THE DEATH WAVE IN NITELLA

III. Transmission

By W. J. V. OSTERHOUT and S. E. HILL

(*From the Laboratories of The Rockefeller Institute for Medical Research*)

(Accepted for publication, November 18, 1930)

When a cell of *Nitella* is cut[1] an electrical response may appear beyond a spot that has been killed by chloroform. This indicates that the electrical changes are due to a mechanical disturbance which travels past the killed spot. If this were a compression wave it would be expected to travel with approximately the speed of sound in water.[2] In order to test this an experiment[3] was arranged as in Fig. 1, the cell being cut at *Z*, and the disturbances at *A* and *B* being recorded by means of separate strings. From the record we can determine the time required to pass from *A* to *B*.

Such a record is shown in Figs. 3 and 4 (the first rapid records of this sort were made by Mr. E. S. Harris). The first downward movement (which we will call *a*) seems to be practically simultaneous at *A* and *B*, indicating a rate of transmission too rapid to be measured under these conditions. It might possibly be that of a compression wave in water[2] such as would naturally be produced by compressing the cell during the act of cutting.

[1] Osterhout, W. J. V., and Harris, E. S., *J. Gen. Physiol.*, 1928–29, **12**, 167, 355.

[2] The speed depends on the elasticity of the wall in relation to the diameter of the cell. E. W. Schroder, (article Hydraulics, p. 284 in Mark's Mechanical Engineer's Handbook, McGraw Hill Book Co., New York and London, 2nd edition, 1924) states that the speed in an ordinary cast iron pipe 2 to 6 inches in diameter is about 4200 feet per second but for a 24-inch pipe it is about 3300 feet per second.

[3] The technique was as previously described (*cf.* Osterhout, W. J. V., and Harris, E. S., *J. Gen. Physiol.*, 1927–28, **11**, 673; 1928–29, **12**, 167, and previous papers. The experiments were performed on *Nitella flexilis*, the average temperature being about 23°C.

In cutting the cell care was taken to communicate no charge from the body of the operator or otherwise. The cell was touched only with materials carefully insulated from the body of the operator and at the same potential as the cell.

This is followed by an upward movement (which we will call *b*, Figs. 2 and 3) the start of which seems to be practically simultaneous at both places but the upward movement of the string is slower at *B* than at *A* since the width of the white line where the curve starts upward[4] is greater in the lower curve than in the upper.

The upward movement is followed by a downward movement (which we will call *c*) starting later at *B* than at *A*. The next move-

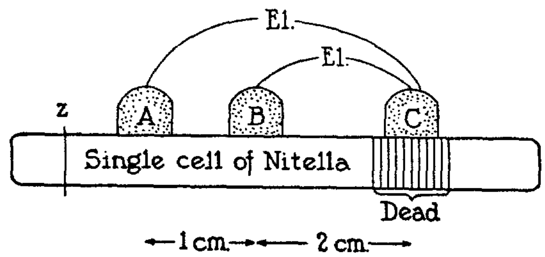

FIG. 1. Diagram to show arrangement of experiments. At *A* and *B* 0.001 M KCl is applied. The protoplasm at *C* is killed by applying 0.001 M KCl saturated with chloroform and the cell is cut at *Z*. The resulting response is shown diagrammatically in Fig. 2.

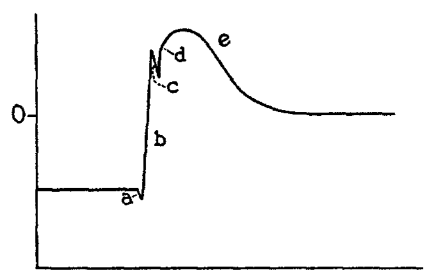

FIG. 2. Diagram to show the electrical responses at *A* due to cutting at *Z* (*cf.* Fig. 1). The duration of movements *a*, *b*, and *c* is exaggerated.

ment is upward, marked *d* in the slower record shown in Fig. 4 (*a* and *b* cannot be seen in this slow record, but *c* can be made out by close inspection in the upper curve and is very clear in the lower one).

[4] When the experiment is arranged as in Fig. 1 but with *C* alive, a complication is sometimes observed when *C* is close to the end of the cell. In such cases transmission to *C* appears slow and the curves have various irregular shapes.

