CHANGES IN RESTING POTENTIAL DUE TO A SHIFT OF ELECTROLYTES IN THE CELL PRODUCED BY NON-ELECTROLYTES

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Experiments[1] on *Nitella* have led to the conclusion that the resting potential is due to the diffusion potential set up by electrolytes in the cell. If this idea is correct we should expect that if these electrolytes are shifted in the cell so as to alter their concentrations in different regions there would be a corresponding effect on the resting potential. The experiments described in this paper show that this is actually the case.[2]

In earlier experiments[3] it was found that when 0.24 M sucrose is applied at the left end of the cell and water is placed at the right, water enters at the right end and moves through the cell to the left end carrying suspended particles as well as solutes with it. Since the electrolytes cannot pass out through the protoplasm (except very slowly) at the left and their concentration increases while at the right end it decreases. Hence if the resting potential depends on the concentration of electrolytes in the cell we might expect it to increase at the left end and decrease at the right end. The experiments described here indicate that this is the case.

In these cells the protoplasm forms a layer about 15 microns in thickness surrounding a large central vacuole over 450 microns in diameter. Outside the protoplasm is a cellulose wall about 15 microns thick. This is very permeable to water and to solutes.[4]

[1] Osterhout, W. J. V., *J. Gen. Physiol.*, 1930, **13,** 715; 1934, **18,** 215; 1943, **26,** 293; *Cold Spring Harbor Symp. Quant. Biol.*, 1940, **8,** 51. Hill, S. E., and Osterhout, W. J. V., *J. Gen. Physiol.*, 1938, **21,** 541. Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1940, **23,** 743; 1940, **24,** 9.

[2] Osterhout, W. J. V., *Proc. Nat. Acad. Sc.*, 1949, **35,** 548. A brief statement of these results has been previously published.

[3] Osterhout, W. J. V., *J. Gen. Physiol.*, 1947, **30,** 439. See also Osterhout, W. J. V., *J. Gen. Physiol.*, 1949, **32,** 553, 559.

[4] The observations were made on *Nitella flexilis*, Ag. The cells were freed from neighboring cells and kept in the laboratory in Solution A (*cf.* Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1933, **17,** 87) at 15°C. ± 1°C. The cells were placed in a room at approximately 25°C. about 24 hours before the experiment, so that their temperature rose gradually to about 25°C. at which temperature the experiments were performed.

At the outer surface of the protoplasm there is a non-aqueous surface layer, called for convenience X, and there is a corresponding non-aqueous layer, Y, at the inner protoplasmic surface. The sap in the vacuole contains about 0.05 M KCl and 0.05 M NaCl (other electrolytes present are in low concentration). The effect of KCl on the resting potential is so great that other electrolytes may be neglected in the discussion.

The importance of KCl lies in the fact that the mobility of K^+ (*i.e.*, U_K) in the inner non-aqueous protoplasmic surface (Y) surrounding the vacuole as well as in the outer non-aqueous protoplasmic surface (X) is much greater than that of Cl^- (*i.e.*, V_{Cl}). Hence the diffusion potential of KCl against these surfaces is very large.

Measurements show[5] that in the outer non-aqueous surface layer of the protoplasm the value of $U_K \div V_{Cl}$ is usually high and the situation is much the same with the inner protoplasmic surface surrounding the vacuole (Y) since if we place sap outside the cell so as to form the chain

Sap | X layer | Protoplasm | Y layer | Sap

we get only a small potential (from zero to 16 mv.) which shows that the inner and outer surfaces are not very different.

EXPERIMENTS

The main axis of the *Nitella* plant consists of a single series of elongated cells joined end to end. Two of these cells were allowed to remain in their natural union and were freed from neighboring cells by cutting.

A pair of such cells was placed[6] on a block of paraffin or of transparent leucite (Fig. 1). The latter had the advantage that it could be substituted for the stage of a microscope so that observations could be made while the electrical measurements were going on. Excavations in the block at A, B, and C were filled with sufficient solution to make good contact with the cell. The length of A was 4 cm., that of A′ 1 cm., that of B 1 cm.; the remainder of cell 1 occupied 1 cm. or more. The length of C was 1 to 3 cm. and the remainder of cell 2 (to the right of C) varied from 3 to 5 cm. At A′ and B′ the cell was protected from evaporation by a layer of vaseline under the cell and a covering of waxed paper smeared with vaseline above the cell. After the waxed paper was placed on the cell its edges were pressed down against the laver of vaseline beneath so as to make a seal to prevent evaporation from the cell.

