

membrane/solution ion distribution mechanism provided by the Donnan equilibrium (Donnan 1924; see also p. 31). The total membrane potential was equal to the sum of two Donnan potentials at the membrane boundaries and a diffusion potential within the membrane. The early development of this fixed charge theory is reviewed by Teorell (1953). It has remained for later workers to modify the theory to include the effects of hydrodynamic flow as well as ion:ion and ion:solvent flux coupling. Further refinements such as path length tortuosity factors and frictional drag coefficients have also been added in analogy with the pore theory equations that were discussed previously.

Recent advances in the fixed charge (also called the extended Nernst-Planck) theory are summarized in several articles (Meares 1974; Schonert 1974; Woermann 1974). A variety of extended Nernst-Planck equations exist in the current literature. The expression of Schlogl (presented in modified form by Woermann 1974) is reproduced in Equation 2, as a useful example of how ionic flows in the apoplast might be simulated (but see also Meares 1974).

$$J_i = C_i v - D_i \left[\frac{dC_i}{dx} + \frac{Z_i C_i F}{RT} \frac{d\bar{\psi}^{(j)}}{dx} \right] \quad (3)$$

with:

$$v = d_h \left(\frac{-d\bar{P}^{(j)}}{dx} - \rho_{el}^{(j)} \frac{d\bar{\psi}^{(j)}}{dx} \right) \quad (4)$$

The various symbols are defined in Appendix D. The limitations on Equation 3 are similar in nature to those placed on the pore theory equation of Verniory et al. (1973). Application of the above ionic

flux equation to the various EPG models would require the same type of assumptions as those previously discussed with respect to nonionic solute flux. Within the framework of Schlogel's equation, the properties of the model membrane are characterized by $-\rho_{el}/F$, C_i , D_i , d_h and $\bar{\psi}$. These symbols represent the density of space charge within the pores, concentration of species per unit volume within the pores, effective diffusion coefficient within the pores, specific mechanical permeability of the membrane, and total electric potential gradient across the membrane. In general, these parameters are functions of the electrolyte concentrations in the bulk phases outside the membrane.

Before concluding this discussion of transport phenomena in the apoplast space, it is necessary to mention the more general flux equations that may be developed by using non-equilibrium thermodynamics (Katchalsky and Curran 1975). These equations include, in a formal way, all of the forces acting on the system and the fluxes of all mobile components. They also allow for the possibility of all types of flux coupling. In most applications these equations are confined to linear relations between forces and fluxes, which implies that they are applicable only to systems that are near equilibrium (Meares 1974). In the case of membrane transport, the forces are often the result of a difference in an intensive variable of the bulk solutions on opposite sides of the membrane. The coefficients which connect these forces with the fluxes are therefore average values across the membrane. In this so called 'discontinuous form' the equations hold only over narrow ranges of concentration that are unlikely to occur in vivo. Recent work by Sauer (cited by Meares 1974) has shown that the linear equations may be

retained for larger concentration gradients if the linear relation between forces and fluxes continues to hold locally within the membrane. However, this does not reduce the amount of experimental data required to determine the large number of 'phenomenological' coefficients necessary to describe the flux of each chemical species. Therefore, in spite of the conceptual elegance of the nonequilibrium-thermodynamic approach, it is likely that biologists will continue to use local differential equation methods for the quantitative correlation of forces and fluxes in complex systems.

The Physical Chemistry of Polyelectrolyte Gels
in Relation to Apoplastic Structure and
Function: Donnan Potentials Revisited

Up to this point, the apoplast has been viewed as a continuum characterized by certain physical features (e.g., pores, fixed charges, etc.) and no real effort has been made to distinguish between the functional roles of the cellulosic and pectic components of the cell wall space. While a certain amount of overlap does exist, it must also be recognized that these two phases each have certain biochemical and biophysical properties that may provide the apoplast with the ability to exhibit a range of dynamical behavior as great as that of the plasma-lemma. In fact, in the opinion of this author, the cell wall should more properly be called the extracellular hydrophilic membrane. In this section of the literature review, relevant aspects of the physical chemistry of the polyuronic acid macroions will be discussed. The cellulose microfibrils, aside from their obvious role in providing physical support for the plant (Preston 1976) have been implicated in

several growth response phenomena. These include cell wall extension and several types of tropistic phenomena (see review by Rayle and Cleland 1977). In addition, the roles of numerous extracellular enzyme complexes (i.e., embedded in the cell wall) are presently under investigation (cf. Mueller and Brown 1980). In terms of the EPG system, the view through the apoplastic 'window' will undoubtedly be controlled, to a great extent, by the electrochemical properties of the cell wall space. The influence of the macroionic constituents of the cell wall will far outweigh that of the cellulose microfibrils in this area. Therefore, it is necessary to subject the former phase to an intensive analysis.

The polyelectrolyte concept provides a coherent framework within which to classify and study biocolloids such as the polyuronic acid gels of the cell wall space. Before proceeding further, a slight digression on terminology may prove useful. In modern terms (van Olphen 1977) a colloidal dispersion is defined as a system in which particles of colloidal dimensions (generally considered to fall between 1 nm and 1 μ m in at least one dimension) are dispersed in a continuous phase of a different composition (often a solvent). If the dispersed particles are suspended as individual 'kinetic units' within a continuous liquid phase, the system is considered to be in the sol state. If the kinetic units become continuous, then the colloidal system is in the gel state. By definition, a gel must necessarily consist of polymers or long chain molecules that can be cross-linked to create a continuous network while maintaining the continuity of the liquid medium. Tanaka (1981) considered the gel phase to be a form of matter intermediate between a

solid and a liquid. In this type of system certain properties are a function of the large amount of interfacial surface area. The interactions between substances at these interfaces mainly involve electrical and other physical forces. The terms polyelectrolyte and macroion are used interchangeably in the biocolloid literature. The physicochemical characteristics of polyelectrolyte gels (biogels) may be explained by fundamental principles that can be derived from the combined theories of polyelectrolytes and of network statistics (Katchalsky 1954). This type of approach utilizes the theoretical knowledge of both the polymer and electrolyte properties of biogels and provides new insight into the biophysical properties of the apoplast space.

While recent work (VanderMolen, Beckman and Rodehorst 1977) has shown unequivocally that the components of the cell wall space form a true gel in vivo, the chemical basis for network cross-linking is not known with certainty. Albersheim (1976) has postulated that rhamnogalacturonan and arabinogalactan (the two major polyelectrolyte components), xyloglucan polymers and probably, hydroxyproline-rich protein are all interconnected by covalent bonds. They form, in effect, a single macromolecule. The cellulose microfibrils are interconnected to the xyloglucan molecules by numerous hydrogen bonds and van der Waals forces with an effective interaction energy as great as that of covalent bonding. Presuming the existence of a three dimensional network structure, it is necessary to examine the forces that act to maintain the biogel state and characterize potential roles for the polyelectrolyte gel in the physiology of the apoplast.

In the cell wall space, the concentration of fixed negative charges resulting from the ionization of the carboxylic acid moieties of pectic polymers far exceeds the concentration of fixed positive charges located on the proteinaceous components of the wall. In fact, the differential is so great that in many plants the apoplast acts solely as a cation exchanger within the range of experimental error (Briggs, Hope and Robertson 1961). Since the concentration of fixed negative charges determined empirically (Walker and Pitman 1976) is fairly high (approximately 0.5 M), intermolecular electrostatic forces will play a major role in the structure of the gel. The electrostatic contribution to the stability of the biogel will be modified by four variables: the degree of ionization of the carboxylic acid (and other) groups which is in turn regulated by the cell wall pH; the dielectric constant of the cell wall solution; the salt concentration in the apoplast space; and the plant water potential. These variables are not independent and it may therefore be expected that the functional relationship between the state of the gel and these internal variables of state will be complex in nature. In general it is reasonable to assume that with a pK in the range of 2.5-3.0, most of the carboxylic acid residues are ionized. The main argument for this is that the pectic acid polymers are extremely hydrophilic and considered to be essentially in aqueous solution. A variety of experimental evidence is available to support this theory (Katchalsky 1954). If this is the case, there is no reason to suppose that the pKs of the carboxylic acids on the polymers will differ appreciably from those of the individual monomers in aqueous solution. The intermolecular electrostatic repulsion

potential may obviously be modified by a change in the dielectric constant of the apoplastic solution. The most important general effect of the salt concentration is its modification of the Debye atmosphere around the charged groups of the polymer network. Changes in ionic concentration will also modify the apparent Donnan distribution of ions between the gel phase and 'bulk' solution. It will be emphasized later in this section that these two phenomena are indistinguishable when analyzed at the mechanistic level of the electrical double layer. Finally, there is the effect of the water potential on the structure of the biogels. Unlike members of the animal kingdom that strive for homeostasis, many plants develop large internal water deficits as a part of their day-to-day metabolism. In the simplest case, the apoplast may be considered to undergo dehydration cycles of varying degrees of severity. Contraction of the dehydrated gel phase may be the basis for the well known diurnal fluctuations in stem diameter measured by water stress physiologists (Slatyer 1967).

A picture has now emerged of the apoplast space as a polyelectrolyte gel. The ionized carboxylic acid groups of the pectic polymers are surrounded by a Debye atmosphere of counter ions. This atmosphere is a function of the ionic strength of the extracellular sap, which may also contain organic solutes such as sucrose, glucose and various smaller molecules. The gel network is interrupted at regular or irregular intervals (depending on whether one is dealing with primary or secondary cell wall material) by crystalline cellulose microfibrils. Having obtained this picture, it is now possible to postulate roles for the biogel in specific aspects of cell wall physiology.

When one discusses the interaction of electrolytes with plant cells, the electric field of any indiffusible or restrained ions must be considered. Indiffusible ions are those which are confined to a particular region by being part of a rigid insoluble structure. A restrained ion is prevented from leaving a phase by a membrane impermeable to it (Briggs, Hope and Robertson 1961). Polyelectrolyte gels make excellent indiffusible ion systems. The quantitative effect of indiffusible ions on the mobile ionic species present in an adjacent electrolyte solution was first elucidated by Donnan (1924, 1979). Hence, the equilibrium conditions that exist when these two phases are in contact is known as the Donnan equilibrium. The polyelectrolyte gel is considered as the Donnan phase. Diffusible ions of the same sign as the fixed ions are termed coions while those of opposite sign are termed counterions. Important measurable effects of a Donnan equilibrium include (Briggs, Hope and Robertson 1961): (a) the counterions are attracted to the region of space adjacent to the indiffusible ions while the coions are repelled; (b) the Donnan phase develops an osmotic pressure in excess of the adjacent electrolyte solution; (c) an electric potential difference will exist between the Donnan phase and the adjacent solution. The sign of the Donnan phase potential is identical with the sign of the indiffusible ions; and (d) the Donnan phase may act as an ion exchange medium for counterions. The equations that describe the fundamental Donnan distribution of ions between two phases are generally developed using the Gibb's equations describing heterogeneous equilibria. Electroneutrality within each phase must be assumed. Plant physiologists consider the Donnan free space to play a significant

role in ion and water movement in plants (Lauchli 1976). In particular, at the root/soil solution interface, the Donnan potential may act to exclude coions from the apoplast space. The water content of the cell wall is influenced by the osmotic concentration of the cell wall sap and is thus a function of the Donnan equilibrium (Lauchli 1976).

Since the original analysis by Donnan (1924) was thermodynamic in nature, it provided no mechanistic basis for the unique features of the observed equilibrium system. As early as 1918, Tolman and Stearn worked out an alternative theory of the swelling effects observed in gelatine based on the assumption of electrical repulsions caused by formation of electrical double layers due to the adsorption of ions at the interfaces of a very fine two phase structure. Proctor (1914) had previously used the theory of Donnan to explain this phenomenon. Donnan himself (1924) pointed out that, "the difference between these two points of view may not be very great." This admonition was apparently not heeded by Katchalsky (1954) who considered that both the Donnan distribution and electric double layer theories were necessary to explain the behavior of polyelectrolyte gels. Two years later, Overbeek (1956) demonstrated that the observed Donnan distribution of ions between a polyelectrolyte gel and an electrolyte solution may be predicted using electrical double layer theory. In this theory, charges on the macroions are compensated for by an excess of counterions and a deficiency of coions (with respect to the bulk electrolyte solution) within the Debye atmospheres of the fixed charges. Using double layer theory and the Poisson-Boltzman equation (see p. 69) to predict these deviations in concentration, Overbeek was able to substantially improve

the fit to experimental data over predictions made using Donnan theory in several cases. He postulated that the Donnan theory predictions are mechanistically equivalent to attributing an average electrical potential to the gel phase.

In spite of the clarity of Overbeek's discussion, modern treatments of the cell wall continue to discuss the behavior of this phase in terms of the Donnan equilibrium (cf. Luttge and Higinbotham 1979). With the exception of Dainty and Hope (1961), who attempted to explain the ion exchange behavior of Chara cell walls in terms of the electrical double layer, most plant physiologists continue to cling to the thermodynamic approach. This is unfortunate in view of the potential insights into cell wall physiology that might be gained from the double layer/polyelectrolyte gel approach. For example, transport pathways through the apoplast could be modified by the excretion of organic solutes into the extracellular space in sufficient quantities to modify the dielectric constant of the biogel solution. The resultant increase in electrostatic repulsion would enlarge the effective pore size thereby allowing for greater flow. A mechanism such as this could play a role in apoplasm-mediated phloem loading in leaves. In a similar manner, modification of the composition of the solvent, pH fluctuations or changes in the ionic strength of the cell wall solution could result in a re-orientation of the gel network. These types of effects have been noted in synthetic polyelectrolyte gels by Tanaka (1981). Local fluctuations in any of the previously mentioned variables could create gradients in cell wall pore size and/or osmotic pressure resulting in directional flow phenomena. It is interesting to consider the possibility that such

polar metabolic phenomena as auxin-mediated cell wall elongation may be triggered by local modifications in biogel structure. For example, a local excretion of auxin might modify the dielectric constant of the apoplastic solution at the cell wall-plasmalemma interphase, inducing the polyelectrolyte phase to swell. This swelling could, in turn, disrupt some form of cell wall/plasmalemma contact triggering the electrogenic H^+ pumping known to occur during wall expansion. Structural constraints within the wall, e.g., the orientation of the cellulose microfibrils, might then direct the propagation of the swelling effect.

