NATURE OF THE ACTION CURRENT IN NITELLA

IV. Production of Quick Action Currents by Exposure to NaCl

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To understand the nature of the action current we must bring it as far as possible under experimental control.

Several methods have already given promising results[1] with *Nitella*. One of these consists in treating cells with NaCl.

The usual action curve of *Nitella* is shown[2] in Fig. 1. It has been suggested[3] that it depends on the movement of K^+ and if this can be manipulated it should be possible to control the action current. An attempt in this direction has met with some success. The principle involved is as follows.

The normal action current appears to flow outward from the sap across the protoplasm, then lengthwise, chiefly in the cellulose wall, and back into the sap (Fig. 3, at *a*). In crossing the protoplasm it passes through an aqueous layer *W* bounded by very thin, non-aqueous layers *X* and *Y*, whose resistance is undoubtedly very high. If *W* could be made more conductive so that the lengthwise flow would take place in it (Fig. 3, at *b*) rather than in the cellulose wall we might expect more rapid recovery and action curves with a single peak.

[1] Unpublished results. See also Osterhout, W. J. V., and Hill, S. E., Some ways to control bioelectrical behavior, in Cold Spring Harbor symposia on quantitative biology, Cold Spring Harbor, Long Island Biological Association, 1936, **4,** 43. Hill, S. E., *Biol. Bull.*, 1937–38, **73,** 362.

[2] The experiments were performed on *Nitella flexilis*, Ag., using the technique described in a former paper (Hill, S. E., and Osterhout, W. J. V., *J. Gen. Physiol.*, 1937–38, **21,** 541). Temperature 18–25°C.

The cells, after neighboring cells had been cut away, were placed in Solution A for some days. They were transferred to 0.01 M NaCl for half an hour or more and then placed on paraffin blocks, using flowing contacts or cup technique, as previously described.

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

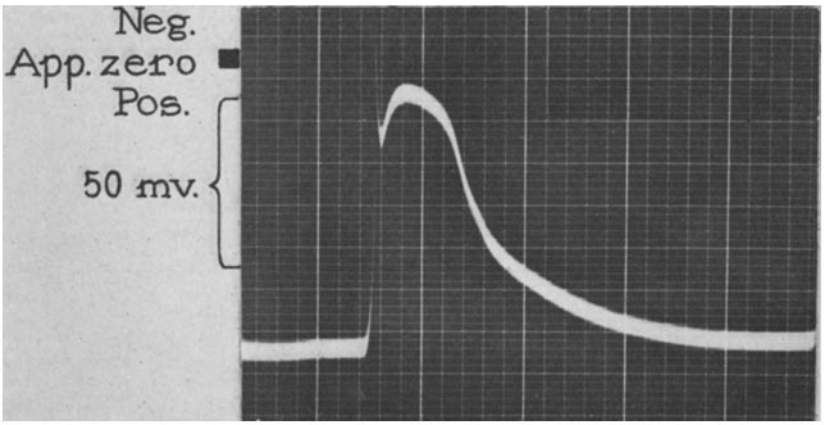

FIG. 1. Shows a normal monophasic action current. The spike goes to zero as frequently happens. (In this case the zero is only approximately known since it is assumed that in contact with 0.01 M KCl the P.D. is zero (as is usually the case): hence it is labelled "App. zero.") This applies to all the other figures except Fig. 8.

The leads were arranged as shown in Fig. 2. The record of *D* in contact with 0.01 M NaCl is shown: that of *C* and *E* (omitted to save space) shows that no change occurred at *F*, which was in contact with 0.01 M KCl.

Heavy time marks 5 seconds apart. Temperature 23°C.

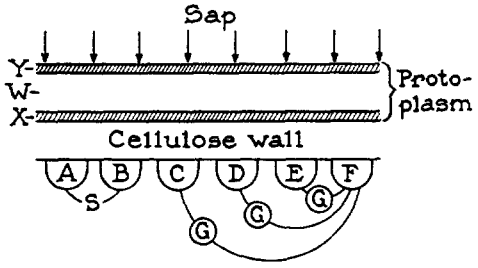

FIG. 2. Diagram to show the arrangement of leads and the hypothetical structure of the protoplasm.

The aqueous layer *W* is bounded by the non-aqueous layers *X* and *Y*. The arrows show the P.D. at *Y* supposedly due chiefly to an outward gradient of K^+ across *Y*. In addition there may be in some cases an outward gradient at *X*.

The electrical stimulus consists of a current entering at *A* and going out at *B*.

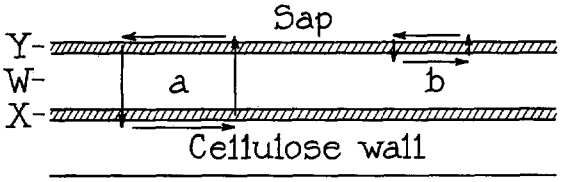

FIG. 3. Hypothetical diagram to show the course of the normal action current (*a*) and the course of the quick action current (*b*).

Both of these expectations are realized when we expose the cell for half an hour or more to 0.01 M NaCl which presumably makes W more conductive.

Let us now consider some of the important factors in this situation.

