# ULTRASTRUCTURAL STUDY ON THE DEVELOPMENT OF CRYSTAL-FORMING SCLEREIDS IN *NYMPHAEA TETRAGONA*

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**Abstract:** The sclereids of *Nymphaea tetragona* Georgi originate sporadically from the fundamental parenchyma. They undergo the following developmental phases: (1) phase of hypertrophic growth and ramification; (2) phase of crystal formation; and (3) phase of sclerification. The distinguishable features of sclereid initials are their large nucleus, dense cytoplasm with abundant organelles and the conspicuous branching of the cells. The formation of calcium oxalate crystals in the cell wall of developing sclereid generally occurs after its shape is attained. Between the plasmalemma and the primary cell wall many crystal chambers are formed, which are bounded by crystal sheath. Mitochondria, dictyosomes, and endoplasmic reticulum often occur in close association with the plasmalemma surrounding the crystal chambers. At maturity, the sclereids possess extremely thick walls and the crystals are embedded between the primary and secondary wall. In plant cells the calcium oxalate crystals are generally located in the central vacuoles.

## INTRODUCTION

Sclereids are found in various plant organs. Their shape and location in a plant body vary in different taxa. But within a given taxon they are specific and some investigators have used them in classification (Mauseth, 1988). Many reports have been concerned with morphological significance of the sclereids as well as their distribution in the plant bodies (Foster, 1944, 1947; Rao and Dave, 1984; Alvin and Rao, 1987; Staff and Clifford, 1987), but little is known about their development at the ultrastructural level (Boyd *et al.*, 1982; Harris, 1983).

In *Nymphaea* the morphology, anatomy and distribution of sclereids have been studied by light microscopy (Guertler, 1905; Gandet, 1960; Chiang and Huang, 1983, 1984), scanning electron microscopy and X-ray microanalysis (Kuo-Huang, 1990). The sclereids of *Nymphaea tetragona* Georgi are found as a form of idioblasts which are sporadically distributed in the parenchymatous tissue and frequently they are around the air channels (Chiang and Huang, 1983). The sclereids in *Nymphaea tetragona* are quite different in their forms, but all of them bear numerous calcium oxalate crystals on the outer surface (Kuo-Huang, 1990). Calcium crystals can often be observed in many plant species (Franceschi and Horner, 1980). Generally, the formation of calcium oxalate crystals is found within vacuoles. However, the crystals of *Nymphae* occur only in the extraplasmic space between the primary and secondary cell wall of the sclereids. Therefore, the differentiation of sclereids in *Nymphae* represents an interesting developmental

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system, especially concerning the crystal formation in the cell wall. This investigation describes the ultrastructural changes of the sclereids in *Nymphaea tetragona* from initiation to maturity.

## MATERIALS AND METHODS

The leaves of *Nymphaea tetragona* Georgi in various stages of development were collected from aquaria in the greenhouse of the Department of Botany, National Taiwan University.

Portions of leaves containing sclereids were dissected into small pieces and routinely fixed in 2.5%-5% glutaraldehyde and 3%-4% paraformaldehyde in 0.1 M sodium cacodylate buffer, followed by postfixation in 1% $OsO_4$ (same buffer), then dehydrated in an acetone series, infiltrated and embedded in Spurr's resin under vacuum (Spurr, 1969). Thin sections for electron microscopy were cut on a Reichert-Jung Ultracut-E by using a diamond knife, stained with uranyl acetate and lead citrate, and examined by a Hitachi H-600 TEM at 75 kV. For light microscopy 1 $\mu$m sections were stained with 1% toluidine blue buffered with borax.

For SEM material was processed as for TEM up to absolute acetone, then dried with a Hitachi Critical Point Dryer (HCP-1), coated with IB-2 ion coater, and examined with the Hitachi S-550 SEM.

## RESULTS AND DISCUSSION

In *Nymphaea tetragona* the sclereids sporadically originate from parenchymatous cells of the fundamental tissue (Chiang and Huang, 1984). They undergo the following developmental phases: (1) phase of hypertrophic growth and ramification; (2) phase of crystal formation; and (3) phase of sclerification (Chiang and Huang, 1984). Within a given section from the leaf of *Nymphaea*, the sclereids are often in different phases of development. As the sclereid initial cells in palisade tissue begin to undergo hypertrophic growth or mification, the sclereids in spongy tissue are already in the phase of crystal formation or sclerification (Chang and Huang, 1984). But in general, all sclereids in a leaf of *Camellia* originate and develop simultaneously (Boyd *et al.*, 1982).

### Phase of hypertrophic growth and ramification

Similarly to other reports (Foster, 1947; Harris, 1983; Alvin and Rao, 1987), the distinguishable feature of sclereid initial cell in *Nymphaea* was its large nucleus and prominent nucleolus (Figs. 1, 6, 7). The large nuclei that characterize large differentiated cell types are common in laticifer initials of *Euphorbia* (Mahlberg and Sabharwal, 1968), and in epidermal trichoblasts of *Hydrocharis* (Cutter and Feldmann, 1970). During the ramification phase the nucleus of sclereid initial displays unique undulation of the nuclear membrane (Figs. 1, 6, 7). In the fibers of *Lolium* (Juniper *et al.*, 1981) and in the foliar sclereids of *Camellia* (Boyd *et al.*, 1982) lobed nuclei are reported, nevertheless in the macrosclereid of seed coat in *Pisum* (Harris, 1983) the nucleus is elongated.

When the initial cells of *Nymphaea* formed their several branches (Figs. 18, 19), large central vacuoles were observed and the cytoplasm was confined to a thin layer around the cell periphery (Figs. 1, 4). The cytoplasm in the branches of

the initial cells (Figs. 1-9) contained numerous organelles as normally associated with actively growing cells. Generally, the plastids in the initial cell were smaller than those in the neighbour mesophyllous parenchymatous cells and they frequently possessed starch grain, few thylakoids and plastoglobuli (Figs. 1, 4). Mitochondria were more numerous in initial cells than in surrounding cells and appeared to have the elongated forms (Figs. 4, 8). Mostly the axes of endoplasmic reticulum, plastids and mitochondria were parallel to the axes of the sclereids or the branches of the sclereids (Figs. 2, 4, 7, 8). Microtubular network was evidently by adjacent to the developing cell wall (Fig. 10).

During this phase of development, the initial cell was surrounded by a thin primary cell wall that was extensible and had a smooth surface (Figs. 18, 19). The branches of the sclereid initial cells could invade adjacent intercellular spaces or even to force their way between neighboring cells by apical intrusive growth (Figs. 2, 4). The primary wall on the branches were thinner than those of the neighboring parenchymatous cell (Fig. 5).

When the sclereid initials of palisade tissue underwent hypertrophic growth, the neighboring palisade parenchymatous cells were divided into two to three rows of cells (Fig. 1).

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## Key for Labels

A: air channel; C: chloroplast; Cr: crystal; D: dictyosome; El: lower epidermis; Er: endoplasmic reticulum; Eu: upper epidermis; Is: intercellular space; M: mitochondrion; Mt: microtuble; N: nucleus; O: oil droplet; P: plastid; Pm: plasmalemma; S: starch grain; Sd: sclereid; V: Vacuole; Wp: Primary cell wall of sclereid initial; Wpp: Primary cell wall of parenchymatous cell; Ws: Secondary cell wall of sclereid.

Figs. 1-10. Transmission electron micrographs of the initial cells of sclereid in *Nymphaea tetragona* during hypertrophic growth and ramification.

Figs. 1, 3. Sclereid initial cell in palisade tissue showing a large nucleus, central vacuole and dense peripheral cytoplasm.

Figs. 2, 4. Sclereid initial cell in spongy tissue showing the branch invading adjacent intercellular spaces or forcing its way between neighboring cells.

Figs. 5-10. Cytoplasm in the branches of initial cells showing an undulating nucleus, many mitochondria, dictyosomes, endoplasmic reticulum, and microtubular network adjacent to the cell wall.

Figs. 11-17, 22. Electron micrographs of the initial cells of sclereid in *Nymphaea tetragona* during crystal formation.

Figs. 11-14. Crystal chambers containing fibrillar materials and bounded by a crystal sheath (▷).

Figs. 15-17, 22. Crystal chambers formed sporadically, the cytoplasm near the chambers showing many mitochondria, dictyosomes and rough endoplasmic reticulum.

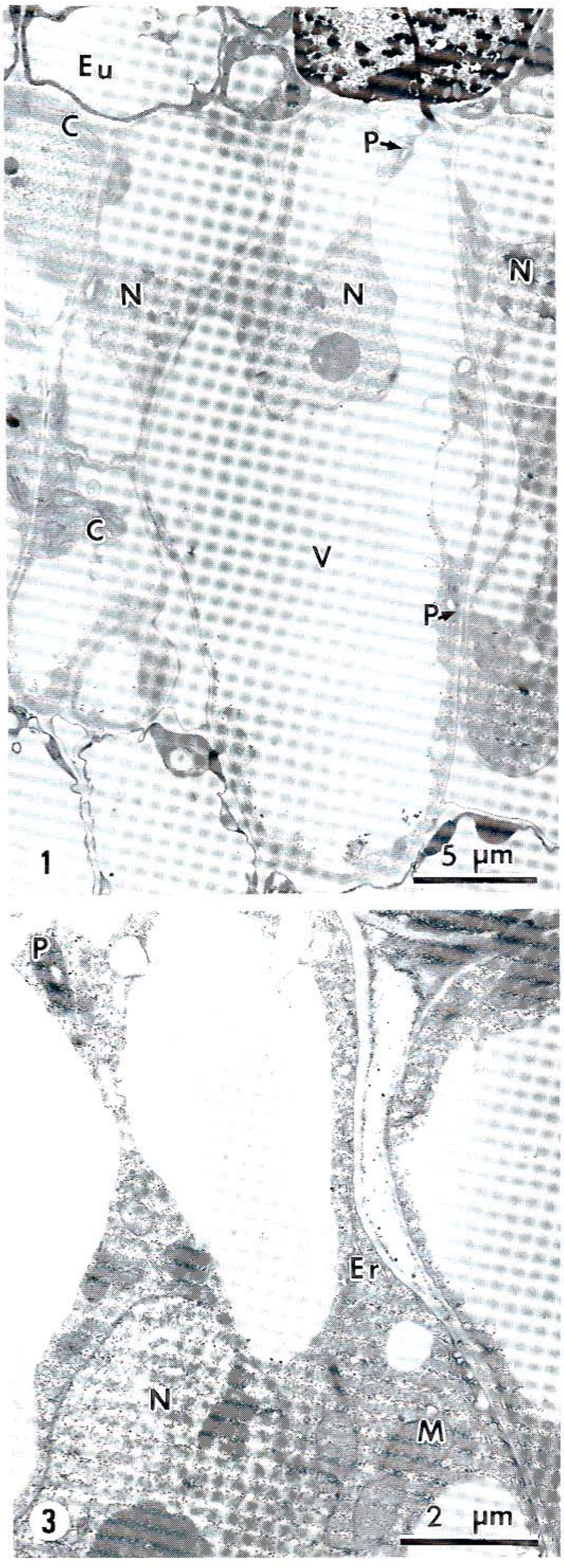
Figs. 18, 19. SEM photographs of the sclereid initial cell in *Nymphaea tetragona* during the late ramification or early crystal formation phase. The initial cell showing branches with smooth outer surface.