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[5] Osterhout, W. J. V., *J. Gen. Physiol.*, 1930, **13,** 715. Osterhout, W. J. V., and Harris, E. S., *J. Gen. Physiol.*, 1928, **11,** 391. Blinks, L. R., *Proc. Nat. Acad. Sc.*, 1949, **35,** 566, see Table I.

[6] The object of using 2 cells is to prevent motion of the sap at the reference electrode C. Similar results were obtained when a single cell was employed and C was placed at the right end of the cell but in this case there was more danger of movement of water at C.

Since the use of metal forceps may cause action currents, bone-tipped forceps or wooden applicators were used in handling the cells.[7] Changes in the solutions at A, B, and C were made by means of pipettes, taking care not to disturb the cell.

A was connected through a calomel electrode, an amplifier,[8] and a recording galvanometer (G) to C, and B had a similar connection to C.[9] The connection with the calomel electrode was made by means of a short string saturated with the solution in the block and extending from this solution to the open tube of the calomel electrode. This open tube was placed a little below the block so that a very slow siphoning of solution from the block to the calomel electrode occurred thus preventing any contamination of the solution in the block by the saturated KCl of the calomel electoode. Any overflow from the calomel electrode fell into a trough placed beneath.

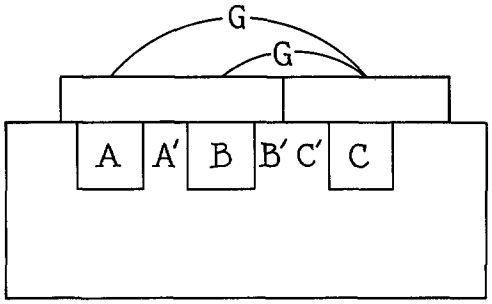

FIG. 1. Shows 2 cells in their natural union on a block of paraffin or of leucite with excavations at A, B, and C filled with solutions which make contact with the cell. At A′, B′, and C′ the cell is in contact with moist air.

A is connected to C through a recording galvanometer (G) and B is similarly connected to C.

At the start 0.001 M KCl was placed at A and at B and 0.01 M KCl at C and a record was made of the potentials. Then 0.001 M KCl at A was replaced by 0.001 M KCl + 0.24 M sucrose.[10] Microscopic observation then showed an immediate move-

[7] In removing the cells from the *Nitella* plant by cutting away neighboring cells, a portion of each neighboring cell was left attached to the living cell to serve as a handle in subsequent manipulation.

[8] Regarding this, see Hill, S. E., and Osterhout, W. J. V., *J. Gen. Physiol.*, 1938, **21**, 541.

[9] We could put the reference spot C on the same cell with A and B but in that case there might be movement of liquid from C to A when sucrose solution was applied at A so that the potential at C might not remain constant. With the arrangement shown in Fig. 1 no movement is seen at C when the cells are observed under the microscope and when 0.24 M sucrose + 0.001 M KCl is applied at A and 0.001 M KCl is applied at B.

[10] The cell is very slightly permeable to sucrose.

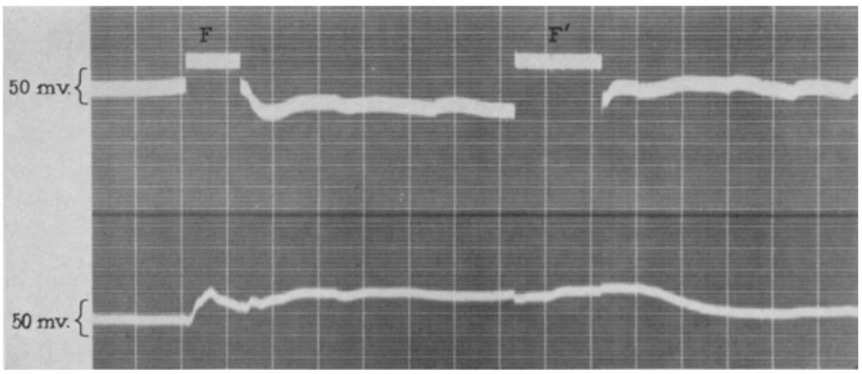

FIG. 2. Photographic record showing changes in potential between A and C (see Fig. 1) in the upper curve and between B and C in the lower curve. A fall in the curve means a rise in potential and a rise in the curve means a fall in potential.

