NOTE ON THE NATURE OF THE CURRENT OF INJURY IN TISSUES

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Previous workers have found the current of injury uniformly negative but experiments on *Nitella*[1] indicate that it can be made either positive or negative according to the method of treatment. It seemed desirable to inquire whether this divergence could be explained, in part at least, by the fact that we employed single cells while previous workers have investigated tissues.

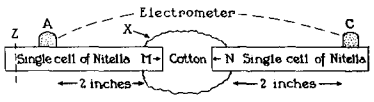

FIG. 1.

FIG. 1. Diagram to show the arrangement of an experiment. Two cells from different plants are placed in contact with a piece of absorbent cotton, *X*, and we then lead off from *A* and *C*. The letters *M* and *N* designate the ends of the cells. One cell is cut at *Z*.

By way of introduction let us consider an experiment[2] arranged as in Fig. 1 which shows two cells of *Nitella* taken from two separate plants and placed in contact with a piece of absorbent cotton. If we place 0.001 M KCl at *A*, and *C*, the cotton being wet with the same solution, we find on cutting the left-hand cell at *Z* that the potential difference of *A* (recorded with reference to *C*) becomes much more negative (Fig. 2), then quickly becomes more positive, after which it gradually approaches zero. This behavior is like that of single cells as described in former papers (in which case the experiment was arranged as shown in Fig. 3).

[1] Osterhout, W. J. V., and Harris, E. S., *J. Gen. Physiol.*, 1927–28, **11**, 673.

[2] The material and technique were as described in previous papers unless otherwise stated. *Cf.* (1) and *J. Gen. Physiol.*, 1927–28, **11**, 391. The experiments were carried out at room temperature averaging about 22° or 23°C.

There is no essential difference between these two cases, because, although the circuit in Fig. 3 passes through A and C and that in Fig. 1 includes[3] A, M, N, and C, the electromotive forces at N and C usually cancel out since they are opposite and almost equal, and only A and M are altered and they may be regarded as corresponding to A and C in Fig. 1.

The situation is different when the experiment is arranged as in Fig. 4. Here we have two cells in their natural union, which consists of a cell wall (W), as shown in Fig. 5; this is only a few microns in

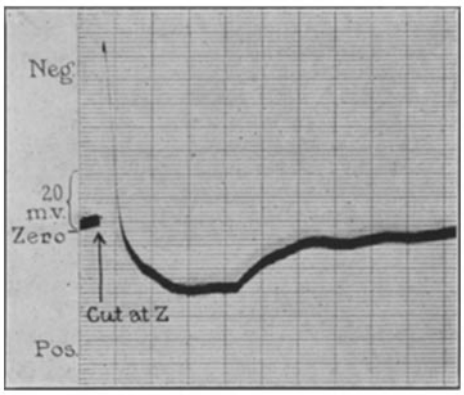

FIG. 2. Photographic record of potential differences, the experiment being arranged as in Fig. 1 with 0.001 M KCl at A, C, and X. When the left cell is cut at Z the curve (which records the state of A with reference to C) shows that A becomes more negative, then more positive, and that the electromotive force then approaches zero. The vertical lines represent 5-second intervals. Selected as typical from 10 experiments.

thickness and in this case is imbibed with tap water. If the left-hand cell is injured so that sap[4] comes out at M, diffuses through the cell

[3] M and N represent the protoplasmic layers at the ends of the cells.

[4] The sap is equivalent in these experiments to 0.05 M KCl. *Cf.* (1). A good method of observing the coming out of sap at M is to arrange an experiment as in Fig. 4 with an additional contact at a spot, B, a little to the right of N. We put 0.001 M KCl at A and B and sap or artificial sap at C. On cutting at Z the A to C curve becomes negative and then positive after which it slowly rises to zero as sap comes in contact with N but the B to C curve does not change unless sap diffuses along to B.

wall,[5] and comes in contact with N it is clear that there is a greater difference between N and C (which is in contact with a cell wall imbibed with 0.001 M KCl). When all the E.M.F. has disappeared from the protoplasm of the cell at the left[6] (as the result of cutting at Z) the positive current will tend to flow from C (in contact with 0.001 M KCl) through the electrometer and the cell at the left (which now acts merely as a conductor) to N which is in contact with sap or a dilute sap (which acts like a solution of KCl more concentrated than 0.001 M). This

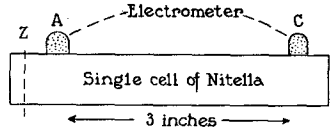

FIG. 3.