The sap contains about 0.05 M KCl and 0.05 M NaCl. The effect[4] of K^+ is so great that other ions may be neglected in the present discussion.

The outward flow of the action current means an outward movement of K^+ and other cations. This may have the following consequences.

(*a*) *Double and Single Peaks.*—The double peak of the typical action current may be due to the following:

The o Movement.—When K^+, moving outward, reaches the outside of Y the concentration gradient of K^+ across Y (on which the P.D. depends) is abolished and in consequence there is a loss of P.D. (negative movement or spike of the action curve) (Fig. 4).

The p Movement.—When K^+ reaches the inside of X it will create an outwardly directed P.D. (positive movement of the action curve). With the completion of o and p we have the first peak of the curve.

The q Movement.—When K^+ reaches the outside of X the positive P.D. across X disappears more or less completely (second negative movement of the curve).

The r Movement.—The process of recovery now sets in, moving K^+ inward across X, *i.e.* back toward the sap and thus reversing the q movement. Thus a positive movement occurs and this together with the q movement, makes the second peak.

On theoretical grounds it might be predicted that soaking the cell in a solution of NaCl would make the protoplasm more conductive so that K^+ would flow mostly in W and not much of it would reach X; hence the q movement would not occur and the curve would have only one peak.

(*b*) *Rapidity.*—This depends on the time of recovery which might be regarded as the time required to move K^+ back into the sap. But if K^+ merely moved into W instead of into the cellulose wall it could return more quickly and thus shorten the recovery time.

[4] This appears to be due to a combination of a high mobility and a high partition coefficient. Even when the mobility of Na^+ becomes relatively high it has much less effect than K^+ because the partition coefficient of the latter is so high.

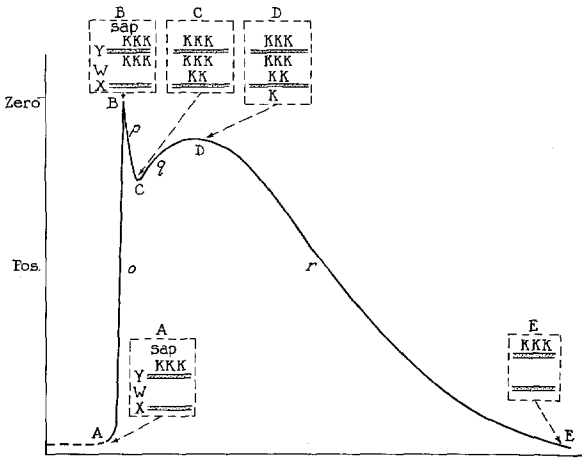

FIG. 4. The unbroken line shows change in P.D. during the action current in *Nitella*, supposedly due to the outward movement of potassium. The broken line shows the P.D. in the resting state, before the outward movement of potassium begins.

In the diagrams the symbol K denotes the outward moving potassium (reduction in its concentration is shown by reduction in the number of symbols). Each stage of its progress is marked by a change in P.D.: for example, in Diagram A the observed P.D. is supposedly due to the gradient of potassium across *Y*; in Diagram B we see that potassium has reached the outer surface of *Y* and in consequence the gradient and the P.D. have disappeared.

The duration of the action current is usually about 15 to 30 seconds.

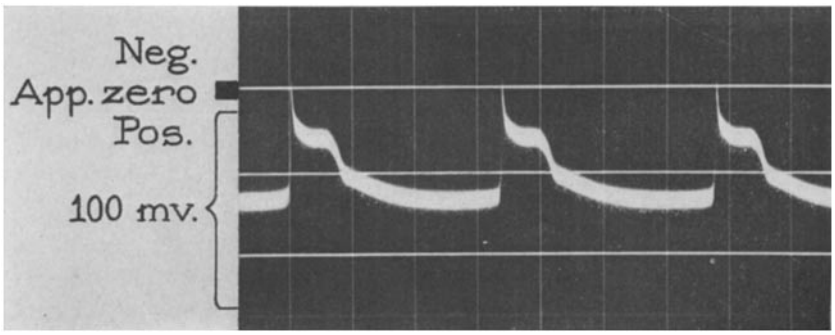

FIG. 5. Shows transitional forms of action current after exposure to 0.01 M NaCl. The record of *E* (in contact with 0.01 M NaCl) is shown (*cf.* Fig. 2): those of *C* and *D* (omitted to save space) show that no change occurred at *F*, which was in contact with 0.01 M KCl.

Heavy time marks 5 seconds apart. Temperature 25°C.