The upper curve shows changes of potential at A. At the start the curve falls showing an increase in potential due to the movement of solutes from B to A. Later on the curve rises showing a decrease in potential due to the reverse movement of solutes from A to B.

The lower curve shows changes of potential at B. At the beginning the curve rises indicating a loss of potential due to the movement of solutes from B to A. Later on the curve falls showing a rise of potential due to the movement of solutes from A to B. These changes are explained in greater detail in the following paragraphs.

At the start A and B were in contact with 0.001 M KCl and were connected through a recording galvanometer (G) with C which was in contact with 0.01 M KCl.

Then the solution at A was removed, thus breaking the electrical connection between A and C and causing the curve to jump to F. During the stretch marked F the potential between A and C was not recorded but at the end of F there was a change in the level of the curve and this marked the resumption of the record of potential between A and C.

During the stretch marked F a solution of 0.001 M KCl + 0.24 M sucrose was poured into A thus making contact with the cell and restoring the electrical connection between A and C. Before this process was completed some drops of the new solution came into contact with the cell at A and caused KCl in the cell to move from B to A thus decreasing the potential between B and C and causing the lower curve to rise before the electrical connection between A and C was restored. This rise continued after the electrical connection between A and C was restored; the rise reached about 38 mv. in 40 seconds after the electrical connection was broken (this rise indicates a partial loss of the potential between B and C).

Since the change of solution at A caused KCl in the cell to move from B to A it increased the potential between A and C thus causing the upper curve to fall below the level it occupied before F. The drop reached about 38 mv. in 30 seconds after the beginning of F.

A little later the solution at A was removed thus breaking the electrical connection between A and C and causing the curve to jump to F'. The solution at A was

ment of particles in the sap from B to A[11] and it is evident from the record that solutes must likewise move from B to A (Figs. 2 and 3) since there was a change of potential at A and at B.

No motion of particles was observed in cell 1 to the right of B or in cell 2 so that no change of potential due to motion is expected at C. If any changes of potential occur at C or at the left end of cell 2 or at the right end of cell 1 they are registered[12] in the record as potential changes which are simultaneous and of equal magnitude in the A curve and in the B curve. They are therefore easy to detect and if they occur the experiment is rejected.

When water enters at B practically no electrolytes pass in with it since they cannot move through the protoplasm except very slowly. Hence the external solution becomes more concentrated but the change is extremely slight due to the large volume applied and its effect may be neglected. In the same way the external solution at A becomes more dilute when water passes out but the effect is negligible.

The cells were examined at the end of the experiment and again 2 days later. Unless they remained normal in appearance and in turgor during this period the experiment was rejected. If the cells were normal 2 days after the experiment they usually lived on indefinitely.

If we replace 0.001 M KCl + 0.24 M sucrose at A by 0.001 M KCl, there is a rapid intake of water at A due to the high concentration of solutes at A which have moved from B to A without being able to escape at A. Hence when we place 0.001 M KCl at

[11] In some cases this did not affect the normal protoplasmic motion which moves in opposite directions on opposite sides of the cell but in most cases the motion from B to A involved the whole of the liquid part of the protoplasm as well as the vacuole. There is apparently very little KCl in the protoplasm so that the movement of the protoplasm need not be considered.

[12] This is because such motion would affect the potential of the protoplasm at the right end of cell 1 and at the left end of cell 2, and both of these are in the recorded circuits. Under normal conditions these potentials are equal and opposite and hence do not affect the record.

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then replaced by 0.001 M KCl causing KCl in the cell to move from A to B so that the potential at A was lowered and in 55 seconds after the beginning of F′ the upper curve rose about 38 mv. above the level it occupied just before F′.

