THE RELATION OF THE STABILITY OF PROTOPLASMIC FILMS IN NOCTILUCA TO THE DURATION AND INTENSITY OF AN APPLIED ELECTRIC POTENTIAL.

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PLATE 3.

Present knowledge concerning the changes which occur in a living plant or animal cell when undergoing stimulation is based upon a complex combination of fact and theory. The dominant idea is that some change occurs in the constitution of the protoplasmic film separating the cell from its external medium. One of the features of this change is generally regarded as a reversible increase in permeability. The thickness of the protoplasmic membrane or limiting layer which is involved is not known, and little attention has been given to the important question as to what extent internal phase boundaries of the cell may be involved in such changes of permeability.

In 1908 Nernst[1] first formulated an approximate but clear statement of the relation of duration and intensity of a constant electric current when employed as a stimulus, in the form of the simplified equation $i \sqrt{t} =$ constant, in which i is the intensity of current and t its duration. Attempts at a greater accuracy of expression of this relationship of time and intensity have been made by Lapicque[2] and especially by Hill,[3] and Lucas.[4] However, from more recent results obtained by Jinnaka and Azuma,[5] with a refined experimental

[1] Nernst, W., *Arch. ges. Physiol.*, 1908, cxxii, 275.

[2] Lapicque, L., *J. physiol. et path. gén.*, 1909, xi, 1009.

[3] Hill, A. V., *J. Physiol.*, 1910, xl, 190.

[4] Lucas, K., *J. Physiol.*, 1910, xl, 225.

[5] Jinnaka, S., and Azuma, R., *Proc. Roy. Soc. London, Series B*, 1922-23, xciv, 49, 71.

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procedure, it appears that neither Nernst's nor Hill's form of the excitation equation expresses the relations sufficiently accurately for amphibian striated muscle.

In view of these facts it would obviously be of interest to know: (1) what happens in a *visible* protoplasmic film when an electric potential is applied to it; and (2) what is the relation of the duration and intensity of the applied electric potential when this visible protoplasmic film is caused to change in a definite observable manner. The observations which we have made upon *Noctiluca*, and report below, bear upon these questions.

Microscopic Structure of the Cytoplasm of Noctiluca.

The cytoplasm consists of an easily observed emulsion structure in which the large discontinuous phase units, which we shall call phase No. 1, consist of a water solution containing among other things a relatively high concentration of acid, first observed by E. N. Harvey[6] and by Lyon.[7] The average diameter of these vacuoles at the periphery of the cell is about 3 to 6 microns, while the larger, inner vacuoles (clearly shown in the photographs of Plate 3) vary roughly between 10 and 50 microns. The second type of readily distinguishable internal phase consists of faintly pink colored granules, the variable size of which appears to depend in part on the state of nutrition of the cell. Most of these granules occur more centrally about the nucleus while the smaller units of the same kind (1 micron ±) occur scattered in all of the peripheral parts of the continuous phase which forms what we shall call the visible protoplasmic films. The granules in these films move from place to place, always remaining in the films. The whole cell is surrounded by a passive, thin, flexible supporting pellicle secreted by the underlying peripheral protoplasmic film which appears to have a thickness of 1 micron or less and is in continuity with the plasma films of the interior. This peripheral plasma film must, therefore, clearly represent the visible part of the mechanism at the surface of the cell which was shown by E. N. Harvey[6] and Lyon[7] to possess certain peculiar selective

[6] Harvey, E. N., *Carnegie Institution of Washington, Pub. No. 251,* 1917, 235.
[7] Lyon, E. P., *Proc. Soc. Exp. Biol. and Med.,* 1922–23, xx, 284.

permeability properties, referred to in what follows. It is the behavior of this system of visible peripheral protoplasmic films, under an applied electric potential,which we wish to consider in this paper. The structure described is partly shown in the photographs in Plate 3.

Evidence for Permeability Increase and Coalescence in the Cytoplasmic Emulsion.