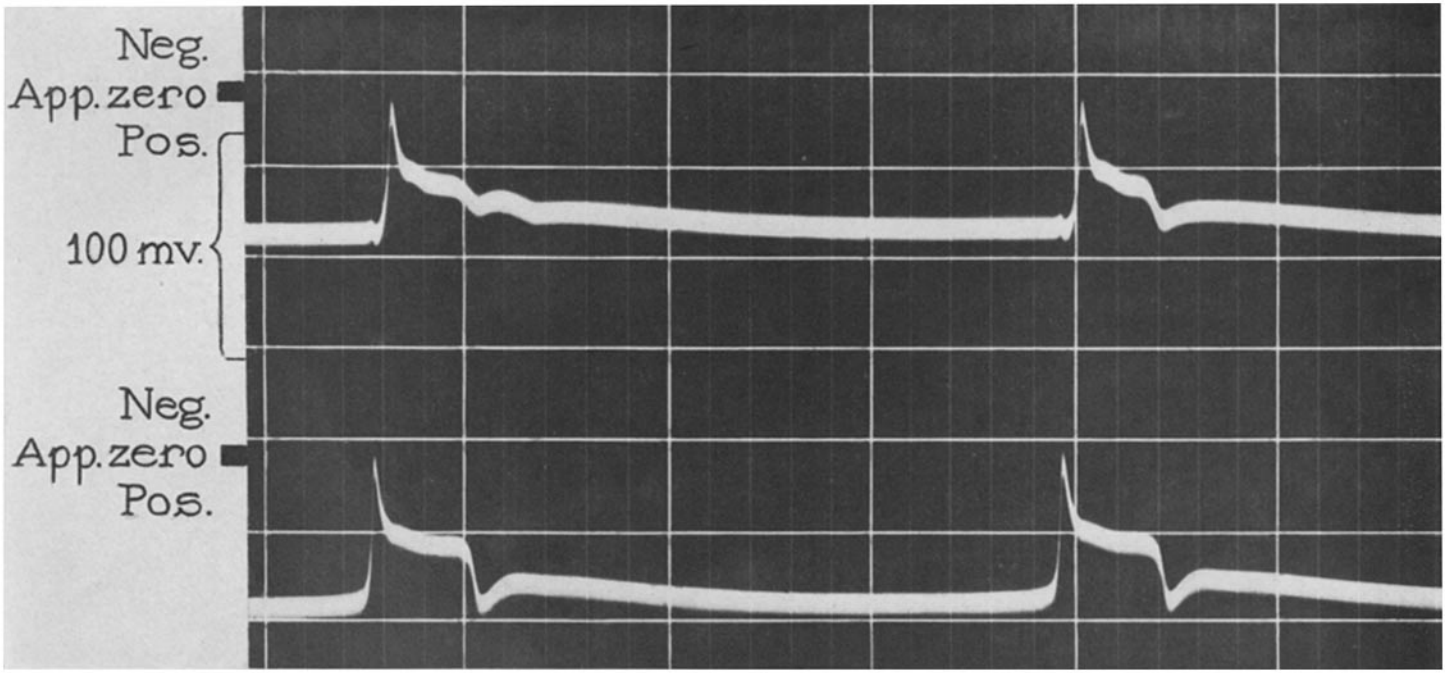

FIG. 6. Shows transitional forms of action current after exposure to 0.01 M NaCl. The records of *D* and *E* (in contact with 0.01 M NaCl) are shown (*cf.* Fig. 2): there are no simultaneous movements indicating changes at *F*, which was in contact with 0.01 M KCl (*F* acts as pacemaker).

The response at *D* (upper string) differs somewhat from that at *E* (lower string).

Heavy time marks 5 seconds apart. Temperature 25°C.

It is possible that the outgoing K^+ may move only a very short distance into *W* before reversing its direction and returning to the sap. The whole process may be very rapid because *Y* presumably becomes very permeable when the action current occurs.[3] The increase in the permeability of *Y* appears to be favored by NaCl since its application lowers the voltage[5] needed for stimulation, which may indicate that *Y* more easily becomes permeable. We also find that the absolute refractory period is shortened by exposure to NaCl. These changes favor the production of rapid action currents.

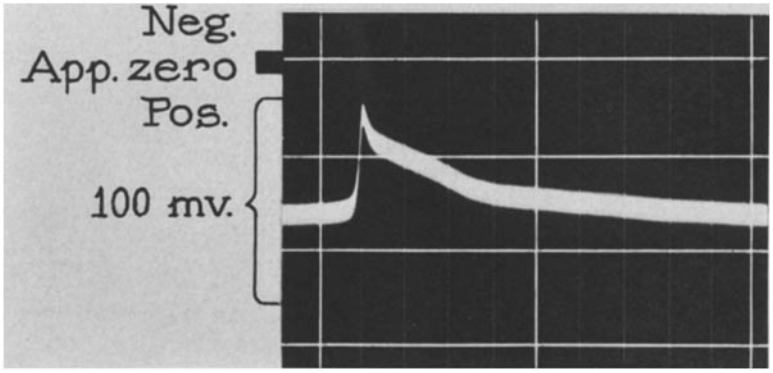

FIG. 7. Shows a transitional form of action current after exposure to 0.01 M NaCl. The record of *D* is shown (*cf.* Fig. 2): those of *C* and *E* (omitted to save space) show that no change occurred at *F*, which was in contact with 0.01 M KCl. Heavy time marks 5 seconds apart. Temperature 25°C.

Various transitional forms between the normal and the rapid action currents are shown in Figs. 5 to 8. Whether all of these are produced by each cell in a fixed order is not known.

The beginning of quick movements is seen in Figs. 9 and 10. These usually occur spontaneously after a time but somewhat prior to this they can as a rule be induced by a brief electrical stimulation (300 mv. D.C. applied for about 0.1 second). It will be noted that the quick movements appear to be superimposed on a slow action curve conforming more or less to the normal pattern.

