Phyton (Austria)	**Vol. 25**	Fasc. 2	225–231	30. 11. 1985

Reversible Dilatation of Endoplasmic Reticulum Cisternae During Pressure and Plasmolysis

By

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With 6 Figures (1 Plate)

Received July 27, 1984

Key words: *Nitella*, endoplasmic reticulum, pressure, plasmolysis

Summary

FOISSNER I. 1985. Reversible dilatation of endoplasmic reticulum cisternae during pressure and plasmolysis. – Phyton (Austria) 25 (2): 225–231, with 6 figures (1 plate). – English with German summary.

The formation of lightmicroscopically visible ER cisternae following the application of pressure or plasmolysis is described in rhizoidal cells of *Nitella flexilis*. The breakdown of the ER cisternae into numerous vesicles during release of pressure or deplasmolysis is shown to be reversible. These observations indicate that the structures which maintain the shape of the elongated ER cisternae are elastic and/or restorable in case of collapse. Their identity with parts of the cytoskeleton is discussed.

Zusammenfassung

FOISSNER I. 1985. Reversible Vergrößerung der Cisternen des endoplasmatischen Reticulums während Druck und Plasmolyse. – Phyton (Austria) 25 (2): 225–231, mit 6 Abbildungen (1 Tafel). – Englisch mit deutscher Zusammenfassung.

Es wird die Bildung von lichtmikroskopisch sichtbaren Cisternen des endoplasmatischen Reticulums in Rhizoidzellen von *Nitella flexilis* während Druck oder Plasmolyse beschrieben. Der Zerfall der ER Cisternen in zahlreiche Vesikel während Druckentlastung oder Deplasmolyse ist reversibel. Diese Beobachtungen weisen darauf hin, daß jene Strukturen, die für die Form der ER Cisternen verantwortlich sind, elastisch und/oder leicht regenerierbar sind. Ihre Identität mit Teilen des Cytoskeletts wird diskutiert.

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1. Introduction

To the author's knowledge, VELTEN 1873 was the first to observe in the living cell elements of the endoplasmic reticulum (ER), which he called „Insucctionskanälchen". After the electron microscopical demonstration of the ER by PORTER & al. 1945 the lightmicroscopical investigations were intensified. Among plant cells the epidermal cells of the inner surface of the scale leaf of *Allium cepa* were probably the most thoroughly examined (URL 1964, URL & BOHLHAR-NORDENKAMPF 1965, BOLHAR-NORDENKAMPF 1966, FELDMANN 1966). The object of our investigations were rhizoids of a characean alga, *Nitella flexilis,* which offer not only good conditions for the analysis of the living cytoplasm but also the opportunity to study singly isolated cells without the influence of a surrounding tissue.

