MICROELECTRODE EXPERIMENTS ON *NITELLA*

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Summary

Tubular glass microelectrodes containing concentrated KCl solutions have been inserted into internodal cells of *Nitella*, under adequate visual control, and the electric potentials of cytoplasm and vacuole measured. The potential of the cytoplasm is found to be close to that of the sap. For one of the groups of cells studied, this potential was − 160 mV referred to the solution bathing the cell when this was 0·0001N KCl. Changes in the KCl concentration of this solution caused the potential of the cytoplasm to change in a similar way to that of the sap.

The resting potential difference between the interior and exterior of the *Nitella* cell is thus presumably located at the cytoplasmic outer membrane, as are the potential changes caused by changing KCl concentrations.

The healing process which involves the sealing off of an inserted electrode has been photographed, and simultaneous potential measurements made.

The significance of these results is discussed in the light of present knowledge of the *Nitella* cell.

I. INTRODUCTION

While considerable progress has recently been made towards an understanding of the relation between potential differences and ion concentrations in nerve and muscle, less is known of plant cells. This is so even for *Nitella*, which, like other algae with large coenocytes ("giant cells"), has been extensively studied. The present state of knowledge has been reviewed by Hope and Robertson (1953). Even the individual p.d.'s across the chief cellular membranes, the plasmalemma and tonoplast (i.e. the cytoplasmic outer membrane and the vacuolar membrane, respectively) are not certainly known. This paper represents an attempt to find these quantities.

The p.d. between the vacuole of *Nitella* internodal cells and the medium outside has been measured by Osterhout (1927, 1929, 1930, 1934, 1936, 1949, 1954) under a variety of conditions. He used a method whereby electrical contact with the vacuole was made at a point where the cytoplasm was killed with chloroform. The method is convenient, but open to the objection that the cells do not survive more than a few hours after the experiment begins. It is not capable of measuring the p.d. between cytoplasm and outside medium, and this quantity is of considerable interest if we are to know how the p.d.'s arise, and what their changes (action potentials, concentration effects, potassium effects, etc.) are due to. Various experimenters have also impaled algal cells on

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coarse capillary tubes, but these have again made contact with the vacuole only. In addition, the normal hydrostatic pressure in the cell is released, leaving it in an abnormal state.

Umrath (1930, 1932, 1934, 1953) has made many experiments on *Nitella* internodes using inserted microelectrodes, and is of the opinion that the p.d. he measured is that between the cytoplasm and the outside medium. His results are rather similar to those of Osterhout on the p.d. between sap and outside medium, and for this reason it is important to know whether the tips of his microelectrodes were in fact in the cytoplasm. The evidence for this in Umrath's papers (Umrath 1930, p. 583) is not entirely convincing, as his use of mature, opaque cells, and a dissecting microscope of limited magnification seems to have made direct visual observation of the position of the microelectrode tip very difficult. It is in any case obviously desirable to measure the vacuolar and cytoplasmic potentials in the same cell, or at least in similar ones and under the same conditions, if they are to be compared. It may be noted that while Osterhout used *Nitella flexilis*, Umrath used *N. mucronata* and *N. opaca.*

In experiments described in this paper, young, transparent *Nitella* internodes were used, and the inserted microelectrode tip observed at high magnification (× 500-1300) in an apparatus of suitable geometry. The location of the tip, either in cytoplasm or sap, could thus easily be distinguished, as seen in the photomicrographs (see Plate 1, Figs. 1 and 2).

The p.d.'s between sap and outside medium, and between cytoplasm and outside medium, were measured on a uniform group of cells of *Nitella* under constant conditions. Often both p.d.'s were measured on the same cell.

It is found that the large p.d. (in these experiments 100-170 mV) between cell interior and outside medium is located at the plasmalemma, the p.d. across the tonoplast being at most a few mV.

The sealing off of an inserted microelectrode, the effects of which on the measured potential were described by Umrath (1932), has been observed and photographed (see Plate 1, Figs. 3 and 4) simultaneously with potential measurements.

In describing the results of these investigations, the arbitrary zero of electric potential is taken as the potential of the solution bathing the cell. Potentials in the cell interior are referred to this zero. Thus in this paper the "potential of the vacuole" is exactly equivalent to the "potential difference between the vacuole and the medium bathing the cell."

II. Methods

Rooted plants of *Nitella* sp. (probably *N. gloeostachys* A.Br.) were grown in a concrete tank in a warm greenhouse, and in glass pneumatic troughs in the laboratory at 15-25°C. The troughs stood at a north window, but an external blind shaded them from direct sunlight for most of the day. The plants were rooted in a 1-cm layer of sand and garden soil at the bottom of the vessels, which were stocked with snails.

