THE BOTANICAL REVIEW

VOL. 60 JULY–SEPTEMBER 1994 NO. 3

# The Excitability of Plant Cells: With a Special Emphasis on Characean Internodal Cells[1]

RANDY WAYNE

*Section of Plant Biology*
*Cornell University*
*Ithaca, New York 14853, USA*



[1]Dedicated to Professor Noburo Kamiya on the occasion of his eightieth birthday.



*The Botanical Review* 60(3): 265–367, July–September, 1994

> Now in the further development of science, we want more than just a formula. First we have an observation, then we have numbers that we measure, then we have a law which summarizes all the numbers. But the real *glory* of science is that *we can find a way of thinking* such that the law is *evident.*
>
> —Richard Feynman (in Feynman et al., 1963)

> Mathematicians may flatter themselves that they possess new ideas which mere human language is as yet unable to express. Let them make the effort to express these ideas in appropriate words without the aid of symbols, and if they succeed they will not only lay us laymen under a lasting obligation, but, we venture to say, they will find themselves very much enlightened during the process, and will even be doubtful whether the ideas expressed in symbols had ever quite found their way out of the equations into their minds.
>
> —James Clerk Maxwell (cited in Agin, 1972)

## I. Abstract

This review describes the basic principles of electrophysiology using the generation of an action potential in characean internodal cells as a pedagogical tool. Electrophysiology has proven to be a powerful tool in understanding animal physiology and development, yet it has been virtually neglected in the study of plant physiology and development. This review is, in essence, a written account of my personal journey over the past five years to understand the basic principles of electrophysiology so that I can apply them to the study of plant physiology and development.

My formal background is in classical botany and cell biology. I have learned electrophysiology by reading many books on physics written for the lay person and by talking informally with many patient biophysicists. I have written this review for the botanist who is unfamiliar with the basics of membrane biology but would like to know that she or he can become familiar with the latest information without much effort. I also wrote it for the neurophysiologist who is proficient in membrane biology but knows little about plant biology (but may want to teach one lecture on ''plant action potentials''). And lastly, I wrote this for people interested in the history of science and how the studies of electrical and chemical communication in physiology and development progressed in the botanical and zoological disciplines.

## Übersicht

Dieser Überblick beschreibt die Grundprinzipen der Electrophysiologie unter Verwendung eines Aktionspotentials in internodalen Zellen der characean Algen als pädagogeshes Mittel. Die Elektrophysiologie hat sich beim Verständnis von Tierphysiologie und entwicklung als wirksames Mittel erwiesen; dennoch ist sie bisher beim Studium der Pflanzenphysiologie und entwicklung praktish vernachlässigt worden. Dieser Überblick ist im Wesentlichen ein schriftlichen Bericht meiner persönlichen Bemühungen in den letzten fünf Jahren die Grungprinzipen der Electrophysiologie zu verstehen, um sie auf das Studium der Pflanzenphysiologie und entwicklung anzuwenden.

Meine Spezialität ist klassische Botanik und Zellbiologie. Elektrophysiologie habe ich durch das Lesen von vielen Physikbüchern für den Laien erlerut; daneben hatte ich auch die Gelengheit, informell mit vielen geduldigen Biophysikern darüber zu sprechen. Ich habe diesen Überblick für den Botaniker geschreiben, der mit den Grundlagen der Membranbiologie nicht vertraut ist, der aber wissen möchte, dass

er/sie ohne allerzu grossen Aufwand mit dem neuesten Stand der Wissenschaft vertraut werden kann. Ich habe ihn weiterhin für den Neurophysiologen geschrieben, der mit der Membranphysiologie wohl vertraut ist, der aber wenig über Pflanzenbiologie weiss (aber velleicht eine Vorlesung über ''Pflanzenaktionspotentials'' halten möchte). Schlieslich habe ich ihn auch für den Leser geschrieben, der sich für die Wissenschaftsgeschichte interessiert und dafür, wie das Studium der Elektrischen und chemischen Kommunikation in der Physiologie und Entwicklung in den botanischen und zoologischen Disziplinen fortgeschritten ist.

－はじめに－

このレビューでは、車軸藻の節間細胞における活動電位の発生に焦点をあて、電気生理学の基礎的な法則について解説する。電気生理学は、動物の生理学・発生学の分野において強力な道具として力を発揮しているが、植物学においては事実上無視され続けている。このレビューは、言うならば、電気生理学の基礎的な法則を理解し、それを植物の生理学・発生学に適用しようとした過去5年にわたる私の個人的な道程をしたためたものである。

私が学を修めたのは、古典的な植物学と細胞生物学である。しかし、私は、数多くの素人向けの物理の本から電気生理学を勉強するとともに、多くの辛抱強い生物物理学者と個人的に話をする恩恵にもあづかった。このレビューの対象は、膜生物学の専門家ではないが、無理をせずに済むなら、最新の情報にもついていきたいと考えている植物学者である。また、膜生物学には詳しいが、植物学の知識には乏しい（が、「植物の活動電位」というタイトルの講義をしてみたいと思っている）神経生理学者にも満足していただけると思う。そして最後に、科学の歴史、特に生理学・発生学において、電気的・化学的情報伝達の研究が、植物学と動物学の分野でそれぞれどの様に進められたかに関心がある方にも興味をもっていただけるであろう。

## Resumen

Este estudio analiza los principios básicos de electrofisiología utilizando la generación de un potencial de acción en células internodiales de algas characean como herramienta pedagógica. Electrofisiología ha demostrado ser un excelente método para el entendimiento de la fisiología y del desarrollo animal pero ha sido ignorada en el estudio de la fisiología y del desarrollo de plantas. Este estudio es, esencialmente, un testimonio de mi experiencia personal durante los últimos cinco años en los que he estado estudiando los principios básicos de electrofisiología para poder aplicarlos al estudio de fisiología y desarrollo de plantas.

Mis estudios se concentraron en botánica clásica y biología celular. He aprendido electrofisiología a base de leer muchos libros de física escritos para gente sin gran conocimiento de la materia, además he tenido el placer de hablar informalmente con muchos biofísicos de gran paciencia. He escrito este estudio para el botánico que

desconoce la base de biología membranal pero al que le gustaría saber que él o ella puede familiarizarse con la información más actual sin mucho esfuerzo. También lo escribí para el neurofisiólogo, experto en biología membranal, pero que no sabe mucho sobre biología de plantas (y que desee enseñar una clase sobre los "potenciales de acción de las plantas"). Y finalmente, escribí esto para gente interesada en la historia de la ciencia y en cómo los estudios de comunicación eléctrica y química en la fisiología y el desarrollo progresaron en las disciplinas zoológicas y botánicas.

## II. Introduction: Plants Sense Environmental Cues and Act Appropriately

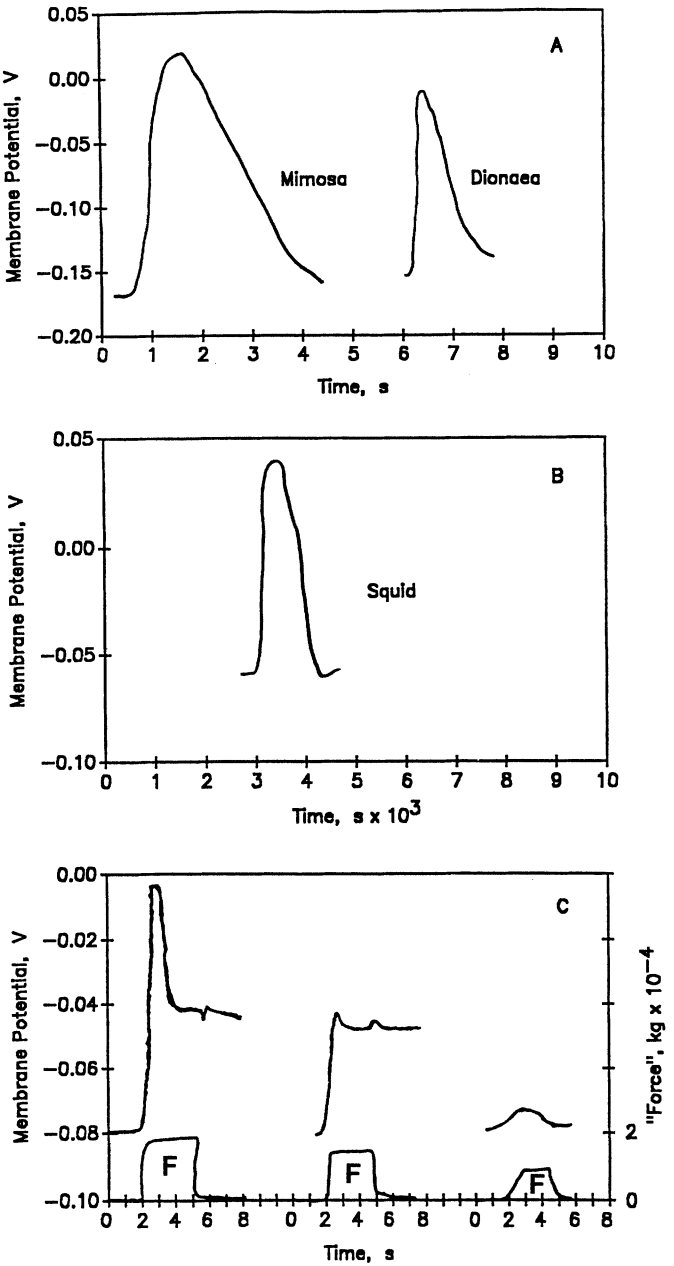
Every day we can observe the fact that plants respond to the environment. For example, plants respond to gravity. "Remember the little seed in the styrofoam cup: The roots go down and the plants go up" (Fulghum, 1989). Plants also respond to light and temperature. In a perennial garden, each variety flowers at a certain time of the year because the plants are capable of identifying the season by detecting both the day length and the temperature. Plants can also perceive the time of day, and many plants have "sleep movements" where they change the orientation of their leaves so that they maximize the absorption of light during the day and minimize the loss of heat at night. Plants adjust their height and leaf shape depending on whether they are growing in the sun or shade. In this case, they detect the difference in the quality of light (i.e., color distribution) that occurs in full sun compared to shade. Plants can determine the direction of light, and they bend toward the light in a way that presents the maximum surface area to the sun to maximize photosynthesis. After sensing the various environmental stimuli, the sensor region must transmit a signal to the responding region. In theory, this signal can be chemical (e.g., hormonal), physical (e.g., electrical or hydraulic), or both (Kudoyarova et al., 1990; Malone, 1993; Appendix B).

It has been known since Charles Darwin's time that many plants, like animals, respond to mechanical stimulation (Bernard, 1974; Darwin, 1893, 1966; Davies, 1987, 1993a, 1993b; Galston, 1994; Jaffe, 1973, 1976; Jaffe & Galston, 1968; Pickard, 1973; Sibaoka, 1966, 1969; Simons, 1981, 1992; Smith, 1788; Thomson, 1932; Wayne, 1993). Insectivorous plants that live in nitrogen- and mineral-depleted areas get their required nitrogen and minerals by capturing and digesting insects. The sundew immobilizes an insect in its mucilage. Subsequently, the mechanical stimulation induced by the insect trying to escape is sensed by the tip of the tentacle. The sensitive tip then produces a series of *action potentials*, that is, pulse-like, propagating electrical disturbances that can communicate information (Fig. 1). In the case of the sundew, the action potentials induce the marginal tentacles to bend, pushing the insect toward

---

→

**Fig. 1.** Action potentials of various organisms. **A.** Action potentials of *Mimosa* (the sensitive plant) and *Dionaea* (Venus fly trap) (redrawn from Sibaoka, 1966). **B.** Action potential of a squid giant axon (redrawn from Hodgkin & Katz, 1949). Note the differences in the ordinates and abscissae in A and B. **C.** Receptor potentials of the sensory (trigger) hair of *Dionaea* induced by a mechanical force (redrawn from Benolken & Jacobson, 1970). The "force" in these experiments is given in kilograms. This is incorrect: Since kilograms are a unit of mass, force should be given in newtons. Force ($F$, in N) can be obtained from the mass ($m$, in kg), according to Newton's Second Law ($F = ma$), by multiplying the mass by its acceleration ($a$, in m $s^{-2}$). However, Benolkin and Jacobson neither estimated nor measured the acceleration in these experiments. Thus, we cannot transform the "force" given in kilograms into proper units. In any case, the first stimulating force ($F$) was greater than threshold and induced an action potential; the second stimulating




force was below the threshold and the resulting membrane depolarization was smaller than the first. The rate of change in the force as well as the magnitude was further reduced in the third stimulating force, and the change in membrane potential was even smaller than the first or second. Thus, a *graded* response occurred in response to subthreshold stimuli. When the stimulus is subthreshold, a receptor potential appears as an electrical replica of the stimulus.

leaf's center (Williams & Spanswick, 1976). Although action potentials are not transmitted to neighboring tentacles, a slower hormonal signal induces them to wrap around the insect and provide a secure trap (Williams & Pickard, 1972a, 1972b). Nearby secretory cells subsequently exude digestive enzymes "to the little stomach thus formed, and the plant makes a meal of its prey" (Pickard, 1973). The lobes of a Venus fly trap capture its prey by closing when an insect walking across it strikes two hairs or one hair twice. This "primitive memory" ensures that the trap closes only around live prey. The trap closes only after the stimulated sensory hairs communicate to the motor cells by means of an action potential (Benolken & Jacobson, 1970; Brown & Sharp, 1910; Burdon-Sanderson, 1873; Burdon-Sanderson & Page, 1876; Di Palma et al., 1961; Hodick & Sievers, 1989; Jacobson, 1965; Stuhlman & Darden, 1950; Williams & Bennett, 1982). John Burdon-Sanderson, an uncle of J. B. S. Haldane, first studied the movements and electrical properties of the Venus fly trap for Charles Darwin (Gillespie, 1970, 1972). Upon closure, the gland cells secrete enzymes that effect digestion of the insect and uptake of nutrients (Rea & Whatley, 1983; Rea et al., 1983; Robins, 1976; Robins & Juniper, 1980; Scala et al., 1968; Schwab et al., 1969).

The leaves of many sensitive plants, including *Mimosa,* fall and look dead and unappealing to a would-be herbivore once the grazing animal touches it (Aimi, 1963; Allen, 1969; Setty & Jaffe, 1972; Tinz-Füchtmeier & Gradmann, 1991; Toriyama, 1954, 1955, 1957, 1967; Toriyama & Jaffe, 1972; Toriyama & Sato, 1968; Turnquist et al., 1993). Leaflet collapse may act as a defense mechanism since the collapse results in an increased exposure of sharp thorns (Eisner, 1981). Touching induces an action potential that propagates from the stimulated region to the rest of the plant (Sibaoka, 1962). This propagated "information" causes the rest of the plant to look unappealing also and to "deploy their thorns." It is also possible that mechanical stimulation by the wind induces an action potential that causes the leaflets to collapse. This reduces the exposed surface from which moisture may be lost (D. F. Grether, pers. comm.).

Plants that do *not* show obvious movements also elicit action potentials (Paszewski & Zawadzki, 1973; Scott, 1962). In *Luffa*, action potentials cause a transient inhibition of growth (Shiina & Tazawa, 1986a). Action potentials may be involved in phloem unloading and, consequently, the distribution of nutrients in plants (Eschrich et al., 1988; Fromm, 1991). Action potentials propagated through the phloem may also be involved in pathogen resistance (Wildon et al., 1992). In various flowers, pollen falling on the stigma generates an action potential that may be involved in subsequent pollination, incompatibility, or maturation processes (Goldsmith & Hafenrichter, 1932; Sinyukhin & Britikov, 1967). At the level of stimulus–response coupling, plants and animal cells undergo many similar processes, including the generation and propagation of action potentials and perhaps stimulation by neurotransmitters, including acetylcholine (Dettbarn, 1962; Fluck & Jaffe, 1974; Tretyn & Kendrick, 1991). Including studies on plant excitability with studies of animal excitability may help us understand the evolution of our own nervous system (Hille, 1984, 1992).

In order to understand the physiological basis of the complex behaviors of animals, neurobiologists took a reductionist approach and began studying the long nerve cells of squids which are so large they were originally thought to be blood vessels (Cole, 1968; Eckert et al., 1988; Hodgkin, 1964; Junge, 1981; Katz, 1966; Lakshminarayanaiah, 1969; Matthews, 1986; Stevens, 1966; Tasaki, 1968; Young, 1936, 1947). Studying simple systems allows the development of techniques and the formulation of theories necessary for understanding more complex systems. Likewise,

in order to understand the complex behaviors of whole plants, some plant physiologists have turned to the giant algal cells, such as *Chara* and *Nitella*, where vegetative and reproductive growth and development, adaptive physiological mechanisms, and nutrient uptake and translocation can be readily studied (Amino & Tazawa, 1989; Andjus & Vucelic, 1990; Barber, 1911a, 1911b; Beilby et al., 1993; Bisson & Bartholomew, 1984; Bisson et al., 1991; Blatt & Kuo, 1976; M. Brooks, 1939; S. Brooks, 1939; Brooks & Brooks, 1941; Brown, 1938; Buchen et al., 1991, 1993; Coleman, 1986; Collander, 1949, 1954; Cote et al., 1987; Dainty, 1962, 1963a, 1963b; Dainty & Ginzburg, 1964a, 1964b, 1964c; Dainty & Hope, 1959; Diamond & Solomon, 1959; Ding et al., 1991, 1992; Dorn & Weisenseel, 1984; Forsberg, 1965; Gertel & Green, 1977; Green, 1954, 1958a, 1958b, 1959, 1960, 1962, 1963, 1964, 1968; Green & Chapman, 1955; Green & Chen, 1960; Gillet et al., 1989, 1992, 1994; Green et al., 1971; Hansen, 1990; Hejnowicz et al., 1985; Hoagland & Broyer, 1942; Hoagland & Davis, 1923a, 1923b; Hoagland et al., 1926; Hodge et al., 1956; Hogg et al., 1968a, 1968b; Homblé & Foissner, 1993; Homblé et al., 1989; Hotchkiss & Brown, 1987; Kamiya & Kuroda, 1956a; Kamiya & Tazawa, 1956; Katsuhara et al., 1990; Katsuhara & Tazawa, 1992; Kersey et al., 1976; Kishimoto, 1957; Kiss & Staehelin, 1993; Kitasato, 1968; Kiyosawa, 1993; Kwiatkowska, 1988, 1991; Kwiatkowska & Maszewski, 1985, 1986; Kwiatkowska et al., 1990; Levy, 1991; Lucas, 1975, 1976, 1977, 1979, 1982; Lucas & Alexander, 1981; Lucas & Dainty, 1977a, 1977b; Lucas & Shimmen, 1981; Lucas & Smith, 1973; Lucas et al., 1978, 1989; MacRobbie & Dainty, 1958; MacRobbie & Fensom, 1969; Maszewski & Kolodziejczyk, 1991; McConnaughey, 1991; McConnaughey & Falk, 1991; McCurdy & Harmon, 1992; Métraux, 1982; Métraux & Taiz, 1977, 1978, 1979; Métraux et al., 1980; Miller & Sanders, 1987; Mimura & Kirino, 1984; Moestrup, 1970; Morrison et al., 1993; Mullins, 1939; Nagai & Hayama, 1979; Nagai & Kishimoto, 1964; Nagai & Rebhun, 1966; Nagai & Tazawa, 1962; Nothnagel & Webb, 1979; Nothnagel et al., 1982; Ogata, 1983; Ogata & Kishimoto, 1976; Ohkawa & Kishimoto, 1977; Ohkawa & Tsutsui, 1988; Okazaki & Tazawa, 1990; Okazaki et al., 1987; Osterhaut, 1927, 1931, 1958; Osterhaut & Hill, 1930a, 1930b; Palevitz & Hepler, 1974, 1975; Pickard, 1969, 1972; Pickett-Heaps, 1967a, 1967b, 1968; Ping et al., 1990; Pottosin et al., 1993; Probine & Barber, 1966; Probine & Preston, 1961, 1962; Reid & Overall, 1992; Reid & Smith, 1988, 1993; Reid et al., 1989; Rethy, 1968; Richmond et al., 1980; Rüdinger et al., 1992; Sakano & Tazawa, 1986; Shen, 1966, 1967; Shepherd & Goodwin, 1992a, 1992b; Shimmen & Mimura, 1993; Shimmen & Yoshida, 1993; Sievers & Volkmann, 1979; Spanswick, 1970; Staves et al., 1992; Steudle & Tyerman, 1983; Steudle & Zimmermann, 1974; Studener, 1947; Sun et al., 1988; Takamatsu et al., 1993; Takatori & Imahori, 1971; Taylor & Whitaker, 1927; Tazawa, 1957, 1964; Tazawa & Kishimoto, 1964; Tazawa et al., 1994; Thiel et al., 1990; Tolbert & Zill, 1954; Trontelj et al., 1994; Tsutsui & Ohkawa, 1993; Turner, 1968, 1970; Volkmann et al., 1991; Vorobiev, 1967; Walker & Sanders, 1991; Wasteneys & Williamson, 1992; Wasteneys et al., 1993; Wayne & Tazawa, 1988, 1990; Wayne et al., 1990, 1992, 1994; Weidmann, 1949a, 1949b; Weisenseel & Ruppert, 1977; Yao et al., 1992; Zanello & Barrantes, 1992, 1994; Zhang et al., 1989; Zhu & Boyer, 1992).

The characean algae, which include *Chara* and *Nitella*, are thought to be the ancestors of all higher plants (Chapman & Buchheim, 1991; Graham, 1993; Graham & Kaneko, 1991; Grambast, 1974; Groves & Bullock-Webster, 1920, 1924; Imahori, 1954; Manhart & Palmer, 1990; Pal et al., 1962; Pickett-Heaps & Marchant, 1972; Wilcox

et al., 1993; Wood & Imahori, 1964, 1965). Indeed, Pliny the Younger considered the charophytes to be members of the genus *Equisetum* (Plinius Secundus, 1469).

The study of excitability in plant cells forms the foundation of the study of excitability in isolated cells. Although excitability in tissues and organs of whole plants and animals were well known since the early work of Luigi Galvani, the electrical phenomena were too complicated to allow much progress (Green, 1953). A giant breakthrough came when electrical measurements were made on single cells. Indeed, action potentials were observed in the isolated internodal cells of *Nitella* in 1898 by Georg Hörmann, using extracellular electrodes, approximately 30 years before they were observed in isolated nerve cells by Adrian and Bronk (1928). In fact, action potentials (also known as negative variations, action currents, and death currents) were so well studied in characean cells by Blinks (1936), Osterhaut (1931), and Osterhaut's colleagues at the Rockefeller Institute, that Cole and his colleagues at the National Institutes of Health began studying excitability in characean cells. It may be surprising to know that Cole and Curtis wrote the following in their 1939 paper "Electrical impedance of the squid giant axon during activity":

> *Recently the transverse alternating current impedance of* Nitella *has been measured during the passage of an impulse which originated several centimeters away (Cole and Curtis, 1938b). These measurements showed that the membrane capacity decreased 15 per cent or less while the membrane conductance increased to about 200 times its resting value. Also this conductance increase and the membrane electromotive force decrease occurred at nearly the same time, which was late in the rising phase of the monophasic action potential. Similar measurements have now been made on Young's giant nerve fiber preparation from the squid (Young, 1936). These were undertaken first, to determine whether or not a functional nerve propagates an impulse in a manner similar to* Nitella, *and second, because the microscopic structure of the squid axon corresponds considerably better than that of* Nitella *to the postulates upon which the measurements are interpreted.*

Characean action potentials can be induced by various stimuli, including suddenly lowered or raised temperature or pressure, UV irradiation, and odorants, as well as by a mechanical stimulation or a depolarizing current (Harvey, 1942a, 1942b; Hill, 1935; Kishimoto, 1968; Osterhaut & Hill, 1935; Staves & Wayne, 1993; Ueda et al., 1975a, 1975b; Zimmermann & Beckers, 1978). As in animal cells, the action potential is an all-or-none response and does not usually depend on the strength of the stimulus. In characean cells there is a refractory period following an action potential, during which a stimulus cannot cause a second action potential. Details of the characean action potential have been reviewed by Kishimoto (1972), Hope and Walker (1975), Beilby (1984), Tazawa et al. (1987), and Tazawa and Shimmen (1987).

The action potential is conducted in both directions away from the place at which the plasma membrane is first depolarized past the threshold. The conduction rate is 0.01–0.4 m $s^{-1}$ (Auger, 1933; Sibaoka, 1958; Umrath, 1933), depending on the conductivity of the medium (Sibaoka, 1958). This is faster than the conduction velocity in sponges (0.003 m $s^{-1}$; Mackie et al., 1983) and slower than the conduction velocity of action potentials in nerves, which is between 0.4 and 42 m $s^{-1}$ (Eckert et al., 1988; Matthews, 1986).

The propagation velocity of an action potential along a cell depends on how rapidly the depolarization (i.e., the decrease in membrane potential) diminishes with distance. The membrane will become depolarized next to the initial site of depolarization, and the potential will increase (i.e., hyperpolarize) with distance away from the initial site of depolarization until the membrane potential is equal to the resting potential. This is known as *electrotonic* transmission. An action potential can be propagated only if sufficient current travels down the cell to depolarize the adjacent membrane below the threshold in membrane potential. A new action potential will begin only if the membrane potential of the adjacent membrane is depolarized past the threshold.

The ionic current responsible for depolarizing the membrane and propagating the action potential can travel through the cytoplasm or across the plasma membrane. The current moves along a potential difference in a manner described by Ohm's Law:

$$I = -E/R' = -EG \tag{1}$$

where

$I$ is current density (in A $m^{-2}$)
$E$ is potential (in V)
$R'$ is the specific resistance (in $\Omega$ $m^2$)
$G$ is the specific conductance (in siemens per $m^2$ (S $m^{-2}$) or $\Omega^{-1}$ $m^{-2}$).

The magnitude of depolarization of the adjacent membrane depends on the amount of current traveling through the cytoplasm, which in turn depends on the relative resistances of the cytoplasm and the membrane. In order to select for the movement of the ionic current through the cell from one end to the other, instead of through the membrane and outside the cell (which would negate the depolarizing effect of the ionic current), the resistance of the cytoplasm must be low and the resistance of the plasma membrane must be high (like an insulated wire). Thus, fast action potentials propagate along nerve cells whose cytoplasmic resistances have been lowered by making their diameters large and whose plasma membrane resistance has been increased by surrounding the axon with a high-resistance myelin sheath. Thus, the difference in propagation velocity between various excitable cells depends on their structure. As in excitable cells in animals, the action potential in characean cells is transmitted from cell to cell (Sibaoka & Tabata, 1981; Spanswick 1974b; Spanswick & Costerton, 1967; Tabata, 1990).

Characean internodal cells exhibit a response to electrical stimulation that is similar to the contraction response skeletal muscles display following an electrical stimulation by nerve cells. In both cases, the response is nicknamed *E–C coupling*. In animal cells, it refers to *e*xcitation–*c*ontraction coupling, where electrical excitation causes muscle contraction (Ebashi, 1976). In characean cells, E–C coupling refers to the fact that *e*lectrical stimulation causes the *c*essation of protoplasmic streaming (Findlay, 1959; Hill, 1941; Hörmann, 1898; Kishimoto & Akabori, 1959).

The protoplasm of characean cells constantly moves around the periphery of the cell like a rotating belt at a rate of $10^{-4}$ m $s^{-1}$, in a process known as protoplasmic streaming (Allen & Allen, 1978; Corti, 1774; Ewart, 1903; Göppert & Cohn, 1849; Kamiya, 1959, 1981; Kamiya & Kuroda, 1956b; Shimmen, 1988; Shimmen & Yokota, 1994). Protoplasmic streaming, which is driven by the same interactions between actin and myosin that cause contraction in muscles, allows the mixing and transport of essential molecules through the protoplasm of large cells where diffusion would be limiting (Fig. 2).

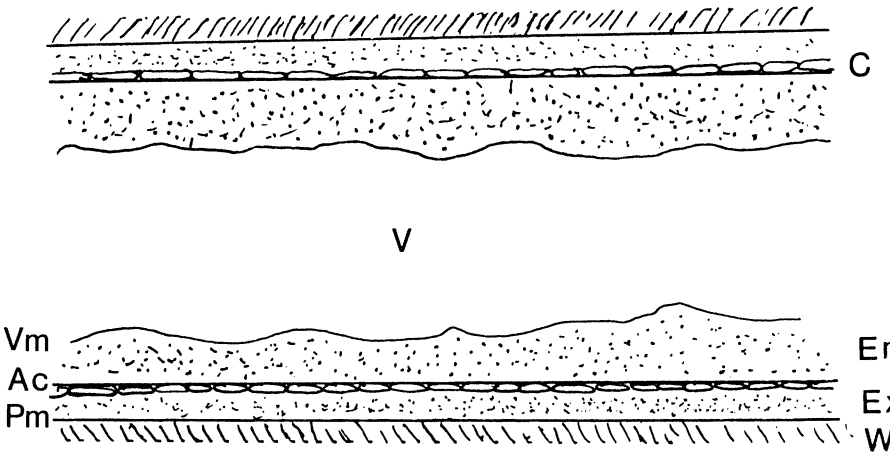



**Fig. 2.** Diagram of characean internodal cell. The cell is essentially a right circular cylinder with a volume equal to $(\pi r^2 l)$] and a surface area equal to $2\pi rl + 2\pi r^2$. The length ($l$, in m) of a "typical" cell depends on the species but may vary from $10^{-2}$ m in *Nitella* to $3 \times 10^{-1}$ m in *Nitellopsis*. The diameter ($2r$, in m) varies from $0.3 \times 10^{-3}$ m in *Nitella* to $10^{-3}$ m in *Chara*. C, chloroplasts; V, vacuole; Ac, actin bundles; Pm, plasma membrane; En, flowing endoplasm; Ex, stationary ectoplasm; W, extracellular matrix (= cell wall). (Redrawn from Tazawa et al., 1987.)

The time it takes for the average particle to diffuse a given distance through a cell can be calculated using Einstein's random walk equation (Berg, 1993; Einstein, 1926; Perrin, 1923; Pickard, 1974):

$$t = x^2/(2D) \tag{2}$$

where

$t$ is the time (in s)
$x$ is the average distance the particles move (in m)
$D$ is the diffusion coefficient (in $m^2\ s^{-1}$).

Note that equation 2 describes the simple case where diffusion is restricted to one dimension. Diffusion can also be described in two dimensions ($x$ and $y$) where $t = r^2/(4D)$ and $r^2 = x^2 + y^2$. In three dimensions ($x$, $y$, and $z$), $t = r^2/(6D)$ and $r^2 = x^2 + y^2 + z^2$ (Berg, 1993).

According to the above equation, the time it takes for a particle to diffuse a given distance is governed only by its diffusion coefficient. The diffusion coefficient of a spherical particle in solution depends on the thermal motion of the particle; consequently, the diffusion coefficient is proportional to temperature. Boltzmann's Constant ($k$, in $J\ K^{-1}$) is the coefficient of proportionality that relates the energy of the particle to the temperature. The kinetic energy $[(1/2)mv^2]$ of a particle moving in three dimensions is a consequence of the thermal motion of the particle and is equal to $(3/2)kT$ where $T$ is the absolute temperature (in K). The velocity of a particle of mass $m_j$ (in kg) is equal to $(3kT/m_j)^{1/2}$. If we consider that the particle is large compared with water, then we can use the equations of fluid mechanics to describe the movement of the particle through an aqueous environment. Thus, the movement of the particle depends on the hydrody-

namic radius of the particle ($r_H$, in m) and the viscosity of the solution ($\eta$, in Pa s). Formally, the diffusion coefficient $D$ is given by the Stokes-Einstein equation:

$$D = kT/(6\pi r_H \eta) \quad (3)$$

where

$D$ is the diffusion coefficient (in $m^2\ s^{-1}$)
$k$ is Boltzmann's Constant ($1.38 \times 10^{-23}$ J $K^{-1}$)
$T$ is the absolute temperature (in K)
$r_H$ is the hydrodynamic radius (in m)
$\eta$ is the viscosity of the solution (in Pa s).

If we assume that the viscosity of the fluid phase of the cytoplasm is 0.004 Pa s (Luby-Phelps et al., 1986) and that most small metabolites have a hydrodynamic radius between $10^{-10}$ and $10^{-9}$ m, then most diffusion coefficients will be between $5 \times 10^{-11}$ and $5 \times 10^{-10}$ $m^2\ s^{-1}$ at 298 K. Thus, a "typical" metabolite will take between 25 and 250 ms to diffuse all the way across a $5 \times 10^{-6}$ m cell, but it will take between $10^3$ and $10^4$ s (2.8 h) to diffuse across a $10^{-3}$ m diam. characean internodal cell and $10^7$ and $10^8$ s (4 months) to diffuse along the long axis of a $10 \times 10^{-2}$ m long characean internodal cell. Diffusion is thus limiting in large cells (i.e., slower than the rates of enzymatic reactions), and mixing by protoplasmic streaming (i.e., convection) becomes necessary. In general, the larger the cell, the more organized the protoplasmic streaming.

In the natural world, cessation of streaming results not from electrical excitation but from the mechanical stimulation of internodal cells by a predator or other objects such as falling sticks. This mechanical stimulation generates a depolarization of the plasma membrane that is known as a *receptor potential* (Kishimoto, 1968). The receptor potential is an amplification mechanism whereby the minuscule mechanical energy of the tactile stimulus is *transduced* by the receptor of the stimulus (in this case a mechanoreceptor) into electrical energy that is amplified in proportion to the magnitude of the initial stimulus. (Energy is not created from this amplification process but is released in the form of ionic currents from the electrical energy already stored across the cell membrane in the form of a membrane potential.) The receptor potential usually lasts as long as the stimulus is present and can be considered an electrical replica of the stimulus. However, the stimulus must be given rapidly enough or the receptor shows a slow decrease in its sensitivity to the stimulus, known as *accommodation* (Kishimoto, 1968; Staves & Wayne, 1993). The actin- or tubulin-based cytoskeleton appears not to be involved in the function of this mechanoreceptor (Staves & Wayne, 1993); however, the involvement of a membrane skeleton (Faraday & Spanswick, 1993) has not been ruled out.

The receptor potential is not self-perpetuating, and thus the depolarization due to the receptor potential decreases with distance away from the activated receptor. [The theoretical description of how potentials and currents spread through cells, known as "cable theory," was originally worked out by Lord Kelvin (1855; Wheatstone, 1855) to describe the electrical properties of the transatlantic telegraph cable. He showed that the potential drops linearly along an insulated cable placed between a battery and ground just like the temperature drops across a substance placed between a heat source and a heat sink.] If the stimulus is great enough and fast enough to cause the membrane potential to depolarize below a certain threshold level, an *action potential* will be

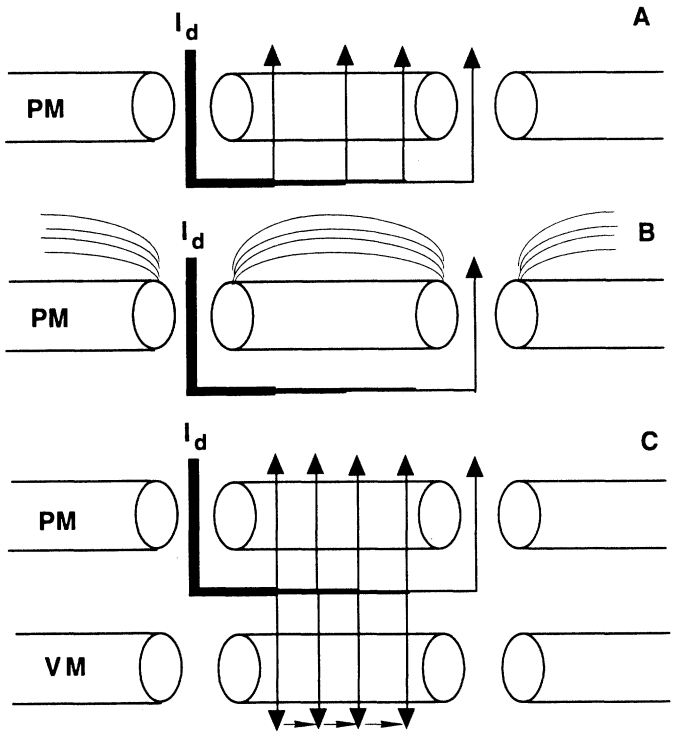



**Fig. 3.** Diagrams of the cable properties of a nerve cell (or other nonvacuolated cell), a myelinated nerve cell, and a characean internodal cell (or other vacuolated cell). **A.** In a nerve cell, the depolarizing current ($I_d$) crosses the plasma membrane (PM) and travels through the cytoplasm and back out through the plasma membrane. The amount of current that moves through the cytoplasm and thus is capable of depolarizing adjacent membrane areas depends on the relative resistance of the cytoplasm and the plasma membrane. The greater the ratio of membrane resistance to cytoplasmic resistance, the greater the amount of depolarizing current that travels through the cytoplasm. The cytoplasmic resistance is inversely proportional to the radius of the axon. If the cytoplasmic depolarizing current is large, then the electrotonic transmission will depolarize an adjacent membrane area enough to surpass a threshold of a voltage-gated channel and initiate an action potential in the adjacent membrane patch. **B.** In a myelinated nerve, the membrane resistance is increased by tightly wrapping Schwann cells around the axon. Schwann cells have membranes that are almost purely lipid, so the axon becomes analogous to a wire wrapped with an insulator. The Schwann cells are discontinuous at points known as the nodes of Ranvier, where the plasma membrane of the axon is in communication with the ions in the external medium and thus, at these points, a sufficient depolarizing current traveling through the cytoplasm will initiate an action potential. **C.** In a *Chara* cell, a depolarizing current can follow many pathways, including the plasma membrane, the cytoplasm, the vacuolar membrane (VM), and the vacuole. A characean internodal cell is thus analogous to an insulated wire within an insulated wire. This makes the pathway of current more difficult to calculate than for (vacuole-less) nerve cells. Note: In all the cases mentioned above, the depolarizing current will travel in all directions so that the current in any direction will be inversely proportional to the resistance in that direction.

elicited (Fig. 3). An action potential is a large transient depolarization of the membrane potential that is self-perpetuating (i.e., regenerative) and thus allows the rapid transmission of information over long distances without any loss of signal strength (information). Thus, receptor potentials are useful for signal transduction within a single small cell, and action

potentials are necessary for signal transmission in a giant cell or between cells.

The action potential generated in one area of a characean cell is responsible for causing the cessation of protoplasmic streaming throughout the cell (Sibaoka & Oda, 1956). When streaming stops, the outer layer of the flowing protoplasm gels (i.e., forms cross-bridges with the actin cables) and prevents any leakage of protoplasm that may occur as a result of a small-animal attack (Kamitsubo et al., 1989; Kamitsubo & Kikuyama, 1992). Moreover, the stimulated cell may become isolated from the neighboring cells since the intercellular passage of substances through small tubes known as *plasmodesmata* is also inhibited. The cessation of streaming inhibits transport through the plasmodesmata indirectly since, in the absence of streaming, unstirred layers form around the openings of the plasmodesmata and arrival of substances at the pore becomes diffusion limited (Ding & Tazawa, 1989). The cessation of cytoplasmic streaming has no effect on plasmodesmatal transport in small cells where diffusion is not limiting (Tucker, 1987).

## III. Recording the Voltage Changes During an Action Potential

The action potential in characean internodal cells can be observed using simple electrophysiological techniques (Fig. 4; Brooks & Gelfan, 1928; Umrath, 1930, 1932, 1934, 1940). According to Alan Hodgkin (1951),

> *The most satisfactory way of recording the electrical activity of a living cell membrane is to insert a small electrode into the interior of the cell and to measure the potential (i.e voltage) of this electrode with reference to a second electrode in the external medium. This method was first employed in plant cells (see Osterhaut, 1931) and has now been widely used in animal tissues.*

When a glass microcapillary electrode is placed into the vacuole and the reference electrode is placed in the external medium, an action potential is observed following stimulation. The action potential appears to be composed of two components: a fast and a slow one (Fig. 5; Findlay, 1970; Findlay & Hope, 1964a; Kishimoto, 1959; Shimmen & Nishikawa, 1988). If we insert two microcapillary electrodes in the cell, we can resolve that these two components are the result of two action potentials; a fast one that occurs across the plasma membrane and a slow one that occurs across the vacuolar membrane (Findlay & Hope, 1964a). The average resting potentials across the plasma membrane and vacuolar membrane are –0.180 V and –0.010 V, respectively. [Note: In both cases the potential on the exoplasmic side of the membrane (E-space) is considered to be zero by convention and the protoplasmic side of the membrane (P-space), i.e., the side that is facing the cytosol, is negative.]

During an action potential, the plasma membrane depolarizes to approximately 0 V and the vacuolar membrane hyperpolarizes to approximately –0.050 V. The changes in the membrane potential, according to the Goldman-Hodgkin-Katz Equation, are due to the ionic currents that flow as a consequence of a change in the membrane permeability of each ion.

The change in membrane permeability that occurs during an action potential can be visualized by measuring the *specific conductance* of the membrane. The specific conductance is an electrical measure of the overall membrane permeability to all ions. The specific conductances of the plasma membrane and vacuolar membrane at rest are 0.83 siemens $m^{-2}$ (S $m^{-2}$) and 9.1 S $m^{-2}$, respectively. The specific conductances

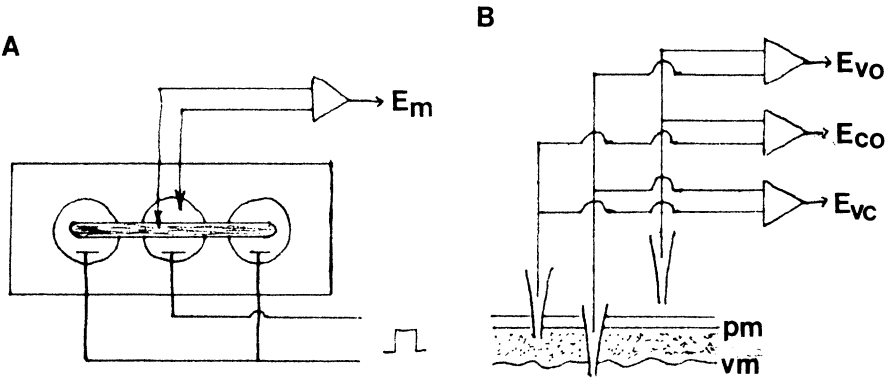



**Fig. 4.** Diagram of setup used to measure membrane potential. **A.** A characean internodal cell is placed in an acrylic block in which three holes (0.4–1 cm diam.) have been drilled. The cell parts situated in a groove in the region between the three wells is coated with silicone grease or petrolatum (Vaseline) so that the three wells are electrically isolated and current can only travel across the plasma membrane and through the cells). A reference electrode (containing ca. 100 mol $m^{-3}$ KCl and 1–2% agar) is placed in the middle chamber and a microcapillary electrode (filled with 3000 mol $m^{-3}$ KCl) is placed in the cytoplasm of the cell part in the middle chamber. The electrical potential across the plasma membrane is measured with an electrometer. The cell is electrically stimulated by applying a depolarizing current to the middle chamber. The current travels through the cell in all directions and is returned to the other terminal of the current generator by way of the wires placed in the two end chambers. Similarly, in order to measure membrane resistance, current pulses (square waves of approximately $10^{-7}$ A, 1 Hz) are applied to the middle chamber. The current travels from the current generator through the cell in all directions and is returned to the other terminal of the current generator by way of the wires placed in the two end chambers. **B.** In order to measure both the plasma membrane potential and the vacuolar membrane potential, two microcapillary electrodes are placed in the cell part in the middle chamber. One electrode is placed in the vacuole and the other is placed in the cytoplasm. Thus, the electrical potential across the plasma membrane ($E_{co}$) can be measured by comparing the voltage measured with the electrode in the cytoplasm with the reference electrode in the bath (0 V by convention). The electrical potential of the vacuolar membrane ($E_{vc}$) is measured by comparing the voltage measured by the electrodes placed in the vacuole and cytoplasm. Using the "patch clamping convention," the voltage of the electrode in the vacuole (or E-space) is considered to be zero and the potential of the cytoplasmic side of the vacuolar membrane is negative. Note that in earlier work, the convention was to consider the cytoplasmic side (P-space) of the vacuolar membrane to be zero, and consequently the vacuolar membrane potential on the vacuolar side was positive. The sum of the potentials of the plasma membrane and vacuolar membranes ($E_{vo}$) can be determined simultaneously by comparing the voltage between the electrode in the vacuole with the reference electrode. Remember that the polarity of the membrane potentials across the vacuolar and plasma membrane are oppositely directed, the sum of the two potentials is less hyperpolarized than the potential across the plasma membrane alone. (Redrawn from Shimmen & Nishikawa, 1988.)

change with time during an action potential (Fig. 6). The peak conductances during the action potential of the plasma membrane and vacuolar membrane are 30 S $m^{-2}$ and 15 S $m^{-2}$, respectively (Cole & Curtis, 1938; Findlay & Hope, 1964a; Oda, 1962). In the squid giant axon, the specific conductance of the plasma membrane increases from 10 S $m^{-2}$ to 400 S $m^{-2}$ during an action potential (Cole & Curtis, 1939). In both *Nitella* and squid, the specific conductance of the plasma membrane increases approximately 40 times during an action potential.

