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SOME ASPECTS OF PHLOEM STRUCTURE AND DEVELOPMENT IN *MARSILEA QUADRIFOLIA* L.

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Summary

Sieve elements in the phloem from rhizomes of ***Marsilea quadrifolia*** **L.** *were examined with the electron microscope. Very young sieve elements are characterized by the presence of the refractive spherules, which arise in the cisternae of the endoplasmic reticulum. As sieve-element differentiation proceeds, the delimiting membranes of many of the refractive spherules begin to fuse with the plasmalemma and the material comprising the spherules is liberated into the region of the wall. At maturity the enucleate, plasmalemma-lined sieve element contains a sparse, parietal network of tubular endoplasmic reticulum, plastids and mitochondria. The sieve-area pores are quite small in size and are lined by the plasmalemma.*

Introduction

Research on the structure and histochemistry of phloem has been and continues to be motivated largely by the desire of investigators to reveal relationships between structure and function—relationships that hopefully will enable them to evaluate the proposed mechanism of phloem transport.

According to the best supported concept, the sieve element attains functional maturity through profound changes in its protoplast, some of which are phenomena of degradation. Nevertheless, the sieve element retains certain characteristics typical of living plant cells. The ultimate interpretation of the relation between the structure of the sieve element and its function of conduction has not yet been brought forward. The evidence is overwhelming, however, that the sieve element carries out the rapid long-distance transport of organic substances after its protoplast undergoes the unique developmental changes and that this activity is regulated in great measure by the relatively unmodified parenchymatic cells associated with the sieve element.

Electron microscopy has attracted a large number of plant biologists to structural research on phloem. During the past ten years enormous strides have been made in understanding the unique structural features of the sieve element, and a fairly uniform picture has emerged for the structure of the angiosperm sieve-tube member and for that of the gymnospermous sieve cell. Nevertheless, little detailed information is available of the phloem in lower vascular plants.

In recent years, use of the electron microscope has created renewed interest in phloem structure in lower vascular plants. The literature now includes information on the ultrastructure of the phloem from each major group of lower vascular plants with the exception of the water ferns[1-11]. The aim of the present study was to contribute information on the ultrastructure of the sieve element in the rhizome of *Marsilea quadrifolia* L., one of the water ferns. This information would be used to provide a basis for comparison of the sieve elements in *Marsilea* with those of other lower vascular plants.

Materials and Methods

Samples of vascular tissue were taken from young and mature rhizomes of *Marsilea quadrifolia*. Segments about 1–2 mm long were immersed in 6% glutaraldehyde in 0.05M sodium cacodylate buffer pH 7.1, and aspirated for 5 h. Tissue was washed with 0.05M cacodylate buffer and postfixed in 2% osmium tetroxide overnight in a refrigerator. All tissues were dehydrated in a series of ethyl alcohol and propylene oxide and embedded in Araldite. Sections were cut on a Sorvall MT–2 ultramicrotome at 300–400 Å, stained with saturated uranyl acetate for 20 min, and post-stained with lead citrate for 10 min. Specimens were examined and photographed with a Hitachi HU-11C electron microscope.

Semi-thin sections at 0.5-1 μ thick were stained with toluidine blue, examined and photographed with on Olympus light microscope.

Results

Brief description of the stele in the stem

Arrangement of the vascular tissue in the prostrate rhizome of *Marsilea quadrifolia* L. is solenostelic. The outer portion of the cortex is lacunate with large air spaces around radiating rows of parenchyma. The inner portion consists of a compact tissue of pith and vascular tissue. The pith is composed of parenchyma cells which become sclerified with increasing age. Fig. 1a shows the arrangement of the vascular tissue with xylem surrounded by internal and external phloem. Portion of the xylem tissue and internal phloem can be seen in Fig. 1b. Xylem has tracheary elements, while phloem is composed of sieve elements and parenchyma cells[12].

Sieve-element ontogeny

Very young sieve elements typically have angular outlines and thin walls (Fig. 2a), and contain all of the protoplasmic components typical of young parenchymatic elements. Mitochondria are numerous and vary in outline from circular to elongate (Fig. 2, 3). They have dense content and contain several well-developed cristae. The plastids are usually oval-shaped and have very few internal membranes in contrast to those of neighboring parenchyma cells whose internal membranes develop into grana