Only young, transparent, internodal cells were used in experiments; they were generally 0·1-0·3 mm in diameter and 3-10 mm long. They were prepared for experiment by cutting off the whorl cells ("leaves"), leaving the apical bud and one basal (mature) internode intact. The preparation was left in 0·0001N KCl solution for about 24 hr after cutting, then mounted on a "Perspex" slide, part of the basal internode being covered with "Vaseline" to secure the preparation in place. A low "Perspex" shelf about 0·4 mm high prevented the experimental cell from moving under the thrust of the microelectrode. This is shown in Figure 1. The "Perspex" slide held 1-2 c.c. of solution bathing the cell, and a steady flow of fresh solution was maintained at 0·3-2 c.c./min as desired. This solution was always 0·0001N KCl, except during experiments on changes of potential caused by changes of external KCl concentration.

Experiments began at least 12 hr after the preparation was mounted on the slide, and continued on each cell for 3-8 days. Cells generally lived indefinitely after the experiments.

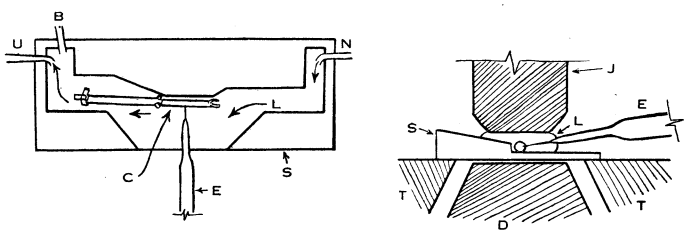

Fig. 1.—(Not to scale). (*a*) Plan of experimental arrangement. (*b*) Section. *C*, *Nitella* cell; *E*, microelectrode; *S*, "Perspex" slide; *L*, bathing solution; *N*, inflow of fresh solution; *U*, outflow of solution; *B*, agar salt bridge; *J*, microscope objective; *T*, microscope stage; *D*, microscope condenser.

The "Perspex" slide was set on the stage of a high-power microscope. The insertion of the microelectrode and the course of the experiment were observed and photographed through a water-immersion objective (× 50) which dipped directly into the bathing solution. A polystyrene collar insulated the objective from the earthed microscope body. A standard low-voltage research lamp was used, fitted with a 3-mm thick heat-filter glass.

Potential differences were measured with an accuracy of ± 3 mV with a valve electrometer. This used a 1S4 valve as an electrometer pentode, operated with low filament, anode, and screen potentials, and biased near the floating grid potential to reduce grid current. The electrometer leads were connected to two symmetrical calomel half-cells. The earthed half-cell was connected to the solution bathing the *Nitella* cell by a 0·1N KCl-agar bridge. The other dipped into the solution filling the microelectrode shank. Experiments indicated that, as expected, the p.d.'s at the KCl-agar junctions were small, and they were therefore neglected. The apparatus was provided with a sufficient electrostatic shield, so that electric pickup effects were negligible.

Microelectrodes of the capillary type were made on a de Fonbrune microforge from "Pyrex" glass tubing of 6 mm outside diameter. While tip diameters of 2-10 μ were used, most were of 3 μ. Tips of smaller diameter than 2 μ were unable to penetrate the cell wall without breaking. An abruptly tapering tip proved to be best, as the hole made in the cell wall was then more effectively sealed. The tips were accordingly drawn in the air stream from the microforge blower.

The shank of the electrode was filled with KCl solution from a pipette, and the solution was forced through to the tip by applying air pressure. The electrode was then used at once, and generally for one insertion only.

In some experiments a microelectrode of 10-20 μ outside diameter was arranged to have a movable glass fibre of 3-5 μ diameter along its axis. The fibre was moved axially by a metal bellows fixed to the micromanipulator holding the electrode. A polythene tube connected the bellows to one of the transmitter pistons of a de Fonbrune micromanipulator. The arrangement is shown in Figure 2. Particles of matter blocking the tip orifice of the microelectrode could be dislodged with the movable plunger.

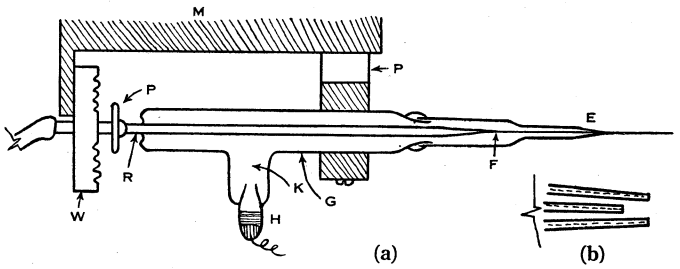

Fig. 2.—(Not to scale). (*a*) General arrangement of microelectrode with plunger. (*b*) Tip of microelectrode enlarged. *E*, Microelectrode; *G*, "Pyrex" glass T-piece; *K*, KCl solution; *H*, calomel half-cell; *R*, "Pyrex" glass rod drawn down to fibre; *F*, glass fibre; *W*, metal bellows; *M*, micromanipulator; *P*, polystyrene insulation.

