INJURY IN RELATION TO CELL ORGANIZATION

By W. J. V. OSTERHOUT

(From the Laboratories of The Rockefeller Institute for Medical Research and the Marine Biological Laboratory, Woods Hole)

(Received for publication, July 24, 1950)

In some cells the protoplasm seems to be so organized that when any part is killed the death of the entire protoplasm quickly follows.

This is true of *Nitella* since previous experiments[1] show that when a small portion of the cell is killed solutes soon escape and the death of the cell soon follows.

Experiments reported in this paper show that when the escape of solutes is delayed there is a corresponding delay in the death of the cell. It would seem that if the escape of solutes could be prevented we might expect the cell to live indefinitely after the death of a part of the protoplasm.

EXPERIMENTAL

Cells of *Nitella* 4 to 6 cm. long were employed[2] using the arrangement shown in Fig. 1. At A the cell was in contact with water; this was separated by a vaseline barrier, C, from a toxic solution placed at B. The relative lengths of A, B, and C were as shown in the figure.

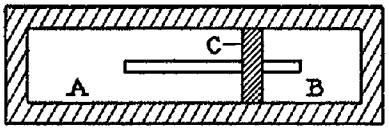

FIG. 1. A diagram showing a cell of *Nitella* with a barrier of vaseline at C separating a solution at A from another solution at B. These solutions are held in place by a barrier surrounding the cell.

When 0.3 M acetic acid was placed at B the protoplasm was killed within a minute as shown by the prompt penetration of acid fuchsin.[3] With 0.01 M HCl at B death usually took place within 4 minutes.

[1] Osterhout, W. J. V., *J. Gen. Physiol.*, 1950–51, **34**, 279.

[2] The observations were made on *Nitella flexilis*, Ag. Regarding the treatment of the cells see Osterhout, W. J. V., *J. Gen. Physiol.*, 1950–51, **34**, 279.

[3] This was 0.3 per cent acid fuchsin in phosphate buffer at pH 7 (the cationic concentration of the buffer was 0.067 M). In normal cells there is no penetration in 1 hour.

When A was in contact with water the death of the protoplasm at B was soon followed by signs of injury at A. The normal movement of the protoplasm (cyclosis) stopped within 2 to 4 minutes and soon afterwards contraction of the chloroplasts was observed at A. Previous experiments have shown that this is due to the ingoing current of water which enters at A and escapes at B.[4]

Within 5 to 10 minutes there was some disintegration and displacement of some of the chloroplasts at A causing a widening of the clear areas between them.[5] This was irreversible since the normal structure could not be restored by creating a current of water in the reverse direction by applying 0.3 M sucrose at A.[4] The protoplasm became progressively disorganized and the whole mass shrank (false plasmolysis). The application of acid fuchsin at an early stage in this process showed that the protoplasm was dead.

Since these effects seemed to be due to a loss of solutes an attempt was made to delay this loss and prolong the life of the protoplasm at A by preventing the entrance of water at A. For this purpose the water at A was replaced by light mineral oil before applying acid at B.

The result was very striking. The protoplasm at A remained normal in appearance and cyclosis continued up to 3.5 hours. It seems possible that the protoplasm at A might remain normal indefinitely if the escape of solutes at B could be entirely prevented. Under the conditions of the experiment they continued to escape slowly. If cyclosis carries acid from B to A this may produce injury.

A similar result can be obtained by using a solution of sucrose at A in place of mineral oil but it is necessary to find the right concentration to prevent any movement of water in the cell. For this purpose the osmotic pressure of the sucrose at A must be equal to the internal osmotic pressure.

DISCUSSION

The results are very instructive in showing how injury in one part of the cell may affect other parts and how this may be controlled.

It may be noted that under certain conditions solutes may escape in such fashion that no deficiency of solutes occurs. We then find that no damage is done. This happened in the experiments of Jacques[6] when cells of *Valonia* and of *Halicystis* were impaled on a capillary into which the solutes escaped as fast as they entered the cells from without. The cells continued to live and electrolytes entered much faster than under normal conditions.

In the experiments on *Nitella* there are alterations in hydrostatic pressure

[4] The contraction of the chloroplasts can often be reversed by the method explained in a previous paper. *Cf.* Osterhout, W. J. V., *J. Gen. Physiol.*, 1946–47, **30,** 229.

[5] For the normal appearance of the chloroplasts see Osterhout, W. J. V., *J. Gen. Physiol.*, 1950–51, **34,** 279.

[6] Jacques, A. G., *J. Gen. Physiol.*, 1938–39, **22,** 147, 757.

in addition to changes in the concentration of solutes. When these are considered the survival of A after the death of B seems all the more remarkable.

The protoplasm forms a layer about 15 microns thick surrounding a large central vacuole in which the hydrostatic pressure normally amounts to about 6.4 atmospheres at 25°C.[7] In consequence the protoplasm is pressed against the cellulose wall which surrounds it and is held firmly in place.

When the solutes escape at B the hydrostatic pressure which holds the protoplasm in place is reduced to zero. As a rule this does not cause much shift in the position of the protoplasm which continues to adhere to the cellulose wall but if it continues to live it does so under very different conditions.

The outer part of the protoplasm is a stiff gel in which the chloroplasts are imbedded. The inner part is a sol which constantly moves up one side of the cell and down on the other side (cyclosis). It seems remarkable that even after the hydrostatic pressure is released the cyclosis continues.

It is evident that the escape of solutes[8] affects the cell in 2 ways: (1) by releasing the pressure which holds the protoplasm in place and (2) by reducing the concentration of solutes below the point which is necessary to maintain the protoplasm in normal condition.

When one end of a uninucleate cell is amputated the part containing the nucleus may survive indefinitely. This is seen in certain animal cells such as ameba and nerve. In these cases the excess of internal over external osmotic pressure is small and there is presumably little escape of solutes. Similar considerations seem to apply to certain multinucleate cells such as *Caulerpa* and *Bryopsis*.

In such cases the cut protoplasm seems to produce a new non-aqueous film at the cut surface so that the escape of solutes is relatively small.

I wish to thank Mr. J. S. Fass for the care and skill he has shown in carrying out these experiments.

SUMMARY

When a part of a *Nitella* cell, A, is covered with water and the rest of the cell, B, is in contact with a toxic solution there is an escape of solutes at B. This is followed by the escape of solutes at A which causes the death of A. Water enters at A, flows along inside the cell, and escapes at B carrying solutes with it. When this is prevented by covering A with mineral oil the escape of solutes at A is delayed and the life of A is correspondingly prolonged. It is remarkable that this occurs in spite of the fact that the hydrostatic pressure inside the cell (turgor) drops from 6.4 atmospheres to zero.

It would seem that A might not be affected by the death of B if the escape of solutes could be prevented.

[7] Osterhout, W. J. V., *J. Gen. Physiol.*, 1948–49, **32**, 553.

[8] Most of the solutes pass out freely through the cellulose wall.