Figs. 20, 21. SEM photographs of the mature sclereid in *Nymphaea tetragona*. The sclereid showing branches with crystals protruded from the outer surface.

Figs. 23-27. Electron micrographs of the sclereid in *Nymphaea tetragona* during sclerification.

Figs. 23-25. Secondary wall deposited between the plasmalemma and the primary cell wall.

Figs. 26, 27. Crystals embedded between the primarimary and secondary wall.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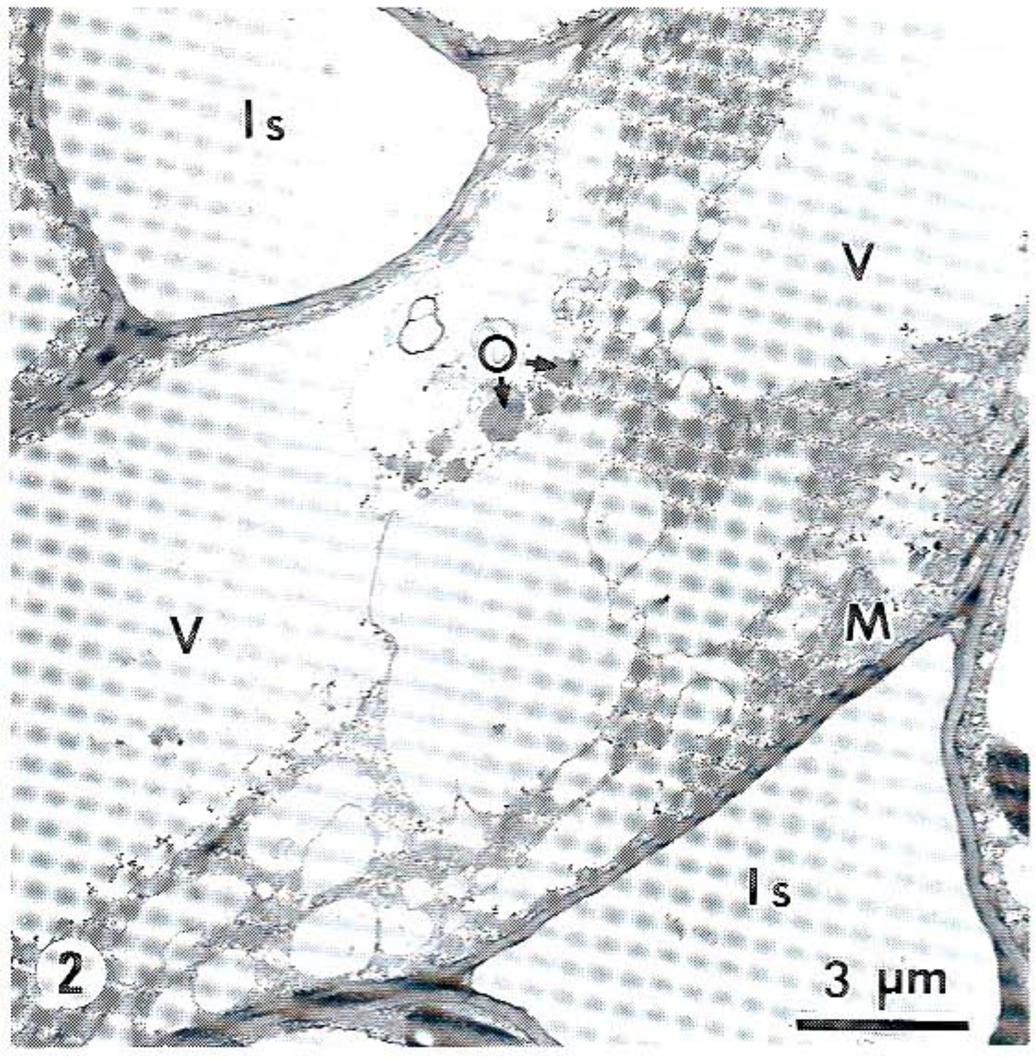
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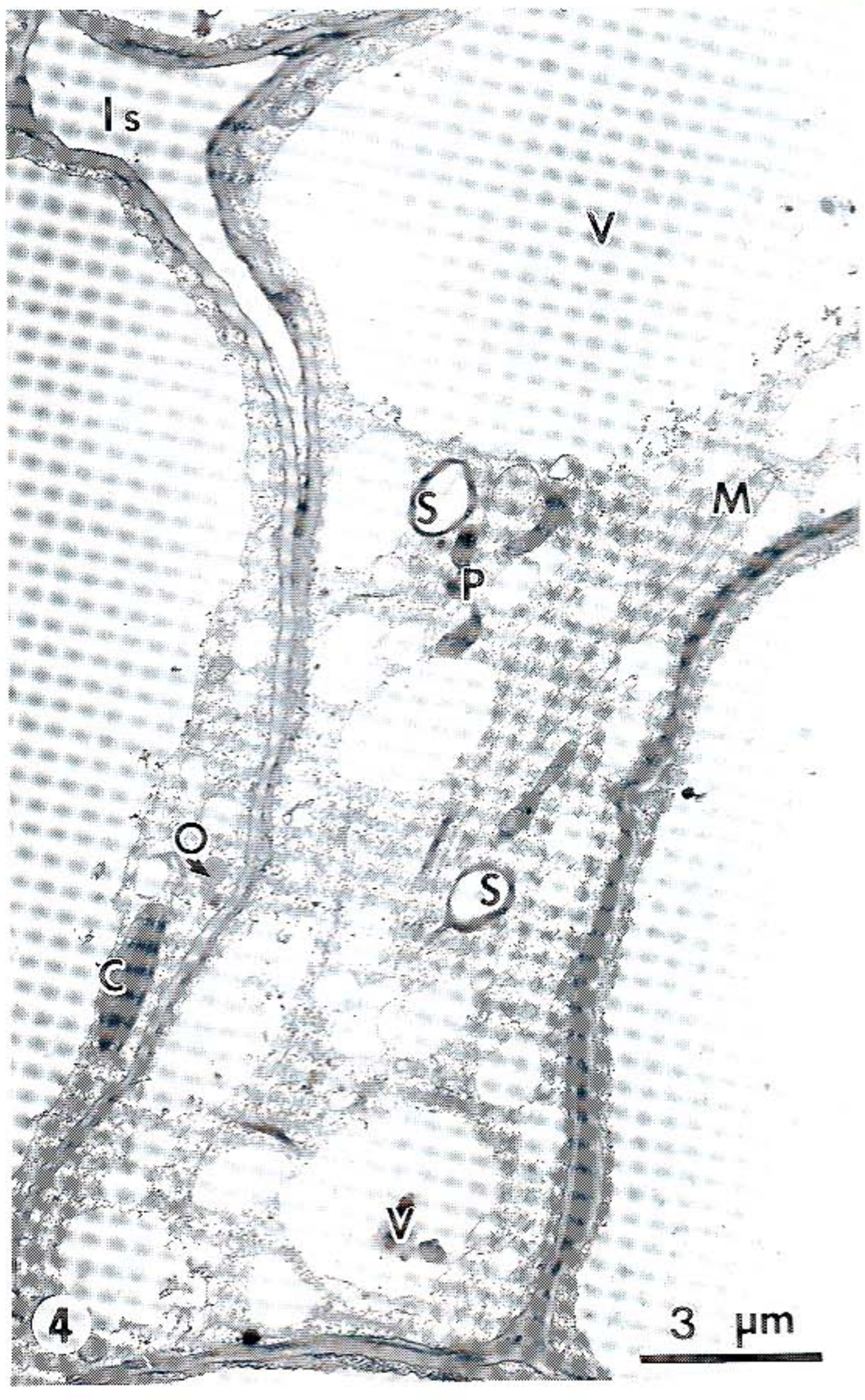
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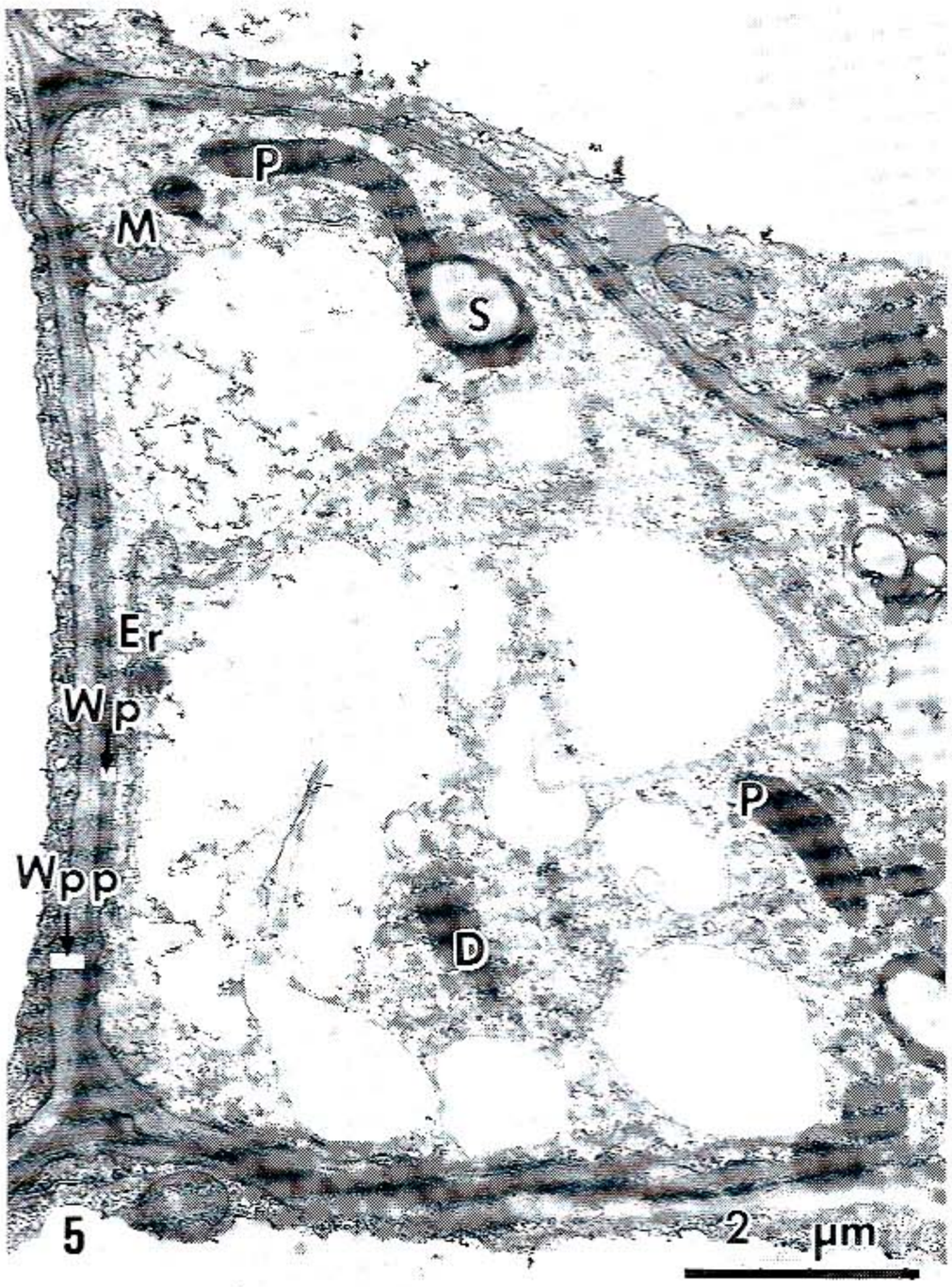
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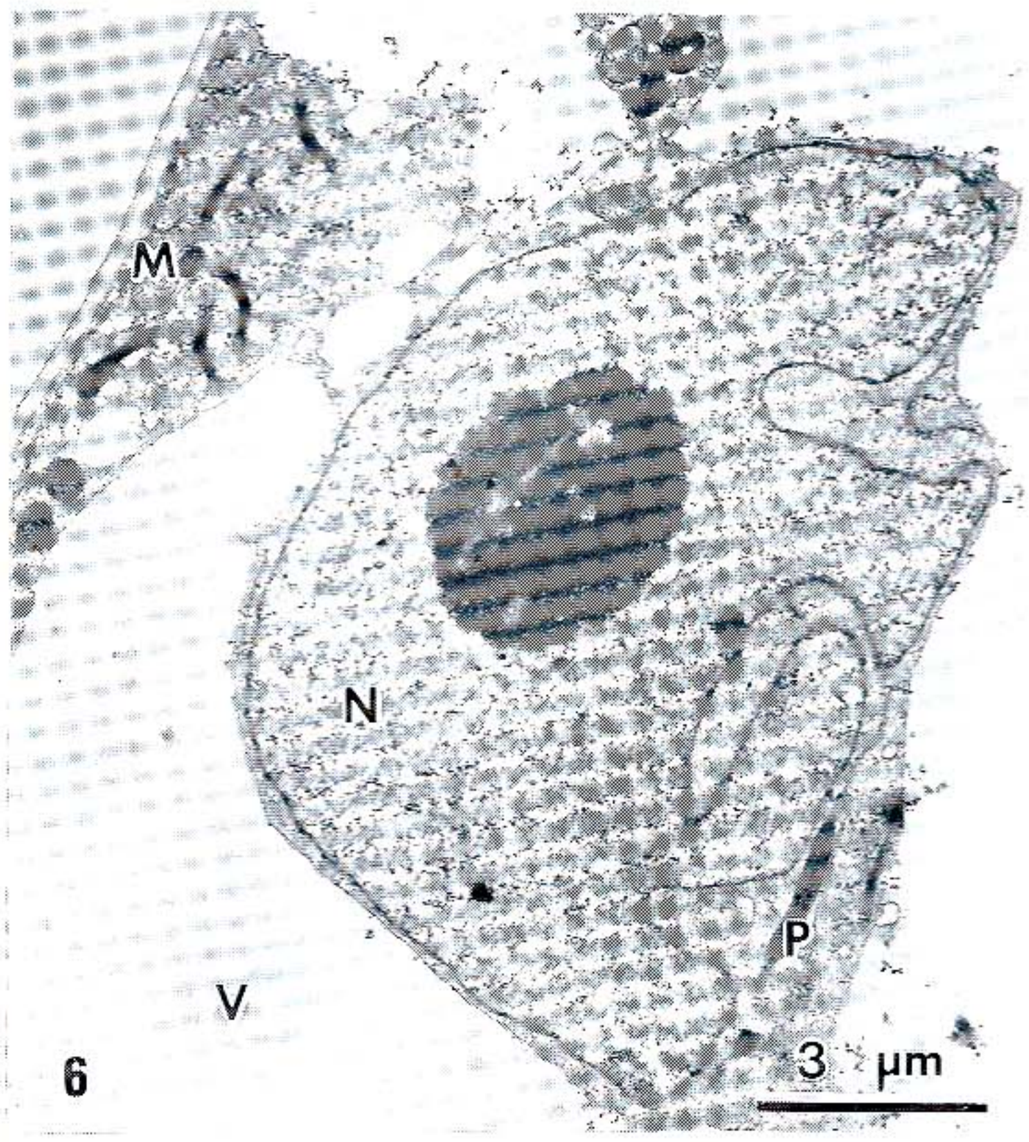
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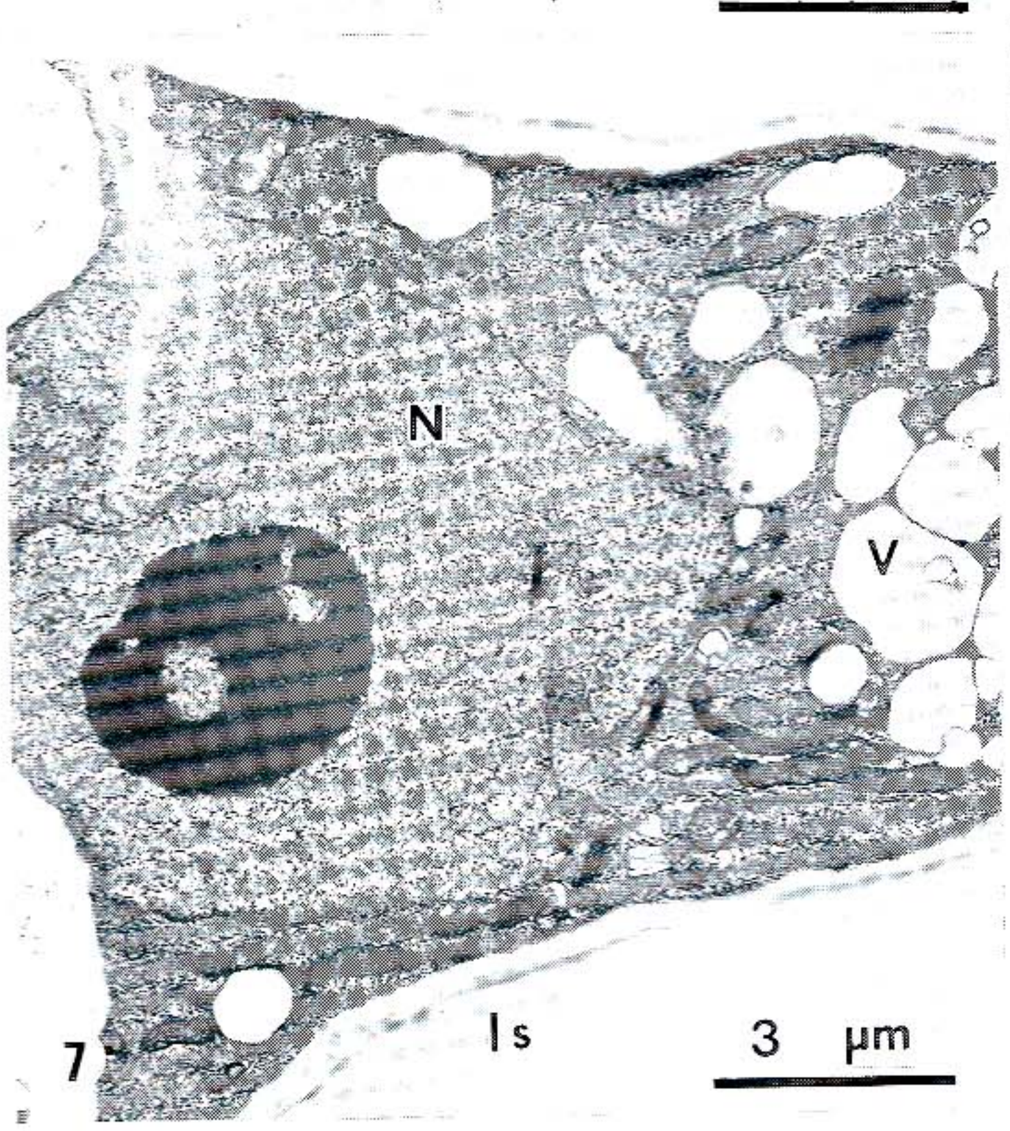
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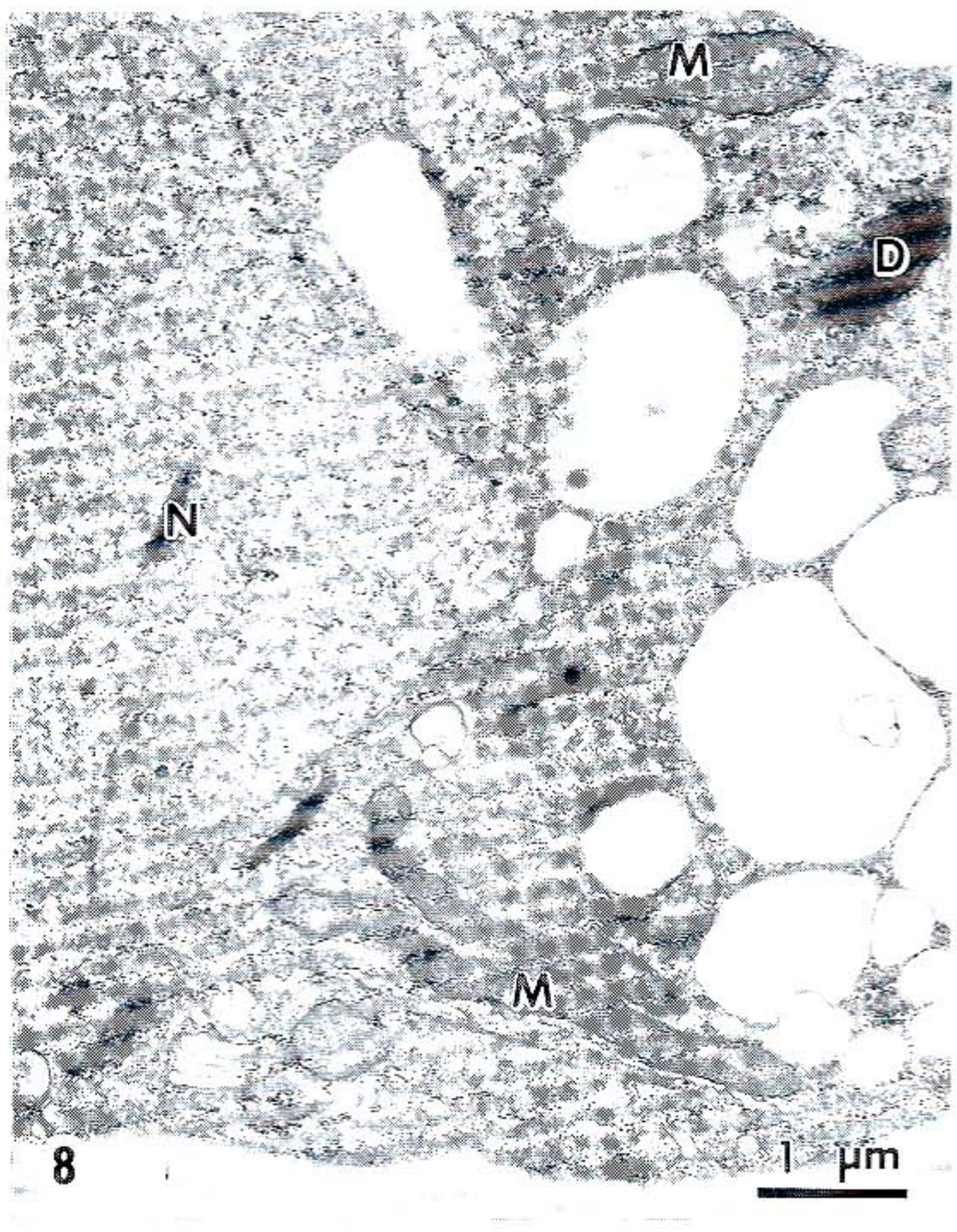
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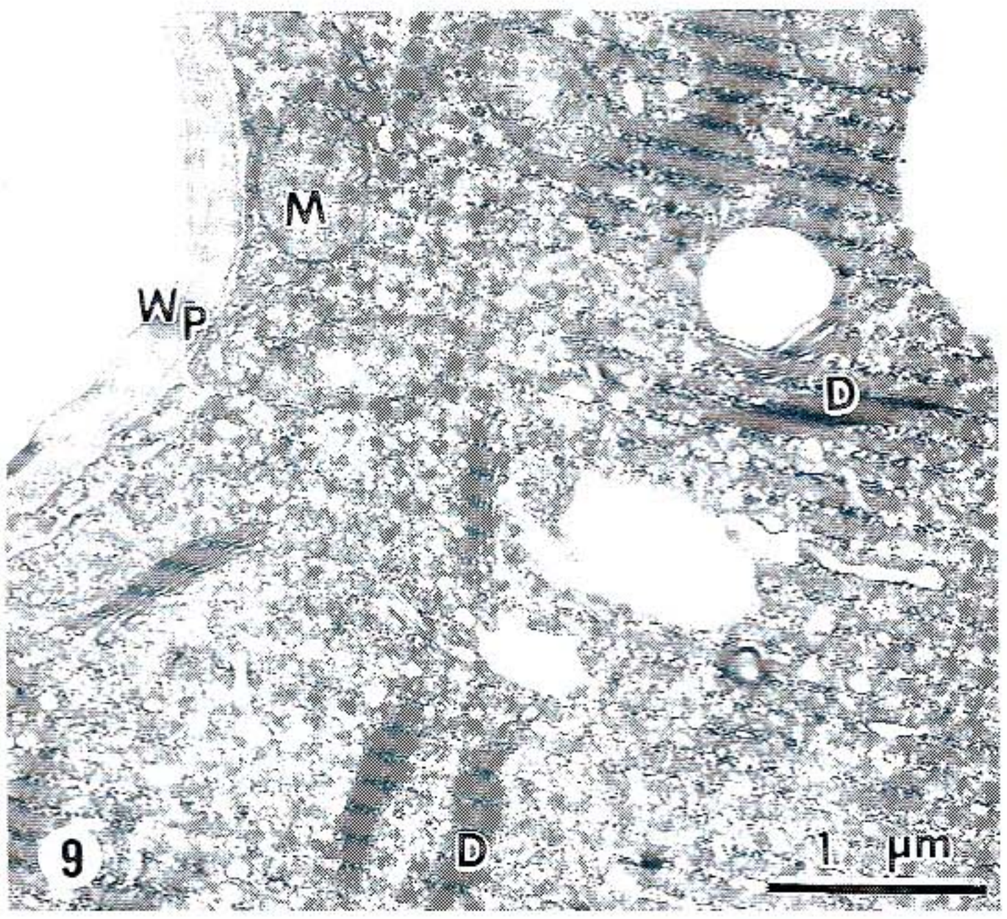
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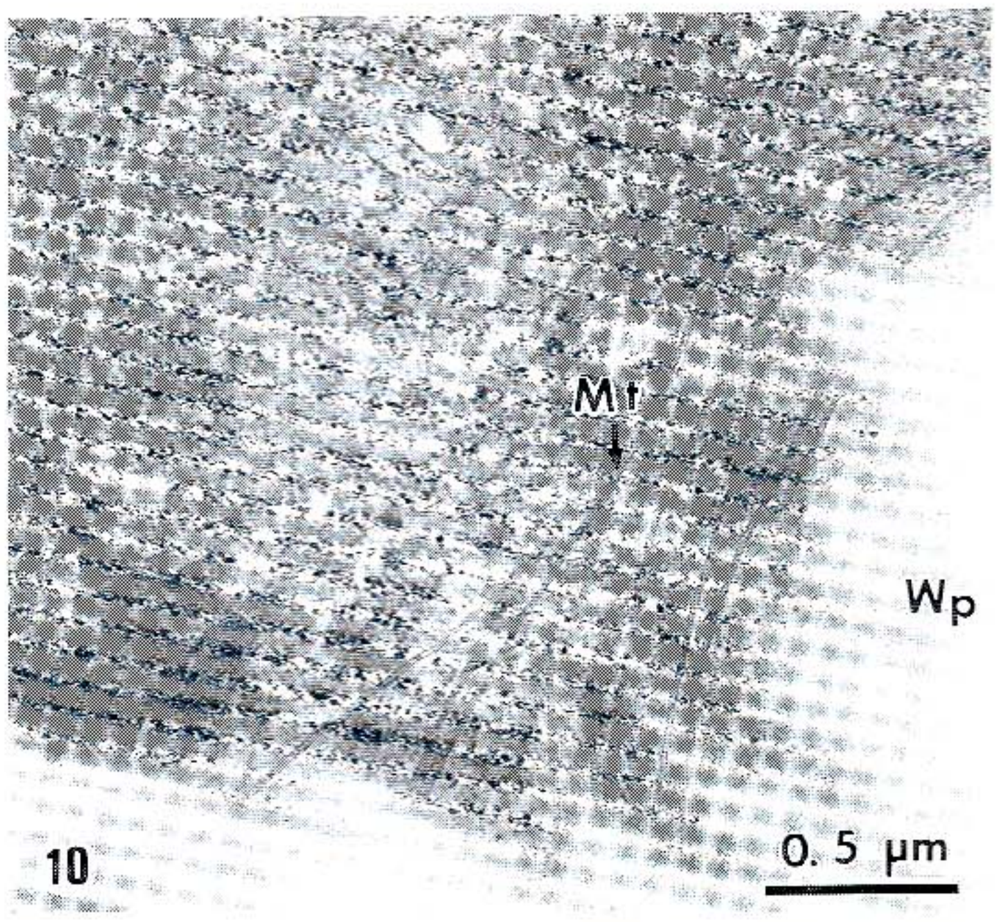