III. Results

(*a*) *Injury of Cell*

The insertion of a microelectrode of course injured the cell, but in general there was very little loss of the cell contents, the electrode filling the gap made in the cell wall. Cyclosis nearly always ceased, but often commenced in 1 min, and was normal after 2-4 min. Cells would generally live indefinitely after the experiment, after as many as six or eight insertions, so they are not considered to have been irreversibly injured by an insertion. This is supported by the facts that the potential of the vacuole reached a steady limiting value within a few minutes in most cases, and that generally reproducible results were obtained by successive insertions into the same cell. The method of avoiding injury to the cell when the electrode was withdrawn is described in Section III (*c*). Re-

sults obtained on cells which died during the experiment were not automatically rejected, but were carefully examined for abnormal features.

(*b*) *Insertions into Cytoplasm and Vacuole*

If a shallow insertion of the electrode were made, i.e. to a depth of 20-30 μ, the tip would generally penetrate into the vacuole, but it would be covered by the cytoplasm as soon as cyclosis began. This is clearly shown in Plate 1, Figures 1 and 2, which were taken immediately after the insertion and 3 min later respectively. This type of insertion was used if the potential of the cytoplasm was to be measured. When the potential of the vacuole was required, the electrode was inserted more deeply, i.e. 50-100 μ. In this case, a considerable time, often an hour or more, would elapse before the electrode tip was covered with cytoplasm, and during this period the potential of the vacuole could be measured. No matter to what depth the electrode was inserted, it was eventually covered with cytoplasm, which crept up until it covered the tip. It was not easy to make insertions to a predetermined depth, and it was not found possible to dispense with high-power visual observations of the electrode tip, if cytoplasm and vacuole readings were to be distinguished.

(*c*) *The Healing Reaction and the Sealing Off of an Electrode*

If the microelectrode did not completely close the hole made in the cell wall, the loss of cell contents was immediately stopped by the accumulation of debris at the hole. The protoplasm then deposited a layer of refractile substance between itself and the debris. This seems to agree with the description by Nichols (1925) for much larger wounds which took longer to heal. The refractile substance was then laid down over the inserted part of the microelectrode, beginning at the cell wall and progressing inwards until the tip was covered. The electrode was then sealed off from the cytoplasm by this layer, which is visible in Plate 1, Figures 3 and 4. It varied in thickness, being sometimes too thin to be clearly seen (as in Plate 1, Fig. 3) and sometimes much thicker as in Plate 1, Figure 4. By comparing its appearance with that of the cytoplasm and of the glass of the electrode, the refractive index of the sealing substance was estimated at 1·45 to 1·5. Such a refractive index might be attained by, for example, a body consisting of more than 60 per cent. of protein, the remainder being water (extrapolating from Barer and Tkaczyk 1954). A seal of moderate thickness was stiff enough to retain its shape when the electrode was withdrawn, so that no loss of cell contents occurred on withdrawal. This was important in obtaining reproducible results from successive insertions into a single cell. The seal was accordingly always allowed to form before each electrode was withdrawn from the cell.

To anticipate the results reported below, an electrode whose tip was in either cytoplasm or sap recorded a potential of about -160 mV. At about the time when the seal was visibly complete over the electrode tip the magnitude of the recorded potential was fairly suddenly reduced by 30-40 mV, and thereafter remained at the lower value. It was by this reduction of the measured potential and by the subsequent inability of the electrode to record action poten-

tials, that Umrath (1932) detected the sealing process. He referred to it as "die Bildung von Plasmalemma" and investigated the time taken for it to occur, with different solutions in the microelectrode. He may have observed it visually.

In the present experiments the time taken for the seal to be completed varied from a minute to several hours, depending largely upon the depth of insertion of the electrode. Generally it was first deposited around the electrode just inside the cell wall, and then progressively towards the tip: if the tip pro-

