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Structure and Development of the Sieve-Cell Protoplast in Leaf Veins of *Welwitschia*[1]

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With 22 Figures

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Summary

Tissue of one-year-old leaves of *Welwitschia mirabilis* was fixed in glutaraldehyde and postfixed in osmium tetroxide for electron microscopy. Mature sieve cells contain nuclei composed of peripherally-distributed chromatin material and an intact envelope with pores. During sieve-cell development many mitochondria become closely associated spatially with the nucleus. In addition to a nucleus and mitochondria, the mature, plasmalemma-lined sieve cell contains plastids and abundant smooth endoplasmic reticulum, which generally occurs in massive aggregates at the sieve areas. Dictyosomes and ribosomes are lacking and a tonoplast is not discernible in mature sieve cells. P-protein is not present at any stage of development.

1. Introduction

Welwitschia mirabilis Hook. is a member of the *Gnetales,* a highly-evolved group of gymnosperm-like plants which possess many advanced anatomical and morphological characteristics (FOSTER and GIFFORD 1959). This probably most bizarre of vascular plants is limited in its distribution to extremely arid deserts along the western coast of southern Africa, in Angola and South-west Africa (RODIN 1953). During its lifetime *Welwitschia* possesses but a single pair of foliage leaves, which grow by means of a perennial basal meristem. Some plants are estimated to be 2,000 or more years old.

Among the advanced anatomical characteristics of *Welwitschia,* and the *Gnetales* in general, is the presence of vessel members in the xylem. Little is known about the phloem in *Welwitschia.* Its sieve elements have been described as very long "sieve-tubes" with oblique end walls (DE BARY 1884,

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Sykes 1910 a, Takeda 1913, Pearson 1929) and gymnosperm-type sieve areas (Takeda 1913, Feustel 1921). Because of the uniqueness of *Welwitschia* among vascular plants and the advanced nature of its xylem, it was decided to study its phloem tissue in detail with the electron microscope. At this time we are reporting on the structure and development of the sieve-cell protoplast in leaf veins of *Welwitschia*.

