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ACTION POTENTIALS IN HIGHER PLANTS

BARBARA G. PICKARD

Biology Department, Washington University
St. Louis, Missouri

INTRODUCTION

Reports that propagating action potentials can be induced in vascular plants have been appearing since the beginning of the century. Surprisingly, these reports have received relatively scant attention, except for those dealing with the specialized, rapid movements of the sensitive plant *Mimosa pudica* L. or its close relatives and of the insectivore *Dionaea.* Recently, reports of localized, spontaneous bursts of putative action potentials have heightened interest in the electrical excitability of plants. The emerging notion that all higher plants might utilize electrical signals in coordinating a variety of daily functions seems ripe for closer examination, and this review will attempt to consolidate the data and ideas which contribute to that point of view. Earlier reviews of action potentials in higher plants were provided by Umrath (1959), Sibaoka (1966, 1969) and Mackie (1970).

SOME REASONABLY WELL CHARACTERIZED ACTION POTENTIALS IN PLANTS WITH RAPID MOTOR ACTIVITY

Dionaea

It is natural that action potentials were first observed in a plant which utilizes them as signals mediating rapid movements of its leaves: in 1873, Burdon-Sanderson described to the British Royal Society how action potentials propagate throughout the bilobed leaf of *Dionaea muscipula* Ellis. When an insect bends certain sensory hairs on the central part of either lobe, and how the action potentials cause the lobes to snap together and (usually) to trap the insect. (Secretory cells of the leaf subsequently provide digestive enzymes to the little stomach thus formed, and the plant makes a meal of its prey.)

Burdon-Sanderson's further publications (1876, 1882, 1888, see also 1911) provided a wealth of detail about the action potentials. He measured rise times of about 0.1 s, durations of about 1 s and rates of propagation of about 200 mm s^{-1}. He observed positive after-potentials recorded both monophasically and diphasically, and showed that propagation occurs across the entire central portion of the leaf blade, but is faster on the abaxial than on the adaxial surface of the leaf and is faster in a direction perpendicular to the midrib than parallel to it. He showed that a second action potential travels faster than the first, but that fatigue ultimately sets in. He demonstrated that conduction velocity is strongly dependent on temperature.

Unfortunately, Burdon-Sanderson's papers were largely forgotten and apparently not read in any detail even by the few subsequent authors who mention them. Of course, the fascinating behavior of *Dionaea* continued to attract attention, and during the past century a relatively large number of papers have dealt with its action potentials; however, because for the most part they contributed merely to the rediscovery of behavior already established by Burdon-Sanderson, in general they need not be reviewed here. An outstanding exception is the careful work of Benolken and Jacobson (1970) on the triggering of electrical activity in the sensory hair. Comparing both intracellular and extracellular recordings, these workers demonstrated a depolarization during deformation of cells at the base of the hair. This receptor potential results in firing of a single action potential, and then rapidly decays. It is not yet certain whether the receptor potential and action potential arise in the same or different cells, but in any event, they are closely coupled. Also, it is worth noting that Sibaoka (1966) has measured action potentials in the cells of the leaf by means of intracellular electrodes.

Drosera

Recently, it has been discovered that *Drosera*, an insectivore which bears flowers closely comparable with those of *Dionaea* but which has leaves of strikingly different appearance, also utilizes action potentials

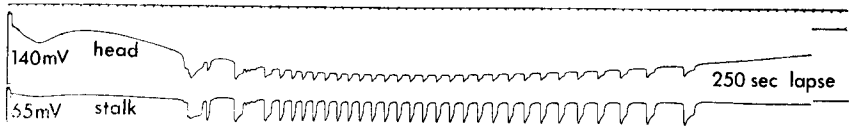

FIG. 1. Simultaneous recordings from the head and lower stalk of a tentacle of *Drosera*; the former shows both a receptor potental and action potentials, while the latter shows the action potentials propagating into the motor region of the stalk (Williams and Pickard, 1972a). 5 s time ticks.

in obtaining its food (Williams and Pickard, 1972a and b). When an insect alights on the mucilaginous head of one of the numerous tentacles of a *Drosera* leaf, its kicking and flailing as it tries to escape from the slime induce a lowering of the potential which can be measured through an electrode inserted in the slime. When this receptor potential falls below a certain value, action potentials are elicited: the greater the voltage drop, the more rapidly the action potentials occur (see Fig. 1). The action potentials travel down the tentacle to its base, where they cause the tentacle with its meaty burden to inflect inward. If the insect is successfully deposited in the center of the leaf, a second, chemotropic mechanism brings still more tentacles into contact with it, and the insect is soon inundated with a secretion containing digestive enzymes.

The action potentials of *Drosera* are slower than those of *Dionaea*, with durations of 3 to 15 s and propagation velocities of about 5 mm s^{-1} at room temperature. They have relatively uniform spikes, variable shoulders or negative after-potentials and variable positive after-potentials (Williams and Pickard, 1972b). All of the living cells of the stalk are excitable (Williams and Spanswick, 1972), and appear to be electrotonically coupled with axial neighbors by plasmodesmata (Williams and Spanswick, 1972; Williams and Pickard, unpubl. obs.). Thus, the voltage fluctuation seen in a single cell during passage of an action potential is a composite of its own excitation and the activities of other cells in the vicinity (Williams and Spanswick, 1972).

The control of initiation of action potentials by receptor potentials in *Drosera* and *Dionaea* suggests that receptor potentials might be rather widely distributed in less conspicuous sensory systems of plants; indeed, receptor potentials might well be of more general importance than action potentials. However, they are not the subject of the present review.

Mimosa

Another plant in which action potentials mediate a conspicuous function is the sensitive plant, *Mimosa pudica*. When an insect sits on a *Mimosa* leaf, thus causing it to vibrate a little, the leaf collapses downward. This startles the insect and encourages it to look elsewhere for lunch; when *M. pudica* grows side by side with a non-motile member of the genus, the latter generally suffers much more from the depredation

of chewing insects (Daniel Janzen, personal communication). The conspicuous motor activity of *Mimosa pudica* and its relatives attracted early electrophysiologists: at the turn of the century, Kunkel (described by Biedermann, 1898) and Bose (1907) showed that electrical fluctuations accompany pulvinar response. Bose also cited, without explanation, a velocity of "transmission of excitatory wave" of 14 mm s^{-1} in the petiole of *Mimosa.* Although these measurements left much to be desired, in 1914 Bose provided a credible measurement of action potentials propagating in *Mimosa* and established their role in causing leaf closure.

On the other hand, in 1916 Ricca provided evidence that leaf closure could be caused by a substance which moves through the stem following certain injurious kinds of stimulation. He cut through a stem, reconnected the pieces by means of a water-filled tube, stimulated a leaf on one side, and showed that an agent inducing leaf closure could pass through the tube. (Note that this has been considered to be a very early demonstration of hormonal activity in plants—e.g. Went and Thimann, 1937.) Several subsequent authors studied and compared these two modes of transmission[1], and their work was culminated in a carefully wrought paper by Houwink (1935).

Houwink showed with great clarity that stimulation of a *Mimosa* stem by a drop of very cold water or by vibration elicits an electrical fluctuation of simple shape which propagates through the bark (presumably, through the phloem) at a velocity of about 20 mm s^{-1} at room temperature; this potential will not pass through severely chilled or damaged cells. Houwink identified it as an action potential. Action potentials will propagate through stems, petioles, and rachises, and into pulvini, but are typically blocked from passing out of pulvini. They may, but sometimes do not, cause the pulvini along and at the end of their path to collapse.

Stimulation by cutting a stem or leaf or by holding a flame close to the tissue causes the appearance of a slowly moving, irregular "variation potential." The variation potential is capable of triggering an action potential, which will propagate ahead of the variation potential. Although as just stated the action potential will in general be blocked from passing through the pulvinus, an action potential may be released on the far side of the pulvinus if the variation potential passes through. The arrival of a variation potential at a pulvinus frequently causes its collapse. One of Houwink's original recordings of an action potential and a variation potential is reproduced in Fig. 2.

The variation potential, like the wound hormone of Ricca, moves with the transpiration stream in the xylem. The wound substance can be obtained in extract, and applied to the base of a cut branch: both wound substance and variation potential will move up the stem. Houwink proposed that the variation potential is caused by the leaking of wound

[1] There is thought to be a third mode, but as it is poorly understood and not known to have electrical correlates, it is not discussed here.