2. Material and Methods

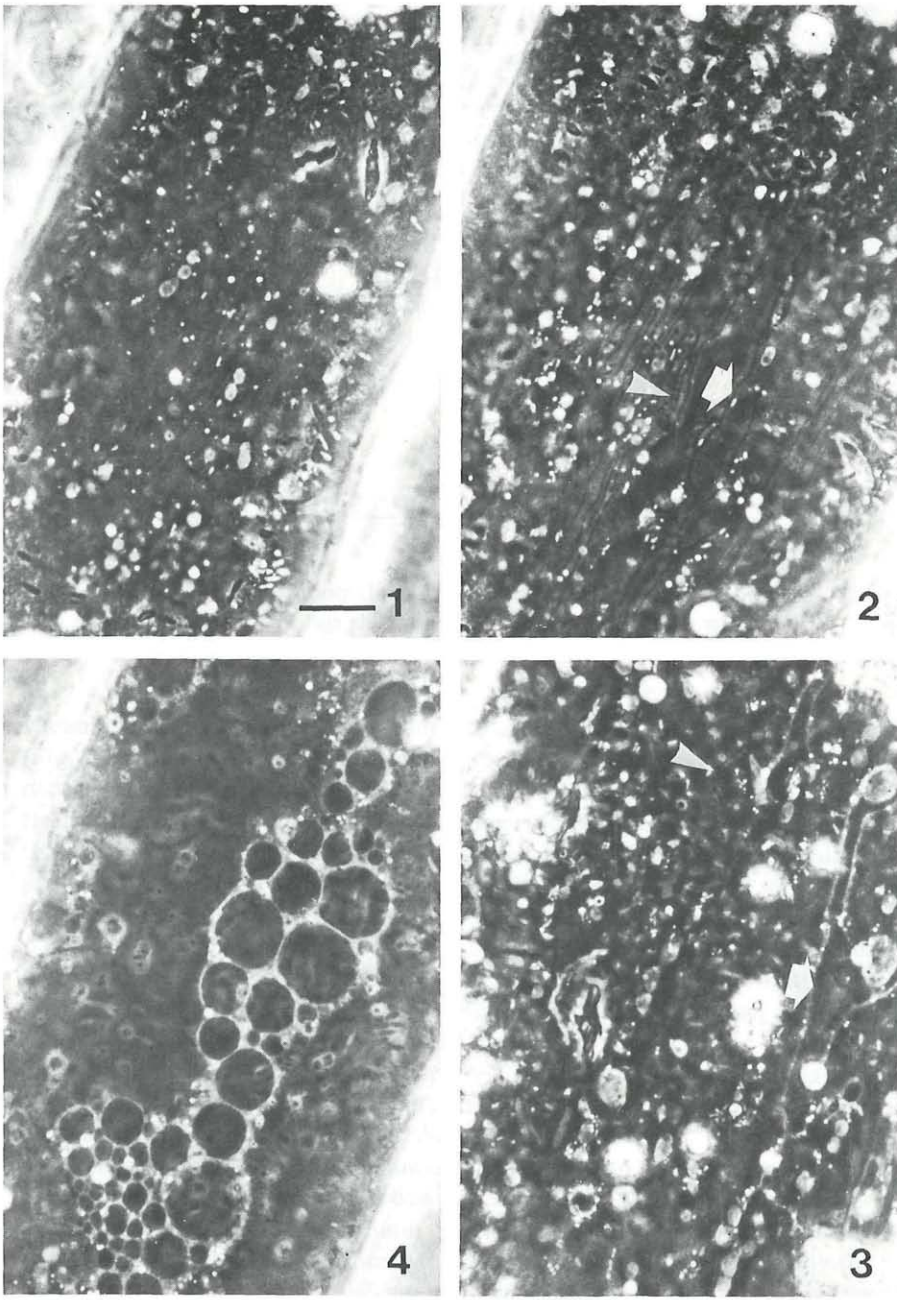
Rhizoidal cells of *Nitella flexilis* were used. The algae were cultured in small aquaria filled with earth and artificial pond water (10^{-6} M NaCl, 10^{-7} M KCl, 10^{-7} M $CaCl_2$). For the pressure experiments single rhizoidal cells were placed in distilled water and covered with a cover-slip to ensure a homogenous compression of the cell. The plasmolysis experiments were performed using a cover-slip supported by vaseline in order to avoid any compression of the cell but allowing perfusion of liquids. All observations were made at 23° C. The microscope used was a Zetopan (Reichert) equipped with anoptral optics. Photographs were made on Ilford XPl 400. Chemicals were purchased from Serva.

3. Results

Using anoptral optics the following cytoplasmic organells can be easily distinguished in a rhizoidal cell: proplastids and subcortical fibrils in the stationary ectoplasm; mitochondria, dictyosomes and spherical inclusions in the streaming endoplasm (see JAROSCH 1961). Shortly after preparation the cytoplasm also contains vesicle- and tube-like vacuoles which in most cases disappear gradually within 30 minutes. This effect neither depends on the pressure of the cover-slip nor on the sort of liquid used for preparation, i. e. either distilled water or culture medium. Only cells without visible cytoplasmic vacuoles were used for the following experiments.

