STUDIES OF ACTION POTENTIALS IN THE VACUOLE AND CYTOPLASM OF *NITELLA*

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Summary

Intracellular microelectrodes have been used to record the action potential in the vacuole and cytoplasm of single cells of the alga *Nitella*. The *Nitella* cells were cultured in White's nutrient solution ($9 \cdot 2 \times 10^{-3}$ g total salts/l) and almost all measurements of the action potential were made on cells in this solution. The resting potentials of the vacuole and cytoplasm with respect to the bathing solution are approximately equal, and have a value of -150 mV.

The action potential in the vacuole, of 93 mV magnitude, was initiated by a short ($0 \cdot 1$ sec) depolarizing pulse of electric current. Two components—a fast component (the spike) of approximately 2 sec duration and a slow component (superposed on the fast component) lasting 5–10 sec, are identified in the action potential.

The action potential in the cytoplasm is almost identical with the action potential in the vacuole. Thus it appears that the action potential occurs almost entirely across the outer cytoplasmic boundary (the plasmalemma).

The effects of long depolarizing and hyperpolarizing current pulses on the vacuole potential have been examined. Measurements of the resistance of the resting cell have been made by passing electric current pulses between vacuole and bathing solution, and recording the change in vacuole potential. The resistance measured with a depolarizing current is less than the resistance measured with a hyperpolarizing current.

I. INTRODUCTION

Although extensive investigations have been carried out on excitatory processes in living cells for more than half a century, it has only been in the last two decades that considerable advances in the explanation of the behaviour of such cells have been made. Not surprisingly, much more has been done in the study of animal nerve cells than has been done in plant cells. Such developments as electronic measuring equipment and intracellular microelectrodes have enabled a very full investigation of many excitatory phenomena, in nerve cells, to be made. A most notable example is the work of Hodgkin, Huxley, and Katz (1952) on the squid axon. However, corresponding advances have not been made for plant cells such as the alga *Nitella*, which exhibit properties similar to those of nerve cells.

The large internode of *Nitella* has long been a subject for studies in excitatory phenomena, but, up to the present, only the action potential in the vacuole has been recorded. The earliest measurements of the action potential (Osterhout 1931, 1934,

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1936, 1944; Hill and Osterhout 1934, 1938) were made by a method in which electrical contact with the vacuole was obtained by killing a region of the cell with chloroform. Cole and Curtis (1938) have also used this method. Although convenient, there is an obvious objection to the method in that the *Nitella* cells only survived for a few hours after such treatment, and were probably in an abnormal condition during the experimental period. Umrath (1930, 1932*a*, 1932*b*, 1934, 1953, 1954), on the other hand, inserted fine glass capillaries (filled with an electrical conducting medium) into *Nitella* cells and was able to measure the vacuole potential without unduly disturbing the cell. Recently Oda (1956) has made measurements of the action potential in *Chara braunii*, using intracellular microelectrodes.

This paper describes the results of measurements of the action potential in *Nitella*, both in the vacuole and in the cytoplasm. These measurements have been made with intracellular microelectrodes similar to those used by Walker (1955) and with improved electrometers and pen-recording equipment.

It is found that the action potential in the vacuole is almost identical with the action potential in the cytoplasm. This potential change is thus located at the outer cytoplasmic boundary, the plasmalemma. As shown by Walker (1955), and confirmed here, the resting potential of the cell is also located at the plasmalemma.

It is known that there is a close relationship between the cytoplasmic streaming and the action potential (Hill 1941). Further observations on this relationship are described in this paper.

Considerable care has been taken to standardize the experiments as far as possible, and so obtain reproducible results from a series of cells. Previous workers did not seem to be greatly concerned with maintaining standard culture methods. For example, Oda (1956) gives results for action potentials in *Chara braunii* bathed in tap water, but does not give the ionic constitution of this bathing solution nor of the original culture solution. A knowledge of these ionic constituents is essential if any theory of the action potential is to be formulated. In this paper, a new and satisfactory method of culturing *Nitella* under controlled conditions is described.

II. Materials, Apparatus, and Methods

In some preliminary experiments, *Nitella* cells of an unknown species were used which had been grown in stagnant cultures. These cultures consisted of a fine river gravel base and tap water (which was changed occasionally). However, it was found that reproducibility of results from cell to cell, and from culture to culture, could not be obtained. Furthermore, the ionic content of each culture solution was not known and was variable. To counteract these difficulties a new and more satisfactory culture method has been evolved. Old-stock cells (grown in a glass-house pond) were placed in a "Perspex" dish, 10 cm deep, and covered to a depth of 1 cm with white quartz gravel. This gravel had been previously treated with boiling distilled water for several days until the diffusion of salts from the gravel was negligible as evidenced by conductivity measurements. A culture solution, similar to that described by White (1943), flowed through the culture bath and maintained a constant environment for the *Nitella* cells. The ionic content of this culture solution

is shown in Table 1. A new growth of cells appeared in 10–14 days. The cultures were kept indoors at 20°C and given 15 hr light per day. These conditions approximated to the most extreme summer conditions that the plant would meet in its natural environment and were chosen to give maximum growth rate. This produced cells which were sufficiently transparent to enable inserted microelectrodes to be seen clearly.