While the specific conductance is an important measure of membrane permeability, it does not tell us which ions are permeating the membrane and carrying the current

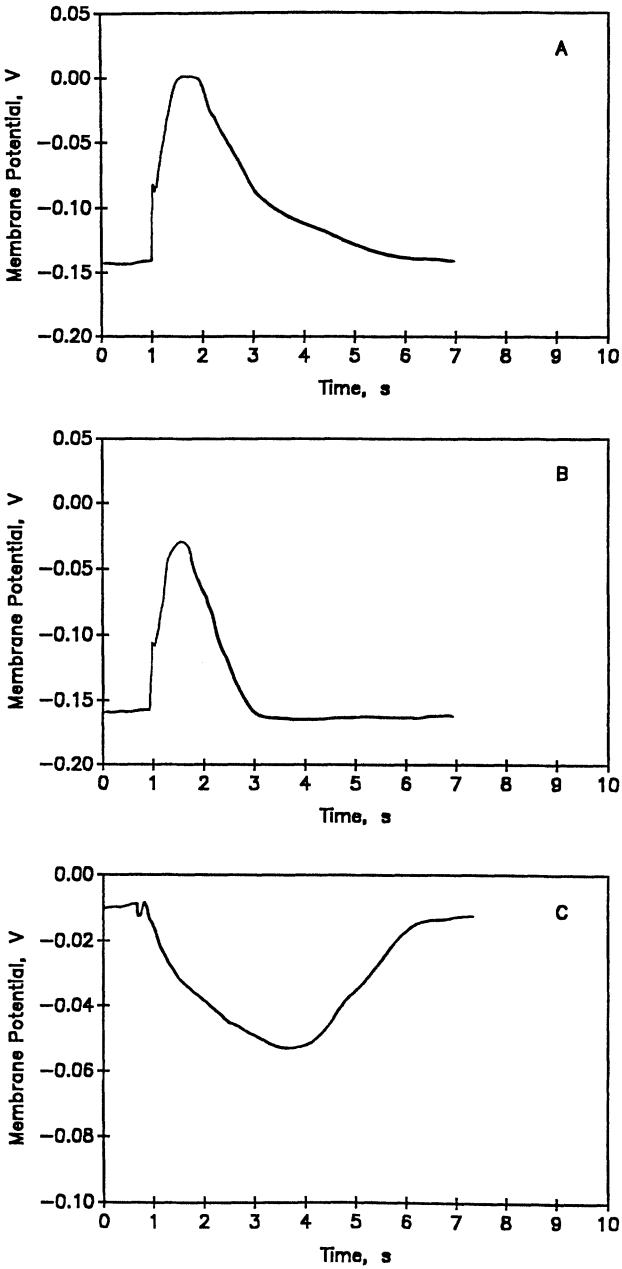



**Fig. 5.** Voltage-time graphs during an action potential in characean internodal cells. An action potential was initiated electrically at 1 second. **A.** The time course of membrane potential changes across both the plasma membrane and vacuolar membrane ($E_{vo}$). **B.** The time course of membrane potential changes across the plasma membrane only ($E_{co}$). **C.** The time course of membrane potential changes across the vacuolar membrane only ($E_{vc}$). (Redrawn from Findlay & Hope, 1964a.)

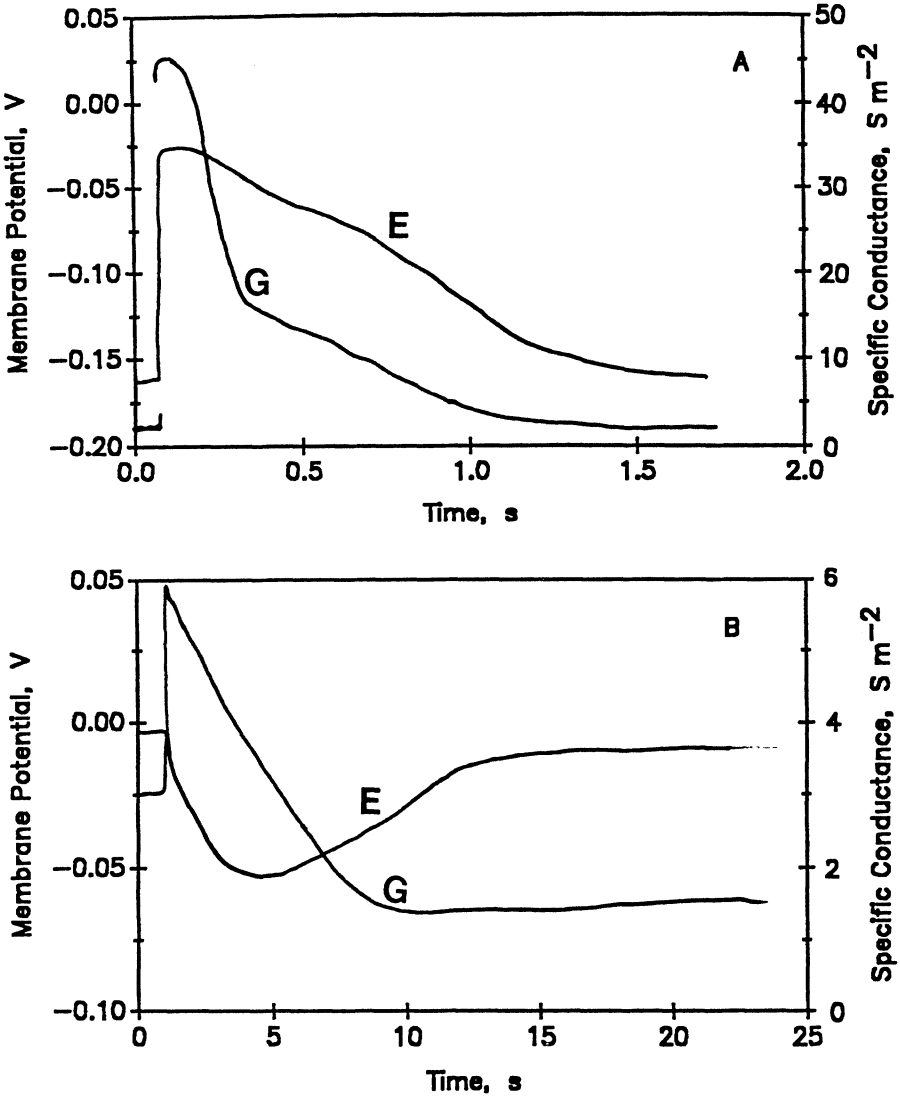



**Fig. 6.** Graphs of conductance changes during an action potential in characean internodal cells. An action potential was initiated electrically at 0 time. **A.** The time course of specific membrane conductance changes across the plasma membrane (*G*). The time course of the plasma membrane potential changes are given for comparison (*E*). **B.** The time course of specific membrane conductance changes across the vacuolar membrane (*G*). The time course of the vacuolar membrane potential changes are given for comparison (*E*). In these cases, the specific membrane conductances were determined from voltage clamping experiments. Note the different time scales in the two graphs. (Redrawn from Findlay & Hope, 1964a.)

that leads to the action potential. Next, we will determine which ions are responsible for carrying the currents that cause the membrane voltage changes that characterize the action potential.

## IV. The Ionic Basis of the Resting Potential

Before we can understand the ionic basis of the action potential, we must first understand the ionic basis of the resting potential and ask, How do the ion concentrations in the cell and extracellular milieu affect membrane potential? The electrical potential across a membrane is determined to a large extent by the difference in ion concentrations across the membrane. Therefore, it is important to know the concentrations of the major ions on each side of a membrane. The concentrations of $Cl^-$, $Na^+$, $K^+$, and $Ca^{2+}$ in the external medium, protoplasm, and cell sap of characean cells as reported by Lunevsky et al. (1983) are given in Table I.

As a comparison, the concentrations of these ions in the protoplasm and the extracellular milieu of a squid axon are also given (Eckert et al., 1988). When we compare the characean internodal cell to the squid axon, we see first that the plant cell has three compartments that bathe two excitable membranes, whereas the animal cell has only two compartments that bathe one excitable membrane. This very fact makes the axon a much simpler system to study since it is geometrically similar to the transatlantic telegraph cable, thus making cable theory easily applied to the analysis of the results. This is the reason that Kenneth Cole and Howard Curtis (1939) began studying the giant squid axon rather than continue with characean internodal cells. Secondly, we see that the $Na^+$ concentration in the external medium is drastically different in the two cell types.

The difference in the concentration of each ion across a differentially permeable membrane creates the driving force for diffusion across the membrane. As each permeant cation diffuses across the membrane, it leaves behind a negative charge (e.g., protein with ionized carboxyl groups) that will tend to attract the cation back to the original side. Thus, there is a *chemical force* that causes the cations to move from high concentrations to low concentrations and at the same time sets up an *electrical force* that tends to move the cations back. Because the membrane is permeable to some ions (e.g., $K^+$) and impermeable to others (e.g., proteins), at equilibrium there is no *net* movement of charge and the chemical force equals the electrical force (see Appendix D). There is thus a stable electrical potential that results from the initial uneven distribution of ions, as was shown by Leonor Michaelis (1925) using dried collodion and apple skins as "the prototype of what we will call a *semipermeable* membrane." The unequal distribution of ions thus gives rise to a transmembrane potential difference that is electrically equivalent to a battery.

The unequal distribution of charge is only possible because the nonconducting lipid bilayer that separates two conducting aqueous solutions acts as a *capacitor*, that is, a component that has the capacity to separate charges (Appendix C). Otherwise, the membrane potential difference (i.e., the battery) would eventually run down. A capacitor is a component that resists changes in voltage when current flows through it (much like a pH buffer resists changes in pH). The *specific membrane capacitance* is a measure of how many charges (ions) must be transferred from one side of the membrane to the other side either to set up or to dissipate a given membrane potential. The specific membrane capacitance of all membranes is approximately 0.01 F $m^{-2}$ (Cole, 1970; Cole & Cole, 1936a, 1936b; Cole & Curtis, 1938; Curtis & Cole, 1937; Dean et al., 1940; Findlay, 1970; Fricke, 1925; Fricke & Morse, 1925; Williams et al., 1964). Using this value, it is possible to calculate the *minimum* number of ions that must diffuse across the membrane in order to establish or dissipate a membrane potential using the following relation:

**Table I**

The ionic composition of characean internodal cells and squid nerves (concentration in mol $m^{-3}$)

| | Characean internodal cell | | | Squid nerve | |
|---|---|---|---|---|---|
| | External | Protoplasm | Vacuole | External | Protoplasm |
| $Cl^-$ | 0.4 | 22 | 162 | 560 | 40–100 |
| $Na^+$ | 0.1 | 5 | 34 | 440 | 49 |
| $K^+$ | 0.1 | 110 | 103 | 22 | 410 |
| $Ca^{2+}$ | 0.1 | < 0.001 | 12 | 10 | < 0.001 |

*Note:* For intact cells the extracellular milieu is easy to control the protoplasmic ion concentration is probably under strict regulation, but the vacuolar ion concentration probably varies tremendously with the age and growing conditions of the cell, and this will affect the shape of the action potential.

$$q_j = -(C_m E_m)/(z_j e) \tag{4}$$

where

$q_j$ is the number of ions that diffuse across a membrane per unit area (in ions $m^{-2}$)
$C_m$ is the specific membrane capacitance (0.01 F $m^{-2}$ = 0.01 C $V^{-1}$ $m^{-2}$)
$E_m$ is the membrane potential set up by the diffusion of ions (in V)
$z_j$ is the valence of the ion
$e$ is the elementary charge ($1.6 \times 10^{-19}$ C $ion^{-1}$)
$q_j z_j e$ is equal to the charge density (in C $m^{-2}$).

Using the equation for membrane capacitance (4) and the equilibrium potentials that we will calculate below, we will see that, at equilibrium, the initial concentrations change by less than 0.001% after the establishment of an equilibrium potential (Plonsey & Barr, 1991). This is important to ensure that there is no violation of the principle of electroneutrality, which states that in a volume greater than a given size (e.g., macroscopic dimensions) the number of positive charges must equal the number of negative charges. Electroneutrality is an outcome of the fact that any net charge sets up an electric field that tends to attract oppositely charged particles (as described by Coulomb's Law) that consequently restore the net charge of the volume to zero.

As a consequence of a membrane capacitance, and the diffusion of ions across a differentially permeable membrane, an equilibrium potential (or Nernst potential) is established (Nernst, 1888, 1889). The resultant equilibrium potential for each ion species is described by the Nernst Equation:

$$E_j = (RT/z_j F) \ln (n_e/n_p) \tag{5}$$

where

$E_j$ is the equilibrium potential for ion $j$ (in V) on the protoplasmic side of the membrane, assuming the potential of the exoplasmic side is 0 V
$R$ is the gas constant (8.31 J $mol^{-1}$ $K^{-1}$)
$T$ is the absolute temperature (in K)
$z_j$ is the valence of ion $j$

**Table II**

The equilibrium potential (in V) of each ion across the plasma membrane and vacuolar membrane of a characean internodal cell and the plasma membrane of a squid nerve.

| | Characean internodal cell | | Squid nerve |
|---|---|---|---|
| | Plasma membrane | Vacuolar membrane | Plasma membrane |
| $Cl^-$ | 0.103 | –0.051 | –0.026 |
| $Na^+$ | –0.100 | 0.049 | 0.075 |
| $K^+$ | –0.180 | –0.002 | –0.075 |
| $Ca^{2+}$ | 0.059 | 0.121 | 0.118 |

$F$ is the Faraday Constant ($9.65 \times 10^4$ coulombs $mol^{-1}$)

$n_e$ and $n_p$ are the concentrations of a given ion on the exoplasmic and protoplasmic sides of the membrane, respectively (in mol $m^{-3}$). We are (simply but wrongly) assuming that the activity coefficients of the ions are identical and equal to 1 on both sides of the membrane. See Appendix D for the derivation of the Nernst Equation.

The equilibrium potentials for each ion across the plasma membrane and the vacuolar membrane calculated from the Nernst Equation (at 298 K) are given in Table II. The equilibrium potentials across the plasma membrane of a squid axon are given for comparison.

The equilibrium potentials of all the ions diffusing across a membrane contribute to the resting membrane potential. David Goldman (1943) and Hodgkin and Katz (1949) showed that at equilibrium the *net flow* of all ions through the membrane is zero and the equilibrium potential is determined by the *permeability* of the membrane to a given ion ($P_j$) according to the following equation known as the Goldman-Hodgkin-Katz Equation:

$$E_d = (RT/F) \ln \frac{P_K [K_e] + P_{Na} [Na_e] + P_{Cl} [Cl_p]}{P_K [K_p] + P_{Na}[Na_p] + P_{Cl}[Cl_e]} \quad (6)$$

where

$E_d$ is the diffusion potential (in V) on the protoplasmic side of the membrane, assuming the potential of the exoplasmic side is 0 V

$R$ is the gas constant (8.31 J $mol^{-1}$ $K^{-1}$)

$T$ is the absolute temperature (in K)

$F$ is the Faraday Constant ($9.65 \times 10^4$ coulombs $mol^{-1}$)

$P_K$, $P_{Na}$, and $P_{Cl}$ are the permeability coefficients of $K^+$, $Na^+$, and $Cl^-$ ions, respectively (in m $s^{-1}$)

$K_e$, $Na_e$, and $Cl_e$ are the concentrations of $K^+$, $Na^+$, and $Cl^-$ on the exoplasmic side of the membrane (in mol $m^{-3}$)

$K_p$, $Na_p$, and $Cl_p$ are the concentrations of $K^+$, $Na^+$, and $Cl^-$ on the protoplasmic side of the membrane (in mol $m^{-3}$). See Appendix E for the derivation of the Goldman-Hodgkin-Katz Equation.

The resting potential of the plasma membrane of characean internodal cells and squid axons is approximately –0.18 V and –0.075 V, respectively, indicating that for both cell types $P_K >> P_{Na} > P_{Cl}$ and the membrane potential is determined to a large extent by the passive diffusion of $K^+$ (MacRobbie, 1962; Spanswick et al., 1967). The greater the permeability to $K^+$, the more hyperpolarized the membrane will tend to be. As we will see later, a hyperpolarized membrane is a stable membrane, compared to a depolarized membrane. Thus increasing the $K^+$ permeability has a stabilizing effect on the membrane. Notice that when $P_K >> P_{Na} > P_{Cl}$, the Goldman-Hodgkin-Katz Equation reduces to the Nernst Equation for $K^+$ (equation 2).

If the plasma membrane potential was only a consequence of the diffusion of $K^+$ from the protoplasm (≈100 mol $m^{-3}$) to the extracellular solution (0.1 mol $m^{-3}$), the cell would have a membrane potential of –0.18 V. According to equation 4, $1.125 \times 10^{16}$ $K^+$ $m^{-2}$ would have to diffuse across the plasma membrane in order to "charge the capacitance" of the membrane to –0.18 V. If we assume that the typical characean internodal cell is a cylinder $0.5 \times 10^{-3}$ m in diameter ($d$) and $3 \times 10^{-2}$ m long ($l$), it has a surface area ($\pi dl$) of $4.7 \times 10^{-5}$ $m^2$. Thus, $5.3 \times 10^{11}$ $K^+$ ions must diffuse across the plasma membrane of one cell to make a membrane potential of –0.18 V. A cylindrical internodal cell with the above dimensions will have a volume ($\pi r^2 l$) of $5.89 \times 10^{-9}$ $m^3$. Thus, there were initially $5.89 \times 10^{-7}$ mol $K^+$ in the cell. Using Avogadro's Number, we see that there were initially $3.5 \times 10^{17}$ $K^+$ in the cell. Thus, only $[(5.3 \times 10^{11})/(3.5 \times 10^{17})] \times 100\% = 0.00015\%$ of the initial $K^+$ ions must diffuse out of the cell in order to charge the capacitance of the membrane and create a membrane potential of –0.018 V.

A closer look at the Goldman-Hodgkin-Katz Equation shows that when $P_{Na}$ and $P_{Cl}$ are nonzero, the resting potential will be less than that predicted by the Nernst potential for $K^+$. However, the plasma membranes of both cell types have electrogenic pumps that pump out cations at the expense of ATP to help generate a more negative membrane potential. Characean cells have a $H^+$-ATPase (Spanswick, 1974a, 1980, 1981) and squid nerves have a $Na^+/K^+$ ATPase (Eckert et al., 1988). The membrane potential of the characean plasma membrane is usually between –0.18 and –0.30 V. The characean plasma membrane has three stable states. The *K-state*, where the membrane potential (more positive than –0.18 V) is determined predominantly by the diffusion of $K^+$; the *pump state*, where the electrogenic $H^+$-pumping ATPase contributes to the resting potential of approximately between –0.18 and –0.30 V; and a third stable state which occurs at high pH where the permeability to $H^+$ or $OH^-$ becomes so large that the diffusion potential is determined by the permeability coefficients for $H^+$ or $OH^-$ ($P_H$ or $P_{OH}$; Beilby & Bisson, 1992; Bisson & Walker, 1980, 1981, 1982). While the plasma membranes of characean cells in the pump state and high pH state are excitable, and cells in the $K^+$-state are inexcitable, we will only talk about the action potential in cells that are just at the interface between the $K^+$-state and the pump state (–0.18 V), since it has been shown that the action potential is mostly due to the changes in the conductance of the passive diffusion channels and not the electrogenic $H^+$-pumping ATPase (Kishimoto et al., 1985).

The vacuolar membrane of characean cells also has an electrogenic $H^+$-pumping ATPase as well as an electrogenic $H^+$-pumping pyrophosphatase (Shimmen & MacRobbie, 1987; Takeshige et al., 1988; Takeshige & Tazawa, 1989) capable of hyperpolarizing the membrane potential to values greater than –0.25 V, yet the vacuolar membrane potential (≈ –0.01 V) is similar to the equilibrium potential for

**Table III**

The electrochemical potential (in V) of each ion across the plasma membrane and vacuolar membrane of a characean internodal cell and the plasma membrane of a squid nerve

| | Characean internodal cell | | Squid nerve |
|---|---|---|---|
| | Plasma membrane | Vacuolar membrane | Plasma membrane |
| $Cl^-$ | –0.283 | 0.041 | –0.049 |
| $Na^+$ | –0.080 | –0.059 | –0.150 |
| $K^+$ | 0 | –0.008 | 0 |
| $Ca^{2+}$ | –0.239 | –0.131 | –0.193 |

If we assume that an ion is moving from a P-space (initial state) to an E-space (final state), we can tell whether the movement is spontaneous (passive, or exergonic) in this direction by multiplying the electrochemical potential with the product of the valence of the ion times the Faraday ($zF$).

When the product of the electrochemical potential and $zF$ is *negative*, cations and anions are accumulated passively (spontaneously) and efflux is active.

When the product of the electrochemical potential and $zF$ is *positive*, cations and anions are accumulated actively and efflux is passive (spontaneous).

In reality, active transport usually results from the energy available from the electrochemical gradient of $H^+$ established by the primary $H^+$-pumping ATPase. Secondary transporters then move the actively accumulated ions across the membrane.

$K^+$ and $Cl^-$, indicating that there is a large permeability to $K^+$ and $Cl^-$ that effectively short-circuits the pumps (Rea & Sanders, 1987; Tyerman, 1992).

The difference between the resting membrane potential ($E_m$) and the equilibrium potential for an ion ($E_j$) across that membrane, known as the *electrochemical potential*, is an indication of whether or not that ion is in passive equilibrium, actively taken up, or actively extruded. Assuming that the membrane potentials across the characean plasma membrane and vacuolar membrane are –0.18 V and –0.01 V, respectively, the electrochemical potentials ($E_m - E_j$) across each membrane can be calculated and are given in Table III. The electrochemical potentials across the plasma membrane of a squid axon are given for comparison (assuming $E_m \approx -0.075$).

At the resting potentials of the characean and squid plasma membrane, $K^+$ is passively (spontaneously) distributed across the plasma membrane [$zF(E_m - E_K) = 0$]; $Cl^-$ is actively (not spontaneously) accumulated against its electrochemical potential gradient [$zF(E_m - E_{Cl}) < 0$] and moves passively out of the cell; $Na^+$ is actively extruded against its electrochemical potential gradient and moves passively into the cell [$zF(E_m - E_{Na}) < 0$]; and $Ca^{2+}$ is also actively extruded against its electrochemical potential gradient [$zF(E_m - E_{Ca}) < 0$] and moves passively into the cell. On the other hand, at the resting potential of the characean vacuolar membrane, $Cl^-$, $K^+$, $Na^+$, and $Ca^{2+}$ are actively extruded from the protoplasm to the vacuole across the vacuolar membrane. The active movement of ions requires energy and is thus an endergonic reaction. The passive movement of ions is an exergonic process which releases energy. This energy can be coupled to endergonic processes to do work, including the cotransport of substances.

In order to better understand the electrophysiology of excitable cells, it helps to draw an *equivalent circuit* that explains the electrical analogs in the membrane. First, let us assume that at the resting potential, the sum of all ionic currents ($\Sigma I_j$, in A $m^{-2}$) is 0

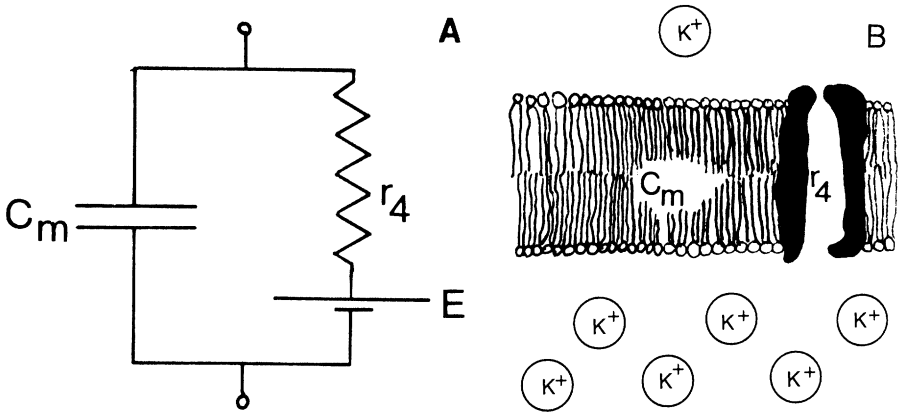



**Fig. 7.** Equivalent circuit and structural interpretation of a *Nitella* plasma membrane. **A.** The equivalent circuit as interpreted by Cole and Curtis (1938). An equivalent circuit is an electrical circuit that is made up of the minimum number of electrical components (resistors, capacitors, batteries, etc.) that, when connected, can mimic complicated electrical (or other) behaviors. $E$ represents the electromotive force of a cell of a battery; $C_m$ represents the membrane capacitance; and $r_4$ represents the resistance of the membrane that is in parallel with the membrane capacitance. **B.** Structural interpretation of the equivalent circuit. The electromotive force or membrane potential ($E$) is determined by the difference in the $K^+$ concentration on both sides of the membrane. The lipid bilayer, by virtue of its thickness ($2.7 \times 10^{-9}$ m) and its relative permittivity (3), separates two conducting layers and thus acts as a capacitor. The conductance (the reciprocal of resistance) is provided to a large extent by the proteinaceous pores through the membrane.

A. The model put forth by Cole and Curtis (1938) includes the observations that the membrane potential is due to an electromotive force (the equilibrium potential due to the unequal distribution of ions) and is equivalent to a battery that is in series with a *conductance* (proteinaceous ion channels) and in parallel with a *capacitor* (as a result of the lipid bilayer). Their model is shown in Fig. 7A and a structural interpretation of the equivalent circuit is given in Fig. 7B.

In order to describe the resting and action potential in giant squid axons, Hodgkin (1964) partitioned the series conductance and electromotive force of the equivalent circuit into a number of conductances (one for $Na^+$, one for $K^+$, and one for the ions that do not contribute to the action potential—the leak) and a number of electromotive forces due to the equilibrium potentials of each ion (Fig. 8).

The contribution of the electromotive force for each ion to the overall membrane potential depends on the ability of that ion to carry current. The fact that the electromotive force and the conductance for an ion are in series means that if the conductance is zero for a given ion, then the electromotive force for that ion will not contribute anything to the membrane potential, even if there is a large electromotive force. Since the conductance for all ions except $K^+$ is so small in the resting cell, the membrane potential is essentially equal to $E_K$ (the equilibrium potential for $K^+$).

The current density ($I_j$, in A $m^{-2}$) carried by each ion depends on both the specific conductance of the membrane for that ion ($g_j$, in S $m^{-2}$) and the electrochemical potential for that ion ($E_m - E_j$). This relationship is just another form of Ohm's Law where the specific membrane conductance for an ion ($g_j$) is equal to the reciprocal of the specific resistance of the membrane to that ion ($r_j$, in $\Omega$ $m^2$).

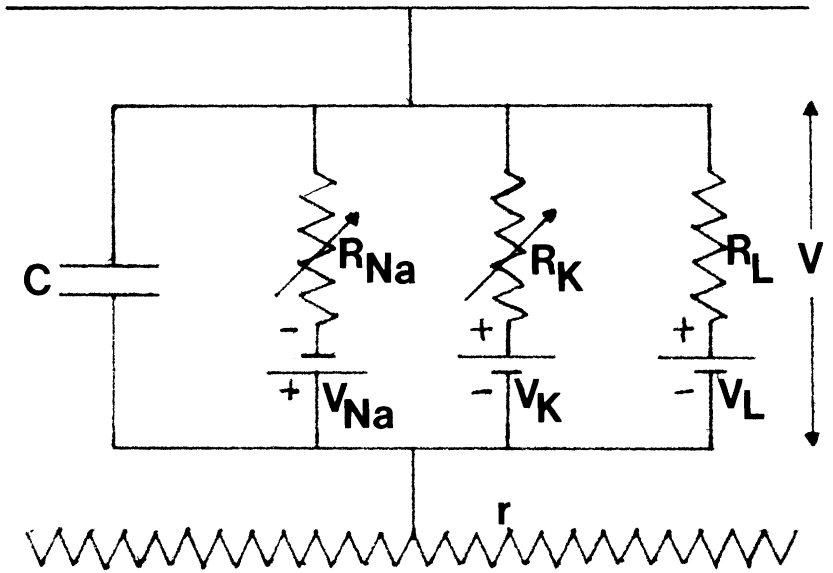



**Fig. 8.** Equivalent circuit of a squid axon according to Hodgkin (1964). $V$ represents the membrane potential. $V_{Na}$, $V_K$, and $V_L$ represent the electromotive force across the plasma membrane (i.e,. the difference between the membrane potential and the Nernst potential) for sodium ions, potassium ions, and ions that do not contribute to the action potential (i.e., the leak), respectively. $R_{Na}$, $R_K$, and $R_L$ represent the (variable) resistance of the plasma to sodium, potassium, and leak ions, respectively. $C$ represents the membrane capacitance that is in parallel to the resistances. The electrical resistance of the cytoplasm, in which the microcapillary electrode resides, is denoted by $r$.

$$I_j = -g_j(E_m - E_j) = -(E_m - E_j)/r_j \tag{7}$$

where

$I_j$ is the current density (in A $m^{-2}$) carried by ion $j$
$g_j$ is the partial specific conductance (in S $m^{-2}$) of the membrane for ion $j$
$E_m$ is the membrane potential (in V)
$E_j$ is the Nernst potential or equilibrium potential (in V) for ion $j$
$r_j$ is the partial specific resistance of the membrane (in $\Omega$ $m^2$) for ion $j$.

We use Benjamin Franklin's convention that positive current is carried by positive charges (Cohen, 1941). So a current carried by $Cl^-$ is negative and a current carried by $Na^+$ or $K^+$ is positive. (Consequently, an *inward current* is carried by the influx of a cation and the efflux of an anion). At rest the sum of all currents equals zero. We can now use the equivalent circuit of the resting membrane to help us understand the action potential. We will see that there is a change in the membrane potential during an action potential because there is a change in the conductance (or the permeability) of the membrane to certain ions.

## V. The Ionic Basis of the Action Potential

The basic principles involved in the generation of an action potential are the same in animal, plant, and fungal cells. The specifics of the action potentials are different

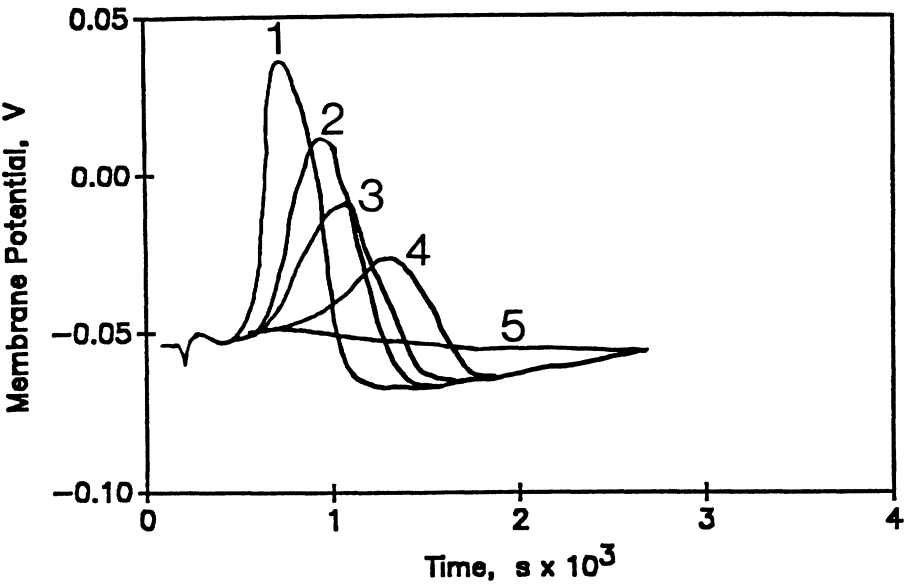



**Fig. 9.** The effect of sodium on the action potential of squid neurons. Trace 1 shows an action potential that was initiated in axons bathed in artificial sea water (ASW) containing NaCl. Traces 2–5 show action potentials initiated after various periods of time after the $Na^+$ in the ASW was then replaced with Choline-Cl. It takes a certain amount of time for the $Na^+$ concentration near the membrane to decrease. (Redrawn from Hodgkin & Katz, 1949.)

and result from the fact that, in general, animal cells live in solutions that are isotonic and are full of ions, while plants and fungi live in dilute solutions that are very close to distilled water. The capability of cells to live in dilute solutions is a consequence of the presence of a rigid extracellular matrix (cell wall) that can support and prevent the lysis of the plasma membrane that otherwise would occur when the osmotic pressure on the two sides of the membrane differ by more than 100 Pa. (Plant and fungal cells can build up pressure differences 1000–10,000 times greater than this value.) Therefore, the "animal type of action potential" is not possible in plant cells (and vice versa), because the ion concentrations surrounding a plant cell are different from those found in the extracellular milieu of animal cells.

Hodgkin and Katz (1949) found that external $Na^+$ is required for the action potential of squid axons. Upon stimulation in normal seawater, the squid axon depolarizes from approximately –0.075 V to +0.04 V at the peak of the action potential. However, when the $Na^+$ is replaced by choline, both the rate of depolarization and the amplitude of the action potential decreases (Fig. 9). Hodgkin and Katz (1949) then proposed the *sodium hypothesis*, which states that the massive depolarization and overshoot of the membrane potential to positive potentials results from an influx of $Na^+$. Using the radioactive tracer $^{24}Na^+$, Keynes (1951) then showed that electrical stimulation causes an 18-fold increase in $Na^+$ influx. Later it was found that tetrodotoxin (TTX), the deadly poison found in and (mostly) removed from the Japanese puffer fish eaten as sashimi, prevents the action potential by specifically blocking $Na^+$ currents in giant axons (Narahashi et al., 1964). Not all animal cells have $Na^+$-based, TTX-inhibited

action potentials. The $Na^+$-based action potentials occur only in higher animals. Evolutionarily, they begin to appear with the Coelenterates. The more ancient and ubiquitous action potentials result from "$Ca^{2+}$ spikes" (Hille, 1984, 1992).

The equilibrium potential for $Na^+$ ($E_{Na}$) is not large enough in characean cells to account for the large depolarization that occurs during an action potential (Williams & Bradley, 1968). Upon stimulation, the characean plasma membrane depolarizes to approximately 0 V. While the electrochemical potential of $Na^+$ will tend to drive $Na^+$ into the cell at the resting potential, it will cease to move passively into the cell once the potential depolarizes to –0.100 V and the electrochemical potential for $Na^+$ approaches 0 V. Thus $Cl^-$ and $Ca^{2+}$ ions have positive equilibrium potentials and are the only ions that are capable of depolarizing the membrane to 0 V. The cation $Ca^{2+}$ can depolarize the membrane by entering the cell, and the anion $Cl^-$ can depolarize the membrane by leaving the cell. Thus, while the action potentials of the squid and characean plasma membrane are similar in terms of the voltage changes, the ions that carry the depolarizing currents are different.

Characean cells have a vacuolar membrane in addition to the plasma membrane. The membrane potential of the vacuolar membrane hyperpolarizes from –0.01 V (protoplasmic side negative) to –0.05 V (protoplasmic side negative) during an action potential. Using similar logic, we see that $Cl^-$ is the only current carrier capable of bringing the vacuolar membrane membrane potential to the –0.05 V it attains at the peak of the action potential. In this case, $Cl^-$ will move from the vacuole to the protoplasm.

## VI. Chloride Efflux Occurs in Response to Stimulation

Using a thermodynamic approach, we have determined that $Ca^{2+}$ and $Cl^-$ ions are capable of carrying the current that leads to the change in the plasma membrane potential that occurs during an action potential in characean cells. There are two ions that are candidates for the charge carrier during the action potential, and there were two schools of thought on which was the charge carrier: One thought that $Ca^{2+}$ was important and the other thought that $Cl^-$ was important. Hope (1961a, 1961b) and Findlay (1961, 1962), working in Australia, noticed that the magnitude of the depolarization that occurred during an action potential depended on the external $Ca^{2+}$ concentration, where the depolarization increased as the external $Ca^{2+}$ concentration increased, and, further, that external $Ca^{2+}$ was required for the action potential (Findlay & Hope, 1964b). They suggested that the action potential was due to an increase in the permeability to $Ca^{2+}$ and $Ca^{2+}$ was the current-carrying ion. However, using $^{45}Ca^{2+}$ as a tracer, they were unable to observe any change in $^{45}Ca^{2+}$ influx (probably due to the difficulty in separating wall-bound $^{45}Ca^{2+}$ from cytoplasmic $^{45}Ca^{2+}$). Thus the role of $Ca^{2+}$ in the action potential remained enigmatic (Hope & Findlay, 1964). Fifteen years later, Hayama et al. (1979) detected a $Ca^{2+}$ influx, again bringing the action of a $Ca^{2+}$ influx into the picture.

On the other hand, Mullins (1962), working in the United States, proposed that the action of $Ca^{2+}$ was to activate the mechanism responsible for $Cl^-$ efflux. Gaffey and Mullins (1958), Mullins (1962), and Hope and Findlay (1964) loaded internodal cells with $^{36}Cl^-$ and measured its efflux in the resting cell and following stimulation. They found that there is an increase in $Cl^-$ efflux during an action potential. Mailman and Mullins (1966) measured the $Cl^-$ efflux using a Ag/AgCl electrode, confirming the

**Table IV**

$Cl^-$ efflux in a characean internodal cell at rest and during an action potential.

| | Efflux (mol $m^{-2}$ $s^{-1}$) |
|---|---|
| Resting cell | $1 \times 10^{-8}$ |
| Excited cell | $100 \times 10^{-8}$ |

data obtained using radioactive tracers and improving on the time resolution from hours using the tracer technique (Hope et al., 1966) to seconds using the electrode technique (Coster, 1966). Typical $Cl^-$ efflux values are given in Table IV.

Given a membrane depolarization of 0.18 V and a specific membrane capacitance of $10^{-2}$ F $m^{-2}$, an increase in the efflux to $1.125 \times 10^{16}$ $Cl^-$ $m^{-2}$ (= $1.87 \times 10^{-8}$ mol $m^{-2}$, using Avogadro's Number as a conversion factor) would be needed to discharge the membrane capacitance and depolarize the membrane to 0 V (equation 4). Assuming that the action potential lasted 1 s, this would be equivalent to an efflux of $1.86 \times 10^{-8}$ mol $m^{-2}$ $s^{-1}$. Thus, the increase in the efflux in response to stimulation is more than enough to overcome the membrane capacitance and account for the depolarization observed during the action potential.

We can estimate the change in membrane permeability to $Cl^-$ ($P_{Cl}$) during excitation using the following formula (Dainty, 1962) based on the Goldman flux equation (Goldman, 1943):

$$J_{Cl} = \frac{P_{Cl}[(z_{Cl}F/RT)E_m]\{n^o_{Cl} - n^i_{Cl}\exp[(z_{Cl}F/RT)E_m]\}}{\exp[(z_{Cl}F/RT)E_m] - 1} \quad (8)$$

where

$J_{Cl}$ is the flux of $Cl^-$ in (mol $m^{-2}$ $s^{-1}$)
$E_m$ is the diffusion potential (in V) on the protoplasmic side of the membrane, assuming the potential of the exoplasmic side is 0 V.
$R$ is the gas constant (8.31 J $mol^{-1}$ $K^{-1}$)
$T$ is the absolute temperature (in K)
$F$ is the Faraday Constant ($9.65 \times 10^4$ coulombs $mol^{-1}$)
$z_{Cl}$ is the valence of $Cl^-$ (–1)
$P_{Cl}$ is the permeability coefficient of the membrane to $Cl^-$ (in m $s^{-1}$)
$n_{Cl}^o$ is the concentration of $Cl^-$ on the exoplasmic side of the membrane (in mol $m^{-3}$)
$n_{Cl}^i$ is the concentration of $Cl^-$ on the protoplasmic side of the membrane (in mol $m^{-3}$). See Appendix E for a derivation of the Goldman-Hodgkin-Katz Equation.

Given the observed fluxes of $Cl^-$ and assuming that the cytosolic and extracellular concentrations of $Cl^-$ remain constant at 22 and 0.4 mol $m^{-3}$, respectively, and the membrane potential is –0.18 V, we find that the *average* permeability coefficient of the membrane for $Cl^-$ rises approximately 100-fold from $4.5 \times 10^{-10}$ m $s^{-1}$ to $4.5 \times 10^{-8}$ m $s^{-1}$ during an action potential.

We can also relate the $Cl^-$ flux ($J_{Cl}$) measured chemically to the conductance of the

membrane ($g_{Cl}$) for $Cl^-$ measured electrically using the following equation (Hodgkin, 1951; Hodgkin & Keynes, 1955; Hope & Walker, 1961; Keynes, 1951; Linderholm, 1952; Smith, 1987; Teorell, 1953; Ussing, 1949a, 1949b; Ussing & Zerahn, 1951):

$$g_{Cl} = (z_{Cl}^2 F^2/RT)J_{Cl} \quad (9)$$

where

$g_{Cl}$ is the partial specific membrane conductance for $Cl^-$ (in S $m^{-2}$)
$z_{Cl}$ is the valence of $Cl^-$ (–1)
$F$ is the Faraday Constant ($9.65 \times 10^4$ coulombs $mol^{-1}$)
$R$ is the gas constant (8.31 J $mol^{-1}$ $K^{-1}$)
$T$ is the absolute temperature (in K)
$J_{Cl}$ is the unidirectional flux of $Cl^-$ (in mol $m^{-2}$ $s^{-1}$).

The result is only approximate since the equation only holds when the $Cl^-$ ions move across the membrane independently of each other and the membrane potential is at the equilibrium potential for $Cl^-$ (i.e., +0.103 V). Many ions probably move through long narrow pores and interact with each other, invalidating the use of this equation (MacRobbie, 1962). Moreover, the membrane potential never reaches the equilibrium potential for $Cl^-$. However, using equation 9, we can approximate the partial specific conductance of the resting membrane ($g_{Cl} = 0.037$ S $m^{-2}$) and of the excited membrane ($g_{Cl} = 3.7$ S $m^{-2}$). Thus $g_{Cl}$ contributes approximately 4% to the overall conductance of the resting membrane and about 12% to the overall conductance of the excited membrane.

Following the massive depolarization, the membrane repolarizes again due to the efflux of $K^+$. This is true for axons as well as for characean cells (Hodgkin & Huxley, 1953; Keynes, 1951). $K^+$ efflux has been determined by measuring $^{32}K^+$ fluxes or by using flame photometry (Kikuyama et al., 1984; Oda, 1976; Spear et al., 1969; Spyropoulos et al., 1961). The $K^+$ efflux is equal to the $Cl^-$ efflux. They are both $1.8 \times 10^{-5}$ mol $m^{-2}$ $impulse^{-1}$ (Oda, 1976). Therefore, during excitation, $P_K$ is approximately $2.3 \times 10^{-5}$ m $s^{-1}$ (calculated using equation 8). This is 1000 times greater than the resting $P_K$ (Gaffey & Mullins, 1958; Smith, 1987; Smith & Kerr, 1987; Smith et al., 1987a, 1987b). The necessity of a $K^+$ efflux for the subsequent repolarization is supported by the observation that a $K^+$-channel blocker, tetraethylammonium (TEA) causes a prolongation of the action potential in some but not all cells (Belton & Van Netten, 1971; Shimmen & Tazawa, 1983a; Staves and Wayne, 1993; Tester, 1988).

