THE EXPERIMENTAL PRODUCTION OF DOUBLE PEAKS IN CHARA ACTION CURVES AND THEIR RELATION TO THE MOVEMENT OF POTASSIUM

BY W. J. V. OSTEHOUT AND S. E. HILL

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

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Chara offers an interesting contrast to Nitella: in the latter the action curve has two peaks but in Chara there is only one.

In Nitella the first peak has been accounted for on the ground that when K⁺, moving outward from the sap, reaches the outer protoplasmic surface it sets up a positive P.D. which makes the action curve fall as soon as the first rise (the spike) is completed. This fall in the curve occurs only when the outer protoplasmic surface is sensitive to K⁺. When it is not sensitive to K⁺ we find no change of P.D. when 0.01 M NaCl in external contact with the cell is replaced by 0.01 M KCl (i.e. no "potassium effect"). We may therefore expect only one peak in the action curve. This situation exists in Nitella when the outer protoplasmic surface is made insensitive to K⁺ by treatment with distilled water.

In Chara the outer protoplasmic surface is normally insensitive to K⁺, i.e. shows no potassium effect, and the action curve has only one peak, as would be anticipated. This is illustrated in Fig. 1.

If the outer surface could be made sensitive to K⁺ we might expect two peaks. This expectation is realized when the outer surface is made sensi-

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2 The effect of K in Nitella and in Chara predominates to such an extent that it alone is mentioned in the following discussion.
4 Presumably the inner protoplasmic surface is sensitive to K⁺ as in Nitella and the outwardly directed P.D. of about 100 mv, usually present when the cell is in pond water is presumably due to the concentration gradient of K⁺ across the inner protoplasmic surface.
5 The cells, after being freed from neighboring cells, stood in the laboratory at 15° ± 1°C. in Solution A (cf. Osterhout, W. J. V., and Hill, S. E., J. Gen. Physiol., 1933-34, 17, 87).

The experiments were performed on Chara coronata, Ziz. (this is an uncorticated Chara with large naked cells like those of Nitella, completely accessible to reagents).

There was no indication of injury in these experiments.
tive to K+ by guanidine which has been used to produce double peaks in leached cells of Nitella.6

The treatment consists in soaking the cells for various periods in 0.1 to 0.001 M guanidine hydrochloride and then testing them for the potassium effect and for irritability.7

In many cases this treatment produces the potassium effect but the time required is very variable. In some cases less than 4 minutes in 0.01 M guanidine hydrochloride8 sufficed: in other cases a much longer exposure was necessary, especially at lower concentrations (0.01 to 0.001 M). The potassium effect is usually less than in Nitella.

Prior to the application of guanidine we find only rounded single peaks (as seen in Fig. 1) and no potassium effect. Guanidine cannot only bring about a potassium effect but, along with this, double peaks appear, such as are seen9 in Figs. 2 to 4.

During this process single peaks may persist for a longer or shorter time at certain places.10 This may be due to the fact that the potassium effect is not fully developed at these places. It appears to depend on a group of organic substances, called for convenience R. When but little R is present in the outer protoplasmic surface the outwardly moving K+

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7 The cells were tested for potassium effect and irritability before the treatment. The potassium effect was always absent; as a rule irritability was present.
8 By this is meant the ability to give propagated action currents when stimulated electrically.
9 NHC(NH₂)₂·HCl.
10 Curves of this form are found in Nitella both in untreated cells and in leached cells treated with various restorative agents.
11 There may even be some fluctuation in the form of the curve at the same spot.
may not produce much positive potential and thus there will be no sudden change in the curve. Moreover the guanidine, penetrating into the aqueous layer of the protoplasm, may tend to act like NaCl in favoring single peaks, or may promote protoplasmic motion and thus make the moving boundary of K⁺ less sharp, as discussed in a previous paper; this would tend to promote single peaks.

The experiments indicate that the movement of potassium is as important in determining the shape of the action curve as in Nitella. The outwardly directed (positive) P.D. normally present in Chara is presumably due chiefly to the outwardly directed concentration gradient of K⁺ across the inner protoplasmic surface. When an action current occurs this surface presumably becomes more permeable. The concentration gradient then disappears and with it the positive P.D. This causes the rise in the action curve (spike). Then K⁺ is carried back into the sap by the forces which normally cause such movement in the resting state of the cell. This produces recovery and the curve falls.

When the outer protoplasmic surface has been made sensitive to K⁺ by treatment with guanidine we observe an additional feature, i.e. on reaching the outer protoplasmic surface K⁺ sets up more or less positive

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12 This is about 100 mV when the cell is in contact with pond water.
13 The P.D. is called positive when the positive current tends to flow from the sap across the protoplasm to the external solution.
15 This is usually more rapid in Chara than in Nitella.
P.D. This may be sufficient to cause a drop in the curve, as in Figs. 2 to 4. Or it may merely halt the course of recovery and so delay the fall of the curve.

As K\(^+\) penetrates the outer protoplasmic surface and its concentration gradient across this surface decreases the positive P.D. will fall off.

**Fig. 3**

**Fig. 4**

Fig. 3. Action curve of a cell of Chara treated with 0.01 M guanidine hydrochloride for 7 minutes and 46 seconds (the potassium effect was produced by this treatment: hence the outer surface was sensitive to K\(^+\)).

The spot recorded, E, was in contact with 0.01 M guanidine hydrochloride and was connected through the galvanometer to a spot G in contact with 0.01 M KCl which kept the p.D. constant approximately at zero. In consequence the action curve is monophasic.

The cell was freed from neighboring cells and kept for 11 days in Solution A at 15 ± 1°C. The record was made at 23°C. Vertical marks 5 seconds apart.

Fig. 4. Action curve of a cell of Chara treated with 0.01 M guanidine hydrochloride for 7 minutes and 46 seconds (the potassium effect was produced by this treatment: hence the outer surface was sensitive to K\(^+\)).

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As the magnitude of this positive P.D. will depend chiefly on the concentration gradient of K\(^+\) across the outer protoplasmic surface it will be affected by a variety of factors. The greatest concentration gradient will be found when K\(^+\) travels outward from the sap in the form of a moving boundary. Anything which tends to disturb this, such as protoplasmic motion, will lessen the concentration gradient across the outer protoplasmic surface and consequently the positive P.D.
The higher the concentration of $K^+$ in the aqueous layer of the protoplasm before stimulation the less will be the effect on the P.D. of the outwardly moving $K^+$.

The P.D. will also depend on the sensitivity of the outer surface to $K^+$ and this in turn will depend on a variety of factors. It is not known how the guanidine acts to sensitize the surface. It might act like certain organic substances ($R$) responsible for the potassium effect. Or it might be a constituent or a catalyst of the reactions by which such substances are formed. Their concentration in the outer protoplasmic surface might fluctuate as the result of diffusion and chemical change.

**SUMMARY**

The action curve in *Chara* seems to depend (as in *Nitella*) on the outward movement of $K^+$ from the sap.

Presumably the increase in permeability in the inner protoplasmic surface and the outward movement of $K^+$ destroy the concentration gradient of $K^+$ across the inner protoplasmic surface. Hence the outwardly directed P.D. disappears, causing the rise (spike) of the action curve.

The outer protoplasmic surface is normally insensitive to $K^+$. But when it is made sensitive to $K^+$ by treatment with guanidine the outwardly moving $K^+$ sets up a positive P.D. on reaching the outer surface and this causes the action curve to fall, producing a peak. Then the curve has 2 peaks, the second being due to the process of recovery.

The action curve thus comes to resemble that of *Nitella* in which the outer protoplasmic surface is normally sensitive to $K^+$. 