

DISTANT EFFECTS OF TOXIC AGENTS

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PLATE 1

(Received for publication, July 14, 1950)

Toxic solutions placed at one end of a *Nitella* cell may produce little or no visible effect at the place of application but signs of injury soon appear at the opposite end of the cell under certain conditions.

The effect evidently depends on the movement of solutes in the cell. The experiment is instructive as throwing new light on the role of such solutes in life processes.

EXPERIMENTAL

Cells¹ 5 to 6 cm. long were employed. A cell was placed on a glass plate (10 × 3 cm.) as shown in Text-fig. 1. Water was placed at A separated by a barrier of vaseline from mineral oil² at C which in turn was separated by a vaseline barrier from a toxic solution at B. A barrier of glass or of vaseline, or of paraffin around the edges of the plate kept the liquids in place. Cover glasses were used when needed.

RESULTS

At the start water was placed at A and B and mineral oil at C. When the water at B was replaced by 0.3 M acetic acid there was little or no change in the visible structure of the protoplasm at B but the green color became somewhat paler except in cells whose color was very pale at the start.

The protoplasm at C remained unchanged in appearance for an hour or more but marked changes began at A within a minute after the acetic acid was placed at B.

¹ The observations were made on *Nitella flexilis*, Ag. The cells were freed from neighboring cells and observed at once or kept in the laboratory in Solution A (*cf.* Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1933-34, **17**, 87) at 15°C. ± 1°C. About 15 hours before use the cells were placed in a large amount of Solution A in a room at about 25°C. and the temperature of the solution rose gradually to about 25°C. and the experiments were performed at about this temperature.

Microscopic observations show that the dimensions of the cell do not change during the experiments so that we may conclude that when a given amount of water enters at one end the same amount escapes at the other end.

The use of metal forceps was avoided.

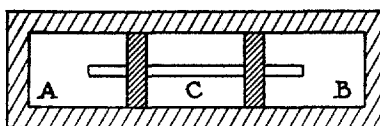
² Mineral oil for medicinal use was employed.

The first visible alteration at A was the shrinkage and rounding up of the chloroplasts. They became spherical instead of ellipsoid³ with the diameter of the sphere less than the smallest diameter of the original ellipsoidal structure.

This was followed by the appearance of small clear areas due to the displacement and disintegration of the chloroplasts. These clear areas became larger and appeared to fuse together to some extent so that the protoplasm took on the appearance shown at *a'* in Fig. 1 of Plate 1. The protoplasmic mass became smaller, presumably as the result of the escape of water from the large central vacuole (false plasmolysis).⁴

This process may continue for half an hour or more. During this time the protoplasm at C remains normal in appearance and at B no further change occurs (Plate 1, Fig. 1, *b'*).

The acetic acid at B causes little or no visible change in the structure of the protoplasm at that point but it renders it permeable and causes death within 1 minute.



Text-Fig. 1. Diagram showing a *Nitella* cell separated by vaseline barriers into three portions A, B, and C. A and B contain solutions and C contains mineral oil. The whole is surrounded by a peripheral barrier which keeps the solutions in place.

This is shown by replacing the acetic acid by a solution of acid fuchsin⁵ which penetrates within a minute or less (in a normal cell it does not penetrate in 1 hour).

A similar test at A shows that the protoplasm does not become permeable until clear areas have been formed in the course of 5 to 10 minutes.

The protoplasmic movement (cyclosis) stops at B almost as soon as the acetic acid is applied and soon afterwards disappears at A but at C it may continue somewhat longer. The acetic acid acts as a good fixative which kills but produces little or no visible change in the structure of the protoplasm. The protoplasm becomes permeable at B so that solutes escape. This can be shown for chlorides by removing some of the external solution surrounding B and testing with AgNO_3 .

³ In many cells the chloroplasts were more elongated than those shown in Plate 1, Fig. 1, *a* and *b*.

⁴ This does not necessarily involve any dehydration of the protoplasm.

⁵ A solution of 0.3 per cent in buffer solution at pH 6.9 was employed. The concentration of cations in the buffer was 0.07 M.

On each side of the cell there is a "white line" over 30 microns in width which is devoid of chloroplasts. The white lines extend the entire length of the cell. In young cells they are nearly straight but in older cells they have a spiral course. These white lines show little or no change at B or C as the result of the treatment. But as the protoplasm at A becomes disorganized the edges of the white line are disturbed.

There is an osmotic drive due to the high internal osmotic pressure which forces water into the cell.⁶ Hence when B is injured water enters at A, travels along inside the cell, and escapes at B. This is easily observed since the water carries with it the large particles suspended in the sap of the large central vacuole.⁷ This stream of water carries solutes with it as shown by the fact that in cells stained with brilliant cresyl blue⁸ the color becomes paler at A.

