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Organization of the cytoplasmic reticulum in the central vacuole of parenchyma cells in *Allium cepa* L.

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Abstract

An elaborate and complex cytoplasmic reticulum composed of fine filaments and lamellae ranging from 0.1 to 4 microns in size is revealed by viewing the central vacuole of onion bulb parenchyma cells with the scanning electron microscope. The larger cytoplasmic strands, visible with the light microscope, are composed of numerous smaller filaments (some tubular) which might explain the observed bidirectional movement of particles in these larger strands. The finely divided cytoplasmic network of filaments is continuous with the parietal cytoplasm inclosing the vacuolar sap. In these highly vacuolated cells the mass of the protoplast is in the form of an intravacuolar reticulum immersed in the cell sap. The probable significance of the vacuolar sap in relation to physiological processes of the cell is discussed.

INTRODUCTION

Well over a century ago botanists described in considerable detail the network of protoplasmic strands with their included organelles in highly vacuolated plant cells (Schleiden, 1845; von Mohl, 1844, 1846; Sachs, 1882). Intrigued by the phenomenon of cytoplasmic streaming, numerous other workers subsequently described the configuration and consistency of transvacuolar cytoplasmic strands with their granular or vacuolar inclusions (see reviews by Seifritz, 1943 and Kamiya 1959). More recently the formation and dynamic transformations of this cytoplasmic reticulum were studied by Mahlberg and his co-workers (Mahlberg, 1962, 1972; Mahlberg and Venketeswaran, 1963).

The size and configuration of transvacuolar strands, although continuously changing in actively metabolizing cells, vary from several mi-

crons in diameter to delicate filaments below the resolution of the light microscope. The development of methods to study the interior of vacuolated cells with the scanning electron microscope has now made it possible to view in minute detail the three-dimensional organization of the protoplast fixed *in situ* (Panessa and Genarro, 1972; Humphreys and Wodzicki 1972). Using these methods, Wodzicki and Humphreys (1973a) and Wodzicki and Brown (1973) revealed the complex, filamentous and lamellate nature of the cytoplasmic reticulum in the vacuole of differentiating pine tracheids. These studies confirmed and extended the earlier microscopical observations of Bailey (1930) who first described the intricate network of cytoplasmic strands in the cambial fusiform initials of *Pinus strobus*. Little is yet known about the physiological significance of this delicate and elaborate organization of cytoplasm in the function or survival of plant cells. According to Küster (1956, 1957) the cytoplasmic configuration reflects the presence of active metabolic interactions between the cytoplasm and vacuolar sap.

For this study of cytoplasmic organization we chose the large parenchyma cells (200—450 μ) of the onion bulb scale *Allium cepa* L. Light microscopy allows one to detect a thin parietal layer of cytoplasm lining the cell wall, and some fine cytoplasmic strands traversing the large central vacuole filled with dilute cell sap. The nucleus is often suspended in the large vacuole by the cytoplasmic strands.

METHODS

Small sections of tissue about 1 mm^2, of the thickness of the bulb scale, were cut under a drop of 2% glutaraldehyde, and fixed for two hours at 4° C in 2% glutaraldehyde buffered to pH 7.2 with 0.1 M sodium cacodylate, then post-fixed for two hours with 2% osmium tetroxide. Subsequently, some of the sections were washed for one hour in several changes of glass-redistilled water, and positioned in shallow wells drilled into the centers of specimen holders (Kent Cambridge type). Each of the sections was frozen in place with a small droplet of water by submersing the specimen holder into liquid nitrogen. The sections were fractured under liquid nitrogen with a razor blade, and freeze-dried under vacuum (10^{-6} torr) overnight. Other blocks of tissue were dehydrated in an ethanol series and embedded in Maraglas as described by Freeman and Spurlock (1962). After trimming, ultra thin sections were obtained, stained with lead citrate and viewed in a Philips 200 transmission electron microscope. The same plastic blocks from which the ultra thin sections were cut were then fractured with a razor blade and viewed with a Cambridge Mark 2A scanning electron microscope, as recently described by Wodzicki and Humphreys (1973b), and Humph-

reys, Wodzicki and Paulin (1973). Both freeze-fractured, and plastic-fractured specimens were coated with a thin layer of gold (200—400 Å) before viewing with the scanning electron microscope.