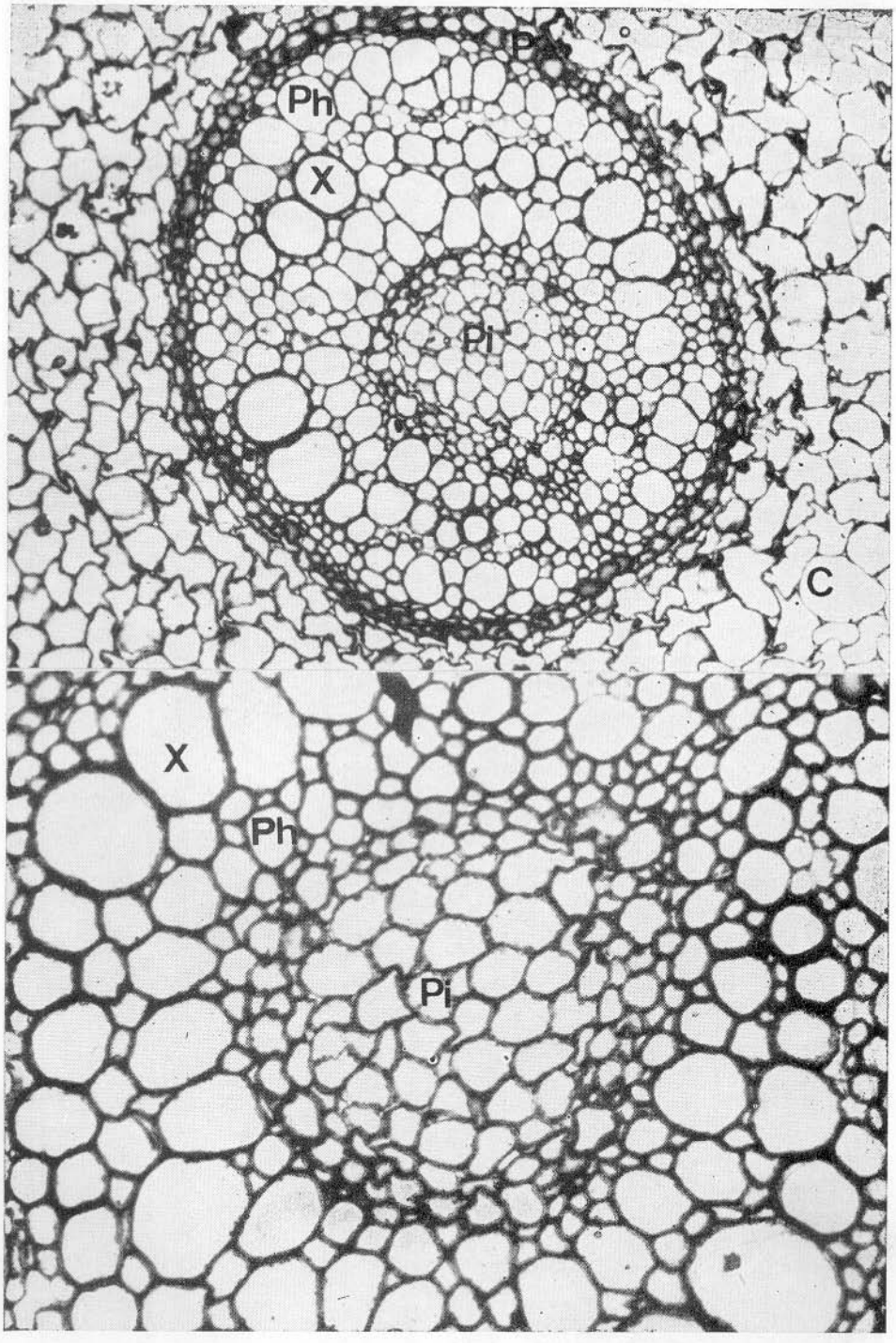


Fig. 1. Photomicrographs of transection of mature stele. a. The arrangement of the vascular tissue with xylem (X) surrounded by internal and external phloem (Ph.) Cortex (C) and pith (Pi) are shown as parts of the stele (300X). b. Details of the internal phloem and central pith (600X).

