MECHANICAL RESTORATION OF IRRITABILITY AND OF THE POTASSIUM EFFECT

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Nitella exposed to distilled water loses its ability to propagate negative variations and to give the normal potassium effect[1] (*i.e.* the action of KCl in making the P.D. more negative). Apparently this is because the distilled water dissolves out of the protoplasm something which may for convenience be called *R*.

Under normal conditions this substance presumably moves into the protoplasm from the sap fast enough to offset the dissolving action of the external solution. But in distilled water the dissolving action is sufficiently accelerated to produce a deficiency of *R*.

It occurred to us that if we could force sap into the protoplasm mechanically, by a pinch, we might succeed in restoring *R* sufficiently to bring back the normal behavior. We have therefore made experiments of this sort.

In some cases the result is very dramatic. Cells which are unable to give any response to electrical stimulation may after a single pinch respond normally. This result cannot always be depended on for if the pinch is too gentle several may be required to produce the desired effect and if it is too vigorous injury may occur. The results, however, seem to show clearly that the normal behavior may be restored by forcing sap into the protoplasm.

The procedure may be illustrated by citing some typical experiments.[2] These were performed on *Nitella flexilis* Ag. at a temperature of 20–22°C.

[1] Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1933–34, **17,** 87, 99, 105.

[2] Unless otherwise stated the technique is that described in previous papers (*cf.* Osterhout, W. J. V., and Harris, E. S., *J. Gen. Physiol.*, 1927–28, **11,** 391. Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1929–30, **13,** 547; 1930–31, **14,** 385, 473; 1933–34, **17,** 87. Blinks, L. R., *J. Gen. Physiol.*, 1929-30, **13,** 361).

Cells which had been freed from neighboring cells were left for several days in a nutrient solution (Solution A[3]) and then kept in distilled water until they lost their irritability and potassium effect (2 days or more).

An experiment with such a cell (arranged as in Fig. 1) is shown in Fig. 2. An electrical stimulus produced no effect since the irritability had been lost, but a pinch produced a mechanical negative variation. As shown in previous papers,[4] this is due to a compression wave pro-

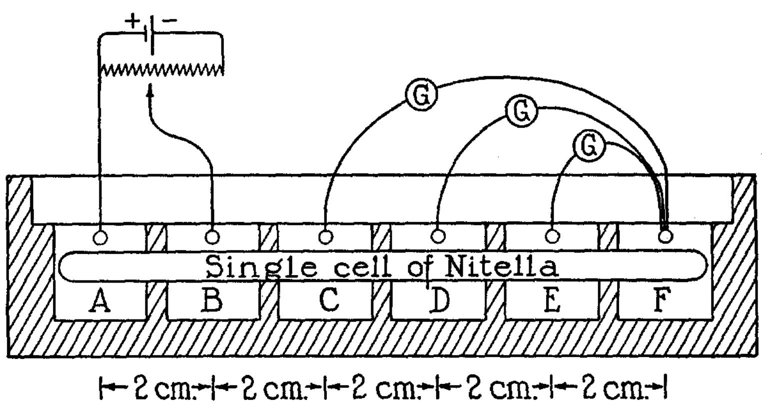

FIG. 1. Arrangement for testing *Nitella* cells. *GGG* represent string galvanometers (3 strings inserted in a single magnetic field of a Type A Cambridge string galvanometer) with vacuum tube amplifiers, arranged as short period voltmeters. Silver-silver chloride electrodes dip in each cup. The *Nitella* cell passes through all of the cups (*cf.* Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1933–34, **17**, 87). Cup *D* had an overflow pipe enabling us to change the solution without interrupting the record by pouring the new solution into the cup.

duced by the pinch. The compression wave travels along the cell, temporarily abolishing the outwardly directed P.D. at each point it

[3] For the composition of this solution see Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1933–34, **17**, 87.

[4] The pinch sets up a compression wave which travels rapidly along the turgid cell the sap of which has an osmotic pressure about 4 atmospheres greater than that of the surrounding solution. *Cf.* Osterhout, W. J. V., and Harris, E. S., *J. Gen. Physiol.*, 1928–29, **12**, 167, 355; Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1930–31, **14**, 385, 473.