The massive efflux of $Cl^-$ and $K^+$ from the cell is accompanied by water loss and a transient decrease in turgor (Barry, 1970a, 1970b). The water loss results in a transient change in the volume of the cell that can be detected with a position transducer (Kishimoto & Ohkawa, 1966) and by laser interferometry (Sandlin et al., 1968).

Now we can begin to account for the membrane potential changes that occur during a characean action potential by the movement of $Cl^-$ and $K^+$. We can make an equivalent circuit to help us visualize the changes in membrane potential that results from the changes in conductance.

Figure 10 shows an equivalent circuit of a characean cell. The resistors symbolize the channels that provide the conducting pathway for a given ion. The batteries represent the electomotive force for each ion due to the unequal distribution of ions (i.e., Nernst potential or equilibrium potential). The capacitor represents the ability of

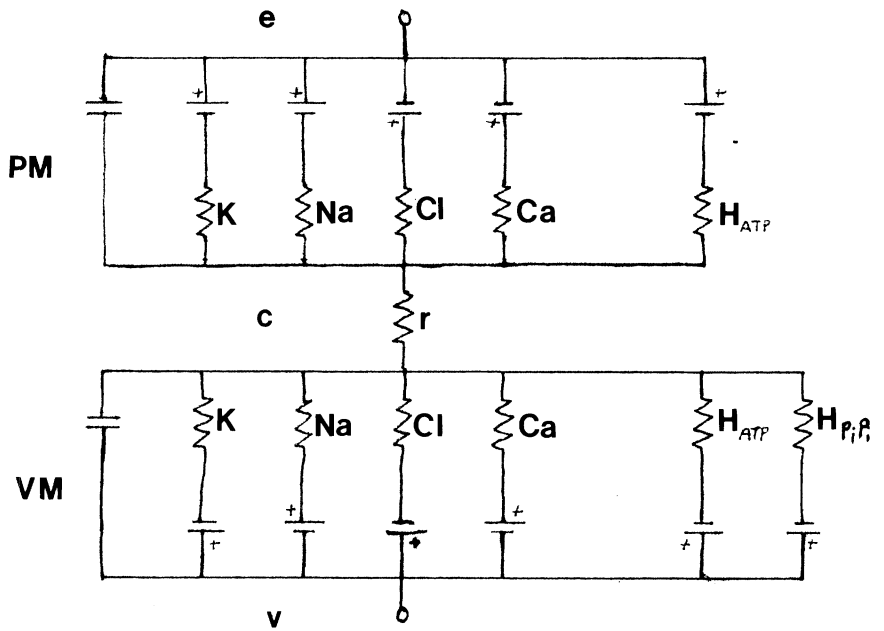



**Fig. 10.** Equivalent circuit of a characean cell. PM and VM represent the plasma membrane and vacuolar membrane, respectively. The external, cytosolic, and vacuolar solutions are represented by *e*, *c*, and *v*, respectively. Both the plasma membrane and the vacuolar membrane have electromotive forces and conductances (1/resistance) for potassium, sodium, chloride, and calcium ions. The two membranes, both with resistances and capacitances, are in parallel. The plasma membrane has a $H^+$-pumping ATPase, which has a conductance. The vacuolar membrane has a $H^+$-pumping ATPase and a $H^+$-pumping pyrophosphatase, both of which have conductances. The electrical resistance of the cytoplasm, in which the microcapillary electrode resides, is denoted by *r*.

the lipid bilayer to separate charge. The plasma membrane has an electrogenic $H^+$-pumping ATPase with an electromotive force of –0.46 V (due to the hydrolysis of ATP; Blatt et al., 1990). The potential difference across the plasma membrane (approximately –0.180 V, inside negative) is defined as the membrane potential of the protoplasmic side minus the membrane potential of the exoplasmic side (defined as 0 V).

The vacuolar membrane has a negative potential difference that is also defined as the membrane potential of the protoplasmic side (–0.010 V) minus the membrane potential of the exoplasmic side (defined as 0 V). The membrane potential of the vacuole is determined to a large extent by the $K^+$-dependent electromotive force since the $K^+$ conductance is so high. This conductance effectively shunts the $H^+$-pumping ATPase and pyrophosphatase on the vacuolar membrane so they do not contribute significantly to the vacuolar membrane potential. Note that the potential differences across the plasma membrane and the vacuolar membrane are oppositely directed.

## VII. Application of a Voltage Clamp to Relate Ions to Specific Currents

Up until now we have been talking about experimental setups where the current is applied as a stimulus and we record the changes in membrane potential. This experi-

ment can be done in reverse by using a voltage clamp, which was designed by K. S. Cole in 1949 to measure the current that flows when the membrane potential is "clamped" to a given voltage (Sherman-Gold, 1993; Standen et al., 1987). No net current flows when the membrane potential is clamped to the resting potential, whereas there is a current flow when the membrane potential is clamped to potentials less negative than the "threshold potential for excitation." When the membrane potential remains constant, the change in the amount of current that flows is a measure of the conductance. The voltage clamp is superior to the traditional current clamp in that it can rapidly clamp the membrane and allow one to distinguish the "instantaneous" currents that flow from the discharge of the membrane capacity (capacity currents) from the time-dependent ionic currents (Hodgkin et al., 1952; Kishimoto, 1964).

The pioneering work on voltage clamping the giant squid axons by Alan Hodgkin, Andrew Huxley, and Bernard Katz established the classical principles of membrane biophysics (Hille, 1984, 1992). When Hodgkin and his colleagues clamped the membrane potential at values less negative than the threshold potential (–0.056 V), they saw a time-dependent current. First there was an inward current and then this was replaced by an outward current (Fig. 11A). They noticed that when they removed the $Na^+$ from the external medium, the early inward current disappeared but the late outward current remained (Fig. 11B). By subtracting the current trace obtained in the absence of $Na^+$ from the one obtained in the presence of $Na^+$, they obtained a difference trace that represented the $Na^+$ current. They assumed that the current trace obtained in the absence of $Na^+$ represented the $K^+$ current. This was confirmed by removing the natural protoplasm by perfusion and substituting it with either $K^+$-containing or $K^+$-free fluid and observing that the late current disappeared when they used $K^+$-free fluid. By dividing the magnitude of the $Na^+$ current or the $K^+$ current at a given time by the membrane potential which was held constant by the clamp, they could calculate the change in $Na^+$ and $K^+$ conductances with time that occurred during an action potential (Hodgkin, 1964).

Hodgkin and his colleagues found that the resting membrane potential results from the fact that $g_K > g_{Na} > g_{Cl}$. The action potential occurs after a stimulus causes an increase in the conductance to $Na^+$ ($g_{Na}$). Thus, upon stimulation, a substantial amount of $Na^+$ moves down its electrochemical potential gradient from the external medium into the axoplasm. This inward current causes a shift in the membrane potential toward the $Na^+$ equilibrium potential (i.e., depolarization). The membrane potential consequently shifts away from the $K^+$ equilibrium potential so that the electrochemical potential gradient now drives an increased efflux of $K^+$. This is accompanied by an increase in the conductance to $K^+$ ($g_K$). The increased conductance for $K^+$ will repolarize the membrane back to its resting level. The repolarization of the membrane to its resting level due to $K^+$ efflux is facilitated by the time-dependent decrease in $g_{Na}$ (i.e., inactivation). Since the $K^+$ efflux overlaps the $Na^+$ influx, the membrane never depolarizes all the way to $E_{Na}$. The peak depolarization is somewhere between $E_K$ and $E_{Na}$ and can be predicted from the Goldman-Hodgkin-Katz Equation using the time-dependent relative permeabilities and concentrations of $K^+$ and $Na^+$ (see Eckert et al., 1988; Junge, 1981; Matthews, 1986).

Lunevsky et al. (1983), working in the former Soviet Union, used a voltage clamp to study the action potential across the plasma membrane of the characean alga *Nitellopsis*. They inserted one electrode in the protoplasm and one in the medium to

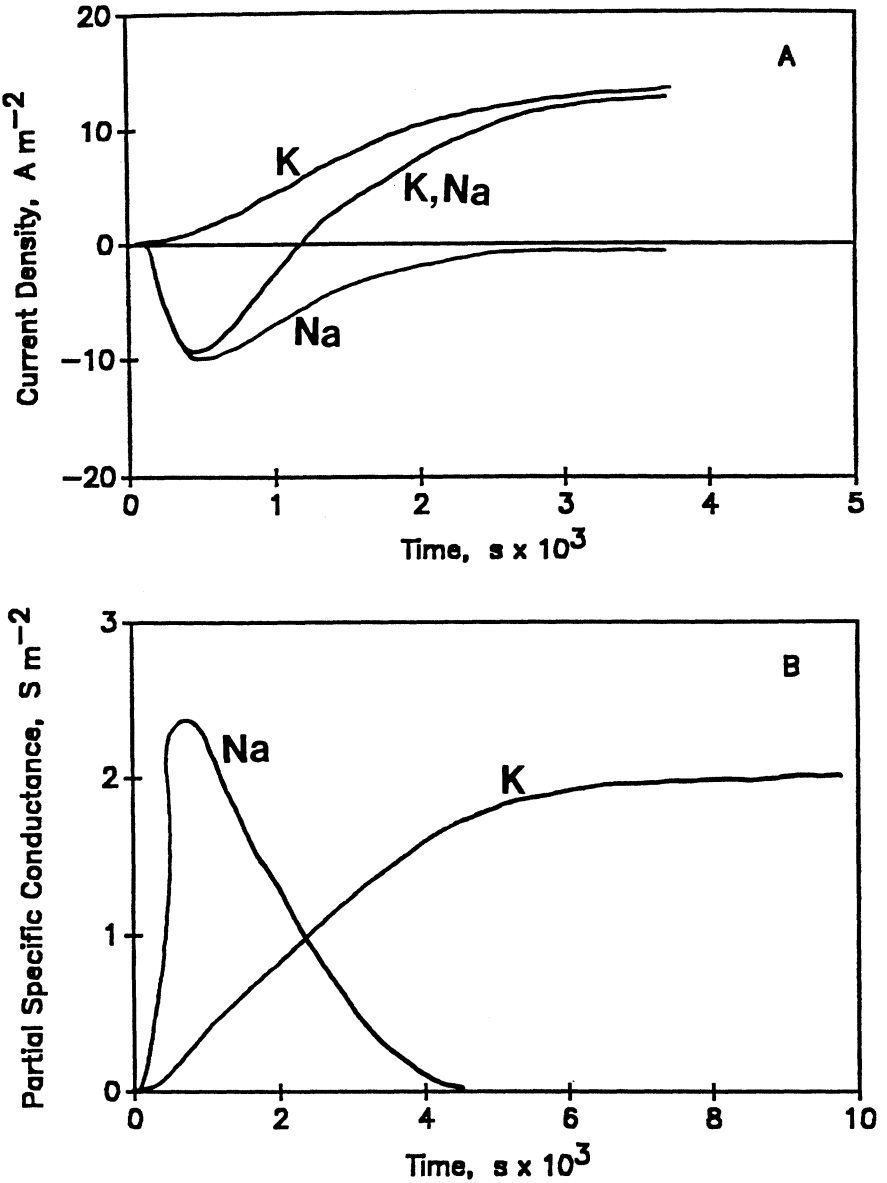



**Fig. 11. A.** Current recordings from a squid axon. Separation of currents carried by $Na^+$ and $K^+$ using a voltage clamp. K,Na represents currents measured in the presence of extracellular $Na^+$ and intracellular $K^+$. K represents the currents measured in the absence of extracellular $Na^+$. (In this case the $Na^+$ was replaced by choline). Na represents the $Na^+$ current, which was obtained by subtracting the $K^+$ current from the total current. Note: By convention, inward currents are always downward. **B.** Change in conductance over time. The conductance for $Na^+$ and $K^+$ were determined by dividing the current density (in A $m^{-2}$) carried by $Na^+$ or $K^+$ by the potential at which the membrane was held by the voltage clamp (0.056 V). (Redrawn from Hodgkin, 1964.)

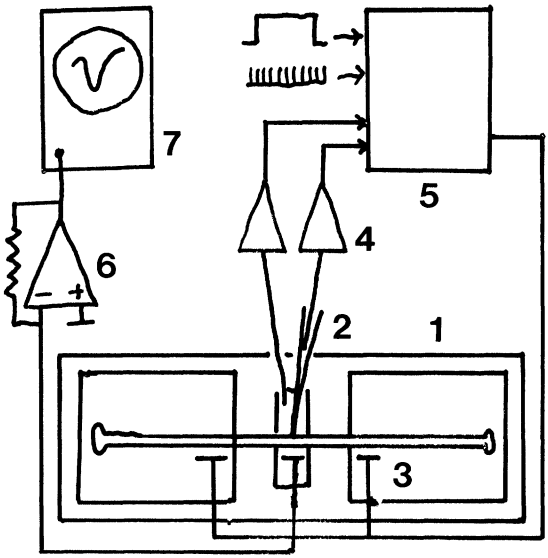



**Fig. 12.** Setup of voltage clamp to study characean cells. An internodal cell is placed in a three-chambered acrylic holder (1). A microcapillary electrode (2), used to record the membrane potential, is placed in the cytoplasm. The voltage is followed by a preamplifier (4) and passed to a voltage clamp amplifier (5). The voltage clamp amplifier compares the membrane potential to the desired potential and outputs current to the Ag/AgCl wires (3) so that the membrane potential is held at a desired value. The current is then passed to a current monitor (6) and an oscilloscope or oscillograph (7) so that the current and voltage can be recorded simultaneously (7). (Redrawn from Lunevsky et al., 1983.)

measure the membrane potential. The membrane is clamped to the desired voltage by passing just enough current through the cell using Ag/AgCl wires (Fig. 12).

Using a step-voltage clamp, where the membrane potential can be varied in a stepwise manner, Lunevsky et al. (1983) obtained a series of current traces that represented current movement through the plasma membrane at each membrane potential (Fig. 13). They resolved their data into three currents: the quick transient, the slow transient, and the steady state current (in addition to the leakage current).

The first, quick transient component lasts for several hundred milliseconds. When the membrane is depolarized to –0.05 V it appears as an inward current. It changes from an inward current to an outward current as the membrane potential is clamped to more and more depolarized values. The *reversal potential* for this current—that is, the voltage where the current is neither inward nor outward ($I_j = 0$ A m$^{-2}$)—is between –0.060 and –0.020 V in artificial pond water (APW). This rapid, transient current could be studied better if we could block the second current, so we'll talk about the second current and then come back to the first.

The second, slow transient current appears when the membrane is depolarized below a threshold between –0.09 and –0.12 V and the maximum peak inward current is seen at potentials 0.01 to 0.02 V more positive than the threshold. The slow transient current exhibits *activation–inactivation kinetics* that are similar to, although slower than, the $Na^+$ current in squid axons. The reversal potential of the slow transient current depends on the external $Cl^-$ concentration. When the external $Cl^-$ concentration is increased

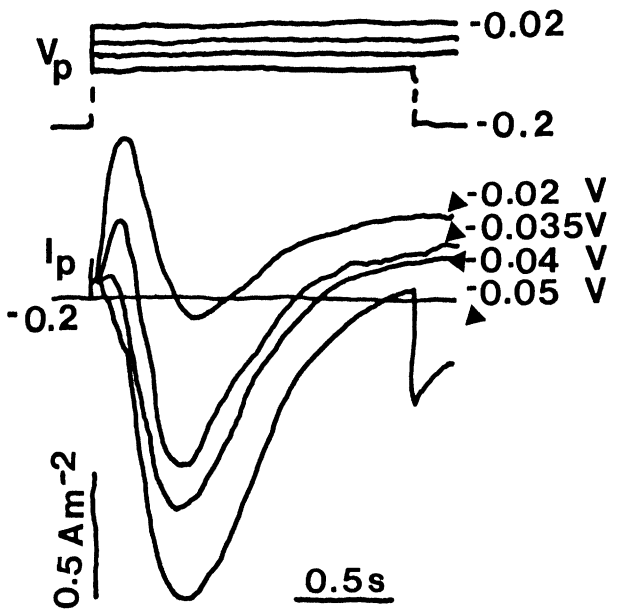



**Fig. 13.** Current recordings from a characean internodal cell. The membrane was clamped at various potentials and the current that flowed through the cell was recorded. $V_p$ and $I_p$ represent the potential of the plasma membrane and the current density through the plasma membrane, respectively. The reversal potential for the first quick transient current is –0.045 V in this cell. (Redrawn from Lunevsky et al., 1983.)

3.25-fold, from 32 mM to 104 mM, the reversal potential changes from –0.010 V to –0.040 V in a manner completely accounted for by the Nernst Equation, as $Cl^-$ is the only ion carrying the current. Further evidence that the second slow transient current is carried by $Cl^-$ comes from the observation that this current is inhibited by the well-known *Cl-channel blockers*, ethacrynic acid and anthracene-9-carboxylic acid (Fig. 14). The peak current carried by $Cl^-$ ions is between 4 and 7 A m$^{-2}$. The net flux can be linked to the observed current using the following equation:

$$J_{Cl} = I_{Cl}/(z_{Cl}F) \tag{10}$$

where

$J_{Cl}$ is the flux of $Cl^-$ (in mol m$^{-2}$ s$^{-1}$).
$I_{Cl}$ is the electric current carried by $Cl^-$ (in A m$^{-2}$)
$z_{Cl}$ is the valence of $Cl^-$ (–1)
$F$ is the Faraday Constant ($9.65 \times 10^4$ coulombs mol$^{-1}$).

This means that at the peak of the action potential the *instantaneous* rate of $Cl^-$ efflux must be $4–7 \times 10^{-5}$ mol m$^{-2}$ s$^{-1}$.

When the $Cl^-$ current is inhibited by ethacrynic acid, it is possible to study only the first quick transient current. The first current rapidly activates in 100–250 ms and slowly inactivates over the next 0.5–1 s (Fig. 15). The channel that passes the first current passes cations, including $Na^+$, $K^+$, and $Ca^{2+}$, although under normal conditions it will pass $Ca^{2+}$ ions selectively.

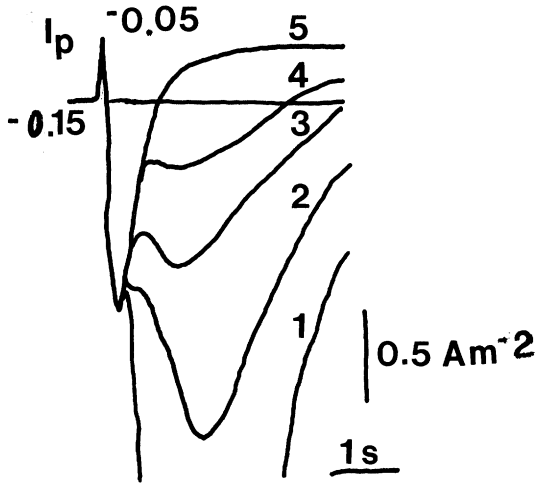



**Fig. 14.** Inhibition of the $Cl^-$ current by the $Cl^-$ channel blocker ethacrynic acid. Trace 1 represents the initial current and traces 2–5 represent currents measured at successive 10-minute intervals after the application of ethacrynic acid. (Redrawn from Lunevsky et al., 1983.)

The voltage-clamp experiments show that membrane depolarization can activate two transient (inward) currents: The first one is carried by $Ca^{2+}$ ions moving into the cell and the second one is carried by $Cl^-$ ions moving out of the cell. Thus, there is an initial quick transient increase in the conductance for $Ca^{2+}$, followed by a slow transient increase in the conductance for $Cl^-$. Following these two transient currents there is a steady state current that probably is carried by $K^+$, as determined by its reversal potential (Fig. 16). The changes in conductances over time have been estimated from the work of Lunevsky et al. (1983). Beilby and Coster (1979a, 1979b) carried out a Hodgkin–Huxley type analysis on their voltage-clamp data which cannot be reconciled at the present time with that of Lunevsky et al. (1983). Hirono and Mitsui (1983) also have done a Hodgkin–Huxley type analysis on the activation–inactivation of the combined $Ca^{2+}$ and $Cl^-$ currents. A complete analysis and reconciliation of the data will have to await new methods for specifically identifying and completely blocking each individual ionic current.

Lunevsky et al. (1983) postulated that the $Cl^-$ channel is activated by an increase in the protoplasmic $Ca^{2+}$ concentration that occurs as a consequence of the activation of the $Ca^{2+}$ conductance. The activation of the $Cl^-$ channel is inhibited when cells are treated with the $Ca^{2+}$ channel blocker, $La^{3+}$ (Tsutsui et al., 1987a, 1987b, 1987c). Moreover, $Ca^{2+}$ is required for the repolarization of the membrane potential which results from $K^+$ efflux across the plasma membrane (Shimmen & Tazawa, 1983a) as well as the activation of a $K^+$ channel responsible for the repolarization of the vacuolar membrane (Katsuhara et al., 1989; Laver, 1992; Laver & Walker, 1991).

Now let's study the excitability of each membrane separately instead of observing two membranes in series. First we'll look at the vacuolar membrane. The vacuolar membrane is studied by gently permeabilizing the plasma membrane (with 5 mM EGTA and no $Ca^{2+}$ at 4°C) so that the plasma membrane becomes completely permeable to ions and small molecules yet keeps macromolecules in the protoplasm

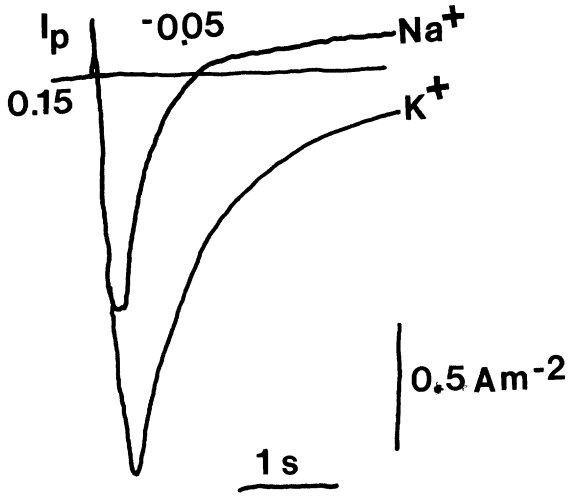



**Fig. 15.** Activation–inactivation of the first current. The $Cl^-$ current has been inhibited in this cell by ethacrynic acid. This transient current represents the passage of monovalent ions (60 mol $m^{-3}$ $K^+$ or $Na^+$) through a cation channel. This channel probably functions as a $Ca^{2+}$ channel under physiological conditions, where the $Na^+$, $K^+$, and $Ca^{2+}$ concentrations are approximately 0.1 mol $m^{-3}$ each. (Redrawn from Lunevsky et al., 1983.)

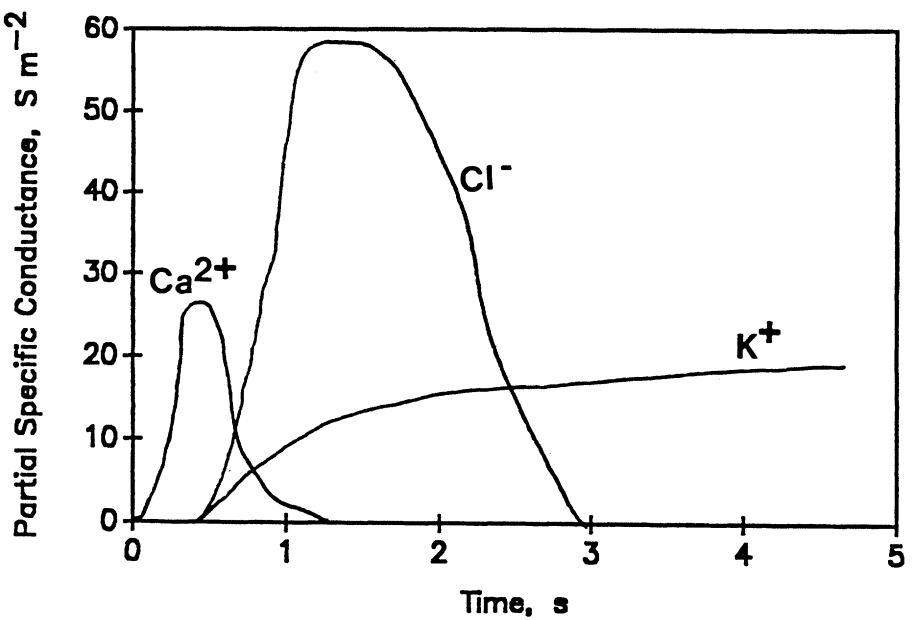



**Fig. 16.** Change in conductance over time. Estimate of partial specific conductances derived from data presented in Figures 13–15.

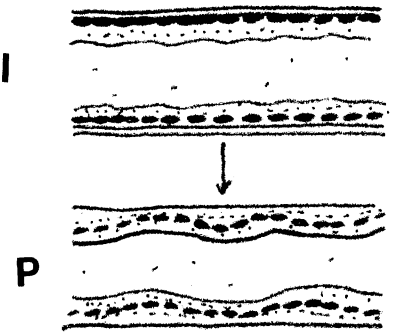



**Fig. 17.** Diagram of a permeabilized cell. *I* and *P* represent intact and permeabilized cells, respectively. In the intact cell, both the plasma membrane and the vacuolar membrane are semipermeable. In the permeabilized cell, only the vacuolar membrane is semipermeable. (Redrawn from Tazawa & Shimmen, 1987.)

(Shepherd & Goodwin, 1989; Fig. 17). Since ATP leaks out (and streaming stops), the cells must be bathed in $Mg^{2+}$-ATP, which results in a reactivation of protoplasmic streaming (Shimmen & Tazawa, 1983b). The action potential of the vacuolar membrane can also be studied on protoplasmic droplets (Kobatake et al., 1975; Takenaka et al., 1975).

## VIII. The Action Potential at the Vacuolar Membrane

In plasma membrane–permeabilized cells of *Chara*, the vacuolar membrane action potential can be activated by increasing the $Ca^{2+}$ concentration of the external medium from 0 to 1 μM (Fig. 18; Kikuyama, 1989). The increased $Ca^{2+}$ concentration on the protoplasmic side of the vacuolar membrane causes the vacuolar membrane to depolarize for as long as the $Ca^{2+}$ is present; however, a short pulse will activate a normal-looking depolarization and repolarization. When the $Cl^-$ concentration on the protoplasmic side is increased, the degree of $Ca^{2+}$-induced depolarization decreases as predicted by the Nernst Equation if $Cl^-$ is the only current-carrying ion. The $Cl^-$ current flows through a specific $Cl^-$ channel, since the $Cl^-$ channel blocker, anthracene–9-carboxylic acid, completely eliminates the $Ca^{2+}$-induced depolarization (Kikuyama, 1989). In order to confirm that a $Cl^-$ channel was actually opening, Kikuyama (1988) measured the flux of $Cl^-$ from the vacuole using a Ag/AgCl electrode. He found that an increase of the $Ca^{2+}$ concentration on the protoplasmic side of the membrane from 0 to 5 μM caused an increase in the flux of $Cl^-$ from the vacuole by approximately $10^{-6}$ mol $m^{-2}$ $s^{-1}$. If the natural cell sap is replaced by perfusion with a $Cl^-$-free cell sap, no $Cl^-$ flux can be measured, indicating that the observed $Cl^-$ flux originates from the vacuole (Kikuyama, 1988).

How is the vacuolar membrane action potential activated in intact cells? In intact cells, it is generated when the plasma membrane has been excited, indicating that there must be a coupling mechanism between the two membranes (Findlay, 1970; Kikuyama & Tazawa, 1976). $Ca^{2+}$ is assumed to be the intracellular coupling agent since:

1. The $[Ca^{2+}]_i$ increases upon excitation from approximately 0.1 to 10 μM as measured by aequorin luminescence (Kikuyama et al., 1993; Kikuyama & Tazawa,

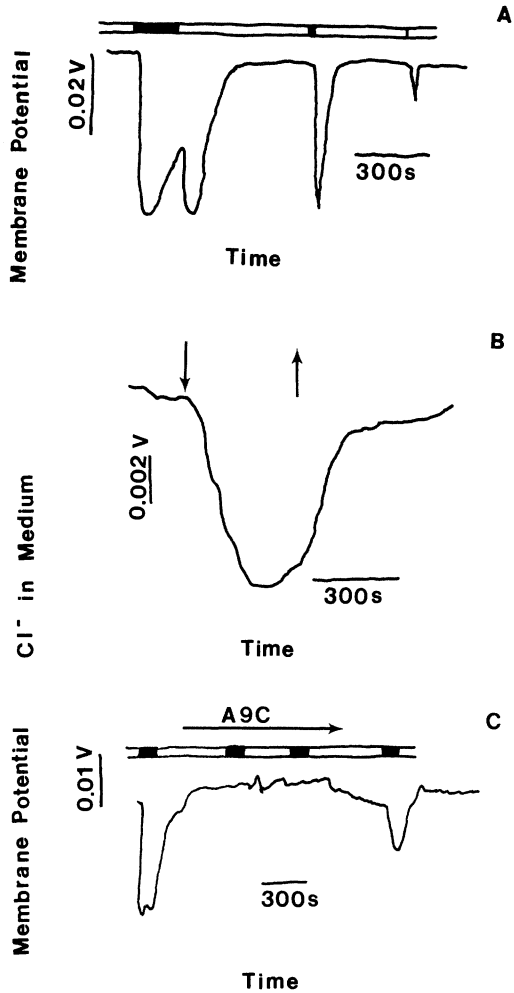



**Fig. 18.** The effect of $Ca^{2+}$ on the vacuolar membrane action potential in plasma membrane permeabilized cells. **A.** The $Ca^{2+}$ concentration on the protoplasmic side of the vacuolar membrane of permeabilized cells was increased at various times (black bars) and the membrane potential was recorded. A downward deflection represents a hyperpolarization. **B.** The $Ca^{2+}$ concentration on the protoplasmic side of the vacuolar membrane of permeabilized cells was increased (down arrow) or decreased (up arrow), and the $Cl^-$ concentration of the medium was measured. The $Cl^-$ concentration was measured with a Ag/AgCl wire and given in volts, where, in accordance with the Nernst Equation, a 10-fold change in $Cl^-$ causes a 0.059 V change in the potential of the wire. **C.** The $Ca^{2+}$ concentration on the protoplasmic side of the vacuolar membrane of permeabilized cells was increased at various times (black bars) and the membrane potential was recorded. This experiment was done in the presence or absence of A-9-C. A downward deflection represents a hyperpolarization. (Redrawn from Kikuyama, 1988, 1989.)

1983; Williamson & Ashley, 1982). The influx of $Ca^{2+}$ increases from $10^{-9}$ mol $m^{-2}$ $s^{-1}$ to $3 \times 10^{-7}$ mol $m^{-2}$ $s^{-1}$ upon excitation (Hayama et al., 1979).

2. Replacement of external $Ca^{2+}$ with $Ba^{2+}$, $Mg^{2+}$, or $Mn^{2+}$ does not inhibit the plasma

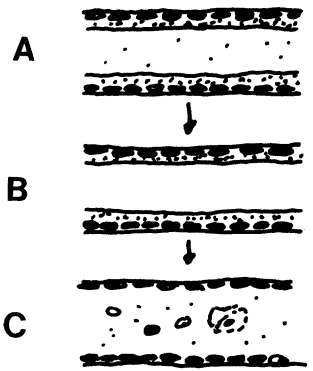



**Fig. 19.** Diagram of a vacuolar membrane-free cell. **A** represents the intact cell, **B** represents a cell whose natural cell sap has been replaced with artificial cell sap, and **C** represents a cell in which the cell sap was replaced with a solution that causes the disintegration of the vacuolar membrane and the dilution of the cytosol. (Redrawn from Tazawa & Shimmen, 1987.)

membrane action potential (Barry, 1968), but inhibits the generation of the vacuolar membrane action potential and the cessation of streaming (Kikuyama, 1986).

3. Microinjection of $Ca^{2+}$ into the protoplasm induces an action potential at the vacuolar membrane (Kikuyama, 1986).

It seems clear that the vacuolar membrane action potential results from the stimulation by $Ca^{2+}$, the "messenger" activated by the plasma membrane action potential.

## IX. The Plasma Membrane Action Potential

The action potential at the plasma membrane can be studied by endoplasm-enriched fragments using centrifugation (Beilby & Shepherd, 1989) or by making vacuolar membrane–free cells (Tazawa & Shimmen, 1987; Williamson, 1975). This is done by cutting off the ends and perfusing the cell with a $Ca^{2+}$-free solution (Fig. 19). The vacuolar membrane subsequently becomes unstable and breaks. The plasma membrane then becomes the only surface membrane. The composition of the two solutions on each side of the plasma membrane can be readily adjusted to study the excitability of the plasma membrane (Tazawa & Shimmen, 1987).

In *rapidly perfused,* vacuolar membrane–free cells, containing the $Ca^{2+}$-chelator EGTA, the $Cl^-$ channel in the plasma membrane is inactivated (Mimura & Tazawa, 1983) and the $Ca^{2+}$ current can be studied in isolation (Fig. 20; Shiina & Tazawa, 1987a). There are at least three classes of $Ca^{2+}$ channels in the plasma membrane of characean internodal cells that are differentiated based on their localization and pharmacological properties (Staves et al., 1993; Wayne et al., 1993). In a resting *Nitellopsis* cell, the $Ca^{2+}$ ($Sr^{2+}$) flux is approximately $130 \times 10^{-9}$ mol $m^{-2}$ $s^{-1}$, and it increases to $1640 \times 10^{-9}$ mol $m^{-2}$ $s^{-1}$ during an action potential. These ionic fluxes can be related to the ionic currents by multiplying the fluxes by $zF$ ($19.3 \times 10^4$ coulombs $mol^{-1}$). Thus, the current resulting from the flux of $Ca^{2+}$ transiently increases from 0.025 A $m^{-2}$ to 0.316 A $m^{-2}$ during an action potential. [Note that these

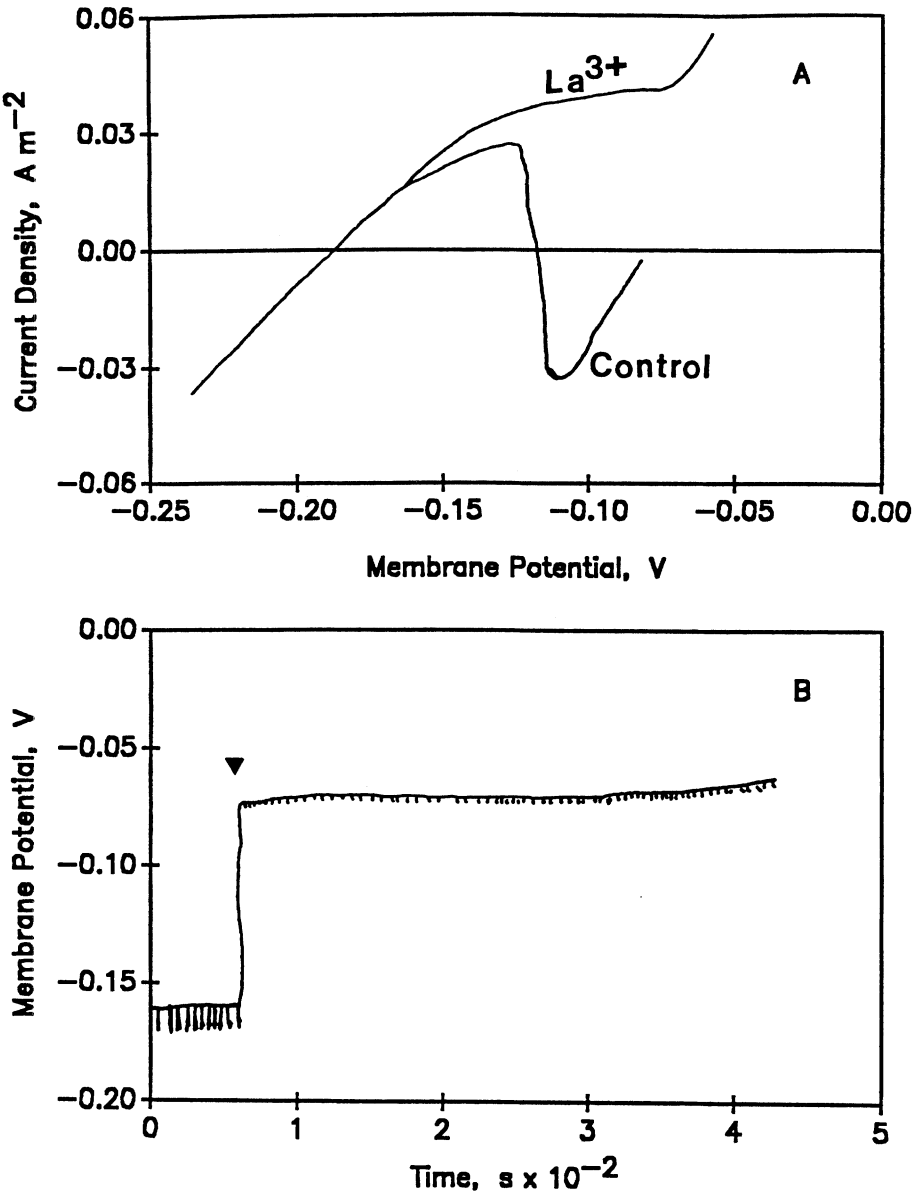



**Fig. 20.** The $Ca^{2+}$ current in rapidly perfused, vacuolar membrane–free cells. **A.** The current density of vacuolar membrane–free cells was measured with a voltage clamp. **B.** The membrane potential of a vacuolar membrane–free cell was measured with a current clamp. The membrane was excited by a depolarizing current. (Redrawn from Shiina & Tazawa, 1987a.)

fluxes observed in tonoplast-free *Nitellopsis* cells are higher than those observed in normal *Chara* cells (Staves, unpublished results)]. The current that passes through the $Ca^{2+}$ channel involved in the generation of the action potential is blocked by $La^{3+}$

(Shiina & Tazawa, 1987a). The shape of the action potential of the plasma membrane is different in vacuolar membrane-free cells compared with intact cells. In vacuolar membrane-free cells the action potential is typically rectangular in shape, indicating that the $Ca^{2+}$ current does not show time-dependent inactivation. This activation may be regulated through the phosphorylation of a $Ca^{2+}$ channel, since the $Ca^{2+}$ channel can be activated only when it is dephosphorylated (Shiina & Tazawa, 1986b; Shiina et al., 1988).

In *slowly perfused* vacuolar membrane–free cells, the plasma membrane $Cl^-$ channel is still functional (Shiina & Tazawa, 1988). In these cells, membrane depolarization causes a large inward current (Fig. 21). $Cl^-$ ions carry the inward current since the large depolarization and the $Cl^-$ efflux are inhibited by the $Cl^-$ channel blocker A-9-C. The $Cl^-$ channel is activated in response to extracellular $Ca^{2+}$ since the $Cl^-$ current and $Cl^-$ efflux require extracellular $Ca^{2+}$ and are inhibited by the $Ca^{2+}$ channel blocker, $La^{3+}$ (Shiina & Tazawa, 1987b).

## X. Identification of the $Ca^{2+}$-Activated $Cl^-$ Channel on the Plasma Membrane Using Patch Clamp Techniques

Up to now we have talked about the specific conductance of the membrane to a given ion, and we have assumed that this conductance is due to the presence of channels. Channels are single protein molecules embedded in the membrane that act like little regulated (i.e., gated) pores through which ions can pass. Recently, the *patch clamp technique* has made possible the visualization of single channels (Hamill et al., 1981; Sakmann & Neher, 1983). With this technique, a tiny piece of the plasma membrane ($\approx 10^{-12}$ $m^2$) can be pulled away from the cell, thus making certain the identity of the membrane in which the channel resides (Fig. 22). The isolated membrane patch no longer has a natural membrane potential, so a voltage clamp is used to set (and vary) the membrane potential to any given value. Typically, channels are activated at certain potentials and a current can be measured. When the channels are closed, no current passes; and when the channels open, a small current in the picoampere range can be observed. It is possible to observe the behavior of single channels opening and closing. Okihara et al. (1991, 1993), using patch clamping techniques, have actually identified the $Ca^{2+}$-activated $Cl^-$ channels in the plasma membrane that are responsible for the action potential (Fig. 23).

The current passing through the channels is observed only when the $Ca^{2+}$ concentration on the protoplasmic side of the membrane is about 1 μM. When the $Ca^{2+}$ concentration is either lower or higher, the current decreases, indicating that a small amount of $Ca^{2+}$ is needed to activate the channel and greater amounts cause it to inactivate. This behavior may account for the kinetics of the "slow transient second" current observed by Lunevsky et al. (1983) in their voltage clamp experiments on intact cells.

The reversal potential of the $Ca^{2+}$-activated current is positive and depends on the $Cl^-$ concentration in a manner predicted by the Nernst Equation if $Cl^-$ is the only ion carrying the current. The $Ca^{2+}$-activated $Cl^-$ channel is also voltage-dependent. This channel is activated upon depolarization to voltages less than –0.16 V, indicating that a membrane depolarization (i.e., receptor potential alone) is necessary but not sufficient for the activation of the $Cl^-$ channel that is responsible for the realization of the massive depolarization and increase in conductance characteristic of the action potential.

The magnitude of the $Cl^-$ current observed ($I_o$) in a patch depends upon the number

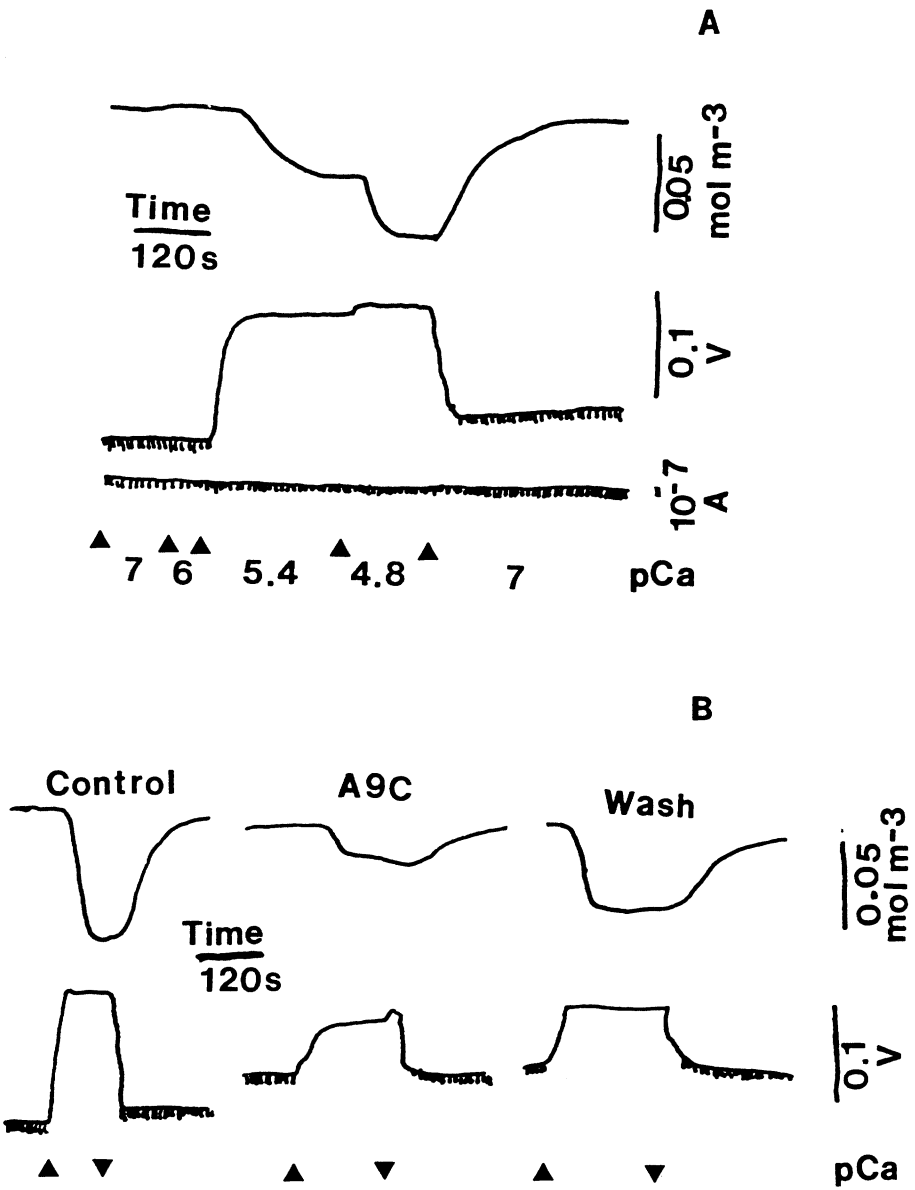



**Fig. 21.** The action potential in vacuolar membrane–free cells. **A.** The effect of $Ca^{2+}$ on the $Cl^-$ efflux and membrane potential. $Cl^-$ efflux was measured with Ag/AgCl wires in the external medium. Membrane potential was measured with a microcapillary electrode. pCa represents the -log[$Ca^{2+}$]. **B.** The effect of A-9-C on $Cl^-$ efflux and membrane potential. (Redrawn from Shiina & Tazawa, 1987b.)