The shrinkage of the chloroplasts at A is evidently due to the movement of water as in experiments previously reported in which the movement was due to a solution of sucrose at B.⁹ The effect was then reversible since the chloroplasts expanded when the movement of water and of solutes was reversed by placing a solution of sucrose at A and water at B.

When we replace the water at A by 0.3 molal sucrose in the present experiment we obtain a reversal of the movement of water as shown by the behavior of the large particles in the vacuole but the chloroplasts do not always expand as much as in the experiment described in a previous paper.⁹ This might be expected since the reversal cannot carry back to A the solutes which have escaped at B and some acetic acid may move from B to A.

If the current of water is reversed without carrying acetic acid from B to A the expansion of chloroplasts at A may take place as described in a previous paper.⁹ This can be accomplished by removing some mineral oil at C and substituting water in its place before applying 0.3 molal sucrose at A. The movement of water then takes place from C to A without carrying any acetic acid from B and solutes return to A.

The injurious action of the removal of solutes from A which is shown by the formation of clear areas is irreversible. The clear areas continue to enlarge with consequent greater disorganization of the protoplasm even when the current is reversed by replacing the water at A by 0.3 molal sucrose.

⁶ The internal osmotic pressure at A is about 6.4 atmospheres at 25°C.

⁷ This is about 0.5 mm. in diameter. The movement of large particles in the vacuole toward B is due to the toxic effect at B and not to the osmotic effect of the acetic acid as the concentration of the acetic acid is too low to produce such an effect by osmotic action. This is evident since the movement does not start at once upon application of acetic acid as would happen if the acetic acid acted osmotically.

⁸ The cells were placed in 0.005 per cent of the dye at pH 8. Cf. Irwin, M., *J. Gen. Physiol.*, 1925-26, **9**, 561; 1926-27, **10**, 75.

⁹ Osterhout, W. J. V., *J. Gen. Physiol.*, 1946-47, **30**, 229.

Some other toxic agents such as 0.01 M HCl and 0.04 M picric acid produce death at B with little or no visible change in the structure of the protoplasm. Others such as 0.67 M formaldehyde and water saturated with chloroform cause changes in appearance during the process of death. In all cases changes similar to those described above were observed at A.

DISCUSSION

It is usually assumed that injury spreads from the region where a toxic agent is applied to neighboring regions and thence to more remote parts. This may happen in *Nitella* when one end of the cell is injured mechanically by cutting or by compression. Apparently a compression wave is set up which travels along the cell producing injury as shown by changes in the electrical potential of the cell.¹⁰

The effects of mechanical injury have been observed to travel slowly along the cell in *Griffithsia*¹¹ in a manner which suggests the spread of a toxic substance. It would seem that such substances play a part when injury to a cell spreads to neighboring cells as seen for example in leaves of *Monotropa*.¹² When observed under the microscope we see that a cut which kills some of the cells causes their nuclei to turn black due to formation of melanin. After a short time this effect spreads from the cut involving more cells as though some substance were diffusing out from the injured region.

The conventional view of the effects of injury does not apply to the experiments on *Nitella* described here. In these experiments injury is caused by the loss of substances at A due to killing a part of the cell at B and the injury does not spread from B to the neighboring regions according to the conventional conception.

Small injuries involving little or no loss of solutes may produce no distant effects. In *Valonia* and in *Halicystis* puncture of the cell by a capillary tube does not cause permanent injury and apparently the effects of the puncture do not spread. In *Nitella* small mechanical injuries which displace or destroy a few chloroplasts do not produce visible effects in neighboring regions. In these cases the loss of solutes is relatively small.

The injury described here accompanies a decrease in the concentration of solutes. There is a decrease in hydrostatic pressure and in this respect it differs from the decrease in concentration of solutes produced in marine organisms by diluting the sea water where an increase in hydrostatic pressure occurs inside the cell.

It may be added that the role of electrolytes inside the cell is very different from their effect in the external solution. A balanced solution of the usual sort

¹⁰ Osterhout, W. J. V., *J. Gen. Physiol.*, 1928-29, **12**, 167, 355; 1930-31, **14**, 385, 473.

¹¹ Osterhout, W. J. V., *Proc. Nat. Acad. Sc.*, 1916, **2**, 237.

¹² Osterhout, W. J. V., *Science*, 1917, **46**, 367.

may not always be present inside the cell as shown by the low concentration of calcium.¹³ In *Valonia* the sap is toxic when applied to the outside of the cell.¹⁴

As the changes at A appear to be due to loss of solutes¹⁵ it is of interest to know what mechanism produces this result. The appearance of the protoplasm suggests a coagulation of proteins. Certain proteins in solution are precipitated by the removal of salts from the solution but the process is reversible which is not the case with the protoplasm.

