

A C T A A D R I A T I C A
INSTITUTI BIOLOGICO-OCEANOGRAPHICI
SPLIT (JUGOSLAVIJA)

No. 4

STIMULATION PLASMOLYSIS ON MARINE ALGAE

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SPLIT 1934
BIOLOŠKO-OKEANOGRAFSKI INSTITUT

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The contraction of the protoplast and its consequent separation from the cell wall is the primary meaning of the term plasmolysis. This phenomenon can be observed to occur under different conditions and is brought about by different causes:

1. Plasmolysis, osmotic plasmolysis, »Reaktionsplasmolyse« (Schütt 44, p. 110). »This is a well-known phenomenon of contraction of the protoplast from the cell wall when the cell is immersed in a solution of higher osmotic concentration than the cell sap.« (Stiles 47, p. 65). A false osmotic plasmolysis which occurs after the death of the cell is described by Schneider (43).
2. »Schrumpfung. Die Plasmolyse ist eine Reaktionswirkung der lebenden Zelle, davon principiell zu unterscheiden ist die Schrumpfung, die der todte Zellkörper oder einzelne Theile desselben als Reaktionswirkung sehr leicht erleiden. Besonders leicht schrumpft der Kern.« (Schütt 44, p. 111.) »Gerinnung des protoplasmatischen Wandbelags — alle Schädlichkeiten« is described already by Hofmeister (12, p. 10). Many unfavourable changes are followed by pseudoplasmolysis (= false plasmolysis).
3. »Werden lebenskräftige Zellen gequetscht, so sieht man hernach häufig den Schlauch von der Membran zurückgezogen, ganz oder stellenweise freilegen. Ich machte diese Beobachtung vorzüglich an Süßwasseralgen« (Nägeli 31, p. 2. Cfr. p. 2., 3.).
4. »Endlich löst sich der Schlauch zuweilen in Folge des normalen Lebensprozesses von der Membran los. Dies

geschieht wohl nur dann, wenn er zur Schwärmzelle wird und heraustritt (Taf. I. 6. 13), oder wenn er eine ruhende Spore bildet (*Oedogonium*, Ausnahmefälle bei *Spirogyra*, *Zygnema* etc.)«. (Nägeli 31, p. 2., »Entwicklungs-plasmolyse«, Schütt 43, p. 109, Pfeffer 35, p. 450.)

5. Since the cohesion between the water in the hydrated cell walls and the water in the protoplast can be broken only by a force greater than 250 atmospheres, the cell wall normally follows the course of the contraction of the protoplast during the dessication of cells. Only in rare cases is a state of plasmolysis to be observed in dried up cells (Nägeli 31).
6. Stimulation-plasmolysis, »Reizplasmolyse«. »In der äusseren Erscheinung der normalen Plasmolyse durchaus ähnlich, unterscheidet sich von dieser scharf dadurch, dass nicht nach physikalischen Gesetzen durch Lösungen, die unter höherem osmotischen Druck als der Zellsaft stehen, das Abheben des plasmatischen Wandbeleges erfolgt, sondern vielmehr durch alle möglichen Reize ausgelöst werden kann.« (Benecke 1, p. 554.)

The older books can only be obtained with great difficulty and therefore extensive quotations from them are made here.

Cells can be stimulated to »plasmolyse« mechanically as by pressure, or by chemical action or light. The first description of this »stimulus plasmolysis« is given by Nägeli (31) who on pages 11 and 12 gives a detailed description of chloroplast contraction in *Spirogyra*. This phenomenon has recently been carefully studied by Gicklhorn (8) and also by Sakamura (40).

Spirogyra orthospira shows stimulus plasmolysis in the following way: »Durch den Druck werden zuerst die diosmotischen Verhältnisse der Zellen verändert, wie man das deutlich aus der allmählichen Abnahme der Turgeszenz sieht (ich verweise noch auf den folgenden Artikel über die Diosmose der Pflanzenzelle). Die Zellflüssigkeit wird durch den vermehrten Austausch dem Wasser ähnlicher. Die Wirkung des Wassers auf das Protoplasma besteht immer darin, dass das letztere das Bestreben zeigt, eine der Kugelform sich nähernde Gestalt anzunehmen. Daher sieht man die Bänder später sich verkürzen und verdicken, und zuletzt in Kugeln sich verwandeln. Das eindringende Wasser wird zuerst auf die

Plasmafäden einwirken und dieselben verkürzen. Da sie mit dem einem Ende an den Kern, mit dem andern an die Bänder angeheftet sind, so bewirken sie die Lostrennung der letzteren, welche ohnehin den Schlauch nur mit den Rändern berühren und ebenfalls schon die Einwirkung des Wassers erfahren haben. Die Einbiegung der Bänder geht so lange fort, bis dieselben den Kern beinahe oder ganz berühren; und sie wirkt ihrerseits natürlich wieder wesentlich zur vollständigen Lostrennung der Bänder selbst mit. Wenn die Zellflüssigkeit durch die Diosmose dem umgebenden Wasser gleich geworden ist und der Grund für die Ausspannung des Primordialschlauches wegfällt, so erfährt auch dieser, den verdichtenden Einfluss des Wassers, und trennt sich von der Membran los. Es geschieht dies aber langsam, weil er den Druck der eingeschlossenen Flüssigkeit zu überwinden hat, d. h. weil eine entsprechende Menge dieser letzteren heraustreten muss«. (Nägeli 31, p. 13.)

