DELAYED POTASSIUM EFFECT IN NITELLA

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

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

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In normal cells of *Nitella* potassium produces a large and rapid change of P.D. in a negative[1] direction. When 0.01 M NaCl is replaced by 0.01 M KCl the P.D. usually becomes less positive[2] by 85 mv. or more. This is called for convenience the potassium effect (Figs. 1 and 3). Since the response to KCl is so rapid we suppose that it occurs at X, the outer non-aqueous protoplasmic surface layer (*cf.* Fig. 2).

The potassium effect usually disappears when cells are placed for 2 or 3 days in distilled water.[3] This removes organic material which may be recovered from the distilled water and returned to the cell, thereby restoring the potassium effect. For convenience this substance[4] (or group of substances) may be called R_P.

When the potassium effect has been lost as the result of exposure to distilled water or for other reasons[5] the replacement of 0.01 M

[1] The P.D. is said to be negative when the positive current tends to flow from the external solution across the protoplasm to the sap. All the effects of KCl described in this paper were fully reversible.

[2] *Cf.* Osterhout, W. J. V., *J. Gen. Physiol.*, 1929–30, **13,** 715.

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

[4] Exposure to distilled water also removes irritability and this appears to be due to another organic substance (or group of substances) which may be called R_A. The reason for supposing that R_P and R_A are different is that the loss of irritability sometimes precedes and sometimes follows the loss of the potassium effect. But it is, of course, possible that we are dealing with a single substance operating under different conditions.

[5] This usually takes place under natural conditions in late spring and early summer, the season of rapid growth (*cf.* Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1933–34, **17,** 105), and may happen in an occasional cell at any time of year.

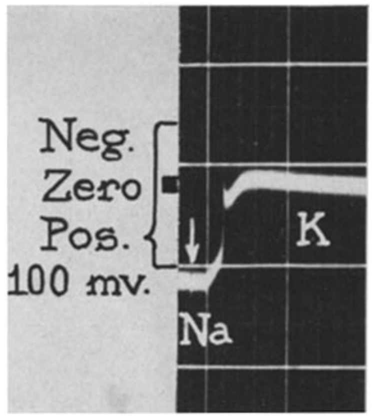

FIG. 1. Shows prompt response to the application of 0.01 M KCl to a normal cell, replacing 0.01 M NaCl at the time marked by the arrow. The curve resembles an action curve. The slight delay presumably represents the time required to diffuse through the cellulose wall.

The leads were arranged as shown in Fig. 2. The record shown is that of *D* (*C* and *E* were omitted): *F* was in contact with 0.01 M KCl which reduces the P.D. approximately to zero.

Heavy time marks are 5 seconds apart. Temperature 21°C.

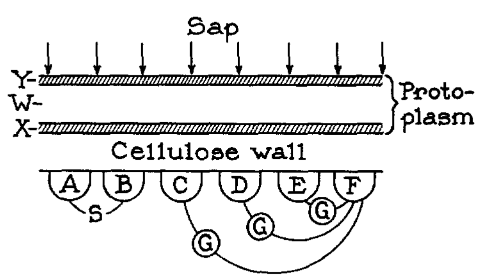

FIG. 2. Diagram to show the arrangement of leads and the supposed structure of the protoplasm which is assumed to consist of an aqueous layer *W*, an outer non-aqueous layer *X*, and an inner non-aqueous layer *Y*.

The arrows show the outwardly directed (positive) P.D. whose seat is supposed to be chiefly at *Y* when the cell is in pond water: hence the P.D. at *X* is regarded as negligible and is not shown. But under some conditions the P.D. at *X* may become important.

Each lead is connected to a separate amplifier and to one string of the 3-string Einthoven galvanometer.

NaCl by 0.01 M KCl usually has little or no effect,[6] but in some cases we obtain the result[7] seen in Figs. 4, 5, and 6. Here the potassium effect seems to be absent when KCl is first applied but appears after some delay as though it had been restored by contact of the cell with KCl.

