NATURE OF THE ACTION CURRENT IN NITELLA

III. Some Additional Features

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This paper describes certain forms of the action current which might be explained by assuming that the outer protoplasmic surface shows no rapid electrical change.

The records (made as previously described[1]) are in all cases monophasic. The cells were arranged as shown in Figs. 1 and 2. When the common contact, *F*, was not killed, simultaneous records[2] of *C*, *D*, and *E* showed that the common contact was quiescent and made no contribution to the recorded changes in P.D. No electrical or mechanical stimulus was applied, though in some cases the solutions used may have acted as chemical stimuli (care was taken to avoid any disturbance due to evaporation).

Let us now consider some of the records. The upward movement in Fig. 3 is due to a partial loss of the P.D. across the protoplasm. This appears to consist of two separate[3] P.D.'s, one at *Y* and another at *X* (Fig. 4). We suppose that in the usual form of negative variation both of these disappear when the action current flows outward. This produces a rapid electrical change in *X* (and makes a second peak in the action curve) as K^+ sweeps across the protoplasm[4] and out through *X* to the cellulose wall in which its lengthwise flow chiefly occurs (Fig. 5*a*).

When the non-aqueous layer *W* offers less resistance (owing to its

[1] Hill, S. E., and Osterhout, W. J. V., *J. Gen. Physiol.*, 1934–35, **18**, 377.

[2] Except in Fig. 14 these records were continuous as shown in previous papers (Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1930–31, **14**, 473, 611). In order to save space the record of only one of these spots is given in each case.

[3] The P.D.'s at *Y* and at *X* appear to depend chiefly on the difference in the concentration of K^+ on their opposite sides. In addition organic substances in *W* may play a part.

[4] *Cf.* Osterhout, W. J. V., *J. Gen. Physiol.*, 1934–35, **18**, 215.

thickness or content of electrolyte) or when[5] X or the cell wall is more resistant, so that the lengthwise flow of the outgoing current occurs chiefly in W (Fig. 5 *b*) instead of in the cellulose wall, no rapid electrical

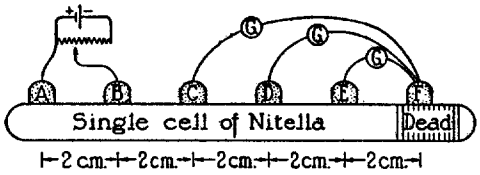

FIG. 1. Arrangement for testing *Nitella* cells. *GGG* represent string galvanometers (three strings inserted in the single magnetic field of a Type A Cambridge string galvanometer) with vacuum tube amplifiers, arranged as short period voltmeters. Absorbent cotton, moistened with the contact solution, connects the cells to saturated calomel electrodes.

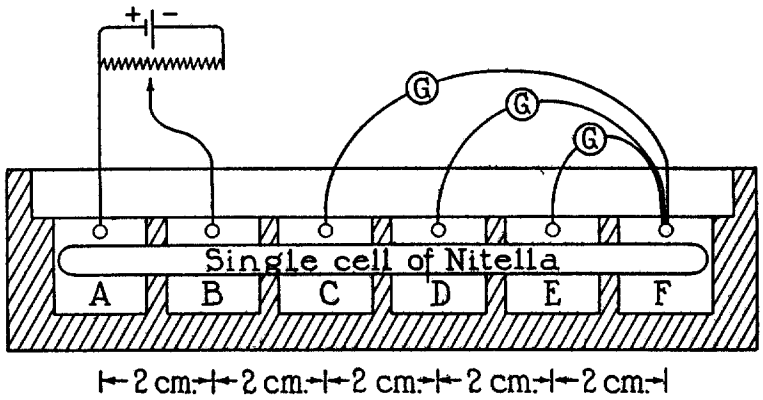

FIG. 2. Diagram of a series of paraffin cups A to F, with a single cell of *Nitella* passing through all of them. (In each partition the *Nitella* cell is sealed in with vaseline.) *GGG* as in Fig. 1. Ag-AgCl electrodes dip into the cups. *Cf.* Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1933–34, **17**, 87.

change is to be expected in X for, after moving outward through Y, K^+ may travel lengthwise in W without reaching X[6] to any considerable extent.

[5] The transverse resistance of X is largely due to polarization (*cf.* Blinks, L. R., *J. Gen. Physiol.*, 1929–30, **13**, 495), but the ohmic longitudinal resistance is doubtless high, since X is presumably non-aqueous and may be only a few molecules thick. The resistance of the cell wall varies according to its content of water.

[6] In general we should expect more electrolyte in the inner portion of W than in the outer since the electrolyte moves into W from the sap and it requires time

Under these conditions we should expect only one peak[7] in the action curve because too little K^+ would reach X to make a second peak. As the outward movement of K^+ would be relatively small its backward movement might be correspondingly quick so that recovery would be rapid. Both of these expectations are realized, as shown in Fig. 3.