THE ELECTROPHYTOGRAM MODELED AS A CHANGING
REDOX POTENTIAL: MODEL I

The Electrochemical Potential

The choice of the system as outlined on page 6 allows the EPG to be modeled as two phases separated by a fixed boundary (the surface of the microprobe) permeable to some of the chemical species but not to others. The condition for the two phases to be in equilibrium with respect to any permeable species "i" is given by Equation (5):

$$\mu_i^{(m)} = \mu_i^{(s)} \quad (5)$$

This type of partial equilibrium is defined as the membrane equilibrium (Guggenheim 1977). The advantage of using the membrane equilibrium condition is that, since only electrons are able to move between the two phases, there is only one chemical species that satisfies the condition expressed in Equation (5), resulting in what Guggenheim (1977) termed a contact equilibrium.

Inherent to the use of the equilibrium concept are the two other major assumptions necessary in order to carry out a thermodynamic analysis of the EPG system. One is that electrons in solution and in a solid may be treated as a chemical species. The other is that the EPG system must be in thermodynamic equilibrium so that the constant T and P condition, $dG = 0$, holds. The assumption of equilibrium also implies that there can be no change in the total number of exchangeable

electrons within the system. The implications of each of these assumptions will now be discussed.

The choice of the electron as the chemical species of interest is dictated by the experimental conditions. For the description of systems containing electrolytes, the concept of chemical potential has been generalized by Guggenheim (1977) to include electric potential resulting in an electrochemical potential ($\tilde{\mu}_1 = \mu_1 + z_1 F\psi$). The extension of the electrochemical potential concept to electrons ($\tilde{\mu}_e$) is not new and has frequently been used to characterize processes in both electrochemistry (Ives and Janz 1961) and solid state electronics (Smith, Janak and Alder 1967). The exact numerical value for $\tilde{\mu}_e$ is more easily calculated in the solid state where $\tilde{\mu}_e$ is the Fermi energy of an electron multiplied by Avogadro's number. However, the existence of $\tilde{\mu}_e^{(m)}$ implies the existence of a corresponding $\tilde{\mu}_e^{(s)}$, although the value of the latter may be much more difficult to determine (see Appendix C).

The condition of a constant number of exchangeable electrons is actually contained within the assumption that thermodynamic equilibrium exists across the membrane during an EPG measurement. Obviously, since the EPG is represented by a time varying function, the entire measured phenomenon cannot be viewed from an equilibrium standpoint. However, to quote from Lewis and Randall (revised by Pitzer and Brewer, 1961): "Of the various possible processes which may occur within a system, some may take place with extreme slowness, others with great rapidity. Hence we may speak of equilibrium with respect to the latter process before the system has reached equilibrium with respect to all processes." In the case of the EPG, one may consider that the time it

takes for electrons to equilibrate between the solution and the probe is very fast ($\leq$ milliseconds) with respect to the time necessary for changes in the bulk solution to show up as a variation in the measured voltage (10-15 minutes). For all practical purposes then, the time dependent EPG may be divided up into a series of "frozen" equilibrium states as described by Guggenheim (1977), where "freezing" is accomplished by shifting to the fast time scale. After the equilibrium voltage measurement is made, the system is allowed to "thaw" by shifting to the slow time scale and changes in composition then take place. During this period of changing composition the system is not in state space and therefore undefined thermodynamically. After a suitable time interval, the system is "frozen" again and another voltage measurement taken.

Biological Work

Thermodynamically, one may view the plant as a multiphase open system capable of exchanging both matter and energy with its surroundings (Katchalsky and Curran 1967; Nobel 1974). Furthermore, the various phases within the plant are capable of exchanging energy in the form of biochemical and biophysical work. This work may be performed in a number of ways. For example, the biosynthesis of protein macromolecules on the ribosomes and their subsequent transport out of the cell may be considered as one phase (the cell) doing work on another (the extracellular fluid) with the final result being the transport of matter.

When an open system is capable of doing non-mechanical (i.e., non-PV) work it is necessary to expand the work term in Equation (6):

$$dU \leq TdS - PdV + \sum \mu_i dn_i \quad (6)$$

where the various terms are defined in the usual manner (Katchalsky and Curran 1967) to include the other possible mechanisms by which energy may be exchanged. Assuming reversibility, one may write in the most general case:

$$dU = TdS - PdV + dW_a + \sum \mu_i dn_i \quad (7)$$

where dW_a stands for all the other types of work that the system is capable of performing. The ' W_a ' term is broken down into a summation of work terms to include such processes as molecular contraction, surface and magnetic effects. A characteristic expression for dW in a system composed of phase j with q chemical components is shown in Equation (8):

$$dW = PdV - \sum_{i=1}^q z_i F E^{(j)} dn_i - \sum_{i=1}^q \mu_i dn_i - \sum_{i=1}^q m_i g dh_i - \sum_{i=1}^q f d\ell_i \quad (8)$$

where

z_i = charge number of species i (unitless, carries the algebraic sign of the coulombic charge on i)

F = Faraday constant (joules volt⁻¹ mole⁻¹)

$E^{(j)}$ = Galvani electrical potential of phase j ($\frac{\text{joules}}{\text{coulomb}}$)

μ_i = chemical potential of i (joules mole⁻¹)

m_i = mass of i (kg)

g = gravitational constant (meters sec⁻²)

h_i = relative height of species i in earth's gravitational field (meter)

n_i = moles of i (1 mole = Avogadro's number of atoms or molecules)

f_i = contractile force (newton)

l_i = length (meter).

Many biologically important atoms and molecules are not electrically neutral, so that their movement through the system may result in a charge displacement equivalent to the flow of an electric current. This phenomenon is well documented for such processes as vision (Hagins 1972) and electron transport coupled oxidative phosphorylation. This type of energy exchange involves both the electrical and chemical work terms from Equation (8). The passive diffusion of uncharged chemical compounds is also well known in the area of physiological chemistry and may be understood in terms of an entropy driven process whereby the system attempts to equilibrate the chemical potential of a given species throughout the entire volume or across several phases, resulting in the performance of chemical work.

With respect to Equation (8), Guggenheim (1977) has shown by means of a simple numerical calculation that when two phases differ infinitesimally in the chemical content of a charged species ' i ', the change in relative energy due to the excess mass of ' i ' is negligible when compared to the electrical effect of the charge excess. In studying the transport of small charged molecules (such as ions) in a biological system, it is reasonable to suppose that the contributions of magnetic and non-PV mechanical work to the total dW term will also be very small. When the non-significant terms are dropped, one is left with Equation (9) for the work term:

$$dW = PdV - \sum_{i=1}^q \sum_{j=1}^p z_i F E^{(j)} dn_i^{(j)} + \sum_{i=1}^q \sum_{j=1}^p \mu_i^{(j)} dn_i^{(j)} \quad (9)$$

where q = number of components and p = number of phases. In terms of the EPG system, $E^{(j)}$ is now the Galvani potential of phase j with respect to infinity.

When dealing with a multicomponent, multiphase system where changes in the concentration of species i can have both chemical and electrical effects on the potential of a given phase to do work, Guggenheim (1977) has suggested the term electrochemical potential (symbolized here by $\tilde{\mu}$) be used. In a multiphase, multicomponent system, adoption of the electrochemical potential concept results in the abbreviated representation of Equation (9)

$$dW = PdV - \sum_{i=1}^q \sum_{j=1}^p \tilde{\mu}_i^{(j)} dn_i^{(j)} \quad (10)$$

where:

$$\tilde{\mu}_i^{(j)} = \mu_i^{(j)} + z_i^{(j)} F E^{(j)} \quad (11)$$

It is important to note that $\tilde{\mu}_i^{(j)}$ is an intensive property with the units energy per mole. The change in energy of the system is now expressed by Equation (12):

$$dU = TdS - PdV + \sum_{i=1}^q \sum_{j=1}^p \tilde{\mu}_i^{(j)} dn_i^{(j)} \quad (12)$$

Electrochemical Equilibrium

With the modified work term, one may now proceed to obtain an expression for electrochemical equilibrium using the method of thermodynamic potentials. This treatment involves the introduction and differentiation of the Gibbs function (Katchalsky and Curran 1967):

$$G = U - TS + PV \quad (13)$$

$$dG = dU - TdS - SdT + PdV + VdP \quad (14)$$

Substituting dU from Equation (12) results in:

$$dG = -SdT + VdP + \sum_{i=1}^q \sum_{j=1}^p \tilde{\mu}_i^{(j)} dn_i^{(j)} \quad (15)$$

Under the biological conditions of constant T and P , the amount of energy available in a system to do useful work is given by the resulting Gibbs function:

$$dG = \sum_{i=1}^q \sum_{j=1}^p \tilde{\mu}_i^{(j)} dn_i^{(j)} \quad (16)$$

Furthermore, at equilibrium $dG = 0$, so that electrochemical equilibrium in a multicomponent, multiphase system is described by the relationship:

$$\sum_{i=1}^q \sum_{j=1}^p \tilde{\mu}_i^{(j)} dn_i^{(j)} = 0 \quad (17)$$

Finally, with the added constraint on the system that there are no chemical reactions between the i species (i.e., $n_i^{(1)} + n_i^{(2)} + \dots + n_i^{(n)} =$ constant for all i) one can demonstrate by the method of Lagrangian multipliers (cf. Zemansky 1943), that Equation (16) may be broken down into the series of equalities:

$$\begin{array}{ccccccc}
\tilde{\mu}_1^{(1)} & = & \tilde{\mu}_1^{(2)} & \dots & = & \tilde{\mu}_1^{(p)} & \\
\vdots & & \vdots & & & \vdots & \\
\tilde{\mu}_q^{(1)} & = & \tilde{\mu}_q^{(2)} & \dots & = & \tilde{\mu}_q^{(p)} &
\end{array} \quad (18)$$

Returning to the EPG system, it can be seen that the condition for equilibrium with regard to exchangeable species results in the reduction of the set of equations in (17) to the single equality ($q = 1$, $p = 2$):

$$\tilde{\mu}_e^{(s)} = \tilde{\mu}_e^{(m)} \quad (19)$$

since only electrons can move between the two phases. The remaining problem is to manipulate Equation (18) into a form such that changes in the concentration variable are given as a function of changes in the measured voltage. In the following sequence of equations, the lowest integer subscript is used to indicate that the value for a variable is at a given point in time; i.e., in one particular frozen equilibrium state:

$$\tilde{\mu}_{e_1}^{(s)} = \tilde{\mu}_{e_1}^{(m)} \quad (20)$$

$$\mu_{e_1}^{(s)} + z_e FE_1^{(s)} = \mu_{e_1}^{(m)} + z_e FE_1^{(m)} \quad (21)$$

likewise:

$$\mu_{e_2}^{(s)} + z_e FE_2^{(s)} = \mu_{e_2}^{(m)} + z_e FE_2^{(m)} \quad (22)$$

therefore:

$$\mu_{e_1}^{(s)} - \mu_{e_1}^{(m)} = F z_e (E_1^{(m)} - E_1^{(s)}) \quad (23)$$

and

$$\mu_{e_2}^{(s)} - \mu_{e_2}^{(m)} = FZ_e (E_2^{(m)} - E_2^{(s)}) \quad (24)$$

However, $E^{(m)} - E^{(s)}$ is the measured electrode potential which we will designate as E_M , therefore:

$$\mu_{e_1}^{(s)} - \mu_{e_1}^{(m)} = FZ_e E_{M_1} \quad (25)$$

and

$$\mu_{e_2}^{(s)} - \mu_{e_2}^{(m)} = FZ_e E_{M_2} \quad (26)$$

Finally, since:

$$\mu_{e_2}^{(m)} - \mu_{e_1}^{(m)} = 0 \quad (27)$$

one may obtain the desired relationship by subtracting Equation (25) from (26) which yields:

$$\mu_{e_2}^{(s)} - \mu_{e_1}^{(s)} = FZ_e (E_{M_2} - E_{M_1}) \quad (28)$$

or, with $Z_e = -1$:

$$\Delta\mu_{e_{2,1}}^{(s)} = -F \Delta E_{M_{2,1}} \quad (29)$$

This equation states that the changes in the measured potential of the macroscopic galvanic cell are proportional to the changes in chemical potential of the electrons in the vicinity of the measuring probe/tissue interface. The analysis has been carried to the extent that the electrical variable $\Delta E_{M_{2,1}}$ is now related to the chemical variable μ_e in the electrolyte surrounding the probe. The next step in the analysis is to investigate in more detail the electron chemical potential in the electrolyte.

The total chemical potential is considered to be composed of two components (Lewis and Randall 1961) as shown in Equation (30):

$$\mu_e^{(s)} = \mu_e^{o(s)} + RT \ln a_e^{(s)} \quad (30)$$

therefore:

$$\Delta\mu_{e2,1}^{(s)} = (\mu_{e2}^{o(s)} - \mu_{e1}^{o(s)}) + RT_2 \ln a_{e2}^{(s)} - RT_1 \ln a_{e1}^{(s)} \quad (31)$$

with: $T_2 \sim T_1$,³ and $\mu_{e2}^{o(s)} - \mu_{e1}^{o(s)} = 0$,

$$\Delta\mu_{e2,1}^{(s)} = RT \ln \frac{a_{e2}^{(s)}}{a_{e1}^{(s)}} \quad (32)$$

substitution of Equation (29) yields:

$$\Delta E_{M2,1} = -\frac{RT}{F} \ln \frac{a_{e2}^{(s)}}{a_{e1}^{(s)}} \quad (33)$$

Analysis of Equation (31) in the case where $T_1 \approx T_2$ has shown that a large $\Delta T_{2,1}$ (20°C in 15 minutes) would be necessary to generate a $\Delta E_{m2,1}$ of more than a few millivolts over the same time period. It may now be seen that further analysis of the measured EPG values requires the representation of a_e in a form from which physiological information may be extracted.

The Relative Electron Activity

Now that the change in measured voltage has been related to a change in the electron activity, it is necessary to interpret this latter variable in terms of the various chemical species existing in

solution. While no one would seriously propose that free electrons exist in either the apoplastic or symplastic plant phases, it is equally misleading to cling to an artificial separation of the processes of acid base equilibria, electron exchange, and complex formation when one is dealing with what Clark (1960) has so aptly described as a 'chemical continuum.' Along these lines, it is useful to consider a transformation of the Nernst equation (Laitinen 1960).

$$E_M = E^O + \frac{RT}{nF} \ln \frac{(Ox)}{(Red)}, \quad (34)$$

where E_M is the electrical potential exhibited by the half-cell in relation to the standard hydrogen half-cell; E^O is the standard half reduction potential (pH = 0) of the redox couple; and ( ) represents the thermodynamic activity of the oxidized and reduced species. Dutton and Wilson (1974) have pointed out that, by converting to the logarithm to the base 10, Equation (34) converts to the form of Equation (35):

$$E_M = E^O + \frac{RT}{nF} 2.3 \log \frac{(Ox)}{(Red)} \quad (35)$$

which, in turn, may be written in a form analogous to the Henderson-Hasselbach equation

$$P_e = P_e + \log \frac{(Ox)}{(Red)} \quad (36)$$

where $P_e = -\log [e] = \frac{E_M nF}{2.3RT}$. Dutton and Wilson (1974) have called P_e the relative electron activity and have further pointed out that the dimensionless P_e might be useful in certain aspects of redox potentiometry.