FIG. 3. Diagram to show the arrangement of an experiment. We lead off from A and C. The cell is cut at Z.

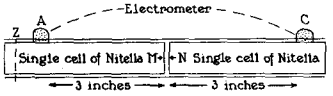

FIG. 4.

FIG. 4. Diagram to show the arrangement of an experiment. Two cells are employed, their natural union being left intact. We lead off from A and C and cut at Z. The letters M and N designate the ends of the cells.

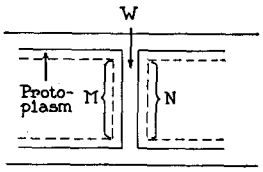

FIG. 5.

FIG. 5. Enlarged view of the point of union of the two cells shown in Fig. 4. The letter W designates the cell wall: inside this is a delicate layer of protoplasm surrounding the sap of the large central vacuole. The letters M and N designate the ends of the cells (as in Fig. 4).

gives a negative current of injury which is wholly due to the uninjured cell and which may last a long time (*i.e.*, until the cell at the right begins to lose its E.M.F. as the result of injury). Possibly this is the sort of negative current of injury observed in some cases by workers who employ groups of small cells. It is quite different from the negative current of injury in a single cell of *Nitella* which usually

[5] The cell wall is very permeable.

[6] There is some P.D. due to the cell wall which would in the present case tend to make A appear somewhat more negative than it actually is. *Cf.* Osterhout, W. J. V., and Harris, E. S., *J. Gen. Physiol.*, 1928–29, **12**, 761.

lasts only a few seconds (*cf.* Fig. 2) after which the cell dies and the protoplasm[6] soon loses its E.M.F.

We assume that if sap did not come out at M[7] we should get a curve like that in Fig. 2, *i.e.*, the changes in the circuit would be confined to A and M: but if sap exudes at M and comes in contact with N the curve will tend to reach a fixed negative value, the time

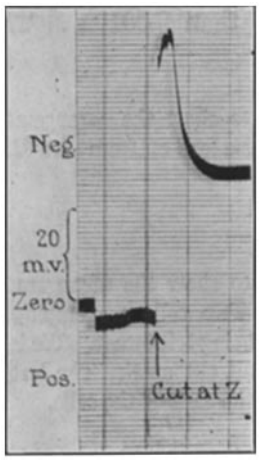

FIG. 6.

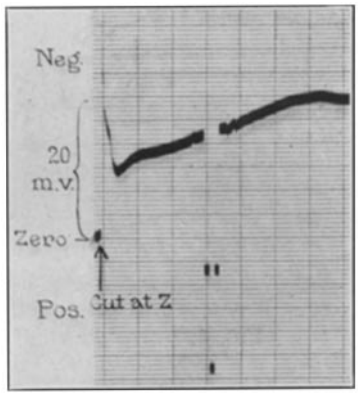

FIG. 7.

FIG. 6. Photographic record of potential differences, the experiment being arranged as in Fig. 4 with 0.001 M KCl at A and C. When the left cell is cut at Z the curve (which records the state of A with reference to C) shows that A becomes more negative after which the E.M.F. falls to a fixed value which is regarded as due to the coming out of sap at M (Fig. 5) which affects N, making it more negative (*cf.* Fig. 8*a*). The vertical lines represent 5-second intervals. Selected as typical from 40 experiments.

FIG. 7. Like Fig. 6 but showing a different result which is regarded as due to the slower exit of sap (*cf.* Fig. 8*b*). Selected as typical from 39 experiments. (Test for reversibility at about 16 seconds.)

depending on the speed with which the sap diffuses across the cell wall from M to N. That this time is variable is evident from Figs. 6 and 7.

[7] At the ends of the cells (*i.e.*, near M and N) there are a few very small cells exterior to the large cells forming the axis of the plant but owing to their small size it is not believed that they affect the observed P.D.