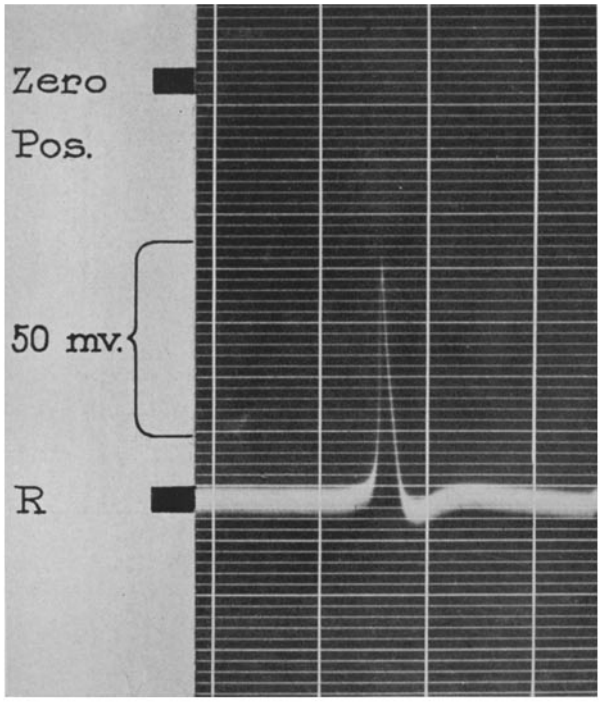

FIG. 3. Photographic record of experiment arranged as in Fig. 1, employing contacts C and F only. F was killed with $CHCl_3$ before the record started, to give monophasic response. C was in contact with 0.001 M KCl. The cell had been kept for 9 days in tap water. The vertical lines are 5 seconds apart. Temperature about 21°C. R represents the complete resting state.

The action curve does not go to zero; it has a single peak and rapid recovery. This would be expected if the curve were due to loss of P.D. at Y (Fig. 4), leaving intact the P.D. at X. There is some after-positivity.

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to carry it across W. Hence the lengthwise current would tend to flow more in the inner than in the outer portion of W.

[7] This would also be true if K reached X and its mobility in X were low, but in that case the resistance of X would be correspondingly high (*cf.* footnote 4).

In this case the curve does not go to zero. This is to be expected if the protoplasm is in the condition shown in Fig. 4, for the upward movement will cease as soon as the P.D. across Y disappears after which there remains[8] the P.D. of X. As the latter is presumably variable the behavior of the action curve would be expected to correspond and this

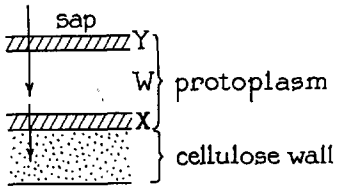

FIG. 4. Hypothetical diagram to show the P.D. in the protoplasm. The arrows show the direction in which the positive current tends to flow. It will be noted that lengthening either arrow would cause the action curve to move downward; *i.e.*, in the direction of the arrow. The length of the arrow is supposed to depend largely on the concentration gradient of K^+ across the layer.

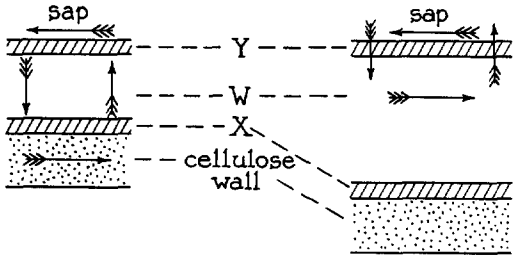

FIG. 5*a* FIG. 5*b*

FIG. 5*a*. Hypothetical diagram to show the flow of the action current under ordinary conditions.

FIG. 5*b*. Hypothetical diagram to show the course of the action current when its outgoing lengthwise flow is largely confined to the protoplasm. This may be due to greater thickness or greater electrolyte content of W or to increased resistance of X or of the cellulose wall.

expectation is fully borne out by observation as the amount by which the curve falls short of zero is quite variable. Occasionally a single peaked curve reaches zero in which case we suppose that there is practically no P.D. across X. This is especially common in *Chara*.[9]

[8] The P.D. of X apparently depends largely on the concentration of K^+ in W as compared with that in the external solution, but it may depend also on organic substances in W. *Cf.* Osterhout, W. J. V., *Ergebn. Physiol.*, 1933, **35**, 1014.

[9] In *Chara* (cf. footnote 4) the mobility of K^+ in X appears to be no greater

It may be added that, as previously explained,[4] the loss of P.D. appears to depend in part on an increase of permeability in Y and if this took the form of an actual breakdown (partial or complete) of Y it might produce a loss of P.D. even if little or no movement of K^+ took place, but such a breakdown would undoubtedly facilitate the outward movement of potassium across Y.

Let us now consider a phenomenon which is observed occasionally in connection with normal action currents but is more common with

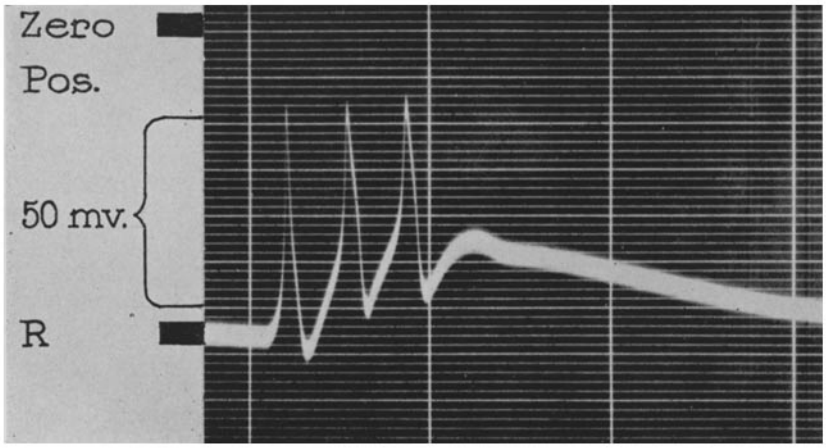

FIG. 6. Photographic record of an experiment arranged as in Fig. 1, employing contacts C and F only. F was killed with $CHCl_3$ before the experiment started, to give monophasic recording at C. C was in contact with Solution A (*cf.* Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1933–34, **17**, 87) in which the cell had been kept for 10 days. The vertical lines are 5 seconds apart. Temperature about 20°. R represents the complete resting state.