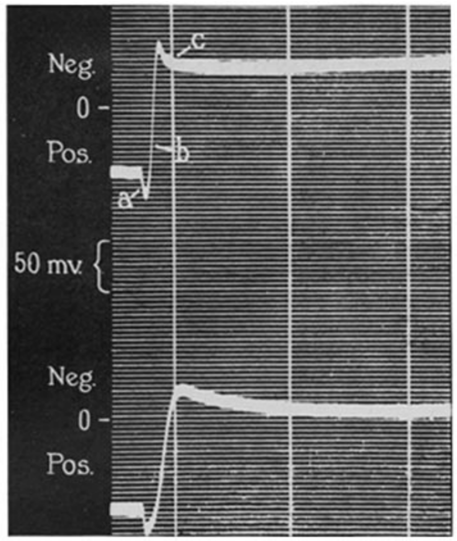

FIG. 3. Photographic record showing the result of an experiment arranged as in Fig. 1. The upper curve records the P.D. of *A* with reference to *C*; the lower curve that of *B* with reference to *C*. Cutting the cell at *Z* produces the movements *a*, *b*, and *c*. The distances between vertical marks represent intervals of one-tenth of a second. At the same time a slower record (Fig. 4) is taken on another galvanometer.

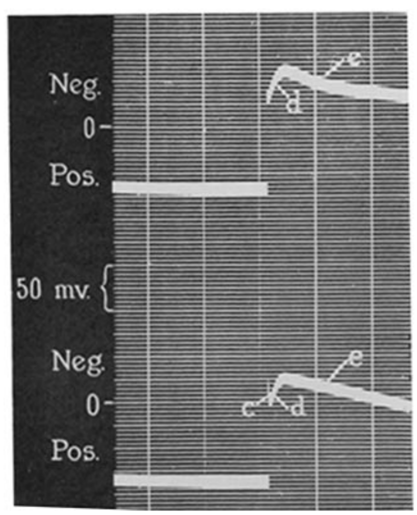

FIG. 4. Slower record of the same experiment as that shown in Fig. 3 (taken simultaneously on another galvanometer). The distances between vertical lines represent 5-second intervals.

This is followed by a downward movement (*e*) which ends by approaching zero.[5]

It is evident from Fig. 3 that the *c* movement starts later at *B* (lower curve) than at *A* (upper curve) and this difference in time will for convenience be called the lag. In general the lag appears to increase with the successive movements, *i.e.* it is greater for *d* than for *c* and still greater for *e*. The lag also appears to increase as the mechanical disturbance travels down the cell. For example, measurements on 15 cells showed that the lag of the *c* movement between *A* and *B*

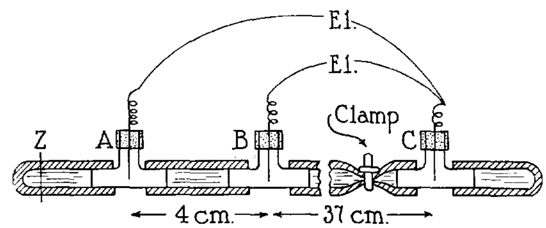

FIG. 5. Diagram of an experiment on a piece of soft rubber tubing with Ag-AgCl electrodes in glass T-tubes at *A*, *B*, and *C*. The tube is filled with tap water (all air bubbles being excluded). Between *B* and *C* the tube is clamped to prevent movement of water at *C*. In some experiments a U-tube filled with sand was substituted for the clamp. The tube was wound at *Z* with copper wire which was connected to the striking hammer to keep the two at the same potential and avoid communicating a charge.

(1 cm. apart)[6] was 0.008 sec. and between *B* and *D* (situated 1 cm. to the right of *B*) was 0.043 sec.

[5] This is clearly shown in previous papers.[1]

Certain of these movements may be lacking and the curve may have only one crest.

In cutting *Chara coronata* and *Nitella flexilis* it was sometimes observed that when the knife remained in contact with the cut so that no sap flowed out no death wave occurred but only a response resembling a negative variation and a second one could be produced by cutting the same cell again. These sometimes traveled with the speed of the death wave, and sometimes with the speed of the negative variation. Such responses will be discussed in a subsequent paper.