If phenol red is added to a suspension of *Noctiluca* cells in sea water no observable change in color of the indicator occurs within at least 20 minutes. However, if the tube is given a strong mechanical shock, a striking color change occurs, indicating loss of acid by some or all of the cells. If this cell suspension is examined under the microscope, some or all of the cells, depending upon the intensity of the shock, are found to have their peripheral cytoplasmic film partly or wholly pulled away from the outermost supporting pellicle. Similarly, as Lyon[7] showed, a brief electric current of sufficient intensity causes the cytoplasm to shrink away from the surrounding pellicle at anode and at cathode, with a simultaneous loss of acid at these points. During these changes the cytoplasm decreases in volume. Careful microscopic examination of this process has shown that this shrinkage occurs as a result of the breakdown of the protoplasmic film or plasma membrane at the periphery of the cytoplasmic emulsion, thus liberating the contents of the peripheral vacuoles of phase No. 1 into the space which forms immediately under the pellicle when shrinkage occurs. The free acid in this space (Figs. 2, 3, and 4, Plate 3) then readily diffuses out through the pellicle, as shown by the indicator. If the electric current is continued this shrinkage progresses until complete or nearly complete coalescence in respect to phase No. 1 has occurred. This picture is quite similar to the precipitation of a coarse viscous emulsion when the process of coalescence occurs at the surface of the emulsion.[8] Now an interesting fact is that the specific gravity of a normal *Noctiluca* cell is less than that of sea water, and therefore it floats at the surface; while the same cell after coalescence of its cytoplasm with respect to phase

[8] Clayton, W., The theory of emulsions and emulsification, Philadelphia, 1923.

No. 1 is denser than sea water and sinks to the bottom of the vessel. From this it is clear that the density of the solution of phase No. 1 is less than that of sea water and yet its effective osmotic concentration must be equal to or greater than that of sea water, providing that the peripheral protoplasmic film separating the solution of phase No. 1 from the sea water does not exert a negative "osmotic force." Facts bearing on this question will be reported in a later paper. The polar coalescence in *Noctiluca* is in certain respects similar to the polar effects of the electric current on *Actinosphærium* and certain other protozoa described long ago by Verworn.[9]

Relation of Duration and Intensity of an Applied Constant Electric Potential to the Process of Anodal Coalescence.

The simplest condition and the only one which will be considered here is that produced when the applied potential is maintained constant throughout its duration (Nernst,[1] Hill,[3] and Lucas[4]). Two experiments performed upon different lots of cells gave practically identical results and are therefore summarized in Table I. Each test was made by noting the duration from the instant when the current was turned on, to the time when one-half of the cells in the field showed beginning of coalescence toward the anode.[10] The duration test for each current intensity was repeated from five to eight times, employing a total of 50 to 90 cells. The values for t given in Table I are therefore averages. Duplicate tests at the same intensity showed a remarkable agreement in the duration of the interval before coales-

[9] Verworn, M., *Arch. ges. Physiol.*, 1895, lxii, 415.

[10] In order to control accurately the density of the electric current flowing through the cells an accurately calibrated wedge-shaped glass chamber was used; the dimensions of which were 14 mm. long, 1.04 mm. deep, 2.2 mm. and 6.25 mm. wide at the two ends respectively. It was provided with a removable glass cover 14 mm. × 10 mm. bearing an accurately calibrated and numbered scale etched on the glass. The current was read to a hundredth of a milliampere on a sensitive ammeter. Products of electrolysis from the electrodes were excluded in a similar manner to that described elsewhere (Lund, E. J., *J. Exp. Zool.*, 1924, xxxix, 357; *Bot. Gaz.*, 1923, lxxvi, 288). Using such an arrangement it is possible to determine quite accurately the threshold potential across any one cell for coalescence. The same section of the wedge was used for all tests reported in this paper.

cence occurred. However, at the extreme lowest and highest intensities of current it was difficult to determine the exact moment when anodal coalescence began in half the cells and, therefore, the first and last values in the table are of somewhat doubtful accuracy. The values for i and t are plotted in the curve in Fig. 1.

The constant k in Nernst's equation is fairly good, in fact better than that which can be obtained from the accurate determinations of Jinnaka and Azuma[5] on frog muscle. It should also be noted that the duration in these experiments is in seconds, while that on muscle by other workers is in 0.001 second. The current density is also apparently much greater than that used by Jinnaka and

TABLE I.

Current intensity. i	Duration. t	$i\sqrt{t} = k$
ma.	*sec.*	
4.20	166 (?)	54.0 (?)
4.90	64	39.2
5.62	38.5	34.8
7.12	30.0	38.9
8.54	20.4	37.5
10.00	16.4	40.4
11.46	13.5	42.0
12.96	10.7	42.3
14.60	9.1	43.9
15.90	9.2	48.1
16.55	7.3	44.9
20.60	5.5± (?)	48.2 (?)