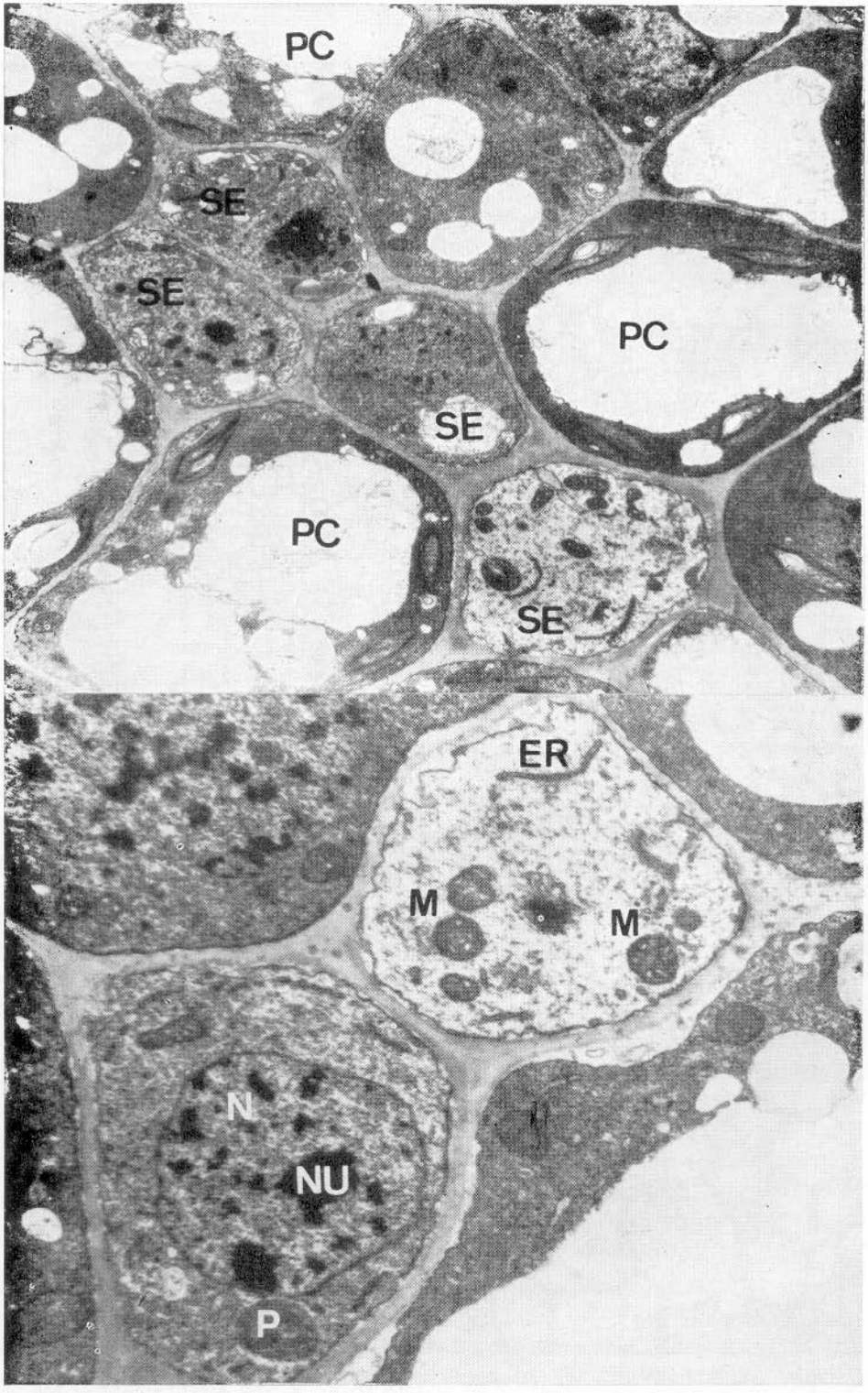


Fig. 2. Transection through portion of young vascular bundle. a. Part of young sieve elements (SE) in association with parenchyma cells (PC) (12,500X). b. Young sieve elements at higher magnification showing various organelles (15,000X). Details: ER, endoplasmic reticulum; M, mitochondrion; N, nucleus; NU nucleolus; P, plastid.