At this time the upper curve was in approximately the same position as at the beginning of the record showing that the increase of potential at A which occurred when KCl moved from B to A was lost when KCl moved back from A to B.

An opposite movement occurred at B (lower curve) so that in 80 seconds after the beginning of F′ the lower curve fell to a level about 50 mv. below the position it occupied just before F′. Its position was then about 12 mv. above the level it occupied at the start of the record.

This shows that the loss of potential at B due to the movement of KCl from B to A was somewhat exceeded by the rise of potential when the back movement from A to B occurred.

Time marks 15 seconds apart.

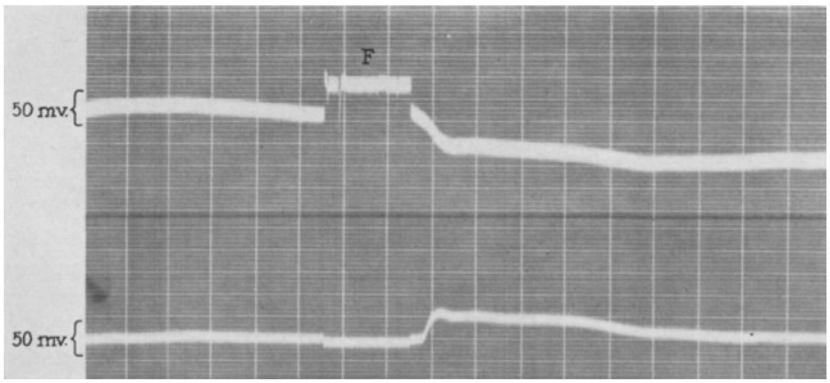

FIG. 3 *a*. As in Fig. 2 but a higher concentration of sucrose was employed causing a greater movement of the curve when applied at A.

At the start A was in contact with 0.001 M KCl and C with 0.01 M KCl. The solution at A was removed thus breaking the electrical connection between A and C and causing the curve to jump to F (regarding F see Fig. 2). A new solution consisting of 0.001 M KCl + 0.4 M sucrose was then applied at A causing KCl in the cell to move from B to A, thus increasing the potential at A and causing the upper curve to fall. In 65 seconds after the start of F the drop amounted to about 50 mv. below the level occupied just before F. The movement of solutes from B to A lowered the potential at B thus causing the lower curve to rise to a higher level than it occupied just before F; the rise reached about 41 mv. in 45 seconds after the beginning of F.

Time marks 15 seconds apart.

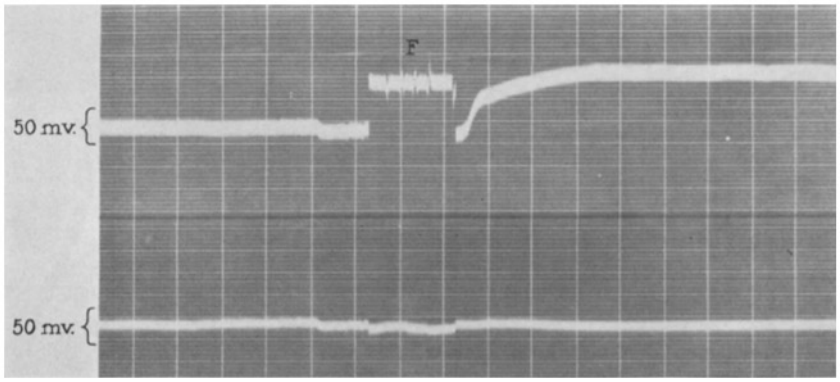

FIG. 3 *b*. Continuation of the record shown in Fig. 3 *a* with 6 minutes of the record omitted. The solution at A (consisting of 0.001 M KCl + 0.4 M sucrose) was removed thus breaking the electrical connection between A and C and causing the curve to jump to F (regarding F see Fig. 2). The solution at A was then replaced by 0.001 M KCl causing solutes in the cell to move from A to B so that the potential at A was lowered and the upper curve rose. In 73 seconds after the start of F the curve rose to a level about 81 mv. above the level just before F.

The level of the curve was then about 31 mv. above the level it occupied just before the beginning of F. This shows that the loss of potential at A due to the movement of KCl from B to A was overcompensated by the subsequent movement of solutes from A to B.