The experimental cell (usually 1·5 cm long) was held on a "Perspex" mount with small pieces of clean petroleum jelly. In almost all experiments described in this paper the cell was bathed with White's solution of the same concentration as the culture medium, flowing continuously at a rate of 2 c.c./min to ensure a constant environment for the cell. It was decided that action potentials be measured in cells of *Nitella* in their culture medium because the main purpose of the investigations

TABLE 1
IONIC CONSTITUENTS OF MODIFIED WHITE'S CULTURE SOLUTION

Ion	Concentration (μ-equiv/l)	Ion	Concentration (μ-equiv/l)
Mg^{2+}	600	SO_4^{2-}	890
Ca^{2+}	240	NO_3^-	200
Na^+	150	Cl^-	90
K^+	170	PO_4^{3-}	40
Mn^{2+}	3·0	BO_3^{3-}	7·8
Zn^{2+}	0·9	I^-	0·45

described in this paper was to ascertain the nature of the action potential of *Nitella* in its normal state before any measurements are made with changed external conditions.

Measurements of the electric potential of the vacuole and cytoplasm of the *Nitella* cell were made with "Pyrex" glass microelectrodes or probes (filled with 0·3N KCl as a conducting medium) similar to those described by Walker (1955). The microelectrodes (of tip diameter between 5 and 8 μ and resistance between 20 and 5 MΩ) were inserted into the *Nitella* cell using fine screw manipulators. Both single- and double-barrelled probes have been used. The double-barrelled probes were made by fusing together, side by side, two pieces of glass tubing (6 mm external dia.), and then drawing them out, first in a gas flame, and then on a de Fonbrune microforge, as is done with single probes. Symmetrical calomel half-cells connected the solution in the probe and a reference point in the bathing solution to the input of one of the electrometers.

The action potential in *Nitella* is initiated when the vacuole potential is depressed below a critical level. This depression can be caused by:

(1) The passage of an electric current from the vacuole to the bathing solution (Osterhout 1931).

(2) An increase in ionic concentration of the external solution (Osterhout 1931).

Action potentials can also be caused by:

(3) A sudden decrease in temperature (Osterhout and Hill 1935).

(4) Mechanical shock (Osterhout 1931).

Of these methods, (1) has been used for the experiments described in this paper. This method of stimulation can be controlled much more easily than other methods, and allows very rapid changes to be made in the plant potential. Generally, in measurements of the action potential, stimulating current pulses of 0·1 sec duration and magnitudes of $10^{-7} - 5 \times 10^{-6}$ A cm^{-2} of cell membrane have been used. This pulse time has been chosen because the stimulating current is removed very soon after the action potential commences and consequently does not confuse the records of the action potential obtained from the plant cell itself. With current pulses shorter than 0·1 sec, there is considerable variability in the strength of pulse needed to initiate the action potential, and consequently these short pulse times have not been used in the present investigation. In Sections III(*c*) and III(*d*) observations have also been made of the action potential with stimulating current pulses lasting several seconds. The cell was stimulated once every 10–15 min. This time was chosen to allow the resting potential to return fully to its normal value, and the cytoplasmic streaming rate to reach its maximum. If the interval between stimulation was 5 min or less, the action potential changed in form after several stimulations.

The stimulating current was applied to the cell through a probe inserted into the vacuole. A high impedance (50 MΩ) potential source was connected to this probe, and to a platinum plate in the bathing solution. The high impedance ensured a constant current flow through the stimulating probe irrespective of changes in cell potential and resistance. The potential source supplied rectangular current pulses of varying current strength and duration. The potential drop across a 10 kΩ resistance in series with the stimulating probe was fed into an electrometer, which enabled the stimulating current to be measured.

In measurements of the vacuolar action potential two probes were inserted into the vacuole of the cell—the stimulating probe and a recording probe.