OBSERVATIONS

The interior of numerous parenchymatous cells exposed by freeze--fracturing and freeze-drying reveal an elaborate reticulum of fine filaments and lamellae (Fig. 1). The network of fine filaments permeating the vacuole, and frequently forming rather dense aggregates, extends to, and integrates with the thin-layer of parietal cytoplasm lining the cell wall (Fig. 3). The thicker strands such as the one diagonally crossing the vacuole in Fig. 2 resemble the cytoplasmic threads visible in the light microscope. This particular strand, approximately 4 microns in thickness, appears to be composed of many fine filaments about 0.1—0.4 microns in diameter when viewed at much higher magnification (Fig. 4). In few cases, a tubular structure of the component strands seem to be revealed (arrows).

One may reasonably ask if, or to what extent, this filamentous cytoplasmic reticulum occupying the vacuole has been altered or artificially created during the process of freeze-drying, and therefore question if this pattern of organization exists *in vivo*, because similar filaments are difficult to reveal using standard techniques of transmission electron microscopy. This difficulty is best resolved by following the same procedures as those for preparing ultra-thin sections by embedding in plastic. Tissue embedded in Maraglas and fractured at room temperature expose cells in several different planes for viewing (Fig. 5), namely: (*a*) a smooth cleavage of the cell wall exposing the outer plasmalemma surface of the protoplast; (*b*) a clean break directly through cell interior; (*c*) an irregular break exposing the interior of the vacuole; and (*d*) a concave cleavage exposing a hollowed-out portion of the vacuole and the inner portion of the parietal cytoplasm. In cells corresponding to (*b*) the cytoplasmic filaments are randomly distributed in the vacuole (Fig. 6). Other cells are fractured in such a way as to expose only remnants of broken cytoplasmic strands and the larger spherical organelles within the vacuole (Fig. 7 and 8). Structural details of cytoplasmic strands seem to be revealed by the smooth fracture of the cell presented in Fig. 5 (*e* — arrow), which is shown magnified in Figs. 9—11. Branching indicates the integrated organization (Fig. 10). At a few places the fracture exposed the ovoid bodies with internal structure, one of which is seen in the micrograph. The tubular structure of some cytoplasmic strands may be observed in Figs. 10 and 11 (arrows). In some of the plastic embedded fractured cells one may obtain a general three-dimensional view of the intravacuo-

lar cytoplasm (Fig. 12). Such fractures reveal the relatively dense, thread-like, network of cytoplasmic strands extending throughout the central vacuole.

Observations of ultra thin sections with the transmission microscope from the same block (Fig. 5) before it was fractured, reveals the structural features of cytoplasmic elements within the central vacuole of similar parenchymatous cells (Fig. 13). These structures enclosed in single or double membranes may be spherical or irregular in shape. Many of them possess some degree of organized internal structure, and in some of the structures, cisternae of the endoplasmic reticulum can be identified. The invaginations of the inner membrane are seen in many places resembling mitochondrial cristate (Fig. 14). Diameters of these segregated structures vary in thickness from 0.1 to 1.0 microns, and the parietal cytoplasm is reduced to a very thin layer lining the cell wall.

DISCUSSION

The remarkable agreement of the results obtained by the two methods used for scanning microscopy in these studies excludes practically the possibility that the demonstrated intravacuolar structure has been artificially created during post-fixation steps of preparation of the tissue. O'Brien et al. (1973) have recently documented the non-coagulant value of glutaraldehyde-OsO_4 fixation. It was shown to stabilize Hecht's threads in plazmolyzed onion epidermis against breakage during dehydration. However, some transformations of the vacuolar membranes resulting in production of membraneous outgrowths were also observed. Although the possibility of artificial creation of the whole complex structure, (such as demonstrated in this study) during fixation did not seem very probable the fresh and glutaraldehyde-fixed tissue was additionally studied with dark-field optics under a light microscope (unpublished). An extremely delicate network of cytoplasmic strands could be observed by this method, but no specific differences between the two types of cells were detected. However, taking into account that observations performed with a light microscope under low magnification do not allow precise conclusions, the reservations implied by the work of O'Brien et al. should not be completely disregarded. These reservations may concern particularly the organization of such structural details as tubular or multifilamentous strands.