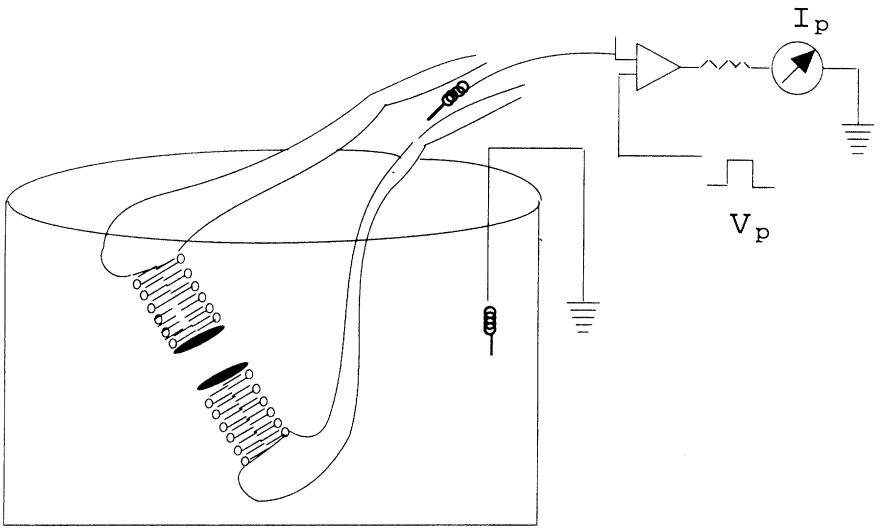



**Fig. 22.** Diagram of a patch clamp setup. A patch of membrane is held by a high-resistance seal to a glass microcapillary pipette. One side of the membrane is exposed to the bath and the other side of the membrane is exposed to the pipette solution. The electrode is connected to a patch clamp amplifier, which in principle is identical to a voltage clamp amplifier except that the currents that flow are smaller (picoampere compared to microampere). The patch clamp amplifier can issue a command to hold the membrane at a given voltage and measure the current that flows at the command voltage [see Hamill et al. (1981) for details].

of $Cl^-$ channels in the patch ($m$), the probability of a channel being open ($P_o$), and the current passed by a single channel ($I_s$) according to the following relation:

$$I_o = bP_oI_s \tag{11}$$

where

$I_o$ is the current passed through the patch (in A)
$b$ is the number of channels in the patch (dimensionless), assuming that all the channels are identical
$P_o$ is the probability of a channel being open (dimensionless)
$I_s$ is the current passed by a single channel (in A).

A kinetic analysis of the results shows that the increase in the observed current induced by an increase in the $Ca^{2+}$ concentration on the protoplasmic side of the membrane from 0.1 to 1 μM results from an increase in the probability of the $Ca^{2+}$-activated $Cl^-$ channel being open. Likewise, the decrease in the current observed when the $Ca^{2+}$ concentration increases from 1 to 10 μM results from a decrease in the probability that the $Ca^{2+}$-activated $Cl^-$ channel will be open. This appears to be part of the molecular mechanism to explain the activation–inactivation kinetics of the

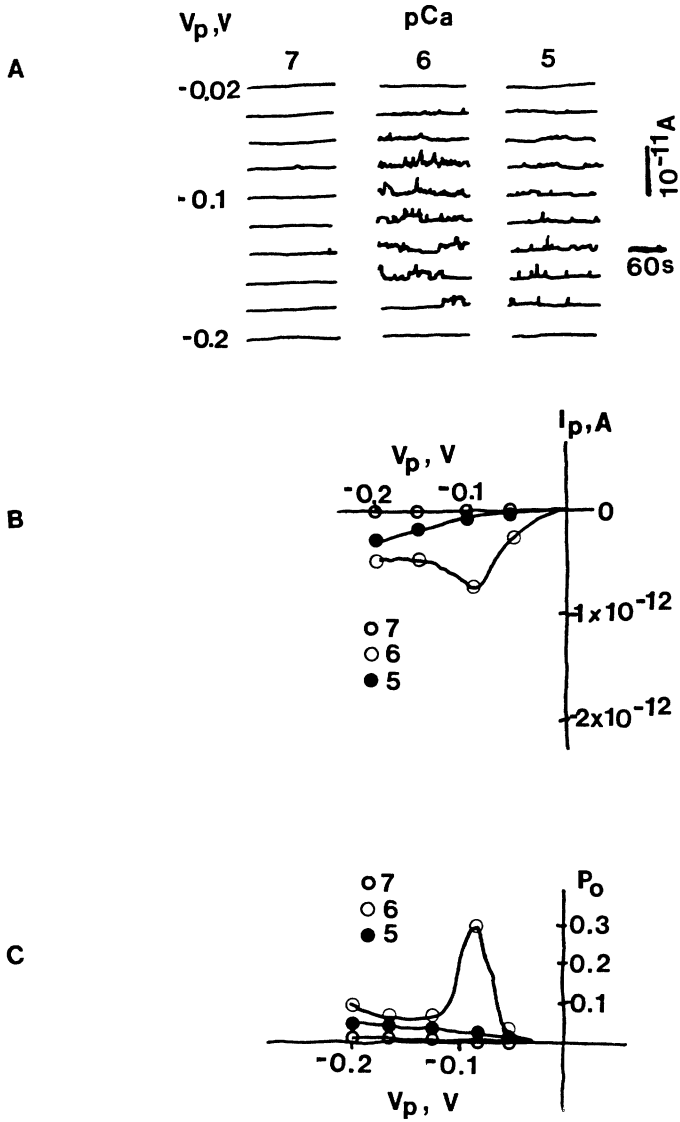



**Fig. 23.** Patch clamping analysis of the $Ca^{2+}$-activated $Cl^-$ channel. **A.** Patch clamp analysis of inside out patches treated with various concentrations of $Ca^{2+}$ in the bath and held at various command voltages. The observed currents are discrete, which is due to the opening and closing of $Cl^-$ channels. There are probably 3 or 4 channels in the patch. **B.** I-V curves of single channels. **C.** The effect of $Ca^{2+}$ on the open probability of the $Cl^-$channel. $V_p$ and $I_p$ represent the electrical potential of the plasma membrane and the current through the plasma membrane, respectively. $P_o$ represents the open probability of the $Cl^-$ channel and pCa = -log ($Ca^{2+}$). (Redrawn from Okihara et al., 1991.)

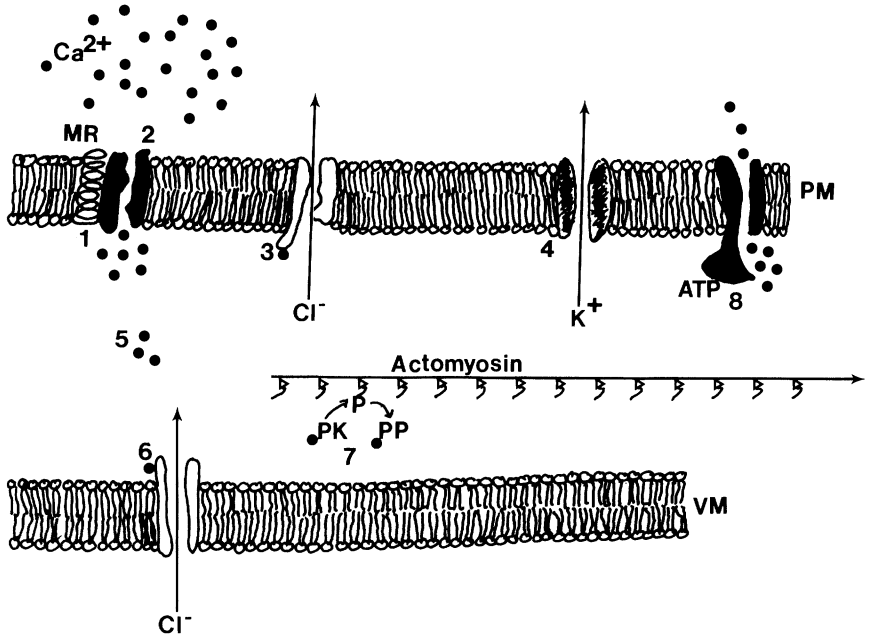



**Fig. 24.** Summary diagram of the generation of an action potential and the subsequent cessation of streaming. A mechanical stimulus, perceived by a mechanoreceptor (MR), causes a depolarization of the plasma membrane known as the receptor potential (1). The receptor potential is probably due to the activation of $Ca^{2+}$ channels (2). If the increase in the intracellular $Ca^{2+}$ concentration is sufficient, the membrane potential further depolarizes when $Ca^{2+}$ and the accompanying depolarization activates the $Cl^-$ channel in the plasma membrane (3). This results in an inward current carried by $Cl^-$ (i.e., $Cl^-$ efflux). Subsequently, the membrane potential moves away from the equilibrium potential for $K^+$. Thus, $K^+$ moves out of the cell down its electrochemical gradient and the membrane potential repolarizes (4). The permeability of the membrane to $K^+$ may also increase as a consequence of the activation of a $Ca^{2+}$-activated $K^+$ channel.

The $Ca^{2+}$ that enters the cell in response to mechanical stimulation then diffuses across the 5–20 μm protoplasm at a velocity of approximately 1 μm $s^{-1}$ (5) and activates the $Cl^-$ channel of the vacuolar membrane. $Cl^-$ moves from the vacuole into the protoplasm, causing a slower vacuolar membrane action potential, which is observed as a hyperpolarization of the vacuolar membrane (6). $Ca^{2+}$ also activates a $K^+$ channel on the vacuolar membrane which results in a repolarization of the vacuolar membrane potential. The transient increase in protoplasmic $Ca^{2+}$ stops streaming as the wave of $Ca^{2+}$ moves through the cell. Protoplasmic streaming stops because the increased $Ca^{2+}$ activates a protein kinase that phosphorylates the heavy chain of myosin and inhibits the interaction of actin and myosin, thus inhibiting the proteins that provide the motive force for streaming (7). Eventually the elevated $Ca^{2+}$ concentration returns to the resting level due to the pumping out of $Ca^{2+}$ by $Ca^{2+}$-ATPases on the plasma membrane and sequestering into internal compartments (8).

inward $Cl^-$ current. Patch clamping has provided a visualization of membrane phenomena that would have been beyond the wildest ideation of Julius Bernstein (1912), the creator of the membrane theory of excitation.

## XI. Summary

A mechanical stimulus causes a depolarization of the plasma membrane known as the receptor potential (Fig. 24). The receptor potential is probably due to the activation

of $Ca^{2+}$ channels. Probably the value of the receptor potential is directly related to the number of $Ca^{2+}$ channels activated. If a sufficient number of $Ca^{2+}$ channels are not activated, the increased $Ca^{2+}$ influx will not cause a large enough increase in the intracellular $Ca^{2+}$ concentration to activate the processes necessary to cause the regenerative action potential, since $Ca^{2+}$ influx must be much greater than the $Ca^{2+}$ efflux brought about by the $Ca^{2+}$-pumping ATPases in the plasma membrane. If the increase in the intracellular $Ca^{2+}$ concentration is sufficient, the membrane potential further depolarizes when $Ca^{2+}$ and the accompanying depolarization activates the $Cl^-$ channel in the plasma membrane. This results in an inward current carried by $Cl^-$ (i.e., $Cl^-$ efflux). Subsequently, the membrane potential moves away from the equilibrium potential for $K^+$. Thus $K^+$ moves out of the cell down its electrochemical gradient and the membrane potential repolarizes. The permeability of the membrane to $K^+$ may also increase as a consequence of the activation of a $Ca^{2+}$-activated $K^+$ channel.

The $Ca^{2+}$ that enters the cell in response to mechanical stimulation then diffuses across the 5–20 μm protoplasm at a velocity of approximately 1 μm $s^{-1}$ and activates the $Cl^-$ channel of the vacuolar membrane. $Cl^-$ moves from the vacuole into the protoplasm, causing a slower vacuolar membrane action potential which is observed as a hyperpolarization of the vacuolar membrane. $Ca^{2+}$ also activates a $K^+$ channel on the vacuolar membrane which results in a repolarization of the vacuolar membrane potential. The transient increase in protoplasmic $Ca^{2+}$ stops streaming as the wave of $Ca^{2+}$ moves through the cell. Protoplasmic streaming stops because the increased $Ca^{2+}$ activates a protein kinase that phosphorylates the heavy chain of myosin and inhibits the interaction of actin and myosin, thus inhibiting the proteins that provide the motive force for streaming (Tominaga et al., 1986). Eventually the elevated $Ca^{2+}$ concentration returns to the resting level due to the pumping out of $Ca^{2+}$ by $Ca^{2+}$-ATPases on the plasma membrane and sequestration into internal compartments.

## XII. Coda

In this paper I have tried to show that work on excitability of plants and particularly of characean internodal cells has advanced considerably over the past century. We are just beginning to know the extent to which action potentials in higher plants are similar to or different from those in characean cells (Hodick & Sievers, 1986; Iijima & Hagiwara, 1987; Iijima & Sibaoka, 1981, 1982, 1983, 1985; Samejima & Shibaoka, 1982; Tazawa, 1984). Likewise, we are beginning to realize the extent to which action potentials may be involved in long-range communication in higher plants (Davies, 1987; Shiina & Tazawa, 1986a; Wildon et al., 1992). Could action potentials communicate some of the information needed for whole-plant development and responses to environmental stimulation? Living organisms use both chemical (e.g., hormonal) and physical (e.g., electrical) messengers for long-range (i.e., cell to cell) information transfer. It is noteworthy that animal physiologists are split approximately 50%/50% between those who study chemical communication and those who study electrical communication, while plant physiologists are split approximately 99%/1% between those who study chemical communication and those who study electrical communication, with a few studying both (Bandurski, 1991; Bates & Goldsmith, 1983; Cleland et al., 1977; Felle et al., 1986; Liu et al., 1991; Lund, 1947; Pickard, 1984). This unequal division among plant physiologists is especially puzzling after we realize that plant electrophysiology played such a prominent role in the early history of electrical

communication. I suggest that there may be five possible explanations that explain the unequal division that has been so prominent during the last half of the twentieth century:

1. the discovery of auxin, a ubiquitous and omnipotent chemical messenger
2. institutional nationalism, racism, and sexism
3. the belief that plant cells do not have a plasma membrane
4. the apprehension of plant physiologists in using physical laws to model biological systems
5. the use of plants in parapsychological work.

Perhaps the split into a chemical thesis of communication and an electrical antithesis of communication in plants resulted from the notion that one type of messenger must be more important than the other (the "one God, one Messenger" theory). Thus, the great successes of the prominent, chemical-oriented plant physiologists Frits Went and Kenneth Thimann (documented in Went & Thimann, 1937) in purifying a chemical messenger (the plant hormone auxin) that caused so many profound physiological and developmental responses left the physical-oriented plant physiologists powerless to continue their work on electrical communication in plants. Indeed, since that time, plant growth and development have almost become synonymous with the study of hormones.

Auxin was discovered in the 1930s, a time when the world was in political turmoil. Perhaps the division of the world into the Allies and Axis exacerbated the birth of the thesis–antithesis conviction. The discovery of auxin coincided with the rise of Adolph Hitler in Germany. While Germany had been the "center" of plant physiological research prior to the discovery of auxin, America (particularly Cal Tech) became the "center" of plant physiological research after the discovery of auxin (Boysen-Jensen, 1936; Brauner & Rau, 1966; Bunning, 1939, 1948, 1953; Goebel, 1920; Haberlandt, 1890, 1901; Kohl, 1894; Nemec, 1901; Pfeffer, 1875; Skoog, 1951; Stern, 1924; von Guttenberg, 1971). Thus the false dichotomy was set up: Choose the physical-oriented plant physiology dogma established by W. Pfeffer's school in Germany or the chemical-oriented plant physiology dogma authorized by F. Went's and K. Thimann's school in America. Interestingly, following World War II, the political alliance between Germany and Japan also led to a scientific alliance, and physical-oriented plant physiology also became prominent in Japan.

I believe it possible that racism and sexism further compounded the problem in America since the results and conclusions of an Indian man (Jagadischandra Bose) and an American woman (Barbara Pickard), the two major proponents of the importance of electrical communication in plants (Bose, 1906, 1913, 1926, 1985; Pickard, 1973, 1984), were somewhat overlooked. Interestingly, the work of Sir J. C. Bose that showed that plants have almost the same life reactions as humans inspired the Nobel Laureate Rabindranath Tagore to dedicate to him a book of poems entitled *Vanavani* (Voice of the forest) (Kripalani, 1962).

Another reason why the study of electrical communication in plants lacked popularity by plant physiologists may have arisen from the belief held by some biophysical plant physiologists through the late 1950s, that plant cells do not have a differentially permeable plasma membrane but only a cytoplasmic surface layer or "plasmalemma" (see Briggs, 1957; Briggs & Hope, 1958; Briggs & Robertson, 1948, 1957; Briggs et al., 1961; Dainty, 1962; Hope, 1951; Hope & Robertson, 1956; Hope & Stevens, 1952; Hope & Walker, 1975; MacRobbie & Dainty, 1958; Robertson, 1992; Walker, 1955, 1957). This

view was championed by the prominent biochemical (Briggs & Haldane, 1925) and biophysical plant physiologist G. E. Briggs, who with R. Robertson wrote in 1957:

> *It is generally accepted that there is, between the vacuole and the outside of a plant cell, a barrier or barriers which offer a high resistance to the passage of many solutes including salts of strong acids and bases. The tonoplast which separates the vacuole from the cytoplasm is accepted as one such barrier, but many assume that there is also another barrier [the plasma membrane] at the outside of the cytoplasm adjoining the cell wall. Although we believe that no such barrier exists for many solutes we do not propose to consider all the evidence in detail, nor is it the purpose of this article to survey the history of what we believe to be erroneous concepts.*

This conclusion came from the application of Ockam's razor (Ockam, 1487; cited in Hope & Walker, 1975). The logic was that since animal cells have only one limiting membrane, it stood to reason that plant cells, too, have only one limiting membrane. It was obvious that plant cells have a visible vacuolar membrane, surrounded by the protoplasmic "free space," so there seemed to be "no reason" to have a second membrane (Hope & Walker, 1975: 32).

Even today many botanists use the term "plasmalemma," which was first used by Mast in 1924 and defined by him in 1926 to signify the outer layer of the protoplasm of *Amoeba* that may or may not be a distinct differentially permeable barrier. Plasmalemma was introduced by Mast (1926) as a noncommittal term to replace controversial terms like *Zellhaut*, *Plasmahaut*, or *Plasmamembran*, used by Pfeffer to indicate a differentially permeable membrane; or *Häutchen* (third layer, pellicle, or pellicula), used by Bütschli, Rhumbler, Schaeffer, Chambers, and Howland to indicate a surface layer surrounding a colloidal or foam-like protoplasm, a protoplasm which itself is capable of causing differential permeability.

Plasmalemma was supposed to be a harmonious word that fit somewhere between Pfeffer's (1877) operational definition of the plasma membrane and Bütschli's (1984) opinion:

> *The surface of the protoplasm has been referred to as the "Hautschicht", "Hautplasma" or best of all "hyaloplasma". . . . I have decided to designate this semipermeable layer [the surface of the protoplasm] as "plasmahaut" or "plasmamembran". . . . The "plasmamembran" can only be defined by its osmotic properties and not by its morphological characteristics. (Translated from Pfeffer, 1877).*

> *I am only doubtful whether the so-called protoplasmic membrane possesses the importance for osmotic processes which Pfeffer ascribes to it; on the ground of which processes Pfeffer was really first led to assume its existence; they are now also supposed by him to prove its presence, even when direct observation shows nothing of it for certain.* (Bütschli, 1984)

"Plasmalemma" was then introduced into the plant literature by Plowe (1931a):

> *Mast (1924) has given us the term "plasmalemma" for the thin external layer*

*which is the "membrane" if an osmotic function exists. This term seems free from the difficulties presented by both "ectoplast" and "plasma membrane." It has seemed permissible, therefore, to extend its use to the botanical world, and to employ it to denote a distinct, differentiated layer on the outer surface of the plant protoplast.*

Incidently, the two papers published in 1931 by Plowe present definitive evidence showing that in the plant cells she studied, the "plasmalemma" was a differentially permeable "membrane" (Plowe, 1931a, 1931b). Given the preponderance of the evidence that the "plasmalemma" of the plant cell is a differentially permeable membrane, it is both meaningful and correct to refer to it as a plasma membrane.

The study of electrical communication in plants requires the application of physical laws to biological systems, and the application of physical laws to organisms usually requires the use of the most simple organisms available or the isolation of single cells. The apprehension of plant physiologists in using simple lower plant systems and physical laws to model biological systems is evident in the following quote by F. C. Steward (1935) in his assessment of Osterhaut's work:

*It is in these operations that undue weight seems to have been given to certain features, not applicable to cells in general, of what is after all an obscure organism* [Valonia].

*Lastly, there seems grave danger that the admittedly ingenious and extensive work devoted to models may divert attention from the fundamental facts that, not only are they far removed from physiological reality, but also that the very principles upon which they are based are such that judgement must be reserved concerning their applicability to the general problem of salt absorption* in vivo.

This view is still prevalent today. As late as 1986, Steward wrote in the *Treatise of Plant Physiology*:

*This chapter [Solutes in Cells] has developed its main theme with reference to cells of angiosperms, that is, the cells of the most highly organized land plants which encounter in nature the most complex environments. The earlier chapter (25) properly gave much attention . . . to the uses made of aquatic plants in investigations on cell physiology, especially in studies which capitalized on special features of their morphology. As, for example, the so-called giant cells of members of the Siphonales and the large internodal cells of* Chara *and* Nitella. *Interesting as these organisms were, and are, the special features that make them attractive experimental objects also render them, from the standpoint of this chapter, atypical and not representative of the plant kingdom as a whole.*

It appears that the philosophy (Osterhaut, 1924) and experiments of W. Osterhaut—the scientist so instrumental in enticing the animal physiologists Curtis and Cole and, later, Hodgkin and Huxley into studying simple systems that could be physically modeled—had the opposite effect on plant physiologists.

In physics, fundamental laws were discovered by choosing simple "ideal" systems like gases or dilute liquids, but not solids, to demonstrate the existence and behavior of atoms (Feynman, 1965, 1985). Hydrogen atoms, not tin atoms, were used to explain the quantum nature of the atom. Likewise, animal behaviorists study the electrical

behavior of isolated nerve cells in order to understand complex behaviors from the knee-jerk response to the regulation of the heartbeat. However, within the scientific realm of plant physiology, the presentation of the "botanical equivalent of an ideal gas in a real world" (i.e., algal cells) has typically met with the reaction that they are "far removed from physiological reality."

While progress is being made in applying the laws of physics to the study of plants at the organ level (Cosgrove, 1993a, 1993b, 1993c; Green, 1981; Niklas, 1992; Silk, 1984; Thompson, 1963) and the suborganellar level (Jean et al., 1989), the single cell has been under-utilized in applying these laws to the study of plant physiology and development. The study of plant growth and development at the single-cell level complements investigations done on the whole plant, isolated organelles, and protein complexes (Delbrück, 1970).

Once a cellular approach is taken, it is very important to identify the cell type that is responding to the physiological or developmental stimulus (Wayne, 1992). This is necessary since the signal transduction chains and response characteristics of a given cell type in a multicellular organism may vary (Hamill et al., 1991). Perhaps the reason that we have no evidence for the involvement of cyclic AMP (cAMP) in plants is because the average cAMP concentration of all cells in seedlings, and not just the cAMP concentration in the responding cells, was assayed (Amrhein, 1977; Brown & Newton, 1973; Elliot & Murray, 1975). This experimental approach would be analogous to treating a whole calf with light or hormones and then grinding it all up to determine whether or not the level of cAMP has changed or not.

I believe that, even today, plant physiologists prefer the vitalistic approach to science rather than the unificationist approach that is based on the hypothesis that there are certain fundamental laws of nature that can describe physics, chemistry, and biology in mathematical form (Brillouin, 1949; Elasser, 1958; Loeb, 1912, 1916; Lotka, 1925). There is still a strong belief that there is a "grave danger" in applying the fundamental laws of physics to plant physiology. Steward was wary of Osterhaut applying electrical theory to membrane transport since membrane transport depended on respiration, a component not included in the Nernst Equation. However, Kitasato and Spanswick later showed the relationship between the product of respiration (ATP) and membrane transport when they discovered the electrogenic pump and the equivalent electrical circuit that includes both the Nernst Equation and the ATP-utilizing electrogenic $H^+$ pump. In my opinion, more progress can be made and has been made by building up a synthetic plant physiology from fundamental principles (unificationist approach) than from describing phenomena and making interpretations without considering whether or not the interpretations obey the laws of physics.

I hope this review inspires some people to apply biophysical principles to the study of plant physiology and development. Using the common language of mathematics and principles of physics allows a ready transference of information between related fields (Rothstein, 1951). Since all measurements are based on physical principles, we have a chance of understanding our instruments as well as understanding the plant if we include biophysical thinking in our approach. When we make theories in plant physiology and development, we are implicitly or explicitly assuming that certain mathematical or physical principles are either true or false. Implicit and wrong assumptions can mislead the field for years.

Interestingly, while sometimes the assumptions of physical principles are met in

biological systems, often they are not (Bohr, 1933; Donnan, 1927; Klieber & Gradmann, 1993; Schrödinger, 1946; Szent-Györgi, 1960). This gives biologists a chance to broaden the current laws of physics or, if you like, "natural laws." According to Brillouin (1949),

> *We have been looking, up to now, for a physiochemical interpretation of life. It may well happen that the discovery of new laws and of some new principles in biology could result in a broad redefinition of our present laws of physics and chemistry, and produce a complete change in point of view.*

This is not so farfetched as it seems when we realize that botanists have already played a leading role in the development of new physical principles. It was the work of the botanist Robert Brown (1828, 1866) on the movement of organelles in the cytoplasm of pollen that gave rise to the acceptance of the reality of atoms and the determination of Avogadro's Number (Einstein, 1926; Perrin, 1923). And it was the work of botanists H. de Vries, C. von Nägeli, W. Hofmeister, and W. Pfeffer that led J. H. van't Hoff to apply the gas laws to dilute solutions and develop the Boyle-van't Hoff Law, which relates concentration to osmotic pressure (Ling, 1984). The botanist Friedrich Reinitzer discoverd liquid crystals (Brown & Wolton, 1979). And the physicist George Stokes used his own studies on the fluorescence of chlorophyll extracted from bay leaves to develop the relationship between absorption and fluorescence known as Stokes' Law (Harvey, 1957; Lloyd, 1924). In fact, the botanist Andrew Caesalpinus, who was the Chair of Medicine at the University of Pisa from 1597 to 1592, was Galileo's teacher (Brewster, 1846).

Some animal physiologists of the nineteenth century, including Adolf Fick, Hermann von Helmholtz, and Jean Poiseuille also contributed greatly to physics. They were members of a "new school of physiologists" who rejected vitalist explanations of life and attempted to describe physiological phenomena in terms of physical and chemical principles. J. B. S. Haldane (1924) and R. A. Fisher (1930) contributed greatly to mathematics in the twentieth century as a consequence of their need to describe genetics quantitatively. These nineteenth- and twentieth-century scientists tried to unite natural history (e.g., botany, zoology, physical geography, geology, and mineralogy) with natural philosophy (e.g., mathematics, physics, astronomy, and chemistry). Natural philosophy and natural history were separated based on the assumption that the former but not the latter was susceptible to mathematical and experimental treatments (Huxley, 1902).

Lastly, although the study of electrical communication in plants had already been ignored by the plant physiology community for years, during the 1960s the study of electrical communication in plants became taboo following the publication of high-profile, irreproducible work that reported that plants that were connected to a lie detector were able to perceive danger and gave a perceptible change in the electrical resistance of a leaf while witnessing the murder of brine shrimp (Backster, 1968; Horowitz et al., 1975).

As a result of the renewed interest in ionic messengers, particularly $Ca^{2+}$, in plant development (Trewavas, 1986), coupled with the successful introduction of the vibrating electrode (Nuccitelli, 1986) and patch clamp techniques to plant physiology (Hamill et al., 1981; Hedrich & Schroeder, 1989), many more plant physiologists are bridging the gap between the chemical-oriented thesis of communication and the

physical-oriented antithesis of communication to form a synthesis that recognizes the importance of both chemical and electrical communication in considering the mechanisms responsible for plant development and the responses of plants to the environment.

I will end with a passage from a letter Benjamin Franklin wrote to Peter Collinson on 29 April 1749:

> *Chagrined a little that we have been hitherto able to produce nothing in this way of use to mankind; and the hot weather coming on, when electrical experiments are not so agreeable, it is proposed to put an end to them for this season, somewhat humorously, in a party of pleasure, on the banks of the Skuylkil. Spirits, at the same time, are to be fired by a spark sent from side to side through the river, without any other conductor than the water; an experiment which we some time since performed, to the amazement of many. A turkey is to be killed for our dinner by the* ***electrical shock****, and roasted by the* ***electrical jack****, before a fire kindled by the* ***electrified bottle****: when the healths of all the famous electricians in* ***England, Holland, France, and Germany*** *are to be drank in* ***electrified bumpers*** *[small thin glass tumblers, nearly filled with wine, and electrified as the bottle. This when brought to the lips gives a shock, if the party be close shaved, and does not breathe on the liquor (Cohen, 1941)], under the discharge of guns from the* ***electrical battery****.*

## XIII. Acknowledgments

Our laboratory work is supported by NASA. Thanks to Drs. G. Bailey, A. Furuno, O. Hamill, M. Lea, C. Leopold, S. Meissner, T. Mimura, K. Niklas, L. Palevitz, M. Rutzke, J. Sáenz-Badillos, R. Spanswick, A. Stachowitz, M. Staves, R. Turgeon, S. Wayne, and S. Williams for their comments on the manuscript. I would also like to thank my parents—for everything.

## XIV. Literature Cited

**Adrian, E. D. & D. W. Bronk.** 1928. The discharge of impulses in moter nerve fibres. I. Impulses in single fibres of the phrenic nerve. J. Physiol. **66:** 81–101.

**Agin, D. P.** 1972. Perspectives in membrane biophysics. A tribute to Kenneth S. Cole. Gordon and Breach Science Publishers, New York.

**Aimi, R.** 1963. Studies on the irritability of the pulvinus of *Mimosa pudica.* Bot. Mag. (Tokyo) **76:** 374–380.

**Allen, N. S. & R. D. Allen.** 1978. Cytoplasmic streaming in green plants. Annual Rev. Biophys. Bioengin. **7:** 497–526.

**Allen, R. D.** 1969. Mechanism of the seismonastic reaction in *Mimosa pudica.* Pl. Physiol. **44:** 1101–1107.

**American Society for Testing and Materials (ASTM).** 1974. Evolution of the International Practical Temperature Scale of 1968. ASTM Special Technical Publication 565. ASTM, Philadelphia, Pennsylvania.

**Amino, S. & M. Tazawa.** 1989. Dependence of tonoplast transport of amino acids on vacuolar pH in *Chara* cells. Proc. Japan Acad. B. **65:** 34–37.

**Amrhein, N.** 1977. The current status of cyclic AMP in higher plants. Annual Rev. Pl. Physiol. **28:** 123–132.

**Andjus, P. R. & D. Vucelic.** 1990. $D_2O$-induced cell excitation. J. Membr. Biol. **115:** 123–127.

**Auger, D.** 1933. Contribution à l'étude de la propagation de la variation électrique chez les Characées. C. R. Séanc. Soc. Biol. **113:** 1437–1440.

**Backster, C.** 1968. Evidence of a primary perception in plant life. Int. J. Parapsych. **10:** 329–348.

**Bandurski, R. S.** 1991. Cell biophysics and plant gravitropism. ASGSB Bull. **42:** 51–64.

**Barber, M. A.** 1911a. A technic for the inoculation of bacteria and other substances into living cells. J. Infect. Dis. **8:** 348–360.

———. 1911b. The effect on the protoplasm of *Nitella* of various chemical substances and of microorganisms introduced into the cavity of the living cell. J. Infect. Dis. **9:** 117–129.

**Barry, P. H.** 1970a. Volume flows and pressure changes during an action potential in cells of *Chara australis*. I. Experimental results. J. Membr. Biol. **3:** 313–334.

———. 1970b. Volume flows and pressure changes during an action potential in cells of *Chara australis*. II. Theoretical considerations. J. Membr. Biol. **3:** 335–371.

**Barry, W. H.** 1968. Coupling of excitation and cessation of cyclosis in *Nitella*: Role of divalent cations. J. Cell. Physiol. **72:** 153–160.

**Bates, G. W. & M. H. M. Goldsmith.** 1983. Rapid response of the plasma-membrane potential in oat coleoptiles to auxin and other weak acids. Planta **159:** 231–237.

**Beilby, M. J.** 1984. Calcium and plant action potentials. Pl. Cell Environm. **7:** 415–421.

——— **& M. A. Bisson.** 1992. *Chara* plasmalemma at high pH: Voltage dependence of the conductance at rest and during excitation. J. Membr. Biol. **125:** 25–39.

——— **& H. G. L. Coster.** 1979a. The action potential in *Chara corallina*. II. Two activation-inactivation transients in voltage clamps of the plasmalemma. Austral. J. Pl. Physiol. **6:** 323–335.

——— & ———. 1979b. The action potential in *Chara corallina*. III. The Hodgkin-Huxley parameters for the plasmalemma. Austral. J. Pl. Physiol. **6:** 337–353.

———, **T. Mimura, & T. Shimmen.** 1993. The proton pump, high pH channels, and excitation: Voltage clamp studies of intact and perfused cells of *Nitellopsis obtusa*. Protoplasma **175:** 144–152.

——— **& V. A. Shepherd.** 1989. Cytoplasm-enriched fragments of *Chara*: Structure and electrophysiology. Protoplasma **148:** 150–163.

**Belton, P. & C. Van Netten.** 1971. The effects of pharmacological agents on the electrical responses of cells of *Nitella flexilis*. Canad. J. Physiol. Pharmacol. **49:** 824–832.

**Benolken, R. M. & S. L. Jacobson.** 1970. Response properties of a sensory hair excised from Venus' fly-trap. J. Gen. Physiol. **56:** 64–82.

**Berg, H. C.** 1993. Random walks in biology. Expanded edition. Princeton University Press, Princeton, NJ.

**Bergethon, P. R. & E. R. Simons.** 1990. Biophysical chemistry. Molecules to membranes. Springer-Verlag, New York.

**Bernard, C.** 1974. Lectures on the phenomena of life common to animals and plants. Vol. 1. Trans. by H. E. Hoff, R. Guillemin & L. Guillemin. Charles C. Thomas, Springfield, IL.

**Bernstein, J.** 1912. Elektrobiologie. Die Lehre von den elektrischen Vorgängen im Organismus auf moderner Grundlage dargestellt. Vieweg, Braunschweig.

**Bialek, W.** 1987. Physical limits to sensation and perception. Annual Rev. Biophysics Biophys. Chem. **16:** 455–478.

**Bisson, M. A. & D. Bartholomew.** 1984. Osmoregulation or turgor regulation in *Chara*? Pl. Physiol. **74:** 252–255.

———, **A. Siegel, R. Chau, S. A. Gelsomino & S. L. Herdic.** 1991. Distribution of charasomes in *Chara*: Banding pattern and effect of photosynthetic inhibitors. Austral. J. Pl. Physiol. **18:** 81–93.

——— **& N. A. Walker.** 1980. The *Chara* plasmalemma at high pH. Electrical measurements show rapid specific passive uniport of $H^+$ or $OH^-$. J. Membr. Biol. **56:** 11967.

——— & ———. 1981. The hyperpolarization of the *Chara* membrane at high pH: Effects of external potassium, internal pH, and DCCD. J. Exp. Bot. **32:** 951–971.

——— & ———. 1982. Control of passive permeability in the *Chara* plasmalemma. J. Exp. Bot. **33:** 520–532.

**Blatt, F. J. & Y. Kuo.** 1976. Absence of biomagnetic effects in *Nitella*. Biophys. J. **16:** 441–444.

**Blatt, M. R., M. J. Beilby & M. Tester.** 1990. Voltage dependence of the *Chara* proton pump revealed by current-voltage measurement during rapid metabolic blockade with cyanide. J. Membr. Biol. **114:** 205–223.

**Blinks, L. R.** 1936. The effect of current flow on bioelectric potential. III. *Nitella.* J. Gen. Physiol. **20:** 229–265.

**Block, S. M.** 1992. Biophysical principles of sensory transduction. Pages 1–17 *in* D. P. Corey & S. D. Roper (eds.), Sensory transduction. Society of General Physiologists. 45th Annual Symposium, Marine Biological Laboratory, Woods Hole, Massachusetts. Rockefeller University Press, New York.

**Bohr, N.** 1933. Light and life. Nature **131:** 421–423, 457–459.

**Bose, J. C.** 1906. Plant response as a means of physiological investigation. Longmans, Green, and Co., London.

———. 1913. Researches on irritability of plants. Longmans, Green, and Co., London.

———. 1926. The nervous mechanism of plants. Longmans, Green, and Co., London.

———. 1985. Life movements in plants. B. R. Publishing, Dehli.

**Boysen-Jensen, P.** 1936. Growth hormones in plants. Translated and revised by G. S. Avery Jr. & P. R. Burkholder. McGraw-Hill, New York.

**Brauner, L. & W. Rau.** 1966. Versuche zur Bewegungsphysiologie der Pflanzen. Springer-Verlag, Berlin.

**Brewster, D.** 1846. The martyrs of science, or The lives of Galileo, Tycho Brahe, and Kepler. John Murray, London.

**Briggs, G. E.** 1957. Some aspects of free space in plant tissues. New Phytol. **56:** 305–324.

——— **& J. B. S. Haldane.** 1925. A note on the kinetics of enzyme action. Biochem. J. **19:** 338–339.

——— **& A. B. Hope.** 1958. Electric potential differences and the Donnan equilibrium in plant tissues. J. Exp. Bot. **9:** 365–371.

———, ——— **& R. N. Robertson.** 1961. Electrolytes and plant cells. F. A. Davis, Philadelphia, Pennsylvania.

——— **& R. N. Robertson.** 1948. Diffusion and absorption in disks of plant tissue. New Phytol. **47:** 265–283.

——— **&** ———. 1957. Apparent free space. Annual Rev. Pl. Physiol. **8:** 11–30.

**Brillouin, L.** 1949. Life, thermodynamics, and cybernetics. Amer. Scientist **37:** 554–568.

———. 1950. Thermodynamics and information theory. Amer. Scientist **38:** 594.

———. 1956. Science and information theory. Academic Press, New York.

———. 1959. Information theory and its applications to fundamental problems in physics. Nature **183:** 501–502.

———. 1964. Scientific uncertainty, and information. Academic Press, New York.

**Brooks, M. M.** 1939. Effect of certain radioactive elements on the metabolism of cells. Proc. Soc. Exp. Biol. **42:** 558–559.

**Brooks, S. C.** 1939. Ion exchanges in accumulation and loss of certain ions by the living protoplasm of *Nitella.* J. Cell. Comp. Physiol. **14:** 383–401.

——— **& M. M. Brooks.** 1941. The permeability of living cells. Gebrüder-Borntraeger, Berlin-Zehlendorf.

——— **& S. Gelfan.** 1928. Bioelectric potentials in *Nitella.* Protoplasma **5:** 86–96.

**Brown, E. G. & R. P. Newton.** 1973. Occurrence of adenosine 3′5′-cyclic monophosphate in plant tissues. Phytochemistry **12:** 2683–2685.

**Brown, G. H. & J. J. Wolton.** 1979. Liquid crystals and biological structures. Academic Press, New York.

**Brown, R.** 1828. A brief account of microscopical observations made in the months of June, July, and August, 1827, on the particles contained in the pollen of plants; and on the general existence of active molecules in organic and inorganic bodies. Richard Taylor, London.

———. 1866. Additional remarks on active molecules. [1829.] Pages 479–486 *in* The miscellaneous works of Robert Brown. The Ray Society and R. Hardwicke, London.

**Brown, S. O.** 1938. Relation between light and the electric polarity of *Chara.* Pl. Physiol. **13:** 713–736.

**Brown, W. H. & L. W. Sharp.** 1910. The closing response in *Dionaea.* Bot. Gaz. **49:** 290–302.

**Buchen, B., Z. Hejnowicz, M. Braun & A. Sievers.** 1991. Cytoplasmic streaming in *Chara* rhizoids. Studies in a reduced gravitational field during parabolic flights of rockets. Protoplasma **165:** 121–126.

———, **M. Braun, Z. Hejnowicz & A. Sievers.** 1993. Statoliths pull on microfilaments. Experiments under microgravity. Protoplasma **172:** 38–42.

**Bunning, E.** 1939. Die Physiologie des Wachstrums und der Bewegungen. Julius Springer, Berlin.

———. 1948. Entwicklungs- und Bewegungsphysiologie der Pflanze. Springer-Verlag, Berlin.

———. 1953. Entwicklungs- und Bewegungsphysiologie der Pflanze. Ed. 3. Springer-Verlag, Berlin.

**Burdon-Sanderson, J.** 1873. Note on the electrical phenomena which accompany stimulation of leaf of *Dionaea muscipula.* Proc. Roy. Soc. London **21:** 495–596.

——— **& F. J. M. Page.** 1876. On the mechanical effects and on the electrical disturbance consequent on excitation of leaf of *Dionaea muscipula.* Proc. Roy. Soc. London **25:** 411–434.

**Bütschli, O.** 1894. Investigations on microscopic foams and on protoplasm. Experiments & observations directed towards a solution of the question of the physical conditions of the phenomena of life. Translated by E. A. Minchin. Adam and Charles Black, London.

**Chapman, R. L. & M. A. Buchheim.** 1991. Ribosomal RNA gene sequences: Analysis and significance in the phylogeny and taxonomy of green algae. Crit. Rev. Pl. Sci. **10:** 343–368.

**Cleland, R. E., H. B. A. Prins, J. R. Harper & N. Higinbotham.** 1977. Rapid hormone-induced hyperpolarization of the oat coleoptile potential. Pl. Physiol. **59:** 395–397.

**Cohen, I. B.** 1941. Benjamin Franklin's experiments. A new edition of Franklin's experiments and observations on electricity. Edited, with a critical and historical introduction, by I. B. Cohen. Harvard University Press, Cambridge, Massachusetts.