The action potential in the cytoplasm was measured in two ways. First, two probes (a single and a double probe) were inserted into the cell. The double probe was inserted 150–200 μ into the vacuole. One barrel of this probe recorded the vacuole potential and the other barrel acted as a stimulating probe. The single probe was inserted 20–40 μ into the vacuole. Shortly after the insertion, the cytoplasm flowed up and over the probe and at the same time, the seal began to form on it (cf. Walker 1955). By moving this probe a little further into the cell every 10 min or so, the tip could be kept clear of the seal and yet remain in the cytoplasm. This movement did not stimulate the cell. The probe could be kept in the cytoplasm for 1–2 hr in this way. In the second method, the single probe was inserted and then pushed to the far side of the *Nitella* cell, until its tip (as seen under a magnification of × 600) was in the cytoplasmic stream. As long as this probe does not

touch the cell wall it will not seal and it can be moved at will from cytoplasm to vacuole and vice versa.

The electrometers, of a new design, were constructed at this University. They had an input impedance of 10^{10} ohms and were characterized by a high degree of stability. On the range 0–10 mV the zero drift was less than 0·5 mV during a 12-hr run. This stability was obtained by the use of an extremely stable potential supply for the filaments of the two input electrometer valves. The D.C. input to the electrometer was applied to the grids of these valves (ME 1400), the screens of which were driven at 1000 c/s, so that the output of this stage was a 1000 c/s signal of amplitude dependent on the D.C. input level. The rectified output of the amplifier (max. 30 V, 10 mA) was fed into an Evershed and Vignoles high-speed pen recorder (chart speed usually 0·25 or 0·5 in/sec). A block diagram of the experimental set up is shown in Figure 1.

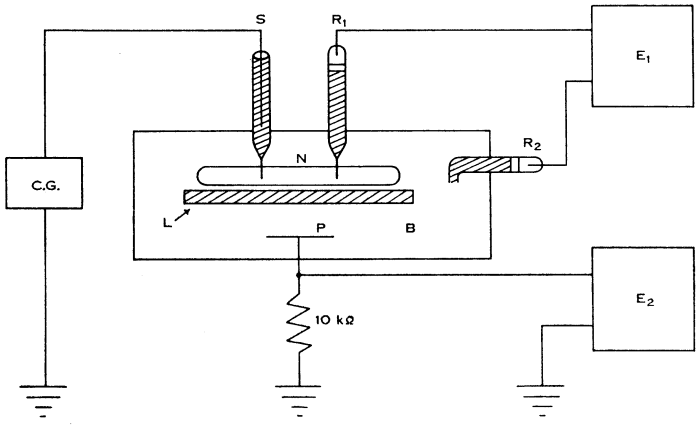

Fig. 1.—Diagram of the experimental set-up (shown in plan) for measuring the action potential in the vacuole. *N*, *Nitella* cell; *S*, stimulating probe; R_1, recording probe; R_2, reference electrode; *L*, small ledge to prevent movement of the *Nitella* cell when the two probes are inserted; *B*, bathing solution; *P*, platinum plate; *C.G.*, stimulating current generator; E_1, electrometer measuring plant potential; E_2, electrometer measuring stimulating current. The cell is observed with a microscope using a water-immersion objective.

III. Results

(*a*) *The Action Potential of* Nitella *Cells in Culture Solution*

(i) *The Action Potential in the Vacuole.*—The action potential (its form being shown in Fig. 2) has been measured in 26 cells in the solution in which they were grown (White's nutrient solution, see Table 1). None of these cells was more than 6 weeks old, and their mean vacuole resting potential was 150 mV with a standard deviation of 5 mV. A depolarizing current pulse of 0·1 sec duration was applied to the cell through a stimulating probe causing the vacuole potential to become less negative. This applied current pulse is called suprathreshold or subthreshold depending on whether or not an action potential is initiated. When the threshold has been passed the action potential is initiated, and will then proceed independently of the stimulating current. The action potential is characterized by a rapid

rise (maximum rate 194 ± 46 mV/sec) in the vacuole potential to a mean value of −50 ± 15 mV, and then a recovery of this potential at a slower rate, to the original resting level. The maximum change in vacuole potential is 93 ± 11 mV. The least negative value of the vacuole potential was reached at a time between 0·5 and 2·0 sec after the stimulating current was applied.

Figure 3 shows action potentials recorded in four cells. Each record is formed by the superposition of several traces obtained from the one cell. It can be seen that in any one cell there is not a great deal of scatter in the action potential traces. However, there is some variation from cell to cell. This is thought to be due mainly to differences in age of cells used.