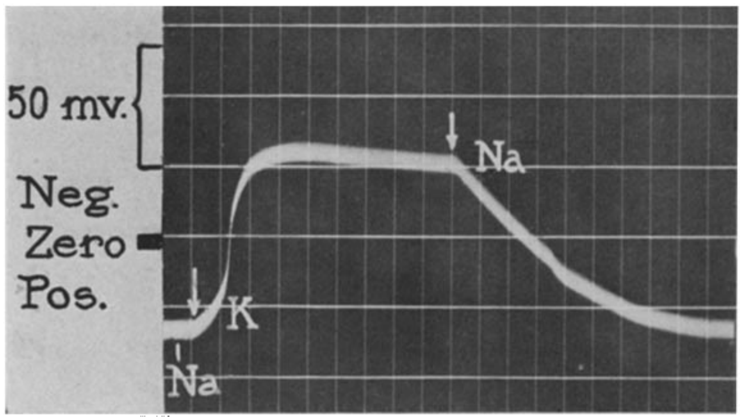

FIG. 3. Response in a normal cell, showing a curve which does not closely resemble the usual action curve, when 0.01 M NaCl is replaced by 0.01 M KCl. *D* (*cf.* Fig. 2) is recorded (*C* and *E* are omitted). Although *D* and *F* are both in contact with 0.01 M NaCl, *D* is positive to *F*, owing to some local difference. *D* becomes negative to *F* as soon as KCl is applied. When 0.01 M NaCl is subsequently applied to *D* the P.D. returns to the original value.

Time marks 5 seconds apart. Temperature 28°C.

It is evident that KCl might do this if it formed a compound which sensitized X to the action of potassium. This compound might be R_P, formed by the reaction $K^+ + Z = R_P$ where Z is an organic substance. (If R_P contains potassium it is easier to understand why it causes the cell to act somewhat like a potassium electrode.)

[6] If there is an effect it may be in the direction of greater or of less positivity according to the condition of the cell.

[7] The experiments were made 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).

The zero in the figures is located on the assumption that 0.01 M KCl reduces the P.D. approximately to zero. This is usually close to the actual condition.

If this were the case we might expect that when KCl is applied no change in P.D. will occur until sufficient R_P is formed[8] to sensitize X and cause a gradual rise in the curve such as is seen in Figs. 4, 5, and 6.

In Figs. 1, 4, and 6 the curve has the appearance of an action curve.

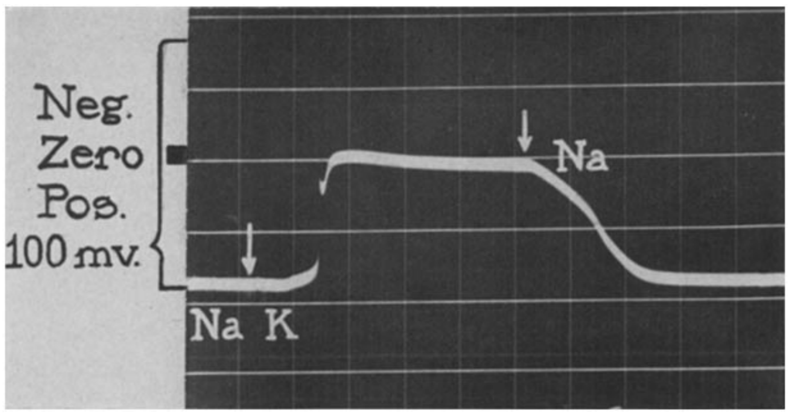

FIG. 4. Shows delayed response to KCl in a cell which had been soaked for 4 days in distilled water: the curve resembles an action curve. At the start the spot recorded (D, Fig. 2) was in contact with 0.01 M NaCl. When this was replaced by 0.01 M KCl the P.D. became less positive after a delay. The application of 0.01 M NaCl returned the P.D. to the original value.

The leads were arranged as in Fig. 2: C and E were omitted: F was in contact with 0.01 M KCl.

Time marks 5 seconds apart. Temperature 22°C.