In Fig. 11 we see the beginning of a long train of action currents.

[5] Although the voltage is lowered the resistance of the cell decreases so as to compensate and permit a flow of current which is not very different.

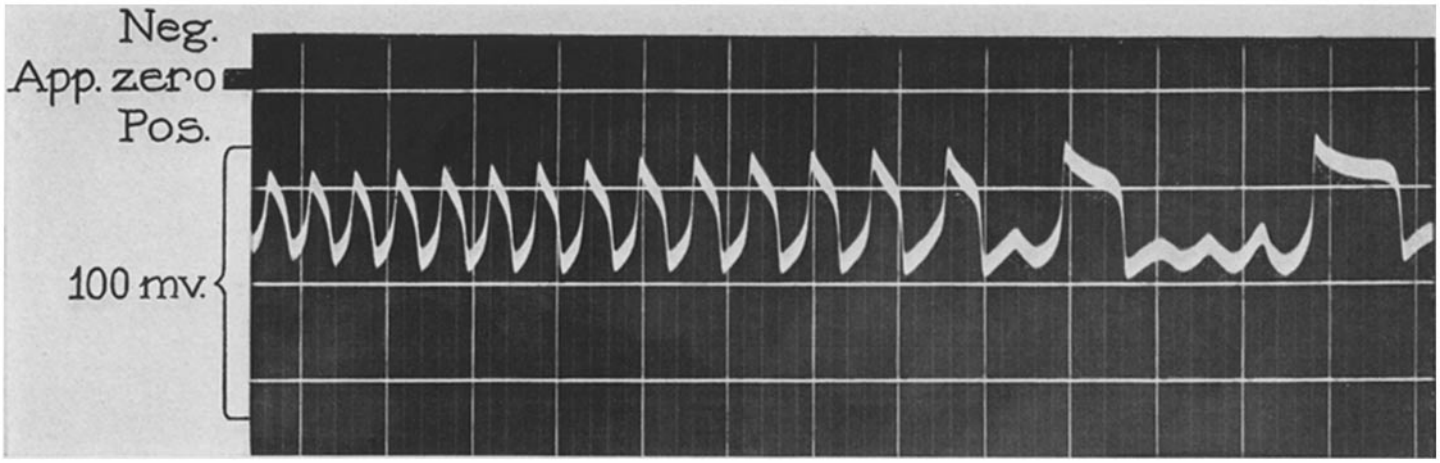

FIG. 8. Shows transitional forms of action current after exposure to 0.002 M NaCl. The record of *C* (in contact with 0.02 M NaCl) is shown: those of *D* and *E* (omitted to save space) show that there was no change at *F*, which was in contact with 0.02 M NaCl (*cf.* Fig. 2). *F* was in contact with 0.02 M NaCl and was kept from changing its P.D. by cooling to about 2°C.
Time marks 5 seconds apart. Temperature 21°C.

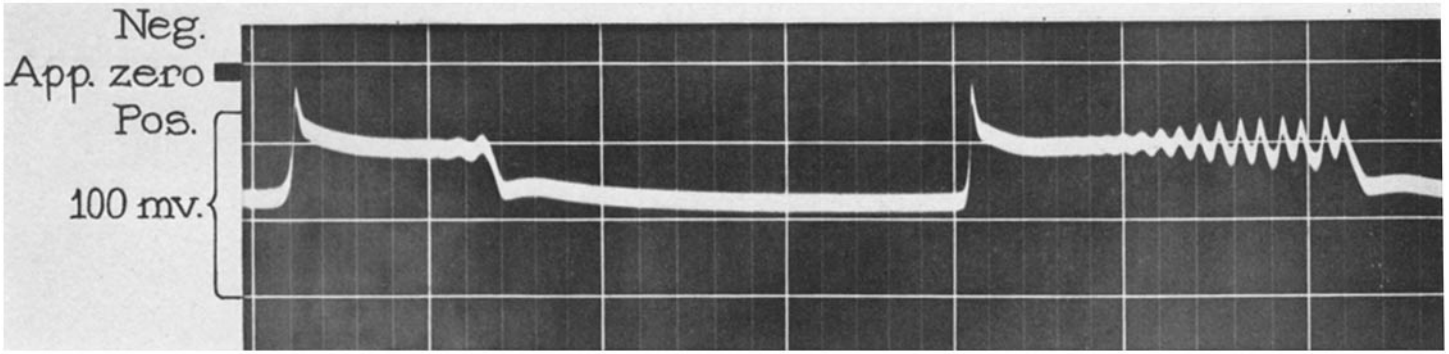

FIG. 9. Shows the beginnings of quick action currents after exposure to 0.01 M NaCl. The record of *C* (in contact with 0.01 M NaCl) is shown (*cf.* Fig. 2): those of *D* and *E* (omitted to save space) show that no change occurred at *F* (in contact with 0.01 M KCl).

Heavy time marks 5 seconds apart. Temperature 25°C.