If the cell wall is imbibed with 0.001 M KCl the situation may be represented diagrammatically as in Figs. 8 *a* and *b*, where the curve[8] labelled *A* to *M* represents the P.D. of *A* with reference to *M*, and the P.D. of *N* with reference to *C* is represented by the curve labelled *N* to *C*. The observed P.D. between *A* and *C* (labelled *A* to *C*) may be regarded as the sum of these two curves and may take a variety of forms.

If before performing the experiment we apply 0.05 M KCl at the joint so that the cell wall between *M* and *N* becomes imbibed with it we may get such a curve as that shown in Fig. 9 and the situation may

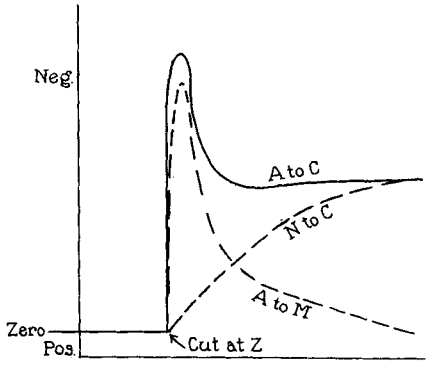

FIG. 8 *a*.

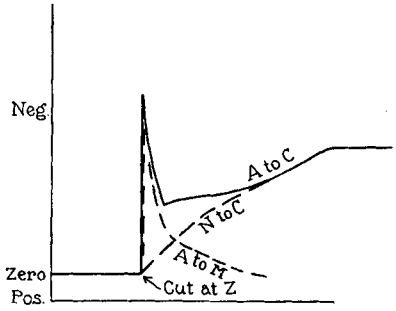

FIG. 8 *b*.

FIG. 8, *a* and *b*. Hypothetical diagram of the changes in P.D. following a cut (at *Z*, Fig. 4) when the cell wall is imbibed with 0.001 M KCl and this solution is also applied at *A* and *C*. The P.D. of *A* with reference to *M* (*cf.* Fig. 4) is represented by the curve "*A* to *M*;" that of *N* with reference to *C* by the curve "*N* to *C*." The observed P.D. ("*A* to *C*") is the sum of these two curves. Figs. 8 *a* and 8 *b* represent two forms of such curves (*cf.* Figs. 6 and 7).

be represented as in Fig. 10. The *A* to *M* curve is positive at the start and the *N* to *C* curve is negative: here too it is found that the curve may take various forms, one of which is indicated in the diagram.

In order to see how far this applies when a larger group of cells is

[8] The curves *A* to *M* in Figs. 8*a* and 8*b* are taken from actual curves obtained in cutting single cells (arranged as in Fig. 3) with 0.001 M KCl at *A* and sap or 0.05 M or 0.1 M KCl at *C*. (*Cf.* Osterhout, W. J. V., and Harris, E. S., *J. Gen Physiol.*, 1928–29, **12**, 355.) In some cases such curves after passing through a negative phase become positive before reaching final equilibrium at zero.

involved experiments were made in the manner shown in Fig. 11. Bundles of plants were employed, the ends of the bundle being allowed

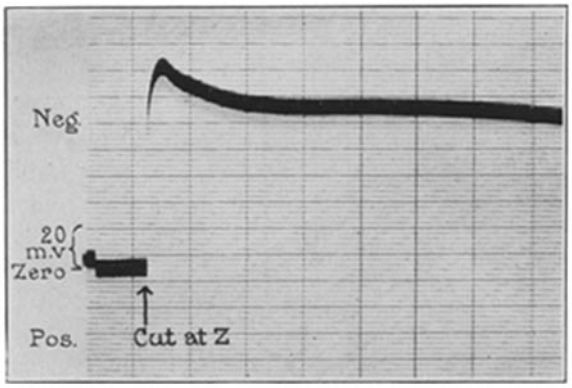

FIG. 9. As in Fig. 6 but the cell wall which separates the cells is imbibed with 0.05 M KCl. Selected as typical from 15 experiments.