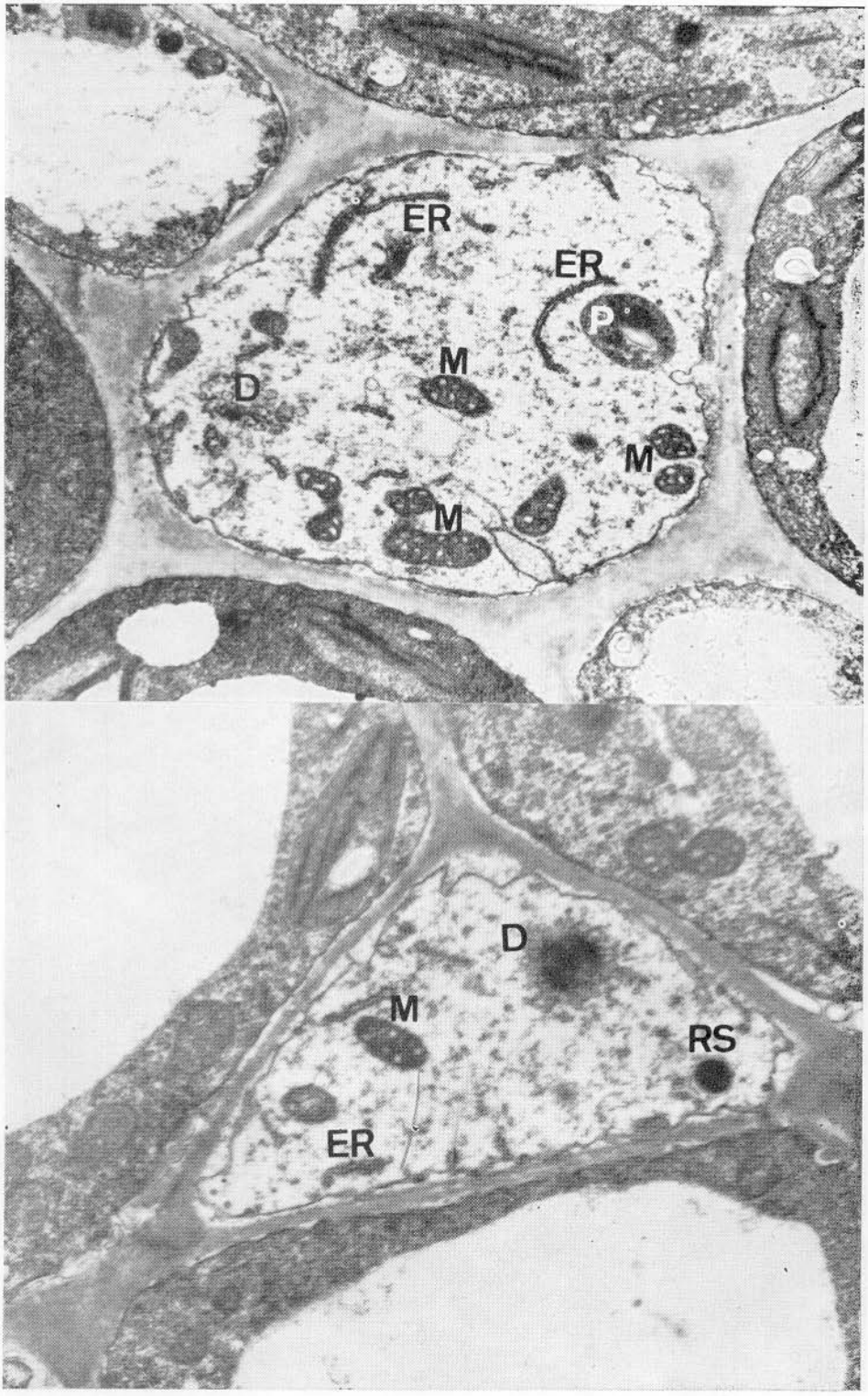


Fig. 3. Transection of immature sieve elements. a. Young element prior to iniation of wall thickening (16,250X). b. Various components of young sieve element (12,500X). Details: D, dictyosome; ER, endoplasmic reticulum; M, mitochondrion; P, plastid; RS, refractive spherule.

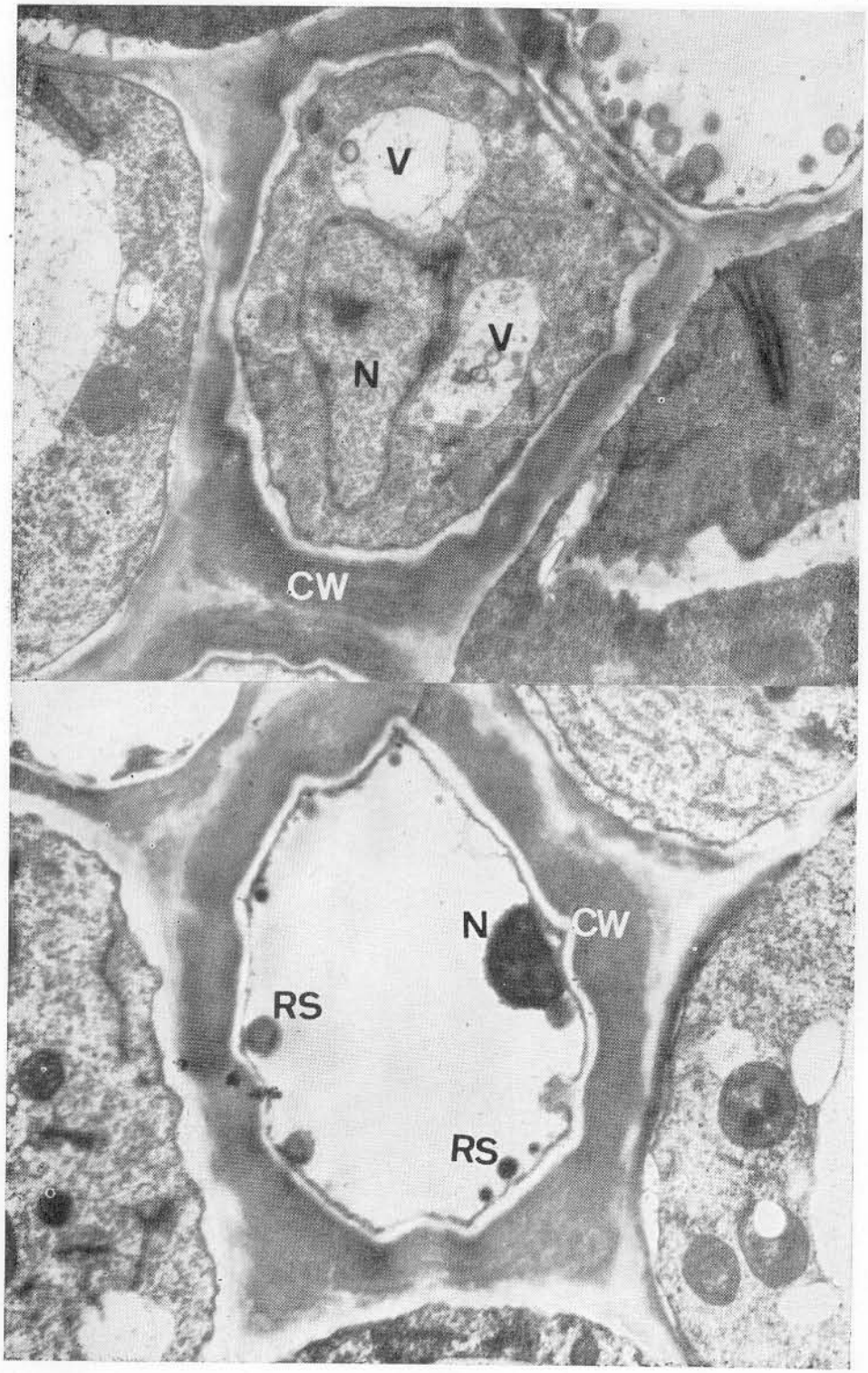
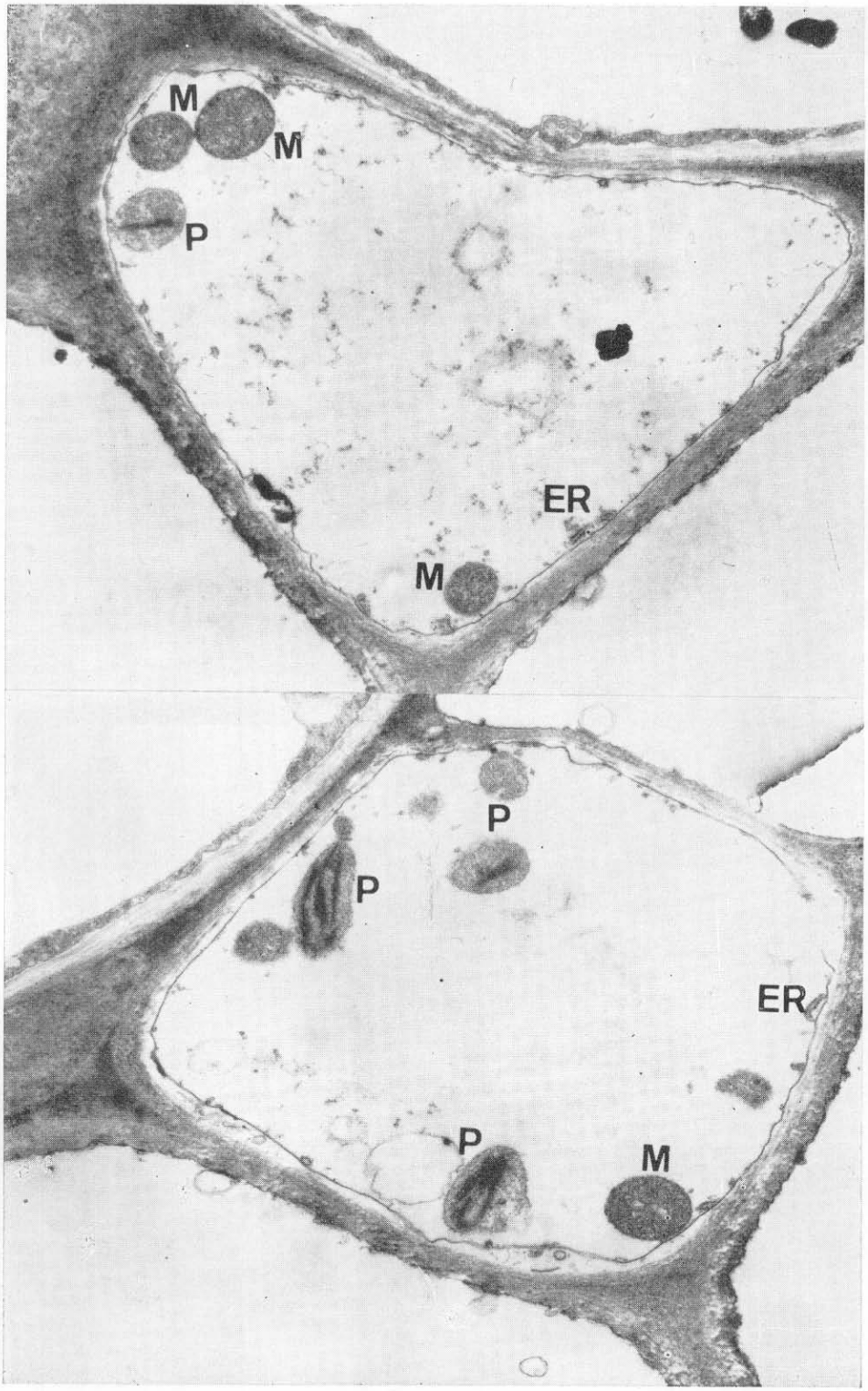