To further emphasize the possible utility of the P_e concept, we have calculated P_e values over a range of electrical potentials that might be measured in biological systems. These values are shown in Table 1.

Aside from its value as a computational tool, the P_e concept has the advantage of being solution oriented as opposed to the electrode potential which is not.

This may be seen from Equation (36), for if P_e represents an electron potential in the same manner that pH represents a proton potential then the P_e is a useful parameter for the characterization of the solution phase. For example, the promotion of an electron across the photosynthetic membrane from Photosystem II to the primary acceptor Q (cf. Kok 1976) involves a change in P_e that may be calculated from Equation (37):

$$\Delta P_e = (E_{M,Q}) \frac{nF}{2.3RT} - (E_{M,PSII}) \frac{nF}{2.3RT} \quad (37)$$

Using the standard oxidation-reduction potentials for Photosystem II and Q given by Kok (1976) (approximately +0.8 and -0.1 volts, respectively) with $n = 1$ and $T = 25^\circ\text{C}$, the calculated $\Delta P_e = -15.2$. For the sake of clarity we feel that the P_e should not be called the relative electron activity as suggested by Dutton and Wilson (1974), but rather should remain the P_e in analogy to the pH. In this paper, the term relative electron activity (which we shall simply call the electron activity with the understanding that the values of any quantity measured as an electrical potential must be relative) is reserved for the effective value of $[e]$ and is symbolized by a_e as in Equation (30).

Table 1. Calculated P_e^0 values, assuming a two electron transfer ($n = 2$) at 25°C .

Electrode Potential (v)	P_e
+1.0	+33.9
+0.8	+27.1
+0.6	+20.3
+0.4	+13.5
+0.2	+ 6.8
0.0	0.0
-0.2	- 6.8
-0.4	-13.5
-0.6	-20.3
-0.8	-27.1
-1.0	-33.9

Electrode potential defined as standard reduction potential against hydrogen electrode.

If the redox couple also behaves as a weak acid or base, so that the half-reduction potential is a function of pH as well as P_e , then the hydrogen ion activity must be included in the calculation of P_e . The treatment presented here is for the case where reduction involves the receipt of a proton as well as an electron (after Dutton and Wilson 1974). Two proton, two electron, as well as other types of interactions have also been considered (Clark 1960). Starting with the modified Nernst Equation:

$$E_M = E^O + \frac{2.3RT}{nF} \log \frac{(Ox)(H^+)}{(Red-H)} \quad (38)$$

The new midpoint electrode potential is

$$E_{\text{midpt.,pH}} = E^O + \frac{2.3RT}{nF} \log(H^+) \equiv E^O - \frac{2.3RT}{nF} \text{pH} \quad (39)$$

and:

$$E_M = E^O - \frac{2.3RT}{nF} \text{pH} + \frac{2.3RT}{nF} \log \frac{(Ox)}{(Red)} \quad (40)$$

define:

$$P_e = E_M \frac{nF}{2.3RT} = -\log[e] \quad (41)$$

In the limit of a dilute solution $[e] \approx a_e^{(s)}$ therefore

$$\frac{E_M nF}{2.3RT} = -\log a_e^{(s)} \Leftrightarrow \frac{E_M nF}{RT} = -\ln a_e^{(s)} \quad (42)$$

Now, returning to the electrophytogram, if one measures the potential (E_M) at two points in time and evaluates the change in a_e that has occurred in terms of Equation (42), the resulting expression shown in Equation (43):

$$\frac{E_{M_2} nF}{RT} - \frac{E_{M_1} nF}{RT} = -\ln a_{e_2}^{(s)} + \ln a_{e_1}^{(s)} \quad (43)$$

is seen to be identical (if $n = 1$) to Equation (33):

$$\Delta E_{M_{2,1}} = -\frac{2.3RT}{nF} \log \frac{a_{e_2}^{(s)}}{a_{e_1}^{(s)}} \equiv -\frac{RT}{F} \ln \frac{a_{e_2}^{(s)}}{a_{e_1}^{(s)}} \quad (33)$$

Returning now to logarithm to the base 10 notation $\Delta E_{M_{2,1}}$ is obtained from Equation (37) as shown in Equation (44):

$$\begin{aligned} \Delta E_{M_{2,1}} &= E_2^0 - 2.3 \frac{RT}{nF} \text{pH}_2 + \frac{2.3RT}{nF} \log \frac{(Ox)_2}{(Red)_2} \\ &\quad - E_1^0 - \frac{2.3RT}{nF} \text{pH}_1 + \frac{2.3RT}{nF} \log \frac{(Ox)_1}{(Red)_1} \end{aligned} \quad (44)$$

Since $E_2^0 = E_1^0$

$$\Delta E_{M_{2,1}} = \frac{2.3RT}{nF} (\text{pH}_1 - \text{pH}_2) + \frac{2.3RT}{nF} \left(\log \frac{(Ox)_2}{(Red)_2} - \log \frac{(Ox)_1}{(Red)_1} \right) \quad (45)$$

$$\Delta E_{M_{2,1}} = \frac{2.3RT}{nF} \left\{ (\text{pH}_1 - \text{pH}_2) + \log \frac{(Ox)_2 (Red)_1}{(Red)_2 (Ox)_1} \right\} \quad (46)$$

Finally, substitution into the logarithm to the base 10 form of Equation (33), with $n = 1$, yields Equation (47):

$$\log \frac{a_{e_2}^{(s)}}{a_{e_1}^{(s)}} = - \left\{ (\text{pH}_1 - \text{pH}_2) + \log \frac{(Ox)_2 (Red)_1}{(Red)_2 (Ox)_1} \right\} \quad (47)$$

It is clear from Equation (47) that changes in the electron activity in the solution adjacent to the probe (in the case where the controlling redox couple engages in a one electron, one proton exchange)

are a function of the changes in both the pH of the solution and the ratio of activities of the two chemical components of the redox couple. The assumption that a single redox couple is controlling the measured electrode potential is, of course, a mechanistic model for the EPG phenomenon. Theoretically any type of redox couple could be involved. However, in biological systems the overwhelming majority of redox reactions involve only one or two electron exchanges. These electron exchanges may or may not be accompanied by protons. Therefore, in addition to Equation (47) other equations must be developed to represent the change in electron activity when the controlling redox couple engages in one electron ($n = 1, H^+ = 0$), two electron ($n = 2, H^+ = 0$), and two electron/two proton ($n = 2, H^+ = 2$) exchanges.

In The first two cases, Equation (34) provides the necessary starting point. The arguments are exactly like those used in developing Equation (47) and the result is given by Equation (48):

$$\log \left(\frac{a_{e_2}^{(s)}}{a_{e_1}^{(s)}} \right) = - \frac{1}{n} \log \frac{(Ox)_2 (Red)_1}{(Red)_2 (Ox)_1} \quad (48)$$

When a redox couple engages in a two electron/two proton exchange, $\Delta E_{M,2,1}$ may be obtained using the equation for E_M developed by Clark (1960) which finally results in Equation (49):

$$\log \frac{a_{e_2}}{a_{e_1}} = \frac{1}{2} \left\{ \log \frac{(K_\alpha K_\beta)_1}{(K_\alpha K_\beta)_2} + \log \frac{(S_o S_r)_1}{(S_r S_o)_1} + \log \frac{T_2}{T_1} \right\} \quad (49)$$

where:

$$T = [(H^+)^2 + K_\alpha (H^+) + K_\alpha K_\beta] \quad (50)$$

and:

$$K_{\alpha} = \frac{[H_2 Red^-] (H^+)}{[H_2 Red]} \quad (51)$$

$$K_{\beta} = \frac{[Red^{2-}] (H^+)}{[H Red^-]} \quad (52)$$

are the apparent ionization constants (note that these apparent ionization "constants" are a function of pH) for the two protonated forms of the reduced species, with:

$$S_r = [H_2 Red] + [H Red^-] + [Red^{2-}] \quad (53)$$

$$S_o = [Ox] \quad (54)$$

the concentrations of the various species involved in the total redox reaction. The pH and temperature are assumed to be constant at the frozen equilibrium points and $T_1 \approx T_2$.

Taken together Equations(47), (48), and (49) provide a basis for modeling the EPG system as a phenomenon resulting from changes in pH and/or the relative concentrations of a single redox couple capable of engaging in one or two electron transfers with or without accompanying proton exchange. In addition to the situations described by these three equations, it is conceivable that two or more redox couples are controlling the measured electrode potential and hence its changes. In the case of such a 'mixed potential' the thermodynamic analyses presented here are not valid and one must turn instead to a kinetic treatment (Bockris and Reddy 1970).

THE ELECTROPHYTOGRAM MODELED AS A BIOELECTRIC
FIELD PHENOMENON: MODEL II

Spatial Charge Separation and the Existence
of Bioelectrical Potentials in Plants

It has been known since the time of Galvani (1791) that changes in the electric potential play an important role in the functioning of a living cell. However, aside from the highly localized transmembrane potentials commonly observed between the inner and extracellular solutions, which may result from either passive or active mechanisms (Nobel 1974), several lines of evidence are now beginning to suggest the possibility that plants are capable of creating regions (i.e., three dimensional volume elements) which contain significant numbers of unbalanced coulombic charges. Due to the magnitude of the electrical forces which must be overcome in order to accumulate an excess of charge in a region of space (Guggenheim 1977), the process of accumulation must be an active one involving the input of metabolic energy. The principal types of experimental data that lend support to this hypothesis are as follows:

1. The existence of electrogenic ion pumps in several types of algae (Luttge and Higinbotham 1979). Although electrogenic pumping of ions will also modify the membrane potential (Nobel 1974), the existence of such a mechanism is an a priori requirement for the accumulation of charge.

2. Treatment of several types of higher plant tissue with the plant hormone indoleacetic acid (IAA) results in the pumping of hydrogen ions from the cytoplasm into the cell wall space (apoplast) (Rayle and Cleland 1977). The phenomenon may be monitored directly with a flat pH microelectrode and can result in a lowering of the pH in the cell wall space from 7 to 4 (Rayle and Cleland 1977). Furthermore, the phototropic response in oat coleoptiles, an IAA mediated phenomenon, results in a change in the measured transverse voltage of several millivolts (Galston and Slayman 1979), a result which strongly implies a net movement of charge. These IAA mediated changes ultimately result in growth response.

3. Several workers (Tanada 1978; Newman and Briggs 1972) have reported on the hormone mediated affinity of plant roots for several types of charged surfaces. In at least one case (Racusen and Miller 1972), the coulombic force between the root tissue and a charged microprobe has been partially quantified.

Although alternative interpretations exist, the possibility that nonmembrane bioelectric potential gradients play a significant role in several physiological processes in plants must now be seriously considered. Therefore, in the following section we will develop a model for the electrophytogram that is purely electrostatic in nature. This model visualizes the volume around the probe (in particular the cell wall space) as being capable of accumulating a net coulombic charge and that the amount of charge present changes as a function of time.

Electrostatic Interactions at the
Probe/Tissue Interface

Figure 2 shows a modification of Figure 1b that allows the probe/tissue interface to be modeled electrostatically as a cylindrical capacitor. The preliminary assumptions of the electrostatic model are:

1. Penetration of the probe into the plant tissue subjects electrons in the probe to electric fields. As a result, the mobile electrons redistribute themselves on the surface of the probe in a manner consistent with the electrostatic condition of zero internal electric field.
2. The charge distribution around the probe is radially symmetric in the xy plane for a unit length in the z direction.
3. The 'frozen equilibrium' condition may be applied (as explained on p. 41) so that the laws of electrostatics may be applied to the analysis.

The relationship between the flux of an electric field out of a closed surface to the amount of charge enclosed within that surface is given (Ramo, Whinnery and Duzer 1965), by Gauss's Law in the form of the surface integral shown in Equation (55):

$$\epsilon_0 \oint \vec{E} \cdot d\vec{s} = q \quad (55)$$

where:

ϵ_0 = the permittivity constant coul² [(nt) (m²)]⁻¹

$\vec{E}$ = the electric field strength (nt coul⁻¹)

$d\vec{s}$ = an infinitesimal element of area (m²)

q = the charge in coulombs.

Figure 2. Cylindrical capacitor model for probe/tissue interface. -- Radial symmetry is assumed in the xy plane, l is a unit increment of length along the z axis.

From symmetry considerations, the Gauss's Law representation of the cylindrical capacitor reduces to Equation (56):

$$E = \frac{q_\ell}{2\pi\epsilon_0 r} \quad (56)$$

where:

E = the electric field strength in the radial direction (nt coul⁻¹)

q_ℓ = the charge density (coul m⁻¹ in the z direction)

r = radius (m).

In the first approximation, ϵ_0 in Equation (56) will be replaced by the permittivity constant of water (Bockris and Reddy 1970). The difference in electric potential between two points in an electric field is given by the line integral shown in Equation (57):

$$\psi_B - \psi_A = - \int_A^B \vec{E} \cdot d\vec{\ell} \quad (57)$$

where:

ψ = the electric potential at a point in the electric field (volt)

$d\vec{\ell}$ = is an infinitesimal distance along the line joining the two points in the electric field (m).

The radial symmetry of the cylindrical capacitor model allows the value of E given in Equation (56) to be substituted into Equation (57) where dr replaces $d\ell$, resulting in Equation (58):

$$\Delta\psi_{r_2, r_1} = - \int_{r_1}^{r_2} \frac{q_\ell}{2\pi r \epsilon_\omega} dr \quad (58)$$

The $\Delta\psi_{r_2, r_1}$ is equivalent (based on the argument presented on page 7) to the measured EPG voltage, ΔE_m . Using a characteristic EPG measurement of 0.1V Equation (58) may be used to calculate the linear charge density along the probe. The value of r_1 becomes the radius of the probe (125 μm) whereas the value of r_2 is a variable that must be determined by the physical environment of the probe within the plant tissue. For the preliminary calculation we shall assume that the probe is surrounded by cell wall material with an average thickness of 1 μm , a reasonable value for dicotyledonous plants (Esau 1960). Allowing the accumulated charges to rest on the plasmalemma side of the cell wall (i.e., on the side of the cell wall distal to the probe), the value of r is set at 126 μm . Finally using an ϵ_ω (at 25°C) of 6.93×10^{-10} coul² [(nt) (m²)]⁻¹ (Bockris and Reddy 1970), one obtains a value for q_i of 5.46×10^{-8} coul m⁻¹.

