STUDIES OF THE INNER AND OUTER PROTOPLASMIC SURFACES OF LARGE PLANT CELLS

I. Plasmolysis Due to Salts

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Electrical studies on *Nitella*[1] show that the outer, non-aqueous, protoplasmic surface (X) differs from the inner (Y). If cell sap is placed outside the cell so as to set up the chain

	X	W	Y	
Sap outside	|	Aqueous protoplasm	|	Sap in vacuole

we find[2] a P.D. of about 16 mv. This would be impossible if the outer and inner protoplasmic surfaces were identical in their properties.

Further evidence of unlikeness is found in plasmolytic experiments. When cells are placed[3] in suitably diluted sea water salts appear to penetrate the outer non-aqueous surface, X, more rapidly than the inner, Y. This increases the salt content and consequently the osmotic pressure of the aqueous layer, W, of the protoplasm so that it withdraws water from the vacuole and increases in thickness[4] (Figs. 1 to 3). Thus the distance between X and Y may become very much greater than in the normal state.

In most cases some separation of X and Y takes place within a few minutes and the cell returns in an equal or shorter length of time to its original state when replaced in tap water, if the alteration has not proceeded too far.

As the process continues the shrinkage of the vacuole may produce a long narrow vacuole (Fig. 2) or it may break up into several spherical or elongated vacuoles (Fig. 3).

[1] *Nitella flexilis*, Ag.

[2] *Cf.* Osterhout, W. J. V., and Harris, E. S., *J. Gen. Physiol.*, 1927–28, **11**, 391. The electrode in contact with X is negative in the external circuit to the electrode in contact with Y.

[3] Sea water 6 parts plus tap water 1 part is suitable in many cases.

[4] 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).

Temperature 25–30°C.

Dead cells do not give these results.

In some cases the outer surface, X, shrinks away from the cell wall. Its position is easily seen as the chloroplasts adhere to it and come away with it when it shrinks away from the cellulose wall.

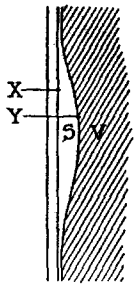

FIG. 1. The beginning of separation of the inner protoplasmic surface Y from the outer X. As the process continues the space, S, between them increases. V, vacuole. Optical section. Diagrammatic.

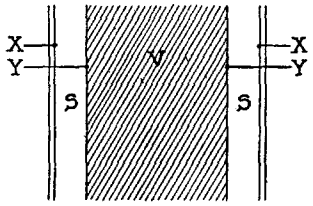

FIG. 2. A later stage of the process indicated in Fig. 1. Diagrammatic.

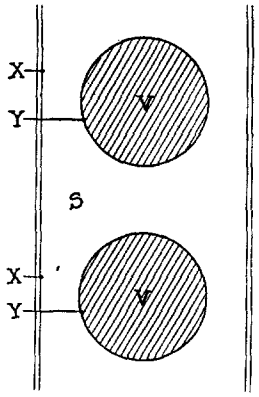

FIG. 3. A later stage of the process indicated in Fig. 2. The central vacuole has broken up into smaller vacuoles. Diagrammatic.

The sea water was employed because it is a balanced solution. The molar proportions of salts were[5] approximately as follows: NaCl 1000, $MgCl_2$ 78, $MgSO_4$ 38, KCl 22, $CaCl_2$ 22. It had a Cl content of 0.52 M.

[5] *Cf.* Osterhout, W. J. V., *Bot. Rev.*, 1936, **2**, 283.

It is probable that the principal salt which penetrates is NaCl which in pure solution produces effects like those of sea water but at a lower osmotic pressure. This indicates that the penetration of NaCl is inhibited to some extent[6] by $CaCl_2$.

These effects are reversible (with no disarrangement of the chloroplasts) if the cells are returned to tap water before the process has gone too far.

These relations may be strikingly demonstrated by staining[7] the vacuole with brilliant cresyl blue or with neutral red but it is possible that these substances may have some direct effect.[8]

Similar and very striking results were obtained with *Chara Braunii*, Gmelin, whose cells are well adapted to such studies. This applies to a considerable extent to *Hydrodictyon reticulatum* (L) Lagerh.

Very small cells of *Valonia macrophysa*, Kütz were kindly furnished by Dr. L. R. Blinks. These were nearly spherical and less than 2 mm. in diameter. When they were removed from Bermuda sea water (with a Cl content of about 0.58 M) and placed in sea water 1 part plus 3 M KSCN about 1 part, small local concavities appeared due to the simultaneous withdrawal of X and Y, without increasing the distance between the two layers.[9] In some places, however, there was a small separation of the two layers. Very careful focussing is required to make sure that such separation has occurred.

The process is reversible if not allowed to go too far.

It would therefore seem that the inner and outer surfaces in *Valonia* differ. This conclusion is in harmony with the results of electrical experiments for when we form the chain[10]

Sap | Protoplasm | Sap

we obtain about 65 mv.[11]

We may therefore conclude that in all these large cells the two non-aqueous protoplasmic surfaces differ in character.

Experiments somewhat similar in nature have been made on small plant cells by various investigators. In most cases these experiments involved very long

[6] *Cf.* Osterhout, W. J. V., *Science*, 1911, **34**, 187. In the present experiments with NaCl the salt furnished by Merck and Co. "for biological use" was employed.

[7] A few minutes in 0.25 per cent of brilliant cresyl blue or 0.05 per cent of neutral red at pH 8.5 is sufficient.

[8] Strugger, S., *Ber. deutsch. bot. Ges.*, 1931, **49**, 453. Drawert, H., *Ber. deutsch. bot. Ges.*, 1938, **56**, 123. Küster, E., *Ber. deutsch. bot. Ges.*, 1940, **58**, 413.

[9] In the normal state the distance between them is less than 10 microns.

[10] See page 139.

[11] Damon, E. B., *J. Gen. Physiol.*, 1931–32, **15**, 525. The electrode in contact with X is negative in the external circuit to the electrode in contact with Y (as in *Nitella*).

exposure to stains which may in itself influence the result,[12] and the effects appeared very slowly and were often not reversible. Balanced solutions were not employed. In no case were electrical measurements made.

The fact that one part of the cell can be made to take water from another is significant and deserves further study.

SUMMARY

In *Nitella, Chara, Hydrodictyon,* and *Valonia* the inner and outer non-aqueous protoplasmic surface layers can be separated by certain plasmolytic agents which penetrate the outer surface more rapidly than the inner and hence raise the osmotic pressure of the protoplasm lying between them and cause it to increase in thickness by taking up water from the central vacuole.

We may therefore conclude that the two surfaces differ. This idea is confirmed by earlier electrical measurements which show that when sap is placed outside the cell the chain

Sap external	Protoplasm	Sap in vacuole

produces an E.M.F. of several millivolts.

[12] For the literature see Hartmair, V., *Protoplasma,* 1937, **28,** 582. See also footnote 8.