Fig. 4. Different stages in nuclear development. a. In differentiating sieve element, the cell wall (CW) has increased in thickness and nuclear degeneration is in progress (15,000X). b. Dictyosomes and ribosomes have disappeared in mature sieve element. Remnant of the degenerate nucleus and most of the cell components occur along the wall (17,000X). Details: N, nucleus; RS, refractive spherules; V, vacuole.



a.

b.

Fig. 5. Transection of mature sieve elements. a. Ribosomes are in the process of degeneration. Mitochondria appear to have fewer cristae and occur along the wall (17,500X). b. The plasmalemma-lined sieve element contains plastids and mitochondria. Most of the ER occur along the wall as a tubular network (12,000X). Details: ER, endoplasmic reticulum; M, mitochondrion; P, plastid.

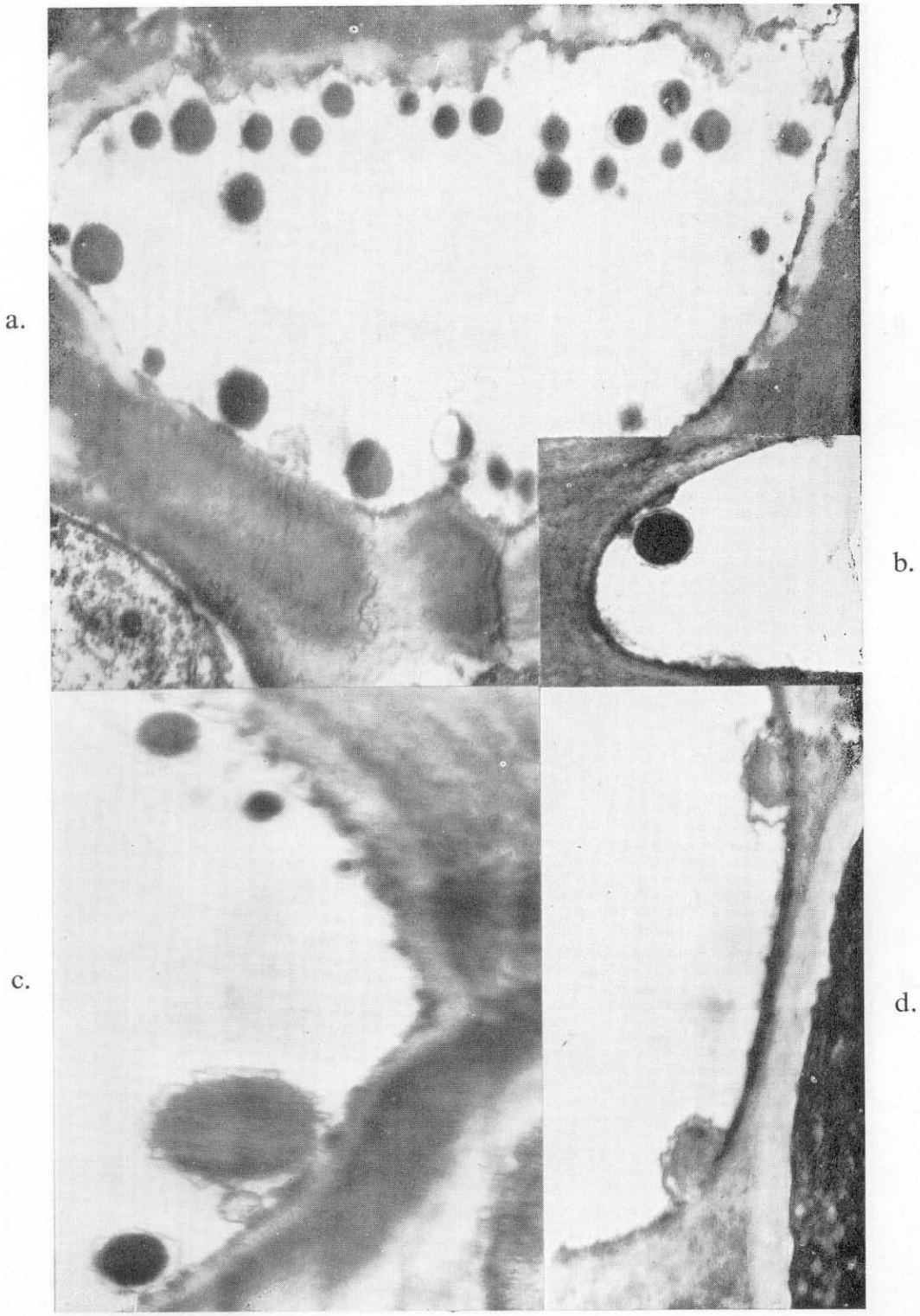


Fig. 6. Stages in refractive spherule differentiation. a. The refractive spherules are similar in appearance to those of immature elements (17,500X). b. Inset shows detail of refractive spherule (31,250X). c. As the sieve element ages, the refractive spherules become less dense and wall-like in appearance (56,250X). d. Finally the delimiting membrane of the spherules fuse with the plasmalemma and their contents are liberated into the region of the wall (31,250X).