»Verletzung oder Quetschung lebender Zellen bewirkt ebenfalls eine Zusammenziehung des Inhalts. Es genügt, eine jüngere Zelle von *Nitella* mit der Spitze einer stumpfen Nadel leicht zu drücken, so leicht, dass die Zellhaut keine Knickung und keinen bleibenden Eindruck erhält, um sofortigen und raschen Rückzug des protoplasmatischen Wandbelegs von der Zellhaut zu veranlassen. Knickung der Gliederzellen von Fäden von grösseren *Spirogyren* und *Oedogonien* hat die nämliche Wirkung«. Hofmeister, 12, p. 10.) On page 303. Hofmeister (12) describes only the behaviour of cut cells of *Nitella* and not stimulus plasmolysis and as is often the case the citations quoted are not correct.

The reversible decrease of turgescence after a mechanical stimulus is described by Janse for *Caulerpa*. (Janse 16, p. 215).

Schütt (44, p. 110) mentions stimulation plasmolysis in *Exuviaella marina* Cienk.: »Reizplasmolyse. Dass überhaupt Plasmolyse, wenn auch mit anderen Mitteln, erreicht werden kann, kann ich jedoch bestätigen, denn abgesehen von den entwicklungsgeschichtlichen Veränderungen bei der Sporenbildung habe ich mehrfach Plasmolyse als Folge eines noch unaufgeklärten Reizzustandes beobachtet. Tafel I, Fig. 1, 5 zeigt als Beispiel eine Zelle in freiwilliger Plasmolyse, unmittelbar nachdem sie auf den Objektträger gebracht war. Durch Zusatz von Süßwasser wurde die Plasmolyse sofort aufgehoben, der Zellinhalt füllte den Panzer wieder vollständig aus, starb aber dann sofort ab. Die mechanische Erklärung dieses Vorganges steht noch aus. Konzentrationssteigerung

des umgebenden Mediums, die sie hätte veranlassen können, war ausgeschlossen, sie ist also wohl als Reizwirkung aufzufassen. Das Eintreten von Plasmolyse in Folge noch unbekannter Reizwirkung steht jedoch nicht vereinzelt da, auch bei andern einzelligen Algen habe ich sie beobachtet, und zwar so häufig, dass mir die Planktonpflanzen ganz allgemein eine gewisse Neigung zur Reizplasmolyse zu besitzen scheinen. Sehr schön habe ich die Reizplasmolyse bei Diatomeen beobachten können. Auch bei *Dinodendron* sah ich dieselbe.« (Schütt 44, p. III.)

Karsten describes a plasmolysis which is however perhaps not comparable with the stimulation plasmolysis we are considering: »Ob der überaus geringe Ueberschuss über die Concentration des Kulturwassers von 0.22 aeq., der bei *Nitzschia longissima* zur Reaktion genügt, den Schluss auf Plasmolyse zulässt, möchte ich unentschieden lassen. Ausser den Angaben Müllers über die grosse Reizempfindlichkeit der Diatomeen kommen hier vielleicht Beobachtungen in Betracht, wie sie unter anderen früher von Hofmeister gemacht sind... Noch eine weitere Beobachtung, die ich hier gemacht habe, ist dabei zu erwähnen. Wenn man nämlich von den zur Beobachtung fertigen *Nitzschia longissima* Individuen durch Fliesspapierstreifen Wasser der ursprünglichen Concentration absaugen liess, so war nach einiger Zeit der Punkt erreicht, dass kein überschüssiges Wasser mehr auf dem Objektträger sich befand. Wurde der Fliesspapierstreifen aber noch nicht gleich entfernt, so musste durch weiteres Wasserentziehen das Deckglässchen fester auf den Objektträger gedrückt werden, also auf darunter liegende Zellen einen gelinden Druck ausüben. Und auch in diesem Falle war ein allgemeines Zurücktreten des Plasma's von der Wand in den *Nitzschia*-Zellen wahrzunehmen. Sowie ein geringer Wasserzusatz dieses festere Andrücken aufhob, war nichts mehr von dem abgehobenem Plasma zu sehen, es hatte sich momentan wieder der Wand angelegt.« (Karsten 20, p. 154, [138].)

The most detailed description of »Reizplasmolyse« or stimulationplasmolysis is given by Benecke (1) on pages 554—555. It is, I believe, the only case on record of full recovery of protoplasm induced to undergo stimulation plasmolysis by the action of light.