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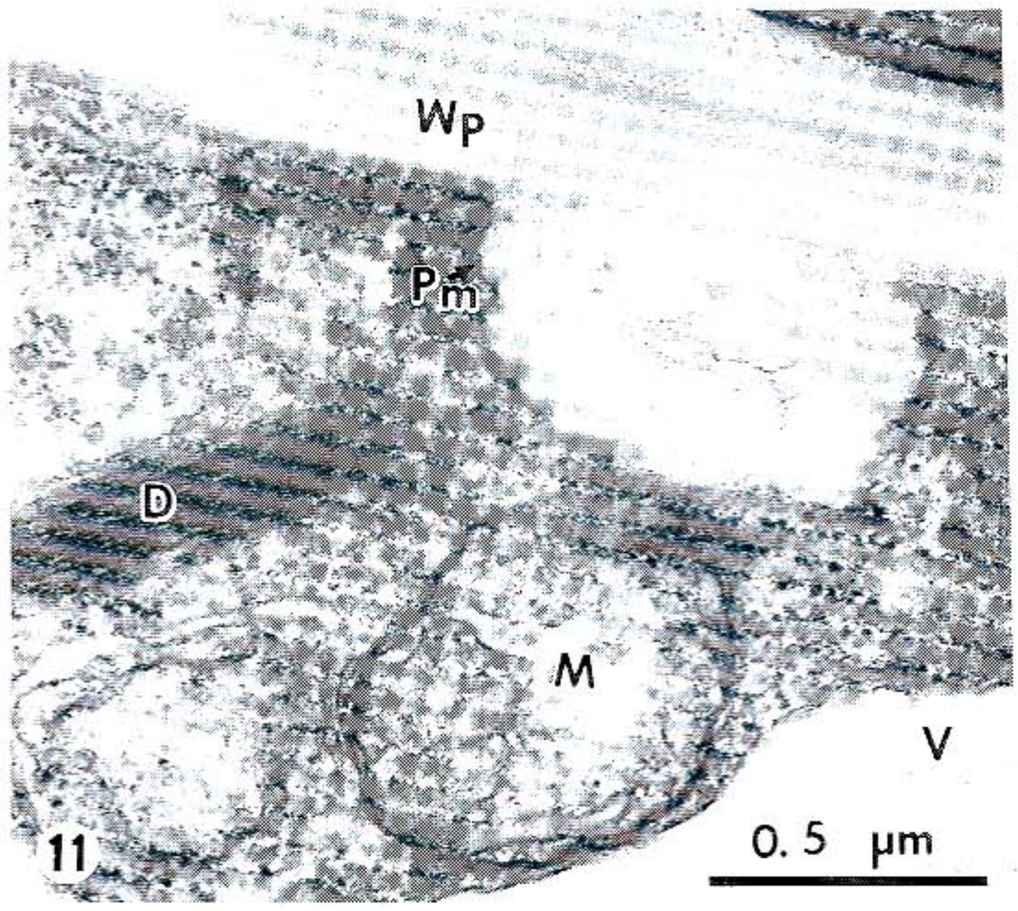
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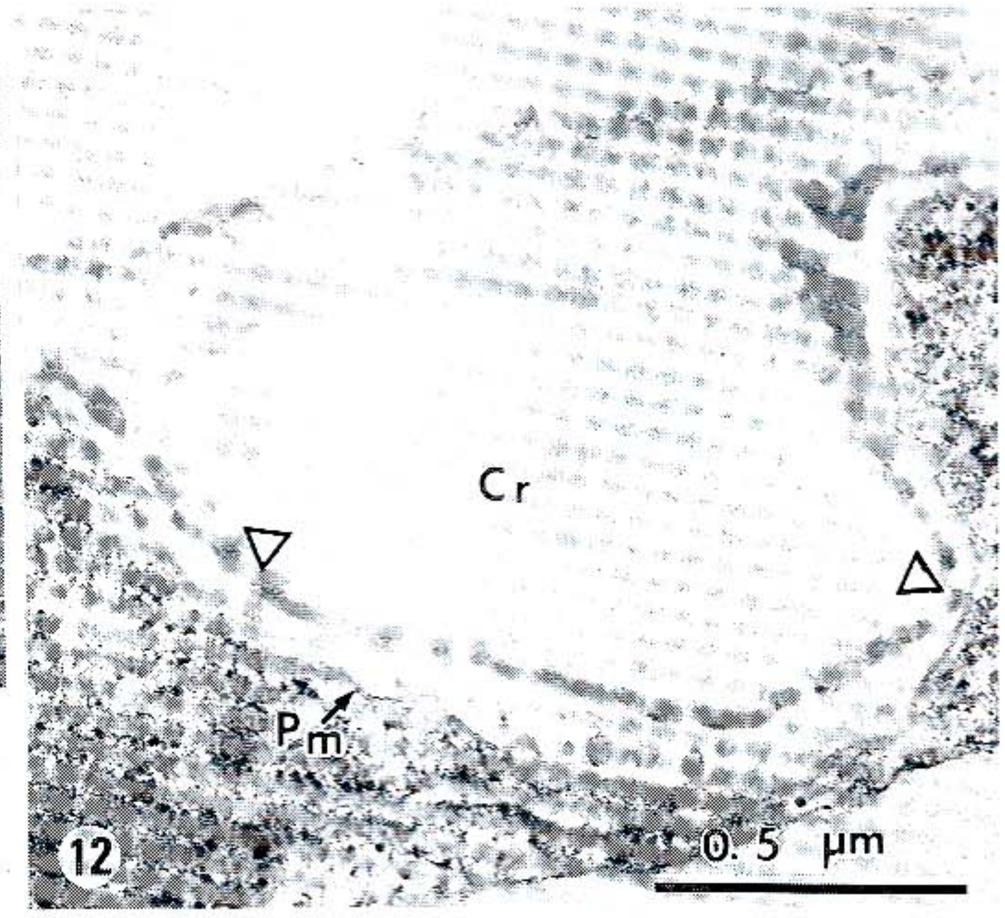
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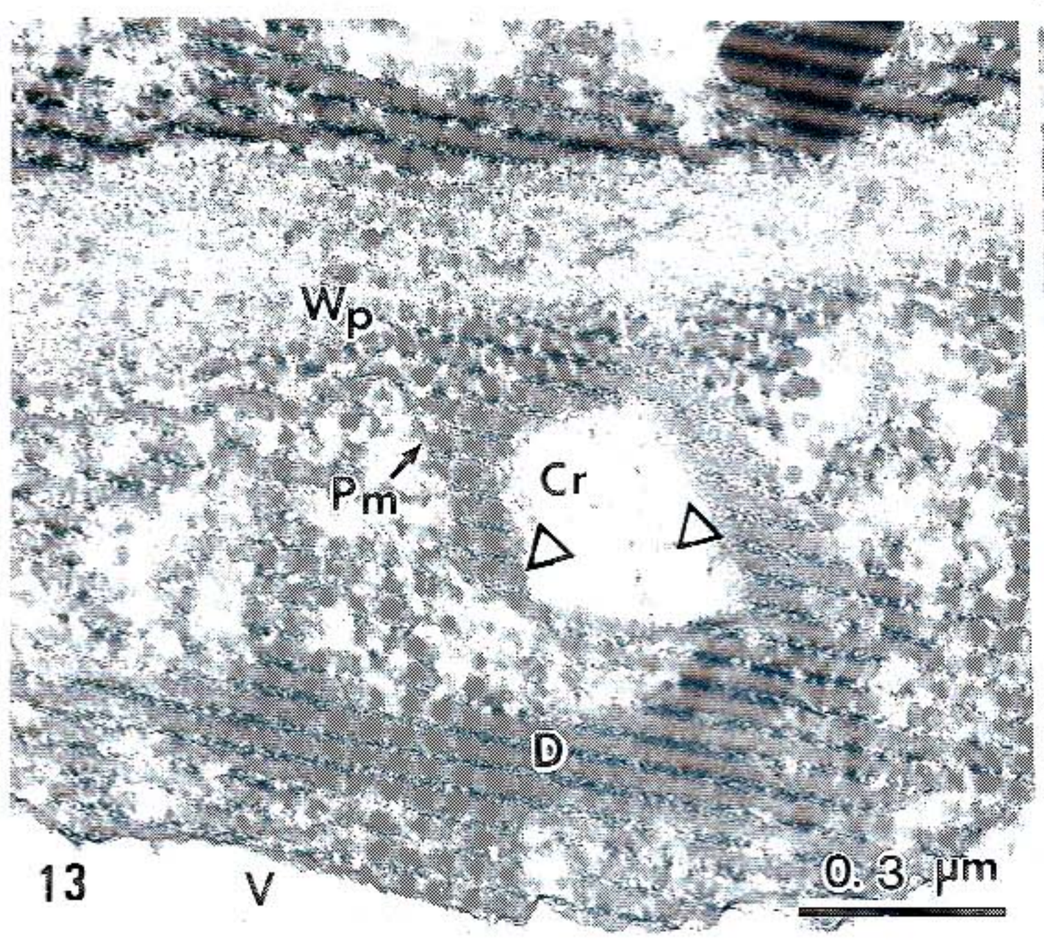
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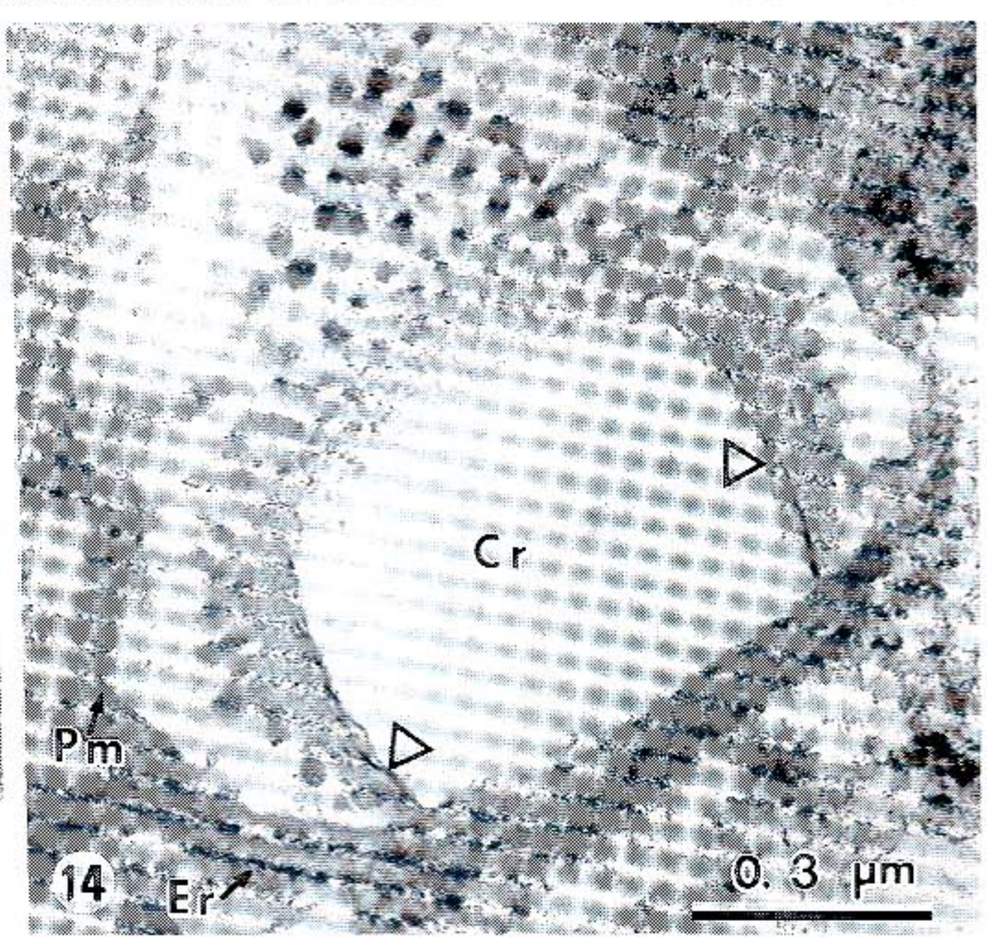
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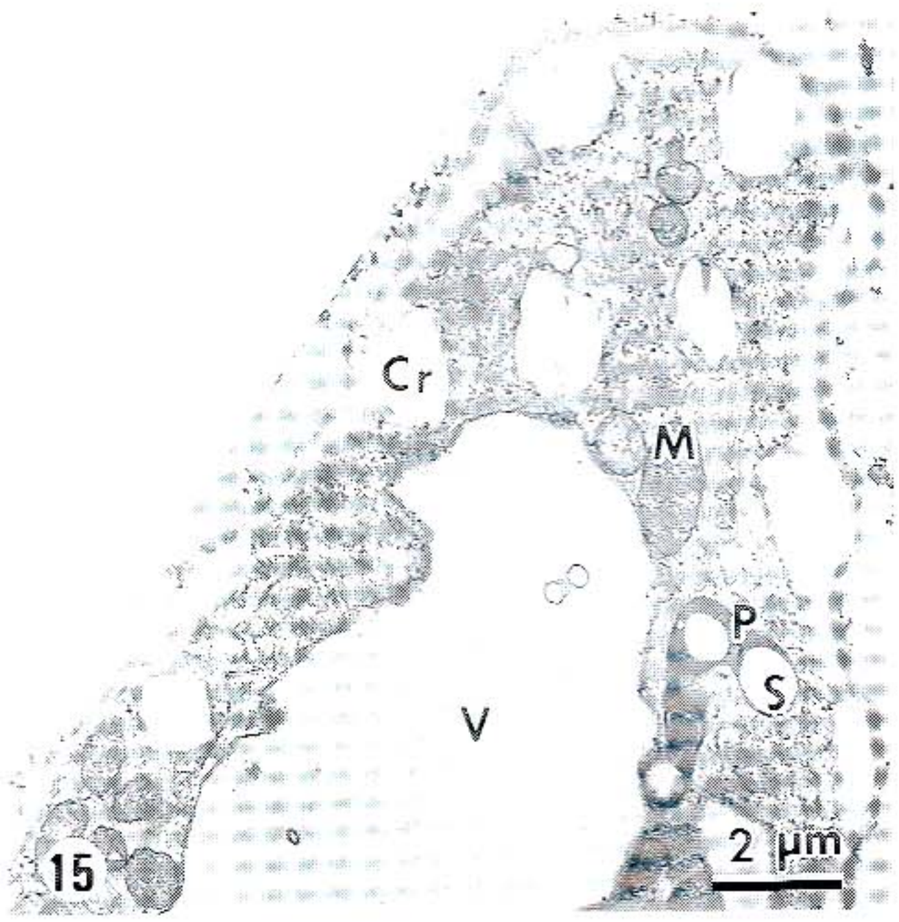
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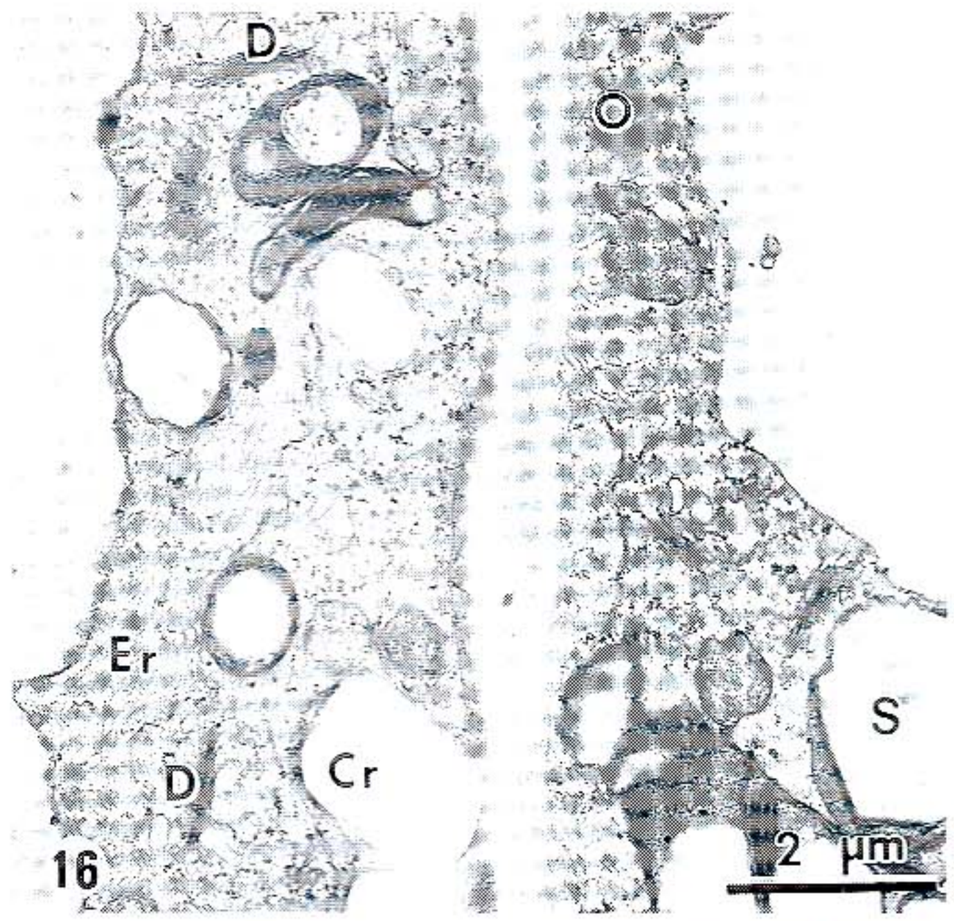
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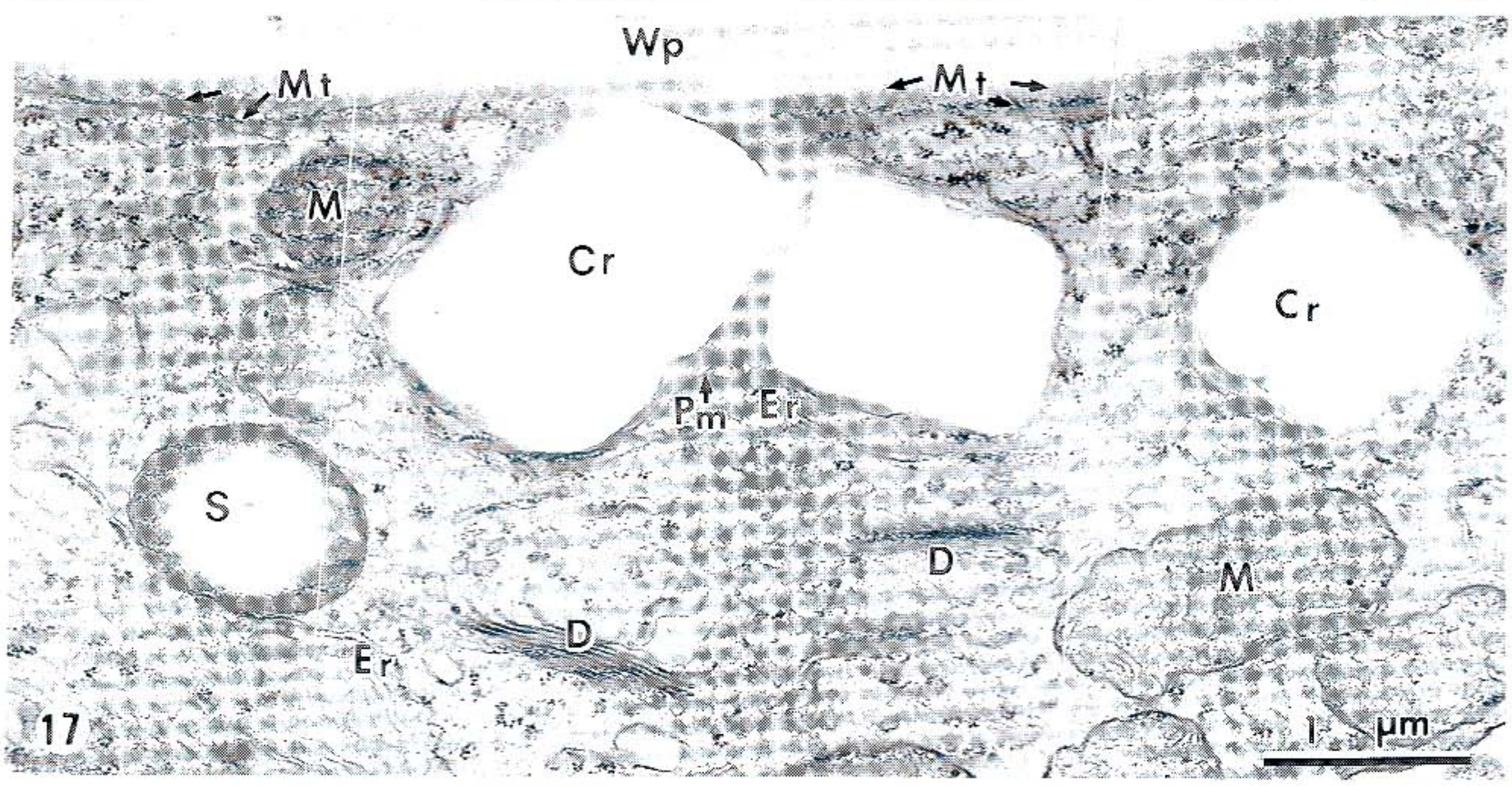
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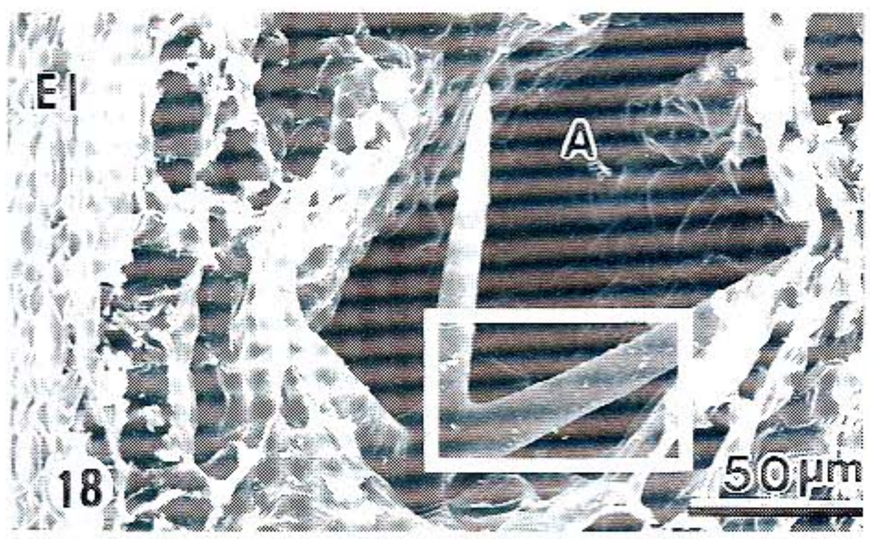
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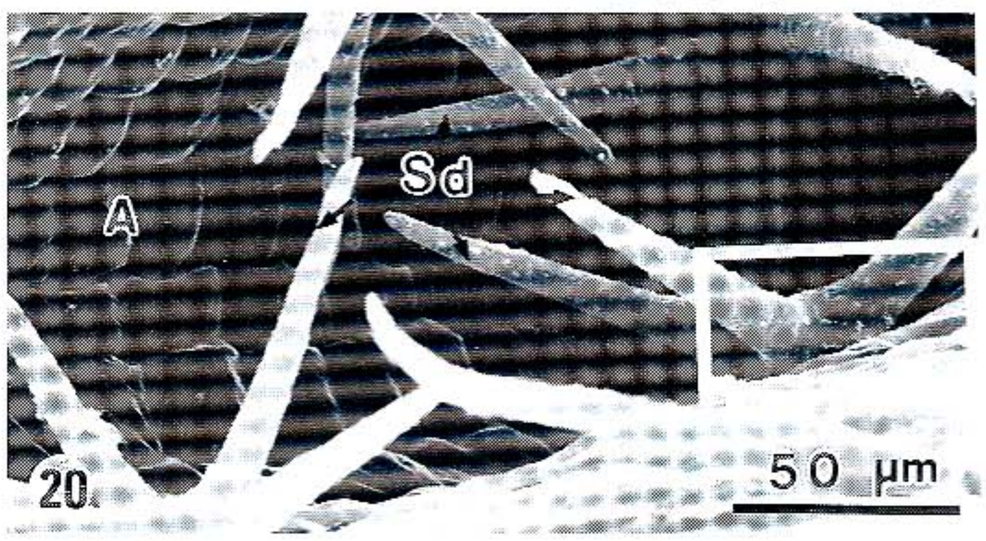
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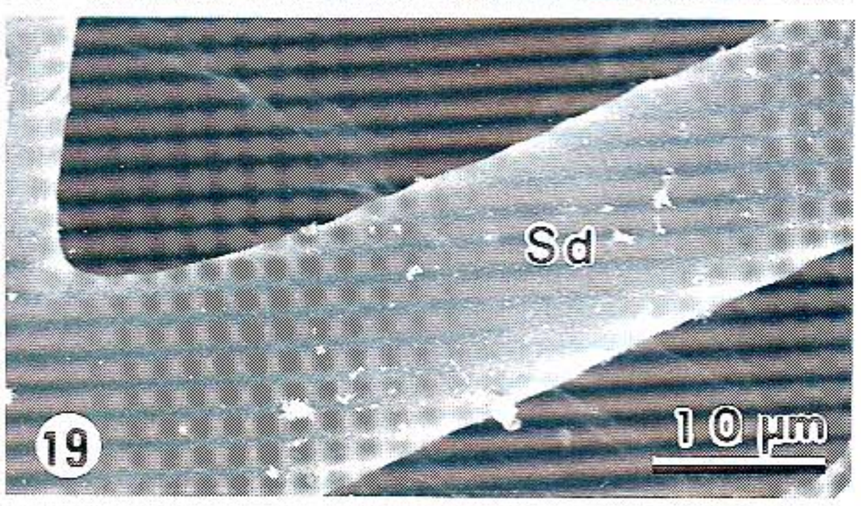
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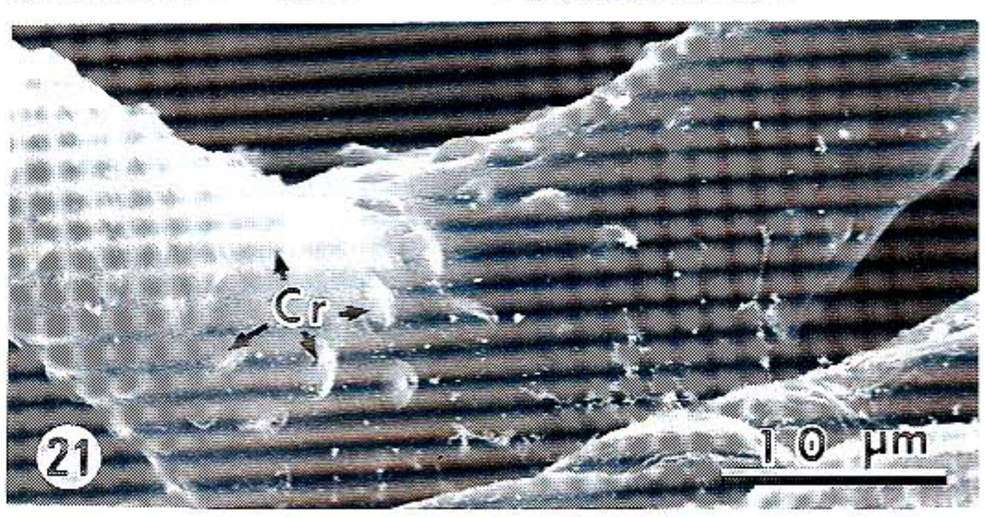
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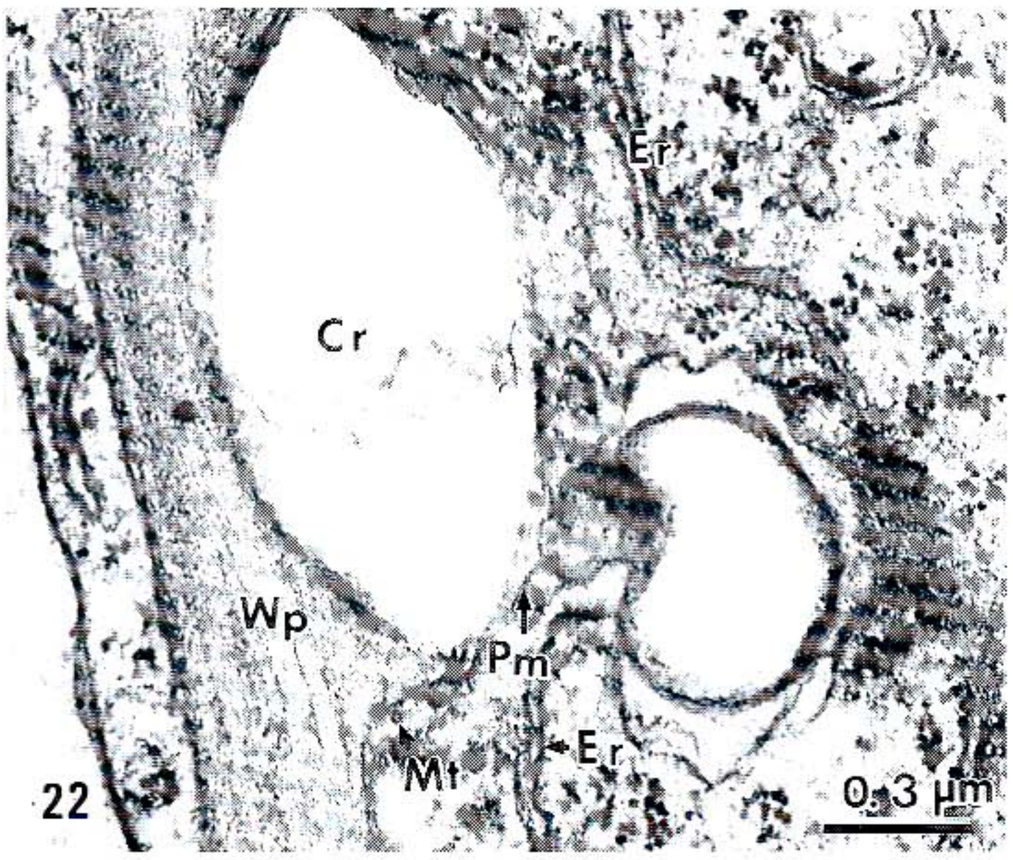