The significance of the extensive intravacuolar reticulum in the large parenchyma cells is fully appreciated only when one envisions the reticulum as a continuously changing, three-dimensional network of branching and coalescing filamentous threads some of which seem to be tubular. Tubuliar transvacuolar strands of cytoplasm have been described already

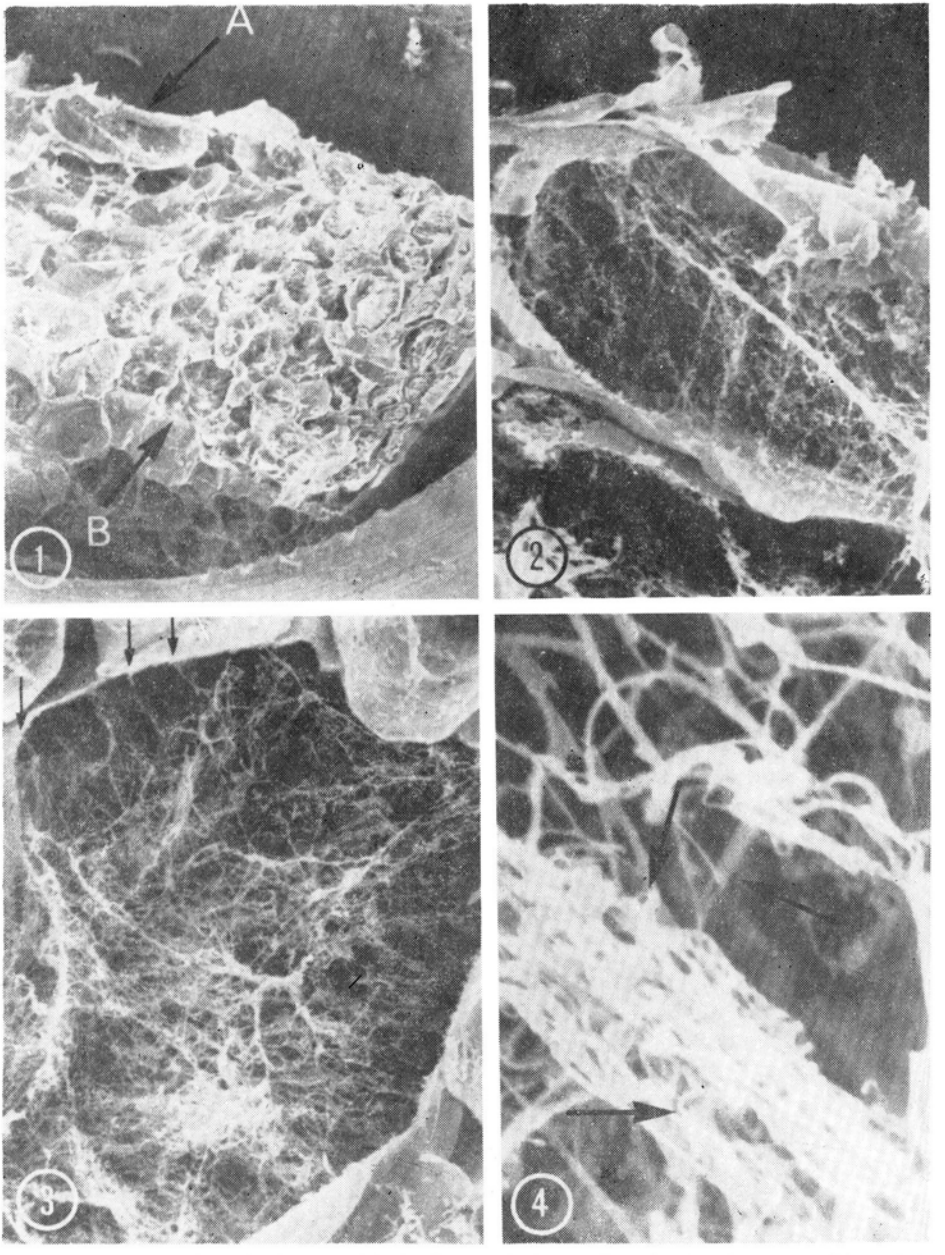


Fig. 1. Freeze-fractured, freeze-dried block of the onion scale tissue positioned in the central well of the aluminum specimen-holder. The cells at A and B are shown at higher magnification in Figs. 2 and 3 respectively. × 50. Fig. 2. Parenchyma cell at A in Fig. 2. Note the network of intravacuolar material and the thicker strand traversing the vacuole. × 250. Fig. 3. Parenchyma cell seen at B in Fig. 2. Arrows indicate the places at which the filaments of intravacuolar reticulum are attached to parietal cytoplasm. × 480. Fig. 4. Magnified portion of the larger cytoplasmic strand seen in Fig. 3 with numerous component strands. Tubular components seen at arrows. × 4600.