A. W. Greeley exposed »*Spirogyra* filaments to lowering of the temperature (from 20° C to 1 or 2°). The filaments were kept at this temperature for about three hours, and then removed

to that of the room. Examination showed that plasmolysis in these filaments had been as perfect as in the others. Regular oval spore like bodies were formed in the centre of each cell (see Fig. 7). Upon removal to the room temperature, the cell gradually took up water, the chromatophores expanded, and the cells resumed their normal appearance... Summary: 4. In *Spirogyra*, a typical plasmolysis can be produced by a reduction of the temperature. 5. This fact makes it probable that a reduction of the temperature and a loss of water have similar effects because the cell loses water when the temperature is lowered, as well as when the concentration of the surrounding medium is raised.» (Greeley 9, p. 127, 128.)

Pfeffer mentions the observations by Schütt, Benecke, Nägeli, Hofmeister and states: »Ob in diesen Fällen Reizwirkungen oder mechanische Verletzungen vorlagen, muss durch fernere Untersuchungen entschieden werden. Ebenso bedürfen die Beobachtungen Schütt's und Benecke's einer kritischen Nachprüfung.« (Pfeffer 35, p. 450.)

The observation made by Prowazek may possibly refer to a stimulation plasmolysis in *Bryopsis*: »Auf stärkere Reize, wie Erschütterungen oder vielfachen, ausgebreiteten Druck zerfällt manchmal das Cytoplasma in den *Bryopsis*-Zellfäden in mehrere Tropfen, in denen oft grosse Vakuolen auftreten, gleichzeitig bildet sich an der Peripherie eine Niederschlagmembran aus, von der sich der Protoplast, abermals unter Fadenbildungen und Fadenausspinnungen zurückziehen kann, um wieder eine neue derartige Membran zu bilden.« (Prowazek 38, p. 740.)

A contraction of the protoplast has often been described in cut cells of *Siphonales*, and in some cases the process resembles plasmolysis contraction. For example in *Vaucheria*, »das unzerstörte Protoplasma zieht sich augenblicklich zusammen« (Hanstein 11). The contraction, recovery, plasma streaming and movements of chloroplasts in cut *Vaucheria*, *Bryopsis* etc. thalli is described by Hanstein (11, p. 48; with the aid of coloured figures, Klemm 23, Winkler 49, Prowazek 37, p. 388, Prowazek 38, p. 739, Oltmanns 32, III, p. 78. n., Jost 19, Küster 26). The literature about woundreactions and regeneration in *Siphonales* is reviewed also by Haberlandt (10). The »Fensterbildung« (Jost 19, Höfler 14), is characteristic of contractions induced both by osmotic and irritant phenomena.

O. Richter (39) was unable to find any plasmolysis in the cells of *Nitzschia putrida* Benecke induced by light or mechanical or chemical stimulation. He did not state whether this difference from the statements of Karsten and Benecke on *Nitzschia longissima* and *N. leucosigma* was on account of the difference in the species or some special physiological conditions.

Janse (17, 18) observed that after mechanical stimulus (rubbing) fluid (cellsap) enters into the intercellular spaces of young roots. The movements of irritable organs in connection with cell contractions were studied by Bünnig (5).

Many experimental results obtained in recent years can be explained as wound plasmolysis. (Cfr. § 3, p. 1. and *Siphonales* p. 5). »Scheinplasmolyse etwa Wundplasmolyse« is described by Höfler (13, p. 158—159, Sep. 60, 61). E. Kemmer describes a »plasmolysis« in the isolated epidermis-tissue of *Rhoeo discolor*: »In pH 5.0 — 4.5 zeigte nach 16 — 20 Stunden die Mehrzahl der Zellen deutliche Grenzplasmolyse, die in manchen Zellen bis zu vollkommener allseitiger Ablösung des Protoplasten von der Zellwand führte. Vielleicht lag eine durch die Säure verursachte »Reizplasmolyse« vor.« Kemmer mentions no recovery and states: »Mit steigender Säure bzw. Alkaleszenz nahm die Lebensdauer rasch ab, z. B. bei pH 4.6 knapp 24 Stunden.« (Kemmer 22, p. 20.) Therefore it seems probable that there was pseudoplasmolysis rather than stimulation plasmolysis. Different cases of protoplasmic contraction after wounding are described by Küster and »systolische und diastolische Veränderungen des Vakuole« by R. Keil (21). »Neuerdings hat Küster (1929) mit Beobachtungen an höheren Pflanzen neue Beiträge zu derselben Frage gebracht. Nach Verwundung mancher Pflanzengewebe tritt spontan Plasmolyse ein (Epidermen von *Rhoeo discolor*, *Sinningia* u. a.) zumal, wenn mechanische Reizung auf die Zellen gewirkt hat (*Rhoeo*). Das Perigon von *Scilla sibirica* zeigt in allen Zellen der abgezogenen Epidermen kräftige Plasmolysen.« (Küster 26, p. 28, Küster 25, p. 165—166.)

Plasmolysis of the cells of *Polygonatum* immersed in diluted cell sap is described by Weber. »Der ganze Erscheinungskomplex der Plasmolyse der Fruchtfleischzellen von *Polygonatum* scheint (also) keineswegs eine einzige, einheitliche Ursache zu haben und bedarf in jeder Hinsicht weiterer Aufklärung.« (Weber 48, p. 439.)