2. Materials and Methods

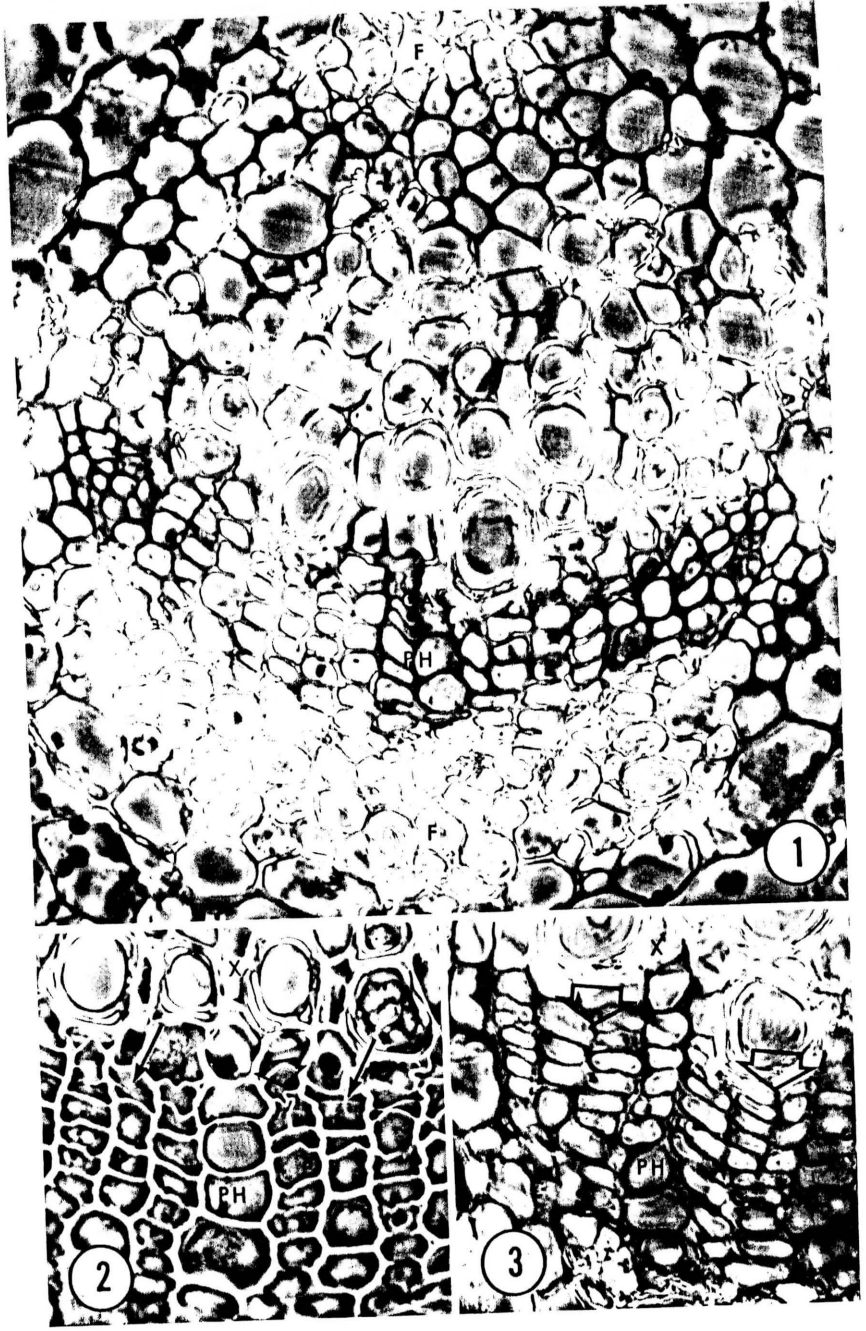
The materials used in this study were obtained from one-year-old, green-house-grown plants. The leaves were severed from the plants just above the basal meristems with sharp razor blades, divided in half into distal and basal portions and then immersed in 0.05 M sodium cacodylate buffer. Each portion was divided into about 2 mm-wide longitudinal strips, which subsequently were diced into pieces about 2 mm square. The tissue was covered with cacodylate buffer during this process. The pieces were then placed in 6% glutaraldehyde in 0.05 M cacodylate buffer for 6 hours at room temperature. After several washes in cacodylate buffer, the tissue was post-fixed in 2% osmium tetroxide in the same buffer in a refrigerator overnight. All tissues were dehydrated in cold ethyl alcohol and embedded in Araldite-Epon. Sections were cut on an LKB ultramicrotome, and viewed and photographed with a Hitachi HU-11 E microscope.

3. Observations

The vascular bundles in the leaf of *Welwitschia* are collateral, the xylem occurring on the adaxial, the phloem on the abaxial side (Fig. 1). Several investigators have reported both primary and secondary vascular tissues in leaves of *Welwitschia* (Bertrand 1874, De Bary 1884, Sykes 1910 a, b, Takeda 1913, Pearson 1929), while others have interpreted the vascular tissues of the leaf to be entirely primary (Strasburger 1891, Rodin 1958). Rodin (1958) pointed out that the criterion used by most investigators to determine the presence of secondary tissues in the leaves of *Welwitschia*, viz., the arrangement of xylem and phloem elements in radial rows, was not adequate for the separation of primary from secondary vascular tissues, and stated that, although he had insufficient material to arrive at a final decision of a "fascicular cambium" in the leaves of *Welwitschia*, he believed none to be present and growth to be strictly primary.