This might occur since irritability often persists after the potassium effect has disappeared.

It has been suggested in a former paper[9] that the application of KCl at a spot D (Fig. 2) might give rise to an action curve at that point by depressing the P.D. sufficiently to cause a discharge from a neighboring point D_1 not in contact with the KCl covering D (and consequently not recorded).

The discharge at D_1 would presumably involve the exit of substances

[8] We suppose that as a rule Z is absent since there is usually no response, not even after a delay.

[9] Hill, S. E., and Osterhout, W. J. V., *J. Gen. Physiol.*, 1937–38, **21**, 541.

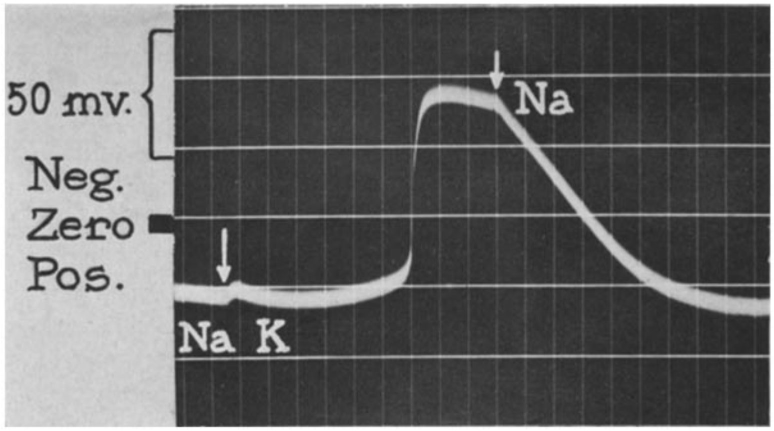

FIG. 5. Shows delayed response when 0.01 M NaCl is replaced by 0.01 M KCl: the curve does not closely resemble the usual action curve. At the start the spot recorded (*D*, Fig. 2) was in contact with 0.01 M NaCl. When this was replaced by 0.01 M KCl the P.D. became less positive after a delay. The application of 0.01 M NaCl returned the P.D. to the original value.

The leads were arranged as in Fig. 2: *C* and *E* were omitted. Although *F* was in contact with 0.01 M NaCl *D* was positive to it at the start (owing to some local difference) but after application of KCl to *D* it became negative to *F*.

Time marks 5 seconds apart. Temperature 28°C.

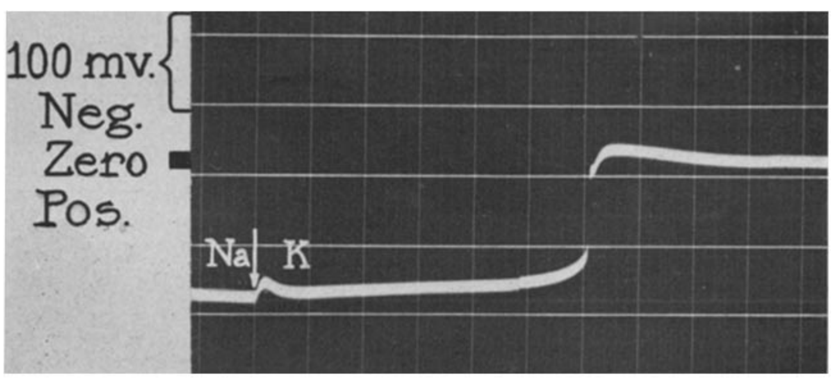

FIG. 6. Delayed response when 0.01 M NaCl was replaced by 0.01 M KCl: the curve resembles an action curve and was propagated to *C* (not shown in the record): *D* is recorded (*E* was omitted): *F* was in contact with 0.01 M KCl.

Time marks 5 seconds apart. Temperature 20°C.

from the sap[10] and these, diffusing[11] along the protoplasm to *D*, might cause the inner protoplasmic surface, *Y* (Fig. 2), to become permeable and allow substances to come out of the sap at *D*. As explained in a former paper this could produce an action current[12] at *D*.