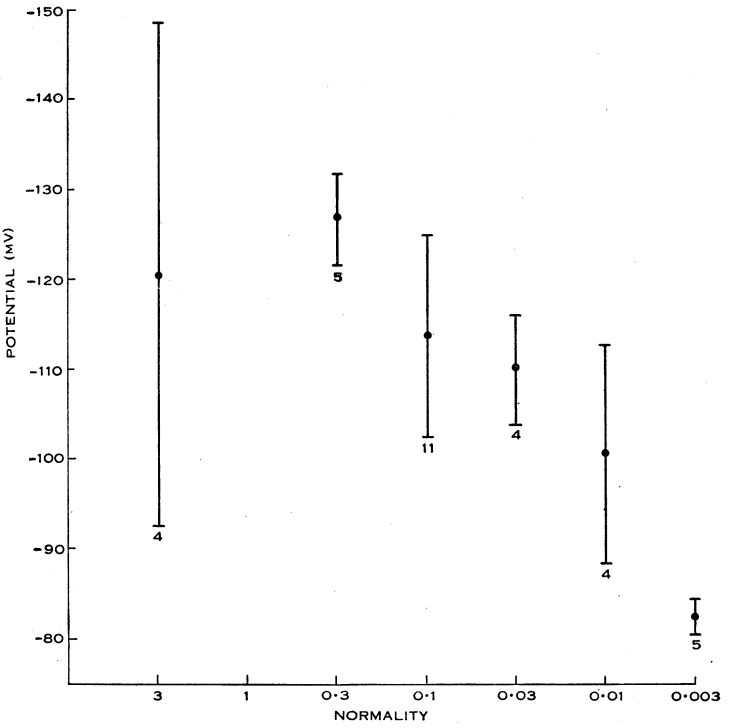

Fig. 3.—Mean potential recorded by an electrode sealed off in the cytoplasm, plotted against the concentration of KCl in the solution filling the electrode. The vertical lines indicate the 95 per cent. confidence limits for the mean of the population sampled: the numerals underneath each point show the number of experiments averaged. Cell bathed in 0·0001N KCl. (See Plate 1, Figs. 3 and 4.)

jected into the vacuole, cytoplasm would creep up it ahead of the seal, which was, of course, not deposited by the sap. In some shallow insertions, however, the seal seemed to be rapidly deposited around the whole electrode tip simultaneously.

As Umrath observed, there was often considerable fluctuation in the measured potential just before and after the seal was completed. This will be discussed below. After some time, however, the potential settled down to a steady value, which was fairly reproducible, and varied with the concentration of KCl

in the electrode in the manner shown in Figure 3. A comparison of Figures 3 and 4 shows that the mean change in potential upon completion of the seal was close to 35 mV with all the KCl concentrations used in the electrodes (Fig. 3 corresponds with the appearance shown in Plate 1, Figs. 3 and 4; and Fig. 4 with that in Plate 1, Fig. 2).

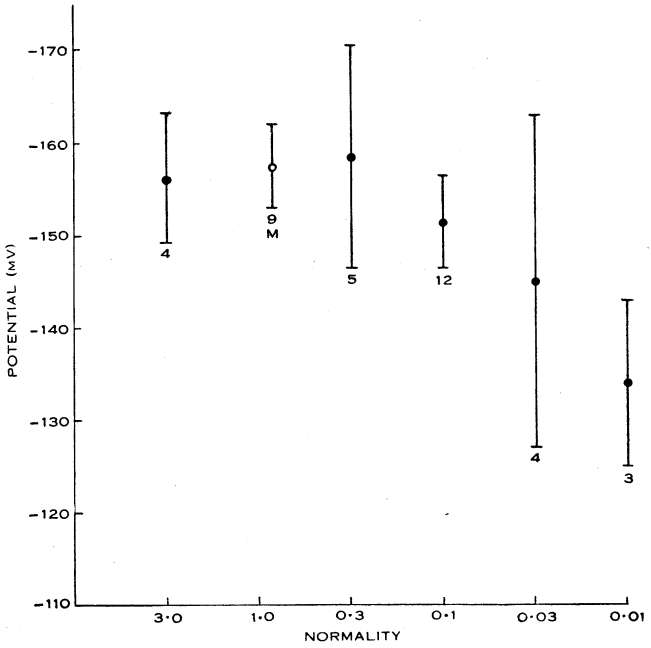

Fig. 4.—Mean potential characteristic of the cytoplasm plotted against the concentration of KCl in the solution filling the electrode. The vertical lines indicate 95 per cent. confidence limits: the numerals show the number of experiments contributing to each mean. The entry marked *M* is the mean of all the experimental values for 3·0 and 0·3N KCl, and is taken as the mean potential of the cytoplasm. Cell bathed in 0·0001N KCl. (See Plate 1, Fig. 2.)

(*d*) *The Potential of the Vacuole*

When the tip of the microelectrode was inserted deeply into the vacuole, the recorded potential increased sharply and then slowly, reaching a steady value in a few minutes or less. This value, about −160 mV, was then maintained until the electrode tip was covered with cytoplasm, except that small slow variations sometimes occurred.