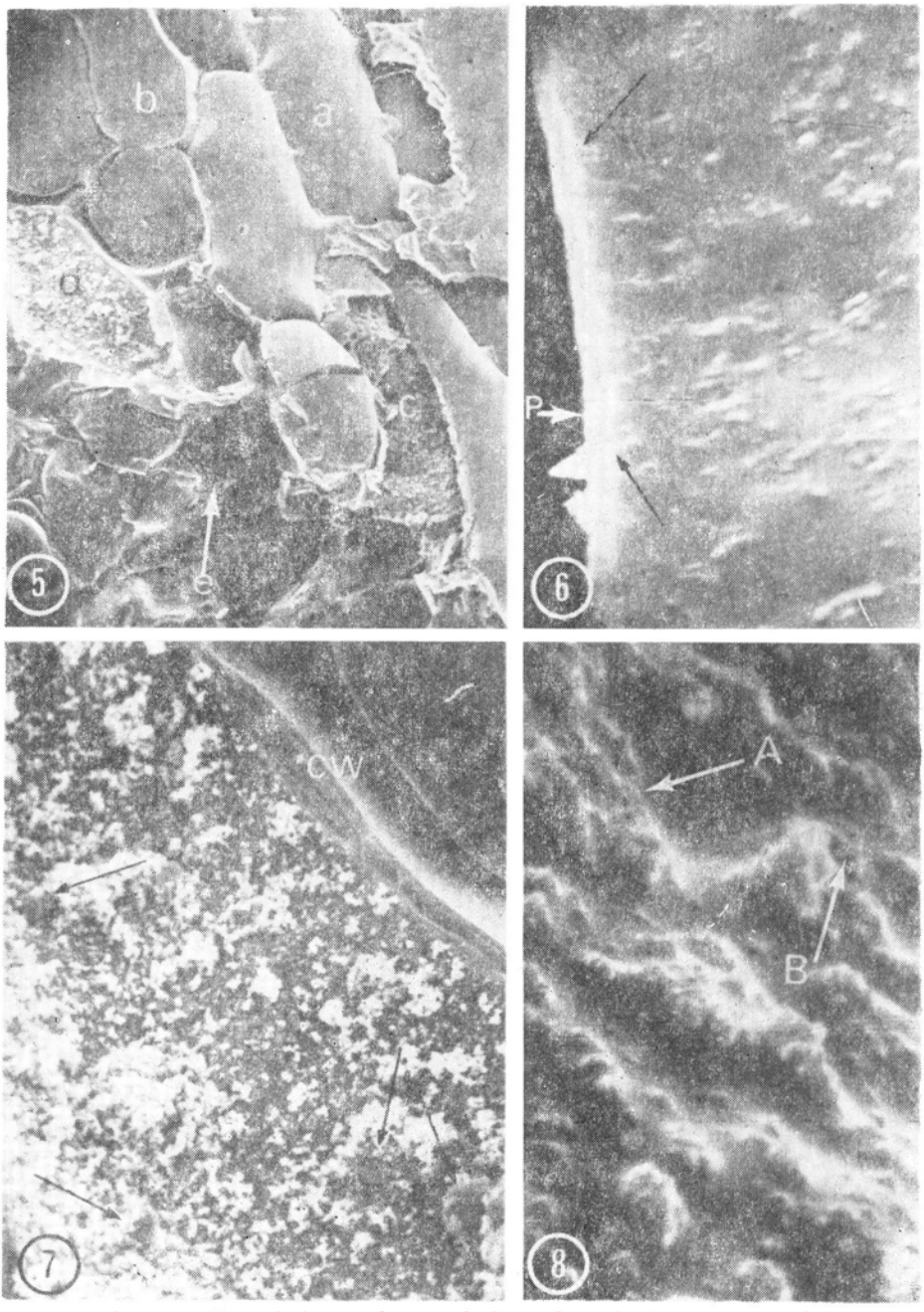
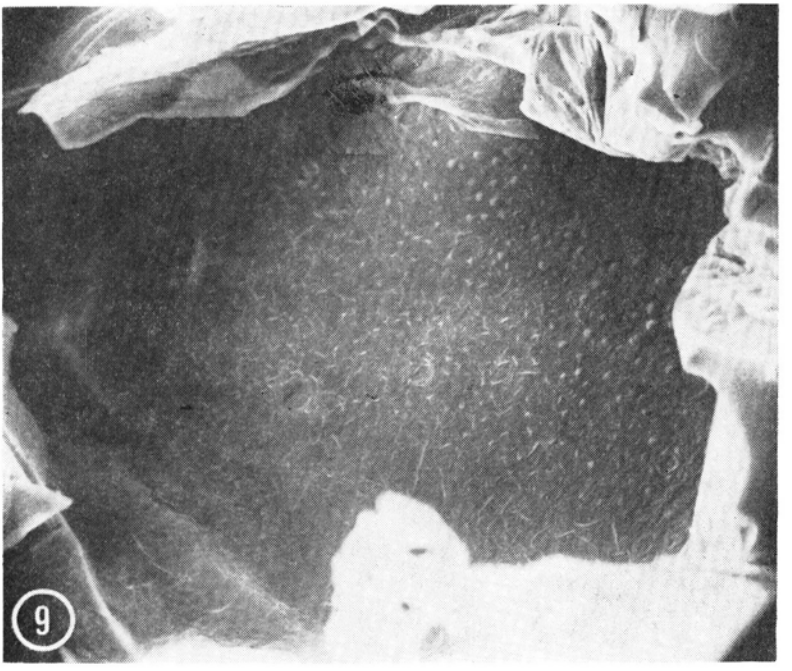