For explanation see Fig. 7.

the form of action current just discussed. This is the production of a series of action currents without complete recovery. An example is seen[10] in Fig. 6.

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than that of Na^+ hence the resistance of X is correspondingly great and the X arrow is probably small.

[10] The suspicion that any part of any of the curves shown in this paper may be instrumental in origin can be ruled out completely. When instrumental disturbances occur they are entirely different in character, being much more rapid, less regular, and of smaller amplitude.

In Fig. 6 the first upward movement is presumably produced by K^+ moving from the sap to the outer surface of Y, thereby reducing the P.D. of Y to zero and causing the Y arrow to disappear. The X arrow is supposed to suffer little alteration because little K^+ is carried across W to X and the curve does not go to zero because after the Y arrow has disappeared the X arrow remains and the distance between zero and the curve at the end of any upward movement measures the

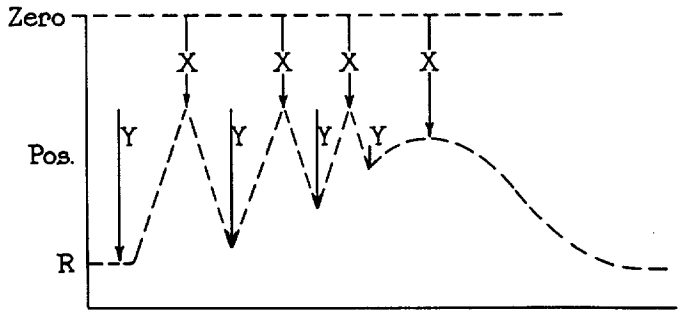


FIG. 7. Hypothetical diagram to show the P.D. across X (X arrow) and across Y (Y arrow): these P.D.'s are presumably due to the potassium gradient across X and Y. We suppose that the X and Y arrows may vary independently because K^+ need not be uniformly distributed across W.

When the first Y arrow has disappeared we suppose that the first upward movement has come to a stop: this applies in all cases, including the last arrow which is very short; in agreement with this the last upward movement is very small. The Y arrow is in all cases regarded as equal to the next upward movement of the curve. The last X arrow is longer than those which precede it presumably because the K^+ gradient across X has increased (the Y arrow is shorter because the K^+ gradient across Y has decreased).

The complete resting state is denoted by R (after the second and third upward movements the curve goes to an incomplete resting state).

length of the X arrow at that time. The length of the Y arrow is given by the extent of the upward movement (assuming that the Y arrow disappears entirely, as is most probable[4]).

After the second action current in Fig. 6 recovery is incomplete.[11]

[11] This recalls the tetanization of muscle and nerve. The incomplete resting state can persist in *Nitella* after action currents have ceased as shown in Figs. 9, 12, and 13. It should be remembered that in these cases no intentional stimulus was applied and under the conditions of the experiment loss of water at any point could not be responsible for the result.

We interpret this to mean that not all of the K^+ which moved into W has returned to the sap and the Y arrow becomes shorter (as shown in Fig. 7) because the K^+ gradient across Y becomes less: as the excess K^+ remaining in W has not yet reached X the X arrow remains unchanged, but a little later some K^+ reaches X and in consequence the X arrow lengthens.

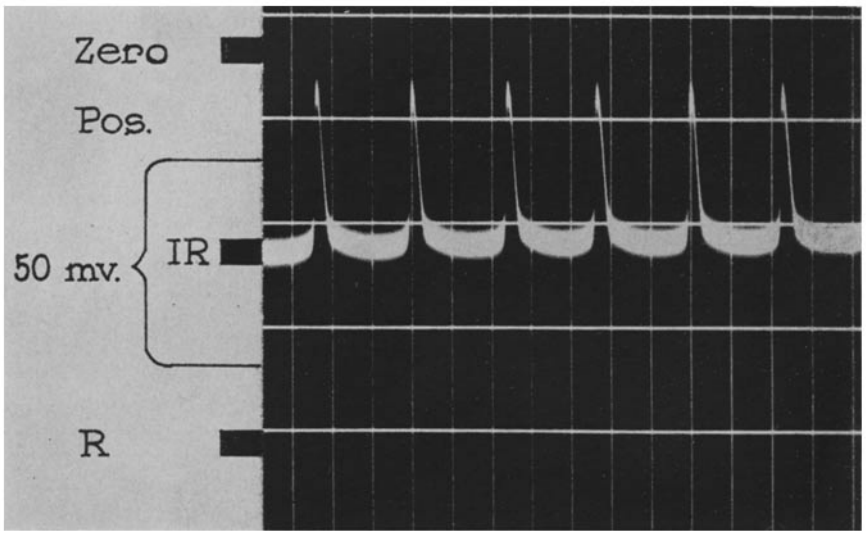


FIG. 8. Photographic record of an experiment arranged as in Fig. 1, employing contacts C and F only. F was not killed, but did not change during the recording. C was in contact with 0.01 M NH_4Cl (pH about 6.0). The cell had been kept for 2 days in Solution A. The vertical lines are 5 seconds apart. Temperature about 21°C. R represents the complete resting state and IR the incomplete resting state.