Azuma.[5] The remarkable fact which should be noted is that here we have a process which is represented by a loss of stability of the plasma films, a deemulsification, exhibiting the same relationships to duration and intensity of an electric current as the typical process of stimulation of the striated muscle cell. In fact the agreement in this case with Nernst's physical theory of stimulation appears to be closer than that for the excitation of muscle. The reason for this closer agreement may be due to the fact that in *Noctiluca* the protoplasmic films which Nernst assumed to exist in the muscle cell, are actually realized. Another reason may be that the distances between the visible protoplasmic films in *Noctiluca* are much greater

than those which could reasonably be assumed to exist between intracellular membranes postulated for the muscle cell, and therefore the factor of distance between the membranes, which Hill showed to
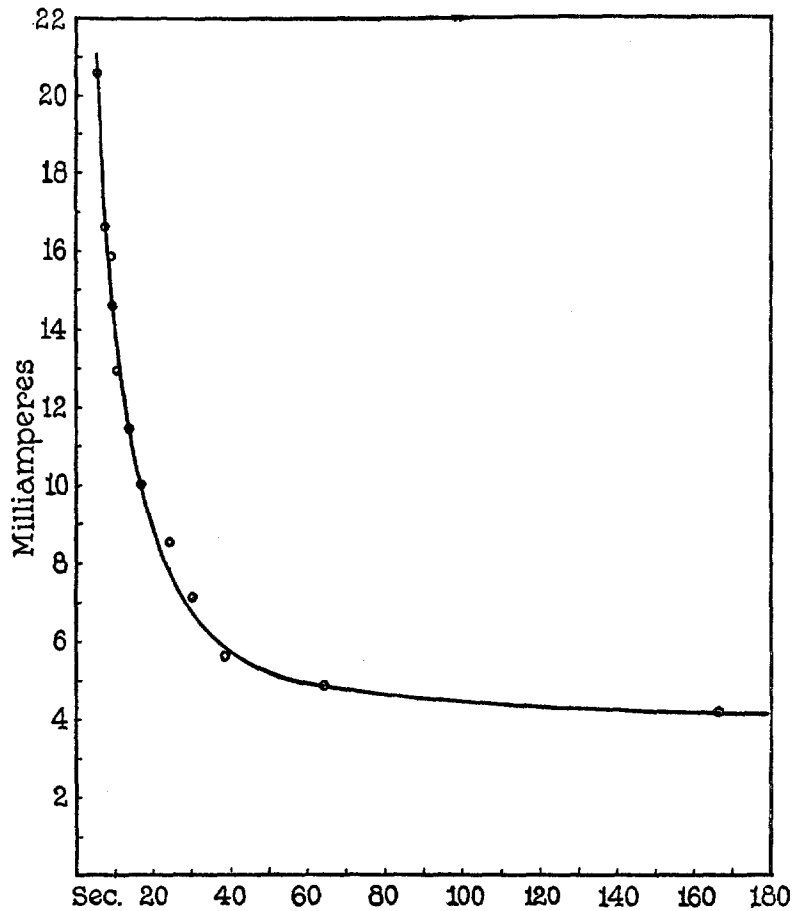

Fig. 1. Curve showing the relation of time and intensity of the constant electric current for coalescence.

be of theoretical importance, is probably less significant in the above experiments on *Noctiluca*.

In typical processes of excitation a living cell usually undergoes a process of recovery. For the present it remains an open question

as to what extent the coalesced cells possess a capacity to recover and therefore whether or not we may consider the changes produced in the plasma films of *Noctiluca* a typical process of excitation. In the writer's opinion the answer to this question at present would therefore merely be a matter of definition. However, the fact which must be emphasized is the striking correspondence between the time-intensity relations in the process of coalescence in *Noctiluca* and in the excitation of the muscle cell.

Another fact which should be noted is that complete coalescence in the plasma films enclosing phase No. 1 does not result in any plainly visible coalescence in the plasma films which surround phase No. 2 (pink granules). Furthermore, E. N. Harvey[6] has shown that *Noctiluca*, after coalescence, continues to respond typically by a flash of light to a mechanical or electrical stimulus. From these facts the significant conclusion would appear to follow that in the process of coalescence described above we have an illustration of *selective coalescence* involving primarily only one visible internal phase (*cf.* below).