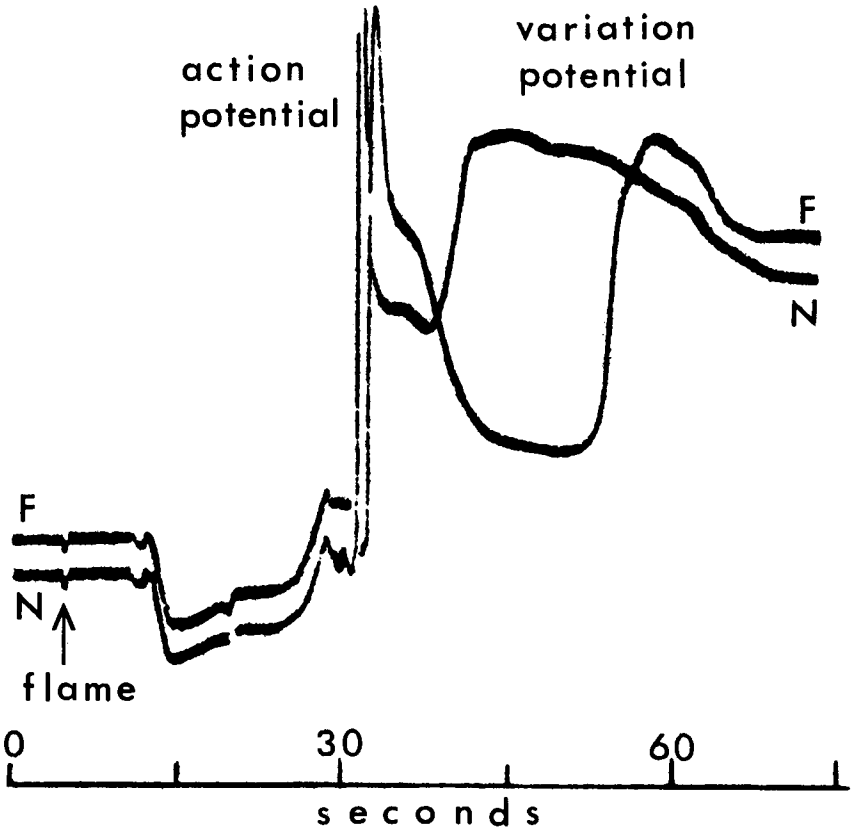

FIG. 2. Photograph (reversed left for right) of one of Houwink's recordings (1935) of flame-stimulated action potential and variation potential propagating along the petiole of *Mimosa*. Recordings taken nearer and farther from site of stimulus labeled N and F, respectively. Lettering has been modified.

hormone from the xylem to adjacent living cells, where it incidentally results in some form of electrical response. He believed that this incidental response might be related to the role which the substance plays in eliciting action potentials and in causing the collapse of pulvini.

Houwink's careful measurements of action potentials have been confirmed and extended by Sibaoka (1953, 1962), who with intracellular electrodes showed that it is in certain of the parenchymatous cells of the phloem and also some parenchymatous cells of the xylem that conduction occurs. These excitable cells have resting potentials of about –160 mV with respect to dilute saline, as compared with the –50 mV resting potentials of surrounding cells. Conduction can take place in either direction along a stem or petiole, but apparently accelerates basipetally and decelerates apically. Sibaoka suggested that the conducting cells are elec-

trotonically coupled through plasmodesmata and cooperate in the passage of an action potential. He showed that localized excitation of small columns of cells soon dies out, but that if a critical number of cells in a large bundle is excited, longitudinal propagation occurs and the wave of excitation spreads electrotonically to excitable cells in other bundles as it passes. The speed of conduction depends on the number of activated cells in the conducting bundle (this may be due to increasing extracellular conductance accompanying loss of ions from the cells). Thus, in the tapering stem or petiole, a basipetally moving action potential encounters increasing numbers of excitable cells and accelerates, while the opposite occurs in acropetal conduction.

The movement of the wound substance of Ricca, and its correlation with the variation potential, have also been confirmed by Sibaoka (1953). Furthermore, assaying by applying test solutions to the cut base of *Mimosa* leaves, Hesse, Banerjee and Schildknecht (1957) have found that sap freshly expressed from the tissue of *Mimosa* contains a highly active factor of low molecular weight which is unstable in air unless the extract is boiled. Partial restoration of activity of the deteriorated factor can be attained by providing a suitable reducing agent. Hesse and coworkers postulate that, as well as containing a stimulatory substance, the tissue has an oxidative enzyme which controls the level of free stimulant in the plant. After reviewing earlier attempts to characterize the stimulatory agent, they provide evidence in support of the view that is is related to *meso*-inositol. Actually, following chromatography, bioassay indicates a whole cluster of chemically related active compounds, and it is not clear whether these are degradation products of a single natural factor or whether they are formed in the plant. Hesse and coworkers also show that closely comparable active agents can be isolated from other plants, including *Thea chinensis* L. (tea). And, incidentally, they make the interesting suggestion that nyctinastic movements of leaves could be accounted for if the substances tended to exist in the oxidized form in the day, and the reduced form at night! Since isolation of the active agent would be an achievement of great interest, and since the evidence to date is of a fragmentary character, it is surprising that there seems to have been little further published effort to characterize the compound. There are rumors that several labs are now at work on the problem, however, so perhaps we may know the formula in the near future.

Other Plants

Because the main focus of this review is on excitability in ordinary plants, it is necessary to omit a great deal of interesting information on plants which carry out rapid movements. It should be noted, however, that plants in several families show rapid elicited motor responses, and frequently these are preceded by an electrical change which may or may not be propagated away from the area of stimulation. Examples are to

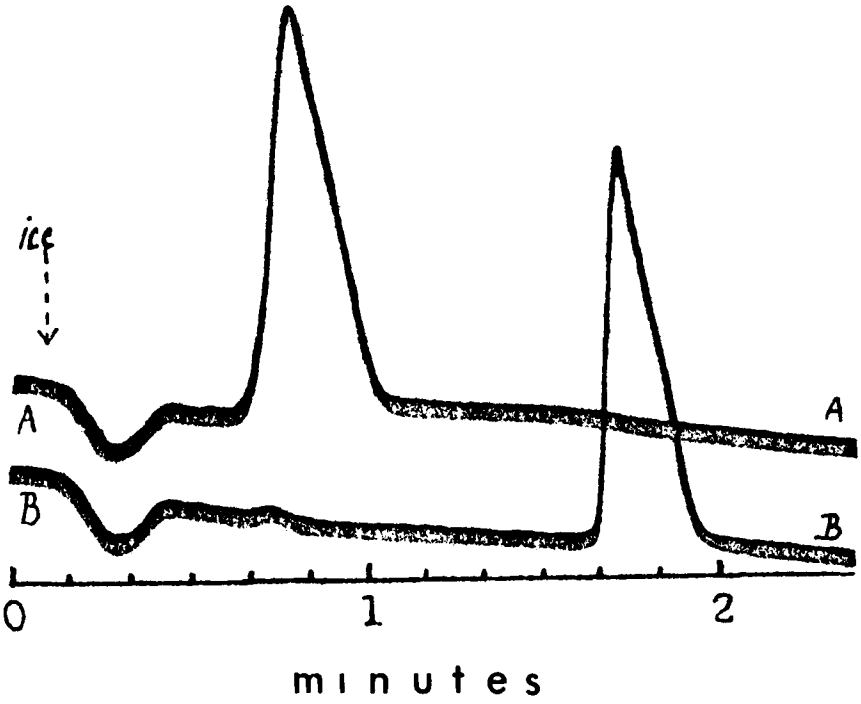

FIG. 3. One of Houwink's galvanometric recordings (1938) of a cold-elicited action potential propagating along a segment of stem of *Naravelia*.

be found in the leaves of the Oxalidaceae (e.g. *Biophytum*), in stigmatic lobes of the Bignoniaceae and presumably stigmatic lobes of the Lentibulariaceae, Scrophulariaceae, and Martyniaceae, and in the contractile filaments of stamens of the Berberidaceae and Tiliaceae and presumably other families such as the Portulacaceae and Compositae. Work on these excitable systems has recently been reviewed by Sibaoka (1969).