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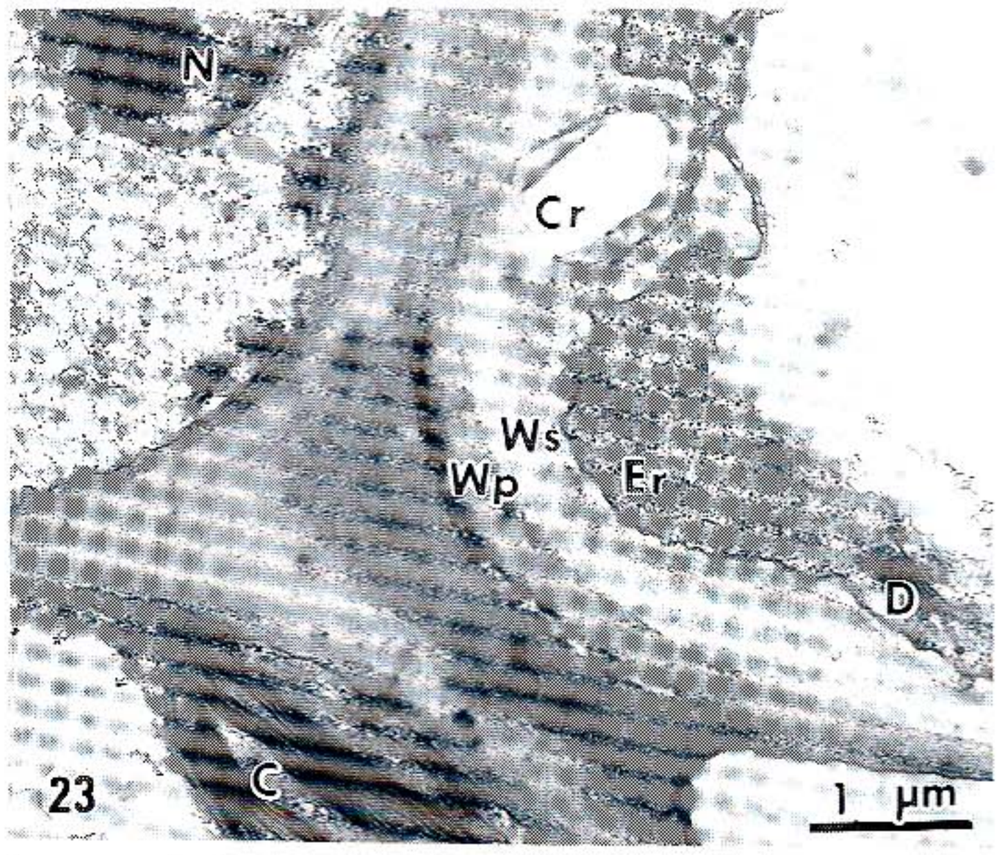