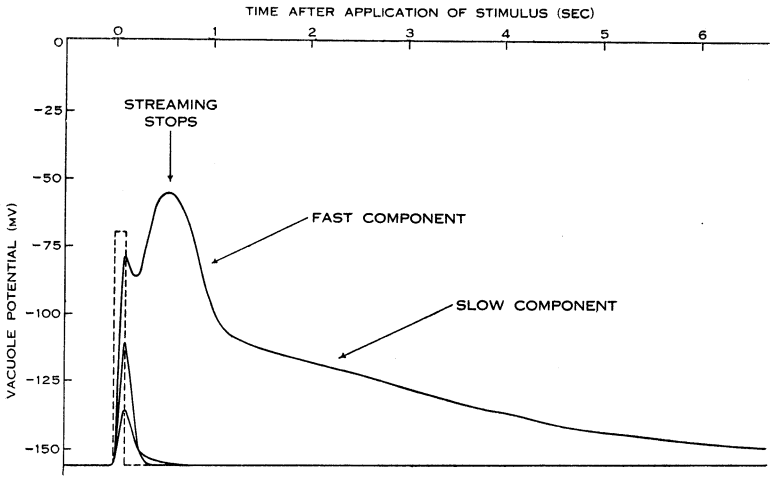

Fig.2.—The action potential measured in the vacuole of a *Nitella* cell. The response of the vacuole potential to two subthreshold pulses and a suprathreshold pulse is shown. The broken line shows the potential applied between the vacuole and the bathing solution by the stimulating current. The two components in the action potential are indicated, as well as the time of stoppage of streaming.

Two components can be identified in the action potential—a fast component and a slow component (Fig. 2). The fast component (which will be called the "spike") is of approximately 1–3 sec in duration, and appears as the fast rise and fall of the vacuole potential which occurs once the action potential has been initiated. The slow component (lasting 5–10 sec) is superposed on the fast component. The decline of this component takes the vacuole potential (from an intermediate value of −100 mV to −120 mV) back to its original resting level.

Results from three cells, which were probably older than most cells used, showed action potentials in which there was a clear separation of the two components. The fast component had occurred normally, but the slow component had been delayed for a short time (Fig. 4).

A few measurements have also been made of the action potential in cells older than 2 months. In these cells it is possible to elicit action potentials of much smaller magnitude with stimuli at, or just above, threshold. Strong stimuli, however,

cause the normal action potential. This is in contrast to the behaviour of young cells, where a stimulating pulse, if it causes an action potential at all, will always initiate an action potential of the normal magnitude.

(ii) *The Action Potential in the Cytoplasm.*—Action potentials have only been recorded in the cytoplasm of young cells 2–3 weeks old because older cells were not sufficiently transparent to allow observation of the probe tip. Measurements have been made with probes in the cytoplasmic stream adjacent to the point of probe insertion, and in the stream opposite to the point of probe insertion. In both cases records have been obtained from five cells. As only one electrometer channel was

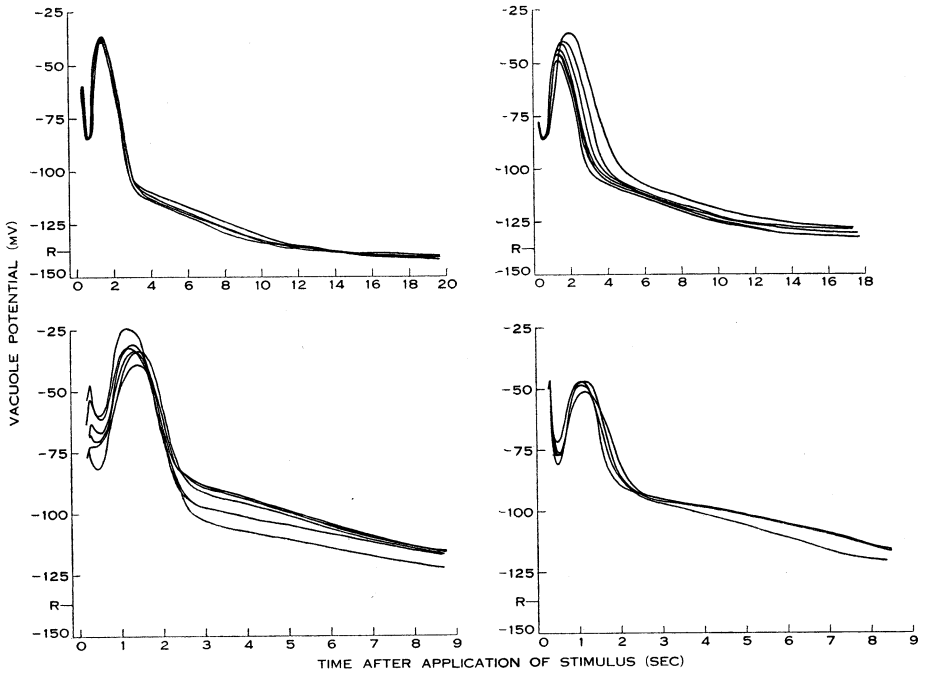

Fig. 3.—Action potentials recorded in the vacuoles of four cells. In the record for each cell, a number of action-potential traces have been superposed. All action potentials have been initiated with a 0·1 sec current pulse. The vacuole potential before the stimulus is applied is shown by the line *R* to the left of the potential axis. Note that the time scale for the two lower traces differs from the scale for the two upper traces.