PROPAGATING ACTION POTENTIALS INDUCED IN ORDINARY PLANTS BY ALTERING THE TEMPERATURE OR OSMOTIC ENVIRONMENT, AND BY CUTTING, MASHING, OR HEATING TISSUE

As a result of his observations on *Mimosa pudica*, Houwink (1935) speculated that action potentials and variation potentials might occur quite generally in the shoots of higher plants, and the unique feature of *Mimosa* is thus not its action potentials but simply the rapid leaf closure which its action potentials trigger. Therefore, Houwink carried out preliminary experiments with two species of *Vitis* (grape) and obtained electrical fluctuations which appeared closely comparable with action potentials he had obtained with *Mimosa*. On the basis of this success,

he undertook further investigations (1938) on woody segments of the tropical vine which he called *Clematis zeylanica* Poir., but which is correctly identified as *Naravelia zeylanica* DC., showing detailed similarity of its action potentials and those of *Mimosa.* (One of his remarkably fine recordings is reproduced in Fig. 3.) Specifically, action potentials stimulated by application of ice water traveled along the stem in either direction with a velocity of about 2 mm s^{-1}, propagated only in the bark, and would not propagate through severely chilled or dead regions of the stem. Though they traveled without decrement in the internodes, they were never observed to cross a node. Houwink further noted that if strands of phloem were teased apart, "submaximal" action potentials would propagate along them, and if a portion of bark was removed and ice water was applied below the resulting wound, "submaximal" action potentials would propagate into and through the tissue above the wound, whereas if ice water was applied above the wound a maximal response could be obtained. Thus, Houwink concluded that in *Naravelia* the action potentials propagate independently along the several phloem strands. In contrast, he found with *Mimosa* that action potentials passing through a divided portion of stem spread into the entire phloem system on entering the intact area, and concluded that in this plant all conducting cells are in some way connected. As already pointed out, in later years, Sibaoka (1966) showed with *Mimosa* that this is indeed the case, and that the communication is by electrotonic spread of current through interfascicular parenchyma.

Because of its simple and decisive character, Houwink's work serves as an excellent introduction to the propagation of action potentials in ordinary plants. However, before the year 1959, at least six other workers reported propagating fluctuations which in some instances appear to have been action potentials and in some instances—extrapolating from Houwink's findings with *Mimosa*—appear to have been variation potentials.

In 1907, Bose, using a D'Arsonval galvanometer, seems to have observed propagating electrical effects in several widely separated genera (*Ficus* and *Artocarpus*, *Cucurbita*, *Corchorus*, "fern"). Bose's habit of generously intermixing non-reproducible data and startling claims with plausible descriptions, and his extremely sketchy manner of describing experimental methods and data, leave the reader baffled about what he can believe; nevertheless, the range of velocities Bose reported (0.5 to 50 mm s^{-1}) corresponds with the ranges for action potentials and variation potentials found by later workers.

A more complete early description of traveling fluctuations in "ordinary" plants is that of Montemartini, also in 1907. He placed his electrodes on the major veins of leaves of *Arum*, *Croton*, *Ficus*, *Inula*, *Rumex*, *Saxifraga*, *Viburnum*, *Phaseolus*, and *Rhynchosia*, and, stimulating by cutting or mashing the leaf at a position some centimeters distant from the electrodes, or by pressing a red-hot rod of glass against the leaf,

measured resulting current flow with a D'Arsonval galvanometer. The response of the instrument is of course slow, but evidently Montemartini was working with slow fluctuations: he reported durations in the range of 2 to 20 minutes. He assessed rates of transmission as ranging from 0.1 to 15 mm s^{-1} and found, in general, that they were greater in the basipetal than in the acropetal direction. It seems quite possible that the fluctuations observed by Montemartini were at least in part variation potentials.

Auger in 1928 utilized the string galvanometer to measure propagating fluctuations in stems of a cucurbit. Auger's brief but fairly detailed report indicates that he stimulated by shocking with an inductorium or by cutting with a razor, and obtained diphasically and monophasically recorded signals of a few seconds' duration and somewhat irregular form traveling about 10 to 60 mm s^{-1}. He reported that both speed and amplitude decrease as the distance of travel decreases, and noted that while fatigue is conspicuous if stimuli are closely spaced, the provision of 15-minute recovery periods following stimulation permits indefinite repetition of the experiment. Auger noted that a wide variety of plants can produce the traveling disturbances, but that none which he tested did it as readily and consistently as the cucurbit. It seems quite likely that Auger was observing action potentials.

The most extensive reports of traveling fluctuations were those of Umrath, reviewed by himself in 1959. Among the plants involved were *Cucumis*, *Cassia*, *Lathyrus*, *Phaseolus*, *Aeschynomene*, and *Phyllanthus*. Umrath's mode of stimulation was to cut, break or burn the tissue. With a capillary electrometer, he observed voltage fluctuations of relatively slow and sometimes irregular time course. Fluctuations did not always return promptly to the baseline. Conduction velocities ranged from 1 to 15 mm s^{-1}, and in some cases Umrath noted that conduction was decremental. It is difficult to evaluate the possible contributions of action potentials and variation potentials to Umrath's published recordings, but it seems likely that both are present.

In 1955, Kawano elicited traveling voltage fluctuations in the petiole of the leaf of sweet potato by burning the blade with a flame. Evidently, as Kawano clearly recognized, the fluctuations were not action potentials, as they were quite irregular voltage shifts without an immediate return to the baseline. No shift was observed to travel through a petiole treated with ice or with boiling water; nevertheless, it may be guessed that the fluctuations are probably to be equated with variation potentials.

Finally, Lou in 1958 observed spreading electrical potentials in *Gingko* and in *Tropaeolum* following damage; it appears that he dealt primarily with variation potentials.

In the 1960's, a new phase of investigation of action potentials was opened by Sinyukhin and coworkers. In three early papers these authors (Gunar and Sinyukhin, 1962; Sinyukhin and Gorchakov, 1966a but sub-

mitted 1961; Siniuchin and Stolárek, 1961)[2] announced that they had recorded action potentials from *Cucurbita pepo* L. and from three other species—*Fagopyrum sagittaeum* Gilib., *Phaseolus multiflorus* Willd., and *Helianthus annuus* L. (In a later paper, Sinyukhin (1964) also reported recordings from *Heracleum sibiricum* L.) They categorized the range of velocities as between about 5 and 30 mm s^{-1}, observed both basipetal and acropetal conduction, and noted that certain treatments produced series of repetitively firing action potentials. Evidently this group, like Auger (1928), found that *C. pepo*, the pumpkin, was the easiest to work with, for further elaboration of the properties of the excitable system were carried out with this plant.

Vyskrebentseva and Sinyukhin (1967) demonstrated that evocation of action potentials is difficult or impossible in potassium-deficient pumpkin plants, and that when propagation occurs it is relatively slow. It was also claimed that an increased leakage of K^+ from root tissue can be measured during stimulation of a sort that normally causes action potentials to propagate through the shoot, and that Ca^{++} disappears from the bathing solution during such stimulation. Unfortunately, not enough information is presented along with the relevant chart and figures to permit a critical evaluation of the data on ionic fluxes; it was not even demonstrated that action potentials do occur in the roots! Recently, Mamulashvili, Krasavina, and Lyalin (1972) have reported that they cannot measure action potentials in roots of *C. maxima* Duchesne stimulated in such a way that action potentials appear in the stem to which they are attached.

Sinyukhin and Gorchakov (1968) supported the idea that propagation occurs in the phloem by dissecting away tissue surrounding the vascular bundles for an axial distance of 10 to 20 mm and showing that the signals could propagate through, and by showing that the signals could not pass a region poisoned with dinitrophenol even though ^{42}K, presumed to move in the xylem, could be shown to pass at a relatively rapid rate. It is surprising that they did not refer to earlier, apparently more definitive, experiments of Sinyukhin (1964) on intracellular recording from the phloem of stems of both *Cucurbita pepo* and *Heracleum sibiricum*. In those experiments, the small parenchymatous cells of the phloem, as well as parenchymatous cells in the vicinity of the protoxylem, were found to have relatively large resting potentials and to be excitable, whereas the sieve cells, described as large in diameter, registered low resting potentials and evidenced no excitability. Of course, it should be kept in mind that the turgid sieve cells are notoriously susceptible to damage (e.g. MacRobbie, 1971).

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[2] It is difficult to sort out the credits, as the three papers have three different coauthors and yet of the total of eight oscillograms provided, four appear in all three papers, two others are redundant in two of the papers and yet another is redundant in a different combination of two papers! In general, the results reported are much the same.