Difference in Threshold Intensities at Cathode and Anode.

As far as could be observed the process of coalescence, at the same current intensity, always began earlier at the anode than at the cathode side of the cell, irrespective of the orientation of the cell. This is well shown in Figs. 2, 3, and 4, Plate 3. The visible changes in the plasma films at anode and at cathode do not appear to be different. We may therefore conclude that the threshold differs at anode and cathode because of the difference in the direction of the current through the membrane. Assuming that the current density is the same at the cathode and anode sides of the cell we would be forced to the conclusion that the peripheral plasma membrane is *asymmetric* with respect to those of its properties which are involved in determining the stability of the film when an electric potential is applied to it. This asymmetry is, of course, to be expected since the constitution of the phases (sea water and internal phase No. 1) are demonstrably different with respect to the pH, density, and perhaps other unknown factors such as inherent electric potential.

This difference in threshold stability of the same membrane depending merely upon the direction of the applied current through the membrane at anode and cathode is obviously of interest in connection with the conditions of unidirectional excitation at the nerve synapse.[11]

In order to bring out more clearly the differences between anodal and cathodal coalescence in the same lot of *Noctiluca* cells, data from one series of tests are given in Table II. Tests were made at the current intensities given in the first column of the table. In all the tests the current was passed through the chamber for 1 minute, at the end of which the number of cells showing coalescence only at

TABLE II.

Current intensity. i	Total cells tested.	Cells showing anode effect only.	Cells showing anode and cathode effect.	Cells affected.	Cells affected at both anode and cathode.
ma.				*per cent*	*per cent*
20.04	59	0	59	100	100
18.64	43	0	43	100	100
17.42	56	0	56	100	100
15.90	68	10	58	100	85.2
14.42	85	17	64	95.2	75.2
12.96	92	32	53	92.4	57.6
10.00	94	43	40	88.2	42.5
8.54	93	49	32	87.1	34.4
7.12	83	52	21	87.9	25.3
5.62	64	41	14	85.9	21.8
4.90	64	29	3	50.0	4.6
4.20	70	4	0	5.7	0

the anode, Column 3, and at both anode and cathode, Column 4, were counted. The total number of cells under observation in each test is given in Column 2. From these figures the per cent of the total number of cells showing anodal and cathodal coalescence were obtained, Columns 5 and 6. These percentages are plotted with respect to the corresponding current intensities as graphs in Fig. 2. The curves bring out some interesting facts. (1) The threshold

[11] For a consideration of the relations which exist between direction of an applied P.D. and the direction of an inherent constant P.D. in an epithelial membrane, the reader is referred to the paper by E. J. Lund, *J. Exp. Zool.*, 1925, xli, 155.

current intensity—the time being constant—is higher for cathodal than for anodal coalescence. (2) The total range of variation of threshold intensities for anodal and cathodal coalescence in the cell population is approximately the same, extending from about 4 to
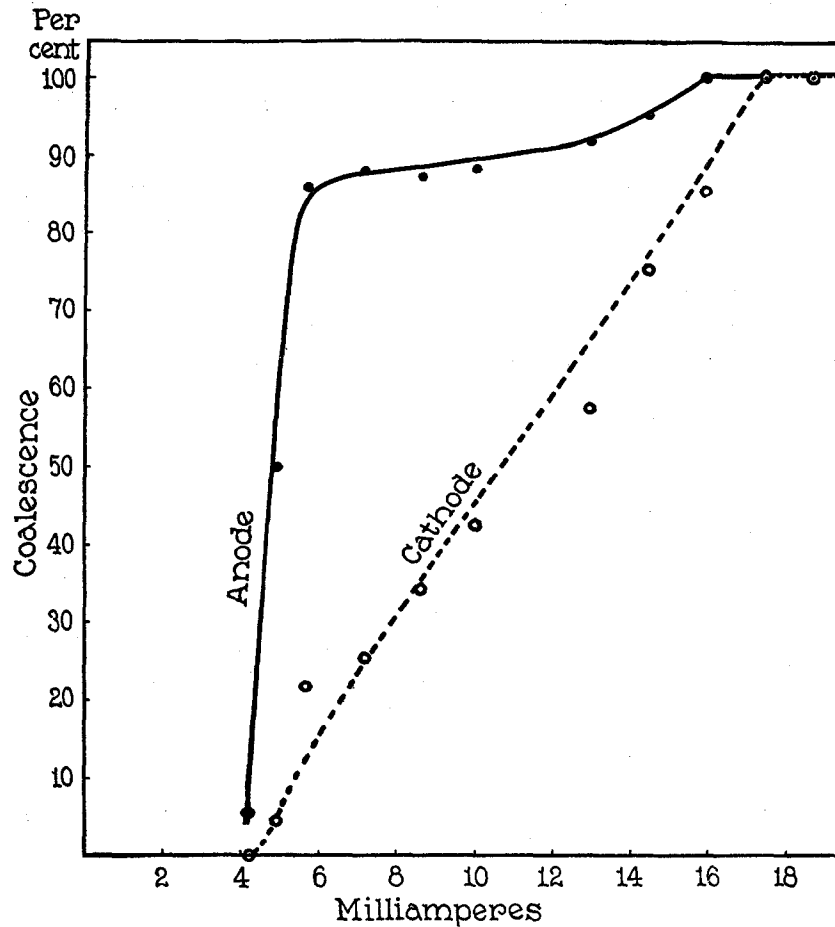