**Cole, K. S.** 1928. Electric impedance of suspensions of spheres. J. Gen. Physiol. **12:** 29–36.

———. 1968. Membranes, ions, and impulses: A chapter of classical biophysics. University of California Press, Berkeley.

———. 1970. Dielectric properties of living membranes. Pages 1–15 *in* F. Snell, J. Wolken, G. Iverson & J. Lam (eds.), Physical principles of biological membranes. Proceedings of the Coral Gables Conference on Physical Principles of Biological Membranes. Gordon and Breach, New York.

——— **& R. H. Cole.** 1936a. Electrical impedance of *Astenas* eggs. J. Gen. Physiol. **19:** 609–623.

——— **&** ———. 1936b. Electrical impedance of *Arbacia* eggs. J. Gen. Physiol. **19:** 625–632.

——— **& H. J. Curtis.** 1938. Electrical impedance of *Nitella* during activity. J. Gen. Physiol. **22:** 37–64.

——— **&** ———. 1939. Electrical impedance of the squid giant axon during activity. J. Gen. Physiol. **22:** 649–670.

**Coleman, H. A.** 1986. Chloride currents in *Chara*—A patch-clamp study. J. Membr. Biol. **93:** 55–61.

**Collander, R.** 1949. The permeability of plant protoplasts to small molecules. Physiol. Pl. **2:** 300–311.

———. 1954. The permeability of *Nitella* cells to non-electrolytes. Physiol. Pl. **7:** 420–445.

**Corti, B.** 1774. Osservazioni microscopiche sulla tremella e sulla circolacione del fluido in una planta acquajuola. Apresso Giuseppe Rocchi, Lucca.

**Cosgrove, D. J.** 1993a. How do plant walls extend? Pl. Physiol. **102:** 1–6.

———. 1993b. Wall extensibility: Its nature measurement and relationship to plant cell growth. New Phytol. **124:** 1–23.

———. 1993c. Water uptake by growing cells: An assessment of the controlling roles of wall relaxation, solute uptake, and hydraulic conductance. Int. J. Pl. Sci. **154:** 10–21.

**Coster, H. G. L.** 1966. Chloride in cells of *Chara australis.* Austral. J. Biol. Sci. **19:** 545–554.

**Cote, R., J. F. Thain & D. S. Fensom.** 1987. Increase in electrical resistance of plasmodesmata of *Chara* induced by an applied pressure gradient across nodes. Canad. J. Bot. **65:** 509–511.

**Curtis, H. J. & K. S. Cole.** 1937. Transverse electric impedance of *Nitella.* J. Gen. Physiol. **21:** 189–201.

**Dainty, J.** 1962. Ion transport and electrical potentials in plant cells. Annual Rev. Pl. Physiol. **13:** 379–402.

———. 1963a. The polar permeability of plant cell membranes to water. Protoplasma **57:** 220–228.

———. 1963b. Water relations of plant cells. Advances Bot. Res. **1:** 279–326.

——— **& B. Z. Ginzburg.** 1964a. The measurement of hydraulic conductivity (osmotic permeability to water) of internodal characean cells by means of transcellular osmosis. Biochim. Biophys. Acta **79:** 102–111.

——— & ———. 1964b. The permeability of the cell membrane of *Nitella translucens* to urea, and the effect of high concentrations of sucrose on this permeability. Biochim. Biophys. Acta **79:** 112–121.

——— & ———. 1964c. The reflection coefficient of plant cell membranes for certain solutes. Biochim. Biophys. Acta **79:** 129–137.

——— **& A. B. Hope.** 1959. The water permeability of cells of *Chara australis* R. Br. Austral. J. Biol. Sci. **12:** 136–145.

**Darwin, C.** 1893. Insectivorous plants. Ed. 2. J. Murray, London.

———. 1966. The power of movement in plants. [1881.] Da Capo Press, New York.

**Davies, E.** 1987. Action potentials as multifunctional signals in plants: A unifying hypothesis to explain apparently disparate wound responses. Pl., Cell Environm. **10:** 623–631.

———. 1993a. Intercellular and intracellular signals and their transduction via the plasma membrane–cytoskeleton interface. Seminars in Cell Biology **4:** 139–147.

———. 1993b. Leaf to root, come in please. The World & I (Oct.): 185–191.

**Dean, R. B., H. J. Curtis & K. S. Cole.** 1940. Impedance of bimolecular films. Science **91:** 50–51.

**Delbrück, M.** 1970. A physicist's renewed look at biology: Twenty years later. Science **168:** 1312–1315.

**Dettbarn, W. D.** 1962. Acetylcholinesterase activity in *Nitella.* Nature **194:** 1175–1176.

**Diamond, J. M. & A. K. Solomon.** 1959. Intracellular potassium compartments in *Nitella axillaris.* J. Gen. Physiol. **42:** 1105–1121.

**Ding, D.-Q. & M. Tazawa.** 1989. Influence of cytoplasmic streaming and turgor pressure gradient on the transnodal transport of rubidium and electrical conductance in *Chara corallina.* Pl. Cell Physiol. **30:** 739–748.

———**, S. Amino, T. Mimura, K. Sakano, T. Nagata & M. Tazawa.** 1992. Quantitative analysis of intracellularly transported photoassimilates in *Chara corallina.* J. Exp. Bot. **43:** 1045–1051.

———**, T. Mimura, S. Amino & M. Tazawa.** 1991. Intercellular transport and photosynthetic differentiation in *Chara corallina.* J. Exp. Bot. **42:** 33–38.

**Di Palma, J. R., R. Mohl & W. J. R. Best.** 1961. Action potential and contraction of *Dionaea muscipula* (Venus' flytrap). Science **133:** 878–879.

**Donnan, F. G.** 1927. Concerning the applicability of thermodynamics to the phenomena of life. J. Gen. Physiol. **8:** 685–688.

**Dorn, A. & M. H. Weisenseel.** 1984. Growth and the current pattern around internodal cells of *Nitella flexilis* L. J. Exp. Bot. **35:** 373–383.

**Duncan, G.** 1990. Physics in the life sciences. Blackwell Scientific Publications, Oxford.

**Ebashi, S.** 1976. Excitation-contraction coupling. Annual Rev. Physiol. **38:** 293–313.

**Eckert, R., D. Randall & G. Augustine.** 1988. Animal physiology. Mechanisms and adaptations. Ed. 3. W. H. Freeman, New York.

**Einstein, A.** 1926. Investigations on the theory of the Brownian movement. Methuen & Co., London.

**Eisner, T.** 1981. Leaf folding in a sensitive plant: A defensive thorn-exposure mechanism? Proc. Natl. Acad. Sci. U.S.A. **78:** 402–404.

**Elasser, W. M.** 1958. The physical foundation of biology. An analytical study. Pergamon Press, New York.

**Elliot, D. C. & A. W. Murray.** 1975. Evidence against an involvement of cyclic nucleotides in the induction of betacyanin synthesis by cytokinins. Biochem. J. **146:** 333–337.

**Eschrich, W., J. Fromm & R. F. Evert.** 1988. Transmission of electric signals in sieve tubes of zucchini plants. Bot. Acta **101:** 327–331.

**Ewart, A. J.** 1903. On the physics and physiology of protoplasmic streaming in plants. Clarendon Press, Oxford.

**Faraday, C. D. & R. M. Spanswick.** 1993. Evidence for a membrane skeleton in higher plants. A spectrin-like polypeptide co-isolates with rice root plasma membranes. Fed. Eur. Biochem. Soc. Lett. **318:** 313–316.

**Felle, H., B. Brummer, A. Bertl & R. W. Parish.** 1986. Indole-3-acetic acid and fusicoccin cause cytosolic acidification of corn coleoptile cells. Proc. Natl. Acad. Sci. U.S.A. **83:** 8992–8995.

**Feynman, R. P.** 1965. The character of physical law. MIT Press, Cambridge, Massachusetts.
———. 1985. QED. The strange theory of light and matter. Princeton University Press, Princeton, New Jersey.
———, **R. B. Leighton & M. Sands.** 1963. The Feynman lectures on physics. Volume I. Addison-Wesley, Reading, Massachusetts.
**Findlay, G. P.** 1959. Studies of action potentials in the vacuole and cytoplasm of *Nitella.* Austral. J. Biol. Sci. **12:** 412–426.
———. 1961. Voltage-clamp experiments with *Nitella.* Nature **191:** 812–814.
———. 1962. Calcium ions and the action potential in *Nitella.* Austral. J. Biol. Sci. **15:** 69–82.
———. 1970. Membrane electrical behavior in *Nitellopsis obtusa.* Austral. J. Biol. Sci. **23:** 1033–1045.
——— & **A. B. Hope.** 1964a. Ionic relations of cells of *Chara australis.* VII. The separate electrical characteristics of the plasmalemma and tonoplast. Austral. J. Biol. Sci. **17:** 62–77.
——— & ———. 1964b. Ionic relations of cells of *Chara australis.* IX. Analysis of transient membrane currents. Austral. J. Biol. Sci. **17:** 400–411.
**Fisher, R. A.** 1930. The genetical theory of natural selection. Oxford University Press, Oxford.
**Fluck, R. A. & M. J. Jaffe.** 1974. The distribution of cholinesterases in plant species. Phytochemistry **13:** 2475–2480.
**Forsberg, C.** 1965. Nutritional studies of *Chara* in axenic cultures. Physiol. Pl. **18:** 275–290.
**Fricke, H.** 1925. The electric capacity of suspensions with special reference to blood. J. Gen. Physiol. **9:** 137–152.
——— & **S. Morse.** 1925. The electric resistance and capacity of blood for frequencies between 800 and 4½ million cycles. J. Gen. Physiol. **9:** 153–167.
**Fromm, J.** 1991. Control of phloem unloading by action potentials in *Mimosa.* Physiol. Pl. **83:** 529–533.
**Fulghum, R.** 1989. All I really need to know I learned in kindergarten. Villard Books, New York.
**Gaffey, C. T. & L. J. Mullins.** 1958. Ion fluxes during the action potential in *Chara.* J. Physiol. **144:** 505–524.
**Galston, A. W.** 1994. Life processes of plants. Scientific American Library, New York.
**Gertel, E. T. & P. B. Green.** 1977. Cell growth pattern and wall microfibrillar arrangement. Experiments with *Nitella.* Pl. Physiol. **60:** 247–254.
**Gibbs, J. W.** 1928. The collected works of J. Willard Gibbs. Vol. I. Longmans, Green and Co., New York.
**Gillet, C., P. Cambier & F. Liners.** 1992. Release of small polyuronides from *Nitella* cell wall. Pl. Physiol. **100:** 846–852.
———, **P. Van Cutsem & M. Voué.** 1989. Correlation between the weight loss induced by alkaline ions and the cationic exchange capacity of the *Nitella* cell wall. J. Exp. Bot. **40:** 129–133.
———, **M. Voué & P. Cambier.** 1994. Conformational transitions and modifications in ionic exchange properties induced by alkaline ions in the *Nitella flexilis* cell wall. Pl. Cell Physiol. **35:** 79–85.
**Gillispie, C. C.** 1970. Dictionary of scientific biography. Vol. II. Charles Scribner's Sons, New York.
———. 1972. Dictionary of scientific biography. Vol. VI. Charles Scribner's Sons, New York.
**Goebel, K.** 1920. Die Entfaltungsbewegungen der Pflanzen und deren teleologische Deutung. Gustav Fischer, Jena.
**Goldman, D. E.** 1943. Potential, impedance, and rectification in membranes. J. Gen. Physiol. **27:** 37–60.
**Goldsmith, G. W. & A. L. Hafenrichter.** 1932. Anthokinetics. The physiology and ecology of floral movements. Carnegie Institute, Washington.
**Göppert, H. R. & F. Cohn.** 1849. Über die Rotation des Zellinhaltes von *Nitella flexilis.* Bot. Z. **7:** 665–673 ff.
**Graham, L. E.** 1993. Origin of land plants. John Wiley & Sons, New York.
——— & **Y. Kaneko.** 1991. Subcellular structures of relevance to the origin of land plants (Embryophytes) from green algae. Crit. Rev. Pl. Sci. **10:** 323–342.
**Grambast, L. J.** 1974. Phylogeny of the Charophyta. Taxon **23:** 463–481.

**Green, P. B.** 1954. The spiral growth pattern of the cell wall in *Nitella axillaris*. Amer. J. Bot. **41:** 403–409.

———. 1958a. Concerning the site of the addition of new wall substances to the elongating *Nitella* cell wall. Amer. J. Bot. **45:** 111–116.

———. 1958b. Structural characteristics of developing *Nitella* internodal cell walls. J. Biophys. Biochem. Cytol. **1:** 505–516.

———. 1959. Wall structure and helical growth in *Nitella*. Biochim. Biophys. Acta **36:** 536–538.

———. 1960. Multinet growth in the cell wall of *Nitella*. J. Biophys. Biochem. Cytol. **7:** 289–296.

———. 1962. Mechanism for plant cellular morphogenesis. Science **138:** 1404–1405.

———. 1963. On mechanisms of elongation. Pages 203–234 *in* Cytodifferential & macromolecular synthesis. Academic Press, New York.

———. 1964. Cinematic observations on the growth and division of chloroplasts in *Nitella*. Amer. J. Bot. **51:** 334–342.

———. 1968. Growth physics in *Nitella*: A method for continuous *in vivo* analysis of extensibility based on a micromanometer technique for turgor pressure. Pl. Physiol. **43:** 1169–1184.

———. 1981. Organogenesis—A biophysical view. Annual Rev. Pl. Physiol. **31:** 51–82.

——— **& G. B. Chapman.** 1955. On the development and structure of the cell wall in *Nitella*. Amer. J. Bot. **42:** 685–693.

——— **& J. C. W. Chen.** 1960. Concerning the role of wall stresses in the elongation of the *Nitella* cell. Z. Wiss. Mikroskop. **64:** 482–488.

———**, R. O. Erickson & J. Buggy.** 1971. Metabolic and physical control of cell elongation rate. Pl. Physiol. **47:** 423–430.

**Green, R. M.** 1953. A translation of Luigi Galvani's *De Viribus Electricitatis In Motu Musculari Commentarius*. Commentary on the effect of electricity on muscular motion. Elizabeth Licht, Cambridge, Massachusetts.

**Groves, J. & G. R. Bullock-Webster.** 1920. The British Charophyta. Volume I. Nitelleae. Ray Society, London.

——— & ———. 1924. The British Charophyta. Volume II. Characeae. Ray Society, London.

**Haberlandt, G.** 1890. Das Reizleitende Gewebesystem der Sinnpflanze. Engelmann, Leipzig.

———. 1901. Sinneorgane im Pflanzenreich. Engelmann, Leipzig.

**Haldane, J. B. S.** 1924. A mathematical theory of natural and artificial selection. Part II. The influence of partial self-fertilization, inbreeding, assortative mating, and selective fertilization on the composition of Mendelian populations, and on natural selection. Proc. Cambridge Philos. Soc. **1:** 158–163.

**Halliday, D. & R. Resnick.** 1970. Fundamentals of physics. John Wiley & Sons, New York.

**Hamill, O. P., J. R. Huguenard & D. A. Price.** 1991. Patch-clamp studies of voltage-gated currents in identified neurons of the rat cerebral cortex. Cerebral Cortex **1:** 48–61.

———**, A. Marty, E. Neher, B. Sakmann & F. J. Sigworth.** 1981. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pflügers Arch. **391:** 85–100.

**Hansen, U.-P.** 1990. Implications of control theory for homeostasis and phosphorylation of transport molecules. Bot. Acta **103:** 15–23.

**Harvey, E. N.** 1942a. Stimulation of cells by intense flashes of ultraviolet light. J. Gen. Physiol. **25:** 431–443.

———. 1942b. Hydrostatic pressure and temperature in relation to stimulation and cyclosis in *Nitella flexilis*. J. Gen. Physiol. **25:** 855–863.

———. 1957. A history of luminescence. From the earliest times until 1900. American Philosophical Society, Philadelphia, Pennsylvania.

**Hayama, T., T. Shimmen & M. Tazawa.** 1979. Participation of $Ca^{2+}$ in cessation of cytoplasmic streaming induced by membrane excitation in characean internodal cells. Protoplasma **99:** 305–321.

**Hedrich, R. & J. I. Schroeder.** 1989. The physiology of ion channels and electrogenic pumps in higher plants. Annual Rev. Pl. Physiol. Pl. Molec. Biol. **40:** 539–569.

**Hejnowicz, Z., B. Buchen & A. Sievers.** 1985. The endogenous difference in the rates of acropetal and basipetal cytoplasmic streaming in *Chara* rhizoids is enhanced by gravity. Protoplasma **125:** 219–230.

**Hildebrand, J. H.** 1963. An introduction to molecular kinetic theory. Chapman & Hall, London.

**Hill, S. E.** 1935. Stimulation by cold in *Nitella*. J. Gen. Physiol. **18:** 357–367.

———. 1941. The relation of streaming and action potentials in *Nitella*. Biol. Bull. **81:** 296.

**Hille, B.** 1984. Ionic channels of excitable membranes. Sinauer Associates, Sunderland, Massachusetts.

———. 1992. Ionic channels of excitable membranes. Ed. 2. Sinauer Associates, Sunderland, Massachusetts.

**Hirono, C. & T. Mitsui.** 1983. Slow onset of activation and delay of inactivation in transient current of *Nitella axilliformis*. Pl. Cell Physiol. **24:** 289–299.

**Hoagland, D. R. & T. C. Broyer.** 1942. Accumulation of salt and permeability in plant cells. J. Gen. Physiol. **25:** 865–880.

——— **& A. R. Davis.** 1923a. The composition of the cell sap of the plant in relation to the absorption of ions. J. Gen. Physiol. **5:** 629–641.

——— & ———. 1923b. Further experiments on the absorption of ions by plants, including observations on the effect of light. J. Gen. Physiol. **6:** 47–62.

———, **P. L. Hibbard & A. R. Davis.** 1926. The influence of light, temperature, and other conditions on the ability of *Nitella* cells to concentrate halogens in the cell sap. J. Gen. Physiol. **10:** 121–146.

**Hodge, A. J., J. D. McLean, & F. V. Mercer.** 1956. A possible mechanism for the morphogenesis of lamellar systems in plant cells. J. Biophys. Biochem. Cytol. **2:** 597–607.

**Hodgkin, A. L.** 1951. The ionic basis of electrical activity in nerve and muscle. Biol. Rev. **26:** 339–409.

———. 1964. The conduction of the nervous impulse. Charles C. Thomas, Springfield, Illinois.

——— **& A. F. Huxley.** 1953. Movement of radioactive potassium and membrane current in a giant axon. J. Physiol. **121:** 403–414.

———, ———, **& B. Katz.** 1952. Measurement of current-voltage relations in the membrane of the giant axon of *Loligo*. J. Physiol. **116:** 424–448.

——— **& B. Katz.** 1949. The effect of sodium ions on the electrical activity of the giant axon of the squid. J. Physiol. **108:** 37–77.

——— **& R. D. Keynes.** 1955. The potassium permeability of a giant nerve fibre. J. Physiol. **128:** 61–88.

**Hodick, D. & A. Sievers.** 1986. The influence of $Ca^{2+}$ on the action potential in mesophyll cells of *Dionaea muscipula* Ellis. Protoplasma **133:** 83–84.

——— & ———. 1989. On the mechanism of trap closure of Venus flytrap (*Dionaea muscipula* Ellis). Planta **179:** 32–42.

**Hogg, J., E. J. Williams & R. J. Johnson.** 1968a. A simplified method for measuring membrane resistances in *Nitella translucens*. Biochim. Biophys. Acta **150:** 518–520.

———, ———, & ———. 1968b. The temperature dependence of the membrane potential and resistance in *Nitella translucens*. Biochim. Biophys. Acta **150:** 640–648.

**Homblé, F. & I. Foissner.** 1993. Electron microscopy and electrophysiology of local cell wall formation in *Chara corallina*. Pl. Cell Physiol. **34:** 1283–1289.

———, **C. Richter & J. Dainty.** 1989. Leakage of pectins from the cell wall of *Chara corallina* in the absence of divalent cations. Pl. Physiol. Biochem. **27:** 465–468.

**Hope, A. B.** 1951. Membrane potential differences in bean roots. Austral. J. Sci. Res., Ser. B. **4:** 265–274.

———. 1961a. Ionic relations of cells of *Chara corallina*. V. The action potential. Austral. J. Biol. Sci. **14:** 312–322.

———. 1961b. The action potential in cells of *Chara*. Nature **191:** 811–812.

——— **& G. P. Findlay.** 1964. The action potential in *Chara*. Pl. Cell Physiol. **5:** 377–379.

——— **& R. N. Robertson.** 1956. Initial absorption of ions by plant tissue. Nature **177:** 43–44.

———, **A. Simpson & N. A. Walker.** 1966. The efflux of chloride from cells of *Nitella* and *Chara*. Austral. J. Biol. Sci. **19:** 355–362.

——— **& P. G. Stevens.** 1952. Electric potential differences in bean roots and their relation to salt uptake. Austral. J. Sci. Res., Ser. B **5:** 335–343.

——— **& N. A. Walker.** 1961. Ionic relations of cells of *Chara australis* R. Br. IV. Membrane potential differences and resistances. Austral. J. Biol. Sci. **14:** 26–44.

——— **&** ———. 1975. The physiology of giant algal cells. Cambridge University Press, Cambridge.

**Hörmann, G.** 1898. Studien über die Protoplasmaströmung bei den Characeen. Verlag von Gustav Fischer, Jena.

**Horowitz, K. A., D. C. Lewis & E. L. Gasteiger.** 1975. Plant "primary perception": Electrophysiological unresponsiveness to brine shrimp killing. Science **189:** 478–480.

**Horowitz, P. & W. Hill.** 1989. The art of electronics. Ed. 2. Cambridge University Press, Cambridge.

**Hotchkiss, A. T. & R. M. Brown Jr.** 1987. The association of rosette and globule terminal complexes with cellulose microfibril assembly in *Nitella translucens* var. *axillaris* (Charophyceae). J. Phycol. **23:** 229–237.

**Huxley, T. H.** 1902. Science and education. American Dome Library Co., New York.

**Iijima, T. & S. Hagiwara.** 1987. Voltage-dependent K channels in protoplasts of trap-lobe cells of *Dionaea muscipula.* J. Membr. Biol. **100:** 73–81.

——— **& T. Sibaoka.** 1981. Action potential in the trap-lobes of *Aldovanda vesiculosa.* Pl. Cell Physiol. **22:** 1595–1601.

——— **&** ———. 1982. Propagation of action potential over the trap-lobes of *Aldovanda vesiculosa.* Pl. Cell Physiol. **23:** 679–688.

——— **&** ———. 1983. Movements of $K^+$ during shutting and opening of the trap-lobes of *Aldovanda vesiculosa.* Pl. Cell Physiol. **24:** 51–60.

——— **&** ———. 1985. Membrane potentials in excitable cells of *Aldovanda vesiculosa* trap-lobes. Pl. Cell Physiol. **26:** 1–13.

**Imahori, K.** 1954. Ecology phytogeography and taxonomy of the Japanese Charophyta. Kanazawa University Press, Kanazawa, Japan.

**Jacobson, S. L.** 1965. Receptor response in Venus' fly-trap. J. Gen. Physiol. **49:** 117–129.

**Jaffe, M. J.** 1973. Thigmomorphogenesis: The response of plant growth and development to mechanical stimulation. Planta **114:** 143–157.

———. 1976. Thigmomorphogenesis: A detailed characterization of the response of beans (*Phaseolus vulgaris* L.) to mechanical stimulation. Z. Pflanzenphysiol. **77:** 437–453.

——— **& A. W. Galston.** 1968. The physiology of tendrils. Annual Rev. Pl. Physiol. **19:** 417–434.

**Jean, J. M., C. K. Chan, G. R. Fleming & T. G. Owens.** 1989. Excitation transport and trapping on the spectrally disordered lattices. Biophys. J. **56:** 1203–1216.

**Joule, J. P.** 1852. On the heat disengaged in chemical combinations. Phil. Mag. Fourth Ser. No. 21. Suppl. Vol. **3:** 481–504.

**Junge, D.** 1981. Nerve and muscle excitation. Ed. 2. Sinauer Associates, Sunderland, Massachusetts.

**Kamitsubo, E. & M. Kikuyama.** 1992. Immobilization of endoplasm flowing contiguous to the actin cables upon electrical stimulus in *Nitella* internodes. Protoplasma **168:** 82–86.

———, **Y. Ohashi & M. Kikuyama.** 1989. Coplasmic streaming in internodal cells of *Nitella* under centrifugal acceleration: a study done with a newly constructed centrifuge microscope. Protoplasma **152:** 148–155.

**Kamiya, N.** 1959. Protoplasmic streaming. Protoplasmatologia. Handbuch der Protoplasmaforschung. Band VIII. Physiologie des Protoplasmas. 3. Motilität. Springer-Verlag, Wein.

———. 1981. Physical and chemical basis of cytoplasmic streaming. Annual Rev. Pl. Physiol. **32:** 205–236.

——— **& K. Kuroda.** 1956a. Artificial modification of the osmotic pressure of the plant cell. Protoplasma **46:** 423–436.

——— **&** ———. 1956b. Velocity distribution of the protoplasmic streaming in *Nitella* cells. Bot. Mag. (Tokyo) **69:** 544–554.

——— **& M. Tazawa.** 1956. Studies on water permeability of a single plant cell by means of transcellular osmosis. Protoplasma **46:** 394–422.

**Katchalsky, A. & P. F. Curran.** 1965. Nonequilibrium thermodynamics in biophysics. Harvard University Press, Cambridge, Massachusetts.

**Katsuhara, M., T. Mimura & M. Tazawa.** 1989. Patch-clamp study on a $Ca^{2+}$-regulated $K^+$ channel in the tonoplast of the brackish characeae *Lamprothamnium succinctum*. Pl. Cell Physiol. **30:** 549–555.

———, ——— & ———. 1990. ATP-regulated ion channels in the plasma membrane of a characean alga, *Nitellopsis obtusa*. Pl. Physiol. **93:** 343–346.

——— **& M. Tazawa.** 1992. Calcium-regulated channels and their bearing on physiological activities in characean cells. Philos. Trans., Ser. B **338:** 19–29.

**Katz, B.** 1966. Nerve, muscle and synapse. McGraw-Hill, New York.

**Kelvin, Lord (William Thomson).** 1855. On the theory of the electric telegraph. Proc. Roy. Soc. London **7:** 382–399.

**Kersey, Y. M., P. K. Hepler, B. A. Palevitz & N. K. Wessells.** 1976. Polarity of actin filaments in Characean algae. Proc. Natl. Acad. Sci. U.S.A.). **73:** 165–167.

**Keynes, R. D.** 1951. The ionic movements during nervous activity. J. Physiol. **114:** 119–150.

**Kikuyama, M.** 1986. Tonoplast action potential of Characeae. Pl. Cell Physiol. **27:** 1461–1468.

———. 1988. $Ca^{2+}$ increases the $Cl^-$ efflux of the permeabilized *Chara*. Pl. Cell Physiol. **29:** 105–108.

———. 1989. Effect of $Ca^{2+}$ on tonoplast potential of permeabilized Characeae cells. Pl. Cell Physiol. **30:** 253–258.

———, **M. Oda, T. Shimmen, T. Hayama & M. Tazawa.** 1984. Potassium and chloride effluxes during excitation of characean cells. Pl. Cell Physiol. **25:** 965–974.

———, **K. Shimada & Y. Hiramoto.** 1993. Cessation of cytoplasmic streaming follows an increase of cytoplasmic $Ca^{2+}$ during action potential in *Nitella*. Protoplasma **174:** 142–146.

——— **& M. Tazawa.** 1976. Tonoplast action potential in *Nitella* in relation to vacuolar chloride concentration. J. Membr. Biol. **29:** 95–110.

——— & ———. 1983. Transient increase of intracellular $Ca^{2+}$ during excitation of tonoplast-free *Chara* cells. Protoplasma **117:** 62–67.

**Kishimoto, U.** 1957. Studies on the electrical properties of a single plant cell. Internodal cell of *Nitella flexilis*. J. Gen. Physiol. **42:** 1167–1187.

———. 1959. Electrical characteristics of *Chara corallina*. Rep. (Annual) Sci. Works Fac. Sci. Osaka Univ. **7:** 115–146.

———. 1964. Current voltage relations in *Nitella*. Jap. J. Physiol. **14:** 515–527.

———. 1968. Response of *Chara* internodes to mechanical stimulation. Rep. Biol. Works **16:** 61–66.

———. 1972. Characteristics of the excitable *Chara* membrane. Advances Biophys. **3:** 199–226.

——— **& H. Akabori.** 1959. Protoplasmic streaming of an internodal cell of *Nitella flexilis*. Its correlation with electric stimulus. J. Gen. Physiol. **42:** 1167–1183.

——— **& T. Ohkawa.** 1966. Shortening of *Nitella* internode during excitation. Pl. Cell Physiol. **7:** 493–497.

———, **Y. Takeuchi, T. Ohkawa & N. Kami-iki.** 1985. A kinetic analysis of the electrogenic pump of *Chara corallina*: III. Pump activity during action potential. J. Membr. Biol. **86:** 27–36.

**Kiss, J. Z. & L. A. Staehelin.** 1993. Structural polarity in the *Chara* rhizoid: A reevaluation. Amer. J. Bot. **80:** 273–282.

**Kitasato, H.** 1968. The influence of $H^+$ on the membrane potential and ion fluxes of *Nitella*. J. Gen. Physiol. **52:** 60–87.

**Kiyosawa, K.** 1993. Loss of osmotic and ionic regulation in *Chara* internodal cells in concentrated KCl solutions. Physiol. Pl. **89:** 499–503.

**Klieber, H.-G. & D. Gradmann.** 1993. Enzyme kinetics of the prime $K^+$ channel in the tonoplast of *Chara*: Selectivity and inhibition. J. Membr. Biol. **132:** 253–265.

**Klotz, I. M.** 1967. Energy changes in biochemical reactions. Academic Press, New York.

**Kobatake, Y., I. Inoue & T. Ueda.** 1975. Physical chemistry of excitable membranes. Advances Biophys. **7:** 43–89.

**Kohl, F. G.** 1894. Die Mechanik der Reizkrümmungen. N. G. Elwert'sche, Marburg.

**Kripalani, K.** 1962. Rabindranath Tagore. A biography. Grove Press, New York.

**Kudoyarova, G. R., I. Yu. Usmanov, V. Z. Gyuli-Zade, É. G. Fattakhutdinov & S. Yu. Veselov.** 1990. Interaction of spatially separated plant organs. Relationship between electric and hormonal signals. Dokl. Bot. Sci. **310:** 13–15.

**Kwiatkowska, M.** 1988. Symplastic isolation of *Chara vulgaris* antheridium and mechanisms regulating the process of spermatogenesis. Protoplasma **142:** 137–146.

———. 1991. Autoradiographic studies on the role of plasmodesmata in the transport of gibberellin. Planta **183:** 294–299.

——— **& J. Maszewski.** 1985. Changes in ultrastructure of plasmodesmata during spermatogenesis in *Chara vulgaris.* Planta **166:** 46–50.

——— **&** ———. 1986. Changes in the occurrence and ultrastructure of plasmodesmata in antheridia of *Chara vulgaris* during different stages of spermatogenesis. Protoplasma **132:** 179–188.

———**, K. Poplonska & K. Zylinska.** 1990. Biological role of endoreplication in the process of spermatogenesis in *Chara vulgaris* L. Protoplasma **155:** 176–187.

**Lakshminarayanaiah, N.** 1969. Transport phenomena in membranes. Academic Press, New York.

**Laver, D. R.** 1992. Divalent cation block and competition between divalent and monovalent cations in the large-conductance $K^+$ channel from *Chara australis.* J. Gen. Physiol. **100:** 269–300.

——— **& N. A. Walker.** 1991. Activation by $Ca^{2+}$ and block by divalent cations of the $K^+$ channel in the membrane of cytoplasmic drops from *Chara australis.* J. Membr. Biol. **120:** 131–139.

**Levy, S.** 1991. Two separate zones of helicoidally oriented microfibrils are present in the walls of *Nitella* internodes during growth. Protoplasma **163:** 145–155.

**Lewis, G. N. & M. Randall.** 1923. Thermodynamics and the free energy of chemical substances. McGraw-Hill, New York.

**Linderholm, H.** 1952. Active transport of ions through frog skin with special reference to the action of certain diuretics. A study of the relation between certain delectrical properties, the flux of labeled ions, and respiration. Acta Physiol. Scand. **27 [Suppl 97]:** 1–144.

**Ling, G. N.** 1984. In search of the physical basis of life. Plenum, New York.

**Liu, Q., K. Katou & H. Okamoto.** 1991. Effects of osmotic stress, ionic stress and IAA on the cell-membrane resistance of *Vigna* hypocotyls. Pl. Cell Physiol. **32:** 1021–1029.

**Lloyd, F. E.** 1924. The fluorescent colors of plants. Science **59:** 241–248.

**Loeb, J.** 1912. The mechanistic conception of life. Chicago University Press, Chicago.

———. 1916. The organism as a whole from a physiochemical viewpoint. G. P. Putnam's Sons, New York.

**Lotka, A. J.** 1925. Elements of physical biology. Williams & Wilkins Co., Baltimore, Maryland.

**Luby-Phelps, K., D. L. Taylor & F. Lanni.** 1986. Probing the structure of cytoplasm. J. Cell Biol. **102:** 2015–2022.

**Lucas, W. J.** 1975. Photosynthetic fixation of $^{14}$carbon by internodal cells of *Chara corallina.* J. Exp. Bot. **26:** 331–336.

———. 1976. The influence of $Ca^{2+}$ and $K^+$ on $H^{14}CO_3^-$ influx in internodal cells of *Chara corallina.* J. Exp. Bot. **27:** 32–42.

———. 1977. Analogue inhibition of the active $HCO_3^-$ transport site in the characean plasma membrane. J. Exp. Bot. **28:** 1321–1336.

———. 1979. Alkaline band formation in *Chara corallina.* Due to $OH^-$ efflux or $H^+$ influx? Pl. Physiol. **63:** 248–254.

———. 1982. Mechanism of acquisition of exogenous bicarbonate by internodal cells of *Chara corallina.* Planta **156:** 181–192.

——— **& J. M. Alexander.** 1981. Influence of turgor pressure manipulation on plasmalemma transport of $HCO_3^-$ and $OH^-$ in *Chara corallina.* Pl. Physiol. **68:** 553–559.

———**, F. Brechignac, T. Mimura & J. Oross.** 1989. Charasomes are not essential for photosynthetic utilization of exogenous $HCO_3^-$ in *Chara corallina.* Protoplasma **151:** 106–114.

——— **& J. Dainty.** 1977a. $HCO_3^-$ influx across the plasmalemma of *Chara corallina.* Divalent cation requirement. Pl. Physiol. **60:** 862–867.

——— **&** ———. 1977b. Spatial distribution of functional $OH^-$ carriers along a characean internodal cell: Determined by the effect of cytochalasin B on $H^{14}CO_3^-$. J. Membr. Biol **32:** 75–92.

——— **& T. Shimmen.** 1981. Intracellular perfusion and cell centrifugation studies on plasmalemma transport processes in *Chara corallina.* J. Membr. Biol. **58:** 227–237.

——— **& F. A. Smith.** 1973. The formation of alkaline and acid regions at the cell surface of *Chara corallina* cells. J. Exp. Bot. **24:** 1–14

———, **R. M. Spanswick & J. Dainty.** 1978. $HCO_3^-$ influx across the plasmalemma of *Chara corallina.* Pl. Physiol. **61:** 487–493.

**Lund, E. J.** 1947. Bioelectric fields and growth. University of Texas Press, Austin.

**Lunevsky, V. Z., O. M. Zherelova, I. Y. Vostrikov & G. N. Berestovsky.** 1983. Excitation of Characeae cell membranes as a result of activation of calcium and chloride channels. J. Membr. Biol. **72:** 43–58.

**Mackie, G. O., I. D. Lawn & M. Pavans de Ceccatty.** 1983. Studies on hexactinellid sponges. II. Excitability, conduction and coordination of responses in *Rhabdocalyptus Dawsini* (Lambe, 1873). Philos. Trans., Ser. B **301:** 401–418.

**MacRobbie, E. A. C.** 1962. Ionic relations of *Nitella translucens.* J. Gen. Physiol. **45:** 861–878.

——— **& J. Dainty.** 1958. Ion transport in *Nitellopsis obtusa.* J. Gen. Physiol. **42:** 35–353.

——— **& D. S. Fensom.** 1969. Measurements of electro-osmosis in *Nitella translucens.* J. Exp. Bot. **20:** 466–484.

**Mailman, D. S. & L. J. Mullins.** 1966. The electrical measurement of chloride fluxes in *Nitella.* Austral. J. Biol. Sci. **19:** 385–398.

**Malone, M.** 1993. Hydraulic signals. Philos. Trans., Ser. B **341:** 33–39.

**Manhart, J. R. & J. D. Palmer.** 1990. The gain of two chloroplast tRNA introns marks the green algal ancestors of land plants. Nature **345:** 268–270.

**Mast, S. O.** 1924. Structure and locomotion in *Amoeba proteus.* Anat. Rec. **29:** 88.

———. 1926. Structure, movement, locomotion, and stimulation in *Amoeba.* J. Morphol. **41:** 347–425.

**Maszewski, J. & P. Kolodziejczyk.** 1991. Cell cycle duration in antheridial filaments of *Chara* spp. (Characeae) with different genome size and heterochromatin content. Pl. Syst. Evol. **175:** 23–38.

**Matthews, G. G.** 1986. Cellular physiology of nerve and muscle. Blackwell Scientific Publications, Palo Alto, California.

**McClendon, J. F.** 1927. The permeability and the thickness of the plasma membrane as determined by electric currents of high and low frequency. Protoplasma **3:** 71–81.

**McConnaughey, T.** 1991. Calcification in *Chara corallina* $CO_2$ hydroxylation generates protons for bicarbonate assimilation. Limnol. & Oceanogr. **36:** 619–628.

——— **& R. H. Falk.** 1991. Calcium-proton exchange during algal calcification. Biol. Bull. **180:** 185–195.

**McCurdy, D. W. & A. C. Harmon.** 1992. Calcium-dependent protein kinase in the green alga *Chara.* Planta **188:** 54–61.

**Mehra, J.** 1994. The beat of a different drum. The life and science of Richard Feynman. Clarendon Press, Oxford.

**Mercer, E. H.** 1981. The foundations of biological theory. John Wiley & Sons, New York.

**Métraux, J.-P.** 1982. Changes in cell-wall polysaccharide composition of developing *Nitella* internodes. Planta **155:** 459–466.

———, **P. A. Richmond & L. Taiz.** 1980. Control of cell elongation in *Nitella* by endogenous cell wall pH gradients. Pl. Physiol. **65:** 204–210.

——— **& L. Taiz.** 1977. Cell wall extension in *Nitella* as influenced by acids and ions. Proc. Natl. Acad. Sci. U.S.A. **74:** 1565–1569.

——— **&** ———. 1978. Transverse viscoelastic extension in *Nitella.* I. Relationship to growth rate. Pl. Physiol. **61:** 131–135.

——— **&** ———. 1979. Transverse viscoelastic extension in *Nitella.* II. Effects of acid and ions. Pl. Physiol. **63:** 657–659.

**Michaelis, L.** 1925. Contribution to the theory of permeability of membranes for electrolytes. J. Gen. Physiol. **8:** 33–59.

**Miller, A. J. & D. Sanders.** 1987. Depletion of cytosolic free calcium induced by photosynthesis. Nature **326:** 397–400.

**Mimura, T. & Y. Kirino.** 1984. Changes in cytoplasmic pH measured by $^{31}$P-NMR in cells of *Nitellopsis obtusa*. Pl. Cell Physiol. **25:** 813–820.

——— **& M. Tazawa.** 1983. Effect of intracellular $Ca^{2+}$ on membrane potential and membrane resistance in tonoplast-free cells of *Nitellopsis obtusa*. Protoplasma **118:** 49–55.

**Moestrup, O.** 1970. The fine structure of mature spermatozoids of *Chara corallina*, with special reference to microtubules and scales. Planta **93:** 295–308.

**Morrison, J. C., L. C. Greve & P. A. Richmond.** 1993. Cell wall synthesis during growth and maturation of *Nitella* internodal cells. Planta **189:** 321–328.

**Mullins, L. J.** 1939. The effect of radiation from radioactive indicator on the penetration of ions into *Nitella*. J. Cell. Comp. Physiol. **14:** 493–495.

———. 1962. Efflux of chloride ions during action potential of *Nitella*. Nature **196:** 986–987.

**Nagai, R. & T. Hayama.** 1979. Ultrastructure of the endoplasmic factor responsible for cytoplasmic streaming in *Chara* internodal cells. J. Cell Sci. **36:** 121–136.

——— **& U. Kishimoto.** 1964. Cell wall potential in *Nitella*. Pl. Cell Physiol. **5:** 21–31.

——— **& L. I. Rebhun.** 1966. Cytoplasmic microfilaments in streaming *Nitella* cells. J. Ultrastruct. Res. **14:** 571–589.

——— **& M. Tazawa.** 1962. Changes in resting potential and ion absorption induced by light in a single plant cell. Pl. Cell Physiol. **3:** 323–339.

**Narahashi, T., J. W. Moore & W. R. Scott.** 1964. Tetrodotoxin blockage of sodium conductance increase in lobster giant axons. J. Gen. Physiol. **47:** 965–974.

**Nemec, B.** 1901. Die Reitzleitung und die reizleitenden Strukturen bei den Pflanzen. Gustav Fischer, Jena.

**Nernst, W.** 1888. Zur Kinetik der in Lösung befindlichen Körper. I. Theorie der Diffusion. Zeitschrift für physikalische Chemie **2:** 613–637.

———. 1889. Die elektromotorische Wirksamkeit der Ionen. Zeitschrift für physikalische Chemie **4:** 129–181.

**Niklas, K. J.** 1992. Plant biomechanics. An engineering approach to plant form and function. Chicago University Press, Chicago.

**Nobel, P. S.** 1991. Physicochemical and environmental plant physiology. Academic Press, San Diego, California.

**Nothnagel, E. A. & W. W. Webb.** 1979. Barrier filter for fluorescence microscopy of strongly autofluorescent plant tissues—Application to actin cables in *Chara*. J. Histochem. Cytochem. **27:** 1000–1002.

———**, J. W. Sanger & W. W. Webb.** 1982. Effects of exogenous proteins on cytoplasmic streaming in perfused *Chara* cells. J. Cell Biol. **93:** 735–742.

**Nuccitelli, R. (ed.).** 1986. Ionic currents and development. Alan R. Liss, New York.

**Ockam, Guillermus.** 1487. Quotlibeta Septum. Impressa Parisii Magistri Petri Rubei. Brunet **4:** 154.

**Oda, K.** 1962. Polarized and depolarized states of the membrane in *Chara Braunii*, with special reference to the transition between the two states. Sci. Rep. Tohoku Imp. Univ., Ser. 4, Biol. **28:** 1–16.

———. 1976. Simultaneous recording of potassium and chloride effluxes during an action potential in *Chara corallina*. Pl. Cell Physiol. **17:** 1085–1088.

**Ogata, K.** 1983. The water-film electrode: A new device for measuring the Characean electro-potential and -conductance distributions along the length of the internode. Pl. Cell Physiol. **24:** 695–703.

——— **& U. Kishimoto.** 1976. Rhythmic change of membrane potential and cyclosis of *Nitella* internode. Pl. Cell Physiol. **17:** 201–207.

**Ohkawa, T. & U. Kishimoto.** 1977. Breakdown phenomena in the *Chara* membrane. Pl. Cell Physiol. **18:** 67–80.

——— **& I. Tsutsui.** 1988. Electrical tolerance (breakdown) of the *Chara corallina* plasmalemma. I. Necessity of calcium. J. Membr. Biol. **103:** 273–282.