The first action current in this series (not shown here) carried the curve upward from the resting state (R) and the figure shows a series of action curves returning each time to an incomplete resting state (IR). This might be interpreted as meaning that approximately the same amount of K^+ moves into the sap each time during recovery but this amount is not sufficient to cause complete recovery. The P.D. across X (X arrow, see Fig. 7) remains constant, presumably because no K^+ moves from the sap to X.

Complete recovery restores the protoplasm to the normal resting state which is designated by R: the incomplete resting state produced by incomplete recovery may be called IR.

When recovery is incomplete we suppose that the K^+ moving into

W does not all return to the sap and it is evident that with successive action currents we may have:

1. The same amount is returned each time so that the degree of recovery remains the same for all the action currents. An example is shown in Fig. 8. Here the first action current (not shown in the

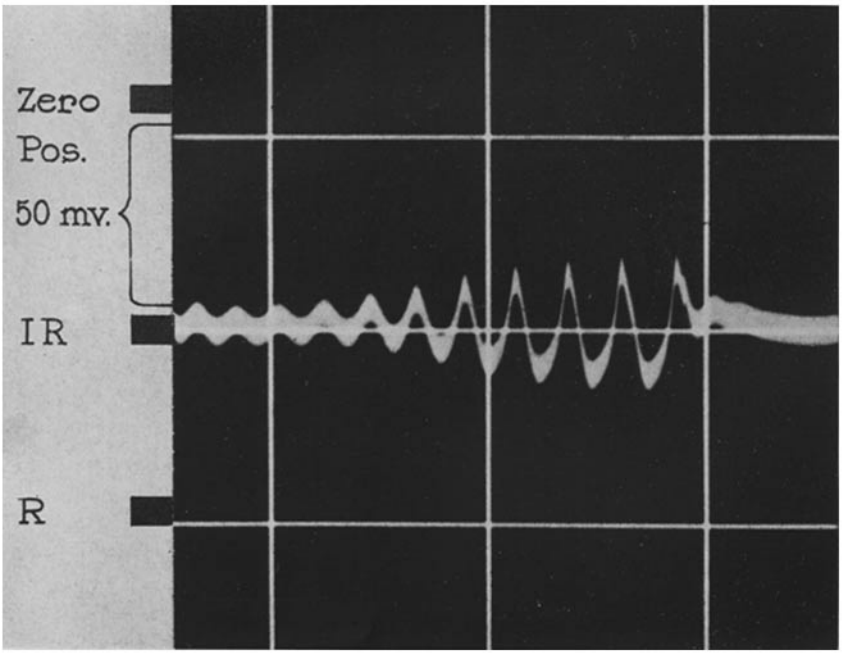


FIG. 9. Photographic record of an experiment arranged as in Fig. 2, employing contacts *D*, *E*, and *F* only. *F* was not killed but did not change during the recording: all spots in contact with 0.01 M NH_4Cl (about pH 6). The cells were kept for 5 days in Solution A. The vertical lines are 5 seconds apart. Temperature about 20°C.

This record might be interpreted as meaning that a little more K^+ is returned to the sap with each successive recovery thus increasing the P.D. across *Y* (*Y* arrow, Fig. 7) and shortening the P.D. across *X* (*X* arrow).

record) carried the curve up from the normal resting state (*R* in the figure) and this was followed by a series of action currents. Recovery was incomplete but its extent was quite uniform.

2. A little more K^+ is returned to the sap with each successive action

current so that the degree of recovery increases as time goes on. Such a case is seen in Fig. 9. Here the decrease of K^+ in *W* not only length-