To obtain a picture of the probe/tissue interface, a typical value for the width of a phloem cell (a type of plant cell that participates in the transport of sugars and other compounds essential to biosynthesis) of 30 μm (Esau 1960) will be used. Although the phloem cell itself is cylindrical and (since its long axis is directed normal to that of the probe) would therefore only contact the cylindrical probe at a point, we may suppose that cell wall material deposited after insertion has created a contiguous probe/tissue interface as shown in Figure 3. For these preliminary calculations, radial symmetry is again assumed. Therefore, one can consider the degree of charge induced on a probe surface strip 30 μm wide and 785.4 μm in circumference, i.e., the charge per 23,562 μm^2 of probe surface area.

Figure 3. The probe/tissue interface model used to estimate the distribution of surface charges on the probe. -- The probe is inserted normal to the stem and therefore normal to the flow of conducting tissue. Contiguity between the probe and the surrounding cells is assumed to occur through the cell wall material.

Having calculated q_l to be 5.46×10^{-8} coul m^{-1} , q_l for a $30 \mu_m$ increment of probe length is then 1.6×10^{-12} coul. Spreading this charge over $23,562 \mu_m^2$ of surface area results in a value of $\sim 7.0 \times 10^{-17}$ coul μ_m^{-2} . Given the elementary charge of 1.6×10^{-19} coul per electron, a final calculation yields the desired value of ~ 437.5 electrons μ_m^{-2} of probe surface area. These calculations show a general picture of the charge that would be induced on the surface of the probe if the EPG measurement resulted from a purely electrostatic interaction between the probe and an electrically charged cell wall.

Pursuing the model one step further, it is possible to calculate the charge density within (or on) the cell wall that is inducing the charge on the probe surface. The relationship between the electric field strength vector at a point and the electric charge density at that point is given (Rambo et al. 1965) by Equation (59):

$$\nabla \cdot \vec{E}_r = \rho_r \quad (59)$$

where:

ρ_r = the charge density at an infinitesimal volume element about a point at radius r (coul m^{-3})

∇ = the gradient operator $(i\frac{\partial}{\partial x} + j\frac{\partial}{\partial y} + k\frac{\partial}{\partial z})$.

With radial symmetry, Equation (59) reduces to Equation (60):

$$\frac{d}{dr} E_r = \frac{\gamma}{\epsilon} \quad (60)$$

where γ is now an equivalent charge density concentrated on the radial side of the volume element at radius r . The γ value retains the dimensions of coul m^{-3} , but may be physically interpreted as a surface by letting the side of the volume element in the r direction go towards

zero about point r as shown in Figure 4. Substitution of the value for E from Equation (56) into Equation (60) and using ϵ_ω at 25°C, one obtains Equation (61):

$$\frac{d}{dr} \frac{q_l}{2\pi\epsilon_\omega r} = \frac{\gamma}{\epsilon_\omega} \quad (61)$$

Cancellation and differentiation results in Equation (62):

$$\frac{-q_l}{2\pi r^2} = \gamma. \quad (62)$$

Using the value of 5.33×10^{-14} coul μm^{-1} for q_l and 126 μm for r (i.e., the side of the 1 μm cell wall distal to the probe) one obtains a value for γ of -7.0×10^{-17} coul μm^{-3} or -473.5 primary charges (the minus sign indicating that these charges are opposite in sign from those on the probe surface).

This, then, is the physical interpretation of the EPG as an electric field phenomenon based on the simplified model of a cylindrical capacitor. Immediate improvement of the model would come from added information about: (a) the three dimensional structure of the probe/tissue interface; (b) the effective permittivity constant. Inherent in this information is the site of charge accumulation and therefore the effective radial value to be used in Equation (56) and Equation (60). Obviously, if charges are accumulated across the cell wall from the probe a value for $\epsilon_{\text{cell wall}}$ will have to be determined. Conversely if charges move through the wall to accumulate in a fluid phase adjacent to the probe then ϵ_{water} or some $\epsilon_{\text{ionic solution}}$ should be used. Future simulations of EPG data with this model and subsequent sensitivity

Figure 4. The physical interpretation of the effect of radial symmetry considerations on the calculation of the charge density within the plant that could induce the charge on the probe surface.

analysis will provide more accurate estimations of r and ϵ . Likewise, anatomical studies now in progress should give us more information on the structure of the probe/tissue interface.

Finally, it will be necessary to determine whether plant cells are capable of accumulating these types of unneutralized charges in a volume element or on a surface area. Analysis of the electrogenic pumping capabilities of certain algae (Luttge and Higinbotham 1979) along with measurement of ion pumping in higher plants (Rayle and Cleland 1977; Keifer and Spanswick 1979) when combined with the structural information obtained from an anatomical study should enable us to begin to address this question.

Concluding Remarks

The analyses presented thus far provide the theoretical framework for modeling the electrophytogram as either a changing redox potential or as a bioelectric field effect. At this point it is not possible to determine which model most accurately depicts the in vivo situation. However, it is of interest to note that the EPG signal is lost upon attempting to draw current from the probe at a rate greater than 1.25×10^9 electrons sec^{-1} (Gensler 1974). The ability of the plants to provide electrons would tend to favor the redox model while the upper limit on the available current source may provide important information about flow processes occurring in the plant. In addition, the success of the EPG technique in monitoring the changes in acidity occurring in a CAM plant (Gensler n.d.) would also seem to imply a redox measuring capability if the probe is acting as a hydrogen electrode.

Further experimental work will be necessary in order to discriminate unequivocally between these two possibilities. Regardless of whether the EPG represents a changing redox potential, an electric field phenomenon, or some combination of the two, the electrical manifestations demonstrated by this measuring technique along with data from other systems in both plants (Luttge and Higinbotham 1979; Galston and Slayman 1979) and animals (Pilla 1974; Hagins 1972) indicates that the study of bioelectric effects will lead to new insights into the mechanisms by which cells carry out their physiological functions.

THE ELECTROPHYTOGRAM MODELED AS A SURFACE
INTERACTION: MODEL III

Image Charge Interactions at the Probe/Tissue
Interface Resulting from the Overlap of
Electrochemical Double Layers

Given an arbitrary charge-bearing surface in a medium containing mobile charges, the electrostatic potential ψ can be calculated from the Poisson equation (see Appendix D for definitions of symbols):

$$\nabla^2 \psi = - \frac{\rho_{el}(x, y, z)}{\epsilon} \quad (63)$$

When the system is in equilibrium, the space charge density ρ can be calculated from the Boltzmann theorem:

$$\rho(x, y, z) = \sum_i e z_i n_i(x, y, z) = \sum_i e z_i n_{i0} \exp \left(\frac{-U(x, y, z)}{kT} \right) \quad (64)$$

The energy $U(x, y, z)$ in the Boltzmann distribution function is composed of a number of terms. If it is assumed that the electrostatic energy contribution $e z_i \psi(x, y, z)$ is much greater than the other terms, Equation (63) becomes:

$$\nabla^2 \psi = - \frac{e}{\epsilon} \sum_{i=1}^n z_i n_i \exp \left(\frac{-e z_i \psi}{kT} \right) \quad (65)$$

Equation (65) is the well known Poisson-Boltzmann (P.B.) equation. This expression forms the foundation of much of the theory of electrochemical double layers and their interactions. The interaction of these double layers, in turn, represents the physiochemical foundation of a good part of colloid chemistry.

If the analysis is limited to one dimension, Equation (65)

becomes:

$$\frac{d^2\psi}{dx^2} = -\frac{e}{\epsilon} \sum_{i=1}^n z_i n_{i0} \exp\left(\frac{-e z_i \psi}{kT}\right) \quad (66)$$

which may be integrated (see Verwey and Overbeek 1948) to yield:

$$\frac{d\psi}{dx} \frac{d^2\psi}{dx^2} = -\frac{e}{\epsilon} \sum_{i=1}^n z_i n_{i0} \frac{d\psi}{dx} \exp\left(\frac{-e z_i \psi}{kT}\right) \quad (67)$$

whence:

$$\left(\frac{d\psi}{dx}\right)^2 = \frac{2kT}{\epsilon} \sum_{i=1}^n n_{i0} \exp\left(\frac{-e z_i \psi}{kT}\right) + C_1 \quad (68)$$

A second integration yields Equation (69):

$$\int \frac{d\psi}{\left\{ \left(\frac{2kT}{\epsilon}\right) \sum_{i=1}^n n_{i0} \exp\left(\frac{-e z_i \psi}{kT}\right) + C_1 \right\}^{1/2}} = \pm \int dx + C_2 \quad (69)$$

Where c_1 and c_2 are evaluated from the appropriate boundary conditions. A detailed discussion for the case $i=2$ is given by Verwey and Overbeek (1948). This specific case of a symmetric electrolyte solution will be used to model the EPG phenomenon as an interacting double layer effect. It is important to note that the application of the P.B. equation to spherical particles was carried out by Derjaguin and Landau at about the same time that Verwey and Overbeek presented their results (discussed by van Olphen 1977). Hence this theory has been termed DLVO theory.

Introducing the restriction of considering only solutions containing ions of one valency, i.e., ions assumed to have originated in a symmetrical electrolyte, one may write : $z_+ = z_- = z$.

Therefore: $n_+ - n_- = n (\exp(-ze\psi/kT) - \exp(ze\psi/kT))$ (70)

but: $\sinh a = \frac{\exp(a) - \exp(-a)}{2}$

therefore: $\rho = -2nze \sinh(ze\psi/kT)$

Since the circumference of the EPG probe is much greater than the dimensions of the double layer, it is reasonable to treat an infinitesimal region of the probe surface as a flat plate. For a flat double layer, ψ is only a function of the x (normal) coordinate. If one now introduces the substitutions:

$$y = ze\psi/kT \quad w = ze\psi_0/kT \quad \kappa^2 = \frac{2ne^2 z^2}{kT} \quad \xi = \kappa x \quad (72)$$

Equation (65) is transformed into the more convenient form:

$$\frac{d^2 y}{d\xi^2} = \sinh(y) \quad (73)$$

With respect to the problem of colloid stability, Equation (73) was applied by Verwey and Overbeek (1948) to a model system composed of two parallel plates (symbolizing two colloidal particles with plane surfaces) immersed in an electrolyte solution and close enough for the double layers to interact and influence each other. Hence, in DLVO colloid stability theory, the quantities of interest are the distribution of charges and the electric potential function in the solution layer between the two plates. In particular, Verwey and Overbeek (1948) examine the limiting and highly symmetrical situation where the particles are identical and of constant surface potential. These assumptions produce boundary conditions necessary to solve Equation (73). Other workers have extended DLVO theory to include nonidentical

particles of either constant surface potential, constant surface charge or one surface of each type (Bierman 1955; Bell and Peterson 1977). However, these types of analyses involve calculating the force between the two charge bearing surfaces. Assumptions must be made about the types of particles involved. For example, Bierman (1955) treated the case where the charge on the particle surface had its origin in the adsorption of potential determining ions from the solution onto the surface. The inclusion of a surface chemical potential term in the equilibrium determination of the force between the two plates leads to the development of an expression for the functional relationship between ψ_0 and x .

In the case of the EPG system, the standard treatments of extended DLVO theory must be modified. For example, it is often argued that the pressure gradient and electrostatic forces between the colloidal particles must cancel (Bierman 1955). However, in the case of the EPG system, the osmotic pressure developed in the solution between the metal surface and the surface of the polyelectrolyte gel (due to the accumulation of ions in the two double layers) will have no effect on the metal probe. Likewise, the rubber elasticity and internal osmotic pressure of the gel will tend to resist the interfacial osmotic pressure buildup. In addition, while the polyelectrolyte gel surface may be considered as one of constant charge (for a given pH and ionic strength), the metal surface is neither of constant charge nor constant potential. Another problem involves the validity of smearing out the charges over the gel (cell wall) surface when, in fact, the distances between charges on that surface are probably of the same order of magnitude as the

distance from the cell wall to the probe surface. As a result of these nonideal conditions care must be exercised in applying extended DLVO theory to the EPG system. On the other hand, it seems inherently reasonable to assume that, since the two surfaces are probably within a Debye length of each other (see p. 86), double layer interactions will occur.

Since Equation (73) is a second degree nonlinear differential equation two boundary conditions are required to obtain a specific solution. Unfortunately, under nonideal (i.e., in vivo) conditions, it is impossible to determine any boundary conditions with certainty. Therefore, several assumptions must be made in order to generate solutions to Equation (73). These assumptions are: (a) the measured EPG voltage is the electric potential of the metal w.r.t. the Ag/AgCl reference electrode; (b) the surface of the cell wall may be treated as a constant charge surface during any given 'frozen equilibrium' measurement period; and (c) the condition of electroneutrality is maintained within the system composed of the probe surface, the cell wall surface and the intervening electrolyte solution. Given these assumptions, the P.B. equation may be solved numerically using the DARE-P continuous-system simulation language (Wait 1977). Equation (73) is integrated once to yield:

$$\left(\frac{dy}{d\xi}\right)^2 = 2 \cosh y + C_1 \quad (74)$$

At the distance l between the two surfaces, $y=y_1$ and:

$$\left(\frac{dy}{d\xi}\right) = \left(\frac{dy}{d\xi}\right)_1 \quad (75)$$

whence:

$$C_1 = \left(\frac{dy}{d\xi}\right)_1^2 - 2 \cosh y_1 \quad (76)$$

therefore:

$$\left(\frac{dy}{d\xi}\right) = -\sqrt{2 \cosh y + C_1} \quad (77)$$

Output from a Computer Simulation

The DARE-P program has been used to generate solutions to Equation (77) for various values of C_1 . An EPG voltage (PSIM) is used to set the initial value for the electropotential of the metal. The output solution gives ψ (or y) as a function of ξ (or x). The question then becomes; at what value of x is the condition $x=1$ achieved? Guidance is furnished by Gauss's law and the requirement for electroneutrality. Assuming that there are no unneutralized charges within the system (see Figure 5), Gauss's law requires that the electric field at the probe surface be equal and opposite to that at the cell wall surface. Therefore, the simulation is run until this condition is met (for a given value of PSIM).