3.1. The effect of mechanical pressure

The cytoplasm of non-pressed cells appears to be homogenous as shown in Fig. 1. Increased pressure by the cover-slip, which was provoked by sucking off the water, leads to the formation of tube-shaped or globular vacuoles in the endoplasm just as observed immediately after preparation (Fig. 3). Their lumen shows the same refraction of light as the central



Figs. 1–4. – Cytoplasm of a rhizoidal cell at different degrees of mechanic pressure (compare with Fig. 6). Fig. 1. No pressure. No cytoplasmic vacuoles visible. Fig. 2. Slight pressure. Formation of vesicle chains (arrow-head) and tubular vacuoles (arrow). Fig. 3. Strong pressure. Enlargement of the vacuoles (arrow), few delicate tubes (arrow-head). Fig. 4. Release of pressure. Formation of giant vesicles. Bar represents 11 μm.

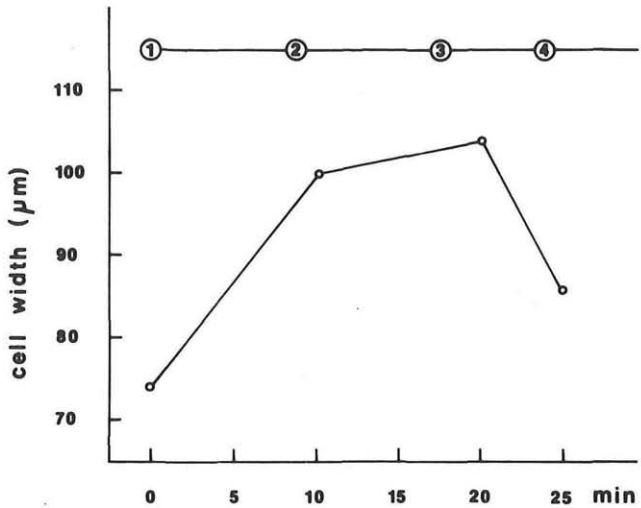


Fig. 5. Cell width as an approximate indication of the mechanic pressure. The numbers on the upper line show the moments when Figs. 1–4 were taken.

vacuole. The vesicles are often joined to each other like a string of pearls (Fig. 2, arrow-head). The tube-shaped elements show local inflations and occasionally branch or fuse with each other. This system of vacuoles is transported and deformed passively during cytoplasmic streaming. Thereby the branching points shift in relation to each other. The elongated vacuoles are arranged parallel to the streaming direction but orientation and shape of the vacuoles persist also in the case of cessation of streaming.

The width of the vacuoles depends on the degree of pressure and varies between parts of a μm and several μm (Fig. 3). The vacuoles of strongly pressed cells appear no longer tube-shaped but flattened. However, even strongly pressed cells contain delicate vacuoles as shown in Fig. 3 (arrow-head) though at a reduced number. The length of the tubes varies and depends on the degree of fusion between single strands. In most cases they form a continuum extending over the whole cytoplasm.

Release of pressure by addition of water results in a sudden breakdown of the tubular system into numerous vesicles (Fig. 4). This process is accomplished within parts of a second. The total volume of the cytoplasmic vacuoles seems to increase. Shortly after, the diameter and accordingly the volume of the vacuoles diminish again. Some of the vesicles burst, others are again transformed into tubes. Normally the vacuoles have disappeared within one hour and the cytoplasm then looks like the cytoplasm in Fig. 1. The cell widths which correspond to the Figs. 1–4 are depicted in Fig. 5 and serve as an approximate measure of the applied pressure. A schematic