According E. G. Pringsheim (36) the appearance of the cells of *Micrasterias rotata* and *M. denticulata* in old cultures resembles the »Reizplasmolyse«, »wobei sich Teile des Protoplasten mit Chromatophorenstücken zusammenballen.« The first stages are reversible under good cultural conditions.

V. S. Iljin (unpublished experiments) observed very pronounced plasmolysis in cooled cells of *Tradescentia zebrina* and *Brassica oleracea capitata rubra* (red cabbage).

Striatella.

In the spring of 1931 (March and April) the very abundant and varied vegetation of marine algae on the island Rab (Jugoslavija) was investigated. In preparations of algae, especially of *Callithamnion*, from the haven (»luka«) in Rab many specimens of the diatom *Striatella* occurred. The cells were often observed to contain contracted protoplasts, resembling an early stage of plasmolysis. The protoplasts were separated from the cell walls and they contracted as they would in hypertonic solutions such as cane sugar dissolved in sea water or concentrated sea water.

In normal sea water which is of course hypotonic to the cells, true osmotic plasmolysis could not occur, and therefore the possibility that the behaviour was an »irritation« or »stimulation« plasmolysis was considered. In July 1933 in Split only occasional cells of *Striatella* were observed in the litoral formation near the Oceanographic Institute. Nevertheless the reaction was frequently seen in these cells and was very marked. Detailed investigations proved that this contraction of the protoplast and apparent plasmolysis is a response to a stimulus of mechanical or chemical nature. When pressure was applied to the coverglass with a needle the protoplast contracted and in from 5 to 30 seconds the cells showed a very considerable degree of apparent plasmolysis.

The specimen agreed very well in the structure of the valvae with the sample in Herbario Musei Nationalis Pragae (Praha)

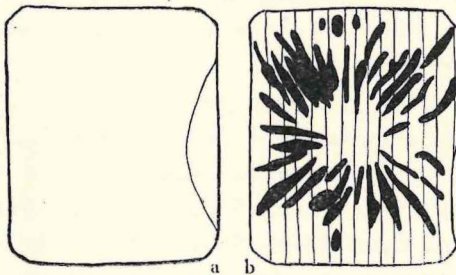


Fig. 1. *Striatella*. Recovery from stimulation plasmolysis. Pressure (stimulation) 10 h 10'. a) 10 h 15', b) 11 h 30'.

N^o 64/1: *Striatella unipunctata* Ag, Cherbourg, M. Jolis, *Striatella* Kütz, Oc. Atl. Syn. *Achnanthes unipunctatus* Grev. *Diatoma arcuatum* Hoffm., *Fragilaria unipunctata* Lyngb., *Tessella arcuata* Erhenb. A matter which is not yet decided is the number of plastids in *Striatella unipunctata* — cfr. partly the contradictory remarks and figures of Kützling (27, Taf. 18, V. 1—4., Taf. 24, VI. 3 ab), Lüders (29), Schmitz (42, p. 19, Taf. fig. 26), Schimper (41, p. 218, 219, Taf. V. Fig. 5, 6), Mitrophanow (30, p. 300), Karsten (20), Senn (45, p. 18, Anm. 1, Taf. IV. Fig. 7, 8), Senn (46), Oltmanns (32, I. p. 152, 158), Hustedt: *Striatella unipunctata* (Lyngbye) Agardh (15 p. 31—33).

All the cells which I was able to observe both in Rab and in Split contained numerous plastids and their shape and position varied according to the physiological stage of cell. The comb or star-like shape of the »unic« protoplast in *Striatella* seems to be an artefact.

As early as 1862 Lüders (29) observed the movements of the chloroplasts in the cells of *Striatella unipunctata* and *Rhipidophora* (*Licmophora*) *elongata*. Later investigations have been made by Schmitz, Schimper, Mitrophanow, Karsten and especially by Senn. Senn describes in detail the movements, grouping and different form of the chromatophores in *Striatella* but he does not mention the contraction of the protoplast. (Senn 45, 46.)

My observations both in Rab and in Split lead me to consider *Striatella* as a very suitable subject with which to study the phenomenon of stimulation

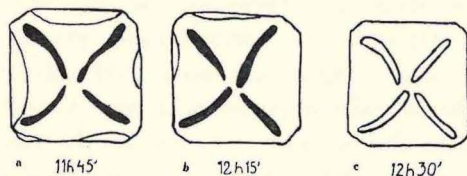


Fig. 2. *Striatella*. Recovery from stimulation plasmolysis. Pressure (stimulation) 11 h 30'. a) 11 h 45', b) 12 h 15', c) (complete recovery) 12 h 30'.

plasmolysis. The contraction was easily observed but difficulties arose when the reversibility of the contraction was questioned. Observation of single individuals through the precesses of contraction and recovery was necessary before any conclusions could be drawn. It is a difficult matter to prevent increase in the concentration of the sea water during protracted observation of such a preparation. It was found best to select individuals of the diatom growing on pieces of *Callithamnion* and

draw their form and the position so that they might be found again.

Between the observations the preparations were placed in moist chambers and the sea water was renewed beneath the coverslip from time to time by the irrigation method. By this means the two processes of contraction nad recovery could be observed successively in single individuals.