The present investigation revealed that secondary growth from a vascular cambium does occur in the leaves of *Welwitschia*. Inasmuch as most of the xylem and phloem elements are arranged in radial files in both primary

Figs. 1–3. Transverse sections of veins as seen with phase microscope. In Fig. 2, a more detailed view of part of the vein shown in Fig. 1, arrows point to recently-formed tangential walls of cambial cells. Anticlinal division of one of the cambial cells has resulted in the formation of two new radial files (arrow head). Four similarly initiated, but older radial files (arrow heads) are shown in part of older vein in Fig. 3. *CA* = cambium, *F* = fibers, *PH* = phloem, *X* = xylem. Fig. 1 ×600, Fig. 2 ×700, Fig. 3 ×730



Figs. 1–3

and secondary tissues it is difficult to delimit these tissues from one another. However, that part or all of some radial files of cells are derived from periclinally dividing cambial initials is quite apparent in all relatively

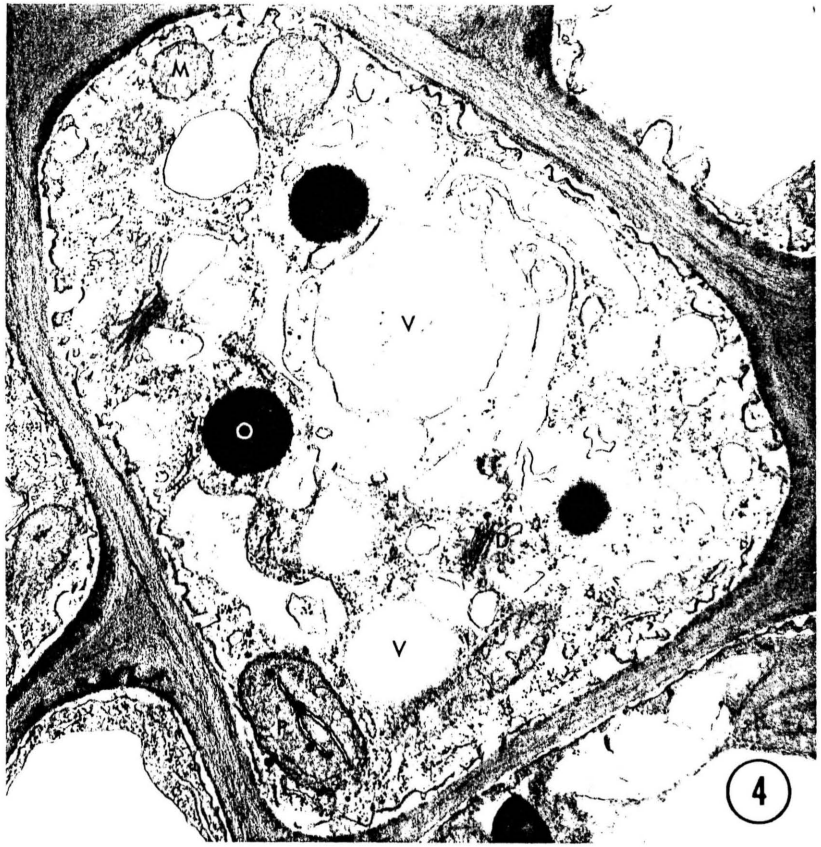


Fig. 4. Transection of procambial cell. Osmiophilic globules (O) are common in procambial cells, but lacking in differentiating sieve elements. *D* = dictyosome, *M* = mitochondrion, *P* = plastid, *V* = vacuole. ×21,300

large veins. In the bundle of Figs. 1 and 2, most of the cambial cells contain recently-formed periclinal or tangential walls. In addition, two, newly-formed radial files of two cells each occur near the middle of the vein (Fig. 2). Increase in numbers of cambial cells and, consequently, of radial files, through anticlinal division of initials is common in the larger veins. Fig. 3 shows part of a vein in which secondary growth has been of longer duration than that of Figs. 1 and 2.