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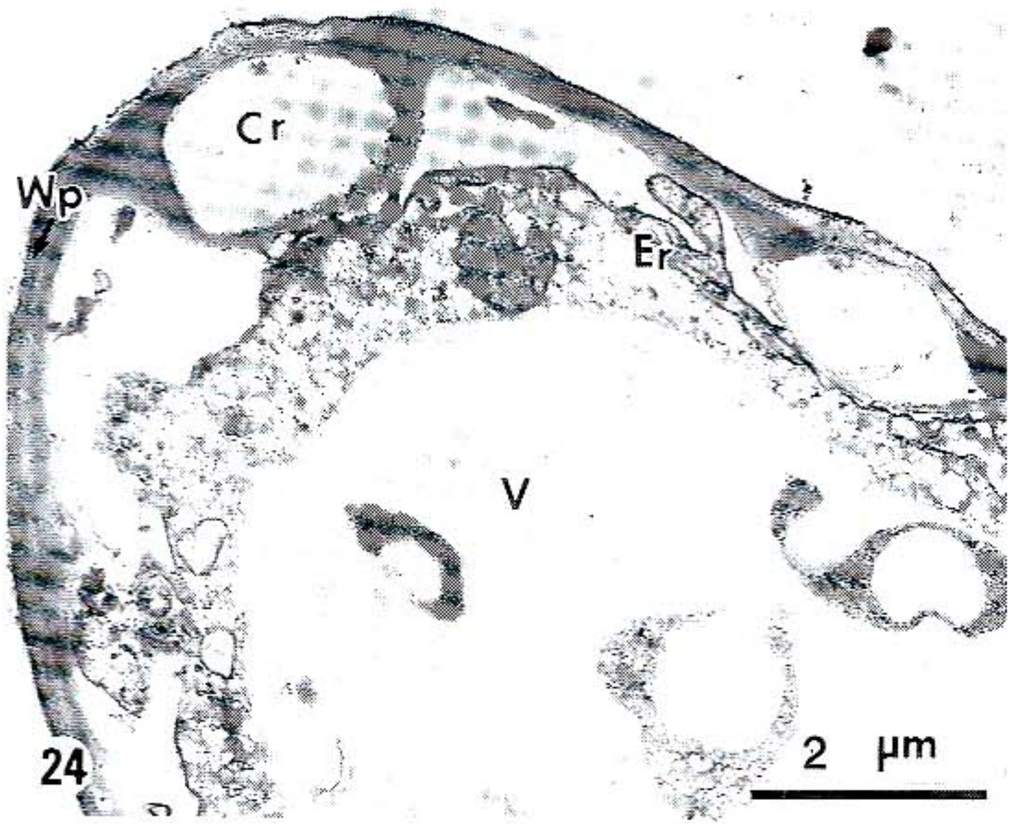