available when these measurements were made, the action potential was recorded alternatively, at 10–15-min intervals, first by the probe in the vacuole and then by the probe in the cytoplasm. By comparing these two measurements it was found that the action potential in the cytoplasm was almost identical with the action potential in the vacuole. There was no significant difference between the heights of the spikes, measured in the cytoplasm and in the vacuole. Although some scatter was apparent in the records of the action potential, particularly in the recovery (the slow component) where the scatter was as large as 15 mV in both the cytoplasm and vacuole measurements, there was no consistent difference between the action

potential traces in these two regions. That is, there was no consistent potential difference across the tonoplast during the action potential.

(iii) *The Action Potential in Relation to Stoppage of Cytoplasmic Streaming.*—A series of observations on *Nitella* cells in White's culture medium has shown that in these cells the cytoplasmic streaming, so characteristic of all members of the Characeae, stops abruptly when an action potential occurs. For a wide range of stimulus strength, the time when the streaming stops corresponds closely to that of the top of the spike. It appears that the stoppage of streaming is in some way dependent on the occurrence of the action potential, and not caused directly by the application of a stimulus. Even in cases where a long current pulse is applied to the cell (see Section III(*c*)(i)) and the action potential is delayed for many seconds after the pulse has commenced, the streaming has always stopped at the top of the spike.

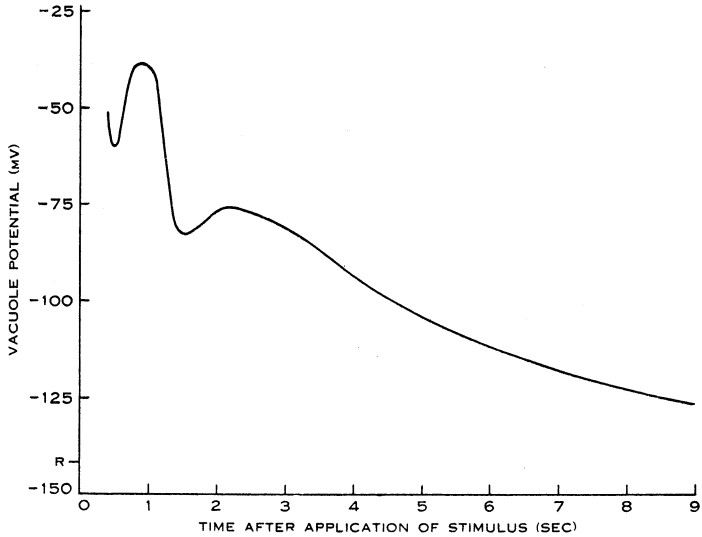

Fig.4.—Record of an action potential in which the fast component and the slow component are clearly separated.

An action potential occurring in a cell in which the cytoplasm is streaming always causes the streaming to stop. No exceptions have been noted in approximately 1500 action potentials observed. On the other hand, action potentials can be initiated in cells in which the cytoplasm is stationary. Subthreshold depolarizing current pulses and hyperpolarizing current pulses both lasting up to 30 sec do not stop streaming although they may slow it down (cf. Hill 1941). Usually the cytoplasm has regained its original streaming rate 2–3 min after an action potential has occurred.

(*b*) *The Action Potential in* Nitella *Cells in Potassium Chloride*

Before measurements were made of the action potential in *Nitella* in its White's culture medium, a preliminary investigation had been made of action potentials

in cells, cultured in White's solution, and transferred to 0·0001N KCl about 24 hr before the experiment began. During the course of an experiment lasting 3–4 hr, in which the cell was stimulated every 10–15 min, the shape of the action potential in the vacuole changed, and its magnitude declined to less than 60 per cent. of the original value, even though the resting potential remained approximately constant This behaviour contrasts with the behaviour of the cell in its culture medium, where the action potentials do not change. It is to be noted that when the solution of 0·0001N KCl bathing the cell was changed back to White's solution the action potential changed to its normal form within 1 hr.