The potential recorded by an electrode in the vacuole will approximate to the potential of the vacuole if the junction p.d. between sap and electrode filling is small. Analyses of *Nitella* sap reported by Brooks and Gelfan (1928) and Osterhout (1931) show it to contain approximately 0·1N Cl^- and 0·05-0·07N

K^+, the balance being largely Na^+, Ca^{++}, Mg^{++}, with a very small content of organic matter; the pH is about 5. Such a solution may be assumed to have a small junction potential against 0·1N or stronger KCl if no membrane separates the two solutions.

The liquid in the electrode interior was in fact separated from the vacuolar sap by particles of matter which blocked the tip. Upon the insertion of the tubular microelectrode into the cell, the high osmotic pressure in the interior (above 6 atm) drove sap into the tip, which was immediately blocked by particles suspended in the sap. Alternatively, the tip was sometimes blocked by a fragment of cell wall.

To test whether these blockages caused an appreciable membrane potential, several microelectrodes of 10 and 20 μ diameter, fitted with plungers (as in Fig. 2) were inserted into cell vacuoles. After the insertion the plunger could be

TABLE 1

POTENTIAL OF VACUOLE

Concentrations of KCl in Electrode	Mean Vacuole Potential* (mV)	95 Per Cent. Confidence Limits (mV)	Number of Experiments
3N	−160	—	1
0·3N	−160	± 6	5
0·1N	−160	± 6	8
0·03N	−162	±30	3
0·01N	−157	±23	3
0·003N	−164	±12	3
All concentrations	−160	± 4	23

* Cells bathed in 0·0001N KCl solution.

moved forward to dislodge momentarily the matter closing the tip. This produced no changes in the measured potential greater than 3 mV.

In Table 1 are shown the results of a number of experiments in which the microelectrode tip was in the vacuole (as in Plate 1, Fig. 1, but deeper). The microelectrodes were filled with KCl solutions of various concentrations. The lack of any trend in the mean potential as the concentration changes suggests that there is no junction p.d. at the electrode tip with any of the concentrations used: accordingly the results are pooled, and a mean vacuolar potential calculated from them.

These results were obtained in spring and early summer 1954, on a uniform group of 11 cells, all young internodes taken from a single culture vessel. The results on the potential of the cytoplasm described below were obtained from the same group of cells, each cell being used for a number of successive insertions.

(*e*) *The Potential of the Cytoplasm*

As mentioned in Section III (*b*) above, a shallow insertion resulted in the tip of the microelectrode being immersed in the flowing cytoplasmic phase (cf. Plate 1, Fig. 2). During the period before the seal was complete, a potential characteristic of the cytoplasm was recorded, and with concentrations of 0·1-3N KCl in the electrode this potential was moderately steady and reproducible. When solutions more dilute than this were used, the seal often formed very rapidly, and even before it appeared complete, transient reductions in the magnitude of the potential were observed. These fluctuations had the same direction and about the same magnitude as the permanent fall of potential due to the seal; they are therefore thought to be due to transient formations of the seal over the electrode tip. Accordingly, in such a case the maximum observed value was taken as the potential characteristic of the cytoplasm.

In Figure 4 the mean potential characteristic of the cytoplasm is plotted against the concentration of KCl in the electrode filling solution. It will be seen

TABLE 2

KCL CONCENTRATION EFFECTS

Potential Measured	Mean Change in Potential* (mV)	Standard Deviation (mV)	Number of Values Averaged
Vacuole	38	8	6
Cytoplasm	35	8	10
Cytoplasm (tip sealed)	37·5	9	14

* As the external KCl concentration is changed from 0·0001N to 0·001N.

that there is a distinct trend in the mean values from − 159 mV at 0·3N to − 134 mV at 0·01N. It is to be expected that the best approximation to the potential of the cytoplasm will be that recorded by an electrode containing strong KCl, and the agreement between the mean values for 3 and 0·3N KCl supports this. Consequently, the mean of all the determinations using electrodes filled with 3 and 0·3N KCl is taken as the mean potential of the cytoplasm in the cells measured. This mean with its 95 per cent. confidence limits, -158 ± 5 mV, is shown as the entry marked *M* in Figure 4.

(*f*) *KCl Concentration Effects*

Changes in the resting potential of the *Nitella* cell, due to changes in the concentration of KCl solution applied to the outside, are well known, and have been investigated by Osterhout (1929) and others. Osterhout concludes that they are changes in the p.d. across the plasmalemma, because of the rapidity with which the new value of the potential is arrived at. This is shown in the records reproduced in Osterhout (1930).

A series of experiments was made in which the concentration of KCl in the solution bathing the cell was changed while the changes in the potential recorded by an electrode in the sap, the cytoplasm, or sealed in the cytoplasm were observed. The new concentration was more slowly established than in Osterhout's experiments, taking 1-2 min (concentration increasing) or 2-3 min (concentration decreasing).