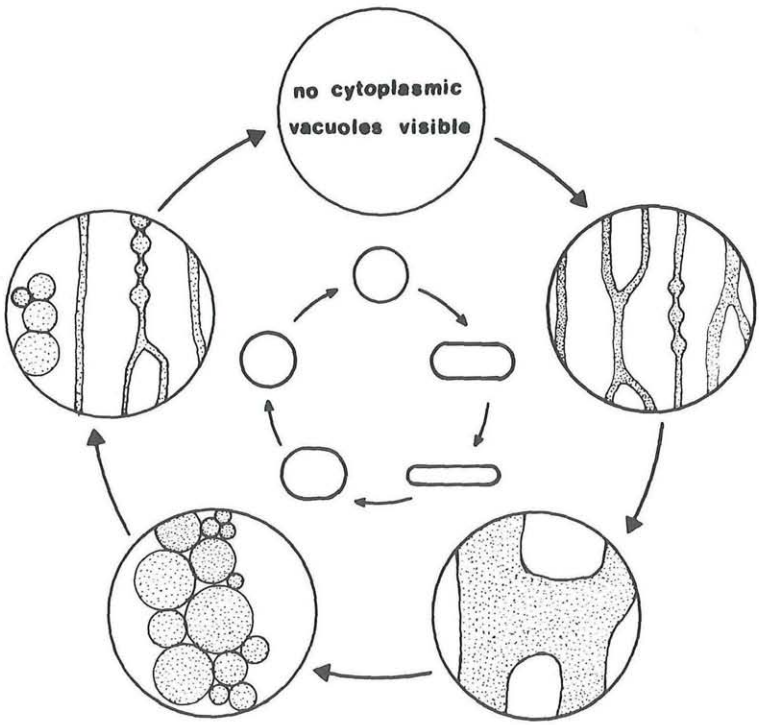


Fig. 6. Changes in the appearance of the cytoplasmic vacuoles during application and release of mechanic pressure (outer circle) and the corresponding cross section of the cell (inner circle).

representation of the processes described above is given in the outer circle of Fig. 6 whereas the inner circle shows the corresponding cross-section of the cell. The formation of cytoplasmic vacuoles as a result of mechanical pressure could also be observed in cells which had previously been placed in various concentrations of carbonylcyanide . m-chloro-phenylhydrazone for 30 minutes.

3.2. Effect of plasmolysing agents

The experiments were performed with 0.3 to 0.38 M solutions of glucose. Higher concentrations are lethal. The addition of the plasmolytices causes the appearance of vacuolar tubes and vesicles just as in pressed cells (compare Fig. 2 and 3). The formation of vacuoles mostly started with the detachment of the protoplast from the wall, rarely before or after that. In contrast to the pressure experiments the diameter of the vacuoles never exceeds 3 μm and decreases again, starting with the end of protoplast

spherulation, which perhaps indicates the onset of osmotic regulation (compare NAKAGAWA & al. 1974). Only delicate tubes and vesicles are visible then. Their volume does not increase when the cells are strongly pressed. Deplasmolysis has the same consequences as release of pressure, i. e. a transient increase of the vacuolar volume and spherulation of the tubes (compare Fig. 3) before the cytoplasm obtains its normal appearance (compare Fig. 1). Plasmolysis and deplasmolysis can be repeated several times showing the same series of events.

The velocity of protoplasmic streaming decreases gradually both during application of pressure and plasmolysis and increases again during release of pressure and deplasmolysis (see also KAMIYA 1959).

4. Discussion

The cytoplasmic vacuoles obviously correspond to extremely enlarged ER cisternae as described in other cells (see the introduction). In our experiments, the ER cisternae became visible during pressure and plasmolysis, which corresponds with the findings of other authors (e. g. URL 1964, 1967, URL & BOLHAR-NORDENKAMPF 1965, BOLHAR-NORDENKAMPF 1966, FELDMANN 1966). The ER cisternae enlarged further in the first stages of regeneration, i. e. release of pressure and deplasmolysis. During each of these processes the cytoplasm presumably contains more water than in its normal state because of a continuous passage of water either from or to the vacuole. We agree, therefore, with BUCKLEY 1965, URL & BOLHAR-NORDENKAMPF 1965 and others that the increased water content of the cytosol causes the enlargement of the intracisternal volume. Accordingly, the enlargement of the ER cisternae shortly after preparation is interpreted as a result of water flow compensating the wall pressure formerly exerted by the neighbour cell, and/or water flow via leakages through the plasmodesmata. Why the water accumulates in the ER cisternae is not clear but it seems to be a passive process since neither the formation nor the disappearance of the enlarged cisternae was affected by an uncoupler which lowers the cytosolic ATP/ADP ratio (GOLLER & al. 1982). In any case we have to expect changes in membrane permeability (KUIPER 1972, ZIMMERMAN & STEUDLE 1978).