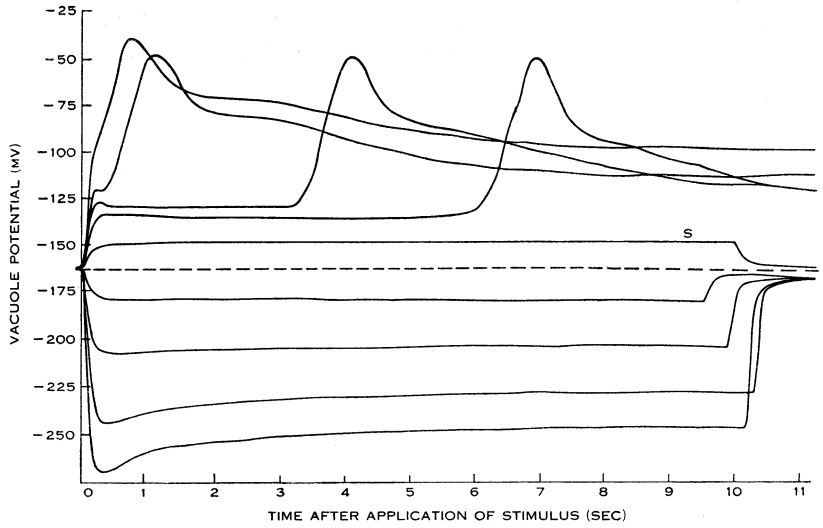

Fig. 5.—Response of the vacuole potential to the application of long depolarizing and hyperpolarizing current pulses of different intensities. The broken line shows the resting potential of the cell. The traces above this line show the response to depolarizing pulses. The curve *S* is a subthreshold response and the four upper curves suprathreshold responses. As the strength of the applied current pulses increases, the action potentials occur at decreasing time intervals from the start of the pulse. The traces below the broken line show the responses to hyperpolarizing pulses of increasing strengths—the strongest pulse producing the largest change in vacuole potential. The hyperpolarizing current pulses have terminated approximately 10 sec after application, but the depolarizing pulses (except the subthreshold pulse) still continue after 11 sec. In this cell, the vacuole potential did not overshoot its resting level when the hyperpolarizing pulse terminated.

(c) *The Effect of Long Current Pulses*

All measurements described in this section were made on cells bathed in White's culture solution.

(i) *Depolarizing Current Pulses.*—Depolarizing current pulses of several seconds duration have been passed between the vacuole and the bathing solution. These pulses, providing they were subthreshold, produced a steady change in the vacuole potential while suprathreshold pulses produced the characteristic action potential.

Measurements were made on 13 cells. Figure 5 shows the potential changes recorded in one of these cells when long current pulses ranging in strength from subthreshold to suprathreshold were applied. It was found that for suprathreshold pulses the time between commencement of the pulse and the top of the spike depended on the strength of the pulse; the stronger the stimulus the sooner the action potential occurred.

An investigation of the time, Δt, between the start of a stimulating current pulse (maintained throughout the action potential) and the top of the spike, and the current density I, yielded a relationship of a hyperbolic nature (Fig. 6). The

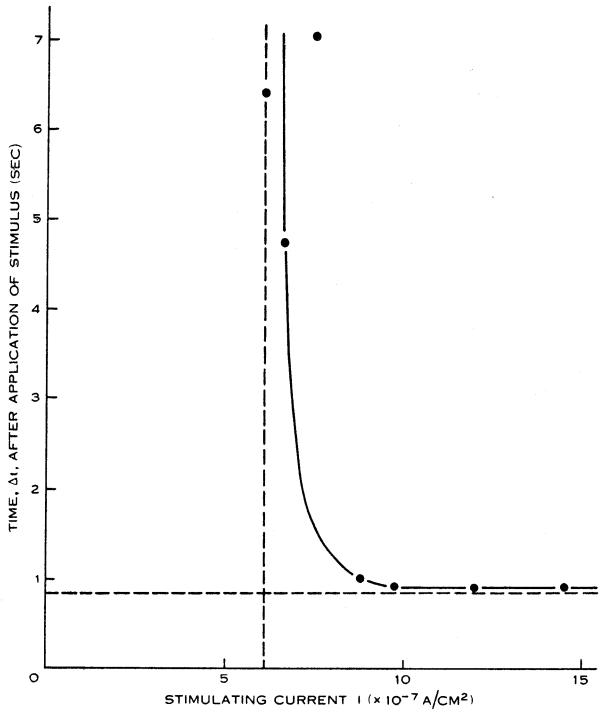

Fig. 6.—Relationship between the time, Δt, from the start of the stimulating current to the top of the spike and the current intensity, I.

asymptote intersecting the current density axis indicates the minimum current density which will produce an action potential, i.e. the threshold current density for long current pulses. For current densities just above this value it is possible to obtain large values of Δt, some being as great as 15 sec. It can also be seen that as pulse strength increases, Δt decreases to a minimum value. This means that there is a limiting time for the action potential to occur, no matter how great the stimulating current. For long current pulses, the threshold potential (the potential the vacuole takes when the stimulating current of threshold intensity is applied) is much closer to the resting potential than is the case for stimulating current pulses of 0·1 sec duration.

In some experiments on old cells, it was found that long current pulses at and just above threshold caused the slow component of the action potential to appear alone. Slightly stronger stimuli caused the slow component to begin by itself, but very soon afterwards the fast component appeared also. With strong stimuli the action potential pattern is normal.