When the contraction was not pronounced complete recovery was made in 45 to 60 minutes. In protoplasts showing a greater degree of contraction the recovery or apparent deplasmolysis took longer. In cells very strongly »plasmolysed« only a partial recovery took place and was followed by a second contraction or the cell contents coagulated without recovering. The chromoplast became disk shaped or spherical and green and the plasma disintegrated.

It is a very remarkable fact that these reactions are very irregular; frequently two adjacent cells behave very differently. In the extreme case some individuals show no contraction while the protoplasts in neighbouring cells are very considerably contracted. The position of the plastids is also variable; and in some cases systrophe of plastids followed, while in others it preceded the

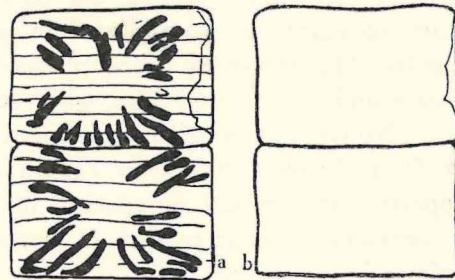


Fig. 3. Striatella. Recovery from stimulation plasmolysis. Pressure (stimulation) 11 h a) 11 h 15', b) 13 h 35'.

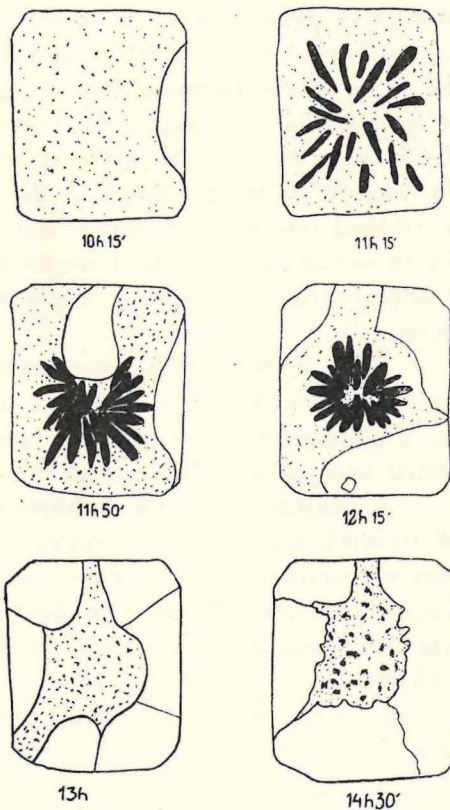


Fig. 4. Striatella. Recovery from stimulation plasmolysis. At 11 h 30' the second stimulus was given by pressing on the cover glass and after pronounced stimulation plasmolysis death occurred.

contraction. Often the plastids remained elongated and scattered in the cell. Generally speaking, the cells with apparently normal and scattered elongated plastids were in good condition and recovered rapidly. The cells with balled plastids in systrophe recovered very slowly and indeed often died while still contracted.

No contraction of the protoplast was observed when the cells of *Striatella* were shaken in a drop of water in a test tube. It appears that chemical stimulation of the cells of *Striatella* causes a contraction similar to that of mechanical stimulus; for instance, »plasmolysis« is induced after 3 seconds over a jar containing ammonia and after 3—5 seconds exposure to ether vapour.

A regular contraction of the protoplasts can be observed with ease in the process of vital staining. The contraction of the protoplast appears and increases if the vacuole first appears coloured or a littel later. If the cells are kept in the staining solution the degree of contraction of the protoplast increases and contraction of the vacuole takes place, the phenomenon of »Entmischung« of the vacuole, that is, the secretion of deeply coloured drops into the vacuole occurs and the cells die. If slightly stained cells are transferred into pure sea water, recovery may take place: at first (in 30—120 minutes) the contents re-expand and then the dye disappears from the vacuole (neutral red, brillantcresylblue, methylenblue).

When a drop of tap water was added to the preparation under a cover slip no contraction occurred, only vacuolisation of the protoplast was observed, the plastids became »balled« and united together and the cells quickly died.

A contraction of the protoplast similar to that in the cells of *Striatella* was observed to result from pressure on the coverglass over preparations of *Licmophora* sp. In these cases however the reaction was less marked. Complete recovery was never observed, when the protoplasts had regained their former volume the cells underwent vacuolisation and died.

In *Rhipidiophora* stimulation plasmolysis was observed to occur in sea water containing a trace of ferric chloride (Split).

Bryopsis and Pseudobryopsis.