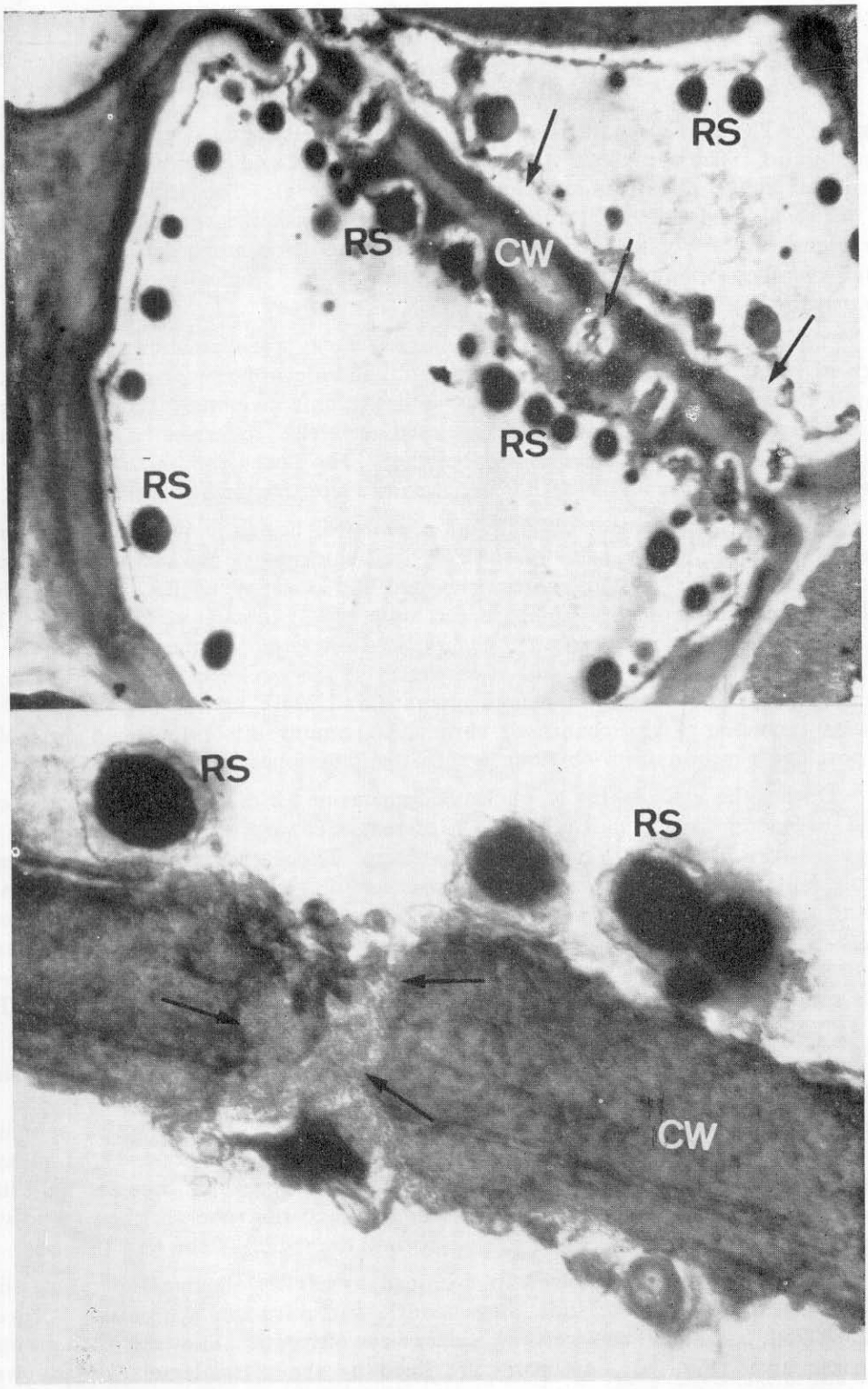


Fig. 7. Sectional view of pores between two mature sieve elements. a. Pores are aggregated into sieve area and some are occluded with callose (unlabelled arrows) (16,250X). b. The pore is filled with numerous, electron-dense, apparently tubular ER. Arrows point to plasmalemma lining pore (50,000X). Details: CW, cell wall; RS, refractive spherule.

(Fig. 3a). The sieve-element plastids have dense matrices and occasionally store few starch grains. The cytoplasm is rich in free ribosomes and contains mostly network of tubular rough endoplasmic reticulum (ER) (Fig. 3a). The ER and dictyosomes are scattered fairly evenly throughout. Fig. 3b shows the face-viewed portion of dictyosome and associated vesicles. The nucleus contains numerous masses of chromatin evenly distributed throughout the nucleoplasm. Nucleoli are apparently few in number (Fig. 2b).

Young sieve elements in *Marsilea quadrifolia* L. are characterized by the presence of refractive spherules. The refractive spherule appears as an electron-dense granule delimited from the cytoplasm by a single unit membrance (Fig. 3b). Serial sections revealed the unit membrane enclosed refractive spherules is the portion of the ER cisterna which is devoid of ribosomes. The flocculent material within the spherules is proteinaceous in nature as it gives a positive protein reaction.

Early in sieve-element differentiation, the wall begins to increase in thickness (Fig. 4a). Concomitantly, with increase in wall thickness a marked increase occurs in the production of dictyosome vesicles and the quantity of ER. At this time, nuclear degeneration begins (compare the state of the nuclei in Fig. 2b and Fig. 4a). The first sign of degeneration generally is migration of the chromatin to the periphery of the nucleus (Fig. 4a). Subsequently, the nucleolus disappears, the envelope ruptures and chromatin masses separate and move toward the cell wall. A significant portion of the condensed chromatin remains intact for some time after the envelope ruptures (Fig. 4b), but eventually it disappears.

During the early stages of nuclear degeneration all of the cellular components found in young sieve elements are still present. Toward the end of nuclear degeneration certain cytoplasmic changes take place. The ribosome population decreases in concomitance with the decrease in numbers of dictyosomes and the reduction in wall thickness (Fig. 5). Fig. 5a shows ribosome degeneration. The ER becomes smooth-surfaced and forms parietal anastomosing network. Dictyosomes and associated vesicles disappear. The remaining organelles—plastids and mitochondria—assume rounded outlines and migrate to a peripheral position within the cell (Fig. 5b). Plastids remain unchanged while mitochondria possess fewer cristae.

There is a large number of refractive spherules in the differentiating sieve elements which also become parietal in position (Fig. 6a). Fig. 6b shows the refractive spherule delimited by a single unit membrane. The refractive spherules also undergo certain modification (Fig. 6c, d). They migrate toward the plasmalemma. Along the way, the material comprising the spherules becomes 'wall-like' in appearance (Fig. 6c). The delimiting membrane of the spherule then fuses with the plasmalemma and its content is discharged into the region of the wall (Fig 6d).