Fig. 5. Fractured tissue of onion bulb scale embedded in Maraglas.

(*a*) Cells with exposed plasmalemma after the cell wall was fractured away; (*b*) fractured cells with a smooth break through vacuole; (*c*) interior of the cell exposed by fracture; (*d*) inner surface of the parietal cytoplasm with remnants of the intravacuolar reticulum after most of the cell content was fractured away. The cell marked (*e*) is shown enlarged in Figs. 9—11. × 200

Fig. 6. Englarged view of the cell shown at (b) in Fig. 5 showing cytoplasmie filaments dispersed throughout vacuole with some being attached to parietal cytoplasm as indicated by arrows. P, parietal cytoplasm × 5000. Fig. 7. View of the fractured cell seen at (*d*) in Fig. 5 showing remnants of cytoplasmic strands. Spherical bodies indicated by arrows are probably plastids. CW, cell wall. × 1000. Fig. 8. Enlarged view of the cell shown at (*c*) in Fig. 5. Traces of structural elements (lamellate, (A) and tubular (B) seen at arrows. × 5000.



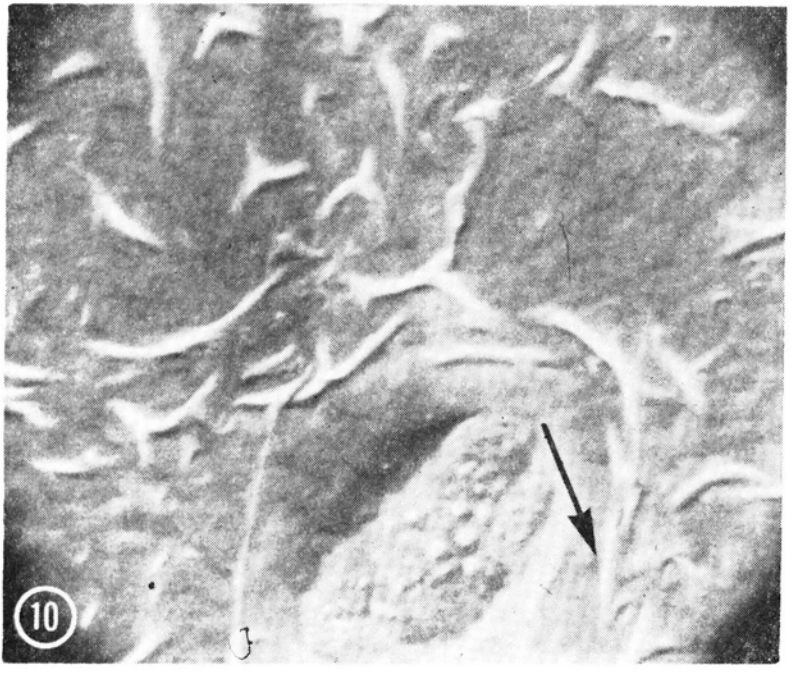
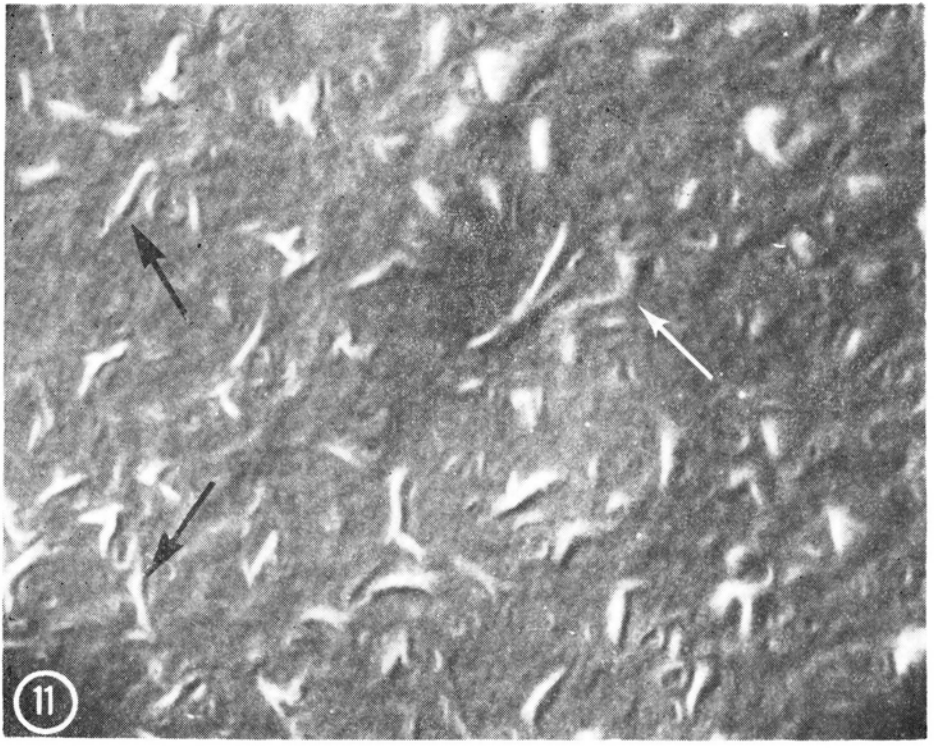