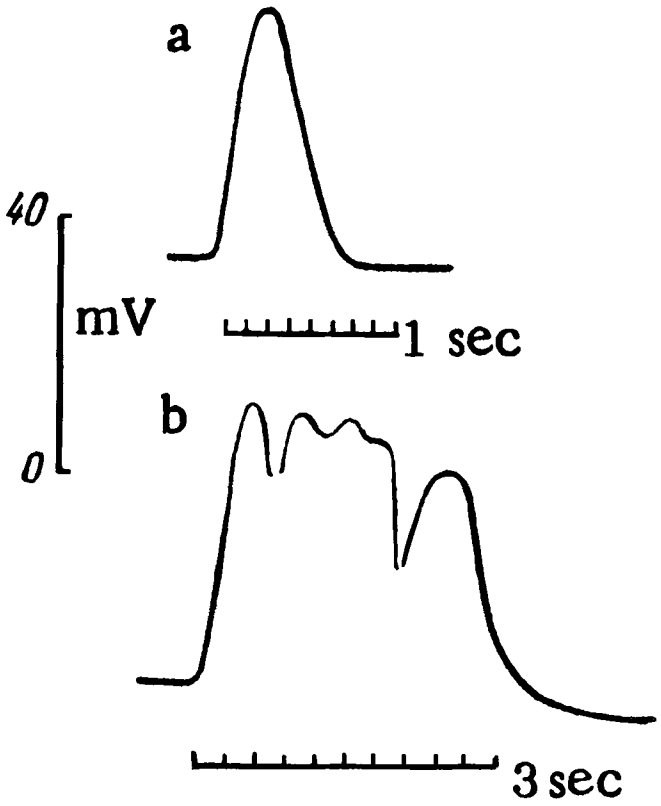

FIG. 4. Illustration by Gunar and Sinyukhin (1963) showing action potentials elicited in *Cucurbita* by a) KCl solution and b) heat.

An especially curious result (see especially Gunar and Sinyukhin, 1963; Sinyukhin and Gorchakov, 1966b) was that the extracellularly recorded shapes of action potentials elicited by different stimuli were different: for example, stimulation of the root system with 10^{-2} to 1 N KCl might produce a spike-shaped action potential in the stem, whereas stimulation of the roots by "thermal denaturation" (a term never clearly defined) might yield a voltage fluctuation with a similar rise-time but with an irregular, slowly descending return phase. Fig. 4 reproduces a typical illustration. Velocities of propagation were reported by Sinyukhin and Gorchakov (1966b) to vary with the nature of the stimulus, also, but it is not clear whether the authors believe the differences to be reproducible.

The finding (Sinyukhin and Gorchakov, 1966a; Siniuchin and Stolárek, 1961) that some stimuli usually produced more than one action potential was further explored (Sinyukhin and Gorchakov, 1966b). Most notably, stimulation with concentrated solutions of KCl caused rhythmic firing.

The rhythms observed were variable and could be quite complex. Although it was supposed in an early investigation (Siniuchin and Stolárek, 1961) that different kinds of stimuli produce different patterns of repetitive firing, no such correlations were found in a more extensive study (Sinyukhin and Gorchakov, 1966b).

When originally looking over all these papers on propagating electrical disturbances—from that of Montemartini in 1907 to that of Sinyukhin and Gorchakov in 1968—it seemed tempting to me to believe that action potentials can propagate in a variety of so-called nonsensitive plants. The well-designed and well-described experiments of Houwink on *Naravelia* seemed especially convincing. But why have all these papers been so generally neglected by plant physiologists? Have people felt that the experiments are not reproducible? I myself, in early attempts to duplicate the work of Sinyukhin's group, met no success (Pickard, 1972). Electrical recording is fraught with possibilities for artifact, and because in most of the papers discussions of technique have been brief, I wondered if artifact could be excluded.

Even assuming the existence of some type or types of propagating electrical disturbance, I felt puzzled in trying to interpret some of the specific findings of the papers. The data seem quite variable and were frequently presented without much supportive detail. Although in none of the studies cited were the results interpreted in terms of the action potentials and variation potentials elucidated by Houwink (1935) with *Mimosa*, they are not obviously incompatible with such an interpretation. For example, in spite of some efforts to demonstrate that propagation of signals requires living tissue, did the propagation of *all* the observed fluctuations require it? Why did the voltage sometimes fail to return to the baseline after a stimulation, as judged from certain illustrations? Why did the shape of the fluctuations seem so variable? With specific reference to Sinyukhin's papers (Gunar and Sinyukhin, 1963; Sinyukhin and Gorchakov, 1966b), did different types of stimulus actually elicit differently shaped action potentials? If real, did these different types of action potentials in fact propagate at different speeds? Did they propagate in different channels of cells, or in the same channels? And if in the same channels, is one dealing with excitable cells which violate the all-or-none rule? As will be discussed in a following section, Sinyukhin and Gorchakov (1968) and Gunar and Sinyukhin (1963) report that signals elicited by different stimuli produce different effects in the leaves, so these last questions are particularly important.

We are not yet in a position to answer all these questions. However, in order to convince ourselves that the basic observations reported in these papers are reproducible and to gain enough insight to justify preliminary interpretation, my laboratory determined to look for action potentials in plants stimulated by sudden changes in temperature, switches of bathing salt solution, mechanical breakage of cells, and burning with a flame. We are delighted to be able to confirm that it is indeed possible

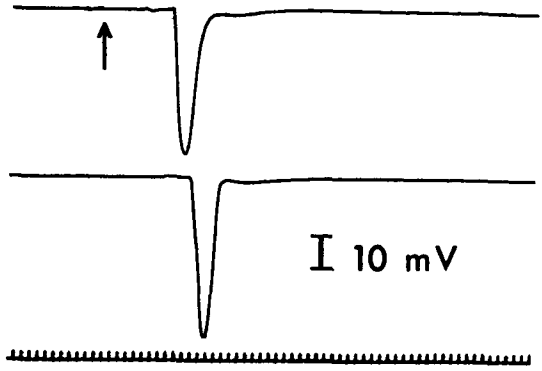

FIG. 5. Original recording of action potential propagating up hypocotyl of seedling shoot of *Cucurbita pepo*. A 10 mm length of the base of the 100 mm hypocotyl was equilibrated in quarter-strength Hoagland's macronutrient solution at 19°C. The wick making extracellular contact for the lower electrode was placed 50 mm from the basal cut, and the upper contact was 5 mm from the cotyledons. Recording equipment was similar to that described by Williams and Pickard (1972a). Stimulation was accomplished by draining the room-temperature solution and replacing it immediately with 14°C solution: replacement is indicated by the arrow. Time ticks, 1 s. Propagation velocity, about 20 mm s^{-1}.

to measure propagating action potentials in several plants, including *Cucurbita pepo*.

However, our efforts immediately suggested one possible reason why the papers on action potentials have not been incorporated into the standard lore of plant physiologists: although we have been able to define a set of conditions for growing and stimulating pumpkin plants which is almost uniformly successful, we are not able to arbitrarily select a plant from a greenhouse, growth chamber, windowsill, or garden and predict whether or not stimulation will produce a response. We are unable even to guess what factors are critical for excitability! Reasonably consistent success with pumpkin, in any case, can be obtained by the use of the cultivar jack-o'-lantern, grown in soil contained in clay pots in a growth chamber at 25°C, with 16 hours light per day from cool white fluorescent tubes yielding a light intensity of about 20 μW mm^{-2} at plant level.