The results indicate that, as a first approximation, there is no difference between the responses of the potentials of vacuole or cytoplasm (see Table 2).

The method of successive vacuolar and cytoplasmic measurements is unsuitable for investigations of detailed differences between the two. It can, however, be fairly concluded that the main potential changes due to KCl concentration changes occur at the plasmalemma, in agreement with Osterhout's conclusion.

It may also be noted that a sealed-off electrode still records these changes with their full magnitude.

As in Osterhout's experiments, the relation between the cell potential and the logarithm of KCl concentration was found to be roughly linear between 0·0001 and 0·01N. The change in potential for ten-fold change in KCl concentration was smaller than that found by Osterhout for most *Nitella* cells, and more like the values he found for cells leached with distilled water (Osterhout 1949). This may be due to the treatment of the cells used here with 0·0001N KCl.

TABLE 3

SUMMARY OF MAIN NUMERICAL RESULTS

Cell Part Potential*	Mean Value (mV)	95 Per Cent. Confidence Limits (mV)
Potential of vacuole	−160	±4
Potential of cytoplasm (p.d. across plasmalemma)	−158	±5
Potential difference across tonoplast	*c.*0	

* All cells bathed in 0·0001N KCl.

IV. DISCUSSION

The main numerical results are summarized in Table 3.

The quoted 95 per cent. confidence limits are derived from the experimental values: they express the uncertainty of the quoted mean due to random experimental errors and the inherent spread in the potentials of the cells measured. In addition there are possible systematic errors of up to 3 mV due to errors in electrometer calibration, and of the same order due to possible small junction p.d.'s at the microelectrode tip. As these latter will probably be different for cytoplasm and sap, they affect the value of the p.d. across the tonoplast. It is, however, concluded that both the resting potential of the cell, and the potential

changes due to changing ionic environment, have their seat at the plasmalemma, the p.d. across the tonoplast and its changes being of a much smaller order of magnitude. This is established for the *Nitella* species used, under the conditions of measurement; but it is probably also true of this species in its natural environment and for other species of Characeae.

These results do not conflict with those of Osterhout on the vacuolar potential in *N. flexilis*, or with those of Umrath on *N. mucronata*. However, both the steady value of the vacuolar potential, and the time course of the measured potential just after an insertion, as described here, are quite different from those reported by Bennett and Rideal (1954). Their paper is primarily concerned with resistance and capacity measurements, but they mention a measurement of vacuolar potential in which a maximum value was reached immediately after the insertion, the potential then falling in a few minutes to about 18 mV (the sign of the potential is not given). It is not stated whether the potential was measured with the Ag/AgCl electrodes used for the resistance measurements, or what solution bathed the cell; so that a detailed examination of the reasons for the differences cannot be made. However, an Ag/AgCl electrode inserted into the vacuole of a *Nitella* cell (say 0·1N Cl^-) might be expected to be more negative than a similar electrode in the bathing solution (say 0·001N Cl^-) by 120 mV (Butler 1951, p. 16) plus the normal vacuolar potential, i.e. by about 300 mV. Bennett and Rideal's result is thus not explained simply on the basis of their use of Ag/AgCl electrodes.

The result reported in the present paper does not provide a basis for deciding which of the various theories of the origin of bioelectric potentials applies to *Nitella*. It is, however, a small part of the body of experimental knowledge which must be built up before such a decision is possible.

The present result does however conflict with the detailed hypothetical scheme of Osterhout (e.g. 1949, p. 555; 1954), as this involves the p.d. across the tonoplast being normally large (equal to the resting potential of the cell) while that across the plasmalemma is normally small. This postulate accounts for the results of his leaching experiments (1934; 1949, p. 552) on the basis that bioelectric potentials in *Nitella* are largely KCl diffusion potentials. In these leaching experiments, the response to externally applied KCl solutions (from which the mobility ratio of K^+ and Cl^- ions can be calculated from diffusion potential theory) was modified drastically by treatment with distilled water, without altering the resting potential (whence the mobility ratio can also be calculated). Osterhout assumes that the resting potential and the potassium response are properties of separate membranes, the tonoplast and plasmalemma respectively, and it is this which now seems untenable. While it is not ruled out, the diffusion potential hypothesis is made more difficult to apply.