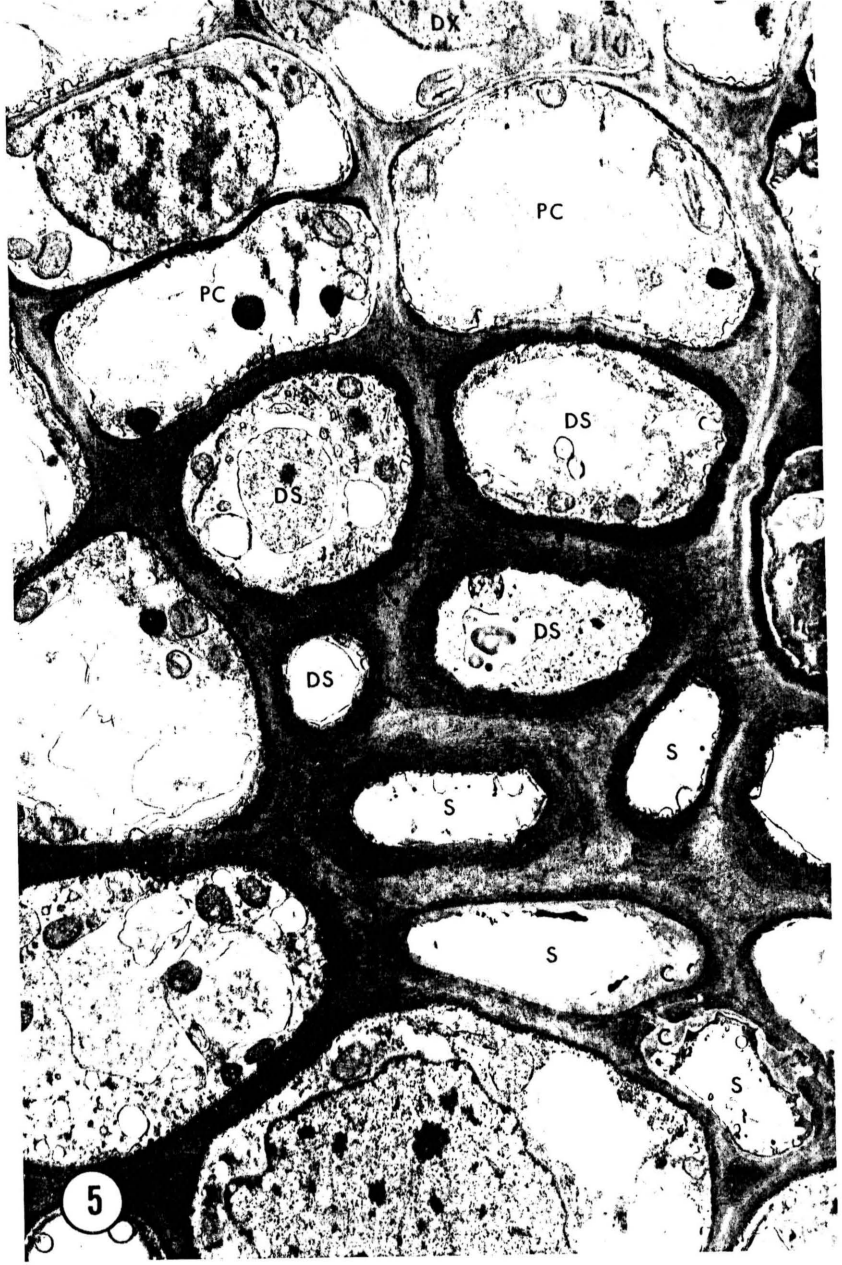


Fig. 5. Transection showing mostly phloem of small vein with sieve cells in various stages of development. The walls of the two oldest sieve cells (below) are completely lined with definitive callose (*C*). Differentiating sieve cells (*DS*) adjacent to procambial cells (*PC*) already have thickened walls. *DX* = differentiating xylem, *S* = sieve cell. ×9,500