When a branch of *Bryopsis* is cut off the protoplasm and plastids instantly contract to a marked degree and protoplasm streams from the wound towards the apex. The contents of the

vacuole however flow to the wound and the cell sap is exuded through the wound into the surrounding water. In a few seconds

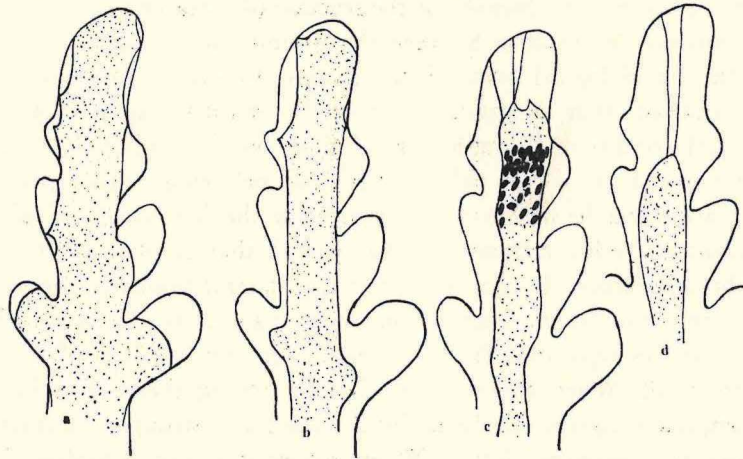


Fig. 5. *Bryopsis plumosa*. At 9 h 30' the branch was cut off. Different stages in the contraction of the protoplast. a) 9 h 32', b) 9 h 37', c) 9 h 48', d) 10 h 15'.

the apical protoplasm and plastids contract and a slow protoplasmic streaming can be observed directed from the apex to the base, but no streaming can be observed in the middle region of the branch. The appearance of the apical portion is the same as in either partial or complete plasmolysis and the protoplast may separate entirely from the cell wall as in plasmolysis or may remain attached by thick threads to the cell wall. Later on the protoplast in the sub-apical regions also contracts and separates from both apical and longitudinal cell walls. After the maximum concentration a very slow recovery (dilatation) occurs. When the contraction is not pronounced recovery is completed in one hour. When

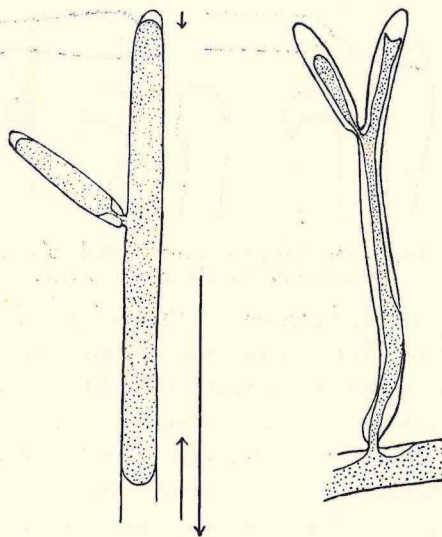


Fig. 6. *Bryopsis plumosa*, contraction of the protoplast in a cuf branch. The two short arrows indicate the streaming of the protoplasm, one long arrow the streaming of the cell sap. The lengths indicate the relative velocities. (Cfr Pro-wazek 38, fig. 2., p. 739.).

the stimulus to »plasmolysis« in strong, the maximum contraction occupies about an hour and dilatation and complete recovery requires some 24 hours. The intensity of the reaction (the degree of contraction) depends on the distance between the wound and the apex, and also on the physiological state of the plants. In many experiments it was noticed that *Bryopsis* is extremely sensitive to changes in external condition. Growth, regeneration, vital staining, the shape and movements of the chloroplasts, and behaviour under plasmolysis are variable within wide limits, and the behaviour of plants brought in freshly contrasts markedly with that of plants cultivated in the laboratory. It is to be noted that lateral branches connected with the main stem react in the same manner as the apex while the branches separated from the main stem by a cell wall do not react at all. When the stimulus is very strong there may be an incomplete recovery succeeded by a second and stronger contraction before full recovery occurs. When recovery is not complete, i. e. when the re-expanded protoplast does not reach to the apex normal regeneration takes place.

The description »der plasmolytischen Kontraktionen, die sich an den Thallusspitzen von *Bryopsis*, zumal an den Spitzen der kleinen Fiederästchen, unter dem Einfluss mechanischen Druckes abspielen« is given by Küster (26. p. 27).

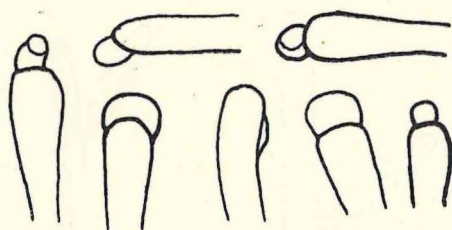


Fig. 7. *Pseudobryopsis* centrifugated 10' after pronounced stimulation plasmolysis.