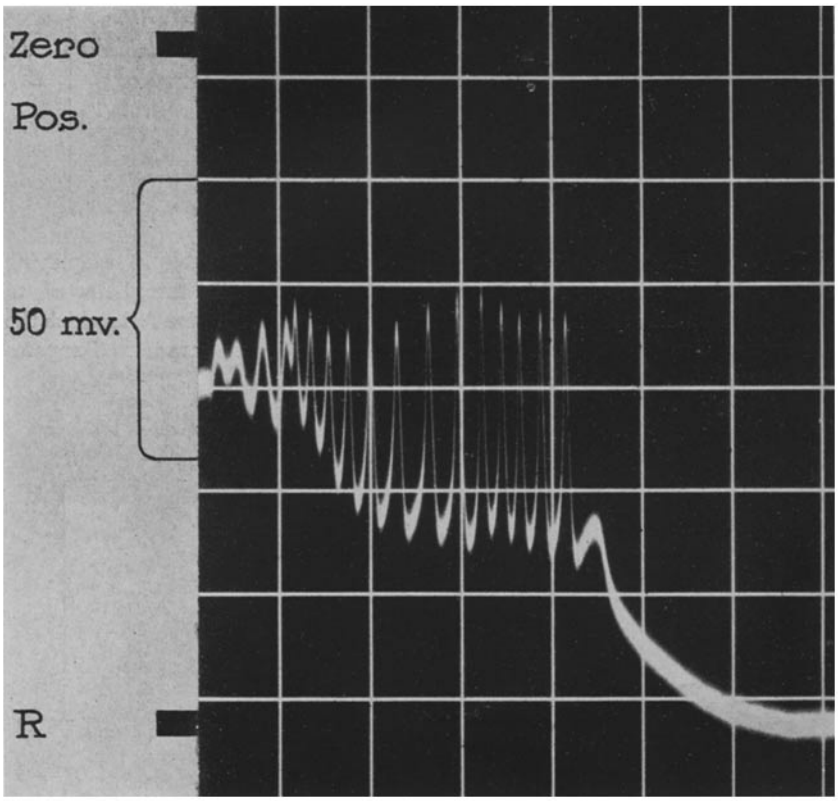


FIG. 10. Photographic record of an experiment arranged as in Fig. 2, employing contacts *C* and *F* only. *F* was killed with $CHCl_3$ before the record started, to secure monophasic recording at *C*. *C* was in contact with 0.01 M NH_4 acetate (pH about 6). The cells had been kept for 29 days in Solution A. The vertical lines are 5 seconds apart. Temperature about 22°C. The initial action curve (not shown here) was due to electrical stimulation at *B* (100 mv. D.C.).

This record might be interpreted as in Fig. 9 except that the P.D. across *X* (*X* arrow) does not shorten, presumably because only the inner part of *W* loses K^+.

ens the *Y* arrow but it also shortens the *X* arrow. In Fig. 10 this shortening of the *X* arrow does not occur, presumably because only the inner part of *W* loses K^+.

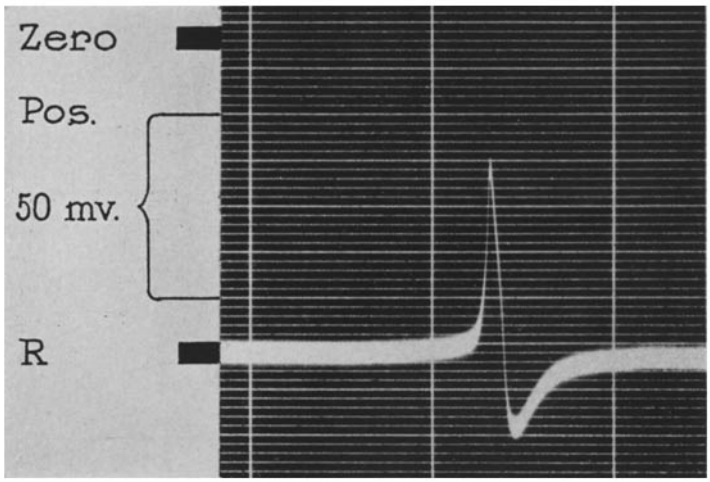


FIG. 11. Photographic record of experiment arranged as in Fig. 1, employing contacts *C* and *F* only. *F* was killed with $CHCl_3$ before the record started, to secure monophasic recording at *C*. *C* was in contact with Solution A, in which the cell had been kept for 10 days. The vertical lines are 5 seconds apart. Temperature about 20°C. *R* represents the complete resting state.

This record shows after-positivity (for discussion see text).

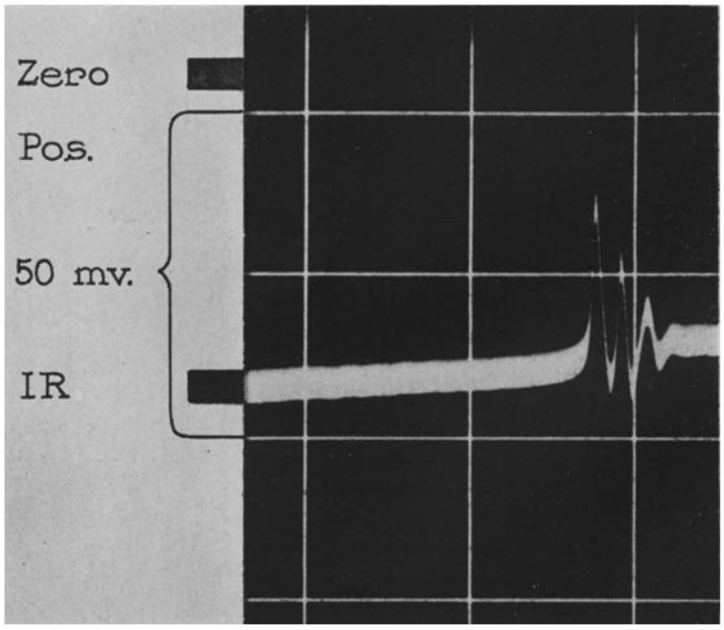


FIG. 12. Photographic record of an experiment arranged as in Fig. 2, employing contacts *C*, *D*, and *F*. *F* was not killed but it did not change during the recording. *C* (the only spot whose record is shown here) was in contact with 0.01 M NH_4Cl (pH about 6) as were also *D* and *F*. The cell had been kept 5 days in Solution A. Temperature about 20°C. The vertical lines are 5 seconds apart.

This record shows a shortening of the *Y* arrow and lengthening of the *X* arrow which might be due to increase of K^+ in *W*.