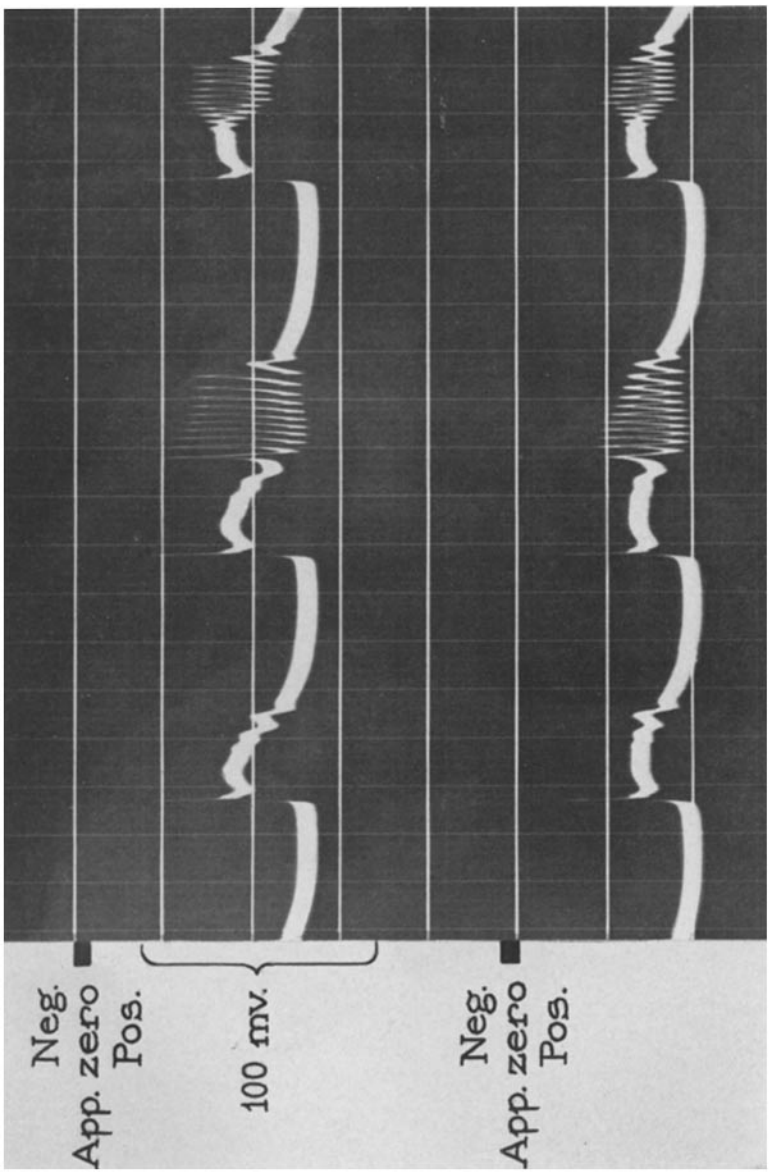

FIG. 10. Shows the beginnings of quick action currents after exposure to 0.01 M NaCl. The records of *C* and *D* (in contact with 0.01 M NaCl) are shown (*cf.* Fig. 2): that of *E* (omitted to save space) shows that no change occurred at *F*, which was in contact with 0.01 M KCl.

Heavy time marks 5 seconds apart. Temperature 25°C.

Such trains may go on without interruption until several hundred have appeared. But as a rule they are interrupted by intervals of rest.[6] Portions of such trains are seen in Figs. 12 and 13. In Fig. 12 the action curves are almost identical at *C*, *D*, and *E* (Fig. 2) but in Fig. 13 this is not the case.

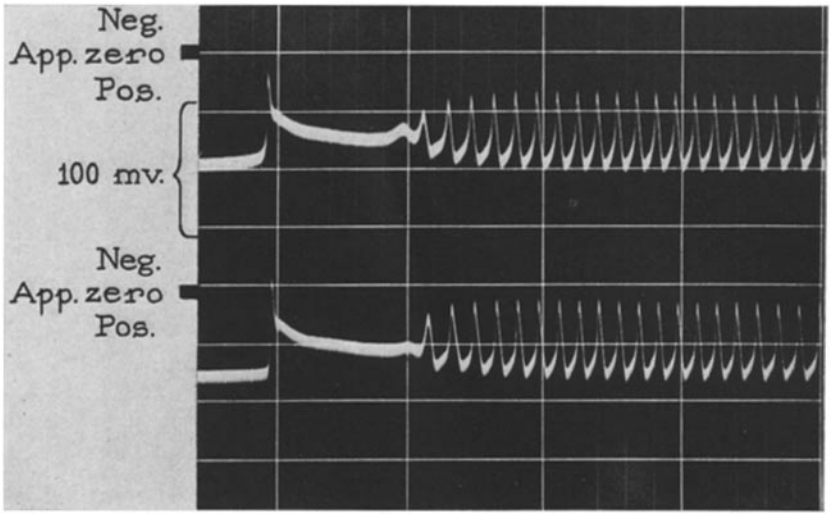

FIG. 11. Shows the beginnings of quick action currents after exposure to 0.01 M NaCl. The records of *C* and *D* (in contact with 0.01 M NaCl) are shown (*cf.* Fig. 2): the absence of any simultaneous movements indicates that no change occurred at *F*, which was in contact with 0.01 M KCl.

The pacemaker is at the left of *C*, as shown by the fact that in each action current the upper string moves first. The responses at *C* (upper string) and *D* (lower string) are very similar.