[6] The distance from the right edge of contact *A* to the left edge of contact *B* is 1 cm. This distance is taken because if an electrical impulse travels from

The facts indicate that the mechanical disturbance loses intensity as it proceeds so that the reactions it starts at each successive spot it reaches proceed the more slowly the greater the distance from the cut.

To learn something of the nature of these electrical changes experi-

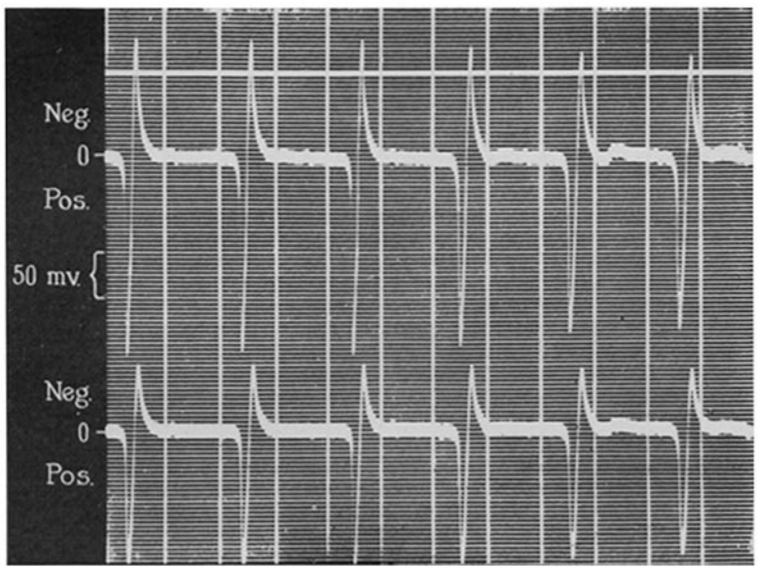

FIG. 6. Photographic record showing electrical responses obtained in an experiment with a soft rubber tube arranged as in Fig. 5. The upper curve records the potential of *A* with reference to *C* and the lower curve that of *B* with reference to *C*. The tube was struck 6 times at *Z*. Each blow produced electrical responses due to the movement of the tap water past the electrodes. The distances between vertical marks represent one-tenth seconds.

ments were made with a rubber tube,[7] inserting glass T-tubes containing Ag-AgCl electrodes about 4 cm. apart,[8] connected to a third electrode (*C*) as shown in Fig. 5. In order to prevent any response in *C*

---

left to right it registers at each contact as soon as it reaches the left edge of the contact, and the time required for transmission to the right edge of the contact is negligible.

[7] An ordinary soft red rubber tube of about 3 mm. internal diameter.

[8] The electrodes were separated by 2 cm. of glass tubing and 2 cm. of rubber tubing.

it was placed 37 cm. away and a clamp[9] was inserted to prevent the compression wave from reaching it. On striking the tube at Z a record was obtained as shown in Fig. 6. Here we find a downward followed by an upward movement and a return to zero. These appear to be practically simultaneous at A and B and may be comparable with movements a and b in the living cell.[10] They may be regarded as due to the movement of liquid (streaming potential?) past the electrodes in the case of the tube or in the case of the cell past protoplasmic surfaces in which little or no alteration has occurred (the protoplasmic surface need not be solid). In both cases the electrical response falls off as the mechanical disturbance decreases.

Do the slower movements (c, d, and e) in the living cell arise from factors not present in the rubber tubing? They might be interpreted as the result of changes in the protoplasm, either structural or due to the inrush of ions into the protoplasm.[11] These changes in the protoplasm seem to proceed at different rates in its outer and inner surfaces since the form of the curve changes decidedly[12] when the solution applied to the outside of the cell is altered (which is not true of the fast movements a and b). The form of the curve has been discussed in previous papers:[1] it changes with distance from the cut in a manner which suggests that the intensity of the mechanical disturbance falls off as it travels.

It might be suggested that the slower movements are connected with an outflow of sap resulting from the cut but these movements are sometimes found when the cell is vigorously bent or pinched[13] without producing an outflow of sap.

[9] The clamp did not introduce sufficient resistance to interfere with the electrical measurement, since the resistance of the measuring instrument is over 30 megohms.