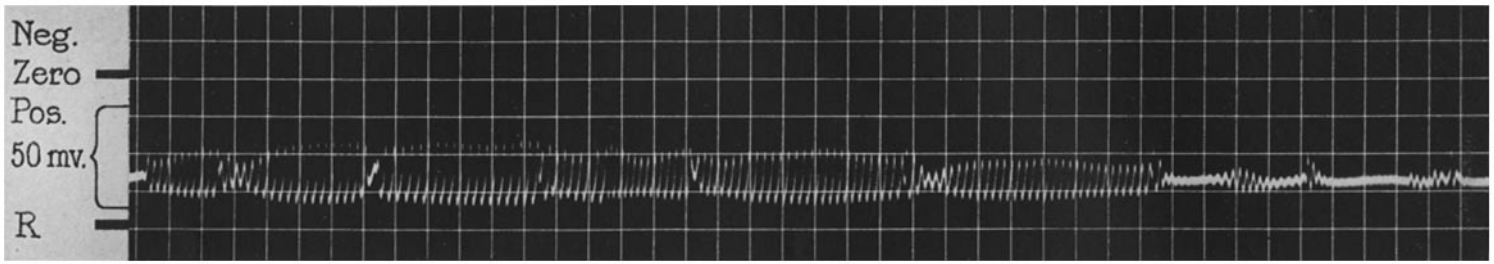


FIG. 13. Photographic record of an experiment arranged as in Fig. 1, employing contacts *C* and *F* only. *F* was killed with $CHCl_3$ before the record started, to give monophasic recording at *C*. *C* was in contact with 0.01 M NH_4 acetate, pH 7.0, in which the cell had been soaked for 24 minutes before the record started; previous to this the cell had been kept in Solution A. The vertical lines are 5 seconds apart. Temperature about 22°C. *R* represents the complete resting state.

The record shows several cases where the P.D. across *Y* (*Y* arrow) increases (as shown by the increasing length of the upward excursion) as the P.D. across *X* (*X* arrow) decreases followed by the reverse relation. This would be expected if the result depended on the decrease of K in *W* followed by an increase.

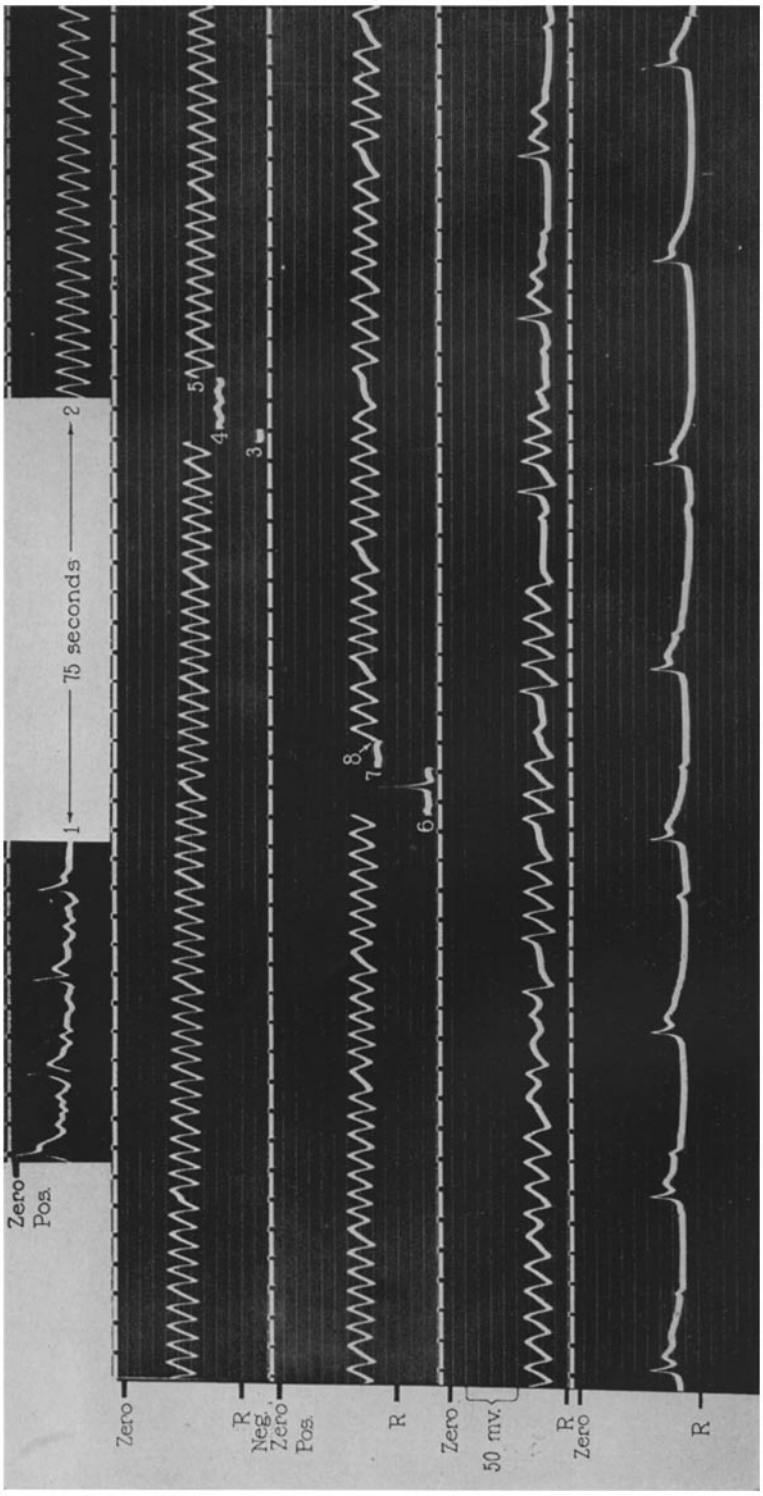

Zero
Pos.
75 seconds
1
2
3
4
5
6
7
8
Zero
R
Neg.
Zero
Pos.
R
Zero
50 mv.
R
Zero
R