True stimulation plasmolysis, was not observed in *Bryopsis plumosa* when studied in Villefranche and in Rab. The reaction was very marked, however in *Pseudobryopsis myura* (J. Ag.) Berthold in Split. The material was collected according to the directions of dr. A. Ercegović on rocks immediately below the »Biološko-oceanografski Institut«. The reaction was very pronounced in most cases and recovery was observed after 6 — 8 hours. The reaction in young shoots was more definite than in older branches. It is interesting to note that *Pseudobryopsis* can be stimulated to »plasmolysis« by centrifuging: plants were centrifuged for 20 minutes (electrical centrifuge Ecco, 2000 revolutions per minute) and no contraction was observed immediately after but in 2 to 5 minutes a stimulation plasmolysis took place, and the protoplasts contracted, remained

unchanged for about an hour and then slowly recovered. A very slow streaming of the protoplasm accompanied by movement of the plastids towards the apex could be observed. When the plants were centrifuged while in this state of »plasmolysis« the reversibility of the contraction was lost. The fluid in the cavity between the contracted protoplast and the apical wall was not forced out by the centrifugal pressure and differently inflated apices resulted, as shown in diagram 7. It must be emphasised that the reaction of stimulation plasmolysis and indeed any other reaction depends on the physiological state of algae, but to determine its stage is very difficult. For example, the material collected at different times at the same place during one week exhibited very variable behavior.

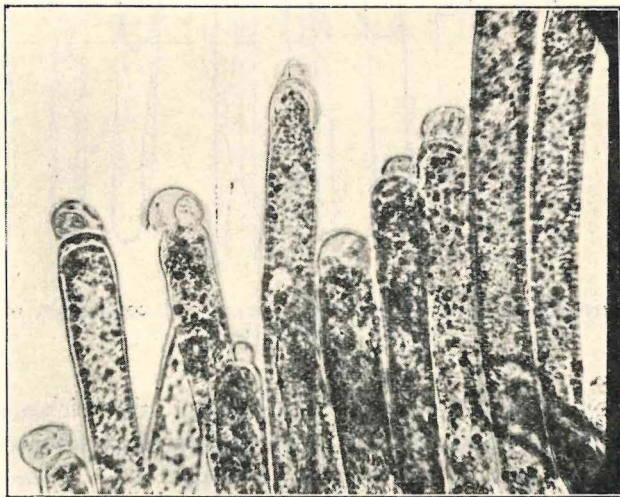


Fig. 8. Pseudobryopsis. Split. 27. VII. 1933. Centrifuged 10 minutes in pronounced stimulation plasmolysis. Then culture in sea water, 7. VIII. fixation with formaldehyd.

Codium.

The structure of *Codium* could be considered as very suitable for similar contractions as observed in *Bryopsis*, but only occasionally in young coenocytes of *Codium bursa* does cutting off the filaments cause regular contraction of the protoplasts (Brun s 4, p. 171, fig. 5, 6. Küster 24, p. 185). It is supposed that in the tallus the cell wall is distended and that when the filaments are cut the tension ceases, the cell wall shrinks and contraction of the

protoplasts and the cell wall proceeds simultaneously. The apex of the cell walls in *Codium bursa* is differentiated into special »cap« and in the contraction of the cell wall, as in plasmolysis, the differentiation can be seen very clearly (50., 51.).

Rhodophyceae.

The trichomes of *Gastroclonium kaliforme* (G. et W.) A r d i s s. (*Chylocladia kaliformis* H o o k) are very sensitive organs and in abnormal conditions are the first organs to die and fall from

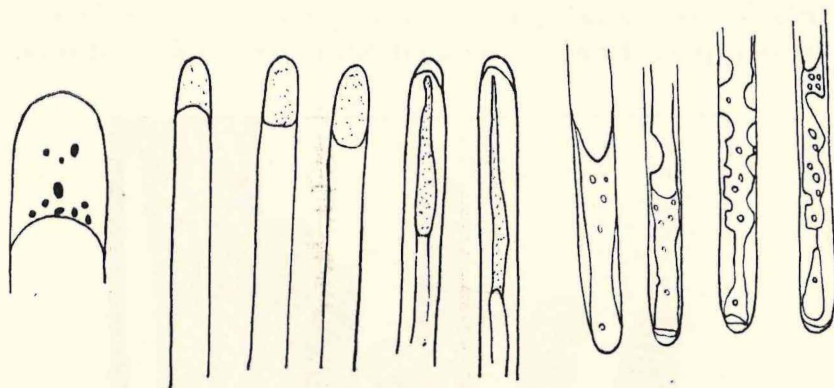


Fig. 9. *Gastroclonium*. Stages in the contraction and coagulation of the protoplast in the trichomes.

the »stems«. When mechanically stimulated, the trichomes show swelling of protoplasm and cell wall resembling pseudoplasmosis. Normally the apex of the trichome is filled with more or less homogeneous protoplasm with a few bright drops or granules near the vacuole. (B e r t h o l d 2, p. 676.) The meniscus of the plasma is perfectly concave while the meniscus of the vacuole is convex. The reaction is most easily observed in the trichomes in the middle region of the thallus; the young trichomes are short and their reaction is not distinct, while the older trichomes die in too short a time. The swelling of the protoplast can be induced by applying pressure to the coverslip and it passes through three phases:

1) The concave meniscus of the protoplast slowly flattens and in some seconds becomes convex.

2) The plasma explosively streams from the apex downwards and at the same time separation of the contents from the apical and

the longitudinal cell walls becomes visible. The inner layers of the walls of the cell apex swell.

3) The meniscus of the protoplast becomes concave and the cell undergoes vacuolisation and dies.