Numerical output from the DARE-P simulation has shown that the equal and opposite field requirement is met at the value of x where $\psi = -(\psi_0)$. This pattern was observed in all simulations. A characteristic output from a simulation is shown in Figure 6. The inversion symmetry of the system is evident and is more accurately described as 'electrostatic mirror' symmetry. In fact, these results may be reasonably interpreted in terms of 'image force' interactions between the probe surface and the charged groups on the polyelectrolyte gel. When discussing image interactions, the metal is replaced by an electrostatic mirror located in the same position as the metal surface (Bockris and Reddy 1970). Therefore, the test (cell wall) charge will have an image

Electroneutrality $\Rightarrow \rho_{\text{tot}} = 0$

Figure 5. The Debye atmosphere at the probe/tissue interface with induced image charges on the probe ($x \leq 2/\kappa$).

Figure 6. Computer simulation of the electric potential function, for a given value of the EPG probe potential using DLVO theory and Gauss's Law.

System parameters: (1) $T = 300\text{k}$; (2) 1:1 symmetric electrolyte at 0.1 M; (3) electric potential at the metal surface, $= + 200\text{ mV}$; (4) C_1 (=AK in the DARE-P program) $\cong 6.6$.

Figure 6. Computer simulation of the electric potential function, for a given value of the EPG probe potential using DLVO theory and Gauss's Law.

charge of equal magnitude and opposite sign as far behind the mirror as the test charge is in front of it. The image interaction between the test charge and the metal is given by the coulombic interaction between the image charge and the test charge (Bockris and Reddy 1970). Changes in the induced image charge will affect the electric potential of the probe. These changes in the induced image charge could result from changes in the distance between the probe surface and the cell wall surface. Alternatively, a modification of the charge on the cell wall surface could also affect the image force interaction. Simulation modeling provides evidence to support the former case. As shown in Figure 7 a variation of 300 mV in measured potential may be accounted for by a change of approximately 2.5 nm in the distance between the two surfaces. It is important to keep in mind that the electropotential values shown in Figure 7 are relative. The actual EPG measurement made with an Ag/AgCl reference electrode would be expected to differ by some constant value. However, it is still valid to conclude that as the two surfaces approach one another, the measured EPG voltage will drive negative (i.e., decrease). This small variation in x could result from a slight expansion or contraction in the volume of the polyelectrolyte gel (see p. 31). It is worth noting that the apparent discontinuity in the slope of ψ shown in Figure 7 is due to the lack of a voltage value at 2nm.

A microscopic picture of the ionic structure at the probe/tissue interface that could account for the exact inversion symmetry seen in Figure 6 is difficult to envision. Only two real possibilities exist: (a) the double layer at the metal surface is equal in charge density and

Figure 7. Computer simulation of the measured EPG potential as a function of the probe/cell wall surface distance. -- Except for the electric potential at the metal surface (now variable), the system parameters are the same as in Figure 6.

opposite in sign to that on the cell wall surface at $x \gg 1/\kappa$; and (b) the double layer at the metal surface is much smaller than that on the cell wall surface at $x \gg 1/\kappa$. In the former case, as wound healing occurred and the two surfaces approached, the Debye atmospheres of the two surfaces would begin to overlap and neutralize one another. This mechanism presupposes that the two surfaces have opposite electrostatic potential values. In order to maintain electroneutrality, image charges on the surface of the probe would interact directly with the charged groups on the cell wall surface. In the second, and more electrochemically reasonable case, only the cell wall surface has a significant Debye atmosphere when the two surfaces are separated. As the two surfaces approach, the Debye atmosphere of the gel would induce an atmosphere of counterions in the thin fluid lamina between itself and the metal surface. This 'counter Debye atmosphere' then would induce an equal and opposite image charge in the metal. This sequence of events is shown in Figure 8. Kinetically one may argue as follows. The Debye atmosphere of the gel surface is not a fixed sheath of ions but rather the result of counterions spending more time, on average, in the region adjacent to the charged groups on the gel. In most treatments of double layer theory there is an infinite (at least relative to the dimensions of the double layer) bulk solution in the direction normal to the surface of interest. Therefore, only a double layer is formed, i.e., the excess of coions in the lamina adjacent to the Debye atmosphere of counterions is 'distributed' in infinitesimal amounts throughout the theoretical bulk solution. This idealized situation is shown in Figure 9. In the case of the EPG system, the distance between the two surfaces

Figure 8. Induction of image charges on the probe surface by the Debye atmosphere.

Figure 9. The difference between the formation of a Debye atmosphere adjacent to a bulk medium (a) and a thin lamina (b).

is of the same magnitude as the Debye length ($\sim 1\text{ nm}$ for a 0.1 M, 1:1 electrolyte solution). This being the case, the anisotropy involved in double layer formation cannot be ignored or 'distributed' away. When the gel surface moves to within $2/\kappa$ of the metal surface, a counter Debye atmosphere is automatically created in the region adjacent to the metal. This phenomenon is a direct result of the dimensions of the interfacial region. As the two surfaces move even closer, the Debye atmospheres will begin to neutralize one another and the charges on the gel surface will be compensated for by the induced image charges on the metal surface. Voltage fluctuations would then be expected to occur only at distances $\lesssim 2/\kappa$. Output from the model shown in Figure 7 tends to support this theory. It may also be seen from Figure 8 that, as the two surfaces approach, the positive mirror charge on the metal surface is expected to increase. The electrons driven from the surface will raise the Fermi energy of the conduction band and drive the electro-potential measured at the other end of the wire in the negative direction. This result is in agreement with the output of the double layer model, i.e., when the surfaces approach the voltage drops.

It may be seen from Figure 7, that beyond $x = 2.5\text{ nm}$ the ψ function can go to $+\infty$ with no further variation in x . This behavior may be interpreted as follows. When the cell wall is withdrawn to a point where image force interactions (including those mediated by the Debye atmosphere) with the probe no longer occur, the functional relationship between the probe electropotential and the distance to the gel surface will break down. In the simulation, this occurs at 2.5 nm when PSIM = 200 mV (see the legend on Figure 6 for other system parameters).

It is reasonable to argue that 2.5nm represents the maximum distance at which image force interactions occur. This being the case, the potential at the metal surface due to the charges on the cell wall will be zero. Choosing an individual charge on the cell wall surface, the condition of zero induced potential at the metal surface implies:

$$\psi_{\text{metal}} = 0 = \frac{4\pi q_{\text{cell wall}}}{(\epsilon_{\text{sln}}) (r \equiv x \sim 2.5 \times 10^{-7} \text{ cm})} \quad (78)$$

The electrostatic system is being used in order to obtain the dielectric constant of the medium. Bockris and Reddy (1970) have argued that the distance at which image forces become significant in vacua is 1×10^5 cm. In analogy with Equation (78) one may write:

$$\psi_{\text{metal}} = 0 = \frac{4\pi q_{\text{cell wall}}}{(\epsilon_0 = 1) (1 \times 10^5 \text{ cm})} \quad (79)$$

Setting these two expressions equal to one another and canceling terms, one is left with Equation (80):

$$\epsilon_{\text{sln}} \cong 40 \quad (80)$$

An effective dielectric constant of approximately 40 is reasonable in a 2.5nm interfacial region composed of a 0.1M (1:1) electrolyte with two highly oriented hydration sheaths at the opposite surfaces.

ANATOMICAL STUDY VIA FREEZE-FRACTURE SCANNING ELECTRON MICROSCOPY

Materials and Methods

Placement of Probes

All experiments were conducted on Gossypium hirsutum L. cv. 'Deltapine 61' grown under field conditions at the University of Arizona Water Resources Center. Probes were inserted into fully elongated petiole or peduncle tissue. Measurements on a large number of plants provided a quantitative relationship between the degree of elongation of the petiole or peduncle proximal to the main stem on an axillary branch and the position of the node from which the axillary branch originated. The measurements involved in establishing this relationship will be reported elsewhere (Rugenstein n.d.). It was determined that at or below the 11th node down from the apical meristem, the proximal peduncle or petiole of the axillary branch was fully elongated.

The working EPG probe is a piece of palladium wire (250 μ m diameter) cut off to a length of 6 to 8 mm and soldered to two strands of #38 wire stripped of their insulation coating and additional strands for a distance of approximately 4.0 cm from the solder joint. Probes were inserted perpendicular to the plant surface to a depth of 2 to 4 mm with a pair of small needle-nosed pliers. No attempt was made to taper the probes. To avoid cantilever action by a probe, the inertia of the wire was reduced after probe insertion by appressing it to the

plant with double sided velcro tape at a point approximately 1 cm down the axillary branch from the petiole or peduncle. These techniques are standard preparative procedure prior to making an EPG measurement.

The insertion wound was allowed to heal for at least 14 days before the petioles or peduncles were harvested for the electron microscope study.

Preparation of the Tissue for Freeze-Fracture Scanning Electron Microscopy

The two strands of wire were gripped with a pair of #7SA Inox approximately 0.5 cm from the solder joint and the wires were cut on the side distal to the probe with a pair of bone cutting shears. Petioles and peduncles were cut from the plant using a scalpel and further trimmed on ice in a glass petri plate filled with water to a distance of 2 to 3 mm on either side of the probe insertion point. The samples were placed immediately in 5% (v:v) glutaraldehyde in wash buffer (0.06 M K_2HPO_4 ; 0.02 M KH_2PO_4 ; pH 7.2) on ice. To aid the initial fixation process, the tissue was subjected to vacuum infiltration for 5 minutes within the first hour. At all times after removal from the plant, the tissue was handled in a liquid medium using #3 Inox to minimize the potential for stress induced damage to the probe/tissue interface.

Glutaraldehyde fixation was carried out for 12 hours followed by 4 hours in wash buffer with two changes at 4C. The specimens were dehydrated by immersion in two changes of 2,2 - dimethoxypropane over 1 hour and then placed in 100% ethanol for 24 hours with several changes. Each specimen was then scored with a fresh scalpel blade on either side and

opposite to the point of probe insertion. The tissue pieces were immersed in liquid N₂ for approximately 10 minutes and then fractured under the liquid N₂ by gripping the opposite ends of the specimen with two small hemostats and exerting a force directed outward and perpendicular to the direction of probe insertion. Fractured specimens were returned to 100% ethanol precooled to -20C and then allowed to warm gradually to room temperature. The material was then transferred to 100% acetone for 12 hours with several subsequent solution changes. Critical point drying was carried out using the Polaron (Ted Pella Inc.) apparatus. Due to the large size of the samples, a prolonged (≥ 12 hours) solvent exchange period was necessary. Otherwise the procedure specified by the manufacturer was followed explicitly. Specimens were mounted on aluminum stubs using either a colloidal carbon or silver base medium, sputter coated at 15 mamp for 1.5 minutes with a Polaron SEM coating unit (Ted Pella Inc.) and examined with an ISI-DS130 scanning electron microscope.

Results

Figures 10 and 11 show the characteristic response of mature (fully elongated) cotton petioles and peduncles to insertion of the probe. It may be seen from Figures 10A, 10B, and 11A that wound regeneration has occurred. Following the penetration wound inward from the epidermis, callus formation is most pronounced in the first ~0.5 mm along the probe and appears to involve primarily redifferentiated collenchyma, cortex and phloem cells. These cells appear smaller than the adjacent tissue and are irregularly oriented (Figure 12). This

Figure 10. Composite of four freeze-fracture scanning electron micrographs (FFSEMs) showing significant aspects of wound healing in a cotton petiole in response to probe insertion.

- (A) Transverse freeze-fracture scanning electron micrograph (FFSEM) of a petiole, through the plane of probe insertion. The probe is seen penetrating a vascular bundle. Disruption of the in vivo longitudinal flow of tissue is limited to less than 0.5 mm normal to the probe in the cortical region of greatest wound healing response and is minimal elsewhere (22x, bar = 1 mm).
- (B) FFSEM showing the lack of wound regeneration in the xylem region. The random orientation of the phloem tissue immediately to the right of the white box indicates a wound regeneration response. The area of greatest regenerative activity is seen in the area between the subepidermal and cortex regions. Inset shows the xylem tissue in greater detail (44x, bar = 500 μ m).
- (C) FFSEM of the progression from physically crushed to intact xylem elements within 3 or 4 cell layers. No wound regeneration is apparent. Inset shows the appression of the cell wall material to the probe surface (598x, bar = 50 μ m).
- (D) FFSEM exemplifying complete wound closure between a crushed xylem element cell wall (CW) and the probe surface (PR) (10,600x, bar = 1 μ m).

Figure 10. Composite of four freeze-fracture scanning electron micrographs (FFSEMs) showing significant aspects of wound healing in a cotton petiole in response to probe insertion.

Figure 11. Composite of four FFSEMs showing significant aspects of wound healing in a cotton peduncle in response to probe insertion.

- (A) Transverse FFSEM of a peduncle through the plane of probe insertion. The pattern of wound regeneration is similar to the petiole. Normal tissue flow is maintained within 0.5 mm of the probe in the cortical regions of greatest regenerative activity. Minimal disruption is observed elsewhere (30x, bar = 500 μ m).
- (B) Analogous to Figure 2, showing the physical disruption of xylem elements in the peduncle and the return to apparently intact tissue within a few cell layers. No wound regeneration is seen (156x, bar = 100 μ m).
- (C) Complete wound closure in the cortex region of the peduncle. Callus tissue is oriented mainly parallel to the probe surface. Inset shows the probe/tissue interface (790x, bar = 10 μ m).
- (D) Enhanced view of the probe/tissue interface showing the apparent contiguity of the two phases. Cell wall (CW), probe surface (PR) (11,500x, bar = 1 μ m).

Figure 11. Composite of four FFSEMs showing significant aspects of wound healing in a cotton peduncle in response to probe insertion.

Figure 12. Composite of four FTSEMs showing additional significant aspects of wound healing in a cotton petiole in response to probe insertion.

- (A) Wound healing response when a probe is inserted into immature petiole tissue. A substantial amount of callus is formed along the entire length of the probe/tissue interface. However, wound closure still occurs (56x, bar = 100 μ m).
- (B) The wound is effectively sealed off from the external environment by the formation of a collar-like structure composed of wound callus in the mature petiole. Inset shows the contiguity of the cell wall material with the probe surface (116x, bar = 100 μ m).
- (C) The degree of involvement by the major tissue types in the wound healing response. The xylem (X) and phloem (P) are labeled for reference to the enlargement shown below (130x, bar = 100 μ m).
- (D) The involvement of the phloem region in wound regeneration is shown in this micrograph (338x, bar = 50 μ m).