Heavy time marks 5 seconds apart. Temperature 25°C.

It happens that the upstroke is slower than the downstroke in these figures, and Fig. 14 indicates that in some cases at least the upstroke involves a certain hesitation when the "base line," *i.e.* the resting potential, is reached. The downstroke in this figure

[6] An example is shown in a previous paper (Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1934–35, **18,** 512 (Fig. 15)). In this case the exposure to NaCl was so short that we may suppose that *W* was already more conductive than usual when the cell was placed in NaCl.

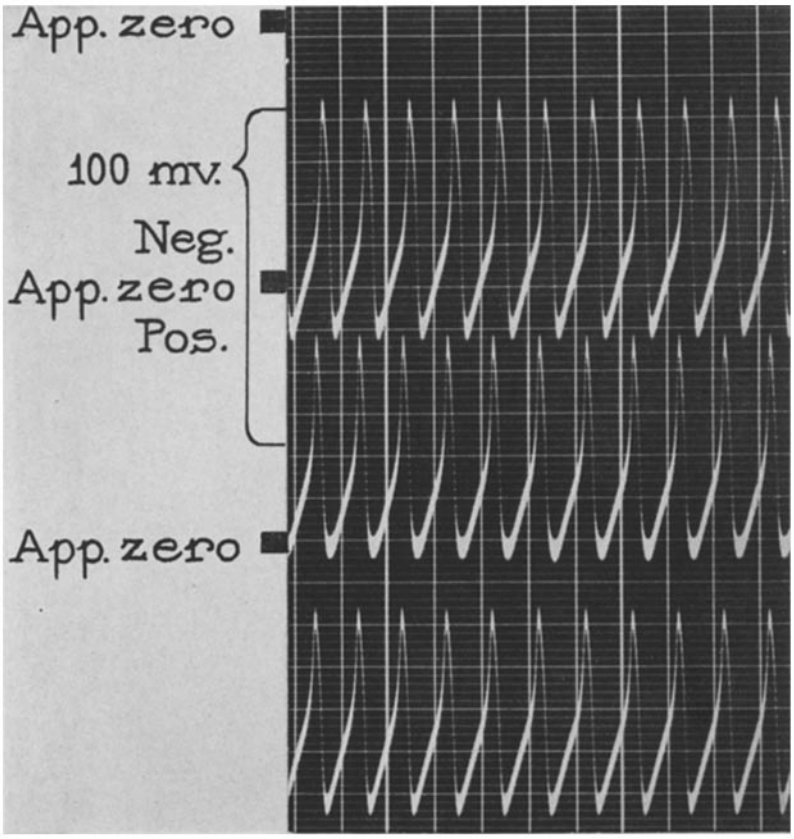

FIG. 12. Portion of a train of quick action currents after exposure to 0.01 M NaCl. The records of *C*, *D*, and *E* (all in contact with 0.01 M NaCl) are shown (*cf.* Fig. 2): the absence of simultaneous movements indicates that no changes occurred at *F*, which was in contact with 0.01 M KCl.

The pacemaker was at the right of *E* as indicated by the fact that *E* (lower string) changes first in each action current. The responses are similar at each spot.

Time marks 1 second apart. Temperature 23°C.

appears to go below the "base line"[7] and then rise, linger at the base line for a brief period, and then go on (*cf.* Fig. 13).

The shapes of the action curves have been discussed in previous

[7] As shown in a previous paper (Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1934–35, **18,** 499) there may be two "base lines," one of which is the "complete" and the other the "incomplete" resting potential.