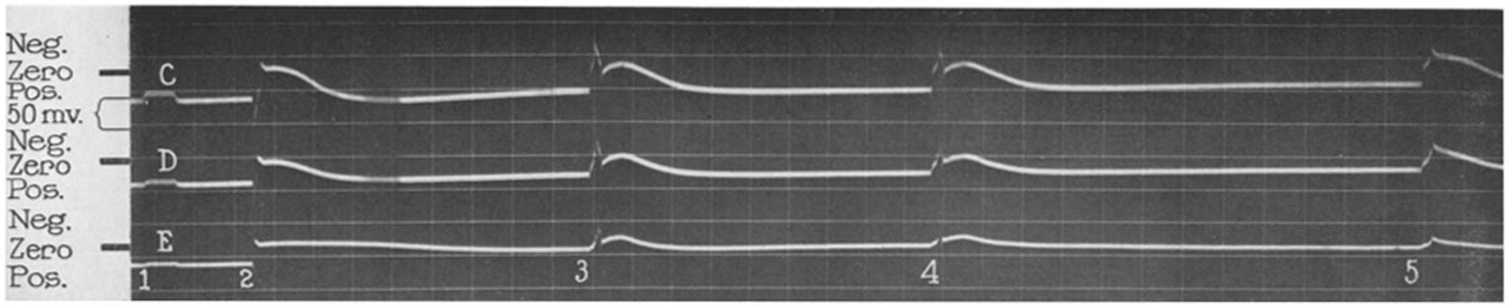

FIG. 2. Photographic record showing the restoration of irritability by a mechanical stimulus. The cell was arranged as in Fig. 1. It had been 2 days in distilled water and in consequence had lost its irritability. All contacts were 0.01 M NaCl.

The top curve shows the P.D. between *C* and *F*, the middle curve that between *D* and *F*, and the bottom curve that between *E* and *F*.

At the place marked 1 on the record an electrical stimulus (300 mv. D.C.) was applied, giving an outgoing current at *B*. This produced no effect (the small temporary rise in the curves is due to spread of current from *B*).

At 2 the cell was pinched between *E* and *F*, producing mechanical negative variations at all points. The beginning of the response at *F* is shown by the simultaneous downward movement at *C*, *D*, and *E*. The remaining portions of the curves are due in part to movement at *F* and in part to movements at the other spots.

At 3, 4, and 5 electrical stimuli produced responses showing that irritability had been restored by the pinch. The responses at 3 and 4 are decidedly diphasic, that at 5 is less so.

The vertical lines are 5 seconds apart. Temperature about 22°C.

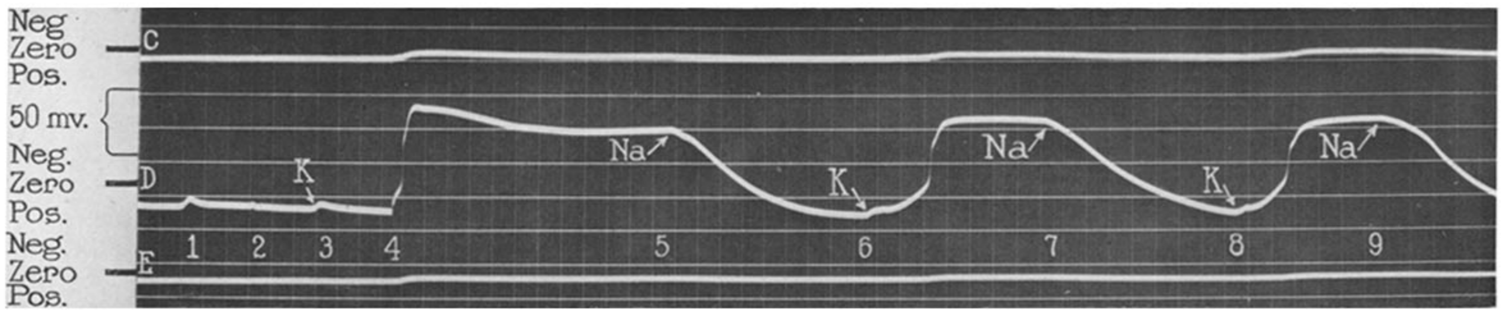

FIG. 3. Photographic record showing the restoration of the potassium effect by a mechanical stimulus. The cell was arranged as in Fig. 1. It had been 8 days in distilled water and in consequence had lost both irritability and the potassium effect. All contacts at the start were 0.01 M NaCl.

The top curve shows the P.D. between *C* and *F*, the middle curve that between *D* and *F*, and the bottom curve that between *E* and *F*.

At the place marked 1 on the record 0.01 M KCl was substituted for 0.01 M NaCl at *D*. This had practically no effect; at 2 this was replaced by 0.01 M NaCl; at 3 this was replaced by 0.01 M KCl, again with practically no effect.

At 4 the cell was gently pinched near *D* and *D* became 83 mv. more negative. There was no recovery because the potassium effect had been restored by the pinch (the level of the curve fell off a little and then became stationary). Then 0.01 M NaCl was applied (5) and brought the curve back to the former level. (The pinch produced no effect at *C*, *E*, or *F* and in consequence the movement of the middle curve is due wholly to changes at *D*. This is not surprising in view of previous experiments which show that the effect of a pinch dies out as the distance from the pinch increases.)