Establishing extracellular electrical contact with either saline wicks (see Pickard, 1972) or saline-filled micropipettes of about 1 μm tip diameter, we find that propagating voltage fluctuations which appear to be action potentials can be measured with equal ease from the hypocotyl, stem, or petiole. Fig. 5 shows a typical example obtained from a hypocotyl which had been severed at its base and inserted into a shallow vial containing a quarter-strength solution of Hoagland's macronutrients at room temperature (19°C). The hypocotyl was stimulated by replacing the 19°C solution with solution chilled to about 14°C (usually, colder

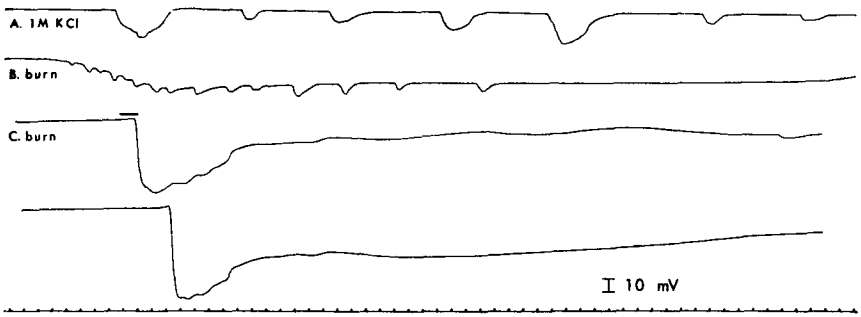

FIG. 6. Voltage fluctuations in *Cucurbita pepo*. Time ticks, 5 s. A) Segment of recording of action potentials elicited in segment of stem by application of 1 N KCl to base. B) Illustrative response to burning of cotyledon by flame; flame removed at beginning of trace. Recorded from hypocotyl just below cotyledon. C) Voltage fluctuations in hypocotyl following flaming of a cotyledon during the period marked by the bar. Upper and lower traces recorded 10 and 70 mm below the cotyledon, respectively. In both B and C, the base of the hypocotyl was continuously bathed in quarter-strength solution of Hoagland's macronutrients.

solution was required). The action potential which resulted had a rise time (10% – 90%) of almost 1 s, an amplitude of about –50 mV, and a propagation velocity of 18 mm s^{-1}. As is not surprising considering the complexity of the conducting tissue and the tissue which surrounds it, the parameters of the action potentials measured at the tips of the two extracellular pipettes are not identical. Even more variability was found when cold-elicited action potentials from different experiments were compared: amplitudes ranged to –75 mV, propagation rates typically varied from about 1 to 30 mm s^{-1}, rise times typically varied from 1 to 5 s and duration, indexed as time between attainment of and return to half-maximal amplitude, typically varied from 1 to 30 s. Large shoulders, sometimes prominent enough to be considered secondary peaks, were occasionally observed. After-potentials of opposite sign were infrequently observed; it is of course difficult to interpret the meaning of these in the extracellular recordings. In general, an action potential appeared larger when detected near the site of stimulation than when detected at a greater distance. Frequently, in stem segments, action potentials could not be detected propagating beyond the stimulated internode, and when they did pass the node they were almost always conspicuously diminished in amplitude. Stimulation of the root system of intact seedlings with saline was as effective as direct stimulation of the shoot in eliciting action potentials in the hypocotyl or stem.

With 1 N KCl, we were also able to reproduce the series of repetitive firings which Sinyukhin and Gorchakov (1966b) induced with either 0.1 or 1.0 N KCl. An illustrative section of a recording is reproduced in Fig. 6A. The larger action potentials seen in this recording channel were also observed 50 mm away at the apical end of the internode but the

smaller ones were not; in this experiment, no action potentials were detected beyond the node. As is always the case in such extracellular recording, it is impossible to know whether the action potentials in fact died out completely, or continued but were not detected for some reason; however, arguing against frequent failure to detect signals which did occur is the observation that an action potential was almost never observed at an electrode distant from the stimulus if it did not appear first at an electrode close to the point of stimulation. If the saline remained continuously in contact with the tissue, fatigue generally became evident in a few minutes or a few tens of minutes. In rare instances, the action potentials kept occurring regularly for fairly long periods—sometimes over an hour. In the illustrated experiment, the repetitive firing was terminated after 30 minutes by replacing the stimulating 1.0 N KCl with the standard solution.

Since Sinyukhin and coworkers reported that saline and heat produced action potentials of different properties, it is particularly interesting to note the effects of thermal treatment in our experiments. Treating the cut base of a hypocotyl or stem with standard solution heated as high at 50°C, action potentials indistinguishable from those elicited by application of chilled solution were often observed. However, 50°C solution was not a particularly effective stimulus, in that about half of the preparations which responded to cold solution applied both before and after the hot solution was applied would not respond to the latter. In contrast, when solution heated above 60°C was applied, or when a flaming match was held under a leaf or stem, voltage fluctuations were almost always observed.

These fluctuations took several forms. Frequently, relatively small drops shaped like action potentials seemed superimposed on a slow, wavering, long-lasting voltage drop. Sometimes, the little presumed action potentials occurred rather regularly while the voltage was low (cf. Fig. 6B). Often, the action potentials propagated ahead of the slow potential. Action potentials sometimes appeared without a slow drop of potential, propagating for various distances along the stem, hypocotyl or petiole. In many instances, on the other hand, the slow drop was unaccompanied by fluctuations which could reasonably be assumed to be action potentials. For slow drops, rates and distances of travel were extremely variable.

Another form of recording resulting from heat treatment is illustrated in Fig. 6C. In this figure, a fairly smoothly and rapidly descending voltage pulse with a large shoulder seemingly composed of small superposed peaks is seen to travel down the hypocotyl at the rate of 5 mm s^{-1}. Following the pulse, the voltage returned only part way toward the baseline, meandering slowly up and down. After about 4 minutes another such pulse appeared, propagating at about the same velocity, and after another 4 minutes there appeared a third such pulse; finally, after about 4 more minutes, meandering ceased and the voltage stabilized at the orig-

inal baseline level. In a common variation of this type of response, the large, slowly propagating pulses had smooth shoulders showing no trace of secondary peaks.

Closely similar responses were observed when plants were stimulated by puncturing, cutting, or mashing tissue. Small stimuli, such as pricking by inserting a pipette of 1 μm diameter, might elicit a single action potential indistinguishable from those caused by cold treatment. Cutting with a razor often gave the same result. Cutting a leaf with a dull pair of scissors, however, or mashing it between the jaws of a pair of pliers tended to give combinations of action potentials and the slower, more irregular fluctuations. In the absence of stimulation, the baseline voltage was remarkably stable.

I tentatively interpret all these results on pumpkin as being in striking analogy with the 1935 findings of Houwink for *Mimosa*. Cold water applied to a region of the stem elicits a single action potential, which is generally large, perhaps, because it arises and propagates synchronously in the several bundles of conductive cells. Application of KCl solution yields similar results, but with repeated firing as long as the stimulus remains and as long as cells recover adequately from their previous excitations. On the other hand, breaking open cells or heating them strongly may be postulated to bring about the release of the wound substance (or substances) which tends to move with the water in the xylem. The wound substance causes local electrical changes as it leaks into living cells in the vicinity of the tracheids and vessels, perhaps by depolarizing them. If the substance diffuses into the excitable tissue, it may set off action potentials. Perhaps sometimes the wound substance builds up very erratically near the bundles of excitable cells, causing only one or a few bundles to fire at a time. Sometimes, when the substance reaches the bundles more uniformly, they all respond synchronously and a large, simple action potential is observed. I would also postulate that stimulation by the wound substance can be so strong that secondary firing of action potentials occurs during the relative refractory period of the initial action potential, resulting in the characteristic large negative peaks with conspicuous shoulders and hence long durations. Fatigue from this great burst of activity could explain the long intervals usually seen between these unusually long-lasting action potentials when they occur in series. Frequent absence of the suggestive little secondary peaks on the shoulder is not surprising if one considers that the extracellular electrodes are summing signals originating in a large number of cells and undergoing capacitative distortion as they travel through the tissue to the area under the tip of the recording pipette.

It should be stressed that much further testing is required to evaluate the validity of this interpretation, and such testing is underway.

In a tentative manner, the interpretation may be extended to the data of Sinyukhin and coworkers. Since they have not described how they applied "heat treatment" or "mechanical damage," since the tendency of

plants to show a variation potential in response to the postulated wound substance may be quite variable, and since we lack information on how consistently and rapidly the baseline was regained following damage-stimulated action potentials, there is ample play for supposing that variation potentials could have been overlooked, and for supposing that the two apparent shapes of action potentials could result from the presence or absence of secondary excitation during the return phase of a single type of action potential. Regarding the suggested differences in rates of travel, I would withhold judgement: the scatter in the rates that we have observed is such that I hesitate to place much credence in averages. Other interpretations must of course be entertained until the excitable system of the pumpkin has been much better studied.

Before the final drafting of this manuscript, two recent papers on action potentials in cucurbits (*C. maxima*) came to my attention: Karmanov, Lyalin, and Mamulashvili (1972) reported that shoulders on the action potentials tend to diminish during propagation, an effect we have not consistently observed in *C. pepo*, and Mamulashvili, Krasavina, and Lyalin (1972) reported that action potentials are not observed in the root when stimulation of that tissue can be shown to elicit action potentials in the stem.