It has been suggested that plant cytoplasm contains a high concentration of immobile anions (e.g. Hope and Robertson (1953), and references cited therein), and that a Donnan equilibrium is set up between the medium surrounding the cell and the cytoplasm. The existence of a large p.d. at the outer cytoplasmic surface, under the conditions of the present experiment, is consistent with such a view. In an attempt to obtain confirmation, the equation of

Teorell (1935), which is based on Donnan considerations, might be used to treat the p.d. observed between an electrode containing 0·3N KCl, inserted into the cytoplasm, and the solution (0·0001N KCl) surrounding the cell. If the mobilities of K^+ and Cl^- are assumed equal in cytoplasm, as is nearly true in water, the observed p.d. of − 158 mV would lead to a value of about 0·06N for the concentration of immobile anions in the cytoplasm. If this result is then used to predict the p.d's which should be observed when other concentrations of KCl are used in the electrode, the agreement with the observed values (Fig. 4) is not good (see Table 4).

Little better agreement with the values given in Figure 4 can be obtained if the mobility of K^+ is assumed less than that of Cl^- (see Table 4); good agreement cannot be obtained for any values of X and u/v. These discrepancies are perhaps not serious, as the mean values in Figure 4 have fairly large confidence limits, and as the exact concentration of KCl in the electrode tip is somewhat uncertain, due to probable diffusion of KCl across the boundary. More serious is the fact that the Teorell equation would require the observed

TABLE 4

COMPARISON BETWEEN OBSERVED POTENTIAL IN CYTOPLASM AND THEORETICAL POTENTIAL CALCULATED FROM TEORELL'S EQUATION

Concentration of KCl in Electrode	Mean Observed Potential in Cytoplasm (Fig. 4) (mV)	Potential (mV) Calculated from Teorell's Equation with Following Values:		
		$X = 0{\cdot}06N$ $u/v = 1$	$X = 0{\cdot}095N$ $u/v = \frac{2}{3}$	$X = 0{\cdot}13N$ $u/v = \frac{1}{2}$
3·0N	−156	−161	−150	−146
0·3N	−158	−158	−158	−158
0·1N	−151	−154	−156	−159
0·03N	−145	−139	−140	−144
0·01N	−134	−115	−115	−119

p.d. to change by 58 mV for each ten-fold change in the concentration of KCl bathing the cell, at the bath concentrations used. Such is not the case for the cells used in these experiments (see Table 2). Osterhout has indeed found this "potassium electrode" behaviour in many *Nitella* cells, but in his leaching experiments has been able to modify this independently of the total p.d. Neither Teorell's equation nor the modified Donnan theory of Vervelde (1953) provide an explanation of these leaching experiments, meeting here the same difficulty as the diffusion potential theory.

As Hope and Robertson have pointed out, we do not know whether there are in plant cells specific ionic pumps, comparable with the sodium pump postulated in nerve fibres. An approach to this problem is the determination of the electrochemical potentials of the various ions in the phases of the system of cell and environment (Ussing 1954). This is approximately achieved if ionic con-

centrations in, and electric potentials of, the various phases can be measured. The present technique has given these potentials for *Nitella,* but ion concentration measurements in the cytoplasm are unfortunately not easy. Work has begun in this Laboratory on the potentials and ion concentrations in the sap of *Nitella* and its environment, as a start on the problem. The concentrations of free ions in the cytoplasm have never been measured satisfactorily, although there are indications that the total concentration is fairly high. Thus Gelfan (1928) found the electric conductivity of *Nitella* cytoplasm to be nearly as high as that of the sap (approx. 0·005 and 0·008 ohm^{-1} cm^{-1} respectively). Bennett and Rideal (1954) quote evidence that the osmotic concentration in the cytoplasm is about equal to that in the sap. A single chemical determination reported by Holm-Jensen, Krogh, and Wartiovaara (1944) suffers from an unknown dilution of the extracted protoplasm with sap: the proportion of ions which are free in solution is also unknown.

The fall in the measured potential, when an inserted microelectrode is sealed off, was explained by Umrath (1932) as due to a combination of high electric resistance of the seal, and electric leakage along the surface of the microelectrode. If this were so, one would not expect the final potential to reach a reproducible value: and changes in the potential of the cytoplasm would produce smaller changes in the potential of a sealed electrode. Reference to Figure 3 shows that the final potential is moderately reproducible; and Table 2 shows that a sealed electrode reproduces, undiminished, changes in the potential of the cytoplasm. It seems most probable that the fall in observed potential when the seal is complete is due to a p.d. across the seal layer itself.

The nature of the substance forming the seal is unknown. It seems unlikely, however, that it is identical with the outer gel-like layer of cytoplasm ("ectoplasm"—Frey-Wyssling 1948, p. 126), as the seal has a clearly marked interface with the liquid cytoplasm (Plate 1, Figs. 3 and 4). No such interface appears to occur between the gel-like and sol-like cytoplasm in *Nitella,* either visually or in the electronmicrographs of Mercer *et al.* (1955). The seal might consist of a more concentrated cytoplasmic gel, of denatured protein, or of a cell wall substance.

