

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

III. EFFECTS OF MERCURIC CHLORIDE ON NITELLA

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(Received for publication, September 29, 1944)

It is a striking fact that when formaldehyde is applied to *Nitella* death occurs sooner at the inner protoplasmic surface than at the outer if the external solution contains relatively little potassium. It would seem possible to produce this effect by means of certain other substances which quickly penetrate to the inner surface. The present paper shows that mercuric chloride is such a substance.

The cells used were 3 or 4 inches long and the reagent covered a region (*A*) 1 cm. long near one end of the cell. The potential between this and a similar area (*B*) at the other end of the cell, in contact with 0.01 M KCl, was recorded.¹

Fig. 1 shows the result of applying 0.01 M HgCl₂ at *A*. At the start the spot was in contact with 0.001 M NaCl and there was a positive² P.D. of 100 mv. due chiefly to the diffusion potential of KCl at the inner non-aqueous protoplasmic surface, *Y*. The loss of this potential as shown by the rise of the curve indicates that the reagent penetrated through the non-aqueous outer protoplasmic surface *X* and the aqueous layer of the protoplasm *W*, and affected the inner protoplasmic surface, *Y*.³

Under the influence of the reagent the inner surface *Y* became completely permeable to KCl and other electrolytes so that the curve rose to zero.

With 0.01 M HgCl₂ this occurs in less than 1 minute: with 0.001 M HgCl₂ the rise is usually slower. These changes are irreversible.

¹The 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. Fig. 1 refers to cells in Lot B, Fig. 2 to cells in Lot A (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.

The solutions here used did not produce plasmolysis.

²The P.D. is said to be positive when the positive current tends to flow from the sap across the protoplasm to the external solution.

³Regarding potentials at *X* and *Y* see Osterhout, W. J. V., *J. Gen. Physiol.*, 1944-45, **28**, 23.

After *Y* has become completely permeable to KCl, as shown by the rise of the curve to zero, we find that the outer non-aqueous surface *X* has not yet become completely permeable since it shows a change in P.D. when we vary the concentrations of electrolytes in contact with it (concentration effect).

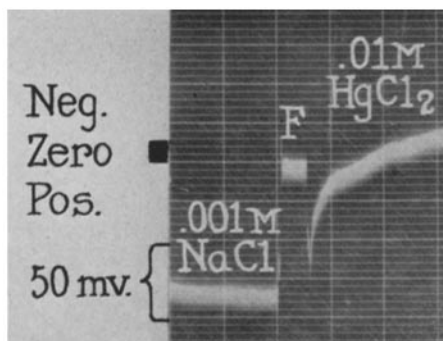


FIG. 1. When HgCl_2 was applied there was a loss of potential, as shown by the rise of the curve.

The record shows the P.D. at a spot *A* which was connected to a spot *B* whose P.D. was zero owing to the application of 0.01 M KCl.

At the start the spot *A* was in contact with 0.001 M NaCl and had a positive P.D. of 100 mv. When the solution was removed from *A*, thus breaking the electrical circuit, the curve jumped to *F*, the "free grid" value of the amplifier.

When 0.01 M HgCl_2 was applied and the spot *A* was again in the circuit the curve rose approximately to zero indicating a complete loss of potential at the inner protoplasmic surface, *Y*. But the outer protoplasmic surface *X* had not become completely permeable for subsequent portions of the record (not reproduced here) showed marked alterations in P.D. at the outer surface when the solutions were changed (these do not occur in a dead cell).

The cell was freed from neighboring cells and kept in Solution A for 4 months at $15^\circ\text{C.} \pm 1^\circ\text{C.}$ An hour before the experiment was started the temperature was raised to 25°C.

Time marks 15 seconds apart.

Before applying HgCl_2 the average change of P.D. on replacing 0.01 M KCl by 0.001 M KCl was 41 ± 2.3 mv. (7 observations). This value became 27 ± 1.4 mv. (6 observations) after the curve had risen to zero under the influence of 0.01 M or of 0.001 M HgCl_2 . The corresponding values for NaCl are 46 ± 1.3 mv. (7 observations) before and 29 ± 5.8 mv. (6 observations) after application of the reagent. These effects gradually disappear.

Hence if we employ permeability as a test of death we may say that death arrives sooner at the inner protoplasmic surface *Y* than at the outer, *X*.

Since this may be due, in part at least, to unlike conditions at *X* and *Y* an attempt was made to make these conditions more nearly equal by raising the