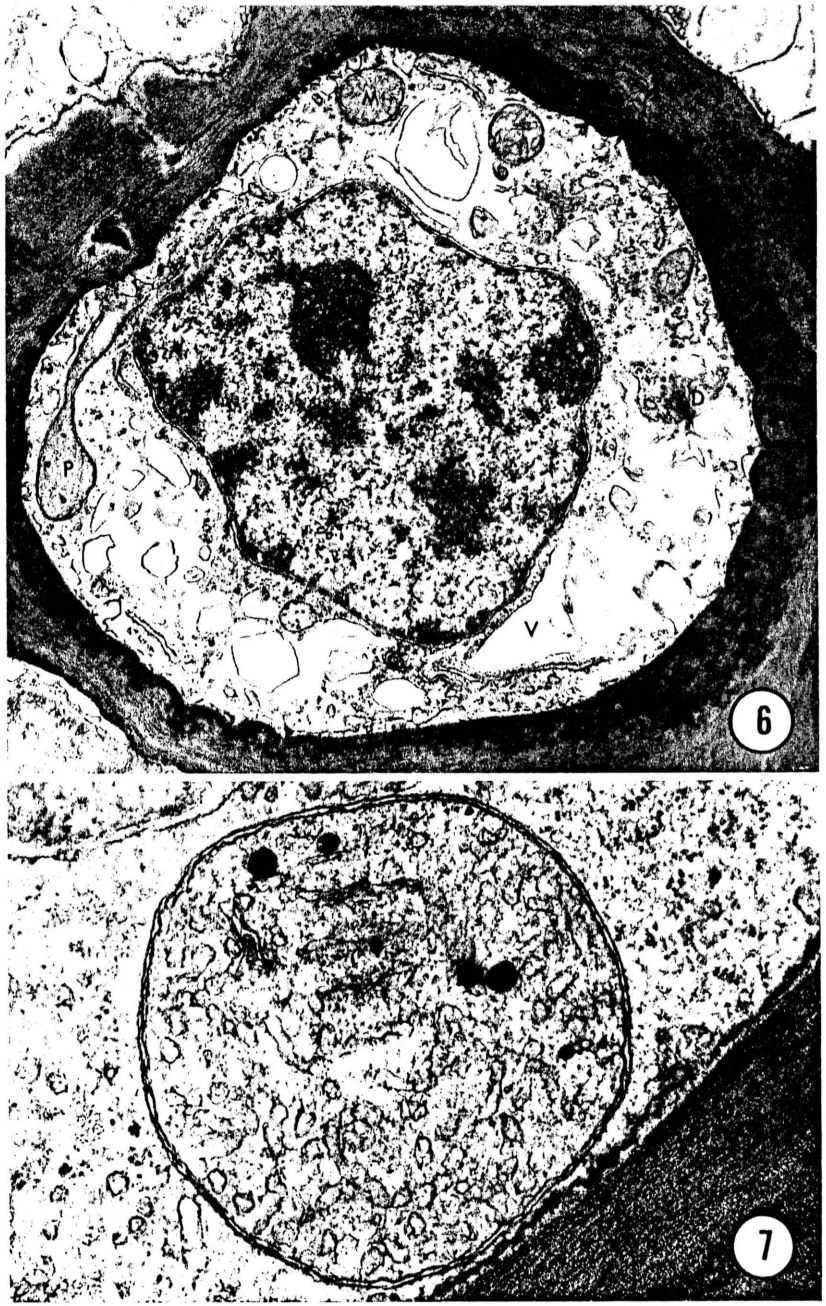
The precursors of the sieve cells, namely, procambial and cambial cells, are essentially similar in ultrastructure, apparently differing only in their degree of vacuolation, the cambial cells being more highly vacuolated than the procambial cells. The procambial cell of Fig. 4 exemplifies the sieve-cell precursors. Many dictyosomes, plastids, and mitochondria are present in these cells. Both plastids and mitochondria contain relatively dense matrices. Small osmiophilic globules occur in the plastids, and relatively large ones, probably lipid droplets, in the cytoplasm. The cytoplasm is rich in free ribosomes and contains many long profiles of rough endoplasmic reticulum (ER). The cytoplasmic matrix appears granular to finely fibrous. Coarser fibrillar material is often found in the vacuoles. Microtubules occur next to the plasmalemma, which borders a relatively thin wall.