(ii) *Hyperpolarizing Current Pulses.*—In a series of experiments, on 13 cells, rectangular hyperpolarizing electric current pulses of several seconds duration were applied to the cell (Fig. 5). Certain features of the response of the vacuole potential to the hyperpolarizing current are characteristic. First, when the current flow commences, the vacuole potential changes (approximately exponentially) to a potential more negative than the resting potential, but then declines to a potential closer to the resting potential. Secondly, when the hyperpolarizing pulse terminates the potential rises exponentially towards the resting level, but in some cases overshoots this level before finally recovering. If, in the case of overshooting, the potential rises above the threshold for stimulation, an action potential occurs.

(*d*) *The Cell Resistance*

Measurements have been made of the resistance between the vacuole and the bathing solution (White's) just outside the cell. Measurements were made on 6 cells. Long subthreshold depolarizing and long hyperpolarizing current pulses of low intensity were applied between the vacuole and the bathing solution and the change in vacuole potential recorded.

While the current flows, the potential recorded by a probe in the vacuole is the sum of the plant potential and the ohmic potential drop across the resistance between the vacuole and the bathing solution. A correction is made for the potential across the bathing solution in the following way. Before the recording probe is inserted into the cell, its tip is placed just outside the cell, in the bathing solution. Electric current is passed by the inserted stimulating probe, and a potential change is shown by the recording probe. The recording probe is then inserted into the cell, and further measurements made. The difference between the internal and external potential measurements is the potential change between the vacuole and a point just outside the cell caused by the current flow.

The cell resistance was defined as the ratio of the potential change to the magnitude of the applied current. For depolarizing current pulses the resistance was constant during the current flow. For changes in vacuole potential up to 40–50 mV, the resistance is approximately linear. For six cells, the mean value of the resistance was $(2{\cdot}7 \pm 1{\cdot}2) \times 10^4\ \Omega\ \mathrm{cm}^2$.

However, for hyperpolarizing constant current pulses, the vacuole potential varies during the application of the pulse (Fig. 5). Nevertheless, the cell resistance defined in terms of either the initial or the final potential change is greater than the resistance determined with a depolarizing current. The resistance defined in terms of the final potential change has a value $(4{\cdot}6 \pm 1{\cdot}2) \times 10^4\ \Omega\ \mathrm{cm}^2$. Figure 7 shows the relationship between the change in vacuole potential, and the applied current, an abrupt change of slope appearing at the resting potential.

Some preliminary investigations have also been made of the change in total cell resistance during an action potential. A suprathreshold current pulse was applied to the cell. This pulse was turned on and off four times per second. At this frequency capacitive effects of the membrane are negligible. The amplitude of the oscillation of the vacuole potential so obtained is the potential drop between the vacuole and the bathing solution, caused by the flow of the applied current of constant amplitude and consequently is proportional to the resistance between the vacuole and bathing solution. A correction can be made for the resistance of the bathing solution, and thus the cell resistance can be determined. The resistance decreases greatly during the rising phase of the action potential and recovers to approximately its normal

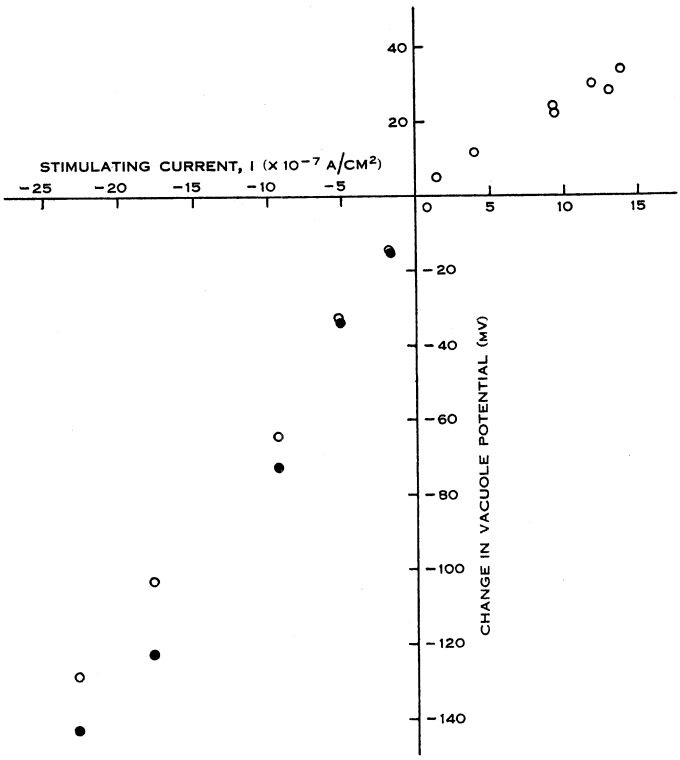

Fig. 7.—Relationship between current flowing between vacuole and bathing solution, and the change in vacuole potential. Depolarizing current is taken as positive. ○ Final change in the vacuole potential. ● Initial change in vacuole potential when a hyperpolarizing current is applied.