At 6, 0.01 M KCl was again applied at *D*, making the P.D. 75 mv. more negative: the P.D. was restored by applying (7) 0.01 M NaCl. At 8, 0.01 M KCl was again applied followed by 0.01 M NaCl at 9.

The vertical marks are 5 seconds apart. Temperature about 21°C.

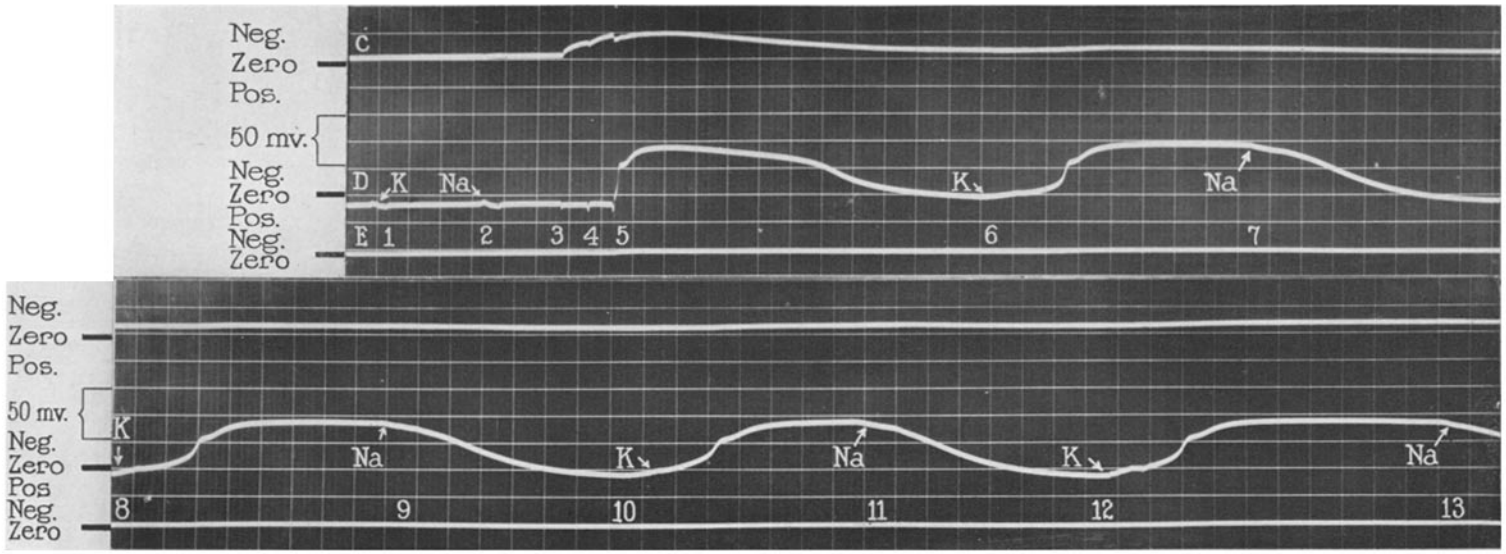

FIG. 4. Photographic record showing the restoration of the potassium effect by a mechanical stimulus. The cell was arranged as in Fig. 1. It had been 7 days in distilled water and in consequence had lost its irritability and the potassium effect. All contacts at the start were 0.01 M NaCl.

The top curve shows the P.D. between *C* and *F*, the middle curve that between *D* and *F*, and the bottom curve that between *E* and *F*.

At the place marked 1 on the record 0.01 M KCl was substituted for 0.01 M NaCl at *D*: this had practically no effect; at 2, 0.01 M NaCl was applied in place of 0.01 M KCl.

At 3 and 4 the cell was gently pinched between *C* and *D* but with no effect (except for a small change at *C*), but at 5 a stronger pinch caused a mechanical negative variation at *D* from which it recovered since it was in contact with 0.01 M NaCl.

At 6 (1 minute later) 0.01 M KCl was applied at *D* and *D* became 50 mv. more negative. There was no recovery because the potassium effect had been restored by the pinch, but when 0.01 M NaCl was applied at 7 the curve returned to the level at the start of the experiment.

At 8, 0.01 M KCl was applied, making the P.D. 50 mv. more negative but when 0.01 M NaCl was applied at 9 the curve returned to the former level. This procedure was then repeated three times (to save space the final drop due to NaCl is not completed on the record).

The vertical marks are 5 seconds apart. Temperature about 22°C.

touches. It probably does this by rupturing the protoplasmic surfaces, the rupture being immediately repaired.