Thickening of the wall of developing sieve cells apparently is a very rapid process, for all cells that could be identified as differentiating sieve cells, from the earliest stages onward, contained conspicuously thickened walls. Fig. 5 shows the difference in wall thickness of sieve cells of various ages, ranging from very young to senescent ones, and of procambial cells of the bundle.

Among the protoplasmic components of differentiating sieve cells, the plastids early show the most marked changes. These include the formation of starch granules, proliferation of internal membranes, and decrease in density of the matrix (Figs. 6 and 7). Osmiophilic globules continue to be found in the plastids, but disappear from the cytoplasm. Many, if not most, of the free ribosomes now occur in aggregates and the production of dictyosome vesicles is high (Figs. 6 and 8). Microtubules, mostly oriented at more or less right angles to the long axes of the cells, parallel the wall inside the plasmalemma. With the exception of the microtubules, the various components of the protoplast, including mitochondria and ER, are more or less randomly distributed throughout the cytoplasm.

The nucleus of the young sieve cell differs little in appearance from that of meristematic cells. It contains clumps of chromatin material, some scattered throughout the granular to finely fibrous matrix, and others located near the nuclear envelope (Figs. 6 and 8). The nuclear envelope contains many pores and its outer membrane commonly is associated with ribosomes. A single nucleolus was observed in some nuclear profiles of young sieve cells.

As differentiation continues, a close spatial relationship develops between the nucleus and many of the mitochondria of the sieve cell. Many mitochondria become closely appressed to the nuclear envelope (Fig. 9), while



Figs. 6 and 7

Fig. 6. Transection of young sieve cell prior to initiation of morphological changes in nucleus. *D* = dictyosome, *M* = mitochondrion, *P* = plastid, *V* = vacuole. ×16,000

Fig. 7. Plastid, with osmiophilic globules and many internal membranes, in young sieve cell. ×48,000