At maturity, the sieve elements are lined by a plasmalemma and tubular network of ER, and contain plastids, mitochondria and refractive spherules. Pores are found on both lateral and end walls of mature sieve elements. They are often grouped into sieve areas (Fig. 7a). All pores are lined by the plasmalemma. Many were occludded with callose while others were filled with numerous, electron-dense membranes, apparently tubular elements of ER (Fig. 7b).

Discussion

Phloem investigators generally agree that the mature sieve element lacks a nucleus at maturity. During the late 1960s and 1970s new reports were made of the presence of nuclei in some mature sieve elements in a wide variety of higher vascular plants, including some conifers, monocotyledons, and dicotyledons[13]. In most cases these nuclei were described as having a degenerated appearance.

The behavior of the nucleus during maturation of the sieve element is quite variable in the lower vascular plants examined thus far. In this study, the nuclear degeneration of *Marsilea quadrifolia* L. sieve element involves the migration of the chromatin to the periphery of the nucleus, the subsequent disappearance of nucleolus, and the rupture of nuclear envelope. Degenerate nuclei do not always persist in mature sieve elements. If they persist, they appear as a mass of condensed chromatin located adjacent to the wall of the sieve elements. In most cases, the chromatin material and nuclear envelope eventually disappear, so that the sieve element is enucleate at maturity. Like the other ferns, *Platycerium* and *Phlebodium*[1], the nuclear chromatin of *Marsilea* undergoes no apparent increase in quantity or density, nor does it accumulate into a more or less continuous mass prior to rupture of the nuclear envelope. On the other hand, nuclear degeneration in other lower vascular plants, *Isoetes*[2-4] and *Equisetum*[5,6], involves the apparent increase in quantity and density of the chromatin material prior to rupture of the nuclear envelope. This is said to be the pycnotic nuclear degeneration which is not observed in the species studied.

The most distinguishing feature of the sieve-element protoplast of most lower vascular plants including *Marsilea* is the presence of highly refractive granules termed refractive spherules. Although reports of the composition of the refractive spherules have varied, most investigators have found the spherules to be proteinaceous in nature[7].

Refractive spherules have been reported to be present in most ferns, such as *Botrychium*, *Marattia*, *Pityrogramma*, and Polypodiaceous ferns[8], and some other lower vascular plants, *Psilotum*[9] and *Equisetum*[5,6]. On the other hand, recent ultrastructural studies of the Lycopsids *(Selaginella*[10], *Isoetes*[2-4], and *Lycopodium*[11]*)* indicated that refractive spherules are lacking in this group of lower vascular plants. However, substantial quantities of crystalline and fibrillar proteinaceous material were encountered in small vacuoles of *Selaginella* and in ER cisternae of *Isoetes*.

In *Psilotum* and *Equisetum*, refractive spherules apparently arise in cisternae of smooth ER. Results of the present study indicate that the refractive spherules in *Marsilea* also develop in dilated portions of the ER. However Evert and Eichhorn[1] have implicated the Golgi apparatus in formation of the refractive spherules in *Platycerium* and *Phlebodium*. Apparently the granular material comprising the refractive spherules in *Platycerium* and *Phlebodium* accumulates in dictyosome-derived vesicles, which eventually fuse with the plasmalemma. In this manner, the refractive spherule is deposited into the region of the wall. Deposition of refractive spherules into the region of the wall was observed in the mature sieve elements of *Marsilea* during the present study.

The development of proteinaceous inclusions in ER cisternae is fairly common in both plant and animal tissues. ER associated protein bodies have been reported in resting cambial cells[14,15], in fleshy cotyledons of exalbuminous seeds[16], in endosperm[17], and in nucellar tissue[18]. These bodies generally are regarded as storage protein. They are not secreted from the protoplast, as are the refractive spherules of *Marsilea* sieve elements. The exact nature and role of the refractive spherules remain to be determined.

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บทคัดย่อ

จากการศึกษาโครงสร้างและการเจริญเติบโตของเนื้อเยื่อที่ทำหน้าที่ลำเลียงอาหาร (phloem tissue) ในพืชชั้นต่ำพวกผักแว่น *(Marsilea quadrifolia)* ด้วยกล้องจุลทรรศน์อิเลคตรอน พบว่า sieve elements ซึ่งเป็นเซลล์ที่สำคัญในการลำเลียงอาหาร มีลักษณะสำคัญคือ ภายในเซลล์มี inclusion ที่มีสารประกอบพวกโปรตีนอยู่เรียกว่า refractive spherules โปรตีนนี้อาจมีความสำคัญเกี่ยวข้องกับการลำเลียงอาหารของพืชชนิดนี้ ใน sieve element ที่เจริญเต็มที่แล้ว พบว่ามีการสลายตัวของพวก organelles และ membrane system ต่างๆ เช่น nucleus, Golgi bodies, ribosomes, microtubules ส่วนที่เหลืออยู่คือ endoplasmic reticulum, plastids, mitochondria, และ refractive spherules ซึ่งจะอยู่ตามขอบเซลล์ การสลายตัวของ organelles นี้เข้าใจว่ามีส่วนเกี่ยวข้องโดยตรงกับการลำเลียงอาหาร เพราะเกิดขึ้นเมื่อ sieve element โตเต็มที่และทำหน้าที่ลำเลียงโดยสมบูรณ์แล้ว