Figure 12. Composite of four FFSEMs showing additional significant aspects of wound healing in a cotton petiole in response to probe insertion.

tissue would be considered as "wound callus" in the terminology of Sussex, Clutter and Goldsmith (1972). However, as one traces the probe/tissue interface further into the region of xylem tissue there is little evidence of wound callus. This may be seen most clearly in Figure 10B. The region outlined in white (shown in enlarged view) contains no visible wound callus, rather there appears to be simple progression of cells with increasing structural integrity as one moves away from the probe/tissue interface. Figures 10C and 11B show this gradient beginning with totally crushed, heavy-walled xylem vessels and returning within a few cell layers to intact xylem. Wound callus adjacent to the probe (Figures 11C and 11D) as well as disrupted vascular tissue (Figure 4) may appear tightly appressed to the probe surface. Within the limits of resolution of the FFSEM techniques, Figures 10D and 11D would be considered as examples of complete wound closure. Regardless of the tissue involved, the contact layer appears to be composed of disrupted, compressed cell residue which has formed into a cicatrice-like layer (Wylie 1930b, 1931).

In order to minimize tissue damage during probe placement, it is necessary to wait until the cell elongation process has finished. A detailed microscopic examination of cotton petiole and peduncle responses to probe insertion at different stages of development has been conducted by Rugenstein (n.d.). It is sufficient here to note that the wound regeneration response in immature tissue involves a large amount of wound callus extending throughout the entire region of probe/tissue interaction (Figure 12A). This observation is in agreement with the results of Sussex et al. (1972). In Nicotiana tabacum, those workers

found that wound meristem size was largest when the wound was made closest to the shoot apex, which corresponds to more juvenile tissue. Rugenstein (n.d.) has also noted this relationship between age and amount of callus formation. In fully elongated petioles and peduncles generation of significant amounts of wound callus appears to be restricted to the cortical parenchyma between the phloem and the collenchyma. In these mature organs the xylem region and the pith seem to respond to the wound with little or no production of new cells (Figures 10A, 11A, 12C). The metaphloem does become involved in the dedifferentiation/redifferentiation response, as shown in Figures 12C and 12D. Figure 13 compares the responses of petioles at different stages of development to probe insertion.

Conclusions from the Study

Lipetz (1970) has stated that in most higher plant tissues, cells adjacent to a wound are stimulated to respond with dedifferentiation and subsequently division and redifferentiation. However, Mollenhauer, Whaley and Leech (1960) did not observe the transmission of this stimulus across even one cell layer. In Gossypium it appears that the degree of wound regeneration that occurs in response to probe insertion is a function of both the type of tissue involved and its physiological age. Sussex et al. (1972) observed some wound tissue derived from the proliferation of the vascular tissue. In Gossypium, the secondary phloem is located between the secondary xylem and the lignified fiber bundles adjacent to the cortex (for a definitive discussion of Gossypium anatomy see Baranov and Maltzev 1937). Figures

Figure 13. Composite of four surface scanning electron micrographs showing the effect of tissue maturity on the wound healing response in a cotton petiole.

The composite represents petioles at axillary positions 7, 5, 2 and 1 (A, B, C and D, respectively) distal to the apical meristem. Two weeks after insertion the petioles were removed and examined directly in the scanning electron microscope.

Figure 13. Composite of four surface scanning electron micrographs showing the effect of tissue maturity on the wound healing response in a cotton petiole.

10B and 12C show that wound regeneration involves all tissue layers down to and including the phloem cells. The wound regeneration response in the xylem tissue ranges from one or two wound callus layers to non-existent (Figures 10B and 11B). Obviously, the mature nonliving tracheary elements cannot regenerate. In addition there is apparently little or no response to wounding by either the fibers or the xylem parenchyma. Lignification of the fibers could play some role in the inhibition of dedifferentiation. A small amount of wound callus is observed adjacent to the probe in the pith region. A detailed analysis of the tissue configuration resulting from wound healing around the probe is presented by Rugenstein (n.d.).

In terms of the electrophytogram system, the data presented here must be interpreted with respect to the degree of wound closure and the extent of the tissue disruption in the region adjacent to the probe. The argument for wound closure at the surface (i.e., at the interface between the plant and the environment) is very strong. Even when immature tissue is wounded, resulting in severe hyperplasia, a collar-like structure is formed around the probe (compare Figures 12A and 12B). This collar of wound callus cells, which may be wound periderm (Esau 1960) depending on the degree of post-wound redifferentiation, is contiguous to the probe surface in the cortical area (Figures 11C, 12A, and 12B). The question remains as to whether the other tissue layers that border the probe are also tightly appressed. There are regions shown in the various transverse fracture faces where the tissue is not contiguous with the probe surface. This is especially obvious in the xylem tissue shown in Figures 10B and 11B. The limitations on

experimental approaches to examining this region with the probe in situ are obvious. It may be argued prima facie that any disturbance caused by the fixation procedure would tend to induce or enhance gaps between the probe and the cell wall material. In particular, cantilever forces applied to the probe, physical stresses created while cutting, or any shrinkage of the cell wall material during the dehydration process would destroy some or all of the contiguity along the interface. It is not possible, using in vitro techniques, to unequivocally resolve the nature of the origin of regions where complete wound closure is not observed.

The extent of tissue disruption is defined for this system as the number of wound induced callus cell layers between the probe and uninjured tissue. Based on the data provided by the transverse fractures, this number may range from a dozen or more layers in the subepidermal wound callus to virtually none in the xylem tissue. It is therefore concluded that insertion of a noble metal microprobe, during the proper developmental stage, does not cause disruption of the tissue configuration of cotton petioles or peduncles to a degree that would interfere with the metabolic functions performed by these organs. This conclusion is supported by the fact that leaves and bolls associated with petioles and peduncles used for continuous EPG measurements continue in their normal cycle of growth and development throughout the season (Gensler n.d.). It is further concluded that, within the limits of the FFSEM system employed, complete wound closure occurs over much of the length of the probe. It is postulated that there is complete wound closure over the entire length of the probe in vivo.

The proper physiological interpretation of the EPG signal depends a great deal on the question of wound closure. This will also be true for future, more sophisticated techniques such as ion-specific microelectrode implants (Wen, Chen and Zemel 1979). In terms of plant electrophysiology, complete wound closure would imply that the probe is monitoring the cell wall 'Donnan free space.' Alternatively, if a gap exists, the probe is immersed in an artificially created 'water free space.' Obviously, both types of conditions could exist simultaneously. These regions will differ significantly in their electrochemical properties. In particular the Donnan free space of the cell wall contains immobile macroions which affect the concentration and distribution of mobile ions in the intrafibrillar solution (Slatyer 1967). When a Donnan phase adjoins an aqueous electrolyte solution (water free space) an electric potential difference (Donnan potential) can exist between the two phases. An artificially created water free space adjacent to the probe in Donnan equilibrium with the cell wall solution would be expected to have an electrical potential opposite in sign to the Donnan phase (Briggs et al. 1961). However, a Donnan potential will not necessarily exist between these two phases. The types and concentrations of mobile and immobile ions as well as possible nonequilibrium conditions must be considered in an analysis of the bioelectrochemistry of the probe/tissue interface.

DISCUSSION

In evaluating the three models it is necessary to separate the probable from the possible in terms of the types of physiological processes necessary to "drive" each model. In particular, Model I involves changes in the concentration (or activity) of one or more chemical species within the apoplast. These changes must range over several orders of magnitude before voltage fluctuations large enough to simulate EPG measurements are produced. Examination of Equations (47), (48) and (49) shows that, in the extreme case, the concentration (activity) of a single chemical species will have to change by six powers of ten to give a $\Delta E_{M2,1}$ of $\sim 300\text{mv}$ (the extreme range of measured EPG voltage changes). In Equation (48) it can be seen (with $n=1$) that the ratio of the concentrations of the redox couple must have a value of 1×10^5 in order to yield a $\Delta E_{M2,1}$ of -297mv . Each component of the ratio could change by one power of ten. Since many biological redox couples also act as acid-base pairs, Equations (48) and (49) may also apply for in vivo conditions. The argument is the same here, although it must be kept in mind that 300mv is in the upper range of the voltage changes observed using the EPG technique. Still, a two or three order of magnitude change in the concentration (activity) of one or both of the members of a redox couple and/or the pH must occur in order to generate a significant change in the voltage. Obviously, there are numerous ways to combine changes in the solution variables to yield a total change that could simulate an in vivo EPG voltage shift.

From the physiological viewpoint, there are several objections to Model I. Firstly, a great deal of data exist to support the hypothesis that, beyond the Casparian strip in the root, the concentration of ions in the xylem (and therefore in the apoplast) is under strict metabolic regulation (Anderson 1976; Cram 1976). Only when the plant is undergoing severe and prolonged water stress, induced by extremely dry soil conditions, is the internal concentration of solutes seen to change dramatically (Anderson 1976). Under the irrigated field conditions where the EPG system is being tested, it is unlikely that this condition will exist. This is especially true of a deep rooted crop such as pecan, a plant with which characteristic EPG voltage fluctuations have been recorded (Gensler n.d.). In addition, it is difficult to identify ionic species in the soil solution that make good candidates for an EPG potential determining redox couple. Very few inorganic redox couples present in the soil and hence in the apoplast, will have the capacity to vary over the range of concentrations required by Equations (47), (48) and (49). For example, in the case of the $\text{Fe}^{3+}/\text{Fe}^{2+}$ couple, both the oxidized and reduced species are present in concentrations which are quite low and of the same order of magnitude (Tisdale and Nelson 1975). Many other candidates have the same shortcoming. In other cases such as:

the concentration of the gaseous component is fixed by its solubility in the aqueous apoplast solution and will not vary except with large

changes in temperature. For example, K_{H_2} (the Henry's Law constant = partial pressure/mole fraction $\times 10^6$) goes from 4.4 to 5.8 as the temperature goes from 0°C to 50°C (Hodgman 1963).

It is also assumed that there are no significant concentrations of organic solutes (e.g., $NADH_2/NAD$) in the apoplast. Therefore, from the physiological standpoint, the most likely inorganic species that could routinely modify the redox potential of the apoplast is the H^+ ion. It has been shown experimentally that, during certain growth processes, the pH of the apoplast undergoes substantial change. During indole-acetic acid-mediated cell wall expansion, the pH of the apoplast can drop from 7 to 5 (Raye and Cleland 1977). This type of effect may also be involved in the phytochrome-mediated electric potential changes observed in plants (cf. Newman and Briggs 1972). It is also known that both photosynthetic and respiratory processes significantly alter the pH of the cell (Raven 1976). This may occur either indirectly as a result of a change in the concentration of dissolved CO_2 or directly through electrogenic pumping of H^+ . However, except in the case of CAM plants, there are no physiological processes in the literature at this time that would cause periodic (e.g., diurnal) fluctuations in the apoplastic pH of mature, fully differentiated tissue to the extent necessary to simulate EPG voltage fluctuations.

The conclusion stated above applies only to presently known or postulated processes in plants. Since it has been shown (Appendix A) that the EPG probe can act as a pH sensitive electrode in vitro, as well as a redox electrode, one cannot dismiss the possibility that the

apoplast does undergo large pH fluctuations. It will be necessary to conduct control experiments before any final conclusion is possible.

From a physiological standpoint, it is almost necessary to dismiss Model II out of hand. Even though many membrane physiologists now consider it probable that there is always an electrogenic component of the total membrane potential (Luttge and Higinbotham 1979), any unneutralized charges pumped through the membrane would, presumably, remain in the vicinity of the pumping site due to the large electric field created in that region. This being the case, charges separated across a $\sim 10\text{nm}$ membrane would exert no appreciable net coulombic force on a metal surface $\geq 1\mu\text{m}$ away from the outer plasmalemma surface. Based on the FFSEM data presented (p. 84), the $1\mu\text{m}$ distance (i.e., at least one cell wall width) appears to be an absolute minimum separation distance between the probe and an active electrogenic site. At this time there is no biophysical mechanism that could account for charge separation over any great distance through an apoplastic diffusion free space. Pethig and Szent-Gyorgyi (1979) have discussed the evidence for electronic conduction in proteins. Ionic and electronic current pathways are known to exist in membranes (Walker and Pitman 1976). In both cases some type of structural constraints exist so that an electrochemical potential gradient can be established.

As previously discussed (p. 31), the cell wall phase is capable of behaving as a polyelectrolyte gel. Teorell (1953) has discussed the possible existence of electric potential differences within this type of material. A lack of experimental evidence precludes speculation about a mechanism for generating electric potential gradients in plant cell

walls. It is necessary to note that if such a mechanism existed, electrogenically produced ions could be separated to a point where coulombic interactions between the probe and unneutralized charges could occur. These interactions would be greatly modified by Debye screening effects. In general, Model II appears to be physiologically untenable at this time.

The interacting double layer explanation for the EPG phenomenon (Model III) is by far the most reasonable. Of the three putative physiological bases for the EPG phenomenon presented here, only Model III is in agreement with any of the essential characteristics of electrophytograms.

1. EPG phenomena have been observed in a wide variety of higher plants ranging from pecan and tomato to cotton. The interacting double layer model depends only on the chemical structure of the primary cell wall. Albersheim (1976) has postulated that the primary cell walls of higher plants are similar in terms of macromolecular infrastructure.

2. EPG phenomena occur in all above ground plant organs where the probe can be placed securely (Gensler 1974, n.d.). As with number one above, this may be considered to result from the uniformity of primary cell wall structure within a given plant.

3. Simultaneous measurements using the EPG technique in conjunction with a linear variable differential transducer shows that stem swelling in Gossypium is accompanied by a drop in the measured EPG voltage (Gensler n.d.). Simulation with Model III shows that the voltage drops when the cell wall approaches the probe surface (Figure 7). This would be expected to occur during stem swelling. The magnitude of the

simulated voltage changes are of the same order of magnitude as those observed in vivo.

4. Attempts to inject current into the plant through an EPG probe have shown that the probe is in an extremely high impedance area (Gensler and Silva-Dias n.d.). Only Model III can simulate the large voltage fluctuations observed in vivo in the absence of electron exchange while allowing for electroneutrality in the apoplast space.

In addition to these correlations with experimental data, there exists the FFSEM observation that the distance between the probe and the cell wall surface is probably in the range of two Debye lengths in vivo. Submicroscopic fluctuations in this distance, induced by dehydration cycles, could generate the observed voltage fluctuations. Gensler and Diaz (n.d.) have determined via linear variable differential transducer analysis that the cotton plant stem can contract down to 75% of its fully hydrated diameter under irrigated field conditions. Other factors that could influence volume fluctuations in a polyelectrolyte gel have been discussed (see p. 31). It is likely that the simple DLVO model applied here does not fully simulate the actual conditions at the probe/tissue interface. In view of the preceding discussion, it appears reasonable to suggest that some type of surface interaction is responsible for many of the electrical characteristics observed with the EPG technique.

