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

II. Mechanical Properties of the Vacuolar Surface

By W. J. V. OSTERHOUT

(From the Laboratories of The Rockefeller Institute for Medical Research)

(Received for publication, May 25, 1944)

The protoplasm of *Nitella* forms a thin layer surrounding a large central vacuole filled with sap. The outer region of the protoplasm, in contact with the cellulose wall and containing the chloroplasts, is motionless. It may form a rather rigid gel which may be caused by centrifugal force to peel off in long stiff strips containing the chloroplasts arranged in straight rows.

The protoplasm inside this layer is usually in motion and is separated from the liquid in the vacuole by a non-aqueous film which is too thin to be visible as a separate membrane, and hence is less than 0.5 micron in thickness. This film will be called *Y*.

When the sap is stained with neutral red[1] this non-aqueous film forms a sharply defined boundary between the deep red vacuole and the colorless protoplasm. It may be pushed violently in and out by the flowing protoplasm which often tends to thicken and become more lumpy under the influence of the stain.[2] Especially at the end of the cell where the stream of protoplasm flows across the end wall (where there are no chloroplasts) we may see that certain large lumps in the protoplasm push the non-aqueous film rapidly in and out (Fig. 1 *a*, *b*, and *c*), so that the observer expects to see it ruptured at any moment. In spite of this not a trace of dye appears in the protoplasm: this indicates that no such rupture occurs.

Mechanical disturbances of the film may occur at various places in the cell. The protoplasmic stream moves up one side of the cell and down the other and the two oppositely moving streams are separated by a surface having at its edges clearly visible white lines on opposite sides of the cell. These white lines are due to the absence of one or more of the rows of chloroplasts which run lengthwise from one end of the cell to the other. One may see particles in the stream approach a white line and come to rest in it for a time and

[1] A solution of 0.01 per cent of dye gives good results: 0.01 per cent of brilliant cresyl blue may also be used (both at pH 8).

[2] This is more striking after the cell has been in contact for several hours with a small volume of 0.01 per cent neutral red at pH 8 under a cover glass sealed at the edges with vaseline.

Downloaded from http://rupress.org/jgp/article-pdf/28/1/17/1239697/17.pdf by guest on 16 April 2021

then return to the stream but very rarely do they cross into the other stream and go in the opposite direction.[3]

At first the white lines may run straight down the cell parallel to its long axis but later they become spirals and there is a corresponding twist of the surface[4] dividing the two oppositely flowing streams so that as one focusses through the cell the white line on the further side cuts that on the nearer side at a definite angle.

If the cell is in an upright position and the flow on the right side of the white line on the nearer surface is upward it is evident that if we could go around and view it from the other side the direction of flow would appear to be downward on the right side. Without performing this maneuver we need only move along the cell (focussing on the nearer surface) until the white line disappears from view in its spiral course

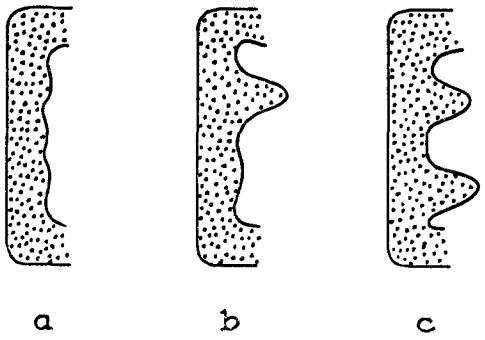

FIG. 1. Sketches at intervals of 5 seconds showing the conformation of the protoplasmic stream at the end of the cell (chloroplasts not shown). Semidiagrammatic.

around the cell: when it reappears further along on the nearer surface we see that the flow on the right side is now in the downward direction.

The protoplasm contains spheres of various sizes some of which are covered with spines, suggesting sphere crystals. These also occur in the sap and it seems evident that they must come in contact with the non-aqueous film.

In the sap we sometimes find gelatinous aggregates which revolve slowly since they extend clear across the vacuole and come in contact with the non-aqueous film on either side so that its motion is communicated to these masses.

The non-aqueous film undergoes a good deal of disturbance when the cell is

[3] When the protoplasm and chloroplasts are squeezed out of the cell (after cutting off one end) the location of the white line may often be detected by an apparent thickening (or thinning) of the cellulose wall.

[4] A model of this may be made by twisting a long narrow strip of cardboard so that each edge follows a spiral course: these edges then correspond to the white lines of the *Nitella* cell.

Downloaded from http://rupress.org/jgp/article-pdf/28/1/17/1239697/17.pdf by guest on 16 April 2021

strongly plasmolyzed by sea water so that the large central vacuole breaks up into a series of smaller vacuoles, leaving the outer non-aqueous surface film, *X*, in its normal position as described in a former paper.[5] This involves a subdivision of the inner surface film, *Y*, yet there is no indication of the escape of dye from the vacuole when the cell is kept under continuous observation. In this process of division the film behaves as though it had true surface tension (the vacuole acts like a drop of oil in water) but we cannot say definitely whether it is a solid or a liquid film.