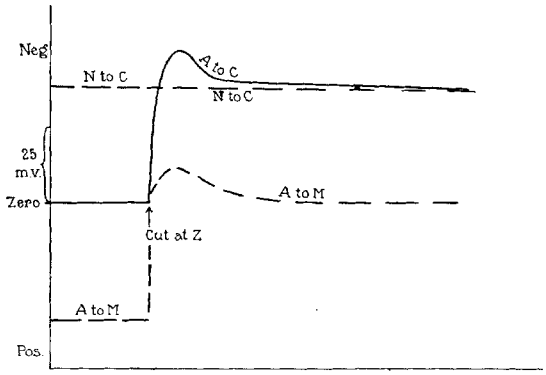

FIG. 10. Hypothetical diagram of the changes in P.D. following a cut (at *Z*, Fig. 4) when the wall separating the two cells is imbibed with 0.05 M KCl and 0.001 M KCl is applied at *A* and *C*. The P.D. of *A* with reference to *M* (*cf.* Fig. 4) is represented by the curve "*A* to *M*," that of *N* with reference to *C* by the curve "*N* to *C*." The observed P.D. "*A* to *C*" is the sum of these two curves. (*Cf.* Fig. 9.)

to dip into two dishes, *A* and *C*, in which were calomel electrodes (the cut was made at *Z*). With *A* and *C* filled with 0.001 M KCl we obtain

curves similar to that in Fig. 12 and the negative current of injury persists for many minutes.

It would seem that if our conception of the process is correct it should enable us to predict what will happen with other concentrations, for example, using the arrangement shown in Fig. 1 with 0.1 M KCl at *A* and *C* and with the cotton at *X* soaked with 0.1 M KCl, we should expect a curve like that obtained with a single cell,[9] since

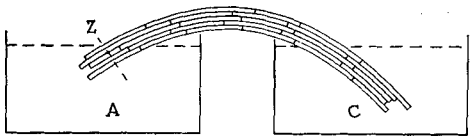

FIG. 11. Diagram to show the arrangement of an experiment in which a bundle of plants was used, the ends of the bundle dipping into the vessels *A* and *C*. The bundle was cut at *Z*.

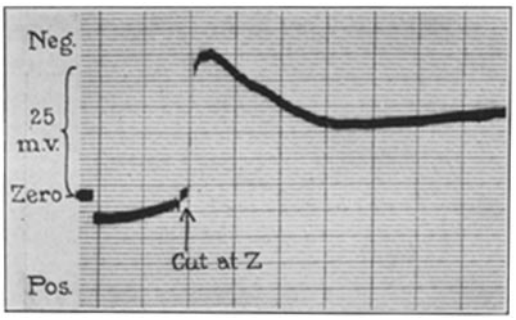

FIG. 12. Photographic record of P.D., the experiment being arranged as in Fig. 11 with *A* and *C* filled with 0.001 M KCl. The vertical lines represent 5-second intervals. Selected as typical from 5 experiments.

the coming out of sap would not affect the result and at the end we should be leading off at two points, *N* and *C*, both in contact with 0.1 M KCl. This is the case as is shown by Fig. 13.

Using the arrangement shown in Fig. 4 we should expect a similar curve at the start but later on, after the E.M.F. of the cell at the left

[9] Even with single cells there is a good deal of variation in the time required for the curve to rise to zero.

has disappeared (as the result of cutting at Z) and sap comes out of M into the cell wall (which is imbibed with tap water) and reaches N the positive current will tend to flow through the electrometer from N (now in contact with sap or dilute sap) to C (in contact with 0.1 M KCl) and as the curve records the P.D. of N with respect to C

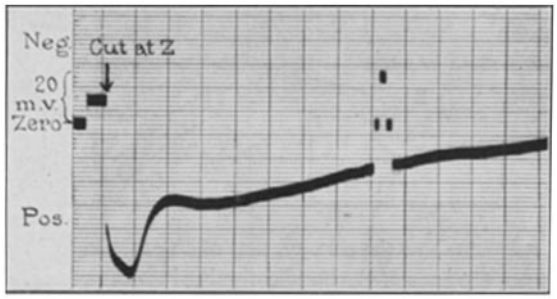

FIG. 13. As in Fig. 2 but with 0.1 M KCl at A and C. Selected as typical from 10 experiments. Test for reversibility at about 45 seconds.