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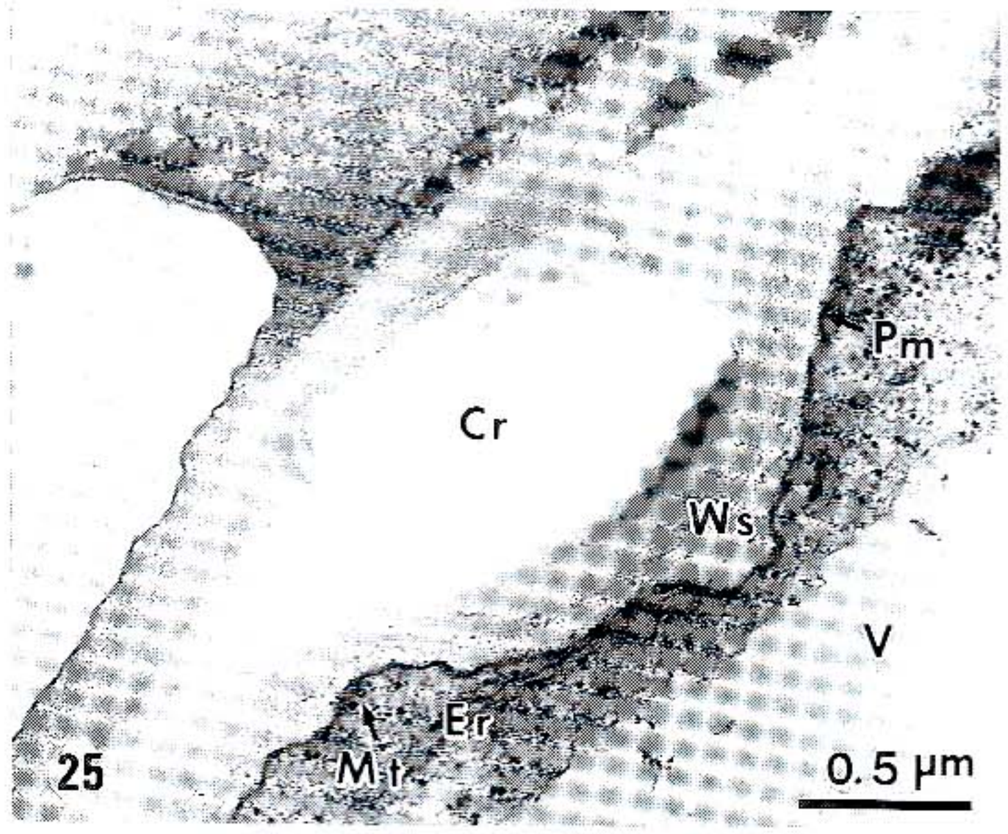


