

DIFFERING RATES OF DEATH AT INNER AND OUTER SURFACES  
OF THE PROTOPLASM

II. NEGATIVE POTENTIAL IN NITELLA CAUSED BY FORMALDEHYDE

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In *Nitella* the normal potential is positive,<sup>1</sup> amounting to about 100 mv. Under the influence of formaldehyde it may gradually disappear, as described in a former paper.<sup>2</sup>

In the present paper it will be shown that there may also be a sudden and striking access of negative potential.

This is illustrated in Fig. 1 which shows the record of a spot *A* which was connected to another spot *C* on the same cell through a recording galvanometer.<sup>3</sup> At first *A* was in contact with 0.001 M NaCl. This was replaced by 0.001 M NaCl plus 0.2 M formaldehyde which caused a drop in the curve, indicating an increase in positive potential. This may be due to the loss of a small negative potential present at the start at the outer non-aqueous protoplasmic surface (called *X*).

After a short period<sup>4</sup> which may be due to the time required for formaldehyde

<sup>1</sup> The potential is called positive when the positive current tends to flow from the sap across the protoplasm to the outside solution.

<sup>2</sup> Osterhout, W. J. V., *J. Gen. Physiol.*, 1944-45, **28**, 23.

<sup>3</sup> The cells, after being freed from neighboring cells, stood in the laboratory at 15°C. ± 1°C. in Solution A (cf. Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1933-34, **17**, 87) for several days. They belonged to Lot B (cf. Hill, S. E., and Osterhout, W. J. V., *Proc. Nat. Acad. Sc.*, 1938, **24**, 312).

The measurements were made on *Nitella flexilis*, Ag., using the technique described in former papers (Hill, S. E., and Osterhout, W. J. V., *J. Gen. Physiol.*, 1937-38, **21**, 541). Temperature 20-26°C. Regarding the amplifier see the reference just cited.

Two spots on the cell, *A* and *B*, were connected to a spot *C* through a recording galvanometer. At the end of the experiment *A*, *B*, and *C* were killed (in this order) by applying chloroform which reduced the P.D. to zero.

It was then possible to ascertain the potential across the protoplasm at *A* and *B* at any previous point on the record on the assumption that *C* had remained constant up to this point (or by correcting for any change). If *C* had changed the amount of alteration could be detected because it would appear as a simultaneous change at *A* and *B* (in the same direction at both).

<sup>4</sup> Regarding latent periods in *Nitella* and other large plant cells see Osterhout, W. J. V., *J. Gen. Physiol.*, 1936-37, **20**, 13; 1939-40, **23**, 569; 1940-41, **24**, 311, 699; *J. Cell. and Comp. Physiol.*, 1941, **18**, 129. Hill, S. E., and Osterhout, W. J. V., *J. Gen. Physiol.*, 1938-39, **22**, 107.

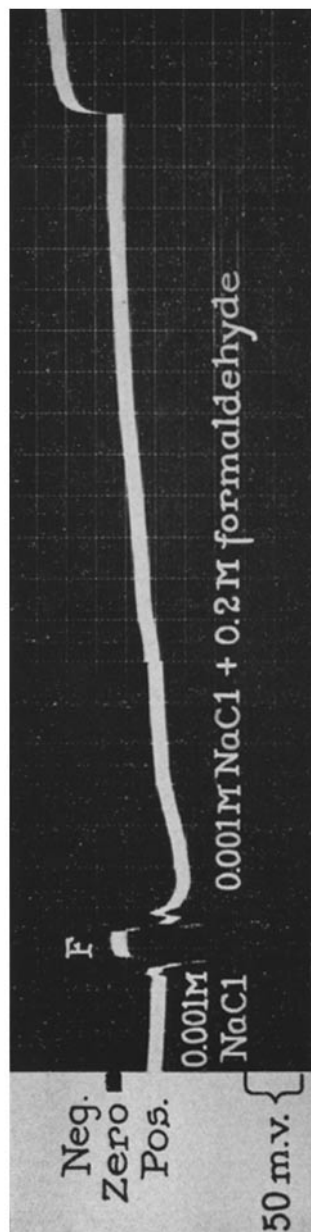


FIG. 1. Shows an abrupt rise near the end of the curve indicating a sudden access of negativity. This occurred when *Y* had become completely permeable to electrolytes as shown by the rise of the curve to zero.

At first the recorded spot was in contact with 0.001 M NaCl. When the solution was removed the spot was no longer in the circuit and the curve jumped to the free grid level "F." Then 0.001 M NaCl plus 0.2 M formaldehyde was applied and the curve resumed its former level and after a dip it rose slowly to zero after which the abrupt rise took place. The recorded spot was connected through a recording galvanometer to another spot in contact with 0.01 M KCl which kept the P.D. constant approximately at zero.