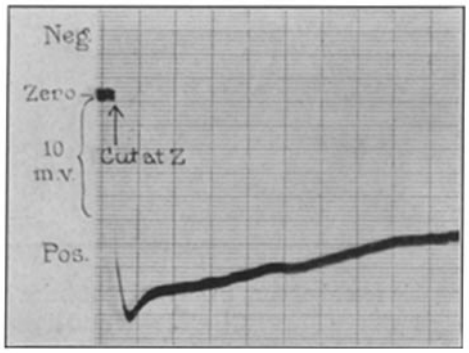

FIG. 14. As in Fig. 6 but with 0.1 M KCl at A and C and with the wall between M and N imbibed with tap water. Selected as typical from 45 experiments.

we shall expect the curve to remain positive. The more slowly the sap comes out of M the more slowly the curve will rise toward zero (this varies greatly) but even when the end wall becomes completely imbibed with sap we shall expect the curve to remain somewhat positive since sap in these experiments is equivalent to 0.05 M KCl.

That these expectations are realized is evident from Fig. 14. In this case the presence of a second cell tends to prolong the positive phase of the current of injury. The result is much the same when we employ the arrangement shown in Fig. 11.

It is evident that whenever the fluid bathing the exterior of the cell is less "negativating" than the fluid contained in the cell (as is the case in *Nitella* when 0.001 M KCl is applied to the exterior) and we lead off from the injured cell to intact cells the action of the latter will tend to prolong in a marked degree the negative phase of the current of injury.

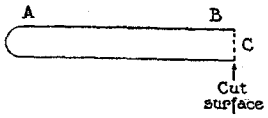

FIG. 15. Diagram of a muscle cut at one end: leading off from A to C gives more P.D. than leading off from B to C.

FIG. 15.

When we employ chloroform the injury spreads much more slowly along the cell and in many cases no spread can be detected for some minutes. In consequence the current of injury in a single cell is more lasting. This more nearly resembles the effect of cutting on muscle and nerve.[10] That there is a slow spread in muscle of the injury due to cutting is indicated by the fact that if we lead off from A to C (Fig. 15) we obtain a greater P.D. than in leading off from B to C but as time goes on this difference becomes less.

We may sum up by saying that whenever we can lead off from two places on the same cell (*Nitella*) or two places on a bundle of elongated cells (muscle and nerve) we may ascertain the current of injury due to the death process in the protoplasm.[11] But when we are not able

[10] The fibers of muscle and nerve differ from *Nitella* in that injury due to cutting does not spread rapidly and hence the current of injury lasts much longer. In *Nitella* there are two protoplasmic surfaces to consider. The experiments of one of us indicate the possibility that this may be true of muscle and nerve.

[11] This has been described for *Nitella* in previous papers. *Cf.* Osterhout, W. J. V., and Harris, E. S., *J. Gen Physiol.*, 1927–28, **11**, 673; 1928–29, **12**, 167, 355. See also Beutner, R., Die Entstehung elektrischer Ströme in lebenden Geweben, Stuttgart, 1920; Bayliss, W., Principles of general physiology, London, 1924, 4th edition; Höber, R., Physikalische Chemie der Zelle und der Gewebe, Leipsic, 1926, 6th edition.

to do this (on account of the small size of the cells) a lasting current of injury may be partly or completely due to the escape of sap which comes in contact with cells[12] which are intact or not sufficiently injured to lose their protoplasmic E.M.F. In plants the cell wall may also play a part.

SUMMARY

Leading off from two places on the same cell (of *Nitella*) with 0.001 M KCl we observe that a cut produces only a temporary negative current of injury.

If we lead off with 0.001 M KCl from any cell to a neighboring cell we find that when sap comes out from the cut cell and reaches the neighboring intact cell a lasting negative "current of injury" is produced. This depends on the fact that the intact cell is in contact with sap at one point and with 0.001 M KCl at the other (this applies also to tissues composed of small cells).

If we employ 0.1 M KCl in place of 0.001 M the current of injury with a single cell is positive (and is more lasting when a neighboring cell is present).

Divergent results obtained with tissues and single cells may be due in part to these factors.

[12] Osterhout, W. J. V., and Harris, E. S., *J. Gen. Physiol.*, 1928–29, **12**, 761.