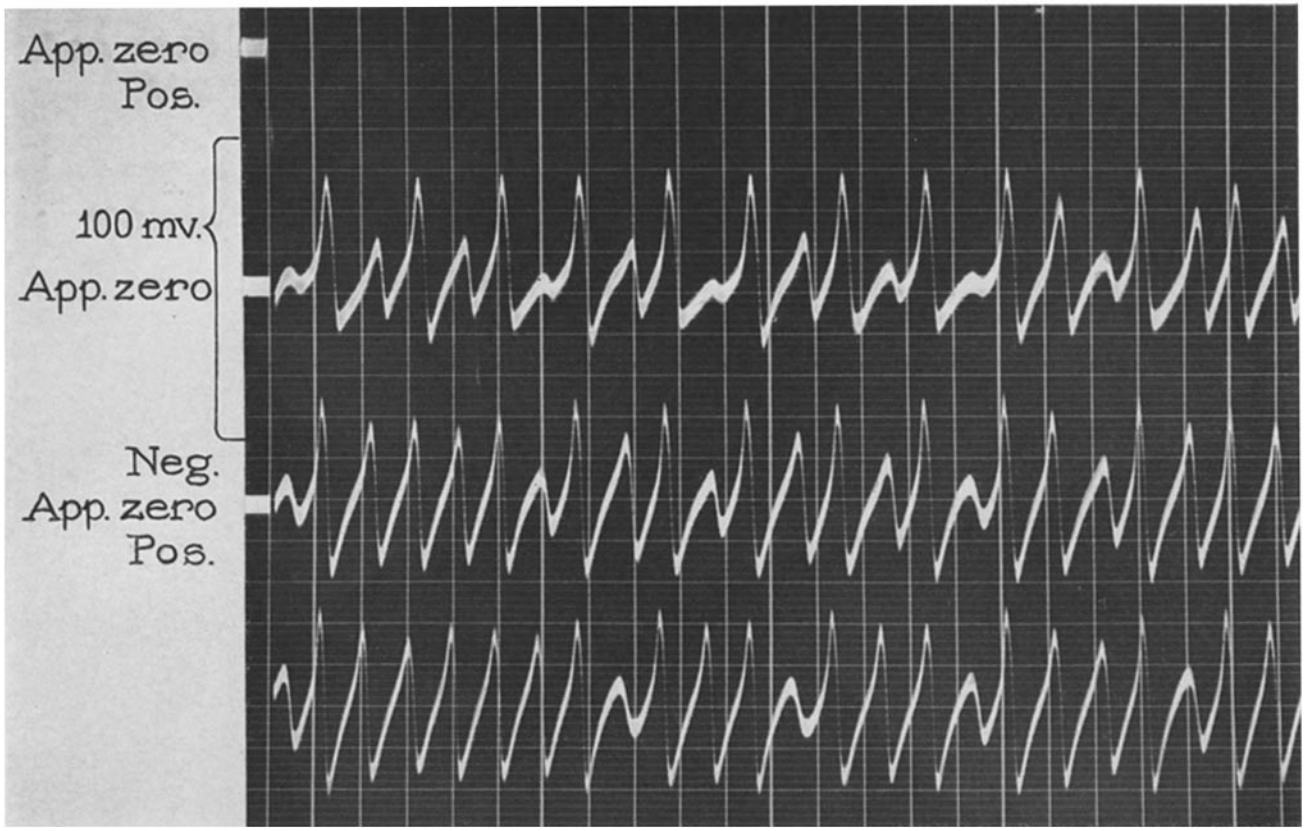

FIG. 13. Portion of a train of quick action currents after exposure to 0.01 M NaCl. The records of *C*, *D*, and *E* (all in contact with 0.01 M NaCl) are shown (*cf.* Fig. 2): the absence of any simultaneous movements shows that no change occurred at *F*, which was in contact with 0.01 M KCl.

The pacemaker was at the right of *E* as indicated by the fact that in each action current the first change was at *E* (lowest string). The responses at the three spots are not identical.

Time marks 1 second apart. Temperature 22°C.

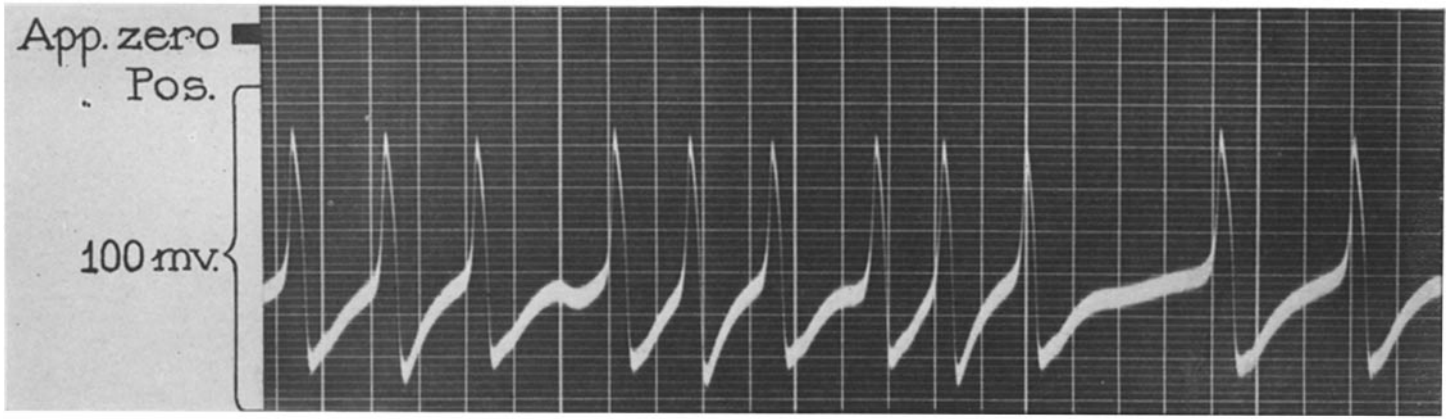

FIG. 14. Shows the tendency to lag in the upstroke as the "base line" or resting potential is approached (in this case there is considerable positive "after potential"). The record of *C* (in contact with 0.01 M NaCl) is shown (*cf.* Fig. 2): those of *D* and *E* (omitted to save space) show that no change occurred at *F*, which was in contact with 0.01 M KCl.

Time marks 1 second apart. Temperature 22°C.

papers[8] which may be referred to for details. It is assumed that K^+ comes out of the sap during the action current and that it goes back into the sap during recovery. If the outward movement of K^+ is restricted or if it fails to reach the outside of X and to destroy any positive potential at X the curve may not go to zero. If the outgoing K^+ does not return completely to the sap recovery will be incomplete. In the present experiments the curves do not go to zero and in the quick movements recovery is often incomplete (Figs. 9 and 10).