The sealing process is of practical importance, as a factor in all microelectrode experiments on *Nitella,* and probably on other plants as well; Umrath (1932, p. 187) mentions its rapid occurrence in experiments with *Spirogyra* and *Elodea* cells. It should be watched for if its occurrence is not to complicate or invalidate the results of such experiments.

V. Acknowledgments

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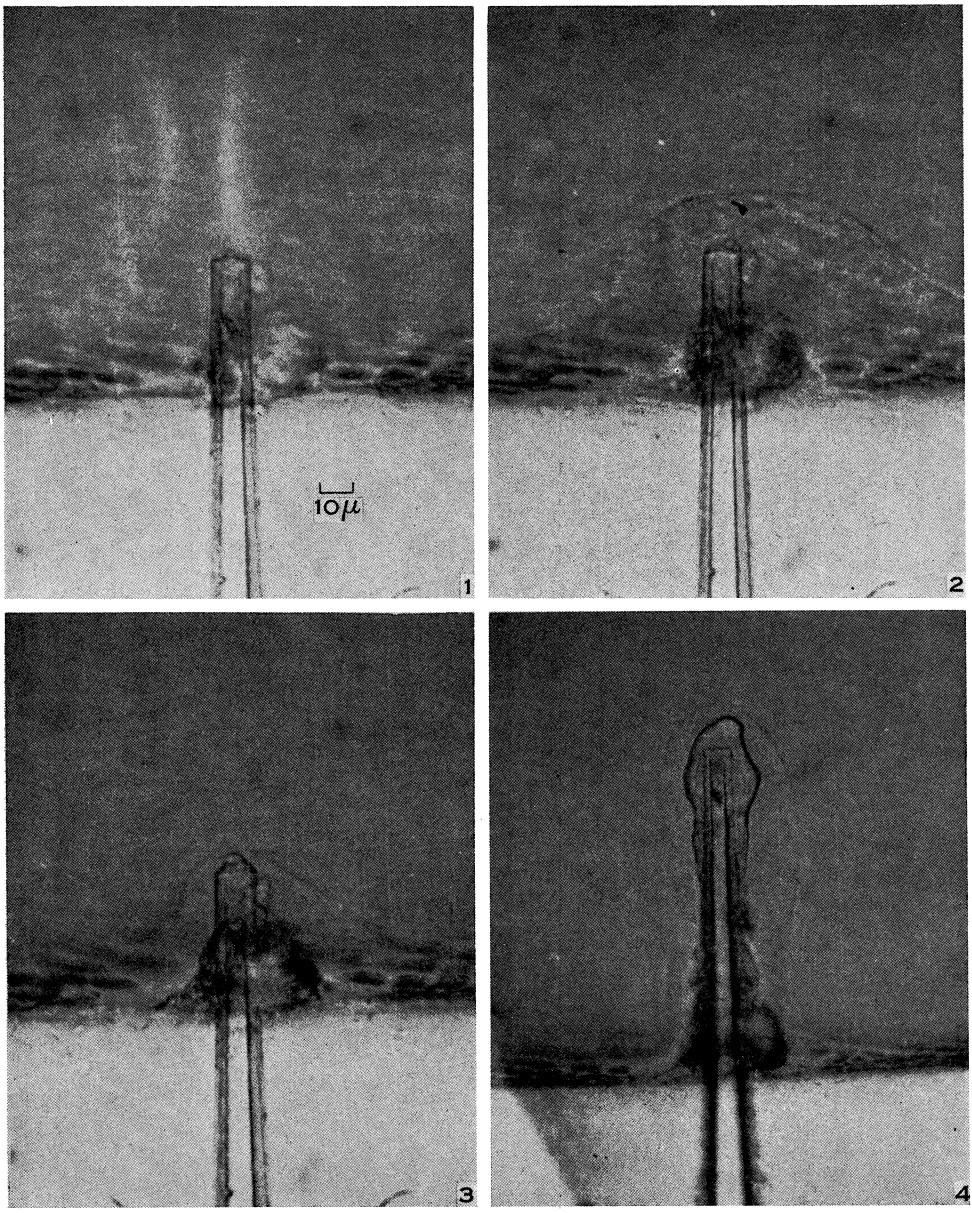
MICROELECTRODE EXPERIMENTS ON *NITELLA*



Photomicrographs of a microelectrode tip inserted into a *Nitella* cell. This tip is 10 μ in diameter, i.e. rather larger than those generally used. Magnification × *c.* 500.
Fig. 1.—Tip in vacuole immediately after insertion. Fig. 2.—Tip covered with cytoplasm, 3 min later. Fig. 3.—Tip covered with barely visible seal, 10 min after insertion. Fig. 4.—Tip covered with thick seal.

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