Fig. 9—10. Enlarged view of the fractured cell seen at (*e*) in Fig. 5. Fig. 9, × 1000. Fig. 10 — Branching intravacuolar filaments and the ovoid body with traces of substructure. Fragment of tubule seen at arrow. × 10 000.



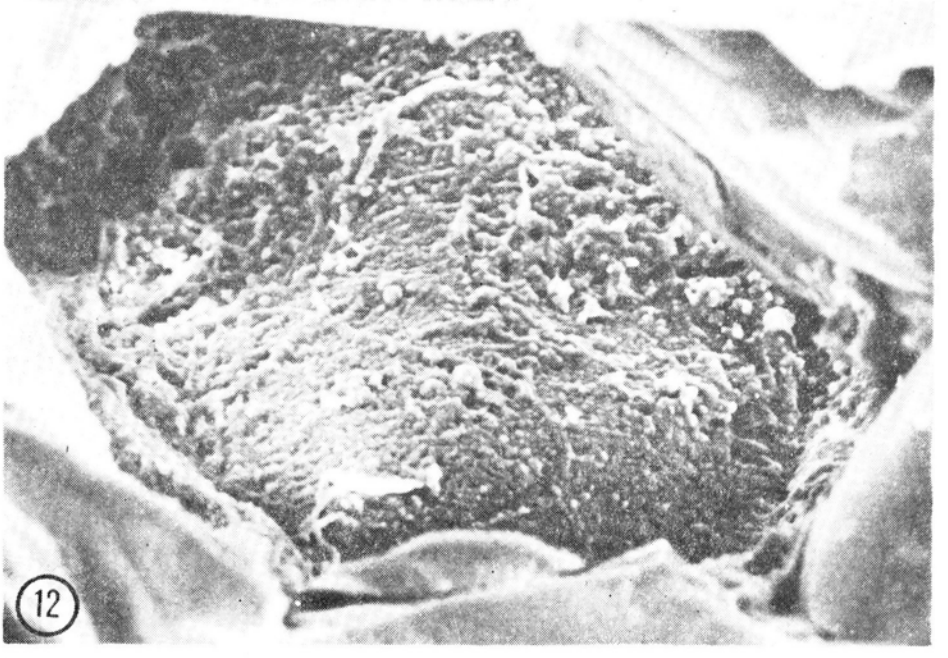
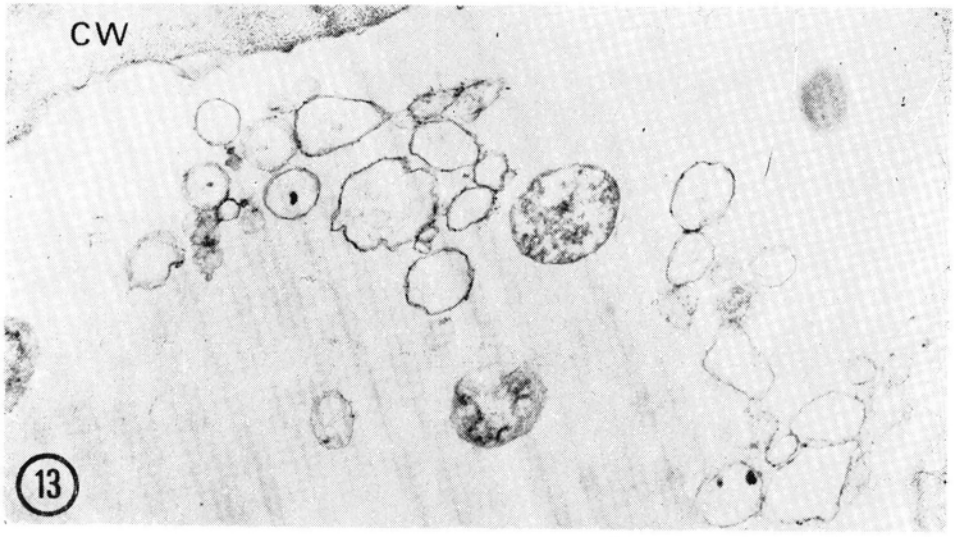