At B there was no drop in the curve such as is seen in Fig. 2. This may be due to irreversible changes produced at B by the violent motion of water. Such changes are occasionally observed with 0.4 M sucrose.

Time marks 15 seconds apart.

A water enters at A and liquid moves from A to B where the solutes are unable to pass out through the protoplasm except very slowly. Thus their concentration decreases at A and increases at B and the motion stops when the internal concentration of solutes becomes equal at A and at B.

The curves in Figs. 2, 3 *a*, and 3 *b*, show that when solutes move from B to A there is an increase in the potential at A because the concentration of KCl in the vacuole at A increases, and in consequence the diffusion potential of KCl across the protoplasm at A becomes greater. The potential at B decreases because the concentration of KCl at B decreases and in consequence the diffusion potential of KCl across the protoplasm at B falls off.

When the sucrose solution at A is replaced by 0.001 M KCl water enters at A, passes along inside the cell, and escapes at B carrying KCl from A to B. In consequence the potential falls off at A and increases at B. These changes in potential are in accordance with expectation.

DISCUSSION

The amount of solutes moving from B to A can be easily calculated. When water enters the cell at B it is because the osmotic drive which forces water into the cell is greater at B than at A. The osmotic drive may be defined as the internal minus the external osmotic pressure. So that we may write $D_B = P_{IB} - P_{OB}$ where D_B is the osmotic drive at B, P_{IB} is the internal and P_{OB} the external osmotic pressure at B.

The internal osmotic pressure as indicated by plasmolytic experiments is about 6.4 atmospheres[13] but for purposes of calculation we may use round numbers and put $P_{IB} = 6$. Since the external osmotic pressure with 0.001 M KCl outside is very small we may write $P_{OB} = 0$. We then have $D_B = P_{IB} - P_{OB} = 6 - 0 = 6$.

At A the external osmotic pressure is approximately 6.4, but, using round numbers, we may write $P_{OA} = 6$. We then have $D_A = P_{IA} - P_{OA} = 6 - 6 = 0$. Hence the osmotic drive is greater at B than at A and water enters the cell at B and escapes at A.

For purposes of calculation we may assume that each cell is divided into sections each of which has a length of 1 cm. Let us assume that the osmotic pressure is due entirely to a single solute, S, and that each section of the cell contains 6 x moles of S, giving an osmotic pressure of 6 atmospheres. Here x is a small number which is constant for any particular cell.

In B we find 6 x mols of S. Let us now transfer one of these to each section of A and then make a similar transfer from A′ to A. Each section of A now contains $6 + 2 = 8$ x mols of S giving an osmotic pressure of 8 atmospheres. Since the external osmotic pressure is 6 atmospheres we have $D_A = 8 - 6 = 2$. We find the same value at A′ and at B where the original value of P_I has fallen from 6 to 2 and the value of P_0 remains at 0 so that $P_I - P_0 = 2 - 0 = 2$.

The value of the osmotic drive is now 2 at A and at B and the motion stops.

[13] All osmotic pressures are taken at 25°C.

During the flow the value of P_{IB} has fallen from 6 to 2 so that the loss is 66.7 per cent. The value of P_{IA} has risen from 6 to 8, an increase of 33.3 per cent.

In order to calculate the effect of these changes on the potentials at A and at B we may proceed as follows. Previous experiments indicate that the potential is due to diffusion potential so that we may employ the Nernst equation[14] which (for 25°C.) may be written:

$$P = 59 \frac{U_K - V_{Cl}}{U_K + V_{Cl}} \log_{10} \frac{a_i(PC)}{a_0(PC)}$$

where U_K is the mobility of K^+ and V_{Cl} is the mobility of Cl^- in X and in Y, which have similar properties. a_i is the activity of KCl in the sap and a_0 the activity in the external solution and PC is the activity partition coefficient of KCl in X and in Y. We may assume that in these cells the potential has the usual value[15] of about 97 mv. and in such cells the value of $U_K \div V_{Cl}$ is about 73. Hence we have[16]

$$P = 59 \frac{73 - 1}{73 + 1} \log_{10} \frac{0.05(PC)}{0.001(PC)}$$

$$= 97 \text{mv.}$$

After the flow is complete and the concentration of KCl in the sap at A has risen 33.3 per cent, *i.e.* from 0.05 to 0.067, we have:

$$P = 59 \frac{73 - 1}{73 + 1} \log_{10} \frac{0.067(PC)}{0.001(PC)}$$

$$= 104.3 \text{ mv.}$$

Since the normal value is 97 mv. this represents a gain of 7 per cent.