The question arises, how can the delicate non-aqueous surface layer of the vacuole survive so much mechanical disturbance.

It might be suggested that the protoplasm adjoining the vacuole is a water-in-oil emulsion (with the oil forming very thin films and the water in small drops only a micron or less in diameter) so that the non-aqueous surface film adjoining the vacuole would be relatively stable and if ruptured would be at once repaired.[6] It is questionable whether in that case we should find the high electrical capacity (1 microfarad per cm.^{2}) observed in *Nitella*,[7] since the successive non-aqueous films might behave like condensers in series. It may also be asked whether such a layer would have the high degree of permeability to water which is found in *Nitella*.

It would seem that such a layer of emulsion could not be more than 2 or 3 microns thick for we see very small granules in the protoplasm, not more than 2 or 3 microns distant from the vacuolar surface, moving much more rapidly than larger granules and spheres equally distant from the vacuolar surface. If the small granules and the large spheres were in an emulsion of this sort we should not expect to see them moving at greatly differing rates.

Even if there were only a single non-aqueous film it is possible that ruptures might occur and be instantly repaired and thus escape detection. It has been suggested that the film is formed of substances which decrease surface tension and hence become trapped in the surface (for if they diffuse into it energy is required to remove them). Such substances might be available for the repair of the film.[8]

The properties of very thin non-aqueous layers between two aqueous systems are not understood. We have little to help us in dealing with this problem. Very thin films of liquids immiscible with water have been prepared

[5] Osterhout, W. J. V., *J. Gen. Physiol.*, 1943–44, **27,** 139.

[6] In this case the surface would probably correspond more nearly with that of *Amoeba* and other protoplasmic surfaces which are subjected to motion.

[7] Blinks, L. R., *J. Gen. Physiol.*, 1936–37, **20,** 229; *Tr. Faraday Soc.*, 1937, **33,** 991. Curtis, H. J., and Cole, K. S., *J. Gen. Physiol.*, 1937–38, **21,** 189.

[8] Quick repair of a ruptured film is familiar to biologists in the well known experiment in which a crystal of copper sulfate is placed in a solution of potassium ferrocyanide.

Downloaded from http://rupress.org/jgp/article-pdf/28/1/17/1239697/17.pdf by guest on 16 April 2021

but they do not seem to have a great degree of mechanical stability. Such films have been made by Danielli[9] and thinner ones have been prepared by Dean, Curtis, and Cole.[10]

The properties of the non-aqueous film surrounding the vacuole are of considerable interest. It is the chief seat of the resting potential of the cell which may amount to 100 mv. or more. If we consider the film to be 0.1 micron thick this would amount to an electrical pressure of 10,000 volts per cm.[11]

It would seem that this is largely due to the diffusion potential of KCl across *Y*. The sap contains about 0.05 M KCl but apparently there is very little K^+ in the aqueous layer of the protoplasm[12] (this layer may be called *W* for convenience). Hence we suppose that there is an outwardly directed concentration gradient[13] of KCl across *Y* and if the mobility of K^+ in *Y* were as much greater than that of Cl^- as it is in *X* it might produce the observed resting potential of 100 mv. or more.[12]

It would also appear that the sudden loss of resting potential which occurs in stimulation is accompanied by a sudden increase in the permeability of *Y*.[14] The ability to produce action currents is lost when cells are leached in distilled water which appears to remove[15] an organic substance (or a group of such substances) called for convenience *R*. This can be recovered and replaced in the cell thus restoring irritability.[16] It can also be restored by blood,[17] by guanidine,[18] and by other substances.

It is also evident from previous work[19] that *Y* has selective permeability.

[9] Danielli, J. F., *J. Cell. and Comp. Physiol.*, 1935, **7,** 393.

[10] Dean, R. B., Curtis, H. J., and Cole, K. S., *Science*, 1940, **91,** 50. Dean, R. B., *Tr. Faraday Soc.*, 1940, **36,** 166. Dean, R. B., in Osterhout, W. J. V., Some models of protoplasmic surfaces, in Cold Spring Harbor symposia on quantitative biology, Cold Spring Harbor, Long Island Biological Association, 1940, **8,** 62.

[11] Under normal conditions the pressure is presumably similar all over the surface so that there is little or no flow of current.

[12] Osterhout, W. J. V., *J. Gen. Physiol.*, 1944–45, **28,** 23.