FIG. 2. For explanation see text.

16 milliamperes. This shows very clearly that the stability of the corresponding films in different cells differs greatly. (3) The forms of the curves differ strikingly from one another. The curve for anodal coalescence shows that in a certain small percentage—10 to

15 per cent—of cells, the plasma films are relatively stable compared to those of the remaining 85 to 90 per cent. This difference is in all probability due to a difference in the age of the cell, because it was very clearly evident during the tests that small young cells had a greater stability (lower susceptibility) than the larger older ones. This difference is illustrated in Figs. 2 and 4, Plate 3.

Evidence for the Existence of Distinct Receptor Mechanisms in the Same Cell.

At present it would appear doubtful in view of the recent results of Jinnaka and Azuma[5] whether more than one receptor mechanism or "substance" (cf. Lucas[4]) has been demonstrated in the frog muscle cell. Our observations on Noctiluca show that the threshold intensity of current for control of the tentacle is much lower than that required for anodal coalescence. The mechanism for light production in the same cell would also appear to differ in this respect from that of anodal coalescence (cf. E. N. Harvey[6]).

A striking case of the occurrence of at least two distinct response mechanisms in the same cell with distinctly different intensity thresholds for excitation by the electric current are the cilia and myonemes of Spirostomum teres. Doubtless many other similar instances could be found. The facts which have been demonstrated in the present paper may have a direct bearing upon the problems relating to the existence of such differences in the intensity thresholds of stimulation for different receptor-affector mechanisms in the same cell; because it appears quite clear that differentiation within the cell is necessarily associated with diversity in constitution of plasma films or phase boundaries which, as shown in this paper, may have different intensity thresholds for excitation.[12]

SUMMARY.

1. The experiments demonstrate that when a constant electric potential of sufficient intensity is applied to Noctiluca, the protoplasmic films which represent a part of the visible continuous phase of the cytoplasm and plasma membrane at the surface of the cell,

[12] Lillie, R. S., Protoplasmic action and nervous action, Chicago, 1923.

become unstable and break down, thus releasing the acid contents of one of the internal discontinuous phases present in the cytoplasm of *Noctiluca*. This process which occurs first at anode then at the cathode side of the cell, appears to be a selective deemulsification or coalescence similar to that at the surface of an emulsion having a viscous continuous phase.

2. The experiments demonstrate that Nernst's equation $i \sqrt{t} = k$ which expresses approximately the relation of duration and intensity of a constant electric current to threshold stimulation of striated muscle, applies equally well to the process of anodal coalescence in *Noctiluca*.

3. Anodal and cathodal coalescence have different thresholds, due to the fact that the semipermeable plasma film at the surface of the cell is asymmetric with respect to the direction of the applied current. Attention is called to the possible relation between this phenomenon and the conditions occurring at the synapse between neurons.

4. The stability of the protoplasmic films in relation to the applied electric potential is greater in young cells than in old cells, or in other words the threshold intensity of the stimulus is higher for young than for old cells.