FIG. 14

It seems possible that the inward movement of K^+ may sometimes raise K^+ in the sap above the normal and so produce the after-positivity seen in Fig. 11. This might be brought about in other ways as suggested elsewhere.[4] It is of comparatively rare occurrence.

3. A little less K^+ is returned to the sap with each successive action current so that the *Y* arrow becomes less and less. We see this in Fig. 6 which also shows that as time goes on some of the K^+ reaches *X* and lengthens the *X* arrow. The same thing is shown more strikingly in Fig. 12: here one might get the impression that the P.D. found at the start (*IR*) is restored by the lengthening of the *X* arrow instead of by the lengthening of the *Y* arrow. If such a procedure restored the true resting state (*R*) it might be termed false recovery to distinguish it from true recovery in which the P.D. is restored by the lengthening of the *Y* arrow. After false recovery action currents could be produced by movements of K^+ across *X*. Evidently false recovery is theoretically possible but it does not seem probable that it plays an important rôle.

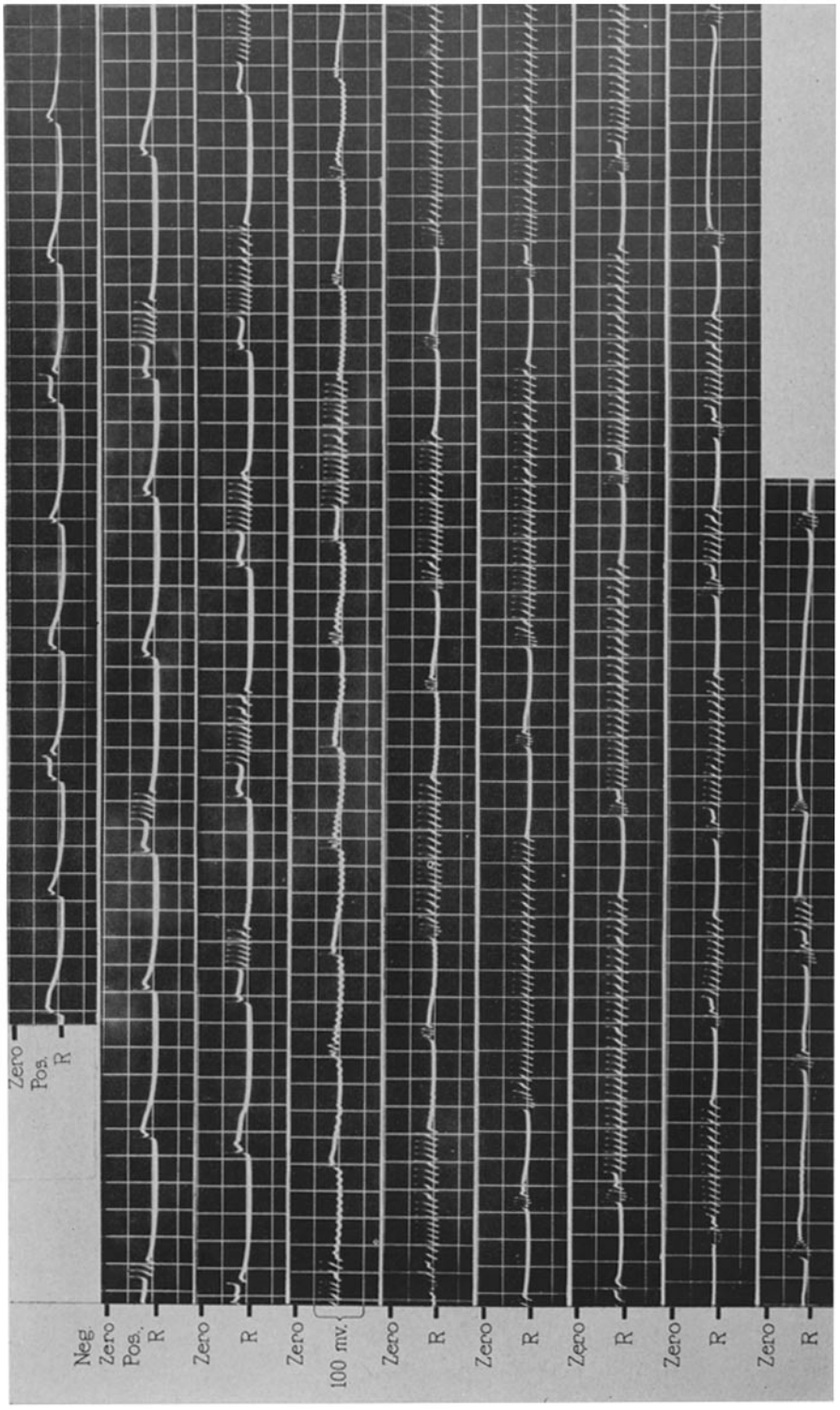
FIG. 14. This photographic record was continuous (except between the places marked 1 and 2): for purposes of reproduction it was cut into strips which are arranged consecutively. The cell was arranged as in Fig. 1. Only one string was used which was connected to *D* except as otherwise noted (*F* was killed with chloroform before the record started). *C* was in contact with 0.001 M $MgCl_2$, *D* with 0.01 M $MgCl_2$, *E* with 0.1 M $MgCl_2$. At 3 the string was switched to *E*; at 4 to *C*; at 5 back to *D*; at 6 to *E*; at 7 to *C*; at 8 back to *D*.