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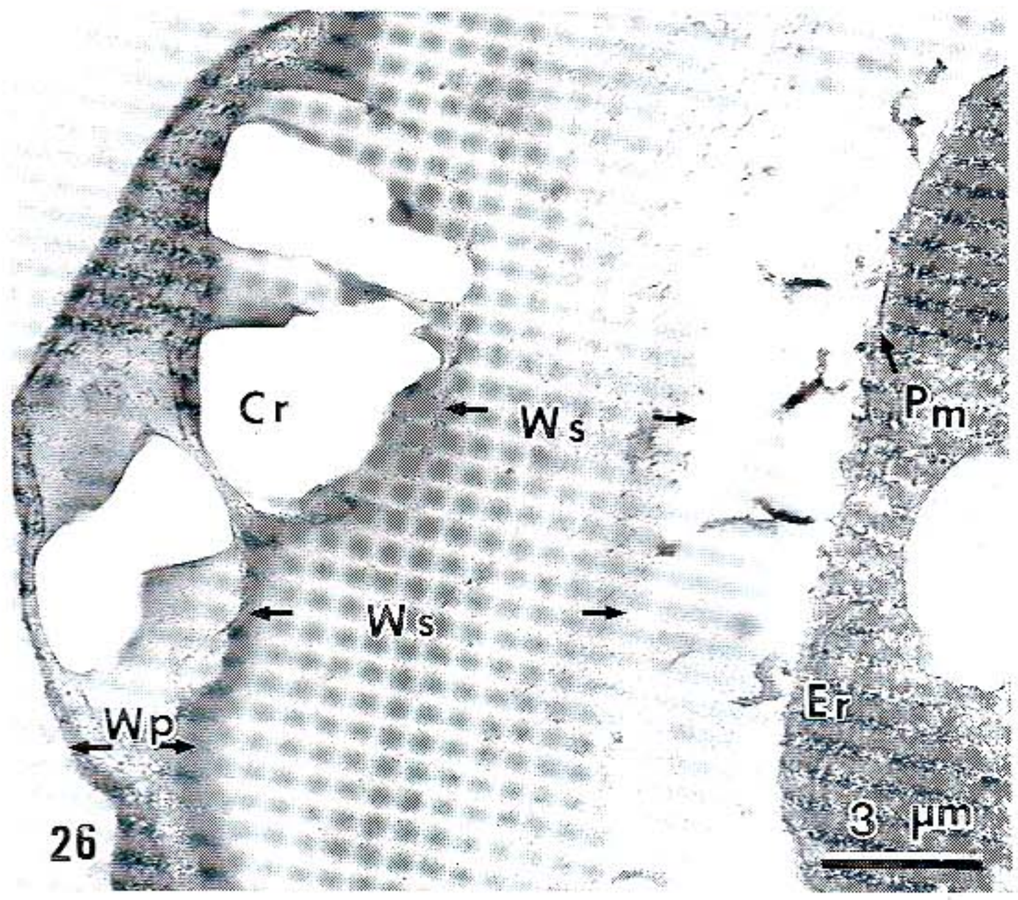