As for instance in *Allium* (FELDMANN 1966), the elongated shape of the ER cisternae in *Nitella* does no only depend on cytoplasmic streaming and points to the existence of supporting structures within or closely at the membranes. The only known structure that could serve as an abutment for the ER is the filamentous network described by FOISSNER & JAROSCH 1981 (compare also ALLEN & al. 1976, WARDROP 1983) in *Nitella* rhizoidal cells. The network shows a predominant longitudinal orientation because it originates in and moves along the subcortical fibrils. If this part of the cytoskeleton is no longer able to withstand the surface tension of the ER cisternae the cell is certainly in a critical state. The tubes will break down

into vesicles, which are a characteristic feature of the pronecrotic cytoplasm (URL 1959, JAROSCH 1961, BOLHAR-NORDENKAMPF 1966, FELDMANN 1966). This occurred during an extreme increase in volume of the ER tubes within a relatively short period of time. The experiment has shown, however, that such changes are reversible in *Nitella* rhizoids. The supporting structures are thus able to recover from collapse within a few minutes or are extraordinarily elastic. The latter property is indeed characteristic for the filamentous network. Since the network is supposed to play an active role in the generation of the motive force (FOISSNER & JAROSCH 1981) the reduced velocity of cytoplasmic streaming could be due to its compression by the increasing ER cisternae.

5. Acknowledgment

I want to thank Prof. R. JAROSCH for valuable discussion. This work was supported by a grant from the Österreichische Nationalbank, Jubiläumsfondsprojekt Nr. 1927.

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Recensiones

MÜLLER H. J. (Ed.) 1984. Ökologie. Bearbeitet von BÄHRMANN R., HEINRICH W., MARSTALLER R., MÜLLER H., SCHALLER G. Studienreihe Biowissenschaften. – Kl.-8° (Taschenbuchformat), 395 Seiten mit 109 Abbildungen, broschiert. – VEB Gustav Fischer Verlag Jena. M 25,–, DM 35,–. – Best.-Nr. 533927 8.

Im selben Jahr, in dem von R. SCHUBERT unter Mitarbeit von 29 Fachwissenschaftern ein großes „Lehrbuch der Ökologie" herausgegeben wurde (vgl. Rezension in Phyton 25 (1): 188, 1985), erschien im selben Verlag unter Mitarbeit von 5 Autoren eine „kleine" Ökologie, wobei ein Autor bei beiden Ökologie – Büchern mitgewirkt hat. Diese „kleine Ökologie" ist aus der Niederschrift einer Vorlesung entstanden und als erste Einführung in die Ökologie gedacht. Der gesamte Stoff wurde in fünf Abschnitten zusammengefaßt und übersichtlich gegliedert. Im Abschnitt 1 wird auf die Entwicklung und Bedeutung der Ökologie eingegangen. Abschnitt 2 behandelt die abiotischen und biotischen Elemente (Bestandteile) des Ökosystems; der Boden wird hier unter die „abiotischen Elemente gereiht! Bei der Besprechung der edaphischen Vikarianz (S. 126) sollte es statt „Urgestein" richtiger „Kristallin" heißen. Der Abschnitt 3 befaßt sich mit den Wechselbeziehungen zwischen den Organismen und den Umweltfaktoren; ausführlich wird dabei auf den Nischenbegriff Bezug genommen, ein Hinweis auf die allelopathischen Wechselbeziehungen zwischen den Organismen wird allerdings vermißt. Abschnitt 4 hat die Struktur, Einteilung, Funktion