Finally, Jerome W. Van Sambeek, in my lab, has found that *Lycopersicon esculentum* Mill., cv. Bonnie Best, (tomato) grown under the same conditions as described for *C. pepo* responds equally consistently, although differences in sensitivity to the several tested stimuli are to be noted. By way of examples, crushing a leaf yields a limited number of small action potentials in the petiole, and burning a leaf yields, in addition to slow fluctuations of the sort which we preliminary identify as variation potentials, a series of surprisingly uniform, slowly falling action potentials. These data, then, lend support to the tentative extension of Houwink's ideas about action potentials and variation potentials to the studies of a variety of plants reported in the literature, emphasizing at the same time that different species may show differences in their electrical responses.

CERTAIN OTHER EVOKED ACTION POTENTIALS

Pollination

The idea that electrical signals result from pollination and carry information down the style ahead of the pollen tube seems to have been proposed for the first time by Lysikov and Dukhovnyi in 1966. These authors placed an extracellular electrode on the style of *Zea mays* L., and shortly after pollination observed both voltage drops of many minutes duration and spikes of variable height and a few seconds duration. Pollen from another species (sunflower) was also said to produce a response, but it could be distinguished from that of maize pollen and did not interfere with the latter. It would be easier to evaluate the report if

propagation had actually been demonstrated by means of paired electrodes, and if extensive controls had been presented to show that the irregular shifts as well as the spikes occurred only after pollination.

A more detailed investigation of action potentials propagating down the style following pollination has been carried out by Sinyukhin and Britikov (1967a and b), who worked with *Lilium martagon* L. and with *Incarvillea grandiflora* Bur. and Franch. and *I. delavayi* Bur. and Franch. These authors report that a few minutes after pollen is placed on the stigma, a sudden drop in potential can be detected by means of an extracellular electrode recording from the stigmatic tissue. All of the published recordings show that the onset of the drop is marked by a spike, although this is never explicitly discussed. Following this, an action potential travels down the style at a velocity of about 30 mm s^{-1}. (Although the method of recording from the style is indicated to be diphasic, evidently several of the illustrations must be exerpted from monophasic recordings. If, however, the velocity is indeed calculated by comparing the occurrence of peaks in diphasic recordings from electrodes as closely spaced as is evident from some of the figures, a large error might be associated with the quoted velocity.)

Moreover, in the case of *Incarvillea*, the stigmatic lobes fold together in response to mechanical stimulation, and an action potential was observed to mediate this closure. The closure may well aid in brushing pollen off visiting insects, and provides a protected chamber in which pollen germination may ensue. However, this mechanically stimulated action potential and the consequent closure do not lower the gross potential of the stigma and do not elicit excitation in the style as does deposition of pollen.

Frictional Stimulation of Seedlings

Like many other sprouts, the seedling shoot of the common garden pea *Pisum sativum* L. responds to frictional stimulation caused by growing through heavy and compacted overlying soil by increasing the shoot diameter, inhibiting the expansion of the delicate plumular leaves, and by keeping the plumule folded back so that the brunt of the seedling's upward thrust is born by the thickened, recurved portion of the stem (Goeschl, Rappaport, and Pratt, 1966; Goeschl, Pratt, and Bonner, 1967). It has been shown (Pickard, 1971) that when the apical portion of the epicotyl is rubbed, small nonpropagating fluctuations resembling action potentials can be detected within a few minutes by means of an extracellular electrode applied to the area, and that these fluctuations tend to become very numerous and to continue, in the case of relatively strong stimuli, for as long as 3 hours. It was suggested that each fluctuation represents the excitation of a single cell, and that the fluctuations serve as a mediational link in the chain of events leading to the ultimate responses. The possibility that they are simply concomitants of other mediational activity was not, however, eliminated.

Elicitation by Pulses of Current

In a preliminary search for signs of excitability, several persons in my laboratory have found that various tissues from many kinds of plants will respond to brief voltage pulses with voltage fluctuations which may often appear as great as 100 mV in amplitude under an extracellular electrode. Frequently, the fluctuations can be elicited only by either negative-going or by positive-going shocks, or may be of different amplitude for shocks of different sign. Invariably, however, the fluctuation itself has a fixed sign for a given recording. The response varies a great deal from situation to situation, sometimes exhibiting a rapid return to baseline with a decay to half-amplitude requiring only a second or so, and sometimes with a return phase lasting for minutes or even tens of minutes. The return phase may be smooth, or may reproducibly show secondary peaks or shoulders. Similar results have been obtained in other laboratories (e.g. Okamoto, 1955; Sinyukhin and Rutkovskii, 1966; Berry and Hoyt, 1943a and b; but see also Gunar et al., 1970). Due to the complications inherent in recording from a tissue system of complex geometry, and due to the possibility that the response may represent merely a passively rectified return of some of the energy of the voltage pulse, it is difficult to interpret the nature of these fluctuations. In situations in which other evidence for the presence of excitable cells was available, I have suggested that the occurrence of relatively rapid responses of simple shape is (Pickard, 1971a) or might be (Pickard, 1972) due to electrically elicited excitation. In spite of the indications that excitability is extremely widespread in plants, the often-observed ability of plant tissue to respond to electrical shocks with patterned voltage fluctuations—especially when these are of slow time-course—cannot be taken as strong evidence for the occurrence of excitability unless the fluctuations meet the tests of elaborate intracellular analysis (or unless, of course, the fluctuations can be seen to propagate).

Incidentally, it should also be noted that other stimuli can produce electrical fluctuations which have been identified on questionable grounds as action potentials. For example, the light- and chemical-induced fluctuations described as action potentials by Maslobrod (1972) and Maslobrod and Lysikov (1972) have such slow time-courses that there is no reason to classify them as excitable signals in the absence of much more elaborate supportive evidence.

SPONTANEOUS, LOCALIZED ACTIVITY

From the foregoing discussion, one might gain the impression that, in the absence of specific, well-defined stimulation of impulses, plants are electrically quiet. Indeed, in my own experience, recordings from experiments in which the relatively large propagating signals are sought are remarkably free of drift or spontaneous deviations. However, if proper precautions to eliminate noise are taken and the amplification of the measuring system is increased ten to a hundred fold, trains of

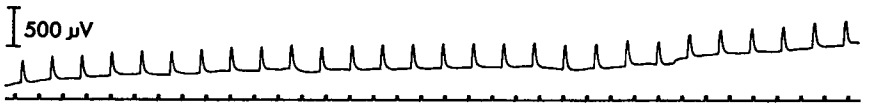

FIG. 7. Spontaneous, repetitive spikes recorded from blade of cotyledon of *Ipomoea* (Pickard, 1972). Time ticks, 1 s.

small, highly patterned voltage fluctuations appear at irregular intervals. I have published such recordings for *Xanthium pennsylvanicum* L., *Pisum sativum* L., and *Ipomoea hederacea* L. (Pickard, 1972), but we have since found them with other plants including the pumpkin.

Fig. 7 presents a photograph of a small portion of a train of repetitive spikes. In this series, the spikes occur about once per 1.5 s, although the frequency changes slowly during the 44 minute duration of the train. From train to train, separation intervals usually varied between 0.1 and 10 s. Train duration varied between 1 s and 2 hours. For the illustrated spikes, the rise time was less than 30 ms; in general, fluctuations of this type had rise times of 1 to 50 ms, and most commonly had durations between 100 and 400 ms. Judging from the size and simplicity of the fluctuations, they may well represent the activity of a single cell, or at most of a small number of cells acting in synchrony. There was no evidence that the signals ever propagated—though propagation would not have been detected if it occurred over a path of only a few cells. On the basis of the shapes and time-courses of the spikes and their regularity within a train, I have suggested that they are action potentials.

Other apparently non-propagating electrical events can also be detected in the leaf or stem. Individual spikes may occur either in isolation, in small groups, or in lengthy bursts of activity. Such fluctuations often have large amplitudes, slower time-courses, and more symmetrical forms than the spikes which occur repetitively in trains, but the ranges of their properties overlap with those of the repetitive spikes.