Fig. 11. Enlarged view of the fractured cell seen at (e) in Fig. 5. Traces of fractured tubules (arrows). × 5000.

Fig. 12. The reticulum of intravacuolar material seen in the fractured cell embedded in Maraglas. Notice the braching strand traversing the vacuole, and numerous globular bodies. × 1000.



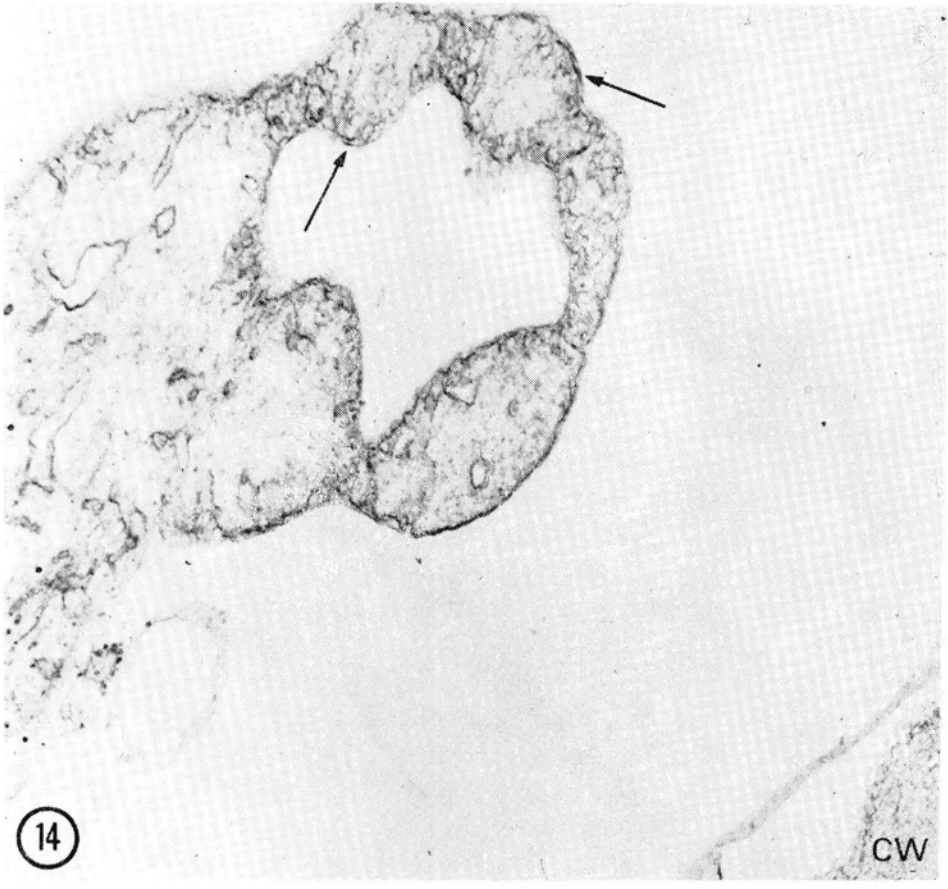


Fig. 13 and 14. Ultra-thin transverse sections of the onion bulb scale parenchyma cells obtained from the same block of tissue fractured in Fig. 5. CW, cell wall. Sections stained with lead-citrate and uranylacetate. Fig. 13 — Intravacuolar cytoplasmic strands viewed in transection. Most of the filaments do not reveal any internal structure. Some, however, possess a denser content than the surrounding vaculor sap of the central vacuole. × 25 000. Fig. 14 — Intravacuolar cytoplasmic strands with internal structure. Double membranes may be seen at arrows. The inner membrane forms deep invaginations and cristae. × 57 000

by Phillips (1926). The thicker strands observed in this study appear to be composed of numerous thinner filaments and tubules. Thus, the observed bidirectional movement of particles in such large strands (Seifritz, 1955; Kamiya, 1959) might be explained as occurring along separate filaments of the same strand, as already discussed by O'Brien and Thimann (1966).