The pinch restored the irritability presumably by forcing sap into the protoplasm. The restoration of irritability is clearly shown by the fact that after the pinch electrical stimulation produced a response in every case.

Can the potassium effect also be restored in the same way? To test this an experiment was made as shown in Fig. 3. The substitution of KCl for NaCl at *D* had no effect because the cell had lost its potassium effect but when the cell was pinched so as to produce a mechanical negative variation at *D* there was no recovery because the pinch restored the potassium effect and in consequence KCl reduced the P.D. to a low value. There was no injury as shown by the fact that when NaCl was applied the P.D. returned to the normal value.

The effect of the pinch in restoring the potassium effect does not disappear at once for subsequent applications of KCl gave typical potassium effects.

A different type of experiment is shown in Fig. 4. Here the cell was pinched with NaCl in contact with *D*: in consequence there was a mechanical negative variation with recovery at *D*. A minute later KCl was applied at *D* and produced a potassium effect, but there was no injury as shown by the application of NaCl which restored the normal P.D. Subsequent applications of KCl showed that the effect persisted.

DISCUSSION

It would seem that when sap is forced into the protoplasm by the pinch,[4] it carries something which is responsible for the potassium effect and for normal irritability. Although for convenience we speak of this as *R*, it is probable that it contains more than one substance since we find, in some cases at least, that when cells are leached with distilled water the potassium effect disappears before the irritability is lost.[5] This is in harmony with the fact that in *Chara coronata* Ziz.[6]

[5] Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1934–35, **18,** 681.

[6] Blinks, L. R., *Proc. Soc. Exp. Biol. and Med.*, 1932–33, **30,** 756. Osterhout, W. J. V., *J. Gen. Physiol.*, 1934–35, **18,** 215.

action currents regularly occur although the potassium effect is normally lacking.

A single pinch may produce only a partial restoration of the potassium effect. In such cases additional pinches may produce more effect but it is possible to overdo the matter and injure the cell (considerable variation exists in this respect).

Let us now consider the rôle of the two protoplasmic surfaces. When we substitute KCl for NaCl the change in P.D. (if it occurs at all) usually takes place within a few seconds which is presumably too short a time for potassium to penetrate through the protoplasm to the inner surface. We therefore suppose that only the outer surface is involved which presumably is not normally in contact with much potassium (either in the external solution or in the protoplasm).

The situation is quite different with the inner surface which is in contact with sap containing about 0.05 M KCl (and 0.05 M NaCl). Since there is presumably not much potassium in the protoplasm there is a marked potassium gradient across the inner protoplasmic surface and we suppose this to be responsible for the outwardly directed P.D. which is characteristic.

The treatment with distilled water which removes the potassium effect from the outer surface does not appear to affect the inner surface in the same way for its outwardly directed P.D. seems not to be lessened. But the inner layer may be affected in such fashion as to prevent the increase of permeability which is necessary for the action current.[6] This would explain why a spot[7] treated with distilled water can act as a block while retaining its outwardly directed potential (100 to 300 mv.).

We therefore suppose that the pinch affects both the inner and outer protoplasmic surfaces. Whether this effect depends wholly on the outward movement of R must be left to future investigation. In the case of the inner protoplasmic surface (which is in contact with the sap) the effect of the movement of R would seem to depend on forcing R into the aqueous layer of protoplasm[8] which lies between the two non-

[7] Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1933–34, **17**, 87.

[8] Even if R is produced in this layer it is presumably leached out by the action of the distilled water.

aqueous protoplasmic surfaces, and so getting *R* on both sides of the inner surface layer. Presumably this layer is temporarily ruptured by the pinch[4] thus allowing *R* and K^+ to pass through after which the rupture is repaired. The outer layer may also be ruptured but this is less likely to happen because it is protected by being adherent to the solid cellulose wall.

SUMMARY

Treatment of *Nitella* with distilled water apparently removes from the cell something which is responsible for the normal irritability and the potassium effect, (*i.e.* the large P.D. between a spot in contact with 0.01 M KCl and one in contact with 0.01 M NaCl). Presumably this substance (called *R*) is partially removed from the protoplasm by the distilled water. When this has happened a pinch which forces sap out into the protoplasm can restore its normal behavior.

The treatment with distilled water which removes the potassium effect from the outer protoplasmic surface does not seem to affect the inner protoplasmic surface in the same way since the latter retains the outwardly directed potential which is apparently due to the potassium in the sap. But the inner surface appears to be affected in such fashion as to prevent the increase in its permeability which is necessary for the production of an action current. The pinch restores its normal behavior, presumably by forcing *R* from the sap into the protoplasm.