It is noteworthy that isolated and irregularly grouped fluctuations have also been detected in the mushroom of *Coprinus curtus* Kalchbrenner (Fuller and Pickard, 1972) and in the sporangiophore of *Phycomyces blakesleeanus* Burg. (Pickard, 1971b). Because the sporangiophore is a very large single cell, it is easy to demonstrate that its fluctuations are of similar shape whether recorded from inside or outside the cell membrane, and to show that adjacent electrodes never detect the same signals unless the tips of the recording pipettes are less than 1 mm part (unpublished data). At this time it is hard to judge whether the fluctuations are action potentials or consequences of other membrane events.

It is gratifying that independent discovery of small voltage flucuations has been made simultaneously by Karlsson (1972a and b) in Denmark. Because Karlssson worked with polarizable electrodes (1972a) and a band-pass filter (1972b), it is impossible to make detailed comparisons of the fluctuations he reports for *Ficus elastica* Roxbg. and the fluctu-

ations I have reported in *Ipomoea*, *Xanthium* and *Pisum*. However, rise times and temporal patterns of occurrence are clearly comparable.

SEQUELAE OF ACTION POTENTIALS

Although coordination by electrical signals in a plant was conclusively demonstrated 30 years before a demonstration of hormonal coordination in a plant was completed, it is the latter rather than the former topic which has figured in the study of control systems in intervening years. However, there is a developing feeling among animal physiologists (e.g. Mackie, 1970; Nelson, Peacock and Minna, 1972; Nelson and Peacock, 1972; Roberts, 1971; Roberts and Stirling, 1971) and protistan physiologists (e.g., Eckert, 1972; Wood, 1970; Ettienne, 1970; Eckert, 1965a and b; Eckert and Sibaoka, 1967; Sibaoka and Eckert, 1967) as well as among some plant physiologists that electrical control mechanisms in cells less specialized than those of animal nervous systems may play coordinating roles much more often than has been generally appreciated. Now that there is abundant evidence for the occurrence of excitable cells in plants, it becomes important to consider what kinds of processes might be under electrical control.

In the case of triggered motor activity—as in *Dionaea*, *Drosera*, and *Mimosa*—it is clear that action potentials produce a sudden decrease in turgor of strategically located cells. Whether the decrease in turgor results directly from changes in permeability of the plasmalemma, from activation of contractile vacuoles, or from some other mechanism is not clear, but it does appear that membranes must be critically involved in the process. Much of the evidence regarding this final phase of the motor responses is reviewed by Sibaoka (1969).

In the case of the friction-elicited putative action potentials of the epicotyl of the pea seedling, it is clear that the penultimate link in the chain of reactions causing morphological response is the release of the hormonal gas ethylene (Goeschl, Rappaport, and Pratt, 1966). It is natural to wonder if the electrical activity causes this release, though there are several possible alternate explanations. Clearly, more work should be carried out on this or related systems; if this speculation is correct, it would be of great interest to establish how an electrical impulse could lead to the appearance of a catalytic chemical agent.

In the case of the pollination-stimulated action potentials which travel down the style to the ovary, Sinyukhin and Britikov (1967a and b) reported that a single action potential can within tens of seconds induce an increase in ovarian respiration. Because of the important implications of the electrically triggered change in oxygen consumption immediately following pollination, it would be very helpful if attempts to confirm and extend this finding were to be made in other laboratories.

Perhaps the most tantalizing of the described consequences of excitation are the fluctuations in the gross potential and in the carbon dioxide exchange immediately following arrival of an action potential

at the leaf of pumpkin, as reported by Gunar and Sinyukhin (1963). These workers enclosed the experimental leaf in a transparent, gas-tight chamber through which a stream of air was continually passed into a CO_2 analyzer. Salt bridges inserted into the chamber permitted differential voltage recording from the upper surface of the leaf blade. Action potentials were then elicited, usually by treatment of the root system with KCl solution or of a nearby leaf with heat, and their arrival in the experimental petiole was monitored.

As the Russian workers interpret their data, within 10–20 s after arrival of a KCl-induced action potential at a leaf blade, a fluctuation of the gross potential can be measured; it may be rather regular or may waver irregularly, but it typically dies out within less than 20 minutes. A similar fluctuation is observed only 4 s after a heat-induced action potential arrives, but the return of the voltage toward the baseline usually proceeds only about one-half of the way and then levels off indefinitely.

Twenty to 200 s after the inception of the change in potential, the amount of CO_2 in the air stream rises or drops for a few minutes by perhaps 10%. The Russians discuss the sign of the change as highly specific for the stimulus with which the action potential was elicited and the presence or absence of illumination. Saline-induced action potentials inhibit photosynthesis and enhance respiration. They point out that in their analysis they treat all changes in CO_2 flux in the light as due to photosynthesis, although the contribution of changes in respiration in the light is not carefully evaluated.

It is difficult to evaluate the paper of Gunar and Sinyukhin (1963) because the methods and data are presented with so little explanation. For example, recording from the leaf is depicted as conducted differentially, with one electrode near and one electrode distant from the petiole: only asymmetries in the leaf can be detected. Thus, a permanent change sweeping across the leaf should appear as a transient on the recording; yet the published transients are interpreted without comment as temporary changes. As a second example, in earlier work from the same laboratory (Sinyukhin and Gorchakov, 1966a; Siniuchin and Stolárek, 1961) it was established that 1 N KCl and heat may elicit trains of action potentials which can last tens of minutes. Such trains are not mentioned by Gunar and Sinyukhin (1963); presumably, lags are calculated from the arrival of the first action potential in a series. Does the number of action potentials which arrive at a leaf influence the magnitude or duration of the transients in gross potential difference and gas exchange? This would seem essential to know.

In view of the interpretation tentatively suggested earlier in this review for the nature of the differences between the electrical responses to KCl solution and heat, one may well wonder whether the different consequences reported by Gunar and Sinyukhin (1963) for KCl- and heat-induced signals might in fact represent responses to action potentials in the case of salt stimulation but to both action potentials and the

variation hormone in the case of thermal stimulation. Sinyukhin and Gorchakov would doubtless speak against such a possibility: they have presented two experiments (1968) which they interpret to mean that action potentials are solely responsible for the transients in leaf potential and CO_2 exchange. First, they measured the rate at which ^{42}K moved up or down the stem and found it to be ten times slower than the respective acropetal and basipetal velocities of action potentials in the identical experiment. However, this demonstration is inconclusive because it remains to be checked that under the precise conditions in which transients of leaf potential and gas exchange were measured the velocity of solution in the xylem was inadequate to carry the variation substance from one heated leaf to its neighbor prior to the observed transients. Second, Sinyukhin and Gorchakov (1968) blocked the propagation of action potentials in the stem just below the target leaf by application of 10^{-2} M dinitrophenol, finding no transients when propagation to the leaf was thus prevented and finding normal transients in controls. However, stimulation was by application of KCl rather than heat, and it is only in the latter case that one would look for transmission of the variation hormone.

Because the experiments of Sinyukhin and coworkers seem likely to be of great importance and because of the uncertainties in the interpretation of their data, our laboratory is currently attempting to confirm that propagating electrical signals indeed bring about transients in gas exchange, and to clarify the nature of the signals eliciting the transients.

Assuming that the existence of the transients can be confirmed, many causal explanations might be considered. The supposition of Gunar and Sinyukhin that photosynthesis and respiration are directly affected is one such explanation. If action potentials and the wound hormone play a significant role in the life of the plant, it seems less likely that photosynthesis or respiration would be directly influenced than that some other cellular process might be enhanced or inhibited and might indirectly influence respiration or photosynthesis by introducing transients in a critical parameter such as the size of a shared pool of substrate.

Changes in stomatal aperture might equally well explain some of the data. In this regard, it is interesting that Umrath (1937, 1959) showed that a series of electric shocks administered to a leaf of *Phaseolus* by means of an inductorium could give rise to an electrical fluctuation which appeared to be an action potential, and further (1959) that such a series of shocks could cause stomatal closing. Also, Koketsu (1923) had earlier brought about closure by shocking leaves of *Tradescantia* or *Rhoeo*, and Pallaghy (1968) has similarly caused closure with *Nicotiana*. Pallaghy believed that he was able to measure the resting potential of guard cells by means of intracellular pipettes, but was unable to induce an excitable response in these cells; therefore, it might appear that in Umrath's experiments the influence of the electric shocks (or of action potentials induced in the epidermis?) was to release a chemical factor

capable of causing rapid stomatal closure. Abscisic acid, which can cause extremely rapid closure (Cummins et al., 1971; Kriedemann et al., 1972) is of course one possible suspect! In all this discussion, the difficulty of interpreting shock-induced voltage fluctuations (see section entitled "Elicitation by Pulses of Current"), and the strong possibility that the shocks induce closure without mediation by normal electrical events should be kept uppermost in the mind.