Developmental processes leading to the formation of the cytoplasmic network in other plant material involves streaming of cytoplasm, coalescence of vacuoles, and outgrowths of cytoplasmic strands from the parietal cytoplasm (Reuter, 1957). Mahlberg and Venketeswaran (1963) found that the formation of new strands was associated with accumulation of cytoplasm in localized areas within the cell and active cytoplasmic streaming. The formation of transvacuolar strands was also observed in isolated mesophyll cells of tobacco grown *in vitro* (Nagata and Takebe, 1970). The growth of individual cytoplasmic filaments and fusion with each other or with the parietal cytoplasm at other points of the vacuole is probably involved in many instances as described by Küster (1957). In addition, formation of stellate vacuoles within the cytoplasm may also account for the development of the cytoplasmic network as suggested by Porter and Machado (1960) and Manton (1962).

The cytoplasmic strands in vacuoles are commonly described as transvacuolar strands, and the term intravacuolar cytoplasm, "intravakuoläres Protoplasma", as introduced by Cholodny (1923) was used to distinguish cytoplasmic inclusions into the vacuole, separated from the parietal cytoplasm. In the case of the finaly divided, and elaborate cytoplasmic network now viewed in the onion parenchyma cells, the identity of the vacuole as the large segregated area in the cytoplasm is lost. In fact, except for the very thin extravacuolar envelop, most of the cytoplasm is located within the vacuole as an integrated network of thin, delicate filaments completely immersed in the vacuolar sap. Therefore, the terminology we have chosen, i.e., "intravacuolar reticulum" seems to be more appropriate than transvacuolar strands in describing this network of interconnecting filaments.

The literature concerning the nature and content of vacuolar sap is extensive (Pisek, 1955), but its function has not been unequivocally determined. By whatever manner cell vacuoles may arise, they are separated from the ground cytoplasm by single membranes, and many workers regard them as being the depository for all excess cellular products. The basic functions ascribed to vacuoles are their important roles in regulating cell turgidity, supplying reserve food materials, serving as sinks for the byproducts of metabolism, and, as such playing a role in detoxification processes (Kramer, 1955). More recently its role in autolysis of segregated cytoplasmic components has been suggested (Matile, 1969).

The organization of the cytoplasm in highly vacuolated plant cells into such an elaborate intravacuolar reticulum provides an extensive surface area of vacuolar membranes which one may logically reason is highly advantageous in the exchange of nutritive substances with the cell sap. The reticulum appears to play a very important role in processes involving the rapid synthesis of secondary cell wall of highly vacuolated differentiating xylem elements (Wodzicki and Humphreys, 1973a; Wodzicki and Brown, 1973). Furthermore, in the highly evolved and rigidly compartmentalized plant cell, the vacuolar sap undoubtedly provides a favorable ultramicroenvironment for the cytoplasm of the cell by buffering it from any rapid deleterious changes in the extracellular environment (see also Walter and Stadelmann 1968). In this sense, the vacuolar sap with its nutritive, osmotic, and detoxification properties accomplishes physiological functions similar to the circulatory system of animals although in a system confined to the individual cell.

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Organizacja retikulum cytoplazmatycznego w centralnej wakuoli komórek miękiszu Alium cepa L.

Streszczenie

Badania przeprowadzone przy pomocy mikroskopu skaningowego ujawniły w centralnej wakuoli komórek miękiszu łuski cebuli retikulum cytoplazmatyczne złożone z delikatnych włókienek i lameli o wymiarach od 0,1 do 4 mikronów. Większe nici cytoplazmatyczne ukazały się jako złożone z licznych cieńszych włókien (także tubul) których obecność może wyjaśnić mechanizm obserwowanego w mikroskopie świetlnym dwukierunkowego ruchu cząsteczek wzdłuż transwakuolarnych nici cytoplazmy. Delikatne włókna siatki cytoplazmatycznej stanowią ciągłość z cienką warstwą cytoplazmy parietalnej otaczającej sok wakuolarny. W tych wysoce zwakuolizowanych komórkach masa protoplastu znajduje się w formie wewnątrzwakuolarnego retikulum zawieszonego w soku komórkowym. W świetle tych obserwacji fizjologiczna rola soku wakuolarnego może być rozpatrywana jako specyficzne ultramikrośrodowisko cytoplazmy.