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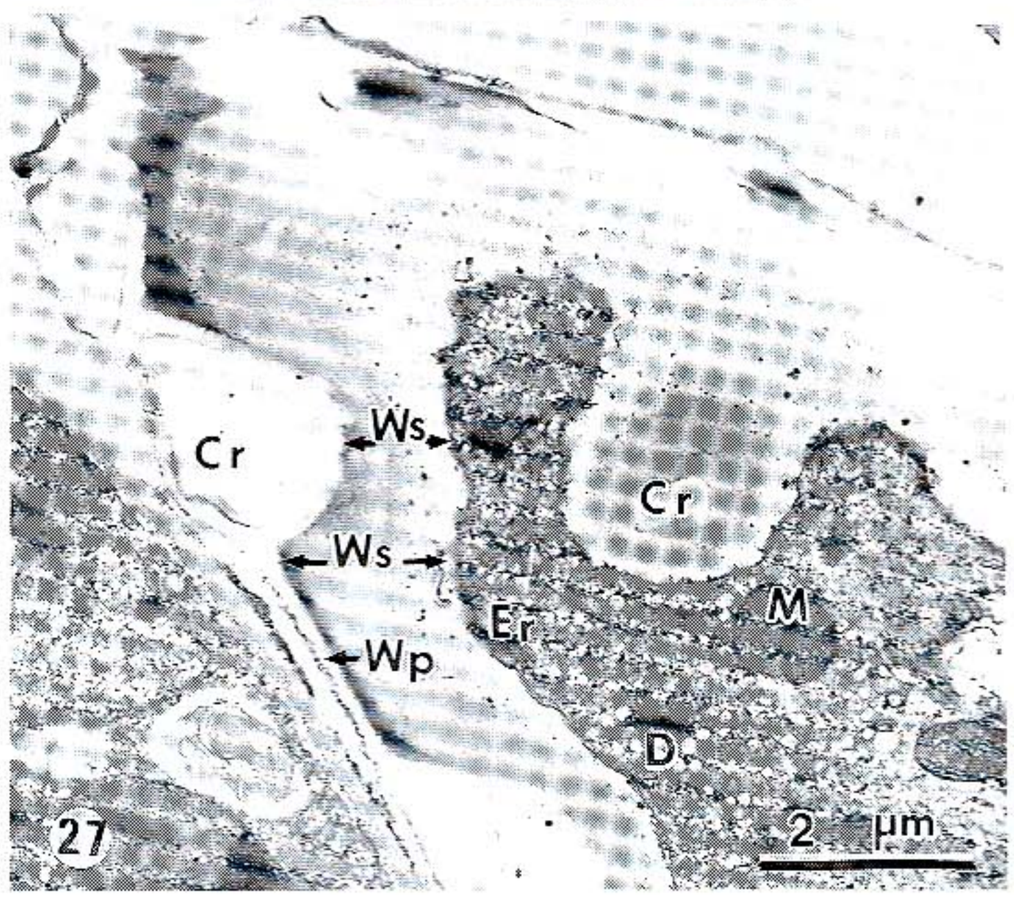


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**Phase of crystal formation**

With the cessation of apical intrusive growth of sclereid in *Nymphaea*, calcium oxalate crystals formed in the cell wall. Between the plasmalemma and primary wall of the sclereid initial cell, many crystal chambers were formed sporadically and almost simultaneously. The crystal chamber contained fibrillar materials (Figs. 11, 13) and was bounded by an electron dense structure (crystal sheath). The crystal sheath lied closely to the plasmalemma and appeared to be developed from the plasmalemma (Figs. 12, 13, 14). As the individual crystal developed, it pressed into the cytoplasm and grew near the cell wall. When part of a crystal chamber was obliquely sectioned, an orderly substructure was discernible (Figs. 13, 14) and gave paracrystalling appearance.

The association of crystal formation with membrane sheathes has been reported (Oladele, 1982), but their precise origin of the sheathes and whose relations with the crystal initiation are not clear.

In the cytoplasm near the crystal chamber, many mitochondria, dictyosomes, and rough endoplasmic reticulum were found. They aften occurred in close association with the plasmalemma surrounding the chamber (Figs. 11-17, 22). Microtubules immediately occurred inside the plasmalemma near the crystal chambers (Figs. 17, 22), though their arrangement didn't follow any particular pattern. They sometimes lied parallel to the plasmalemma and were the other times perpendicular to it (Fig. 17). Crystals underwent periclinal and anticlinal growth, and then showed a tetragonal shape (Figs. 20, 21). In the thin sections of sclereids, many excavations in the cell wall were observed (Figs. 15-17). It was possible that the crystals were lost during the preparations and were leaving cavities in the wall.

**Phase of sclerification**

After the formation of crystals, the secondary cell wall was deposited between the plasmalemma and primary cell wall (Figs. 23-27). As the wall increased in thickness, the cytoplasm contained numerous vesicle-producing dictyosomes and rough endoplasmic reticulum (Figs. 23, 24, 27). The vesicles originated from the dictyosomes could be involved in secondary cell wall formation of sclereids (Harris, 1983). The thickening cell wall made contact with the crystal chambers, and subsequently the crystals were protruded (Figs. 20, 21). However, they were still embedded between primary and secondary wall (Figs. 26, 27). A sharp distinction between primary and secondary wall could be detected. The cell wall, just below the crystals, were thinner than the adjacent cell wall (Fig. 27). Wall thickening continues until mature sclereids possessed extremely thick walls.

In electron micrographs of maturing sclereids in *Nymphaea* (Figs. 24, 26), a distinct hyaline region between the plasmalemma and the secondary cell wall was commonly observed. This region was similar to that described in developing fibers of *Coleus* (Pizzolatol and Heimsch, 1975), and in sclereids of *Camellia* (Boyd *et al.*, 1982). It may be associated with the plasmalemma and cell wall of the sclereid during its development (Marchant and Robards, 1968).

The formation of insoluble calcium crystals is interpreted as a solution for maintaining low cytoplasmic calcium concentration in plant cells (Franceschi and Horner, 1980). The calcium oxalate crystals are generally present in the central vacuoles. The deposition of crystals in the cell wall rather than cytoplasmic

vacuoles is not common among the flowering plants, though it occurs sometimes in conifers. The plant cell wall has dynamic properties, because it could undergo secondary changes and can be as a reservoir for secondary metabolites. Therefore, the primary cell wall of sclereids in *Nymphaea* could act as a sink for the metabolic end products. In many plants oxalic acid is metabolized very slowly or not at all, and is considered to be an end product of metabolism (Borowitzka, 1984). Probably it is secreted sporadically through the plasmalemma and then formed calcium salts with calcium ion in the cell wall (Oladele, 1982). The fact that the crystals are localized rather than formed across the whole cell wall of sclereids may be due to local discharge of oxalic acid from the cytoplasm.

## ACKNOWLEDGEMENTS

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# 睡蓮厚壁細胞發育過程超微構造研究

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## 摘 要

睡蓮厚壁細胞分散地起源於基本組織的薄壁細胞，其發育過程可分爲：(1) 細胞增大與分枝化期；(2) 結晶體形成期；以及 (3) 厚壁化期。厚壁起源細胞的特徵爲大的細胞核、濃細胞質、與明顯的分枝。通常當厚壁起源細胞達到成熟形態，不再行頂端浸入生長時，其細胞壁便開始形成草酸鈣結晶體。許多結晶腔分散地形成於細胞膜與初生細胞壁之間，結晶腔外覆結晶鞘，而細胞質內的粒線體、高網體、及內網體與結晶腔臨近之細胞膜密切相關。成熟的厚壁細胞具有很厚的細胞壁，結晶體存在於初生細胞壁與次生細胞壁之間，此與一般植物之草酸鈣晶體存在於液胞內之情形不同。