At the start *D* is 55 mv. positive to *F* and the first action current reduces this to zero; this action curve has a double peak. The subsequent action curves do not go to zero and after a time only one peak is observed. But at the end of the record a double peak again appears. Otherwise the behavior resembles that in Fig. 8.

From the behavior of the string when switched to *C* and *E* we may infer that these spots were not continuously behaving like *D*.

Temperature about 20°C. Previous to the record the cell had been kept for 15 days in Solution A. The time marks (at the upper margin) are 5 seconds apart. *R* denotes the complete resting state.

The regularity of the oscillations in some parts of the record might raise the suspicion that they were due to instrumental causes but this has been excluded by a careful study of all the possible instrumental disturbances. It will also be noticed that toward the end these oscillations pass over into action currents which are more nearly normal.


Zero
Pos.
R
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Zero
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R
Zero
R
Zero
100 mv.
Zero
R
Zero
R
Zero
R
Zero
R
Zero
R
Zero
R

FIG. 15

4. In such cases as those shown in Fig. 13 we have the situation described under (2) followed by that described under (3); *i.e.*, a decrease of K^+ in *W* followed by an increase.

These considerations would lead us to expect that as K increased in *W* the *Y* arrow would shorten and in proportion as K^+ reached *X* the *X* arrow would lengthen: also that if K decreased in *W* the *X* arrow would shorten (or in some cases remain constant[12]) and the *Y* arrow would lengthen. But we should not expect both *X* and *Y* arrows to lengthen or shorten simultaneously. These expectations are fully borne out by observation.

Figs. 14 and 15 show interesting cases of a transition from a double peaked to a single peaked action current. One way of accounting for this would be to assume that some of the K^+ and Na^+ which moves into *W* is not returned during the process of recovery[13] and that in consequence *W* becomes more conductive so that the action current becomes single peaked. An increase of organic ions in *W* might have the same effect. The opposite process would change a single to a double peak as seen in the last part of the record shown in Fig. 14.

The action curves in these two figures can be interpreted in the manner already indicated. They recall some of the records obtained by Adrian[14] and by Hoagland[15] with nerve.

FIG. 15. This photographic record was continuous and for purposes of reproduction was cut into strips which are arranged consecutively. The cell was arranged as in Fig. 2: only *E* is shown; *C* and *D* were recorded but are not shown here: they prove that the record was monophasic since there was no activity at *F* (*F* was not killed). *C* was in contact with 0.01 M NH_4Cl (pH about 6), *D* with 0.01 M CsCl, *E* with 0.01 M NaCl.

The first action curves are double peaked (the curves do not go to zero), but after a time single peaks appear; then double peaks recur and are followed by single peaks; this happens several times. Later on bursts of single peaks occur which recall some of the records obtained by Adrian and by Hoagland with nerve.

Temperature about 22°C. Previous to the record the cells had been kept for 15 days in Solution A. Time marks are 5 seconds apart.

This record also shows several of the features discussed earlier in the paper.

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[12] It would remain constant if the concentration of K diminished in the inner part of *W* but not in the outer part.

[13] This may or may not lengthen the *X* arrow, depending on how much K^+ reaches *X*.

[14] Adrian, E. D., The basis of sensation, London, Christophers, 1928.

[15] Hoagland, H., *J. Gen. Physiol.*, 1932–33, **16**, 695, 715.

In conclusion it may be desirable to state that the forms of the action curve here described are relatively infrequent and constitute less than 5 per cent of the curves observed by us. The first cases of this sort were observed in 1925 in experiments carried out by E. S. Harris in collaboration with the senior author and additional ones have been noted from time to time up to the present.

When three places on the cell were recorded it was found that in some cases the action current was confined to one spot but in other cases it was propagated to the other spots where it was sometimes quite similar but in other cases varied in form and amplitude.

The hypothesis outlined in this paper seems to involve nothing improbable and may be useful in bringing under a single point of view numerous otherwise puzzling forms of the action curve.

SUMMARY

Several forms of the action curve are described which might be accounted for on the ground that the outer protoplasmic surface shows no rapid electrical change. This may be due to the fact that the longitudinal flow of the outgoing current of action is in the protoplasm instead of in the cellulose wall. Hence the action curve has a short period with a single peak which does not reach zero.

On this basis we can estimate the P.D. across the inner and outer protoplasmic surfaces separately. These P.D.'s can vary independently.

In many cases there are successive action currents with incomplete recovery (with an increase or decrease or no change of magnitude).

Some of the records resemble those obtained with nerve (including bursts of action currents and after-positivity).