All this leads to the central question of whether the propagating action potentials do play a significant role in the life of the ordinary plant. There is as yet no evidence which bears directly on the issue, and experimentation in this area must currently be justified by the hope that such behavior would not have evolved independent of a function. However, many possible roles invite exploration.

For example, the signals might coordinate responses to changes in weather or soil hydration (by watering dry potted plants of several species, we have confirmed the Russian finding (Gunar and Sinyukhin, 1963; Sinyukhin and Gorchakov, 1966b) that wetting dry roots is an extremely effective way to produce action potentials in the stems; we did not observe slow fluctuations).

A most intriguing possibility is suggested by the recent finding of Green and Ryan (1972) that when insects chew on a leaf of potato or tomato, a message to synthesize a proteinaceous inhibitor of trypsin and chymotrypsin spreads through the damaged leaf and then to neighboring leaves. Mechanical wounding can be substituted for the insect. Since in my laboratory Jerome Van Sambeek has recently confirmed our suspicion that wounding tomato leaves can produce action potentials, work is underway to check whether the movement of either of the electrical responses can be unequivocally associated with movement of the factor inducing the synthesis of the protein.

It is provocative that propagation seems to occur in the parenchyma of the phloem. Several unidentified messengers seem to pass along the phloem, including the flower evocating factor. Much evidence speaks for the chemical nature of the factor, of course, but attempts to identify a hormone have been no more effective than attempts to detect an electrical signal!

More attractive is the possibility that electrical signals regulate the function of the phloem. Could the propagating action potentials influence translocation of sugar and other solutes? The trains of small, repetitive spikes which have been observed in shoots but not roots might also be located in cells associated with the phloem. Could it be possible that these provide information about the loading of the sieve tubes by the transfer cells? Is it possible that they influence the permeability of the critical membranes in the phloem for key substances which might regulate motile behavior of the much-discussed fiber system in the sieve tubes? A role for this system in accelerating the movement of sugar and other substances in the phloem is an important possibility (e.g.

MacRobbie, 1971), yet the critical observation of Coulson, Cataldo, Christy and Swanson (1972) that ATP is not required in the petiole while translocation is occurring through it indicates that motility cannot be based on a myofibrillar-type mechanism. Indeed, the isolated fibrillar protein does not behave as an ATPase (Kleinig et al., 1971). However, it is quite sensitive to the presence of Ca^{++}, and this leads to the notion that the fibrils might belong to the new category of contractile protein discovered in the stalk of the ciliate *Zoothamnion geniculatum* Ayrton by Weis-Fogh and Amos (1972). This protein does not respond to ATP, but contracts in the presence of extremely low amounts of Ca^{++}. It is suggestive that Ca^{++} is not transported in the sieve tubes, but is found in the cells surrounding them (Epstein, 1971). Perhaps in the phloem there is an electrical control of Ca^{++} flux reminiscent of the well-known control by the sarcoplasmic reticulum in striated muscle!

These speculations are likely to prove far-fetched, but it seems important at this stage of the study of electrical signaling in plants to let the imagination range freely. If the (putative) roles of the signals were obvious, doubtless they would have been discovered long ago.

Finally, it should be pointed out that the most important facet of plant electrophysiology may well be the opportunity to study the interactions of electrical and chemical signals. Do action potentials release hormones? Short-range control substances? Are they themselves released by hormones or short-range control substances? Do they affect membrane-localized processes of the cell directly? These fundamental questions were posed long ago for neural systems, but have been difficult to answer in detail because of the small diameter and intricate geometry of neurons and neurosecretory cells. Perhaps the questions will be more amenable to study with the larger cells and simpler organization of plant tissue.

ACKNOWLEDGMENTS AND NOTES

Research and preparation of manuscript made possible by National Science Foundation Grant GB-8262. I am grateful for the enthusiastic assistance of Walter R. Taylor and the valuable cooperation of Jerome W. Van Sambeek. Two further publications came to may attention after the final typescript was prepared. V. V. Gorchakov, Dokl. Timiryazevsko Sel'skokhoz. Akad. 70: 101 (1961) is cited by Karmanov et al. (1972) as having demonstrated action potentials in cucurbit stems (cf. Gunar and Sinyukhin, 1961; Sinyukhin and Gorchakov, 1966a; Sinyukhin and Stolárkek, 1961). I. I. Gunar and L. A. Panichkin are also cited by Karmanov et al. as having demonstrated that water flux produces electrical fluctuations which can interfere with the measurement of action potentials (Izv. Timiryazevsk. Sel'skokhoz. Akad. 5: 3, 1970).

NOTE ADDED IN PROOF

Of great interest is the recent discovery by M. G. K. Jones, A. Novacky and V. H. Dropkin [Membrane potentials of transfer cells. Pl. Physiol.

(Lancaster) 51 suppl.: in press.] that spontaneous trains of action potentials (25 mV depolarization, recovery 5 to 60 s) can be recorded intracellularly from giant cells induced in the roots of *Impatiens balsamina* by the root knot nematode *Meloidogyne incognita.* These multinucleate transfer cells have been described by Jones and D. H. Northcote (Multinucleate transfer cells induced in *Coleus* roots by the root-knot nematode, *Meloidogyne arenaria.* Protoplasma 75: 381–395. 1972).

Mary Ellen Pusateri, Walter R. Taylor, and I hope that Sinyukhin and Britikov (1967a, b) will publish a fuller account of the methods by which they measured propagating action potentials triggered by pollination in the style of two species of *Incarvillea* and of *Lilium martagon.* Working with *Lilium longiflorum* Thunb., we have recorded from 17 styles for periods ranging up to 7 hours after pollination. Extracellular pipettes were embedded in the tissue as described by Sinyukhin and Britikov, and great care was taken to prevent accidental pollination before the beginning of the experiment. Similar experiments were also carried out on three plants in the same family as *Incarvillea* (*Catalpa speciosa* Warder, *Paulownia tomentosa* Baillon, and *Campsis radicans* Seem.) as well as on some plants not closely related to those used by Sinyukhin and Britikov. Although some spontaneous activity was observed, in no case was evocation of a propagating action potential recorded.

Some critical Russian papers earlier escaped my attention. Regarding phloem function, G. P. Molotok, E. A. Britikov, and A. M. Sinyukhin (Proc. Acad. Sci. USSR, Bot. 181: 122–125, 1968) assert that secretion in the floral nectaries of *Tilia cordata* Mill., which is triggered by mechanical stimulation, is mediated by successive action potentials in the phloem bundles and secretory cells. If the observation is validated, it reinforces the hypothesis that certain component processes of translocation may in general be controlled by electrical signals. Still more general support comes from work of V. A. Opritov and V. A. Kalinin (Soviet Pl. Physiol. 17: 643–648, 1970; see also Soviet Pl. Physiol. 17: 256–259). These authors, in addition to reporting that a wave of free radical formation accompanies propagation of an action potential in fodder beet, *Beta vulgaris* L. cv. Eckendorf Yellow, report that basipetal conduction of an action potential results in increased acropetal movement of ^{14}C-labeled glycine, ^{32}P-labeled phosphate, and $^{45}Ca^{++}$. Although surprisingly few data are presented, they attribute the movement to translocation in the phloem. They do not remark on the incompatibility of their results with the generally accepted idea that calcium does not move in the phloem.

Another consequence of the initiation of action potentials in the shoot (of *Vicia sativa* L.) is reported to be increased uptake of phosphate by the root system. (V. A. Opritov, V. O. Krauz, and V. M. Treushnidov, Soviet Pl. Physiol. 19: 961–967, 1972). Further exploration of this initial finding would be desirable.

There is yet another 1961 announcement of propagating action po-

tentials in a higher plant: G. D. Dechev and T. K. Pangelova (Biophysics 6: 42–48) presented somewhat ambiguous recordings of action potentials in *Trifolium*.

Readers exhaustively interested in the literature of excitability will wish to check the following publications: Proc. Acad. Sci. USSR, Bot. 168: 89–91; Proc. Acad. Sci. USSR, Bot. 183: 180–182; Soviet Pl. Physiol. 17: 845–850.

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