5. Attention is called to the occurrence in the same cell of different receptor-affector mechanisms having a corresponding difference in intensity threshold when an electric current is acting as a stimulus.

EXPLANATION OF PLATE 3.

Fig. 1. Normal unstimulated cells of *Noctiluca*.

Figs. 2 and 3. Two stages in the process of anodal coalescence.

Fig. 4. Coalescence of the films toward the cathode begins subsequently to the coalescence of the films toward the anode. Note the absence of effect in the small cells in Figs. 2. and 4.

PLATE 3.
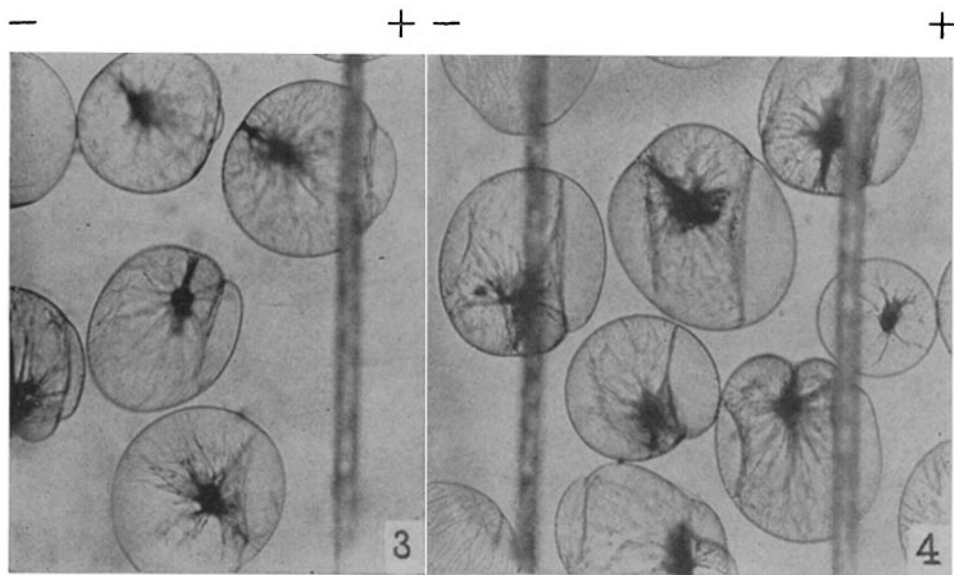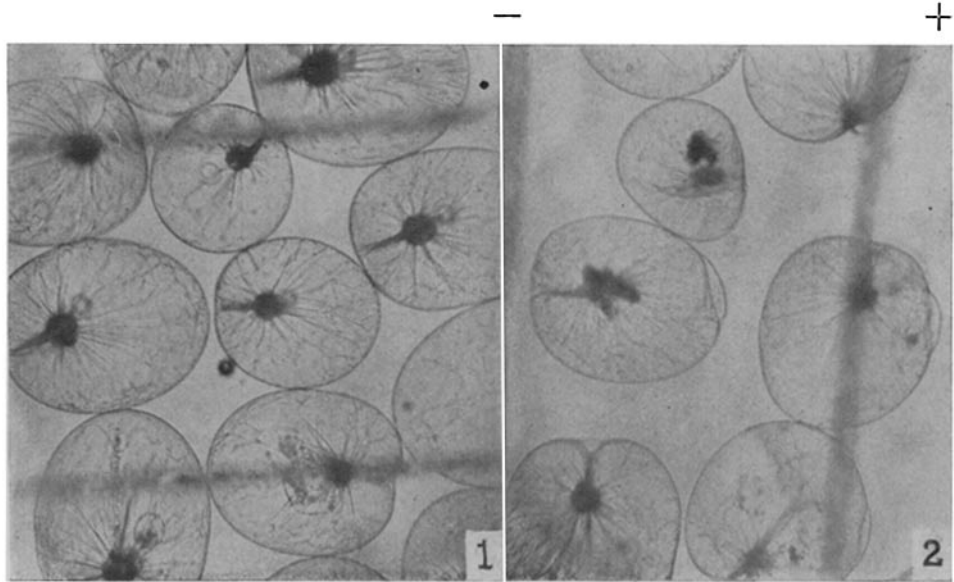
(Lund and Logan: Protoplasmic films in *Noctiluca*.)