The cell was freed from neighboring cells and kept in Solution A for 33 days at  $15^{\circ}\text{C.} \pm 1^{\circ}\text{C.}$  and then placed in 0.001 M NaCl for an hour at  $25^{\circ}\text{C.}$  before the experiment was performed.

Time marks 15 seconds apart.

to penetrate through the aqueous layer of the protoplasm (called *W*) to the inner non-aqueous protoplasmic surface (called *Y*) the curve began to rise, indicating a loss of the positive potential at *Y*.

The positive potential at *Y* is due to a concentration gradient of electrolytes, mostly of KCl, across *Y*. The concentration of KCl in the sap is about 0.05 *M* but in *W* it appears to be relatively low.<sup>2</sup>

As *Y* becomes more permeable KCl moves out into *W* thus lessening the concentration gradient and the potential across *Y*. This is a slow process, as shown by the slow rise of the curve. In some cases KCl reaches *X* very gradually and apparently diffuses out through *X* so that the concentration gradient of KCl across *X* is always small and any positive potential across *X* due to KCl is also small.<sup>5</sup>

It thus appears that the permeability of *Y* to KCl increases under the influence of formaldehyde and causes a gradual loss of potential and that as the process continues *Y* becomes permeable to certain other substances which diffuse outward and produce negative potential when they arrive at *X*. If this negative potential is a diffusion potential it is evident that in general the anions of these substances must have a higher mobility than the cations in *X*.

It is not surprising that the final step in the process (*i.e.* the step which releases the negativating substances) should involve a sudden change, as shown by the abrupt rise of the curve.

If the substances producing negative potential at *X* exist in the sap they must set up much less negative potential against *Y* than against *X* but it is probable that they do not exist as such in the sap but are formed in *W* by a combination between formaldehyde and certain substances coming from the sap. (Such results have been found only in experiments with formaldehyde although other substances can cause *Y* to lose its potential.)

These substances appear to diffuse out through *X*, causing the curve to fall. The fact that it falls slowly indicates that *X* has not become completely permeable (Fig. 2).

At this time *X* may show some potassium effect; *i.e.*, some change of potential when 0.01 *M* KCl is replaced by 0.01 *M* NaCl. Also good concentration effects may be obtained now and later on with NaCl and KCl as described in a former paper.<sup>2</sup> This also shows that *X* has not become completely permeable.

It may be added that if chloroform<sup>6</sup> is applied at this time so that *X* becomes completely permeable the negative potential disappears and the curve falls to zero.

<sup>5</sup> Occasionally it may be noticeable, as shown in a former paper (see footnote 2). It may be larger in the action current where KCl comes out rapidly, *cf.* Osterhout, W. J. V., *J. Gen. Physiol.*, 1934-35, **18**, 215.

<sup>6</sup> A saturated solution of chloroform in 0.001 *M* NaCl.

The idea that organic substances in  $W$  may set up a potential finds support in experiments on *Halicystis*. Here the external solution is nearly the same as the sap but the potential amounts to about 68 mv. positive.<sup>7</sup> This is changed to about 40 mv. negative by treatment with ammonia.<sup>8</sup> It would seem that organic substances are responsible for these potentials and they are probably produced in  $W$ .

These facts lead us to assume that the sudden rise of the curve is due to an effect on  $X$  caused by organic substances passing out from the sap through  $Y$  and combining with formaldehyde in  $W$ .

As the permeability of  $Y$  increases under the influence of formaldehyde it eventually becomes completely permeable to electrolytes so that it loses all of its potential. It would seem that after it becomes permeable to KCl the final change may make it permeable to all sorts of substances, leading to the abrupt rise of the curve as in Fig. 1. It is not surprising that the final step in this process may be sudden, as in Fig. 1, because  $Y$  is a thin non-aqueous film between two aqueous solutions and is subject to the forces of surface tension and therefore to sudden rupture when a certain stage is reached. But even without such a rupture a sudden increase in permeability is conceivable.

Such a sudden change may occur earlier in the process and this is especially apt to happen when solutions are changed; *e.g.*, from 0.001 M NaCl plus formaldehyde to 0.001 M NaCl (Fig. 2). In this case the mechanical disturbance may play a part but there are also changes in osmotic pressure and in chemical composition.

Some other suggestions may now be considered.