Many variations in the details of the reaction occur but cannot be described here. A reaction similar to that in the trichomes of *Gastroclonium* was observed in the trichomes of *Corallina* and in the apical trichomes of Red Alga *Nemalion*. The incrustated thalli of *Corallina* however, are very hard, and the thalli of *Nemalion* very mucilaginous, so that investigation of the reaction of these algae is extremely difficult.

Discussion.

The explanation of the phenomenon of stimulation plasmolysis is not easy. The only thing about which all the authors agree is the irregularity of the reaction. This irregularity may indicate that intracellular conditions rather than the externally applied stimulus control the phenomenon.

»Es sei noch hinzugefügt, das die Reizplasmolyse nicht immer erzielt werden kann, sondern offenbar eine, wahrscheinlich durch äussere Umstände bedingte Disposition der Zelle für ihr Zustandenkommen Bedingung ist« (B e n e c k e r, p. 555).

It is very difficult to express quantitatively the stimulus for this type of plasmolysis: it can as yet only be said that if the stimulus is too weak the reaction fails and if it is too strong the reaction goes too far and is not reversible and the cell dies within a short time. It cannot be doubted that not only water but also the solution of the cell sap is filtered out through the protoplast; i. e. that there is an increase in permeability.

In the year 1913 W. J. V. O s t e r h o u t (33) described some very interesting cases of protoplasmic contraction resembling plasmolysis which are caused by pure distilled water (root hair cells of *Zostera*, hairs of *Polysiphonia violacea*): »The cause of these effects lies in an increase in the permeability of the plasma membrane (and likewise of internal cell membranes), as the result of which some or all of the substances which maintain the osmotic pressure of the cell diffuse out; the protoplasm then shrinks as the result of loss of water from the vacuoles, which in consequence

become smaller (as is shown in figs. 3 — 5). This is often followed by an apparent »coagulation« of the protoplasm, which is sometimes evidenced by the assumption of irregular outline.«

The increase of permeability which is indicated appears to confirmed the conclusions of Janse and of Bü n n i n g: »Wird also bei der Reizung ein Teil des Zellsaftes ausgestossen, so kann dieses nur geschoben, wenn die Vakuolenwand ihre Eigenschaft der Semipermeabilität verliert und also für alle Substanzen permeabel geworden ist.« (Janse 18, p. 19.) »Infolge seismischer Reizung entsteht eine Permeabilitätserhöhung. Erst wenn die Permeabilitätserhöhung einen bestimmten Grad erreicht hat, wird durch den Wandruck Zellsaft (nicht reines Wasser) ausgepresst.« »Die Reizrezeption besteht nicht in einer Thixotropie. Manche Gründe sprechen dafür, dass die Rezeption in einer Zerreissung der semipermeablen Membran besteht und dass die Erregung (also die zum Verlust der Semipermeabilität führende Permeabilitätserhöhung) entsteht, weil das Plasma nach der Zerreissung der semipermeablen Membran mit den Zellsaft in Berührung kommt. Die Permeabilitätserhöhung nach mechanischer Reizung wird nicht durch einen Quellungsvorgang bedingt, wahrscheinlich aber durch eine Koagulation der semipermeablen Membran.« (Bü n n i n g 5, p. 82.)

The »Aggregation« (Ch. Darwin) shows clearly, that the chemical (or mechanical ?) stimulus induces sudden changes in the protoplasm and contraction of the vacuole and also coagulation (»aggregated masses«, Darwin, »Ballung der Protoplasmas«; Bokorny 3).

Coagulation induced by mechanical stimulus (Rühren) is described by H. Freundlich and H. Kroch (6). »Mechanische Koagulation« of the protoplasm of *Bryopsis* is described by Lepeschkin (29). In the cells of *Nitella* a coagulation of protoplast is described in microdissection (T. Petérffy u. G. Yamahara 34).

The contraction of the protoplast during vital staining shows what a great influence some substances can have on the protoplasm even when they are not accumulated in the protoplasm; only the cell sap became coloured, no staining was apparent in the cytoplasm.

The writer wishes to express his thanks to Prof. dr. B. Němec for helpfull advice during all the work, to Prof. dr. V. Vokuk, Direktor of the Biološko-oceanografski Institut in Split for the kind hospitality he was granted with as well as for the

supply of materials and commodities necessary for the performance of the work. He must thank Mr. A. Machař for his kindly making it possible for him to stay and work in Rab.

Summary.

1. The bibliography of stimulation plasmolysis («Reizplasmolyse») is summarised.
2. Stimulation plasmolysis in the cells of *Striatella unpunctata*, *Rhipidiophora*, *Bryopsis*, *Pseudobryopsis* is described.
3. The best method of inducing chemical stimulation of *Striatella* for this type of «plasmolysis» is vital staining. The protoplasm reacts although the vacuole alone appears to take up the dye.
4. Wound plasmolysis and some similar phenomena of coagulation of the protoplast in the trichomes of marine algae are described.
5. Stimulation plasmolysis of intact cells must be distinguished from wound plasmolysis of cut cells.
6. As a possible explanation of the contraction of the protoplast in stimulation plasmolysis a sudden increase in permeability is suggested.

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