[10] When these electrodes were 40 cm. apart the rate of transmission was found to be about 80 meters per second.

[11] Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1929–30, **13**, 459, 547. Osterhout, W. J. V., Electrical phenomena in the living cell, in Harvey Lectures, 1929–30, The Williams and Wilkins Co., Baltimore, 1931, 169. Blinks, L. R., *J. Gen. Physiol.*, 1930–31, **14**, 127.

[12] When 0.001 M KCl is outside, the d movement is negative (upward); when 0.1 M KCl is outside, the d movement is positive (downward). Osterhout, W. J. V., and Harris, E. S., *J. Gen. Physiol.*, 1928–29, **12**, 167, 355.

[13] This will be discussed in a subsequent paper. A vigorous bend or pinch (with no outflow of sap) may produce a true death wave.

The electrical disturbance may pass to a neighboring cell as noted by Jost,[14] but the question arises whether the electrical response in the second cell is a death wave or a negative variation. A death wave might possibly result from a mechanical disturbance in the second cell but if this were lacking a negative variation might start as the result of (1) a mechanical disturbance insufficient to produce a death wave, or (2) a flow of current between the first and second cells, as elsewhere discussed.[15]

Some of the differences between death waves[16] and negative variations may be considered in this connection.

1. The death wave can pass a killed spot while the negative variation cannot except by the aid of a salt bridge.[15] On this basis the responses in the second cell must be regarded as negative variations, as shown by experiments in which a spot was killed in the middle of the second cell.

2. The death wave always shows a marked decrement in the intensity and speed of the response as it travels along: since this is not true of the response in the second cell it would appear to be a negative variation. The decrement shown by the death wave must be due to the fact that the mechanical disturbance becomes less intense as it travels along the cell and that the protoplasmic response falls off[1] as the intensity of the mechanical disturbance diminishes.[17]

3. Leading off near the cut from a spot in contact with 0.001 M KCl or tap water we observe a change in the sign of the P.D. across the

[14] Jost, L., *Sitzungsber. Heidelberger Akad. Wissensch. Math.-naturw. Klasse*, 1927, Abhandl. 13. Jost reports that the stimulus due to cutting may pass to a second, third, or a fourth cell. With our technique we have not seen any response (death wave or negative variation) in a third or fourth cell. When we used his technique we apparently got the result reported by Jost, but we interpret it somewhat differently. We suppose that when all the cells but one in the chain (in their natural union) are in the same reservoir of tap water a cut in any cell in the reservoir may be registered by the electrometer whether it causes a response in a neighboring cell or not.

[15] Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1929–30, **13**, 547.

[16] It will be shown in a subsequent paper that pinching or bending may produce responses intermediate in character between death waves and negative variations.

[17] Not only the amplitude but the speed of the response falls off, as mentioned above.

protoplasm when the cut is made:[1] if the spot is far enough away from the cut this does not occur but the curve goes to zero. In either case there is no recovery (*i.e.* the curve does not return to its original position). The response in the second cell does not show a change of sign and does show recovery.

4. The apparent speed of transmission of all the movements of the death wave is much greater than that of the negative variation. On this ground also the response in the second cell seems to be a negative variation since it is only about 2 cm. per second.

It would therefore seem that the response in the second cell is not a death wave but it is not necessary to conclude that it is due to a flow of current between the two cells (as elsewhere discussed[15]), since this seldom occurs in the absence of a salt bridge. It seems more probable that a mechanical impulse reaches the second cell and produces an electrical disturbance which continues down the cell as a propagated negative variation. This will be discussed in a subsequent paper.

SUMMARY

Cutting a cell of *Nitella* sets up a series of rapid electrical responses, transmitted at a rate too rapid to be measured by means of our records. These are followed by slower responses whose speed falls off as the distance from the cut increases, as though they were caused by a mechanical disturbance whose intensity falls off as it travels.

The faster responses seem to be due to the motion of sap past protoplasmic surfaces which have suffered little or no alteration (they seem to be similar to the electrical changes following a blow on the end of a soft rubber tube containing Ag-AgCl electrodes). The slower responses appear to be due to alterations in the protoplasm and are usually irreversible.