It may be noted in passing that when F (Fig. 2) is in contact with 0.01 M KCl it frequently appears to act as a pacemaker, as would be expected since the P.D. at F would be approximately zero[9] and it has been found that this condition easily sets up action currents. But changes in the location of pacemakers are often observed and when a rhythm is once established it sometimes continues even when the supposed pacemaker is changed or blocked off. The question of pacemakers will be discussed elsewhere.[10]

In this connection attention may be called to the long trains of action currents produced, according to Brink and Bronk,[11] by treating the sciatic nerve of the frog with Ringer's solution free from calcium.

The response of the cell to NaCl presents several interesting aspects among which are the following.

1. Prolonged Exposure to NaCl.—After some hours in 0.01 M NaCl the irritability may disappear for an hour or more so that the cell can no longer be stimulated electrically.[12] To what extent this depends on the previous production of a large number of rapid action currents is an open question.

After some hours irritability returns and the action currents tend

[8] Hill, S. E., and Osterhout, W. J. V., *J. Gen. Physiol.*, 1934–35, **18,** 377. Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1934–35, **18,** 499.

[9] *Cf.* Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1929–30, **13,** 459.

[10] Regarding pacemakers, see Auger, D., Comparaison entre la rythmicité des courants d'action cellulaires chez les végétaux et chez les animaux, *Actualités scient. et indust.*, 314, Paris, Hermann et Cie, 1936.

[11] Brink, F., Jr., and Bronk, D. W., *Proc. Soc. Exp. Biol. and Med.*, 1937–38, **37,** 94.

[12] This refers to electrical stimulation produced in the usual way. In such cases there may be a response at the cathode which is not propagated.

to show the normal recovery time of 15 to 30 seconds. The shape of the action curves frequently resembles those in Figs. 5 to 7.

Evidently a new factor enters into the situation which prevents the production of rapid action currents in spite of the presence of NaCl. This might come about if organic electrolytes were leached out of *W* thus increasing its resistance.

It may be added that at this stage the cells look normal and may continue to do so even after an exposure of several weeks to 0.01 M NaCl.

2. Effects of Calcium.—The addition of calcium suppresses the quick action currents.[13] If instead of 0.01 M NaCl we use a mixture of 0.01 M NaCl + 0.0005 M $CaCl_2$ no rapid action currents appear. In the presence of this concentration of calcium the voltage necessary for stimulation and the absolute refractory period do not fall off.

As might be expected no quick action currents occur in 0.005 M $CaCl_2$.

It seems possible that the addition of $CaCl_2$ inhibits the penetration of NaCl from the external solution while NaCl moves from *W* into the sap under the action of the forces which are constantly producing such a movement;[14] the result would be that the conductivity of *W* would fall off.

It may be of interest in this connection to call attention to the experiments of Chao[15] who found that in *Limulus* Na^+ increases and Ca^{++} decreases the rate of heartbeat when applied to the dorsal median nerve cord or ganglion where the rhythm originates.

3. Penetration of Salts.—It is interesting to note that certain salts, such as NH_4Cl and LiCl which might be expected to penetrate as readily as NaCl, have somewhat the same effect as NaCl in producing quick action currents.

We find that NaSCN which might be expected to penetrate more rapidly than NaCl produces a quicker and more pronounced effect.

But Na_2SO_4 which might be expected to penetrate more slowly produces little or no effect: quick action currents are produced by

[13] This also happens in nerve. See footnote 11.

[14] *Cf.* Osterhout, W. J. V., *Ergebn. Physiol.*, 1933, **35**, 967; *Bot. Rev.*, 1936, **2**, 283.

[15] Chao, I., *Biol. Bull.*, 1933, **64**, 358.

$(NH_4)_2SO_4$ but this may be due to the penetration of undissociated NH_3. ($MgSO_4$ has no effect.)

4. Variability.—Very irritable cells (with a low threshold for electrical stimulation) quickly give rapid action currents[16] when placed in 0.01 M NaCl. Some cells require an exposure of only 15 minutes to 0.01 M NaCl to produce quick action currents and occasionally cells are found which require an even briefer exposure or none at all.[8] In these cases we may suppose that W is more conductive than usual owing to the presence of organic or inorganic electrolytes. In some cases we may have to do with electrolytes which have come out of the sap during an action current and have not gone back completely (incomplete recovery).

Cells with a high threshold require a longer exposure and in some cases fail altogether to produce rapid action currents.

SUMMARY

Treatment of *Nitella* with NaCl greatly reduces the time required for the action current and produces an action curve with one peak instead of the customary two. The time may be reduced to 0.6 second in place of the usual 15 to 30 seconds.

This might be expected if the treatment increased the conductivity of the aqueous part of the protoplasm. The experiments favor this idea although they do not prove its correctness.

This effect is prevented by calcium, possibly because calcium inhibits penetration of salts. That penetration is an important factor is indicated by the fact that salts which might be expected to penetrate rapidly have the most effect. Thus NaSCN is more effective than NaCl but Na_2SO_4 has little or no effect. The action of NH_4Cl and LiCl is similar to that of NaCl.

[16] Cells which have been freed from neighboring cells and then kept in the laboratory for several weeks seem to be especially favorable.