Control experiments to test the various models are necessary. To test Model I, one must determine changes in the oxidation-reduction and pH of the apoplast fluid. An important first step in this process has already been undertaken by Gensler and Silva-Dias. Using the

technique of cyclic voltammetry, they are attempting to determine directly the types of redox couples present on the probe surface. In addition, both the redox potential and the pH of the apoplast fluid could be determined using a microextraction method similar to that employed by Wright and Fisher (1981) to measure the electropotential of phloem sap in Salix. These workers inserted a micro-salt-bridge into phloem sap exuding from an aphid stylet. Exudation occurs because the phloem sap is under positive hydrostatic pressure. Conversely, if a small hollow tube were inserted into the apoplast and the external end placed under vacuum, fluid exudation would presumably occur (it might occur without the vacuum). To simulate EPG conditions a 250 μ m tube sheathing a somewhat narrower rod could be inserted. After wound healing, the central rod could be withdrawn and the tube connected to a vacuum system. Exuded fluid could be assayed directly with an EPG probe, Ag/AgCl reference electrode set-up. Alternatively, the exudate could be analyzed quantitatively for the presence of redox couples. The pH of the exudate could be measured directly or by fluoremetric techniques if mixed with certain indicator molecules (Witt 1975). Finally, tubes could be inserted to different depths sequentially along the longitudinal axis of a stem or petiole. A functional EPG probe could also be inserted. Electropotential measurements on the exudates could then be compared to EPG measurements relative to the same reference electrode. Changes in the measured exudate electropotential or large variations in pH occurring simultaneously with EPG fluctuations would tend to support Model I.

It is difficult to visualize experiments to test for electro-neutrality within the apoplast space. However, it is possible to propose a simple experiment to determine whether electrogenically produced membrane potentials can be "seen" by an EPG probe a cell wall thickness away. Using the experimental set-up described by Nobel (1974), large (2-10 cm in length, 0.5-2.0 mm in diameter) internodal cells of Chara and/or Nitella may be mounted in a channel or trough. An EPG probe could then be placed against (longitudinal axes parallel) or slightly within (longitudinal axes normal) the cell wall of the alga. An action potential may then be triggered by illuminating the cell with ultraviolet light (Findlay and Hope 1976). As an additional control, the action potential may be monitored directly via the method of Walker (1955). In order to measure action potential-induced electrochemical changes in the apoplast with the EPG probe it would be necessary to modify either the EPG data acquisition system or the method of Walker (1955). In particular, the time scale for discrete EPG sampling would have to be greatly decreased (e.g., from once every 15 minutes to every microsecond) or a continuous voltage signal recorded. Alternatively, a prolonged series of action potentials could be maintained via a constant input of the proper stimulus. The extracellular space would, presumably, take on a new time average electropotential under this condition relative to the same region adjacent to a resting membrane. This new electrogenically induced steady state could be sampled on any time scale by the EPG technique. Electrogenic modification of a membrane potential represents the only known physiological mechanism for generating an extracellular region of unbalanced charge. Failure of the EPG probe to

sense the action potential would argue strongly against Model II. Unfortunately, the converse conclusion does not hold since the pH and ionic strength of the apoplast space may also be changing during the propagation of the action potential. Therefore, changes in these two parameters would need to be monitored.

An obvious control experiment for Model III is easy to visualize but difficult to design. It involves inserting an EPG probe that has been modified in such a manner that the cell wall could only approach the metal surface to within a distance much greater than two Debye lengths while allowing the electrolyte solution to reach the metal surface. It might be possible to coat the probe surface with a thin ($\sim 1\mu\text{m}$) layer of porous ceramic (or other type) material. Since the coating could interfere with probe sensitivity it would be necessary to conduct additional control experiments. For example, the coated probe could be tested for pH and ionic strength sensitivity in vitro (see Appendix A).

Another alternative would be to sheath the probe in a fine nylon mesh. This type of material is thick enough ($\geq 10\mu\text{m}$) to prevent double layer effects, assuming that the cell wall material could not bulge through the openings in the mesh. This possibility could be checked to some extent via FFSEM. The logistics of the actual sheathing operation would be involved. The mesh is easily fused with heat. It might be possible to place the probe between two appressed sheets of mesh and simply trace the outline of the wire with a micro-soldering iron using some type of micromanipulation apparatus.

SUMMARY

The purpose of this study was to determine what metabolic or biophysical processes known to occur in plants could result in the electrical potential fluctuations of hundreds of millivolts measured by the EPG technique. A preliminary anatomical study demonstrated unequivocally that the noble metal probe was totally surrounded by cell wall material. In addition, FFSEM evidence strongly implied that, in attempting to achieve complete wound closure, the surface of the probe was placed within a few nanometers of the cell wall surface. This being the case, the EPG phenomenon was postulated to be a measurement of some electrochemical property of the apoplast space within the plant.

Three theoretical models have been developed to provide a physiological basis for electrophytograms. The first model considers the EPG as a variable electrode potential resulting from changes in the populations of redox couples and/or the pH within the cell wall. The physiological feasibility of this model depends on the types of flow processes occurring in the apoplast. An extensive review of the current literature on transport in plants failed to turn up any metabolic process that would routinely modify the redox potential of the apoplast. Significant (i.e., 1 pH unit or more) fluctuations in the H^+ concentration of the apoplast may result from electrogenic H^+ pumping during photosynthesis. However, no definitive data exist at this time to confirm this so-called pH-stat mechanism in higher plants (Raven 1976).

Current models for xylem and phloem transport do not involve large scale changes in the concentrations of ionic redox couples or pH in the apoplastic region adjacent to these tissues.

A second model was developed to deal with the possibility of unbalanced charges within the apoplast region. At the present time, no physiological mechanism exists by which such charges could move into the cell wall space. However, our knowledge of the biophysical properties of this region is still quite limited. In particular, this author postulated that the physicochemical properties of the polyelectrolyte gel phase may give the cell wall many of the characteristics of an ionic membrane. Recent work by VanderMolen et al. (1977) on vascular gelation clearly demonstrates a capability for dynamic apoplast behavior previously considered unlikely by plant physiologists.

The third model for the physiological basis of electrophytograms involves surface (double layer) interactions between the probe and the polyelectrolyte gel component of the cell wall. It is successful in simulating the observation that stem swelling is accompanied by a drop in the measured EPG voltage. This model also explains why similar EPG phenomena are observed in a wide variety of higher plants under a variety of environmental conditions. The interacting double layer model can also account for the extremely high resistance to current injection observed during attempts to employ the techniques of cyclic voltammetry with the EPG system. This model is further supported by electron microscopic data showing the cell wall surface no more than 10nm from the metal surface. Considering the extreme hygroscopic nature of the cell wall polymers, a certain amount of contraction had

undoubtedly occurred during preparative ethanol dehydration. This being the case, it is probable that the two surfaces are within two Debye lengths of one another in vivo. At these types of distances, it is reasonable to propose that surface effects will play an important if not dominant role in the electrochemistry of the system.

It is, of course, possible that several physiological processes are occurring simultaneously so that the voltage fluctuations are a result of effects predicted by two or all three of the models.

APPENDIX A

RESPONSE OF THE EPG PROBE TO CHANGES IN IONIC STRENGTH AND pH IN VITRO

In order to observe the effect of changes in ionic strength and pH on the voltage measurement by the EPG system, in vitro measurements were made. The experimental set-up used a 400 ml beaker containing 200-300 ml of solution. The solution was stirred continuously with a magnetic stir bar. An EPG probe was immersed in the solution after passage through a piece of glass tubing that had been taped to a pH electrode. The two porous ceramic plugs of the reference electrode (actually two Ag/AgCl electrodes) were also immersed. The complete experimental set-up is shown in Figure A-1.

Using this set-up, pH measurements could be made simultaneously with EPG voltage measurements. In order to account for drift in the reference electrode, the voltage between the Ag/AgCl electrode connected to the actual EPG measuring system (i.e., that formed the Galvanic cell with the palladium microprobe) and the second Ag/AgCl electrode present in the reference electrode was monitored continuously.

The experiment consisted of placing a salt solution of known molarity into the beaker and then varying the pH between ~4 and ~9. The initial pH of the salt solution was adjusted down to 4 with an acid solution containing the identical anion (e.g., HCl for KCl, etc.). Because of a low buffering capacity in many cases, titration to exact

Figure A-1. Experimental set-up for measuring in vitro EPG voltages.

pH values could not always be achieved. In an approximate manner, the pH was raised to ~ 9 in 1 pH unit increments. The digital voltage measurement was read after 5 minutes at a given pH value unless it was the first measurement on a new solution, in which case 15-30 minutes was allowed for the voltage to stabilize. After achieving a pH ~ 9 , the solution was back titrated to ~ 4 using a base containing the identical cation (e.g., KOH for KCl, etc.).

The results of these experiments are shown in Table A-1. They may be summarized as follows: (1) in all cases, the EPG probe was pH sensitive. The response appeared linear with an increase in pH (decrease in H^+ concentration) resulting in a decrease in the measured voltage. As the pH varied from ~ 4 to ~ 9 , a variation in electric potential of $\geq 100mV$ was observed in almost all cases; (2) in all cases except that involving a divalent cation, a decrease in ionic strength resulted in an increase in measured voltage. In general, decreasing the ionic strength from 0.1M to 0.001M resulted in a voltage increase of $\geq 100mV$. In all cases, the voltage measurements were within the range of in vivo EPG measurements (i.e., 0-400mV positive w.r.t. the reference electrode). It is of interest to note that increasing the ionic strength of the solution will result in a decrease in $1/K$ (the Debye length) at the probe/solution interface. This results in a drop in the measured EPG voltage in vitro.

Further experiments have demonstrated that EPG probes made from different materials (Palladium, stainless steel, carbon) gave different voltages when immersed in the same salt solution. These voltages varied from $\geq 100mV$ (stainless steel) to $\geq 100mV$ (Palladium). Since there were

Table A-1. In vitro EPG voltage measurements.

	Solution & pH (± 0.1)	R ₁ w.r.t. R ₂ (mV)	EPG w.r.t. R ₁ (mV)
0.1M KCl	4.4	17.9	218.0
	5.7	17.8	190.0
	6.6	17.6	180.0
	7.4	17.6	174.0
	8.6	17.6	150.0
	9.3	17.5	150.0
	-	-	-
	7.2	17.5	167.0
	6.6	17.4	180.0
	5.0	17.4	205.0
	4.5	17.4	219.0
0.01M KCl	4.3	20.1	307.0
	5.8	20.1	272.0
	7.0	20.2	237.0
	8.2	20.4	217.0
	8.9	20.4	190.0
	8.1	20.4	217.0
	6.6	20.4	254.0
	5.2	20.6	318.0
	4.1	20.5	350.0
0.001M KCl	4.6	19.4	360.0
	5.3	19.6	340.0
	6.5	19.9	300.0
	7.5	20.0	270.0
	8.4	20.1	240.0
	9.0	20.1	212.0
	8.5	20.5	222.0
	-	-	-
	6.5	20.7	315.0
	5.0	21.1	360.0
0.1M K ₂ HPO ₄	9.1	21.4	155.0
	8.2	21.6	184.0
	7.4	21.6	200.0
	6.7	21.6	234.0
	5.7	21.6	256.0
	4.5	21.4	306.0
	5.0	21.4	300.0
	6.2	21.4	236.0
	7.4	21.4	218.0
	8.3	21.4	192.0
	10.9	21.4	72.0

Table A-1---continued In vitro EPG voltage measurements.

Solution & pH (± 0.1)		R ₁ w.r.t. R ₂ (mV)	EPG w.r.t. R ₁ (mV)
0.001M K ₂ HPO ₄	9.2	21.6	172.0
	8.0	21.6	207.0
	6.8	21.7	262.0
	5.4	22.1	330.0
	3.7	22.4	415.0
	5.4	22.2	307.0
	6.8	22.4	274.0
	8.3	21.9	212.0
	9.0	21.4	190.0
0.1M Na ₂ SO ₄	4.3	18.0	290.0
	5.5	17.9	260.0
	6.8	18.2	180.0
	7.8	18.2	130.0
	9.0	18.4	100.0
	7.5	18.7	135.0
	6.6	18.6	185.0
	5.4	18.0	250.0
	4.6	18.5	297.0
0.01M Na ₂ SO ₄	4.4	17.4	340.0
	5.6	17.8	275.0
	6.5	17.8	250.0
	7.8	17.5	170.0
	8.5	17.5	162.0
	9.1	17.6	137.0
	7.4	17.5	192.0
	6.5	17.8	261.0
	5.6	17.8	275.0
0.001M Na ₂ SO ₄	4.4	17.8	342.0
	4.4	16.6	370.0
	5.5	16.3	300.0
	6.4	16.4	265.0
	7.8	16.3	210.0
	8.7	16.3	190.0
	7.3	16.6	252.0
	6.2	17.0	293.0
	5.1	17.0	350.0
0.1M Mg(NO ₃) ₂	4.0	16.8	400.0
	4.4	15.2	350.0
	5.5	15.2	300.0
	6.3	15.2	250.0
	7.8	15.2	183.0
	8.6	15.2	160.0
	7.8	15.3	180.0
	5.1	15.3	320.0

Table A-1--continued In vitro EPG voltage measurements.

Solution & pH (± 0.1)		R ₁ w.r.t. R ₂ (mV)	EPG w.r.t. R ₁ (mV)
0.01M Mg(NO ₃) ₂	4.4	15.6	340.0
	5.6	15.6	280.0
	6.4	15.6	225.0
	7.7	15.6	192.0
	8.7	15.6	138.0
	7.7	15.6	202.0
	6.4	15.6	250.0
	5.8	15.8	295.0
	4.4	15.6	350.0
0.001M Mg(NO ₃) ₂	4.4	15.5	360.0
	5.6	15.4	275.0
	6.7	15.4	195.0
	7.7	15.4	185.0
	8.6	15.4	145.0
	6.2	15.5	260.0
	4.7	15.6	352.0

no significant concentrations of redox couples present in the various KCL solutions, the measured EPG voltages were apparently the result of double layer phenomena. It is important to note that the potential of Palladium was positive in every salt solution measured. This may be the reason why EPG voltages measured in vivo are always positive w.r.t. the reference electrode.