Among the substances[13] thus issuing from the sap would be[14] R_P which would sensitize *X* to the action of potassium and thus restore the potassium effect. Hence after the action current *D* would show no recovery of the original P.D. (this lack of recovery is evident, *e.g.* in Figs. 3, 4, and 6).

We suppose that the form of the curve depends upon (1) the external KCl tending to produce negativity at *X*, (2) the increase in the permeability of *Y* producing negativity by short-circuiting, (3) the movement of K^+ out of the sap and then back into the sap,[15] and (4) the sensitizing action of R_P formed at *X* or moving from the sap to *X*.

Evidently the interplay of these variables might produce a variety of curves. When the cell is incapable of producing action currents

[10] Osterhout, W. J. V., *J. Gen. Physiol.*, 1934–35, **18,** 215. Even when KCl is in contact with the cell the outward movement of K^+ might produce the dip if it were accompanied by R_P which sensitizes the inner surface of *X* first.

[11] The time required for this diffusion would increase the delay between the application of KCl and the appearance of the action current. But it might be very brief since the distance between *D* and D_1 might be only a few microns.

[12] As a rule we see no indication that an action current at D_1 is propagated to *C* and *E* (Fig. 2) probably because it involves only a small change of P.D. But the action current at *D* involving a larger change of P.D. is frequently propagated.

[13] Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1934–35, **18,** 681; Hill, S. E., and Osterhout, W. J. V., *J. Gen. Physiol.*, 1934–35, **18,** 687. After R_P has come out of the sap as the result of an action current which is propagated along the whole cell the application of KCl at any spot usually produces the potassium effect without any delay.

[14] If the application of KCl would make R_P come out of the sap in any way other than that suggested the end result would doubtless be the same.

[15] *Cf.* Osterhout, W. J. V., *J. Gen. Physiol.*, 1934–35, **18,** 215. Presumably K^+ moves back into the sap, to some extent at least, at *D* and with it goes some of the K^+ from the external solution. But as a rule R_P does not appear to move back into the sap to any such extent for its effect on *X* may remain for some time (*cf.* Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1934–35, **18,** 681): perhaps the fact that R_P is produced in the cell has something to do with this. Its tendency to move into the sap may be less than that of K^+.

we might expect curves like those in Figs. 3 and 5, but it is not certain that these are not action curves.

Let us now consider another point of view. It might be suggested that the delay occurs because K^+ is unable to affect X but must penetrate through the aqueous layer of the protoplasm, W, to Y in order to produce negativity. But in that case we should expect the production of negativity to occur in every case, instead of occasionally, and the negativity should not at once begin to disappear when KCl is replaced by NaCl, as actually happens (Figs. 3, 4, and 5).

Moreover, we should not expect an action curve as seen in Figs. 4 and 6 since the characteristic dip after the spike is supposed to be due to the outward movement[10] of K^+.

In conclusion we may say that the facts can be explained by assuming that when the potassium effect has disappeared there is not enough R_P in X to make it respond to K^+ but that in some cases an organic substance Z is present in sufficient amount to combine with K^+ and produce enough R_P to sensitize X to K^+.

Regarding the nature of Z little can be said but it is of interest to find that blood[16] acts as though it might contain such a substance. When the potassium effect has been removed by distilled water so that no potassium effect can be secured even after a delay the application of blood[16] sometimes produces a delayed potassium effect in a few seconds: a longer application of blood may then produce the potassium effect with less delay.

SUMMARY

In normal cells of *Nitella* replacement of NaCl by KCl makes the P.D. much less positive: this is called the potassium effect.

Cells which have lost the potassium effect usually show little or no change of P.D. when NaCl is replaced by KCl but an occasional cell responds after a delay.

It seems possible that the delay may be largely due to the time required for potassium to combine with an organic substance, thus forming a compound which sensitizes the protoplasmic surface to the action of potassium.

[16] This was oxalated human blood plasma diluted with 4 parts of water. *Cf.* Osterhout, W. J. V., *J. Gen. Physiol.*, 1935–36, **19**, 423.