At B the value of S and consequently the amount of KCl falls and the loss amounts to 67.7 per cent and the concentration of KCl changes from 0.05 M to 0.017 M. Hence we have:

$$P = 59 \frac{73 - 1}{73 + 1} \log_{10} \frac{0.017(PC)}{0.001(PC)}$$

$$= 70.1 \text{ mv.}$$

Since the normal value is 97 mv. this represents a loss of 28 per cent.

[14] Hill, S. E., and Osterhout, W. J. V., *J. Gen. Physiol.*, 1938, **21,** 541. Osterhout, W. J. V., *J. Gen. Physiol.*, 1930, **13,** 715. There appears to be very little KCl in the protoplasm and its action may be neglected since its effect on X will be equal and opposite to its effect on Y because the electrical properties of X and Y are similar.

[15] This value is not indicated in Figs. 2, 3 *a*, and 3 *b* because the location of the zero line is not exactly known and therefore does not appear in the figures. This makes no difference to the values derived from the rise and fall of the curves since the calibration of 50 mv. is shown.

[16] For convenience we use concentrations in place of activities.

The observed gain of potential at A is usually more than the calculated and this is to be expected since the water rushing out on all sides carries KCl to the protoplasm where it is trapped because it cannot escape (except very slowly) and its concentration becomes greater than in the center of the vacuole which is about 450 microns in diameter. Since the calculation does not take this into account it is less than the observed value.

At B the water rushing in from all sides carries KCl away from the protoplasm to the center of the vacuole so that the concentration is higher in the center than in contact with the protoplasm. Since the calculated loss of potential does not take this into account it is less than the observed.

The sudden rush of water causes a sudden change in potentials at A and at B as shown in Figs. 2 and 3 *a*.

It may be suggested that these effects are due to modifications of the protoplasm rather than to changes in the concentration of electrolytes. It has been shown in a previous paper[17] that an ingoing current of water can produce contraction of the chloroplasts but the loss of potential at B takes place much sooner than the contraction of the chloroplasts. The gain in potential at A occurs before the expansion of the chloroplasts which occurs when water escapes at A as liquid moves from B to A. The rise of potential at A occurs without any change in the chloroplasts. When the potential at A decreases with the ingoing current at A in the reverse movement this occurs some minutes before the contraction of the chloroplasts at A.

It has been shown in a previous paper[18] that changes in the appearance of the protoplasm occur in some cases with a very strong ingoing or outgoing current of water. But in the experiments here described there was no indication of such changes.

It therefore does not seem probable that the changes in potential are due to alterations in the protoplasm.

The experiments indicate that a shift of the solutes in the cell causes changes in potential in the expected direction but the chief factor is not so much the shift along the length of the cell as the shift from the surface of the vacuole toward its center or *vice versa*. These changes are reversible and can be carried out without injury to the cell.

SUMMARY

Experiments on *Nitella* indicate that the resting potential is due chiefly to the outwardly directed diffusion potential of electrolytes which is set up at the inner, non-aqueous, protoplasmic surface surrounding the vacuole. We might therefore expect that any change in the concentration of these electrolytes would affect the resting potential. The experiments described here indicate that this expectation is justified.

[17] Osterhout, W. J. V., *J. Gen. Physiol.*, 1947, **30,** 229.

[18] Osterhout, W. J. V., *J. Gen. Physiol.*, 1948, **31,** 291.

When a sucrose solution is applied at one end of the cell and water is placed at another spot, water enters at the latter, passes along inside the cell, and escapes into the sucrose solution, but the electrolytes are unable to escape into the sucrose solution (except very slowly) so that the concentration of electrolytes increases in the region in contact with the sucrose solution. Hence the potential at this spot increases. At the other spot where the water enters, the concentration of electrolytes decreases and the potential at this spot falls off.

The changes can be carried out reversibly without injury to the cell.