(1) It might be thought that the substances moving out from the sap cause the negativity by increasing the negativating action of the NaCl outside of  $X$ . But the NaCl moving out from the sap and diffusing out through  $Y$  to  $X$  might be expected to create an opposing potential at  $X$ . There is no increase in the mobility of  $\text{Na}^+$  and consequently of its negativating action at this time: this is shown by measurements of the concentration effect of NaCl (*e.g.* by substituting 0.01 M for 0.001 M NaCl in contact with  $X$ ). Moreover we find the same effect when we apply formaldehyde dissolved in distilled water instead of in 0.001 M NaCl.

(2) It might be suggested that the gradual rise of the curve in Fig. 1 is due to gradual growth of negative potential at  $X$  due to the outward movement of substances from the sap which after combining with formaldehyde in  $W$  come in contact with  $X$ . In the meantime the potential at  $Y$  might suffer little or no change until it suddenly disappeared, producing a sudden rise of the curve.

Such a sudden rise occurs in the action current<sup>9</sup> but the resulting curve

<sup>7</sup> Blinks, L. R., *J. Gen. Physiol.*, 1932-33, **16**, 147.

<sup>8</sup> Blinks, L. R., *J. Gen. Physiol.*, 1933-34, **17**, 109.

<sup>9</sup> Cf. Osterhout, W. J. V., *J. Gen. Physiol.*, 1934-35, **18**, 215.

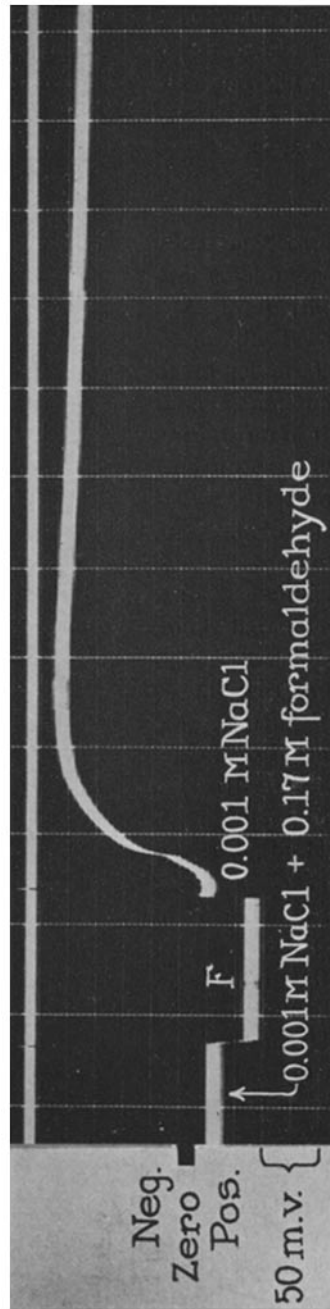


FIG. 2. Shows an abrupt rise of the curve indicating a sudden access of negativity. This occurred before *Y* had become completely permeable since it took place before the curve had risen to zero.

At first the recorded spot was in contact with 0.001 *M* NaCl and the curve rose slowly 27 mv. before the beginning of the record placed in contact with 0.001 *M* NaCl + 0.17 *M* formaldehyde and the curve rose slowly 110 mv. and then began to fall slowly.

When the solution was removed the spot was no longer in the circuit and the curve jumped to the free grid level "F." Then 0.001 *M* NaCl was applied and the curve rose abruptly 110 mv. and then began to fall slowly.

The recorded spot was connected through a recording galvanometer to another spot in contact with 0.001 *M* KCl; at this spot the p.d. remained constant.

The cell was freed from neighboring cells and kept in Solution A for 2 hours at about 25°C. before the experiment was made. Time marks 15 seconds apart.

differs from that seen in Fig. 1, since it has a sharp point of inflection at the apex which appears to be due to the outward movement of KCl causing the curve to rise and then to fall as KCl comes in contact with  $X$ . The curve in Fig. 1 is just what would be expected if substances with relatively rapid anions were diffusing out to  $X$ .

If a gradual growth of negative potential at  $X$  occurred we might expect it sometimes to carry the curve above zero before the sudden change but this does not occur. When, as often happens, there is no sudden change<sup>2</sup> but only a slow rise of the curve to zero it is natural to suppose that this is due to a gradual increase in the permeability of  $Y$  and this applies also to the first part of the curve in Fig. 1.

In some cases the abrupt rise of the curve shows a loss of potential greater than the total potential at the start as in Fig. 2. Evidently this cannot be entirely due to loss at  $Y$ .

#### SUMMARY

A previous paper showed that when the inner protoplasmic surface has lost its potential under the influence of formaldehyde the outer surface can still respond to changes in the concentration of electrolytes.

The present paper indicates that after the inner surface has lost its potential there may be a sudden development of negative potential at the outer surface due to substances coming out of the sap and combining with formaldehyde.