**Okazaki, Y. & M. Tazawa.** 1990. Calcium ion and turgor regulation in plant cells. J. Membr. Biol. **114:** 189–194.

———**, Y. Yoshimoto, Y. Hiramoto & M. Tazawa.** 1987. Turgor regulation and cytoplasmic free $Ca^{2+}$ in the alga *Lamprothamnium*. Protoplasma **140:** 67–71.

**Okihara, K., T. Ohkawa & M. Kasai.** 1993. Effects of calmodulin in $Ca^{2+}$-dependent $Cl^-$-sensitive anion channels in the *Chara* plasmalemma: A patch clamp study. Pl. Cell Physiol. **34:** 75–82.

———, ———, **I. Tsutsui & M. Kasai.** 1991. A $Ca^{2+}$- and voltage-dependent $Cl^-$-sensitive anion channel in the *Chara* plasmalemma: A patch-clamp study. Pl. Cell Physiol. **32:** 593–601.

**Onsager, L.** 1931a. Reciprocal relations in irreversible processes. I. Physical Review **37:** 405–426.

———. 1931b. Reciprocal relations in irreversible processes. II. Physical Review **38:** 2265–2279.

———. 1969. The motion of ions: Principles and concepts. Science **166:** 1359–1364.

———. 1970. Possible mechanisms of ion transit. Pages 137–141 *in* F. Snell, J. Wolken, G. Iverson & J. Lam (eds.), Physical principles of biological membranes. Proceedings of the Coral Gables Conference on Physical Principles of Biological Membranes. Gordon and Breach, New York.

**Orlózi, L.** 1991. Entropy and information. SPB Academic Publishing, The Hague, Netherlands.

**Osterhaut, W. J. V.** 1924. The nature of life. The Colver Lectures in Brown University. 1922. Henry Holt, New York.

———. 1927. Some aspects of bioelectrical phenomena. J. Gen. Physiol. **11:** 83–99.

———. 1931. Physiological studies of single plant cells. Biol. Rev. **6:** 369–411.

———. 1934. Nature of the action current in *Nitella*. I. General considerations. J. Gen. Physiol. **18:** 215–227.

———. 1958. Prefatory chapter. Studies of some fundamental problems by the use of aquatic organisms. Annual Rev. Physiol. **20:** 2–12.

——— **& S. E. Hill.** 1930a. Negative variations in *Nitella* produced by chloroform and potassium chloride. J. Gen. Physiol. **13:** 459–467.

——— & ———. 1930b. Salt bridges and negative variations. J. Gen. Physiol. **13:** 547–552.

——— & ———. 1935. Positive variations in *Nitella*. J. Gen. Physiol. **18:** 369–375.

**Pal, B. P., B. C. Kundu, V. S. Sundaralingam & G. S. Venkataraman.** 1962. Charophyta. Indian Council of Agricultural Research, New Delhi.

**Palevitz, B. A. & P. K. Hepler.** 1974. Actin in the green alga, *Nitella*. Proc. Natl. Acad. Sci. U.S.A. **71:** 363–366.

——— & ———. 1975. Identification of actin *in situ* at the ectoplasm-endoplasm interface of *Nitella*. J. Cell Biol. **65:** 29–38.

**Paszewski, A. & T. Zawadzki.** 1973. Action potentials in *Lupinus angustifolius* L. shoots. J. Exp. Bot. **24:** 804–809.

**Perrin, J.** 1923. Atoms. D. Van Nostrand Co., New York.

**Pfeffer, W.** 1875. Die Periodischen Bewegungen der Blattorgane. Engelmann, Leipzig.

———. 1877. Osmotische Untersuchungen. Engelmann, Leipzig.

**Pickard, B. G.** 1973. Action potentials in higher plants. Bot. Rev. **39:** 172–201.

———. 1984. Voltage transients elicited by sudden step-up of auxin. Pl. Cell Environm. **7:** 171–178.

**Pickard, W. F.** 1969. The correlation between electrical behavior and cytoplasmic streaming in *Chara braunii*. Canad. J. Bot. **47:** 1233–1240.

———. 1972. Further observations on cytoplasmic streaming in *Chara braunii*. Canad. J. Bot. **50:** 703–711.

———. 1974. Hydrodynamic aspects of protoplasmic streaming in *Chara braunii*. Protoplasma **82:** 321–339.

**Pickett-Heaps, J. D.** 1967a. Ultrastructure and differentiation in *Chara* sp. I. Vegetative cells. Austral. J. Biol. Sci. **20:** 539–551.

———. 1967b. Ultrastructure and differentiation in *Chara* sp. II. Mitosis. Austral. J. Biol. Sci. **20:** 883–894.

———. 1968. Ultrastructure and differentiation in *Chara* (*fibrosa*). IV. Austral. J. Biol. Sci. **21:** 655–690.

——— **& H. J. Marchant.** 1972. The phylogeny of the green algae: A new proposal. Cytobios **6:** 255–264.

**Ping, Z., T. Mimura & M. Tazawa.** 1990. Jumping transmission of action potential between separately placed internodal cells of *Chara corallina*. Pl. Cell Physiol. **31:** 299–302.

**Plinius Secundus, C.** 1469. Historiae Naturalis. libri XXXVII. Venetiis.

**Plonsey, R. & R. C. Barr.** 1991. Bioelectricity. A quantitative approach. Plenum Press, New York.
**Plowes, J. Q.** 1931a. Membranes in the plant cell. I. Morphological membranes at protoplasmic surfaces. Protoplasma **12:** 196–220.
———. 1931b. Membranes in the plant cell. II. Localization of differential permeability in the plant protoplast. Protoplasma **12:** 221–240.
**Pottosin, I. I., P. R. Andjus, D. Vucelic & G. N. Berestovsky.** 1993. Effects of $D_2O$ on permeation and gating in the $Ca^{2+}$-activated potassium channel from *Chara*. J. Membr. Biol. **136:** 113–124.
**Probine, M. C. & N. F. Barber.** 1966. The structure and plastic properties of the cell wall of *Nitella* in relation to extension growth. Austral. J. Biol. Sci. **19:** 439–457.
——— **& R. D. Preston.** 1961. Cell growth and the structure and mechanical properties of the wall in internodal cells of *Nitella opaca*. I. Wall structure and growth. J. Exp. Bot. **12:** 261–282.
——— & ———. 1962. Cell growth and the structure and mechanical properties of the wall in internodal cells of *Nitella opaca*. II. Mechanical properties of the walls. J. Exp. Bot. **13:** 111–127.
**Raisbeck, G.** 1963. Information theory. An introduction for scientists and engineers. MIT Press, Cambridge, Massachusetts.
**Rea, P. A. & D. Sanders.** 1987. Tonoplast energization: Two proton pumps, one membrane. Physiol. Pl. **71:** 131–141.
——— **& F. R. Whatley.** 1983. The influence of secretion elicitors and external pH on the kinetics of D-alanine uptake by the trap lobes of *Dionaea muscipula* Ellis (Venus's flytrap). Planta **158:** 312–319.
———, **D. M. Joel & B. E. Juniper.** 1983. Secretion and redistribution of chloride in the digestive glands of *Dionaea muscipula* Ellis (Venus's flytrap) upon secretion stimulation. New Phytol. **94:** 359–366.
**Reid, R. J. & R. L. Overall.** 1992. Intercellular communication in *Chara*: Factors effecting transnodal electrical resistance and solute fluxes. Pl. Cell Environm. **15:** 507–517.
——— **& F. A. Smith.** 1988. Measurement of the cytoplasmic pH of *Chara corallina* using doubled-barrelled pH microelectrodes. J. Exp. Bot. **39:** 1421–1432.
——— & ———. 1993. Toxic effects of the ionophore A23187 on *Chara*. Pl. Sci. **91:** 7–13.
———, ——— **& J. Whittington.** 1989. Control of intracellular pH in *Chara corallina* during uptake of weak acid. J. Exp. Bot. **40:** 883–891.
**Remington, R. E.** 1928. The high frequency Wheatstone bridge as a tool in cytological studies; with some observations on the resistance and capacity of the cells of the beet root. Protoplasma **5:** 338–399.
**Rethy, R.** 1968. Red (R), far-red (FR) photoreversible effects on the growth of *Chara* sporelings. Z. Pflanzen Physiol. **59:** 100–102.
**Richmond, P. A., J.-P. Métraux & L. Taiz.** 1980. Cell expansion patterns and directionality of wall mechanical properties in *Nitella*. Pl. Physiol. **65:** 211–217.
**Robertson, R. N.** 1992. A dilettante Australian plant physiologist. Annual Rev. Pl. Physiol. Pl. Molec. Biol. **43:** 1–24.
**Robins, R. J.** 1976. The nature of the stimuli causing digestive juice secretion in *Dionaea muscipula* Ellis (Venus's flytrap). Planta **128:** 263–265.
——— **& B. E. Juniper.** 1980. The secretory cycle of *Dionaea muscipula* Ellis. I–V. New Phytol. **86:** 279–327, 401–422.
**Rothstein, J.** 1951. Information, measurement, and quantum mechanics. Science **114:** 171–175.
**Rüdinger, M., P. Hierling & E. Steudle.** 1992. Osmotic biosensors. How to use characean internode for measuring the alcohol content of beer. Bot. Acta **105:** 3–12.
**Sakano, K. & M. Tazawa.** 1986. Tonoplast origin of the envelope membrane of cytoplasmic droplets prepared from *Chara* internodal cells. Protoplasma **131:** 247–249.
**Sakmann, B. & E. Neher (eds.).** 1983. Single-channel recording. Plenum Press, New York.
**Samejima, M. & T. Sibaoka.** 1982. Membrane potentials and resistances in excitable cells in the petiole and main pulvinus of *Mimosa pudica*. Pl. Cell Physiol. **23:** 459–465.
**Sandlin, R., L. Lerman, W. Barry & I. Tasaki.** 1968. Application of laser interferometry to physiological studies of excitable tissues. Nature **217:** 575–576.

**Scala, J., D. Schwab & E. Simmons.** 1968. The fine structure of the digestive gland of Venus's-fly-trap. Amer. J. Bot. **55:** 649–657.

**Schrödinger, E.** 1946. What is life? The physical aspect of the living cell. Cambridge University Press, Cambridge.

**Schwab, D. W., E. Simmons & J. Scala.** 1969. Fine structure changes during function of the digestive gland of Venus's-flytrap. Amer. J. Bot. **56:** 88–100.

**Scott, I. H.** 1962. Electricity in plants. Sci. Amer. **207:** 107–117.

**Setty, S. & M. J. Jaffe.** 1972. Phytochrome-controlled rapid contraction and recovery of contractile vacuoles in the motor cells of *Mimosa pudica* as an intracellular correlate of nyctinasty. Planta **108:** 121–131.

**Shannon, C. E. & W. Weaver.** 1949. The mathematical theory of communication. University of Illinois Press, Urbana, Illinois.

**Shen, E. Y. F.** 1966. Oospore germination in two species of *Chara*. Taiwania **12:** 39–46.

———. 1967. Microspectrophotometric analysis of nuclear DNA in *Chara zeylanica*. J. Cell Biol. **35:** 377–384.

**Shepherd, V. A. & P. B. Goodwin.** 1989. The porosity of permeabilized *Chara* cells. Austral. J. Pl. Physiol. **16:** 231–240.

——— & ———. 1992a. Seasonal patterns of cell-to-cell communication in *Chara corallina*. Klein ex Willd. I. Cell-to-cell communication in vegetative lateral branches during winter and spring. Pl. Cell Environm. **15:** 137–150.

——— & ———. 1992b. Seasonal patterns of cell-to-cell communication in *Chara corallina*. Klein ex Willd. II. Cell-to-cell communication during the development of antheridia. Pl. Cell Environm. **15:** 151–162.

**Sherman-Gold, R.** 1993. The axon guide for electrophysiology and biophysics laboratory techniques. Axon Instruments Inc., Foster City, California.

**Shiina, T. & M. Tazawa.** 1986a. Action potential in *Luffa cylindlica* and its effects on elongation growth. Pl. Cell Physiol. **27:** 1081–1089.

——— & ———. 1986b. Regulation of membrane excitation by protein phosphorylation in *Nitellopsis obtusa*. Protoplasma **134:** 60–61.

——— & ———. 1987a. $Ca^{2+}$-activated $Cl^-$ channel in plasmalemma of *Nitellopsis obtusa*. J. Membr. Biol. **99:** 137–146.

——— & ———. 1987b. Demonstration and characterization of a $Ca^{2+}$ channel in tonoplast-free cells of *Nitellopsis obtusa*. J. Membr. Biol. **96:** 263–276.

——— & ———. 1988. Calcium-dependent chloride efflux in tonoplast-free cells of *Nitellopsis obtusa*. J. Membr. Biol. **106:** 135–140.

———, **R. Wayne, H. Y. L. Tung & M. Tazawa.** 1988. Possible involvement of protein phosphorylation/dephosphorylation in the modulation of $Ca^{2+}$ channels in tonoplast-free cells of *Nitellopsis*. J. Membr. Biol. **102:** 255–264.

**Shimmen, T.** 1988. Characean actin bundles as a tool for studying actomyosin-based motility. Bot. Mag. (Tokyo) **101:** 533–544.

——— **& E. A. C. MacRobbie.** 1987. Characterization of two proton transport systems in the tonoplast of plasmalemma-permeabilized *Nitella* cells. Pl. Cell Physiol. **28:** 1023–1031.

——— **& T. Mimura. (eds.).** 1993. Giant cells of the Characeae. Model systems for studying cell motility and membrane transport. Proceedings of a symposium honoring the 80th birthday of Professor Noburo Kamiya, 4–6 September 1993. International Seminar House, Otsu, Japan.

——— **& S.-I. Nishikawa.** 1988. Studies on the tonoplast action potential of *Nitella flexilis*. J. Membr. Biol. **101:** 133–140.

——— **& M. Tazawa.** 1983a. Activation of $K^+$-channel in membrane excitation of *Nitella axillaformis*. Pl. Cell Physiol. **24:** 1511–1524.

——— & ———. 1983b. Control of cytoplasmic streaming by ATP, $Mg^{2+}$, and cytochalasin B in permeabilized Characeae cell. Protoplasma **115:** 18–24.

——— **& E. Yokota.** 1994. The biochemistry of cytoplasmic streaming. Int. Rev. Cytol. In press.

——— **& S. Yoshida.** 1993. Analysis of temperature dependence of cytoplasmic streaming using

tonoplast-free cells of Characeae. Protoplasma **176:** 174–177.
**Sibaoka, T.** 1958. Conduction of action potential in the plant cell. Trans. Bose Res. Inst. Calcutta **22:** 43–56.
———. 1962. Excitable cells in *Mimosa.* Science **137:** 226.
———. 1966. Action potentials in plant organs. In: Nervous and hormonal mechanisms of integration. Symp. Soc. Exp. Biol. **20:** 49–73.
———. 1969. Physiology of rapid movements in higher plants. Annual Rev. Pl. Physiol. **20:** 165–184.
——— **& K. Oda.** 1956. Shock stoppage of the protoplasmic streaming in relation to the action potential in *Chara.* Sci. Rep. Tohoku Imp. Univ., Ser. 4, Biol. **22:** 157–166.
——— **& T. Tabata.** 1981. Electrotonic coupling between adjacent internodal cells in *Chara braunii*: Transmission of action potentials beyond the node. Pl. Cell Physiol. **22:** 397–411.
**Sievers, A. & D. Volkmann.** 1979. Gravitropism in single cells. Pages 567–672 *in* W. Haupt & M. E. Feinleib (eds.), Physiology of movements. Vol. 7 of A. Pirson & M. H. Zimmermann (eds.), Encyclopedia of plant physiology. New Series. Springer, Berlin.
**Silk, W. K.** 1984. Quantitative descriptions of development. Annual Rev. Pl. Physiol. **35:** 479–518.
**Simons, P. J.** 1981. The role of electricity in plant movements. New. Phytol. **87:** 11–37.
**Simons, P.** 1992. The action plant. Blackwell, Oxford.
**Sinnott, E. W.** 1946. Botany principles and problems. Ed. 4. McGraw-Hill, New York.
**Sinyukhin, A. M. & E. A. Britikov.** 1967. Action potentials in the reproductive system of plants. Nature **215:** 1278–1230.
**Skoog, F. (ed).** 1951. Plant growth substances. University of Wisconsin Press, Madison, Wisconsin.
**Smith, J. E.** 1788. Some observations on the irritability of vegetables. Philos. Trans. **78:** 158–165.
**Smith, J. R.** 1987. Potassium transport across membranes of *Chara.* II. $^{42}K$ fluxes and the electrical current as a function of membrane voltage. J. Exp. Bot. **38:** 752–777.
——— **& R. J. Kerr.** 1987. Potassium transport across membranes of *Chara.* IV. Interactions with other cations. J. Exp. Bot. **38:** 788–799.
———, **F. A. Smith & N. A. Walker.** 1987a. Potassium transport across membranes of *Chara.* I. The relationship between radioactive tracer influx and electrical conductance. J. Exp. Bot. **38:** 731–751.
———, **N. A. Walker & F. A. Smith.** 1987b. Potassium transport across membranes of *Chara.* III. Effects of pH, inhibitors and illumination. J. Exp. Bot. **38:** 778–787.
**Spanswick, R. M.** 1970. Electrophysiological techniques and the magnitudes of the membrane potentials and resistances of *Nitella translucens.* J. Exp. Bot. **21:** 617–627.
———. 1974a. Hydrogen ion transport in giant algal cells. Canad. J. Bot. **52:** 1029–1034.
———. 1974b. Symplastic transport in plants. Symp. Soc. Exp. Biol. **28:** 127–137.
———. 1980. Biophysical control of electrogenic pumps in the Characeae. Pages 305–316 *in* R. M. Spanswick, W. J. Lucas & J. Dainty (eds.), Plant membrane transport: Current conceptual issues. Elsevier/North Holland Biomedical, Amsterdam.
———. 1981. Electrogenic ion pumps. Annual Rev. Pl. Physiol. **32:** 267–289.
——— **& J. W. F. Costerton.** 1967. Plasmodesmata in *Nitella translucens*: Structure and electrical resistance. J. Cell Sci. **2:** 451–464.
———, **J. Stolarek & E. J. Williams.** 1967. The membrane potential of *Nitella translucens.* J. Exp. Bot. **18:** 1–16.
**Spear, D. G., J. K. Barr & C. E. Barr.** 1969. Localization of hydrogen ion and chloride ion fluxes in *Nitella.* J. Gen. Physiol. **54:** 397–414.
**Spiridonov, O. P.** 1986. Universal physical constants. MIR Publishers, Moscow.
**Spyropoulos, C. S., I. Tasaki & G. Hayward.** 1961. Fractionation of tracer effluxes during action potential. Science **133:** 2064–2065.
**Standen, N. B., P. T. A. Gray & M. J. Whitaker (eds.).** 1987. Microelectrode techniques. The Plymouth Workshop handbook. Company of Biologists Ltd., Cambridge.
**Staves, M. P. & R. Wayne.** 1993. The touch-induced action potential of *Chara*: Inquiry into the ionic basis and the mechanoreceptor. Austral. J. Pl. Physiol. **20:** 471–488.
———, ——— **& A. C. Leopold.** 1992. Hydrostatic pressure mimics gravitational pressure in characean cells. Protoplasma **168:** 141–152.

———, ——— & ———. 1993. Three distinct types of $Ca^{2+}$ channels are involved in gravisensing and E-C coupling in *Chara*. Pl. Physiol. **102:** S–14.

**Stein, W. D.** 1986. Transport and diffusion across cell membranes. Academic Press, San Diego, California.

———. 1990. Channels, carriers, and pumps. An introduction to membrane transport. Academic Press, San Diego, California.

**Stern, K.** 1924. Elektrophysiologie der Pflanzen. Springer, Berlin.

**Steudle, E. & S. D. Tyerman.** 1983. Determination of permeability coefficients, reflection coefficients, and hydraulic conductivity of *Chara corallina* using the pressure probe: Effects of solute concentration. J. Membr. Biol. **75:** 85–96.

——— **& U. Zimmermann.** 1974. Determination of the hydraulic conductivity and of reflection coefficients in *Nitella flexilis* by means of direct cell-turgor pressure measurement. Biochim. Biophys. Acta **332:** 399–412.

**Stevens, C. F.** 1966. Neurophysiology: A primer. John Wiley & Sons, New York.

**Steward, F. C.** 1935. Mechanism of salt absorption by plant cells. Nature **135:** 553–555.

———. 1986. Solutes in cells: Their responses during growth and development. Pages 551–594 *in* F. C. Steward (ed.), Plant physiology. A. Treatise. Vol. 9, Water and solutes in plants. Academic Press, Orlando, Florida.

**Studener, O.** 1947. Über die elektroosmotische Komponente des Turgors und über chemische und Konzentrationspotentiale pflanzlicher Zellen. Planta **35:** 427–444.

**Stuhlman O., Jr. & E. B. Darden.** 1950. The action potentials obtained from Venus's-flytrap. Science **111:** 491–492.

**Sun, G.-H., T. Q. P. Uyeda & T. Kuroiwa.** 1988. Destruction of organelle nuclei during spermatogenesis in *Chara corallina* examined by staining with DAPI and anti-DNA antibody. Protoplasma **144:** 185–188.

**Szent-Györgi, A.** 1960. Introduction to a submolecular biology. Academic Press, New York.

**Szilard, L.** 1964. On the decrease of entropy in a thermodynamic system by the intervention of intelligent beings. Behavioral Sci. **9:** 301–310.

**Tabata, T.** 1990. Ephaptic transmission and conduction velocity of an action potential in *Chara* internodal cells placed in parallel and in contact with one another. Pl. Cell Physiol. **31:** 575–579.

**Takamatsu, A., T. Aoki & Y. Tsuchiya.** 1993. $Ca^{2+}$ effect on protoplasmic streaming in *Nitella* internodal cell. Biophys. J. **64:** 182–186.

**Takatori, S. & K. Imahori.** 1971. Light reactions in the control of oospore germination of *Chara delicatula*. Phycologia **10:** 221–228.

**Takenaka, T., T. Yoshioka & H. Horie.** 1975. Physiological properties of protoplasmic drops of *Nitella*. Advances Biophys. **7:** 193–213.

**Takeshige, K. & M. Tazawa.** 1989. Determination of the inorganic pyrophosphate level and its subcellular localization in *Chara corallina*. J. Biol. Chem. **264:** 3262–3266.

———, ——— **& A. Hager.** 1988. Characterization of the $H^+$ translocating adenosine triphosphatase and pyrophosphatase of vacuolar membranes isolated by means of a perfusion technique from *Chara corallina*. Pl. Physiol. **86:** 1168–1173.

**Tasaki, I.** 1968. Nerve excitation. A macromolecular approach. Charles C. Thomas, Springfield, Illinois.

**Taylor, C. V. & D. M. Whitaker.** 1927. Potentiometric determinations in the protoplasm and cell-sap of *Nitella*. Protoplasma **3:** 1–6.

**Tazawa, M.** 1957. Neue Methode zur Messung des osmotischen Wertes einer Zelle. Protoplasma **48:** 342–359.

———. 1964. Studies on *Nitella* having artificial cell sap. I. Replacement of the cell sap with artificial solutions. Pl. Cell Physiol. **5:** 33–43.

———. 1984. Excitable membranes—Plants. Cell Struct. Function **9:** S47-S50.

———, **& U. Kishimoto.** 1964. Studies on *Nitella* having artificial cell sap. II. Rate of cyclosis and electric potential. Pl. Cell Physiol. **5:** 45–59.

——— **& T. Shimmen.** 1987. Cell motility and ionic relations in characean cells as revealed by internal perfusion and cell models. Int. Rev. Cytol. **109:** 259–312.

———, ——— **& T. Mimura.** 1987. Membrane control in the characeae. Annual Rev. Pl. Physiol. **38:** 95–117.

———, **T. Yoko-o, T. Mimura & M. Kikuyama.** 1994. Intracellular mobilization of $Ca^{2+}$ and inhibition of cytoplasmic streaming induced by transcellular osmosis in internodal cells of *Nitella flexilis*. Pl. Cell Physiol. **35:** 63–72.

**Teorell, T.** 1953. Transport precesses and electrical phenomena in ionic membranes. Prog. Biophys. Biophys. Chem. **3:** 305–369.

**Tester, M.** 1988. Blockade of potassium channels in the plasmalemma of *Chara corallina* by tetraethylammonium, $Ba^{2+}$, $Na^{+}$ and $Cs^{+}$. J. Membr. Biol. **105:** 77–85.

**Thiel, G., E. A. C. MacRobbie & D. E. Hanke.** 1990. Raising the intracellular level of inositol 1,4,5-trisphosphate changes plasma membrane ion transport in characean algae. E.M.B.O.J. **9:** 1737–1741.

**Thompson, D. W.** 1963. On growth and form. Ed. 2. Cambridge University Press, Cambridge.

**Thomson, J. A.** 1932. Scientific riddles. Williams & Norgate, London.

**Tinz-Füchtmeier, A. & D. Gradmann.** 1991. Laser-interferometric re-examination of rapid conductance of excitation in *Mimosa pudica*. J. Exp. Bot. **41:** 15–19.

**Tolbert, N. E. & L. P. Zill.** 1954. Photosynthesis by protoplasm extruded from *Chara* and *Nitella*. J. Gen. Physiol. **37:** 575–589.

**Tominaga, Y., R. Wayne, H. Y. L. Tung & M. Tazawa.** 1986. Phosphorylation-dephosphorylation is involved in $Ca^{2+}$-controlled cytoplasmic streaming of Characean cells. Protoplasma **136:** 161–169.

**Toriyama, H.** 1954. Observational and experimental studies of sensitive plants. II. On the changes in motor cells of diurnal and nocturnal conditions. Cytologia **19:** 29–40.

———. 1955. Observational and experimental studies of sensitive plants. VI. The migration of potassium in the primary pulvinus. Cytologia **20:** 367–377.

———. 1957. Observational and experimental studies of sensitive plants. VIII. The migration of colloidal substance in the primary pulvinus. Cytologia **22:** 184–192.

———. 1967. A comparison of the *Mimosa* motor cell before and after stimulation. Proc. Japan Acad. **43:** 541–546.

——— **& M. J. Jaffe.** 1972. Migration of calcium and its role in the regulation of seismonasty in the motor cell of *Mimosa pudica*. L. Pl. Physiol. **49:** 72–81.

——— **& S. Sato.** 1968. Electron microscope observation of the motor cell of *Mimosa pudica* L. I. A comparison of the motor cell before and after stimulation. Proc. Japan Acad. **44:** 702–706.

**Tretyn, A. & R. E. Kendrick.** 1991. Acetylcholine in plants: Pressence, metabolism and mechanism of action. Bot. Rev. **57:** 33–73.

**Trewavas, A. J. (ed.).** 1986. Molecular and cellular aspects of calcium in plant development. Plenum Press, New York.

**Tribus, M. & E. C. McIrvine.** 1971. Energy and information. Sci. Amer. **225:** 179–188.

**Trontelj, Z., R. Zorec, V. Jazbinsek & S. N. Erné.** 1994. Magnetic detection of a single action potential in *Chara corallina* internodal cells. Biophys. J. **66:** 1694–1696.

**Tsutsui, I. & T. Ohkawa.** 1993. N-Ethylmaleimide blocks the $H^{+}$ pump in the plasma membrane of *Chara corallina* internodal cells. Pl. Cell Physiol. **34:** 1159–1162.

———, ———, **R. Nagai & U. Kishimoto.** 1987a. Role of calcium ion in the excitability and electrogenic pump activity of the *Chara corallina* membrane I. Effects of $La^{3+}$, EGTA, and calmodulin antagonists on the action potential. J. Membr. Biol. **96:** 65–73.

———, ———, ——— **&** ———. 1987b. Role of calcium ion in the excitability and electrogenic pump activity of the *Chara corallina* membrane II. Effects of $La^{3+}$, EGTA, and calmodulin antagonists on the current-voltage relation. J. Membr. Biol. **96:** 75–84.

———, **R. Nagai, T. Ohkawa & U. Kishimoto.** 1987c. Effects of divalent cations on the excitability and on the cytoplasmic streaming of *Chara corallina*. Pl. Cell Physiol. **28:** 741–751.

**Tucker, E. B.** 1987. Cytoplasmic streaming does not drive intercellular passage in staminal hairs of *S. purpurea*. Protoplasma **137:** 140–144.

**Turner, F. R.** 1968. An ultrastructural study of plant spermatogenesis. Spermatogenesis in *Nitella*. J. Cell Biol. **37:** 370–393.

———. 1970. The effects of colchicine on spermatogenesis in *Nitella*. J. Cell Biol. **46:** 220–233.

**Turnquist, H. M., N. S. Allen & M. J. Jaffe.** 1993. A pharmacological study of calcium flux mechanisms in the tannin vacuole of *Mimosa pudica*. L. motor cells. Protoplasma **176:** 91–99.

**Tyerman, S. D.** 1992. Anion channels in plants. Annual Rev. Pl. Physiol. Pl. Molec. Biol. **43:** 351–373.

**Ueda, T., K. Kurihara & Y. Kobatake.** 1975a. Response of *Nitella* internodal cell to chemical stimulation. J. Membr. Biol. **25:** 271–284.

———, **M. Muratsugu, K. Kurihara & Y. Kobatake.** 1975b. Olfactory response in excitable protoplasmic droplet and internodal cell of *Nitella*. Nature **253:** 629–630.

**Umrath, K.** 1930. Untersuchungen über Plasma und Plasmaströmung an Characeen. IV. Potentialmessungen an *Nitella mucronata* mit besonderer Berücksichtigung der Erregungserscheinung. Protoplasma **9:** 576–597.

———. 1932. Die Bildung von Plasmalemma (Plasmahaut) bei *Nitella mucronata*. Protoplasma **16:** 173–188.

———. 1933. Der Erregungsvorgang bei *Nitella mucronata*. Protoplasma **17:** 259–300.

———. 1934. Der Einfluss der Temperatur auf das elektrische Potential, den Aktionsstrom und die Protoplasmaströmung bei *Nitella mucronata*. Protoplasma **21:** 229–334.

———. 1940. Über die Art der elektrischen Polarisierbarkeit und der elektrischen Erregbarkeit bei *Nitella*. Protoplasma **34:** 469–483.

**Ussing, H. H.** 1949a. The active ion transport through the isolated frog skin in the light of tracer studies. Acta Physiol. Scand. **17:** 1–37.

———. 1949b. The distinction by means of tracers between active transport and diffusion. Acta Physiol. Scand. **19:** 43–56.

——— **& K. Zerahn.** 1951. Active transport of sodium as the source of electric current in the short-circuited isolated frog skin. Acta Physiol. Scand. **23:** 110–127.

**van Ness, H. C.** 1983. Understanding thermodynamics. Dover Publications, New York.

**Volkmann, D., B. Buchen, Z. Hejnowicz, M. Tewinkel & A. Sievers.** 1991. Oriented movement of statoliths studied in a reduced gravitational field during parabolic flights of rockets. Planta **185:** 153–161.

**von Guttenberg, H.** 1971. Bewegunsgewebe und Perzeptionsorgane. Gebrüder Borntraeger, Berlin.

**von Neumann, J.** 1963. Design of computers, theory of automata and numerical analysis. Vol. 5 of A. H. Taub (ed.), Collected works. Pergamon Press, New York.

**Vorobiev, L. N.** 1967. Potassium ion activity in the cytoplasm and the vacuole of cells of *Chara* and *Griffithsia*. Nature **216:** 1325–1327.

**Walker, N. A.** 1955. Microelectrode experiments on *Nitella*. Austral. J. Biol. Sci. **8:** 476–489.

———. 1957. Ion permeability of the plasmalemma of the plant cell. Nature **180:** 94–95.

——— **& A. B. Hope.** 1969. Membrane fluxes and electric conductance in characean cells. Austral. J. Biol. Sci. **22:** 1179–1195.

——— **& D. Sanders.** 1991. Sodium-coupled solute transport in charophyte algae: A general mechanism for transport energization in plant cells? Planta **185:** 443–445.

**Wasteneys, G. O., B. E. S. Gunning & P. K. Hepler.** 1993. Microinjection of fluorescent brain tubulin reveals dynamic properties of cortical microtubules in living plant cells. Cell Motility and Cytoskeleton **24:** 205–213.

——— **& R. E. Williamson.** 1992. Microtubule organization differs between acid and alkaline bands in internodal cells of *Chara* but bands can develop in the absence of microtubules. Planta **188:** 99–105.

**Wayne, R.** 1992. What remains of the Cholodny-Went theory? While there is probable cause to take it to court, there is not a preponderence of evidence to throw it out. Pl. Cell Environm. **15:** 791–792.

———. 1993. The excitability of plant cells. Amer. Sci. **81:** 140–151.

———, **T. Mimura & T. Shimmen.** 1994. The relationship between carbon and water transport in single cells of *Chara corallina*. Protoplasma (in press).

———, **M. P. Staves & A. C. Leopold.** 1990. Gravity-dependent polarity of cytoplasmic streaming in *Nitellopsis*. Protoplasma **155:** 43–57.

———, ——— & ———. 1992. The contribution of the extracellular matrix to gravisensing in characean cells. J. Cell Sci. **101:** 611–623.

———, ——— **& M. Rutzke.** 1993. Inhibitors of gravisensing and E-C coupling differentially reduce $Sr^{2+}$ ($Ca^{2+}$) influx across the plasma membrane of *Chara* internodal cells. Pl. Physiol. **102:** S–14.

——— **& M. Tazawa.** 1988. The actin cytoskeleton and polar water permeability in Characean cells. Protoplasma **[Suppl. 2]:** 116–130.

——— & ———. 1990. Nature of the water channels in the internodal cells of *Nitellopsis*. J. Membr. Biol. **116:** 31–39.

**Weidmann, S.** 1949a. Subthreshold rectifier properties of *Nitella*. Acta Physiol. Scand. **19:** 218–229.

———. 1949b. Initiation of break response in *Nitella*. Acta Physiol. Scand. **19:** 230–236.

**Weintraub, M.** 1951. Leaf movements in *Mimosa pudica*. New Phytol. **50:** 357–382.

**Weisenseel, M. H. & H. K. Ruppert.** 1977. Phytochrome and calcium ions are involved in light-induced membrane depolarization in *Nitella*. Planta **137:** 225–229.

**Went, F. W. & K. V. Thimann.** 1937. Phytohormones. Macmillan, New York.

**Wheatstone, C.** 1855. An account of some experiments made with the submarine cable of the Mediterranean electric telegraph. Proc. Roy. Soc. London **7:** 328–333.

**Wilcox, L. W., P. A. Fuerst & G. L. Floyd.** 1993. Phylogenetic relationships of four charophycean green algae inferred from complete nuclear-encoded small subunit rRNA gene sequences. Amer. J. Bot. **80:** 1028–1033.

**Wildon, D. C., J. F. Thain, P. E. H. Minchin, I. R. Gubb, A. J. Reilly, Y. D. Skipper, H. M. Doherty, P. J. O'Donnell & D. J. Bowles.** 1992. Electrical signalling and systemic proteinase inhibitor induction in the wounded plant. Nature **360:** 62–65.

**Williams, E. J. & J. Bradley.** 1968. Voltage-clamp and current-clamp studies on the action potential in *Nitella translucens*. Biochim. Biophys. Acta **150:** 626–639.

———, **R. J. Johnson & J. Dainty.** 1964. The electrical resistance and capacitance of the membranes of *Nitella translucens*. J. Exp. Bot. **15:** 1–14.

**Williams, S. E. & A. B. Bennett.** 1982. Leaf closure in the Venus flytrap: An acid growth response. Science **218:** 1120–1122.

——— **& B. G. Pickard.** 1972a. Properties of action potentials in *Drosera* tentacles. Planta **103:** 193–221.

——— & ———. 1972b. Receptor potentials and action potentials in *Drosera* tentacles. Planta **103:** 222–240.

——— **& R. M. Spanswick.** 1976. Propagation of the neuroid action potential of the carnivorous plant *Drosera*. J. Comp. Physiol. **108:** 211–223.

**Williamson, R. E.** 1975. Cytoplasmic streaming in *Chara*: A cell model activated by ATP and inhibited by cytochalasin B. J. Cell Sci. **17:** 655–668.

——— **& C. C. Ashley.** 1982. Free $Ca^{2+}$ and cytoplasmic streaming in the alga *Chara*. Nature **296:** 647–650.

**Wood, R. D. & K. Imahori.** 1964. A revision of the Characeae. Volume I. Cramer, Weinheim.

——— & ———. 1965. A revision of the Characeae. Volume II. Cramer, Weinheim.

**Yao, X., M. A. Bisson & L. J. Brzezicki.** 1992. ATP-driven proton pumping in two species of *Chara* differing in salt tolerance. Pl. Cell Environm. **15:** 199–210.

**Young, J. Z.** 1936. Structure of nerve fibres and synapses in some invertebrates. Cold Spring Harbor Symp. Quant. Biol. **4:** 1–6.

———. 1947. The history of the shape of a nerve-fibre. Pages 41–94 *in* W. E. Le Gros Clark & P. Medawar (eds.), Essays on growth and form. Presented to D'Arcy Wentworth Thompson. Oxford University Press, Oxford.

**Young, P.** 1987. The nature of information. Praeger, New York.

**Zanello, L. P. & F. J. Barrantes.** 1992. Blockade of the $K^+$ channel of *Chara contraria* by $Cs^+$ and tetraethylammonium resembles that of $K^+$ channels in animal cells. Pl. Sci. **86:** 49–58.

——— & ———. 1994. Temperature sensitivity of the $K^+$ channel of *Chara*. A thermodynamic analysis. Pl. Cell Physiol. **35:** 243–255.

**Zhang, W., H. Yamane, N. Takahashi, D. J. Chapman & B. O. Phinney.** 1989. Identification of a cytokinin in the green alga *Chara globularis*. Phytochemistry **28:** 337–338.

**Zhu, G. L. & J. S. Boyer.** 1992. Enlargement in *Chara* studied with a turgor clamp. Pl. Physiol. **100:** 2071–2080.

**Zimmerman, U. & F. Beckers.** 1978. Generation of action potentials in *Chara corallina* by turgor pressure changes. Planta **138:** 173–179.

## V. Appendix A: List of Abbreviations

$a$ is $(-K_j u_j z_j e l dx)$
$b$ is the number of channels in a patch (dimensionless)
$c$ is the speed of light ($3 \times 10^8$ m $s^{-1}$; Spiridonov, 1986)
$d$ represents an infinitesimal difference
exp is the base of the natural logarithm (2.72)
$e$ is the elementary charge ($1.60 \times 10^{-19}$ coulombs per particle; Spiridonov, 1986)
$f$ represents a function
$f(x)$ is $1/[\exp(z_j F E_m/RT) - 1]$ where $x = E_m$ and is a constant when $E_m = E_j$
$g$ is the acceleration due to gravity (9.80 m $s^{-2}$) on the surface of the earth on an object of mass $m_j$ (in kg), and is derived from the gravitational constant ($G_g$, $6.67 \times 10^{-11}$ N $m^2$ $kg^{-2}$; Spiridonov, 1986), the mass of the earth ($m_e$, $5.98 \times 10^{24}$ kg) and the radius of the earth ($r_e \approx 6.38 \times 10^6$ m) using the equivalence of the following formula for force: Force $= m_j g = G_g(m_e m_j / r_e^2)$
$g_j$ is the partial specific conductance of the membrane for substance $j$ (in S $m^{-2}$)
$h$ is Planck's Constant ($6.65 \times 10^{-34}$ J s; Spiridonov, 1986)
$h'$ is a height (in m)
i is a subscript or superscript that represents "in"
$j$ is a subscript that represents substance j
$j' = \sqrt{(-1)}$
$k$ is Boltzmann's Constant ($1.38 \times 10^{-23}$ J $K^{-1}$; Spiridonov, 1986)
$k' = (n_o/n_i) = \exp(z_j \beta E_j)$
$l$ is length (in m)
$m$ is molecular mass of a substance (in kg $mol^{-1}$)
$m_j$ is the mass of substance $j$ (in kg)
$n_j$ is the concentration of substance $j$ (in mol $m^{-3}$)
$n_e$ is the concentration of a given ion on the exoplasmic side of the membrane (the E-space, in mol $m^{-3}$)
$n_p$ is the concentration of a given ion on the protoplasmic side of the membrane (the P-space, in mol $m^{-3}$)
o is a subscript or superscript that represents "out"
$q$ is the number of charged particles that move across an area (in $m^{-2}$)
$q'$ is the number of charged particles (dimensionless)
$q_j$ is the number of ions $j$ that move across an area (in $m^{-2}$)
$r$ is the radius (in m)
$r_H$ is the hydrodynamic radius (in m)
$r_j$ is the specific resistance of the membrane for substance $j$ (in $\Omega$ $m^2$)
$t$ is time (in s)
$u$ is the mobility (in $m^2$ $J^{-1}$ $s^{-1}$)
$u_m$ is the molar mobility constant (in mol $m^2$ $J^{-1}$ $s^{-1}$)
$u'$ is the mobility (in $m^2$ $V^{-1}$ $s^{-1}$)
$v$ is velocity (in m $s^{-1}$)
$x$ is a distance (in m)

$z$ is the valence of an ion or electron (dimensionless)
$z_a$ is the valence of an anion (–)
$z_c$ is the valence of a cation (+)
$z_j$ is the valence of substance $j$ (dimensionless)

$A$ is the area (in $m^2$)
$C$ is the specific capacitance (in F $m^{-2}$)
$C_m$ is the specific membrane capacitance ($\approx 10^{-2}$ F $m^{-2}$)
$C'$ is capacitance (in F)
$D$ is the diffusion coefficient (in $m^2$ $s^{-1}$)
$D_j$ is the diffusion coefficient of substance $j$ (in $m^2$ $s^{-1}$)
$E$ is an electric potential (in V)
$E_d$ is the diffusion potential determined by the G-H-K Equation (in V)
$E_t$ is the total driving force on an ion (in V)
$E_m$ is a membrane potential (in V)
$E_j$ is the equilibrium potential of ion $j$ (in V)
$E'_t$ is the potential (in V) at time $t$
$E_o$ is the potential (in V) at time 0
$E_\infty$ is the potential (in V) at time infinity
$F$ is Faraday's Constant ($9.65 \times 10^4$ coulombs $mol^{-1}$; Spiridonov, 1986)
$F'$ is force (in N)
$F^*$ is the electric force between two charged bodies (in N)
$G$ is the specific conductance of the membrane (in S $m^{-2}$)
$G'$ is Gibbs's free energy (defined as an intrinsic quantity, in J $mol^{-1}$)
$H$ is enthalpy (defined as an intrinsic quantity, in J $mol^{-1}$)
$I$ is electrical current density (in A $m^{-2}$ = coulombs $m^{-2}$ $s^{-1}$)
$I'$ is electrical current (in A = coulombs $s^{-1}$)
$I_o$ is the current passed through a patch (in A)
$I_s$ is the current passed by a single channel (in A).
$I_j$ is electrical current density carried by ion $j$ (in A $m^{-2}$)
$I^*$ is information (in J $K^{-1}$)
$J_{tot}$, $J_n$ are the total net flux (in mol $m^{-2}$ $s^{-1}$)
$J_p$ is the net flux due to pressure (in mol $m^{-2}$ $s^{-1}$)
$J_g$ is the net flux due to gravity (in mol $m^{-2}$ $s^{-1}$)
$J_d$ is the net flux due to diffusion (in mol $m^{-2}$ $s^{-1}$)
$J_{el}$ is the net flux of charged particles (in mol $m^{-2}$ $s^{-1}$)
$J_j$ is the net flux of substance $j$ (in mol $m^{-2}$ $s^{-1}$)
$J_w$ is the net volume flow of water (in $m^3$ $m^{-2}$ $s^{-1}$)
$J_K$ is the net flux of $K^+$ (in mol $m^{-2}$ $s^{-1}$)
$J_{Na}$ is the net flux of $Na^+$ (in mol $m^{-2}$ $s^{-1}$)
$J_{Cl}$ is the net flux of $Cl^-$ (in mol $m^{-2}$ $s^{-1}$)
$J_i$ is the influx (in mol $m^{-2}$ $s^{-1}$)
$J_o$ is the efflux (in mol $m^{-2}$ $s^{-1}$)
$K_j$ is the partition coefficient of substance $j$ (oil/water; dimensionless)
$KE$ is kinetic energy (in J)
$L_p$ is the hydraulic conductivity (in m $s^{-1}$ $Pa^{-1}$)
$N_A$ is Avogadro's Number ($6.02 \times 10^{23}$ $mol^{-1}$)
$P$ is pressure (in Pa)

$P_j$ is the permeability coefficient of substance $j$ (in m $s^{-1}$)
$P_o$ is the probability of a channel being open (dimensionless)
$R$ is the gas constant (8.31 J $mol^{-1}$ $K^{-1}$; Spiridonov, 1986)
$R'$ is the specific resistance (in $\Omega$ $m^2$)
$R_m$ is the specific membrane resistance (in $\Omega$ $m^2$)
$S$ is entropy (defined as an intrinsic quantity, in J $mol^{-1}$ $K^{-1}$)
$T$ is absolute temperature (in K); with absolute temperature scales, the "value of a degree" is independent of temperature and 273.16 K is defined as the triple point of water (American Society for Testing and Materials, 1974)
$TI^*$ is the thermal information (in J $mol^{-1}$)
$T'$ is tension (in N $m^{-1}$)
$V$ is volume (in $m^3$)
$V'_j$ is the partial molal volume of a substance (in $m^3$ $mol^{-1}$)
$Y$ is $J_j dx / u_j z_j e dE$
$Z$ is the total specific impedance due to resistors and capacitors (in $\Omega$ $m^2$)
$Z_R$ is the impedance due to resistors (in $\Omega$)
$Z_C$ is the reactance (in $\Omega$)

$ß = F/RT = e/kT$
$\Delta$ represents an infinitesimal difference between state variables
$\pi = 3.14$
$\Sigma$ represents "the sum of"
$\varepsilon$ = relative permittivity (dimensionless)
$\varepsilon_o$ is the permittivity of a vacuum ($8.85 \times 10^{-12}$ F $m^{-1}$)
$\eta$ is the viscosity (in Pa s)
$\mu$ is the chemical potential (in J $mol^{-1}$)
$\mu_j$ is the chemical potential of substance $j$ (in J $mol^{-1}$)
$\mu_j^\circ$ is the chemical potential of substance $j$ in the standard state (in J $mol^{-1}$)
$\tau$ is the membrane time constant (in s)

Note: All units are given in SI units and all constants are rounded off to two decimal places or less.