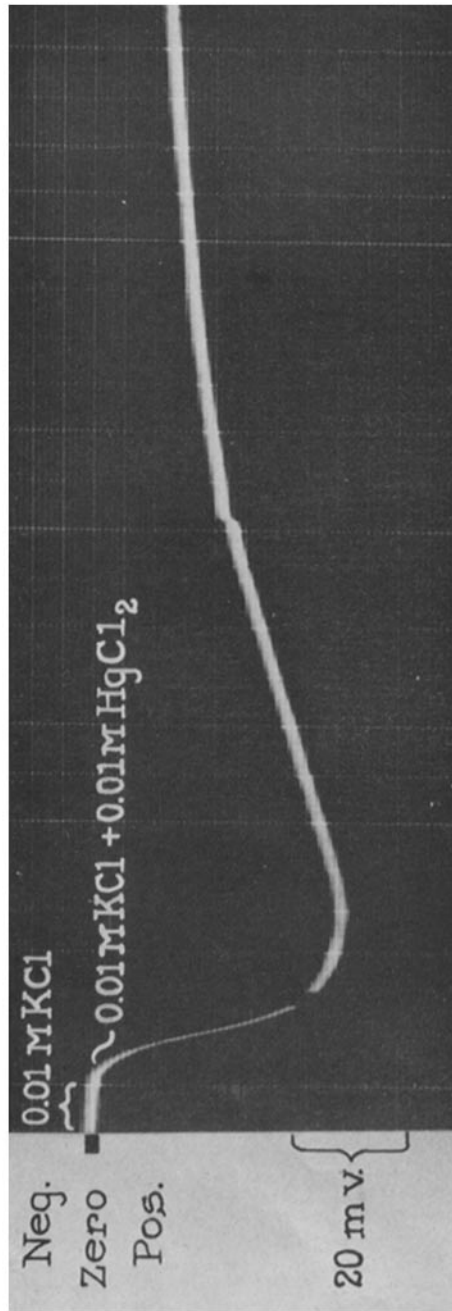


FIG. 2. Shows that when the outer protoplasmic surface X was in contact with 0.01 M KCl it was affected sooner by the addition of HgCl_2 than was the inner protoplasmic surface Y .

At the start the recorded spot A had a negative potential at X which was equal to the positive potential at Y so that the total potential was zero (the spot A was connected through the galvanometer to another spot B whose P.D. was kept at zero by the application of 0.01 M KCl).

When HgCl_2 was applied, X was affected first and the loss of negative potential at X caused the curve to fall. Later the positive potential at Y began to fall off more rapidly than the negative potential at X and the curve began to rise. Eventually the curve reached zero (not shown in the figure) indicating a total loss of potential. In this case the change of solutions was made without breaking the electrical circuit since a continuous flow was maintained (*cf.* Hill, S. E., and Osterhout, W. J. V., *J. Gen. Physiol.*, 1937-38, 21, 541).

The cell was freed from neighboring cells and kept for 24 hours in tap water at $15^\circ\text{C.} \pm 1^\circ\text{C.}$ An hour before the experiment was started the temperature was raised to 25°C.

Time marks 5 seconds apart.

The time of death was longer than in Fig. 1. This may be due in part to the fact that the cells were from different lots collected in different localities.

concentration of KCl in contact with X to 0.01 M since the concentration of KCl in the sap in contact with Y is about 0.05 M. It was then found that death occurred sooner at X than at Y , as shown in Fig. 2. Here the applied reagent was 0.01 M HgCl_2 + 0.01 M KCl which produced at the start a p.d. of zero mv. due to an outwardly directed (positive²) p.d. at Y balanced by an equal negative p.d. at X (both due to the diffusion potential of KCl).

In this case X was affected first,⁴ as shown by the loss of negative potential and the consequent fall of the curve: later the action on Y began to predominate so that the curve began to rise. It reached zero when both X and Y had become completely permeable. This change was irreversible.

During the gradual loss of potential at Y , as shown in Fig. 1, there may be no great change at X in respect to permeability, as indicated by the concentration effect. But at the same time the potassium effect falls off showing that X has been altered. The potassium effect is the change in p.d. in a positive direction when 0.01 M KCl is replaced by 0.01 M NaCl. Before treatment with HgCl_2 it was 31 ± 2.8 mv. (7 observations). After HgCl_2 had caused the curve to rise to zero the potassium effect disappeared in most cases and in others had a very low value. This change is presumably due to loss of a group of organic substances, called for convenience R , as described in previous papers.⁵

The results present a striking parallel to those obtained with formaldehyde as described in a former paper.⁶ It is evident that in both cases the rate of death may differ at the inner and outer surfaces of the protoplasm and that this can be controlled by changing the experimental conditions.

It may be added that earlier experiments⁷ with chloroform gave a similar result in showing that the concentration of KCl determines the behavior. The solutions were saturated with chloroform in all cases. When the external solution contained 0.05 M KCl or less Y was killed first but when it contained 0.1 M KCl X was killed first.

Even when X and Y are under the same conditions they do not behave alike in all respects. When both are in contact with sap the difference in p.d. between the two surfaces is about⁸ 15 mv.

⁴ This is presumably because the reagent reaches X first. As might be expected, the distance to which the curve descends varies greatly with different cells.

⁵ Cf. Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1933-34, **17**, 87, 99, 105.

⁶ Osterhout, W. J. V., *J. Gen. Physiol.*, 1944-45, **28**, 23.

⁷ Osterhout, W. J. V., Some aspects of permeability and bioelectrical phenomena, in *Molecular physics in relation to biology*, *Bull. Nat. Research Council*, No. 69, 1929, 170.

⁸ Osterhout, W. J. V., and Harris, E. S., *J. Gen. Physiol.*, 1927-28, **11**, 391.

SUMMARY

The inner and outer protoplasmic surfaces in *Nitella* may differ greatly in behavior. When 0.01 M HgCl_2 is applied externally death arrives first at the inner surface. But when 0.01 M HgCl_2 + 0.01 M KCl is applied death takes place sooner at the outer surface. Since 0.01 M KCl by itself is not toxic its effect may be to condition the surface layer chemically or by means of the diffusion potential it sets up (this may amount to 100 mv.).

These surfaces consist of non-aqueous films forming the boundaries of a layer of aqueous protoplasm not over 10 microns in thickness.

These and earlier experiments with formaldehyde and with chloroform show clearly that it is possible to control the behavior of the protoplasmic surfaces so that when a toxic agent is applied it may produce death more rapidly at the inner or at the outer surface according to experimental conditions.