[13] By analogy with the behavior of the outer protoplasmic surface we may suppose that K^+ is the most important cation in this connection: accordingly the other cations are neglected for convenience in discussion. *Cf.* Osterhout, W. J. V., *J. Gen. Physiol.*, 1929–30, **13,** 715. Hill, S. E., and Osterhout, W. J. V., *Proc. Nat. Acad. Sc.*, 1938, **24,** 312.

[14] Osterhout, W. J. V., *J. Gen. Physiol.*, 1934–35, **18,** 215; 1943–44, **27,** 61. Cole, K. S., and Curtis, H. J., *J. Gen. Physiol.*, 1938–39, **22,** 37.

[15] Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1933–34, **17,** 87.

[16] Osterhout, W. J. V., and Hill, S. E., *Proc. Soc. Exp. Biol. and Med.*, 1934–35, **32,** 715; *Science*, 1935, **81,** 418.

[17] Osterhout, W. J. V., *J. Gen. Physiol.*, 1935–36, **19,** 423.

[18] Osterhout, W. J. V., *J. Gen. Physiol.*, 1940–41, **24,** 7.

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

Downloaded from http://rupress.org/jgp/article-pdf/28/1/17/1239697/17.pdf by guest on 16 April 2021

The dye does not escape because Y is not very permeable to H^+ and in consequence the vacuolar sap is more acid than the protoplasm in W. Irwin[20] has shown that it is chiefly the difference between internal and external pH which determines the taking up of basic dyes, such as neutral red.

It is also of interest to note that Y differs in some respects from the corresponding non-aqueous layer X at the outer surface of the protoplasm.[21]

Furthermore, according to the results of Blinks[22] and of Curtis and Cole,[23] either X or Y, or both, must have a high electrical resistance and capacity.

Evidently, these non-aqueous surface layers present a variety of interesting problems to the physicist as well as to the chemist.

Methods

The investigation was made on *Nitella flexilis* Ag. The cells were freed from neighboring cells and kept at 15°C. ± 1°C. in Solution A.[24] They were then placed (at about 25°C.) in 0.01 to 0.05 per cent neutral red or brilliant cresyl blue (National Aniline and Chemical Co.) dissolved in distilled water. In some cases they were left in 10 ml. of the stain for a few minutes, then rinsed in distilled water, and observed. In other cases they were placed on a slide in the stain and covered with a cover glass, 1 × 2.5 inches, with the edges sealed with vaseline. In this condition protoplasmic movement may continue for 2 or 3 days.

Generally speaking the youngest cells at the growing tip of the plant stain more deeply. This may indicate that their vacuolar sap is more acid since Irwin has shown[20] that staining of the vacuolar sap with brilliant cresyl blue and presumably with other basic dyes, such as neutral red, depends largely on this factor.

SUMMARY

The vacuolar surface of *Nitella* is covered with a non-aqueous film too thin to be visible as a separate membrane. The motion of the protoplasm may subject this film to a good deal of mechanical disturbance.

[20] Irwin, M., *J. Gen. Physiol.*, 1925–28, **8,** 147; *Proc. Soc. Exp. Biol. and Med.*, 1928–29, **26,** 125; 1931–32, **29,** 995.

[21] Osterhout, W. J. V., and Harris, E. S., *J. Gen. Physiol.*, 1927–28, **11,** 391. Regarding the properties of X see Osterhout, W. J. V., *Physiol. Rev.*, 1936, **16,** 216; *Tr. Faraday Soc.*, 1937, **33,** 997; *J. Gen. Physiol.*, 1939–40, **23,** 171, 429; 1943–44, **27,** 91. Osterhout, W. J. V., and Hill, S. E., Some ways to control bioelectrical behavior, in Cold Spring Harbor symposia on quantitative biology, Cold Spring Harbor, Long Island Biological Association, 1936, **4,** 43.

[22] Blinks, L. R., *J. Gen. Physiol.*, 1929–30, **13,** 495; 1936–37, **20,** 229.

[23] Curtis, H. J., and Cole, K. S., *J. Gen. Physiol.*, 1937–38, **21,** 189. Cole, K. S., and Curtis, H. J., *J. Gen. Physiol.*, 1938–39, **22,** 37.

[24] *Cf.* Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1933–34, **17,** 87.

Downloaded from http://rupress.org/jgp/article-pdf/28/1/17/1239697/17.pdf by guest on 16 April 2021

Apparently this does not rupture the film for no dye escapes into the protoplasm as the result of such disturbance when the vacuolar sap is deeply stained with neutral red or brilliant cresyl blue.

When the deeply stained central vacuole breaks up into several smaller vacuoles, leaving the outer protoplasmic surface in its normal position, there is no evidence of the escape of dye into the protoplasm through the film surrounding the vacuole.

Downloaded from http://rupress.org/jgp/article-pdf/28/1/17/1239697/17.pdf by guest on 16 April 2021