## XVI. Appendix B: Information Theory, Thermodynamics, and Cell Communication

The development and adaptive behavior of multicellular organisms depends on communication between cells. Communication is the faithful transfer of information. Information transfer can occur at many levels: between the environment and the cell, between cells in a tissue, between tissues, and between organs. In order to get a better understanding of communication and methods of communication, we are going to define information in thermodynamic terms. It appears that information theory (Bialek, 1987; Block, 1992; Mehra, 1994; Mercer, 1981; Orlózi, 1991; Raisbeck, 1963; von Neumann, 1963; Young, 1987) may provide a sturdy framework for understanding the information transfer involved in plant growth and development.

According to Brillouin (1949, 1950, 1956, 1959, 1964), Shannon and Weaver (1949), Szilard (1964), Rothstein (1951), Tribus and McIrvine (1971), and von Neumann (1963), information can be considered equivalent to negative entropy (or

negentropy) where entropy is equivalent to the number of possible states. The greater the number of possible states, the greater the entropy and the greater the lack of information.

$$I^* = -\Delta S \tag{B.1}$$

where

$I^*$ is information (in J $K^{-1}$)
$\Delta S$ is entropy (in J $mol^{-1}$ $K^{-1}$)

It is useful to think about information in energy units. In order to do this we multiply by the absolute temperature ($T$, in K).

$$TI^* = -T\Delta S \tag{B.2}$$

where

$TI^*$ represents thermal information (in J $mol^{-1}$)

We can relate the thermal information to Gibbs's free energy using the following formula:

$$\Delta G' = \Delta H - T\Delta S \tag{B.3}$$

where

$\Delta G'$ is the Gibbs free energy (in J $mol^{-1}$)
$\Delta H$ is the enthalpy (in J $mol^{-1}$)
$T$ is the absolute temperature (in K; and is assumed to be constant)
$\Delta S$ is the entropy (in J $mol^{-1}$ $K^{-1}$; Bergethon & Simons, 1990; Klotz, 1967; Lewis & Randall, 1923; van Ness, 1983).

I have defined Gibbs's free energy as an intrinsic quantity. This is in keeping with the spirit of Josiah Gibbs, who defined it in terms of free energy ($\psi$, in kcal) per implied mass of 1 kg. Gibbs used kilograms instead of moles because in the nineteenth century, when the atomic and formula masses were constantly being revised, moles were not reliable units compared to the mass of a substance (Lewis & Randall, 1923).

Since $TI^* = -T\Delta S$,

$$TI^* = \Delta G' - \Delta H. \tag{B.4}$$

We can relate thermal information to the chemical potential of a system since $\Delta G'$ (defined as an intrinsic quantity) is related to the chemical potential according to the following definition:

$$\Delta G' = \Delta\mu \tag{B.5}$$

where

$\Delta G'$ is Gibbs's free energy (in J $mol^{-1}$)
$\Delta\mu$ is the chemical potential (in J $mol^{-1}$)

The chemical potential as conceived by Gibbs (1928) describes the contribution of

every type of energy (including concentration effects, mechanical energy, etc.) to the system. Thus,

$$\Delta\mu = \Delta\mu^{\circ} + RT\,\Delta(\ln n) + zF\Delta E + P\Delta V + F'\Delta x + \Delta T'A + mg\Delta h' + \ldots \quad \text{(B.6)}$$

where

$\Delta\mu$ is the difference in the chemical potential (in J $mol^{-1}$) of the system in the final state minus the initial state
$\mu^{\circ}$ is the chemical potential of the system in the standard state (in J $mol^{-1}$) and $\Delta\mu^{\circ}$, and is assumed to be equal to 0 J $mol^{-1}$
$R$ is the gas constant (8.31 J $mol^{-1}$ $K^{-1}$)
$T$ is the absolute temperature (in K)
$\Delta(\ln n)$ is the difference in the natural log of the concentration of a substance in the final state minus the initial state (dimensionless)
$z$ is the valence of a substance (dimensionless)
$F$ is the Faraday Constant ($9.65 \times 10^4$ coulombs $mol^{-1}$)
$\Delta E$ is the electrical potential difference of the system containing the substance in the final state minus the initial state (in V or J $coulomb^{-1}$)
$V$ is the partial molar volume of a substance (in $m^3$ $mol^{-1}$)
$\Delta P$ is the difference in the pressure on the system containing the substance in the final state minus the initial state (in Pa)
$F'$ is the translational force applied to the system (in N)
$\Delta x$ is the difference in the dimension of the system containing the substance in the final state minus the initial state (in m $mol^{-1}$)
$A$ is the surface area of the system (in $m^2$ $mol^{-1}$)
$\Delta T'$ is the difference in the tension of the surface of the system containing the substance in the final state minus the initial state (in N $m^{-1}$)
$m$ is the molecular mass of a substance (kg $mol^{-1}$)
$g$ is the acceleration due to gravity (9.80 m $s^{-2}$)
$\Delta h'$ is the difference in height of the system containing the substance in the final state minus the initial state (in m).

Thus, by combining equations B.4 and B.5, and since $\Delta\mu^{\circ} = 0$ J $mol^{-1}$, we get

$$TI^{*} = RT\,\Delta(\ln n) + zF\Delta E + P\Delta V + F'\Delta x + \Delta T'A + mg\Delta h' - \Delta H. \quad \text{(B.7)}$$

Equation B.7 tells us that electrical, mechanical, and gravitational energy, as well as chemical energy, can be used for information. This information can be used to direct plant growth and development (Sinnott, 1946). Thus, in thermodynamic terms, information can be released or stored following a change in the concentration of a substance [$\Delta(\ln n)$], a change in electrical potential ($\Delta E$), or a change in volume ($\Delta V$), distance ($\Delta x$ or $\Delta h$), and/or tension ($\Delta T'$). $\Delta H$ (at constant pressure) represents energy converted into heat. The thermal information of the message is positive only if the energy in the stimulus (or stimuli) is greater than $\Delta H$, the level of thermal noise in the system. At room temperature (298 K), the minimum heat content of (or thermal noise in) the system is equal to (3/2)$RT$, or 3714.6 J $mol^{-1}$. The energy absorbed by any one receptor must be greater than (3/2)($R/N_A$)$T$ or (3/2)$kT$ which is $6.2 \times 10^{-21}$ J.

Information can also be released and stored following a change in other types of energy, including light energy [$hN_A\Delta\nu$ where $h$ is Planck's Constant ($6.65 \times 10^{-34}$ J s), $N_A$ is Avogadro's Number ($6.02 \times 10^{23}$ mol$^{-1}$), and $\Delta\nu$ is the difference in frequency of the light absorbed or radiated (in s$^{-1}$), assuming that the frequency of the initial state is 0], or by changing the sensitivity of a receptor (protein) to substance ($n$), an electrical potential difference ($\Delta E$), mechanical deformation ($P$, $F'$ and $\Delta T'$).

The concept of energy has changed over the past 200 years since Count Rumford (Benjamin Thompson) observed that the heat produced during the boring of a cannon was roughly equivalent to the mechanical energy expended. Later, James Joule determined the quantitative relationship between mechanical energy and heat by measuring the change in temperature of water in which a paddle wheel was turning (Joule, 1852). Joule also determined the relationship between electrical energy and heat by measuring the change in temperature of water as electricity was passed through it. Other experiments showed the equivalence of chemical energy and heat by adding reactants to a solution (Joule, 1852). These and other experiments led to the principle of the equivalence beween various types of energy and the powerful concept of the conservation of energy. This review treats electrical energy and chemical energy as equivalent in providing information needed for plant growth and development, and focuses on the methods and results gained from studies on how electrical energy plays a part in information transfer in plant cells.

## XVII. Appendix C: Membrane Capacitance

A capacitor is composed of an insulating layer sandwiched between two conducting layers (Cole, 1928; Horowitz & Hill, 1989). Capacitance ($C'$ in F) is the property of a membrane that resists changes in voltage. It is a measure of the charge in coulombs that must be added to the membrane in order to raise its potential by 1 V. The magnitude of the capacitance is dependent on the electrical properties of the insulating layer or dielectric ($\varepsilon\varepsilon_0$, in F m$^{-1}$), the area of one side of the insulating layer ($A$, in m$^2$), and the thickness of the insulating layer ($x$, in m). The capacitance can be determined from the following equation:

$$C' = (\varepsilon\varepsilon_0)A/x \qquad \text{(C.1)}$$

where

$C'$ = capacitance (in F)
$\varepsilon$ = relative permittivity of the insulating layer (3 for lipids and 80 for water, dimensionless)
$\varepsilon_0$ is the permittivity of a vacuum ($8.85 \times 10^{-12}$ F m$^{-1}$)
$A$ is the surface area of one side of the insulating layer (in m$^2$)
$x$ is the thickness of the insulating layer (in m).

Since the capacitance depends on the surface area of the capacitor, we usually talk about the specific capacitance ($C$, in F m$^{-2}$) which is normalized with respect to area and is equal to $C'/A$. The specific capacitance of many membranes is approximately 0.01 F m$^{-2}$ (Cole, 1970; Cole & Cole, 1936a, 1936b; Cole & Curtis, 1938; Curtis & Cole, 1937; Dean et al., 1940; Findlay, 1970; Fricke, 1925; Fricke & Morse, 1925; Williams et al., 1964).

The specific membrane capacitance ($C_m$, in F $m^{-2}$) is thought to be a property of the nonconducting lipid bilayer since it can be accounted for by the properties of the bilayer (Cole, 1970). Assuming the relative permittivity of lipids is approximately 3 and the thickness of the lipid bilayer is approximately $2.7 \times 10^{-9}$ m, the specific membrane capacitance will be approximately $10^{-2}$ F $m^{-2}$, according to equation C.2.

$$C_m = (\varepsilon\varepsilon_o)/x. \tag{C.2}$$

The capacitance of a material or membrane can be determined algebraically from the definition of a capacitor given in equation C.1 or C.2, respectively. These equations can also be used to determine the identity of the dielectric material or the area of the membrane if the specific capacitance is known.

The capacitance can be measured empirically since the capacitance is defined as the amount of charge per unit area (in coulombs $m^{-2}$) that can be separated when an electrical potential ($E$, in V) is placed across two conducting elements that are separated by a nonconducting substance.

When 1 V is placed across a 1 F $m^{-2}$ capacitor, 1 coulomb of positive charge builds up on the plate of the capacitor connected to the positive terminal of the battery and 1 coulomb of negative charge builds up on the plate of the capacitor connected to the negative terminal of the battery (Horowitz & Hill, 1989). Conversely, capacitance can be defined as the number of either positive or negative charges per unit area that must be separated in order to establish an electrical potential of 1 V.

Since the capacitance relates the charge separation to an electrical potential, we get the following relation:

$$C = -(zeq)/E \text{ or } E = -(zeq)/C \tag{C.3}$$

where

$C$ is the specific capacitance (in F $m^{-2}$)
$z$ is the valence of the charged particle
$e$ is the elementary charge ($1.6 \times 10^{-19}$ coulombs)
$q$ is the number of charged particles that move to one side of the capacitor (in $m^{-2}$)
$E$ is the electrical potential difference across the capacitor (in V).

If we differentiate equation C.3 with respect to time, we get

$$dE/dt = -d[(zeq)/C]/dt. \tag{C.4}$$

Since $C$ is a constant, we can take it out of the differential and we get

$$dE/dt = -(1/C)[d(zeq)/dt]. \tag{C.5}$$

We can simplify. Since $d(zeq)/dt$ is equal to the amount of charge moving per unit area per unit time (in coulombs $m^{-2}$ $s^{-1}$), it is equal to the current density ($I$, in A $m^{-2}$).

$$dE/dt = -I/C \tag{C.6}$$

where

$C$ is the specific capacitance (in F $m^{-2}$)
$(zeq)/dt$ is equal to current density ($I$, in A $m^{-2}$).

Thus a capacitor determines how long it will take a given current to cause a change in voltage. By combining equation C.6 with Ohm's Law,

$$I = -E/R' \tag{C.7}$$

where $R'$ is the specific resistance (in $\Omega$ $m^2$), we see that

$$dE/dt = -E/(R'C) \text{ or } (dE/E) = -dt/(R'C) \tag{C.8}$$

We can integrate equation C.8 to determine the potential difference at any time $t$ (in s). Remember that for the indefinite integral, $\int dE/E = \ln E$ +/– constant of integration, $\ln x - \ln y = \ln (x/y)$, ln and exp are inverse functions, and $\exp(\ln x) = x$; thus,

$$\ln E'_t - \ln E_0 = -[t/(R'C)] \text{ or } E'_t = E_0 \exp[-t/(R'C)] \tag{C.9}$$

where

$t$ is the time (in s)
$E'_t$ is the potential (in V) at time $t$
$E_0$ is the potential (in V) at time 0
$R'$ is the specific resistance (in $\Omega$ $m^2$)
$C$ is the specific capacitance (in F $m^{-2}$).

Thus, the membrane potential ($E_m$) will not change instantaneously in response to a change in current, but will change logarithmically in a manner that depends on $R_mC_m$. $R_mC_m$ determines the time it takes for the current to charge the membrane capacity. For this reason, $R_mC_m$ is called the *membrane time constant* ($\tau$, in s; Duncan, 1990). When we are considering the time dependence of how membrane potentials respond to a change in current, it is important to take into consideration both the capacitive currents and the ionic currents. Since in a typical characean cell $R_m$ is approximately 1 $\Omega$ $m^2$ and $C_m$ is about $10^{-2}$ F $m^{-2}$, the membrane time constant is approximately 10 ms. Thus it takes 10 ms to reach 0.63 $E_\infty$ where $E_\infty$ is the potential (in V) at time infinity. It takes 20 ms to reach 0.86 $E_\infty$; 30 ms to reach 0.95 $E_\infty$; 40 ms to reach 0.98 $E_\infty$; and 50 ms (or $5R_mC_m$) to reach 0.99 $E_\infty$.

Equation C.8 is essentially a restatement of Coulomb's Law, which relates the force ($F^*$, in N) to the distance between two charged spherical bodies. Coulomb's Law is given by the following equation:

$$F^* = (zeq')(zeq')/(4\pi x^2 \varepsilon_0 \varepsilon) \tag{C.10}$$

where

$F^*$ is the force of attraction (when $F^*$ is negative) or repulsion (when $F^*$ is positive) between two spherical bodies (in N)
$q$ is the number of charges (dimensionless)
$z$ is the valence (dimensionless)
$e$ is the elementary charge ($1.6 \times 10^{-19}$ coulombs)

$x$ is the distance between the two charged bodies (in m)
$\varepsilon$ = relative permittivity of the insulating layer (dimensionless)
$\varepsilon_0$ is the permittivity of a vacuum ($8.85 \times 10^{-12}$ F m$^{-1}$).

Let's rearrange equation C.10:

$$[(F^*x)/(zeq')](4\pi x\varepsilon_0\varepsilon) = (zeq'). \tag{C.11}$$

Now we can see that $(F^*x)/(zeq')$ is equivalent to the potential (in V) between the two spheres and can be designated $-E$ (in V). $(F^*x)/(zeq')$ is defined as $-E$ since positive current moves spontaneously toward negative potentials.

If we assume that the capacitance ($4\pi x\varepsilon\varepsilon_0$, in F) is constant and we differentiate equation C.11 with respect to time, then after removing the capacitance from the differential (because we assume that it is constant) we get

$$-(dE/dt)\,(4\pi x\varepsilon\varepsilon_0) = d(zeq')/dt \tag{C.12}$$

where $d(zeq)/dt$ is the positive current that flows between the two spheres and can be designated $I'$ (in A). Thus,

$$-(dE/dt)\,(4\pi x\varepsilon\varepsilon_0) = I' \tag{C.13}$$

and

$$dE/dt = -I'/(4\pi x\varepsilon\varepsilon_0). \tag{C.14}$$

$4\pi x$ represents the "average" length (in m) of the lines of force between the two point spheres. The shortest line between the two spheres is a straight line $x$ m long. The other lines are curved and get longer (and the force gets weaker) as they approach infinity. If we line up many point spheres to form plates, the lines of force reinforce each other so that in essence the "average" length of the lines of force is equal to the distance between the plates ($x$, in m). Thus, equation C.14 becomes

$$dE/dt = -I'/(x\varepsilon\varepsilon_0), \tag{C.15}$$

and thus

$$dE/dt = -I'/C' \tag{C.16}$$

where $C'$ is the capacitance (in F), defined in equation C.1, and relates the change in voltage over time to the current.

Up to now, we have made the assumption that the current is *constant.* However, the magnitude of the current can vary. The *impedance* (in $\Omega$) is a property of the membrane that relates the change in current to the change in voltage when the membrane is clamped by a sinusoidally varying voltage (Horowitz & Hill, 1989; McClendon, 1927; Remington, 1928). While the magnitude of the current is proportional to the magnitude of the voltage (according to Ohm's Law), there will be a time delay if the circuit contains a capacitor. A capacitor introduces a time delay between the change in voltage and the change in current. The time delay (also known as the phase change) introduced

by the capacitor depends on the frequency of the voltage. When the frequency is 0 Hz (i.e., direct current, d.c.), the frequency-dependent resistance, or *reactance,* of the capacitor is infinite, and current will not pass through the capacitor; consequently, no time delay or phase change is observed.

The time delay introduced by the capacitor can be determined by comparing the sine wave of the output current with the sine wave of the input voltage. Although this is conceptually easy, it is mathematically relatively difficult and so we use the algebra of complex numbers to represent the two sine waves and to determine the phase relationship.

Complex numbers have a real part and an imaginary part. In the case of impedance, the real part relates to the impedance due to the resistors in the circuit [$Z_R$ (in $\Omega$) = $R$ where $R$ is the resistance of the resistors], and the imaginary part relates to the impedance due to capacitors in the circuit [i.e., reactance $Z_C$ (in $\Omega$) = $1/j'\omega C'$ where $j' = \sqrt{(-1)}$, $\omega$ is the frequency of the voltage change (in $s^{-1}$), and $C'$ is the capacitance of the capacitor (in F)]. Only the imaginary part of the impedance introduces a time delay or phase change. Thus, the imaginary impedance is the "frequency-dependent resistance" that introduces a phase delay in the current relative to the voltage change.

Thus, the specific resistance ($R'$, in $\Omega$ $m^2$) is the factor that relates the current to the voltage in a d.c. circuit, and the specific impedance ($Z$, in $\Omega$ $m^2$) is the factor that relates the current to the voltage in an alternating current (a.c.) circuit.

## XVIII. Appendix D: Derivations of the Nernst Equation

The Nernst Equation is a formula that gives us information on how two different forces (chemical and electrical, each in N $mol^{-1}$) act on a concentration of particles (mol $m^{-3}$) to give us a certain distribution of particles at equilibrium (Nernst, 1888, 1889; Nobel, 1991; Stein, 1986, 1990). It is assumed that the movement of each particle is independent of the movement of any other particle. The Nernst Equation describes the electrical potential gradient that may arise when the initial distribution is uneven.

Like any robust equation, the Nernst Equation can be derived from many starting points. One way is based exclusively on *irreversible thermodynamics* (Katchalsky & Curran, 1965). Irreversible thermodynamics, in contrast to classical thermodynamics, considers what happens to a system as it approaches equilibrium instead of what happens at equilibrium.

We derive the Nernst Equation from irreversible thermodynamics by assuming that all fluxes are a result of an applied force (in N $mol^{-1}$) on the particles that are moving:

$$\text{Flux} \propto \text{Force}. \tag{D.1}$$

We also assume that the force must act on a given amount of substance in a given volume (i.e., concentration, in mol $m^{-3}$). Thus,

$$\text{Flux} \propto \text{Force} \times \text{Concentration}. \tag{D.2}$$

The units of the product of force × concentration are N $m^{-3}$ or force per unit volume. According to the principles of irreversible thermodynamics, a phenomenological

coefficient exists to relate a flux to a given force per unit volume (Onsager 1931a, 1931b, 1969, 1970): That is,

$$\text{Flux} = \text{Coefficient (Force per unit volume).} \tag{D.3}$$

The permeability coefficient of a substance $j$ ($P_j$, in m $s^{-1}$) relates the flux of that substance to the difference in its concentration. The permeability coefficient is a function of the diffusion coefficient ($D_j$, in $m^2$ $s^{-1}$) and the partition coefficient ($K_j$, dimensionless). The partition coefficient is the ratio of the solubility of the substance in water and oil or any hydrophobic substance that best represents the membrane in question (Stein, 1986). The partition coefficient relates the concentration of a particle in the aqueous solution surrounding the membrane to the concentration of the particle in the "oily" membrane (oil/water). The permeability also depends on the distance ($x$, in m) over which the concentration drop occurs. The permeability coefficient is given by the following equation:

$$P_j = (D_j K_j / x). \tag{D.4}$$

In biology, various phenomenological coefficients exist that relate a given type of movement (flux) to a driving force. In addition to the permeability coefficient ($P_j$, in m $s^{-1}$), which relates the flux of the substance ($J_j$, in mol $m^{-2}$ $s^{-1}$) to the concentration gradient ($dn_j$, in mol $m^{-3}$),

$$P_j = -J_j / (dn_j), \tag{D.5}$$

other phenomenological coefficients exist. These include the specific conductance of the membrane ($G$, in S $m^{-2}$) which relates the flux of charge or current density ($I$, in A $m^{-2}$ or coulombs $m^{-2}$ $s^{-1}$) to the electrical potential difference ($dE$, in V). [This is related to Ohm's Law where the coefficient is $R$ (specific resistance in $\Omega$ $m^2$) and $R' = 1/G$.]

$$G = -I/dE. \tag{D.6}$$

We also have the hydraulic conductivity ($L_p$, in m $s^{-1}$ $Pa^{-1}$) which relates the volume flow of water ($J_w$, in $m^3$ $m^{-2}$ $s^{-1}$) to the difference in pressure ($dP$, in Pa).

$$L_p = -J_w / dP. \tag{D.7}$$

Since the concentration of water, $n_w$ (in mol $m^{-3}$), is approximately 55,000 mol $m^{-3}$ (55 M), we can multiply $J_w$ by $n_w$ to get the flux (in mol $m^{-2}$ $s^{-1}$).

This is related to the Hagen-Poiseuille Law, where the volume flow of water ($J_w$, in $m^3$ $m^{-2}$ $s^{-1}$) is related to the difference in pressure ($dP$, in Pa) by the coefficient $\pi r^2 / 8 l \eta$, where $r$ is the radius of the tube (in m), $l$ is the length of the tube (in m), and $\eta$ is the viscosity of the fluid (in Pa s).

Irreversible thermodynamics does not tell us anything about the mechanism of movement. But it does specify very clearly the *direction* of movement and whether or not the movement in a given direction is *spontaneous*. Figuring out the direction of movement with respect to the direction of the force can be somewhat confusing, so I will begin by defining the important conventions and concepts.

1. Fluxes occur as a result of a force.
2. The magnitude of the flux depends on the magnitude of the force and the magnitude of the phenomenological coefficient.
3. The direction of the net flux is determined exclusively by the chemical potential in each compartment.
4. The net flux always occurs from higher chemical potential to lower chemical potential.
5. Since the difference in chemical potential always is defined as the chemical potential of the final state minus the chemical potential of the initial state, the net flux always occurs in a direction exactly opposite to the difference of the chemical potential. That is, the flux is *spontaneous* (i.e., passive) when the difference in the chemical potential is negative.

The chemical potential ($\mu_j$, in J mol$^{-1}$) is defined as

$$\mu_j = \mu_j^{\circ} + RT\ln n_j + z_jFE + V'_jP + m_jgh' + \ldots \quad \text{(D.8)}$$

where

$\mu_j^{\circ}$ is the chemical potential of substance $j$ in the standard state (in J mol$^{-1}$). The standard state is defined as 298 K, 1 molar, zero electrical potential, 0.1 MPa of pressure and zero height above sea level. The other terms describe how the chemical potential of the substance changes when it is in "nonstandard" conditions. (In standard conditions, all other terms become zero.)

$n_j$ is the concentration of the substance (in mol m$^{-3}$)

$z_j$ is the valence of the substance (dimensionless)

$V'_j$ is the partial molar volume of the substance (im m$^3$ mol$^{-1}$). This is the differential change in volume of a solution when a differential amount of solute is added. To visualize this, consider how much the volume of a $10^{-3}$ m$^3$ (one liter) solution of water changes when you add one mole of solute. This value is typically close to $3 \times 10^{-5}$ m$^3$ mol$^{-1}$ (for NaCl and KCl, it is about $2.7 \times 10^{-5}$ and $3.7 \times 10^{-5}$ m$^3$ mol$^{-1}$, respectively).

$m_j$ is the mass of the substance (in kg)

$E'$ is the electrical potential of the compartment (in V)

$P$ is the hydrostatic pressure (above or below 0.1 MPa) of the compartment (in Pa)

$h'$ is the height of the substance above or below sea level (in m)

$F$ is the Faraday Constant ($9.65 \times 10^4$ coulombs mol$^{-1}$)

$R$ is the gas constant (8.31 J mol$^{-1}$ K$^{-1}$)

$T$ is the absolute temperature (in K)

$g$ is the acceleration due to gravity (9.80 m s$^{-2}$).

Since, when we talk about cells, we will be talking about the distribution of ions between compartments that are both at essentially the same pressure and height, we can eliminate the last two terms and simplify the equation:

$$\mu_1 = \mu^{\circ} + RT\ln n_1 + zFE_1 \quad \text{(D.9a)}$$
$$\mu_2 = \mu^{\circ} + RT\ln n_2 + zFE_2 \quad \text{(D.9b)}$$

where $\mu_1$ and $\mu_2$ refer to the chemical potentials of substance $j$ in compartments 1 and 2, respectively.

It may seem that the hydrostatic pressure that develops across a plant cell can be so large that we should not eliminate the pressure term. In fact, we can, as I will show you. If we have a solute with a partial molar volume ($V'_j$) of $3 \times 10^{-5}$ $m^3$ $mol^{-1}$ and a hydrostatic pressure of 0.5 MPa acting on the solution on one side of the membrane (inside the cell) and a hydrostatic pressure of 0 MPa acting on the solution outside, the difference in the chemical potential in the two compartments will be –15 J $mol^{-1}$. By comparison, if we have a 10-fold concentration difference between two compartments, the difference in the chemical potential $RT\ln(n_2/n_1)$ of those compartments is –5702 J $mol^{-1}$; or if we have a –0.05 V difference in voltage across the membrane, the difference in the chemical potential ($zF\Delta E$) of the two compartments is –4825 J $mol^{-1}$. Therefore, we can eliminate the pressure term since it contributes less than1% to the chemical potential.

Thus, it is valid to consider the electrochemical potential of each compartment to be equal to equations D.9a and D.9b.

The difference in chemical potential ($\mu_2 - \mu_1$) is defined as the chemical potential of the final state (which, for a spontaneous process, is the state with the lowest or least positive chemical potential) minus the initial state (which, for a spontaneous process, is the state with the highest or most positive chemical potential). Under conditions where a change in enthalpy is negligible, this means that we are defining the difference in chemical potential to be the state with higher entropy minus the state with lower entropy.

$$(\mu_2 - \mu_1) = (\mu^\circ + RT\ln n_2 + zFE_2) - (\mu^\circ + RT\ln n_1 + zFE_1). \tag{D.10}$$

$\mu_2 - \mu_1$ is negative for a spontaneous, passive, or exergonic process; positive for a nonspontaneous, active, or endergonic process; and zero for a process which is at equilibrium.

Let's consider the flux of an uncharged particle where $z = 0$. The difference in the chemical potential ($\mu_2 - \mu_1$) is then given by

$$(\mu_2 - \mu_1) = (\mu^\circ + RT\ln n_2) - (\mu^\circ + RT\ln n_1). \tag{D.11}$$

We can simplify the equation using algebra and the rules of logarithms:

$$(\mu_2 - \mu_1) = (RT\ln n_2) - (RT\ln n_1) \tag{D.12}$$
$$(\mu_2 - \mu_1) = RT(\ln n_2 - \ln n_1) \tag{D.13}$$
$$(\mu_2 - \mu_1) = RT[\ln (n_2/n_1)] \tag{D.14}$$

When $n_1 > n_2$, the flux is from $n_1$ to $n_2$. The initial state is $n_1$. The final state is $n_2$ and the difference in chemical potential is always written (by convention) as final state minus initial state. The net flux due to diffusion ($J_d$, in mol $m^{-2}$ $s^{-1}$) is proportional to the difference in the force per mole (in N $mol^{-1}$) in the final state minus the initial state. The force per mole is equal to the difference in chemical potential ($d\mu$, in J $mol^{-1}$) over the distance ($dx$, in m) the concentration gradient exists. The flux is related to the force by the following relation:

$$J_d \propto (-d\mu/dx). \tag{D.15}$$

The *gradient* of the chemical potential (in J $mol^{-1}$ $m^{-1}$ or N $mol^{-1}$, which is force per mole) is defined as the difference in chemical potential divided by the distance. The gradient in chemical potential is opposite in sign to the *drop* in chemical potential. The drop is defined as the chemical potential of the initial state minus the chemical potential of the final state, divided by a distance.

The flux also depends on the concentration of substance ($n$, in mol $m^{-3}$) the force acts upon:

$$J_d \ \alpha(n)(-d\mu/dx). \tag{D.16}$$

Note: $(n)(-d\mu/dx)$ is in units of force per unit volume (N $m^{-3}$) and $(u/N_A)$ are the constants of proportionality that relate the flux to the force per unit volume.

$$J_d = (u/N_A)(n)(-d\mu/dx) \tag{D.17}$$

where

$N_A$ is Avogadro's Number ($6.02 \times 10^{23}$ $mol^{-1}$)

$u$ is the mobility (in $m^2$ $J^{-1}$ $s^{-1}$). The mobility can also be given in units of velocity (in m $s^{-1}$) per unit force (in N) and thus relates the velocity of a particle to the force exerted on it.

Since the derivative of $\ln(n_2/n_1) = dn/n = (n_2 - n_1)/n$ where $n_1$ and $n_2$ are the concentrations at points 1 and 2, respectively and $n$ is the concentration of the whole system at equilibrium which is equal to the concentration at the midpoint between points 1 and 2. Since $RT$ is constant, the derivative of $RT$ is $RT$; then

$$d\mu = dRT \ln(n_2/n_1) = (RT)(1/n)(n_2 - n_1), \tag{D.18}$$

and equation D.17 becomes

$$J_d = (u/N_A)(n)(-RT)(1/n)(n_2 - n_1)/dx. \tag{D.19}$$

Rearrange terms:

$$J_d = -(u/N_A)(RT)(n)(1/n)(n_2 - n_1)/dx. \tag{D.20}$$

Simplify, since $(n)(1/n) = 1$:

$$J_d = -(u/N_A)(RT)(n_2 - n_1)/dx. \tag{D.21}$$

We will use this form of the equation to derive the Nernst Equation. This equation relates the flux of particles ($J_d$, in mol $m^{-2}$ $s^{-1}$) to the force per volume on the particles that results from a concentration gradient [$RT(n_2 - n_1)/dx$, in N $m^{-3}$] and the mobility of the particle ($u/N_A$, in mol $m^2$ $J^{-1}$ $s^{-1}$). Sometimes $u/N_A$ is referred to as the molar mobility constant ($u_m$, in mol $m^2$ $J^{-1}$ $s^{-1}$).

We can derive the usual form of Fick's Law from this equation since $R/N_A = k$, where $R$ is the gas constant (8.31 J $mol^{-1}$ $K^{-1}$), $N_A$ is Avogadro's Number ($6.02 \times 10^{23}$ $mol^{-1}$), and k is Boltzmann's Constant ($1.38 \times 10^{-23}$ J $K^{-1}$). Thus,

$$J_d = -(ukT)[(n_2 - n_1)/dx]. \quad \text{(D.22)}$$

Using Einstein's definition of the diffusion coefficient,

$$D = ukT, \quad \text{(D.23)}$$

we can put equation D.22 in the following form:

$$J_d = -(D)[(n_2 - n_1)/dx], \quad \text{(D.24)}$$

which, by substituting $dn$ for $(n_2 - n_1)$, can be written

$$J_d = -(D)(dn/dx), \quad \text{(D.25)}$$

which is the usual form of Fick's First Law. It states that the driving force for the flux comes from the difference in chemical potential. In this case, the difference in chemical potential comes exclusively from the difference in concentration. The diffusion coefficient relates the magnitude and direction of the flux to the magnitude and sign of the concentration gradient.

In the above derivation we assumed that $n_1$ and $n_2$ are not equal and that either $z$ or $dE$ equals zero. However, if $n_1 = n_2$, and if $z$ and $V$ are not 0, the difference in chemical potential $(\mu_2 - \mu_1)$ is given by

$$(\mu_2 - \mu_1) = (\mu^\circ + zFE_2) - (\mu^\circ + zFE_1). \quad \text{(D.26)}$$

Since $\mu^\circ - \mu^\circ = 0$,

$$(\mu_2 - \mu_1) = (zFE_2) - (zFE_1)\,. \quad \text{(D.27)}$$

Like the flux of uncharged particles, the flux of charged particles ($J_{el}$, in mol $m^{-2}$ $s^{-1}$) is also proportional to the negative difference in chemical potential in two compartments ($-d\mu$):

$$J_{el} \propto (-d\mu/dx). \quad \text{(D.28)}$$

Remember that the gradient of the chemical potential (in units of J $mol^{-1}$ $m^{-1}$ or N $mol^{-1}$, which is force per mol) is defined as the difference in the chemical potential divided by the distance. The gradient in chemical potential is opposite in sign to the drop in chemical potential.

We call the flux $J_{el}$ since the only driving force is electrical. The flux also depends on the concentration of substance ($n$, in mol $m^{-3}$) the force acts upon:

$$J_{el} \propto (n)(-d\mu/dx). \quad \text{(D.29)}$$

$(u/N_A)$ are the constants of proportionality that relate the flux to the force per unit volume. $N_A$ is Avogadro's Number ($6.02 \times 10^{23}$ $mol^{-1}$) and $u$ is the mobility in units of ($m^2$ $J^{-1}$ $s^{-1}$). Thus,

$$J_{el} = (u/N_A)(n)(-d\mu/dx). \quad \text{(D.30)}$$

According to equation D.27, $d\mu = (zFE_2) - (zFE_1)$; thus,

$$J_{el} = (u/N_A)(n) - [(zFE_2 - zFE_1)/dx]. \quad \text{(D.31)}$$

We can simplify the equation using algebra:

$$J_{el} = -(u/N_A)(n)(zFE_2 - zFE_1)/dx \quad \text{(D.32)}$$
$$J_{el} = -(u/N_A)(n)(F)(zE_2 - zE_1)/dx \quad \text{(D.33)}$$
$$J_{el} = -(u)(F/N_A)(n)(zE_2 - zE_1)/dx. \quad \text{(D.34)}$$

We will use this form of the equation to derive the Nernst Equation. This equation relates the flux of particles ($J_{el}$, in mol $m^{-2}$ $s^{-1}$) to the force per unit volume on the particles that result from an electrical gradient [$Fn(zE_2 - zE_1)/dx$, in N $m^{-3}$] and the mobility of the particle ($u/N_A$, in mol $m^2$ $J^{-1}$ $s^{-1}$). Sometimes $u/N_A$ is referred to as the *molar mobility constant* ($u_m$, in mol $m^2$ $J^{-1}$ $s^{-1}$).

When $zE_1 > zE_2$, the flux is from $E_1$ to $E_2$. $E_2$ is the final state. The difference in chemical potential is always written (by convention) as final state – initial state. Thus, the flux is proportional to the negative difference in chemical potential.

(Note: Since both $z$ and $E$ can be either positive or negative, we have an inherently confusing situation. For spontaneous processes, the *initial* state is always the *most positive* value of the product $zE$ and the *final* state is always the *least positive* value of the product $zE$. With this convention, then, the difference (final – initial) is always negative for a spontaneous, passive, exergonic flux. When the difference is positive, the flux is nonspontaneous, active, or endergonic.)

For example, for a monovalent cation ($z_c$ = +) in an electric field that is between 0 V at $A$ and 100 V at $B$, $z_cE_A = 0$ V and $z_cE_B = 100$ V. The difference in electrochemical potential is $z_cE_A - z_cE_B = 0$ V - 100 V = –100 V. The gradient is from $A$ to $B$. The flux will be spontaneous from $B$ to $A$ but will require energy for transport from $A$ to $B$. The passive flux will be proportional to the negative difference in the electrochemical potential.

For a monovalent cation ($z_c$ = +) in an electric field that is between 0 V at $A$ and –100 V at $B$, $z_cE_A = 0$ V and $z_cE_B = -100$ V. The difference in electrochemical potential is $z_cE_B - z_cE_A = -100$ V – 0 V = –100 V. The gradient is from $B$ to $A$. The flux will be spontaneous from $A$ to $B$ but will require energy for transport from $B$ to $A$. The passive flux will be proportional to the negative difference in the electrochemical potential.

For a monovalent anion ($z_a$ = –) in an electric field that is between 0 V at $A$ and 100 V at $B$, $z_aE_A = 0$ V and $z_aE_B = -100$ V. The difference in electrochemical potential is $z_aE_B - z_aE_A = -100$ V – 0 V = –100 V. The gradient is from $B$ to $A$. The flux will be spontaneous from $A$ to $B$ but will require energy for transport from $B$ to $A$. The passive flux will be proportional to the negative difference in the electrochemical potential.

For a monovalent anion ($z_a$ = –) in an electric field that is between 0 V at $A$ and –100 V at $B$, $z_aE_A = 0$ V and $z_aE_B = 100$ V. The difference in electrochemical potential is $z_aE_A - z_aE_B = 0$ V – 100 V = –100 V. The gradient will be from $A$ to $B$. The flux will be spontaneous from $B$ to $A$ but will require energy for transport from $A$ to $B$. The passive flux will be proportional to the negative difference in the electrochemical potential.

For electron transport (redox) reactions on the mitochondrial membrane, thylakoid membrane, or plasma membrane, we use the *reduction potential* for $E$ and –1 for $z$ to see which way electrons flow spontaneously. In this way, negative differences

mean the flux is spontaneous. (Do not use the oxidation potential, which is just the reduction potential $\times -1$.)

We can derive the usual form of Ohm's Law from equation D.34 since $e = F/N_A$, where $e$ is the elementary charge ($1.6 \times 10^{-19}$ coulombs), $F$ is the Faraday Constant ($9.65 \times 10^4$ coulombs mol$^{-1}$), and $N_A$ is Avogadro's Number ($6.02 \times 10^{23}$ mol$^{-1}$):

$$J_{el} = -(u)(n)(e)(zE_2 - zE_1)/dx. \tag{D.35}$$

Factor out $z$:

$$J_{el} = -(u)(n)(e)(z)(E_2 - E_1)/dx. \tag{D.36}$$

Since we will be talking about electrons, let $z = -1$.

$$J_{el} = (u)(e)(n)(E_2 - E_1)/dx\,. \tag{D.37}$$

Multiply both sides by $F$ where $F$ is the Faraday Constant ($9.65 \times 10^4$ coulombs mol$^{-1}$).

$$(F)J_{el} = F(u)(n)(e)(E_2 - E_1)/dx\,. \tag{D.38}$$

Note that $(F)J_{el}$ is in units of coulombs m$^{-2}$ s$^{-1}$ or A m$^{-2}$ and is thus current density ($I$); $(u)(c)(e)(1/dx)$ is in units of coulombs$^2$ m$^2$ J$^{-1}$ s$^{-1}$, which is $\Omega^{-1}$ m$^{-2}$ or S m$^{-2}$, and is thus the specific conductance or the reciprocal of the specific resistance ($G = 1/R'$). The potential difference $(E_2 - E_1)$ is in units of volts = $(E)$. Thus,

$$I = E/R' \text{ or } E = IR', \tag{D.39}$$

which is Ohm's Law for the flow of electrons (where $z = -1$) and 1 A = 1 coulomb/s; 1 V = 1 J/coulomb and 1 $\Omega$ = 1 Js/(coulomb)$^2$ in SI units.

For the flow of positive current, Ohm's Law is written

$$I = -E/R'. \tag{D.40}$$

We can now use equations D.21 and D.34 to derive the Nernst Equation. The flux of a particle due to more than one driving force (e.g., concentration, electrical, pressure, gravity, etc.) is

$$J_{tot} = J_d + J_{el} + J_p + J_g + \ldots \tag{D.41}$$

Note that each flux may be in a different direction. Thus, while the individual fluxes may be large, the total net flux may be small or zero.

In the case of a particle with only two driving forces (concentration gradient and electrical gradient), we have the following situation:

$$J_{tot} = J_d + J_{el} \tag{D.42}$$

where

$$J_d = -(u/N_A)(RT)(n_2 - n_1)/dx \tag{D.21}$$
$$J_{el} = -(u)(F/N_A)(n)(zE_2 - zE_1)/dx. \tag{D.34}$$

Thus,

$$J_{tot} = [-(u/N_A)(RT)(n_2 - n_1)/dx] + [-(u)(F/N_A)(n)(zE_2 - zE_1)/dx]. \quad (D.43)$$

This equation, according to irreversible thermodynamics, describes the initial flux of a particle given the initial conditions. As the flux continues, the concentration gradient and the electrical potential gradient changes and we have to keep modifying the equation to determine either the flux or the mobility coefficient. For this reason, this equation is usually used for two special cases: the *starting condition* or at *equilibrium.* The solution to this equation at equilibrium is known as the Nernst Equation. The Nernst Equation relates the electrical forces to the chemical forces at equilibrium.