value before the slow component of the action potential starts to decline. However, accurate measurements of the minimum resistance are difficult to obtain, because the amplitude of the potential oscillation becomes very small when the cell resistance decreases. From measurements made on six cells, it was found that the cell resistance decreased to possibly less than 1 per cent. of the resting resistance (cf. Cole and Curtis 1938), although not much weight is given to this value. The

use of a voltage clamp method (Hodgkin, Huxley, and Katz 1952) for measuring the cell resistance during activity is at present under consideration.

IV. Discussion

In Section III it was seen that the action potential in a *Nitella* cell is located at the outer cytoplasmic boundary—the plasmalemma. Walker (1955) has shown that the resting potential also is located at the plasmalemma. Thus, when the cell is in both resting and active conditions, practically no potential difference is maintained, or appears, across the inner cytoplasmic boundary—the tonoplast. These results make untenable the theory put forward by Osterhout (1927, 1934) to account for the action potential in *Nitella*, in which it was assumed that the major part of the resting potential of the cell was located at the tonoplast, and that this potential changed during activity.

It appears that the tonoplast does not play a direct role in the steady and transient electrical behaviour of the cell. Its importance is most probably in relation to ion-carrier processes. For *Nitellopsis obtusa*, MacRobbie and Dainty (1958) have suggested that a Cl^- pump is located at the tonoplast. Although the *Nitella* used in experiments described in this paper grows in fresh water, while *Nitellopsis obtusa* grows in brackish water, an analysis of vacuolar sap, made in this Laboratory and elsewhere shows that Cl^-, and probably other ions, are not in electrochemical equilibrium between the vacuole and outside medium, and consequently need to be actively transported into the vacuole. It is likely that the tonoplast is involved in such transport.

MacRobbie and Dainty (1958) have also shown that in *Nitellopsis obtusa* the resting flux of K^+, Na^+, and Cl^- ions across the tonoplast is 20–100 times lower than the flux of these ions across the plasmalemma. A similar situation probably obtains in the *Nitella* used in experiments described in this paper. As also shown in this paper, the action potential occurs across the plasmalemma, and consequently involves considerable changes in ionic fluxes across this membrane but probably not across the tonoplast. Hence, the tonoplast appears to play little direct part in the short term electrical changes which comprise the action potential. One is thus led to the conclusion that a three-phase system, consisting of cytoplasm–plasmalemma–outside medium, controls the transient electrical behaviour of the cell. Unfortunately, very little information on the ionic composition of the cytoplasm is available.

In Sections III(*a*) and III(*b*) it was seen that in *Nitella* cells in their culture medium the action potential has two components. In young cells both components always occur, but in some older cells it is possible to obtain the slow component by itself. It is possible that future investigations will be able to associate with each component the movement of a particular ion across the plasmalemma. This has been done successfully in squid axon (Hodgkin, Huxley, and Katz 1952) where it has been shown that the movement of Na^+ ions into the axon causes the initial rise in membrane potential, and a later movement of K^+ ions outwards causes the recovery with an overshoot past the resting level.

Osterhout (1934, 1936, 1944, 1947) and Osterhout and Hill (1935) have obtained records of action potentials in *Nitella flexilis* which show two components, but the

first component was very short (0·5 sec) and the slow component had a greater magnitude than that measured by this author. Measurements of action potentials in *Nitella opaca* and *Nitella mucronata* made by Umrath (1932*a*, 1932*b*, 1953, 1954) also suggest two components. Records obtained by Oda (1956) on *Chara braunii* and by Sibaoka (1958) on *Nitella flexilis* show two components clearly. On the other hand, the action potential in *Nitella flexilis* recorded by Cole and Curtis (1938) does not show two components.

For *Nitella* grown in White's solution and in tap water, the magnitude of the action potential measured in these culture media is always less than the resting potential, and consequently the vacuole potential does not become positive. However, some measurements of action potentials (not described in this paper) which have been made on cells in White's solution 10–30 times the strength of the culture solution show some overshoot of the zero potential. Fujita and Mizuguchi (1955) have reported overshooting by 20 mV of the zero potential level in *Nitella*, and Oda (1956) observed overshoots of 50 mV in *Chara braunii*, but in the latter case, no details are given of the concentrations of culture solution and bathing solutions.

V. Acknowledgments

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VI. References

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