The sap of the *Nitella* cells employed contains about 0.05 M NaCl and about 0.05 M KCl. After they have been removed by the current of water entering at A and the cell has died so that it becomes permeable to salts we can replace the NaCl and KCl at A by covering this region with a solution of 0.05 NaCl + 0.05 KCl. But this does not produce any changes in the protoplasm which suggest a restoration to the normal condition. This indicates an irreversible process such as we find to a considerable extent in the denaturation of proteins.

We do not at present know of any case except in the living organism in which the lowering of the concentration of solutes produces irreversible changes comparable with those described here. It would be interesting to know if the changes described here resemble those found in marine organisms when placed in dilute sea water.

We may conclude that any local injury, no matter how small, which permits the escape of solutes may be fatal. If there are regions of the cell which are unusually sensitive they will play an important role in this connection and the effects of toxic agents will be enhanced accordingly.

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

SUMMARY

Toxic solutions applied at one end of a *Nitella* cell 6 cm. long may produce little or no visible change in the structure of the protoplasm at the place of application but if the opposite end is covered with water its protoplasm soon disintegrates. If the middle of the cell is covered with mineral oil this region remains normal in appearance for half an hour or more.

The result is due to the movement of substances in the cell. The loss of substances at the end where the toxic agent is applied results in loss at the opposite end if it is covered with water since water enters and travels along inside the cell carrying substances with it. This causes injury at the spot where the water enters.

¹³ Osterhout, W. J. V., *Bot. Rev.*, 1936, **2**, 283.

¹⁴ Osterhout, W. J. V., *J. Gen. Physiol.*, 1924-25, **7**, 561.

¹⁵ It may be asked whether the current of water entering at A causes mechanical displacements which affect the result but this does not appear probable.

The conception developed here differs fundamentally from the usual view that the effects of injury spread gradually from the region where the toxic agent is applied to the immediately adjoining regions and thence to more remote places.

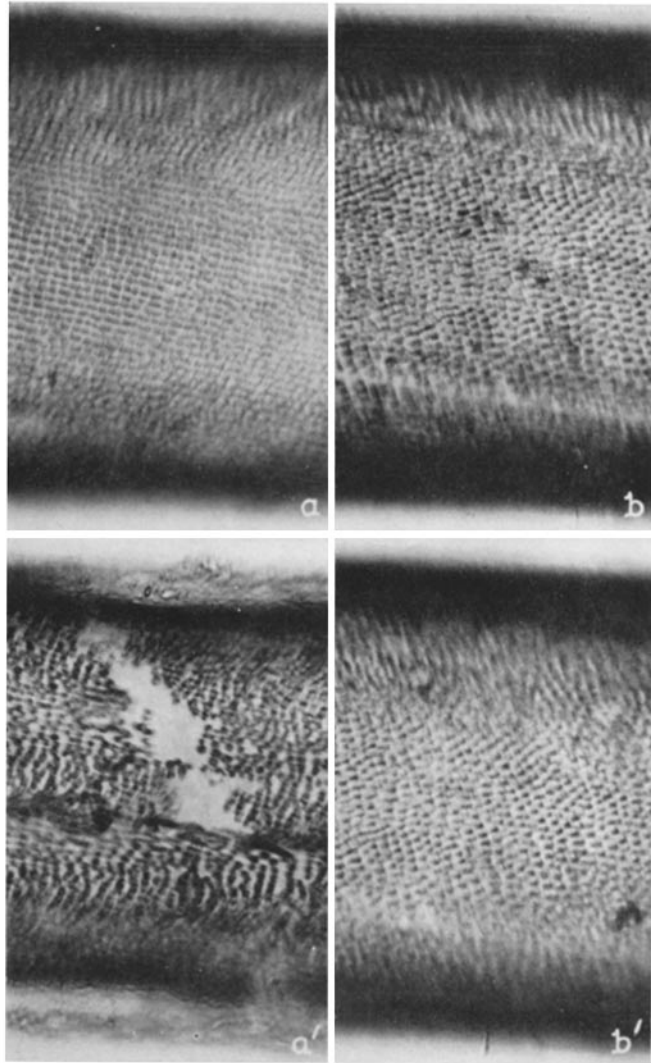
The change produced by loss of substances produces an interesting pattern which deserves study.

EXPLANATION OF PLATE 1

FIG. 1. At *a* is shown the appearance of the protoplasm at A and at *b* the appearance of the protoplasm at B before B was covered with 0.3 M acetic acid. At *a'* is shown the appearance of the protoplasm at A and at *b'* the appearance of the protoplasm at B after B has been covered for 30 minutes with 0.3 M acetic acid. All photographs were made from the same cell.

Before acid was placed at B both A and B were covered with water and during treatment of B with acid A was covered with water and C was covered with mineral oil.

It is evident that the toxic effect of acetic acid at B has produced little or no change in the structure of the protoplasm at B but marked alterations have occurred at A.



(Osterhout: Distant effects of toxic agents)