At equilibrium, the net flux is zero. That is, $J_{tot} = 0$.

$$J_{tot} = [-(u/N_A)(RT)(n_2 - n_1)/dx] + [-(u)(F/N_A)(n)(zE_2 - zE_1)/dx] = 0. \quad (D.44)$$

After rearranging terms, we get

$$-(u)(F/N_A)(n)(zE_2 - zE_1)/dx = (u/N_A)(RT)(n_2 - n_1)/dx. \quad (D.45)$$

Divide both sides by $uzF/N_A$ to get

$$-(n)(E_2 - E_1)/dx = (RT/zF)(n_2 - n_1)/dx. \quad (D.46)$$

Divide both sides by $n$ to get

$$-(E_2 - E_1)/dx = (RT/zF)(1/n)(n_2 - n_1)/dx. \quad (D.47)$$

Integrating equation D.47 will allow us to solve for the membrane potential that results from the diffusion of ion *j* if we know the concentration of the ion on both sides of the membrane. In integrating this equation we are assuming that the electrical potential gradient is linear across the membrane. Integrate the concentration across the membrane thickness ($dx$) from side 1 to side 2. Remembering that, for the definite integral $\int_2^1 (1/n)(dn) = \ln n$, and using the Fundamental Theorem of Calculus, we get

$$-(E_2 - E_1) = (RT/zF)\ln(n_2/n_1). \quad (D.48)$$

This equation tells us that the electrical gradient will always be the opposite, in sign, of the concentration gradient for a cation.

If $n_2$ and $E_2$ are always defined as the concentration on the external side of the membrane ($n_o$, in mol $m^{-3}$) and the electrical potential on the external side of the membrane ($E_o$, in V), respectively, and if $n_1$ and $E_1$ are always defined as the concentration on the protoplasmic side of the membrane ($n_i$, in mol $m^{-3}$) and the electrical potential on the protoplasmic side of the membrane ($E_i$, in V), respectively, then

$$-(E_o - E_i) = (RT/zF)\ln(n_o/n_i), \quad (D.49)$$

and since by convention $E_o = 0$ V, then

$$-(0 - E_i) = (RT/zF)\ln(n_o/n_i). \quad \text{(D.50)}$$

We can simplify to

$$E_i = (RT/zF)\ln(n_o/n_i), \quad \text{(D.51)}$$

which is the common form of the Nernst Equation.

We can also derive the Nernst Equation from statistical mechanics by starting with Fick's Law (which describes the motion of particles in a concentration gradient) and Ohm's Law (which describes the motion of charged particles in an electric field). While thermodynamics gives us information about the direction of the overall process, statistical mechanics can be used to postulate a mechanism for the process.

According to Fick's Law, there will be a tendency for the particles in any two groups of particles in communication with each other to mix. The net vector of motion will be from the dense group to the sparse group (or opposite in direction to the gradient in chemical potential). The magnitude of the flux depends on the magnitude of the concentration gradient and the magnitude of the diffusion coefficient ($D$, in $m^2\ s^{-1}$).

$$J_d = -D\ (dn/dx). \quad \text{(D.25)}$$

The average flux is proportional to temperature. This is reasonable, since diffusion is a consequence of the thermal motion of particles. Therefore,

$$D \propto kT \quad \text{(D.52)}$$

where $k$ is Boltzmann's Constant ($1.38 \times 10^{-23}\ J\ K^{-1}$) and $T$ is the absolute temperature (in K). Boltzmann's Constant relates the translational energy of the particle to the temperature.

The average translational kinetic energy ($KE$, in J) of a particle is equal to $(3/2)kT$. Hildebrand (1963) and Halliday and Resnick (1970; 382) show that $(3/2)kT$ is equal to $(1/2)mv^2$, where $m$ is the mass of the particle (in kg) and $v$ is the root mean square velocity of the particle (in $m\ s^{-1}$). The higher the temperature, the more energy a particle has and the faster it moves. The coefficient that relates $D$ to $kT$ is the mobility $u$ (in units of $m^2\ J^{-1}\ s^{-1}$).

$$D = ukT; \quad \text{(D.23)}$$

thus,

$$J_d = -ukT(dn/dx). \quad \text{(D.53)}$$

The mobility is a frictional component that relates the velocity of a particle ($v$, in $m\ s^{-1}$) to the force ($F'$, in N):

$$v = uF'. \quad \text{(D.54)}$$

This is the form of Stoke's Law which states that the friction of a spherical particle depends on its hydrodynamic radius ($r_H$, in m) and the viscosity ($\eta$ in Pa s) of the

continuous medium through which it travels. (A continuous medium implies that the size of the moving particle is much larger than the size of the particles that constitute the medium.) That is,

$$v = F'/(6\pi r_H \eta). \tag{D.55}$$

By substituting D.55 into D.54, we see that

$$u = 1/(6\pi r_H \eta). \tag{D.56}$$

That is, the mobility is inversely proportional to the hydrodynamic radius of a spherical particle and the viscosity of the medium through which it moves. Thus, after substituting D.56 into D.23 we obtain the following equation:

$$D = kT/(6\pi r_H \eta), \tag{D.57}$$

and consequently,

$$J_d = -[(kT)/(6\pi r_H \eta)](dn/dx). \tag{D.58}$$

Thus, the flux ($J_d$) of particles is proportional to the concentration gradient ($dn/dx$) and its kinetic energy (related to $kT$), and is inversely proportional to its size (related to $r_H$) and the viscosity of the medium through which it moves ($\eta$).

We can look at the flux of particles induced by an electrical gradient in a similar manner. The flux of particles ($J_{el}$, in mol $m^{-2}$ $s^{-1}$) is related to the current density (I, in A $m^{-2}$ or coulombs $m^{-2}$ $s^{-1}$) by the following relation:

$$J_{el} = I/(zF) \tag{D.59}$$

where F is the Faraday Constant ($9.65 \times 10^4$ coulombs $mol^{-1}$) and $z$ is the valence of the ion (dimensionless).

Now use Ohm's Law for the flow of positive current:

$$I = -E/R'. \tag{D.40}$$

Divide both sides by $zF$:

$$I/(zF) = -E/(R'zF). \tag{D.60}$$

We will see that equation D.60 will be transformed into the form of a typical flux equation since $I/zF$ is equal to $J_{el}$ and $E/(R'zF)$ is equal to $uzen(dE/dx)$. In order to show this I will use dimensional analysis—that is, write out the units of each term and values of each constant:

$$\begin{aligned}&(\text{coulombs m}^{-2}\,\text{s}^{-1})/(9.65 \times 10^4\ \text{coulombs/mol})\\ &= -(\text{V})/[(\text{m}^2\ \text{Js/coulombs}^2)(9.65 \times 10^4\ \text{coulombs/mol})].\end{aligned} \tag{D.61}$$

Multiply both sides by 9.65 x $10^4$:

$$\begin{aligned}&(\text{coulombs m}^{-2}\,\text{s}^{-1})/(\text{coulombs/mol})\\ &= -(\text{V})/[(\text{m}^2\ \text{Js/coulombs}^2)(\text{coulombs/mol})].\end{aligned} \tag{D.62}$$

Cancel coulombs on both sides:

$$(\text{mol m}^{-2}\ \text{s}^{-1}) = -(\text{V})/[(\text{m}^2\ \text{Js/coulombs})(\text{mol})^{-1}]. \quad \text{(D.63)}$$

Multiply the right-hand side by one ($m^2/m^2$):

$$(\text{mol m}^{-2}\ \text{s}^{-1}) = -(\text{m}^2/\text{m}^2)(\text{V})/[(\text{Js/coulombs})(\text{mol})(\text{m}^2)]. \quad \text{(D.64)}$$

Rearrange the terms on the right:

$$(\text{mol m}^{-2}\ \text{s}^{-1}) = -(\text{V/m})(\text{m}^2/\text{Js})(\text{coulombs})(\text{mol/m}^3). \quad \text{(D.65)}$$

Thus, the whole equation (in terms of units) is

$$(\text{mol m}^{-2}\ \text{s}^{-1}) = -(\text{V/m})(\text{m}^2/\text{Js})(\text{coulombs})\ (\text{mol/m}^3); \quad \text{(D.66)}$$

or, in terms of symbols,

$$J_{el} = -(dE/dx)(u)(e)(n) \quad \text{(D.67)}$$

where

$J_{el}$ is the flux (in mol $m^{-2}$ $s^{-1}$)
$dE/dx$ is the electrical potential gradient or electric field (V $m^{-1}$)
$u$ is the mobility (in $m^2$ $J^{-1}$ $s^{-1}$)
$e$ is the elementary charge ($1.6 \times 10^{-19}$ coulombs)
$n$ is the concentration (in mol $m^{-3}$).

Note: $ue$ is also called the mobility ($u'$, but is given in units of $m^2$ $V^{-1}$ $s^{-1}$).

Equation D.67 describes the flow of monovalent cations. In order to describe the flow of any ion we must multiply $e$ by the valence ($z$).

$$J_{el} = -uzen(dE/dx). \quad \text{(D.68)}$$

Equation D.68 is in the form of a flux equation that relates the flux to the force of the electrical potential gradient ($zendE/dx$, in N mol $m^{-3}$) and the mobility of the ion ($u$, in $m^2$ $J^{-1}$ $s^{-1}$).

Now we are ready to derive the Nernst Equation from Fick's Law and Ohm's Law. The flux described by Fick's Law of diffusion equals

$$J_d = -ukT(dn/dx), \quad \text{(D.21)}$$

and the flux described by Ohm's Law is equal to

$$J_{el} = -uzen(dE/dx), \quad \text{(D.68)}$$

and the total flux ($J_{tot}$) is equal to

$$J_{tot} = J_d + J_{el}\ . \quad \text{(D.42)}$$

After substituting equations D.21 and D.68 into D.42, we get

$$J_{tot} = [-ukT(dn/dx)] + [-uzen(dE/dx)] \quad \text{(D.69)}$$

At equilibrium, $J_{tot} = 0$:

$$J_{tot} = [-ukT(dn/dx)] + [-uzen\ (dE/dx)] = 0. \quad \text{(D.70)}$$

Thus, after rearranging terms we get

$$-ukT(dn/dx) = uzen(dE/dx) \quad \text{(D.71)}$$

Divide both sides by *uzen*:

$$-[(ukT)/(uze)](1/n)(dn/dx) = (dE/dx) \quad \text{(D.72)}$$

Cancel *u*:

$$-[(kT)/(ze)](1/n)(dn/dx) = (dE/dx). \quad \text{(D.73)}$$

Integrating the above equation will allow us to solve for the membrane potential that results from the diffusion of ion *j* if we know the concentration of the ion on both sides of the membrane. In integrating this equation we are assuming that the electrical potential gradient is linear across the membrane. Integrate the concentration across the membrane thickness (*dx*) from outside to inside. Remembering that the definite integral $\int_0^i (1/n)(dn) = \ln n$, and using the Fundamental Theorem of Calculus, we get

$$-[(kT)/(ze)] \ln(n_i/n_o) = dE = E_i - E_o \quad \text{(D.74)}$$

Since $-\ln [x/y] = \ln [y/x]$,

$$[(kT)/(ze)] \ln (n_o/n_i) = dE = E_i - E_o. \quad \text{(D.75)}$$

Define $E_o = 0$ (by convention):

$$[(kT)/(ze)] \ln (n_o/n_i) = E_i. \quad \text{(D.76)}$$

Multiply the left side by one $(N_A/N_A)$:

$$(N_A/N_A)[(kT)/(ze)] \ln (n_o/n_i) = E_i. \quad \text{(D.77)}$$

Since $N_A k = R$ and $N_A e = F$, we get

$$E_i = (RT/zF) \ln (n_o/n_i), \quad \text{(D.51)}$$

which is the familiar form of the Nernst Equation.

## XIX. Appendix E: Derivation of the Goldman-Hodgkin-Katz Equation

The flux equations for electrolytes and the Goldman-Hodgkin-Katz Equation are calculated from the general flux equation (based on Fick's and Ohm's Laws). Remember, for a nonelectrolyte the permeability coefficient ($P_j$) is defined by the following equation:

$$J_j = P_j\,(dn_j) \tag{E.1}$$

where

$J_j$ is the net flux of a substance (in mol $m^{-2}$ $s^{-1}$)
$dn_j$ is the difference in the concentration of a substance across a membrane (in mol $m^{-3}$),

and

$$P_j = K_j u_j kT/(x) \tag{E.2}$$

where

$K_j$ is the partition coefficient (oil/water) and is dimensionless
$u_j$ is the mobility of the nonelectrolyte (in $m^2$ $J^{-1}$ $s^{-1}$)
$k$ is Boltzmann's Constant ($1.38 \times 10^{-23}$ J $K^{-1}$; $= R/N_A$)
$R$ is the gas constant (8.31 J $mol^{-1}$ $K^{-1}$)
$N_A$ is Avogadro's number ($6.02 \times 10^{23}$ $mol^{-1}$)
$T$ is the absolute temperature (in K)
$x$ is the thickness of the membrane (in m),

and, as defined by Einstein (1926),

$$D_j = u_j kT \tag{E.3}$$

where $D_j$ is the diffusion coefficient (in $m^2$ $s^{-1}$).

However, in order to determine the permeability of an electrolyte, we must include the electrical force as well as the concentration gradient in the equation (as we did in Appendix D).

$$J_j = [-u_j kT(dn_j/dx)] - [u_j n_j z_j e(dE/dx)]. \tag{E.4}$$

We must also replace $n_j$ and $dn_j$ with $K_j n_j$ and $K_j dn_j$ where $K_j$ is the partition coefficient for the electrolyte, since the concentration in the membrane is not equal to the concentration we measure in the bulk aqueous solutions surrounding the membrane but is related to that concentration by the partition coefficient ($K_j$, dimensionless). Water and the hydrophobic solvent that best mimics the properties of the membrane in question should be used to determine the partition coefficient (Stein, 1986).

$$J_j = [-u_j kTK_j(dn_j/dx)] - [u_j K_j n_j z_j e(dE/dx)]. \tag{E.5}$$

We will define the permeability coefficient of an electrolyte by going through a series of algebraic manipulations of this basic equation.

Now we'll move the electrical term $[u_j K_j n_j z_j e(dE/dx)]$ to the left side of the equation:

$$J_j + u_j K_j n_j z_j e(dE/dx) = -u_j kTK_j(dn_j/dx). \tag{E.6}$$

Factor out $u_j z_j e(dE/dx)$ from the left side:

$$u_j z_j e(dE/dx)\{[(J_j dx)/(u_j z_j edE)] + n_j Kj = u_j kTK_j(dn_j/dx). \tag{E.7}$$

Divide both sides by $[(J_j dx)/(u_j z_j edE)] + n_j K_j$:

$$u_j z_j e(dE/dx) = -u_j kTK_j(dn_j/dx)/\{[(J_j dx)/(u_j z_j edE)] + n_j K_j\}. \quad \text{(E.8)}$$

Multiply both sides by $dx/(u_j kT)$:

$$\begin{aligned}&(dx/u_j kT)[u_j z_j e(dE/dx)]\\ &= (dx/u_j kT)\{-u_j kTK_j(dn_j/dx)/[(J_j dx)/(u_j z_j edE) + n_j K_j]\}.\end{aligned} \quad \text{(E.9)}$$

Define ß = $e/kT = F/RT$ and cancel like terms:

$$(z_j ß)(dE) = -\{K_j(dn_j)/[J_j dx/u_j z_j edE + n_j K_j]\}. \quad \text{(E.10)}$$

If the term $(J_j dx/u_j z_j edE)$ is defined as $Y$ and all its parts are *constant,* we can easily integrate across the membrane (from the outside to the inside) assuming that $dE/dx$ is linear. This is known as the "constant field assumption." We must use the substitution rule of integral calculus to get the definite integral $\int_0^i [K_j dn_j/(K_j n_j + Y)] = \ln (K_j n_j + Y)$ and the Fundamental Theorem of Calculus. Then

$$(z_j ß)(dE) = -\{\ln[K_j n_j^{i} + (J_j dx/u_j z_j edE)] - \ln[K_j n_j^{o} + (J_j dx/u_j z_j edE)]\}. \quad \text{(E.11)}$$

Rearrange terms and remember $\ln x - \ln y = \ln (x/y)$:

$$(z_j ß)(dE) = -\ln\{[K_j n_j^{i} + (J_j dx/u_j z_j edE)]/[K_j nj^{o} + (J_j dx/u_j z_j edE)]\}. \quad \text{(E.12)}$$

Remove ln by exponentiating both sides:

$$\exp[(z_j ß)(dE)] = -\{[K_j n_j^{i} + (J_j dx/u_j z_j edE)]/[K_j n^{jo} + (J_j dx/u_j z_j edE)]\}. \quad \text{(E.13)}$$

Rearrange the right side, remembering that $-\ln [x/y] = \ln [y/x]$:

$$\exp [(zjß)(de)] = [K_j n_j^{o} + (J_j dx/u_j z_j edE)]/[K_j nj^{i} + (J_j dx/u_j z_j edE)]. \quad \text{(E.13a)}$$

Multiply both sides by $K_j n_j^{i} + (J_j dx/u_j z_j edE)$:

$$\exp[(z_j ß)(dE)][K_j n_j^{i} + (J_j dx/u_j z_j edE)] = [K_j n_j^{o} + (J_j dx/u_j z_j edE)]. \quad \text{(E.14)}$$

Distribute the terms on the left side:

$$\begin{aligned}&K_j n_j^{i}\exp[(z_j ß)(dE)] + \{(J_j dx/u_j z_j edE)\exp[(z_j ß)(dE)]\}\\ &= [K_j n_j^{o} + (J_j dx/u_j z_j edE)].\end{aligned} \quad \text{(E.15)}$$

Put all concentration terms on the right side:

$$\begin{aligned}&(J_j dx/u_j z_j edE)\exp[(z_j ß)(dE)] - (J_j dx/u_j z_j edE)\\ &= \{K_j n_j^{o} - K_j n_j^{i}\exp[(z_j ß)(dE)]\}.\end{aligned} \quad \text{(E.16)}$$

Factor out $J_j$ from the left side:

$$J_j[(dx/u_jz_jedE)\exp[(z_jß)(dE)] - (dx/u_jz_jedE)] = \{K_jn_j^{o} - K_jn_j^{i}\exp[(z_jß)(dE)]\}. \quad \text{(E.17)}$$

Divide both sides by $(dx/u_jz_jedE)\exp[(z_jß)(dE)] - (dx/u_jz_jedE)$:

$$J_j = \{(K_jn_j^{o} - K_jn_j^{i}\exp[(z_jß)(dE)])/[(dx/u_jz_jedE)\exp[(z_jß)(dE)] - (dx/u_jz_jedE)\}. \quad \text{(E.18)}$$

Factor out $dx/(u_jz_jedE)$ on the right side:

$$J_j = \{(K_jn_j^{o} - K_jn_j^{i}\exp[(z_jß)(dE)])/[(dx/u_jz_jedE)\exp[(z_jß)(dE)] - 1]\}. \quad \text{(E.19)}$$

Multiply the right side by one (ß/ß):

$$J_j = (ß/ß)\{(K_jn_j^{o} - K_jn_j^{i}\exp[(z_jß)(dE)])/[(dx/u_jz_jedE)\exp[(z_jß)(dE)] - 1]\}. \quad \text{(E.20)}$$

Factor out $K_j$:

$$J_j = (K_jß/ß)\{(n_j^{o} - n_j^{i}\exp[(z_jß)(dE)])/[(dx/u_jz_jedE)\exp[(z_jß)(dE)] - 1]\}. \quad \text{(E.21)}$$

Since $ß = F/RT$,

$$J_j = (ßK_jRT/F)\{(n_j^{o} - n_j^{i}\exp[(z_jß)(dE)])/[(dx/u_jz_jedE)\exp[(z_jß)(dE)] - 1]\}. \quad \text{(E.22)}$$

Define the permeability coefficient of an electrolyte to be $P_j$, in m $s^{-1}$:

$$P_j = (K_ju_jeRT)/(Fx). \quad \text{(E.2)}$$

Note that this is equal to $K_ju_je/(ßx)$, which is equal to $K_ju_jRT/(N_Ax)$, which is in turn equal to $K_ju_jkT/x$. Thus, the permeability coefficient of an electrolyte is identical to the permeability coefficient of a nonelectrolyte.

$$J_j = P_j(z_jß)(dE)\{(n_j^{o} - n_j^{i}\exp[(z_jß)(dE)])/(\exp[(z_jß)(dE)] - 1)\}. \quad \text{(E.23)}$$

Since we integrated from the outside (initial state) to the inside (final state), $J_j$ is defined as $J_i - J_o$ where the net flux equals the influx minus the efflux. Thus $J_j$ is the net flux of substance $j$ out of the cell. When $J_j$ is positive (and the membrane potential is negative), the net passive (spontaneous) flux of both cations and anions is *into* the cell. When $J_j$ is negative (and the membrane potential is negative), the net passive (spontaneous) flux is *out of* the cell for both anions and cations.

The Goldman-Hodgkin-Katz Equation characterizes the membrane potential that develops across a membrane as a result of the diffusion of ions down their concentration gradients (through a membrane with a specific permeability coefficient for each ion).

In order to derive the Goldman-Hodgkin-Katz Equation, we assume that the flux of cations is equal to the flux of anions. Thus at equilibrium the principle of electroneutrality is maintained. That is,

$$J_K + J_{Na} = J_{Cl} \quad \text{(E.24)}$$

or

$$J_K + J_{Na} - J_{Cl} = 0. \quad \text{(E.25)}$$

Define the fluxes of the major ions using equation E.23:

$$J_K = P_K\,(z\beta)(dE)\{[K^o - K^i\exp[(z\beta)(dE)]]/[(\exp[(z\beta)(dE)] - 1)]\}, \quad \text{(E.26a)}$$

$$J_{Na} = P_{Na}\,(z\beta)(dE)\{[Na^o - Na^i\exp[(z\beta)(dE)]]/[(\exp[(z\beta)(dE)] - 1)]\}, \quad \text{(E.26b)}$$

$$J_{Cl} = P_{Cl}\,(z\beta)(dE)\{[Cl^o - Cl^i\exp[(z\beta)(dE)]]/[(\exp[(z\beta)(dE)] - 1)]\}. \quad \text{(E.26c)}$$

Since $z_K = 1$, $z_{Na} = 1$, and $z_{Cl} = -1$, then

$$J_K = P_K\,(\beta)(dE)\{[K^o - K^i\exp[(\beta)(dE)]]/[(\exp[(\beta)(dE)] - 1)]\}, \quad \text{(B.27a)}$$

$$J_{Na} = P_{Na}\,(\beta)(dE)\{[Na^o - Na^i\exp[(\beta)(dE)]]/[(\exp[(\beta)(dE)] - 1)]\}, \quad \text{(B.27b)}$$

$$J_{Cl} = P_{Cl}\,(-\beta)(dE)\{[Cl^o - Cl^i\exp[(-\beta)(dE)]]/[(\exp[(-\beta)(dE)] - 1)]\}. \quad \text{(B.27c)}$$

Simplify $J_{Cl}$:

$$J_{Cl} = -P_{Cl}(\beta)(dE)\{[Cl^o - Cl^i\exp[(-\beta)(dE)]]/[(\exp[(-\beta)(dE)] - 1)]\}. \quad \text{(E.28)}$$

Set $J_K + J_{Na} - J_{Cl} = 0$ according to equation E.25:

$$\{P_K(\beta)(dE)[(K^o - K^i\exp[(\beta)(dE)])/[(\exp[(\beta)(dE)] - 1)]]\}$$
$$+ \{P_{Na}(\beta)(dE)[(Na^o - Na^i\exp[(\beta)(dE)])/[(\exp[(\beta)(dE)] - 1)]]\}$$
$$+ \{P_{Cl}(\beta)(dE)[(Cl^o - Cl^i\exp[(-\beta)(dE)])/[(\exp[(-\beta)(dE)] - 1)]]\} = 0. \quad \text{(E.29)}$$

Divide all terms by $\beta(dE)$:

$$\{P_K[(K^o - K^i\exp[(\beta)(dE)])/[(\exp[(\beta)(dE)] - 1)]]\}$$
$$+ \{P_{Na}[(Na^o - Na^i\exp[(\beta)(dE)])/[(\exp[(\beta)(dE)] - 1)]]\}$$
$$+ \{P_{Cl}\,[(Cl^o - Cl^i\exp[(-\beta)(dE)])/[(\exp[(-\beta)(dE)] - 1)]]\} = 0. \quad \text{(E.30)}$$

Simplify since $1/\{\exp[(-\beta)(dE)] - 1\}$ is equal to $-\exp[(\beta)(dE)]/\exp[(\beta)(dE)] - 1$; then

$$\{P_K[(K^o - K^i\exp[(\beta)(dE)])/[(\exp[(\beta)(dE)] - 1)]]\}$$
$$+ \{P_{Na}[(Na^o - Na^i\exp[(\beta)(dE)])/[(\exp[(\beta)(dE)] - 1)]]\}$$
$$+ \{P_{Cl}(-\exp[(\beta)(dE)])[Cl^o - Cl^i\exp[(-\beta)(dE)]/[(\exp[(\beta)(dE)] - 1)]\} = 0. \quad \text{(E.31)}$$

Cancel $1/\{\exp[(\beta)(dE)] - 1\}$ from each term:

$$\{P_K\,[(K^o - K^i\exp[(\beta)(dE)])]\} + \{P_{Na}\,[(Na^o - Na^i\exp[(\beta)(dE)])]\}$$
$$+ \{P_{Cl}(-\exp[(\beta)(dE)])[(Cl^o) - Cl^i\exp[(-\beta)(dE)])]\} = 0. \quad \text{(E.32)}$$

Distribute the permeability coefficients:

$$P_K K^o - P_K K^i \exp[(ß)(dE)] + P_{Na} Na^o - P_{Na} Na^i \exp[(ß)(dE)] + P_{Cl}(-\exp[(ß)(dE)])(Cl^o) - P_{Cl} Cl^i(-\exp[(ß)(dE)])(\exp[(-ß)(dE)]) = 0. \quad \text{(E.33)}$$

Simplify, remembering that $-\exp[(ß)(dE)]\exp[(-ß)(dE)] = -1$; thus,

$$P_K K^o - P_K K^i \exp[(ß)(dE)] + P_{Na} Na^o - P_{Na} Na^i \exp[(ß)(dE)] + P_{Cl}(-\exp[(ß)(dE)])(Cl^o) - P_{Cl} Cl^i(-1) = 0. \quad \text{(E.34)}$$

Simplify, since $(-1)(-1) = 1$:

$$P_K K^o - P_K K^i \exp[(ß)(dE)] + P_{Na} Na^o - P_{Na} Na^i \exp[(ß)(dE)] + P_{Cl}\{-\exp[(ß)(dE)]\}(Cl^o) + P_{Cl} Cl^i = 0. \quad \text{(E.35)}$$

Move the negative in the fifth term outside the parentheses:

$$P_K K^o - P_K K^i \exp[(ß)(dE)] + P_{Na} Na^o - P_{Na} Na^i \exp[(ß)(dE)] - P_{Cl}\{\exp[(ß)(dE)]\}(Cl^o) + P_{Cl} Cl^i = 0. \quad \text{(E.36)}$$

Rearrange terms:

$$P_K K^o + P_{Na} Na^o + P_{Cl} Cl^i = P_K K^i \exp[(ß)(dE)] + P_{Na} Na^i \exp[(ß)(dE)] + P_{Cl}\{\exp[(ß)(dE)]\}(Cl^o). \quad \text{(E.37)}$$

Factor out $\exp[(ß)(dE)]$:

$$P_K K^o + P_{Na} Na^o + P_{Cl} Cl^i = \exp[(ß)(dE)]\{P_K K^i + P_{Na} Na^i + P_{Cl} Cl^o\}. \quad \text{(E.38)}$$

Divide both sides by $P_K K^i + P_{Na} Na^i + P_{Cl} Cl^o]$.

$$(P_K K^o + P_{Na} Na^o + P_{Cl} Cl^i)/(P_K K^i + P_{Na} Na^i + P_{Cl} Cl^o) = \exp[(ß)(dE)]. \quad \text{(E.39)}$$

Remove the exponent by taking the natural log:

$$\ln [(P_K K^o + P_{Na} Na^o + P_{Cl} Cl^i)/(P_K K^i + P_{Na} Na^i + P_{Cl} Cl^o)] = (ß)(dE)\,. \quad \text{(E.40)}$$

Divide both sides by ß:

$$dE = (1/ß)\{\ln [(P_K K^o + P_{Na} Na^o + P_{Cl} Cl^i)/(P_K K^i + P_{Na} Na^i + P_{Cl} Cl^o)]\}. \quad \text{(E.41)}$$

Replace $1/ß$ with $RT/F$:

$$dE = (RT/F)\{\ln [(P_K K^o + P_{Na} Na^o + P_{Cl} Cl^i)/(P_K K^i + P_{Na} Na^i + P_{Cl} Cl^o)]\}. \quad \text{(E.42)}$$

Since we integrated from outside to inside and the Fundamental Theorem of Calculus

states that $\int_{o}^{i} \times dx = i - o$, the membrane potential $dE$ is defined as $E_i - E_o$. Since $E_o = 0$ by convention, $dE = E_i$. Thus,

$$E_i = (RT/F)\{\ln [(P_K K^o + P_{Na} Na^o + P_{Cl} Cl^i)/(P_K K^i + P_{Na} Na^i + P_{Cl} Cl^o)]\}, \quad (E.43)$$

which is the common form of the Goldman-Hodgkin-Katz Equation and describes the electrical potential of the membrane as a consequence of the diffusion of ions down their electrochemical potential gradient and the permeability of the membrane to each ion.

The Goldman-Hodgkin-Katz Equation is extremely useful in calculating permeability coefficients; however, it assumes that the permeability coefficient is *independent* of membrane potential and the concentration of the permeating ion—that is, we assume the ions move through the membrane independently. If these assumptions are not met, the equation must be modified.

## XX. Appendix F: Derivation of the Hodgkin Equation That Relates Conductance to Flux.

The Hodgkin Equation (text equation 9) relates the unidirectional flux of an ion ($J_i$ or $J_o$, in mol $m^{-2}$ $s^{-1}$) measured chemically to the partial specific conductance of the membrane for an ion ($g_j$, in S $m^{-2}$) measured electrically (Walker & Hope, 1969).

First we must define the partial specific conductance. The partial specific conductance of a membrane is defined from Ohm's Law:

$$g_j = -I_j/E_t \quad (F.1)$$

where

$g_j$ is the partial specific conductance (in S $m^{-2}$)
$I_j$ is the net current density carried by the ion (in A $m^{-2}$)
$E_t$ is the total electrical driving force on the ion (in V).

The minus sign is included in equation F.1 so that the conductance is defined as positive when the driving force is negative and a cation is carrying the current flowing into the cell.

The total electrical driving force on the ion ($E_t$, in V) is defined as the difference in the membrane potential ($E_m$, in V) and the equilibrium potential, or Nernst potential, for that ion ($E_j$, in V), calculated using the Nernst Equation, where

$$E_j = (RT/z_j F) \ln (n_o/n_i) \quad (F.2)$$

where

$E_j$ is the Nernst potential or equilibrium potential for an ion (in V)
$R$ is the gas constant (8.31 J $mol^{-1}$ $K^{-1}$)
$T$ is the absolute temperature (in K)
$z_j$ is the valence of the ion (dimensionless)
$F$ is the Faraday Constant ($9.65 \times 10^4$ coulombs $mol^{-1}$)
$n_o$ and $n_i$ are the concentrations of ion $j$ on the exoplasmic side of the membrane (E-space) and protoplasmic side of the membrane (P-space), respectively (in mol $m^{-3}$).

Thus, the total driving force ($E_t$, in V) is defined by

$$E_t = E_m - E_j \tag{F.3}$$

where

$E_t$ is the driving force (in V)
$E_m$ is the membrane potential (in V)
$E_j$ is the Nernst potential or equilibrium potential of the ion (in V).

Thus, the partial specific conductance can be expressed like so:

$$g_j = -I_j/(E_m - E_j). \tag{F.4}$$

However, experience tells us that most ionic currents are not linear throughout the whole range of possible membrane potentials, and thus a partial specific conductance must be defined for a small range in membrane potentials ($dE_t$). Thus,

$$g_j = -dI_j/dE_t, \tag{F.5}$$

or, after expanding the $dE$ term,

$$g_j = -dI_j/d(E_m - E_j). \tag{F.6}$$

We can also define the driving force ($E_t$, in V) as

$$E_t = (1/z_j ß) \ln \{\exp[(E_m - E_j)z_j ß]\}, \tag{F.7}$$

since

$$E_j = (1/z_j ß) \ln (n_o/n_i), \tag{F.2a}$$
$$\ln (n_o/n_i) = E_j z_j ß, \tag{F.2b}$$
$$(n_o/n_i) = \exp(E_j z_j ß). \tag{F.2c}$$

Substituting equation F.2c into F.2 yields

$$E_j = (1/z_j ß) \ln [\exp(E_j z_j ß)]. \tag{F.8}$$

Thus,

$$E_m - E_j = E_m - (1/z_j ß) \ln [\exp(E_j z_j ß)] \tag{F.9}$$

and

$$d[E_m - E_j] = d\{E_m - (1/z_j ß) \ln [\exp(E_j z_j ß)]\}. \tag{F.10}$$

Since $\ln [\exp(x)] = x$,

$$d[E_m - E_j] = d[E_m - (1/z_j ß)(E_j z_j ß)]. \tag{F.11}$$

After rearranging the right side of equation F.11, we get the differential voltage change:

$$d(E_m - E_j) = (1/z_j ß)d[(z_j ß)E_m - (z_j ß)E_j] \quad \text{(F.12)}$$

where $(z_j ß)E_m - (z_j ß)E_j$ is a dimensionless number that characterizes the magnitude of the driving force for an ion.

Next we must define the current carried by the ion. The current carried by ion $j$ ($I_j$, in A $m^{-2}$) is related to the net flux according to the following definition:

$$I_j = z_j F J_n \quad \text{(F.13)}$$

where

$I_j$ is the current density carried by the ion (in A $m^{-2}$ or coulombs $m^{-2}$ $s^{-1}$)
$z_j$ is the valence of the ion (dimensionless)
$F$ is the Faraday Constant ($9.65 \times 10^4$ coulombs $mol^{-1}$)
$J_n$ is the net flux of the ion (in mol $m^{-2}$ $s^{-1}$).

Now, to relate the partial specific conductance to the net flux of an ion, we substitute equation F.13 into equation F.5 to get

$$g_j = -d(z_j F J_n)/d(E_m - E_j)\ . \quad \text{(F.14)}$$

And substituting equation F.12 into F.14, we get

$$g_j = -d(z_j F J_n)/(1/z_j ß)d[(z_j ß)E_m - (z_j ß)E_j]. \quad \text{(F.15)}$$

Since $z_j$ and $F$ in the numerator are constants, let's take them out of the differential:

$$g_j = -z_j F d J_n/(1/z_j ß)d[(z_j ß)E_m - (z_j ß)E_j]. \quad \text{(F.16)}$$

Combine constants outside the differential:

$$g_j = -z_j F/(1/z_j ß)\{dJ_n/d[(z_j ß)E_m - (z_j ß)E_j]\}. \quad \text{(F.17)}$$

Simplify the constants outside the differential (since $ß = F/RT$):

$$g_j = -(z_j^2 F^2/RT)\{dJ_n/d[(z_j ß)E_m - (z_j ß)E_j]\}. \quad \text{(F.18)}$$

Simplify the constants inside the differential:

$$g_j = (z_j^2 F^2/RT)\ \{dJ_n/d[(z_j ß)(E_m - E_j)]\}. \quad \text{(F.19)}$$

In order to determine the partial specific conductance we must thus know the voltage dependence of the flux $\{dJ_n/d[(z_j ß)(E_m - E_j)]\}$. As this may not be an easily attainable or known quantity, we can simplify the equation by assuming that the membrane potential equals the Nernst potential or equilibrium potential for the ion. Thus, when we take the limit of equation F.19 as $d(E_m - E_j)$ goes to zero, we find

$$g_j = (z_j^2 F^2/RT)J_i, \quad \text{(F.20a)}$$
$$g_j = (z_j^2 F^2/RT)J_o \quad \text{(F.20b)}$$

where

$g_j$ is the specific membrane conductance for the ion (in S $m^{-2}$)
$z_j$ is the valence of the ion (dimensionless)
$F$ is the Faraday Constant ($9.65 \times 10^4$ coulombs $mol^{-1}$)
$R$ is the gas constant (8.31 J $mol^{-1}$ $K^{-1}$)
$T$ is the absolute temperature (in K)
$J_i$ and $J_o$ are the unidirectional fluxes (influx and efflux, respectively) of the ion at equilibrium (in mol $m^{-2}$ $s^{-1}$).

Below is the derivation of equation F.20 from equation F.19:

First, let's replace $J_n$ with $J_i - J_o$:

$$g_j = -(z_j^2F^2/RT)\{d(J_i - J_o)/d[(z_j\beta)(E_m - E_j)]\}. \quad \text{(F.21)}$$

Now we must define the net flux $J_n$ (in mol $m^{-2}$ $s^{-1}$) as $J_i - J_o$ (in mol $m^{-2}$ $s^{-1}$) where $J_i$ and $J_o$ represent the influx and efflux of an ion, respectively. This equation was derived in Appendix E.

$$J_n = J_i - J_o = -(K_j u_j z_j e E_m/dx)\{1/[\exp(z_j\beta E_m) - 1]\}[n_o - n_i\exp(z_j\beta E_m)]. \quad \text{(F.22)}$$

$J_i$ is obtained when $n_i = 0$; thus,

$$J_i = -(K_j u_j z_j e E_m/dx)\{1/[\exp(z_j\beta E_m) - 1]\}(n_o). \quad \text{(F.23)}$$

$J_o$ is obtained when $n_o = 0$; thus,

$$J_o = -(K_j u_j z_j e E_m/dx)\{1/[\exp(z_j\beta E_m) - 1]\}[n_i\exp(z_j\beta E_m)]. \quad \text{(F.24)}$$

In order to simplify the algebra in the following expressions, let's define:

$(-K_j u_j z_j e/dx) = a$
$1/[\exp(z_j\beta E_m) - 1] = f(x)$ where $x = E_m$ and is a constant when $E_m = E_j$.

Equations F.23 and F.24 become

$$J_i = (aE_m)[f(x)](n_o) \quad \text{(F.25)}$$

and

$$J_o = (aE_m)[f(x)][n_i\exp(z_j\beta E_m)]. \quad \text{(F.26)}$$

Substituting equations F.25 and F.26 into equation F.21 gives

$$g_j = -(z_j^2F^2/RT)\{d(aE_m)[f(x)]\}[n_o - n_i\exp(z_j\beta E_m)]/dz_j\beta(E_m - E_j). \quad \text{(F.27)}$$

Take the constants $z_j\beta$, $aE_m$, and $f(x)$ out of the differential (remember that $f(x)$ is a constant when $x = E_j$):

$$g_j = -(z_j^2F^2/RTz_j\text{ß})\{(aE_m)[f(x)]\}\ d\ [n_o - n_i\exp(z_j\text{ß}E_m)]/d(E_m - E_j). \quad \text{(F.28)}$$

Define $k' = (n_o/n_i)$:

$$g_j = -(z_j^2F^2/RTz_j\text{ß})\{(aE_m)[f(x)]\}dn_i[k' - \exp(z_j\text{ß}E_m)]/d(E_m - E_j). \quad \text{(F.29)}$$

Remove the constant $n_i$ from the differential:

$$g_j = -(z_j^2F^2/RTz_j\text{ß})\{(aE_m)[f(x)]\}n_i d[k' - \exp(z_j\text{ß}E_m)]/d(E_m - E_j). \quad \text{(F.30)}$$

According to the Nernst Equation,

$$E_j = (RT/z_jF)\ln(n_o/n_i). \quad \text{(F.2)}$$

Thus,

$$E_jz_j\text{ß} = \ln(n_o/n_i) \quad \text{(F.2b)}$$

and

$$n_o/n_i = \exp(z_j\text{ß}E_j). \quad \text{(F.2c)}$$

Thus, since $k'$ was defined as $n_o/n_i$,

$$k' = \exp(z_j\text{ß}E_j). \quad \text{(F.31)}$$

Substituting equation F.31 into equation F.30 yields

$$g_j = -(z_j^2F^2/RTz_j\text{ß})\{(aE_m)[f(x)]\}n_i d(\exp[z_j\text{ß}E_j] - \exp[z_j\text{ß}E_m])/d(E_m - E_j)]. \quad \text{(F.32)}$$

Multiply $\exp(z_j\text{ß}E_j) - \exp(z_j\text{ß}E_m)$ by $-1$ to get $\exp(z_j\text{ß}E_m) - \exp(z_j\text{ß}E_j)$, which will set up a correct derivative. Then multiply $-(z_j^2F^2/RTz_j\text{ß})$ by $-1$ so the whole equation remains equivalent.

$$g_j = (z_j^2F^2/RTz_j\text{ß})\{(aE_m)[f(x)]\}n_i d(\exp[z_j\text{ß}E_m] - \exp[z_j\text{ß}E_j)]/[d(E_m - E_j)]. \quad \text{(F.33)}$$

Now $d[\exp(z_j\text{ß}E_m) - \exp(z_j\text{ß}E_j)]/[d(E_m - E_j)]$ is a derivative that fits the definition of a derivative:

$$g'(x) = \lim_{x \to a} [g(x) - g(a)]/(x - a)$$

Now we determine the derivative of $d[\exp(z_j\text{ß}E_m) - e(z_j\text{ß}E_j)]/[d(E_m - E_j)]$ as $E_m \to E_j$. Remember, if $g(x) = \exp(z_j\text{ß}x)$ then $g'(x) = z_j\text{ß}\exp(z_j\text{ß}x)$.

$$\lim_{E_m \to E_j} d[\exp(z_j\text{ß}E_m) - e(z_j\text{ß}E_j)]/[d(E_m - E_j)] = z_j\text{ß}\exp(z_j\text{ß}E_m)$$

$$g_j = (z_j^2F^2/RTz_j\text{ß}\{(aE_m)[f(x)]\}n_i[z_j\text{ß}\exp(z_j\text{ß}E_m)]. \quad \text{(F.34)}$$

Cancel $z_j ß/z_j ß$:

$$g_j = (z_j F^2/RT)\{(aE_m)[f(x)]\}n_i[\exp(z_j ß E_m)]. \quad \text{(F.35)}$$

At the limit as $E_m$ approaches $E_j$, $E_m$ becomes $E_j$, and so equation F.35 becomes

$$gj = (z_j{}^2 F^2/RT)\{(aE_j)[f(x)]\}n_i[\exp(z_j ß E_m)]. \quad \text{(F.36)}$$

Since, according to equation F.26, $J_o = (aE_m)[f(x)][n_i \exp(z_j ß E_m)]$; and at the limit where $E_m$ approaches $E_j$,

$$J_o = (aE_m)[f(x)][n_i \exp(z_j ß E_m)] = (aE_j)[f(x)][n_i \exp(z_j ß E_j)]; \quad \text{(F.37)}$$

then after we substitute equation F.37 into equation F.36 we get

$$g_j = (z_i{}^2 F^2/RT)(J_o). \quad \text{(F.20b)}$$

And since at equilibrium $J_o = J_i$, then

$$g_j = (z_i{}^2 F^2/RT)(J_i). \quad \text{(F.20a)}$$

Equations F.20a and F.20b are valid only in determining the partial specific conductance when the fluxes are measured at the equilibrium potential for that ion—that is, when the membrane potential equals the Nernst potential for that ion.

When both the fluxes and the conductances are measured at the equilibrium potential but are not equal when applying equations F.20a or F.20b, then the assumptions that underlie one of the equations are not true. Typically, this is because the independent movement of ions assumed in the derivation of equation F.22 from Fick's Law and Ohm's Law does not occur. If the ions bind to an asymmetrical channel or a symmetrical channel that has multiple binding sites within the pore, then the independence assumption will be violated and the flux will not be proportional to the ion concentration at the equilibrium potential. See Hodgkin and Keynes (1955) for an excellent explanation of the assumption of independence.