APPENDIX B

DARE-P CONTINUOUS-SYSTEM MODELING PROGRAM FOR THE SIMULATION OF MODEL III AND CHARACTERISTIC OUTPUT

DARE PORTABLE (VERSION 4.4)

```

$M3
$D1
  Y. = - SQRT ( 2. * ( COSH (Y) ) + AK )
  X = T/AKDEB
  PSI = ( Y* AKB * TEMP ) / ( V * E )
  Q = COSH ( Y )
END
  TEMP = 300.
  AKB = 1.3805*10.**(-23)
  V = 1.
  E = 1.6 * 10. ** ( -19)
  AKDEB = 1. * 10. ** ( 9 )
  PCELL = -0.027
  YC = (PCELL * V * E ) / (AKB * TEMP)
  PSIM = 0.500
  Y = ( PSIM * V * E ) / ( AKB * TEMP )
  FIELDG = (( 8.10 * 10. ** (7)) * V * E ) / ( AKB *
    TEMP * AKDEB
  AK = -2, * COSH ( YC ) + ( FIELDG ) ** 2
  XMAX = 2.5 * 10. ** ( -9 )
  TMAX = 2.5
END
  LIST Y,X,PSI,T,Q
END

```


Table B-1. Characteristic output from the simulation of model.

LIST Y, X, PSI, T, Q				
Time	Y	X	PSI	Q
0.	1.93167E+01	0.	5.00000E-01	1.22488E+08
2.50000E-02	8.76334E+00	2.50000E-11	2.26834E-01	3.19771E+03
5.00000E-02	7.38362E+00	5.00000E-11	1.91120E-01	8.04699E+02
7.50000E-02	6.56901E+00	7.50000E-11	1.70035E-01	3.56331E+02
1.00000E-01	5.98966E+00	1.00000E-10	1.55039E-01	1.99642E+02
1.25000E-01	5.53955E+00	1.25000E-10	1.43388E-01	1.27284E+02
1.50000E-01	5.17069E+00	1.50000E-10	1.33840E-01	8.80211E+01
1.75000E-01	4.85769E+00	1.75000E-10	1.25738E-01	6.43669E+01
2.00000E-01	4.58521E+00	2.00000E-10	1.18685E-01	4.90168E+01
2.25000E-01	4.34360E+00	2.25000E-10	1.12431E-01	3.84987E+01
2.50000E-01	4.12620E+00	2.50000E-10	1.06804E-01	3.09792E+01
2.75000E-01	3.92825E+00	2.75000E-10	1.01680E-01	2.54189E+01
3.00000E-01	3.74625E+00	3.00000E-10	9.69693E-02	2.11927E+01
3.25000E-01	3.57752E+00	3.25000E-10	9.26019E-02	1.79063E+01
3.50000E-01	3.42000E+00	3.50000E-10	8.85246E-02	1.53011E+01
3.75000E-01	3.27206E+00	3.75000E-10	8.46951E-02	1.32017E+01
4.00000E-01	3.13236E+00	4.00000E-10	8.10791E-02	1.14858E+01
4.25000E-01	2.99983E+00	4.25000E-10	7.76488E-02	1.00660E+01
4.50000E-01	2.87357E+00	4.50000E-10	7.43806E-02	8.87831E+00
4.75000E-01	2.75283E+00	4.75000E-10	7.12554E-02	7.87538E+00
5.00000E-01	2.63699E+00	5.00000E-10	6.82568E-02	7.02131E+00
5.25000E-01	2.52549E+00	5.25000E-10	6.53707E-02	6.28851E+00
5.50000E-01	2.41787E+00	5.50000E-10	6.25852E-02	5.65554E+00
5.75000E-01	2.31374E+00	5.75000E-10	5.98898E-02	5.10555E+00
6.00000E-01	2.21275E+00	6.00000E-10	5.72755E-02	4.62509E+00

APPENDIX C

THE RELATIVE ELECTRON POTENTIAL AS AN ALTERNATIVE TO THE REDOX POTENTIAL FOR CHARACTERIZING AN ELECTROLYTE SOLUTION

As discussed on page 46, if a metal electrode is placed into a solution containing redox couples and the system is allowed to come to equilibrium, the electrochemical potential of the electron species must be equal in the two phases. This condition is represented in Equation (83):

$$\tilde{\mu}_e^{(m)} = \tilde{\mu}_e^{(s)} \quad (83)$$

From this relationship it is possible to continue on and relate the electron activity (a_e) to the measured redox electrode potential (see p. 46). The question then arises of whether the $\tilde{\mu}_e$ concept may be extended to a generalized electrolyte solution where the types and concentrations redox couples are unknown. This situation is analagous to measuring the pH of an unknown solution. Even though free protons do not exist, the pH concept has proven useful in characterizing electrolyte solutions. Likewise, the $\tilde{\mu}_e$ concept may be useful, particularly when charges in in vivo electrode potentials are measured. In these types of experimental systems, the chemical nature of the animal or plant fluid is usually unknown.

The usefulness of $\tilde{\mu}_e$ as an alternative indicator of the redox potential of a solution has already been considered by Gerischer (1969,

1972). His discussion centers on electron transfers between two distinguishable quantum states resulting from relatively weak interactions. Since such transitions occur rapidly ($\leq 10^{-13}$ sec), he treats the electron transfer analogously to a spectral transition (i.e., by applying the Franck-Condon principle). Assuming a closed system and neglecting interactions with phonons during the transition, he obtains the simplest equation for the description of electron transfer between a metal and a solution:

$$\nu_e \sim \int K(U_e) \cdot D_- \cdot D_+ (U_e) dU \quad (84)$$

where:

ν_e = the rate of electron transfer

U_e = the electron energy

$K(U_e)$ = the transfer frequency as a function of U_e

$D_+(U_e)$ = the density of occupied electron states with energy U_e

$D_-(U_e)$ = the density of unoccupied electron states (holes) with energy U_e

An obvious hindrance to any extension of this analysis is the need to define the meaning of the density of electron quantum states in an electrolyte solution. As previously discussed (p. 46), the condition of electronic equilibrium between the metal and the solution combined with the energy band model in the solid state argues strongly for the existence of electron energy levels within the electrolyte. Gerischer (1969) has also confronted this problem and has concluded that the existence of electron energy levels in an electrolyte solution may be demonstrated by means of a thermodynamic cycle. For comparison with the solid state, the energy terms in electrolytes must be related to the

same reference state, the free electron in vacuo. This allows the energy levels of an electron donor (reducing agent) or acceptor (oxidizing agent) to be directly related to the electronic work function of the donor or the electron affinity of the acceptor. The thermodynamic cycle of Gerischer (1969) will not be reproduced here. He concludes that the value $-\Delta G_{\text{vac}}^{\circ}$ (identical to $-\Delta G^{\circ}$ for the reaction; $\text{Red}_{\text{solv}} \rightleftharpoons \text{Ox}_{\text{solv}}^{+} + e_{\text{vac}}^{-}$) represents the mean free energy of electrons in the redox electrolyte and is equal to the Fermi energy of the redox system. At electrochemical equilibrium, the Fermi energy of the solution is equal to the Fermi energy of the metal.

In the thermodynamic analysis, individual energy levels were used to demonstrate the free energy relations in a cycle defining electron energy levels in an electrolyte. Gerischer (1969, 1972) recognized that it was necessary to extend the analysis to account for fluctuations in the energy levels of donor and acceptor states. These fluctuations result from changes in the interaction energy of solvation, particularly in a polar liquid. Solute:solute interactions may also occur but are generally neglected in dilute solutions. As a result, the energy levels of the donor and acceptor states must be described by distribution functions, $W(U)$, which characterize the statistics of electrons in donor orbitals or of virtual electrons (holes) in acceptor orbitals in some microstate of interaction with the solvent. The $W(U)$ functions represent the time averages of electron (hole) energies for a single donor (acceptor) molecule or the instantaneous energy distribution over a large population of donors (acceptors) (McQuarrie 1973). Assuming that interactions cause fluctuations in electronic energy levels in both

directions around a most probable energy level ($U_{e,m.p.}^0$), Gerischer (1972) derives a distribution function:

$$w(U) = \frac{1}{(4 LkT)^{1/2}} \exp \left[\frac{-(U - U_{e,m.p.}^0)^2}{4 LkT} \right] \quad (85)$$

The density of states at a particular energy level U is then given by the product of the concentration of the respective species and the distribution function:

$$\begin{aligned} D_-(U) &= [\text{Red}] w_{\text{Red}}(U) \\ \text{or:} \quad D_+(U) &= [\text{Ox}] w_{\text{Ox}}(U) \end{aligned} \quad (86)$$

This is the quantum statistical interpretation of the electron energy levels in an electrolyte solution containing a single redox couple which undergoes the reaction, $\text{Red} \rightleftharpoons \text{Ox}^+ + e^-$, in equilibrium with a metal electrode. The quantum mechanics of electron transfer in this type of system are treated in detail by Bockris and Khan (1979). Meier (1968) and Myamlin and Pleskov (1967) discuss electron transfer across the semiconductor/electrolyte interface. With respect to the more complex in vivo system, the general theory should remain valid. The presence of several chemically distinct redox couples will contribute to the total number of electron states. It is also possible that two species may have electron states close enough in energy to form a single degenerate state. In this case the expression for the density of electron states with energy U would be of the form:

$$D_-(U) = \sum_{i=1}^n [\text{Red}]_i w_{\text{Red}_i}(U) \quad (87)$$

In the case of a solution containing a heterogeneous mixture of redox couples, the definition of the mean free energy of electrons in the electrolyte must also be modified. If an electron is dropped into the solution from the vacuum level a reduction will take place. But which species will be reduced? If only one electron is dropped in, the oxidized species with the greatest electron affinity is the likely candidate for reduction. Therefore, in order to obtain the mean free energy, reductions must be performed on every oxidized species present as shown in Equation (88):

so that:
$$G_{\text{vac, ave}}^{\circ} = - \sum_{i=1}^n \Delta G_i^{\circ} \quad (89)$$

This situation becomes even more complex if two (or more) electron and/or H^{+} exchanging redox couples enter the solution. However, this increase in complexity will not affect the validity of the general treatment of Gerischer (1969, 1972). For example, in the case of a redox couple that also undergoes protonation/deprotonation, one may still write:

Multiple electron and electron/proton exchanges may be handled as a sequence of one electron or one electron/one proton exchanges. Even though an Ox species in one reaction becomes a Red for the next, the electrons involved (and hence their energy levels) are chemically distinct.

APPENDIX D

SYMBOLS AND ABBREVIATIONS

w.r.t.	With respect to
P.D.	Potential Difference
$\tilde{\mu}_e^{(j)}$	Electron electrochemical potential in phase j (joule mole ⁻¹)
FFSEM	Freeze-fracture scanning electron microscopy
E_m	Galvanic cell potential (volt)
$\Delta E_{m2,1}$	Difference of galvanic cell potentials at time 2 and time 1 (volt)
r_H	The effective distance from which electrons may be exchanged or electric field effects occur between the probe and the media(m)
J_s	Solute flow (mol cm ⁻² sec ⁻¹)
D_s°	Free diffusion coefficient in solvent (m ² sec ⁻¹)
K_1, K_2	Wall correction factors (dimensionless)
$C_s^{(j)}$	Solute concentration in phase j (mol ml ⁻¹)
J_v	Solvent volume flow (ml m ⁻² sec ⁻¹)
S_d, S_f	Stearic hindrance restriction factors (dimensionless)
$\bar{C}_s$	Mean solute concentration in pores (mole ml ⁻¹)
$\bar{V}_s$	Partial molar volume of solute (ml)
F_{sw}°	Molar frictional coefficient in infinite medium (joule sec m ⁻¹ mole ⁻¹)
$\Delta P_{2,1}$	Hydrostatic pressure difference phases two and one (nt m)

v	Streaming velocity ($\text{ml m}^{-2} \text{ sec}^{-1}$)
P_e	$E_m nF/RT$
z_i	Charge number (electrochemical valency) of i
ψ	$[(H^+)^2 + K_a (H^+) + K_a K_\beta]$
K_a, K_β	Apparent ionization constants (concentration unit)
S_r, S_o	Total concentrations of redox couples
ϵ_w, ϵ_o	Permittivity constant in water or <u>in vacuo</u> ($\text{coul}^2 [\text{nt m}^2]^{-1}$)
$\vec{E}$	Electric field vector (nt coul^{-1})
$\vec{ds}$	Infinitesimal area element (m^2)
q	Electrostatic charge (coul)
E	Electric field strength scaler (nt coul^{-1})
q_1	Linear charge density (coul m^{-1})
d_1	Infinitesimal length element (m)
r	Radius (m)
∇	Gradient operator $\left(i \frac{d}{dx} + j \frac{d}{dy} + k \frac{d}{dz} \right)$
γ	Modified charge density (coul m^{-3})
ψ, ψ_o	Electrostatic potential at a point or at the origin of a coordinate system (volt)
e	Electron charge (coul)
U_i	Total energy of i (joule)
k	Boltzmann constant ($\text{joule molecule}^{-1} \text{ } ^\circ\text{K}^{-1}$)
D_i	Effective diffusion coefficient of species i in the membrane ($\text{m}^2 \text{ sec}^{-1}$)
F	Faraday constant ($\text{joule volt}^{-1} \text{ mole}^{-1}$)
R	Gas constant ($\text{joule mole}^{-1} \text{ } ^\circ\text{K}^{-1}$)
T	Kelvin temperature ($^\circ\text{K}$)

$\bar{\psi}^{(j)}$	Average electrical potential in j (volt)
$\bar{p}^{(j)}$	Average pressure in phase j (nt m ⁻²)
d_h	Specific mechanical permeability (m ³ kg ⁻¹ sec ²)
$\rho_{el}^{(j)}$	Density of space charge in phase j (coul m ⁻³)
$\mu_i^{(j)}$	Chemical potential of i in phase j (joules mole ⁻¹)
m_i	Mass of i (kg)
g	Gravitational constant (m sec ⁻²)
h_i	Relative height of i in earths gravitational field (m)
n_i	Moles of i
f_i	Contractile force on i (newton)
l_i	Length of i (m)
$a_{e_t}^{(j)}$	Relative electron activity at time t in phase j (concentration unit)
E_o	Standard half-cell reduction potential (volt)
Ox	Oxidized chemical species
Red	Reduced chemical species
y	A dimensionless number proportional to $(=ze\psi/kT)$
z	A dimensionless number proportional to $o (= ze \psi_o/kT)$
κ^2	Debye constant squared $(= 2ne^2 z^2/kT)$
ξ	A dimensionless distance parameter $(= \kappa x)$
ν	Rate of electron transfer (sec ⁻¹)
$K(U_e)$	Transfer frequency as a function of U_e (dimensionless)
$D_{\mp}(U_e)$	Density of occupied (-) or unoccupied (+) electron states with energy U_e
ΔG	Change in free energy (joule mole ⁻¹)
$W(U)$	Statistical mechanical distribution function

L Solvent sheath reorganization energy (joule mole⁻¹)

Red		
solv	Red	Reduced species in association with its most probable solvation sheath
Ox		
solv	Ox	Oxidized species in association with its most probable solvation sheath

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