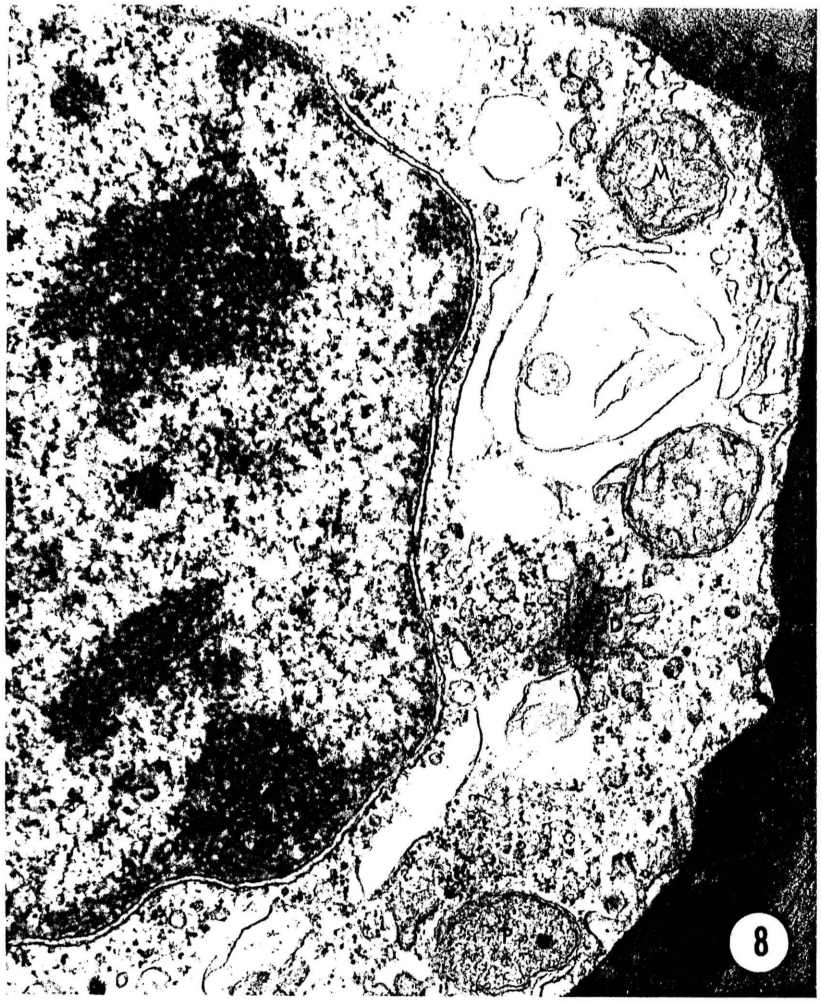


Fig. 8. Part of young sieve cell of Fig. 6 at higher magnification. Portions of the nuclear envelope are associated with ribosomes. *D* = dictyosome, *M* = mitochondrion, *P* = plastid. ×37,000

others are nearly or entirely surrounded by the nucleus. At about the same time portions of the inner membrane of the nuclear envelope develop protrusions into the nuclear matrix so that, in some profiles parts of the nucleus appear holey (Figs. 9 and 10).

Aggregates of smooth ER now begin to appear throughout the cytoplasm of the sieve cell (Fig. 11). Superficially, they appear to be aggregates of vesicles,

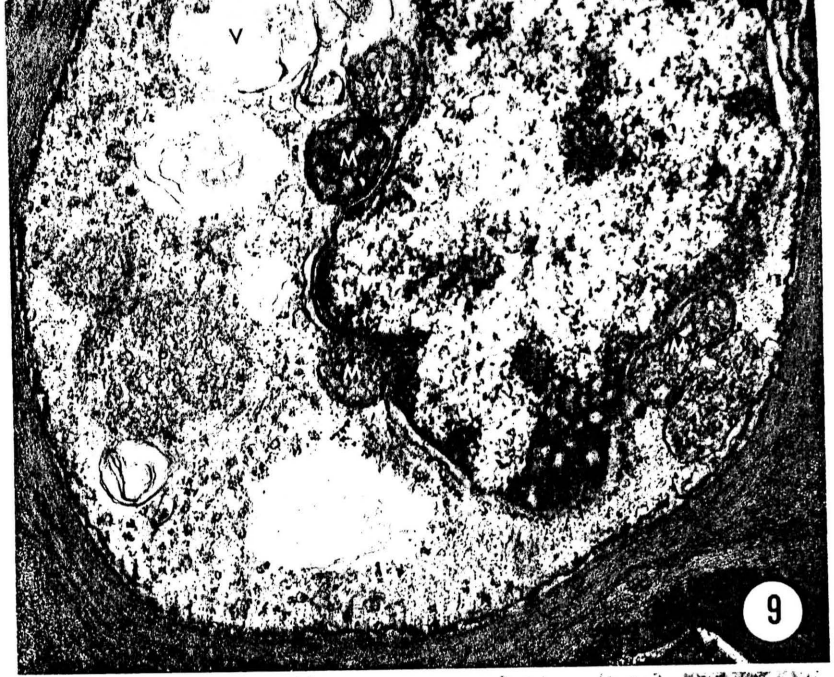


Fig. 9. Immature sieve cells with mitochondria closely associated with nucleus. The lower portion of nucleus has a holey appearance (see Fig. 10). *M* = mitochondrion, *P* = plastid, *V* = vacuole. ×25,400

Fig. 10. Part of nucleus in immature sieve cell showing holey appearance resulting from protrusion of portions of inner membrane of nuclear envelope into the nuclear matrix. ×60,000

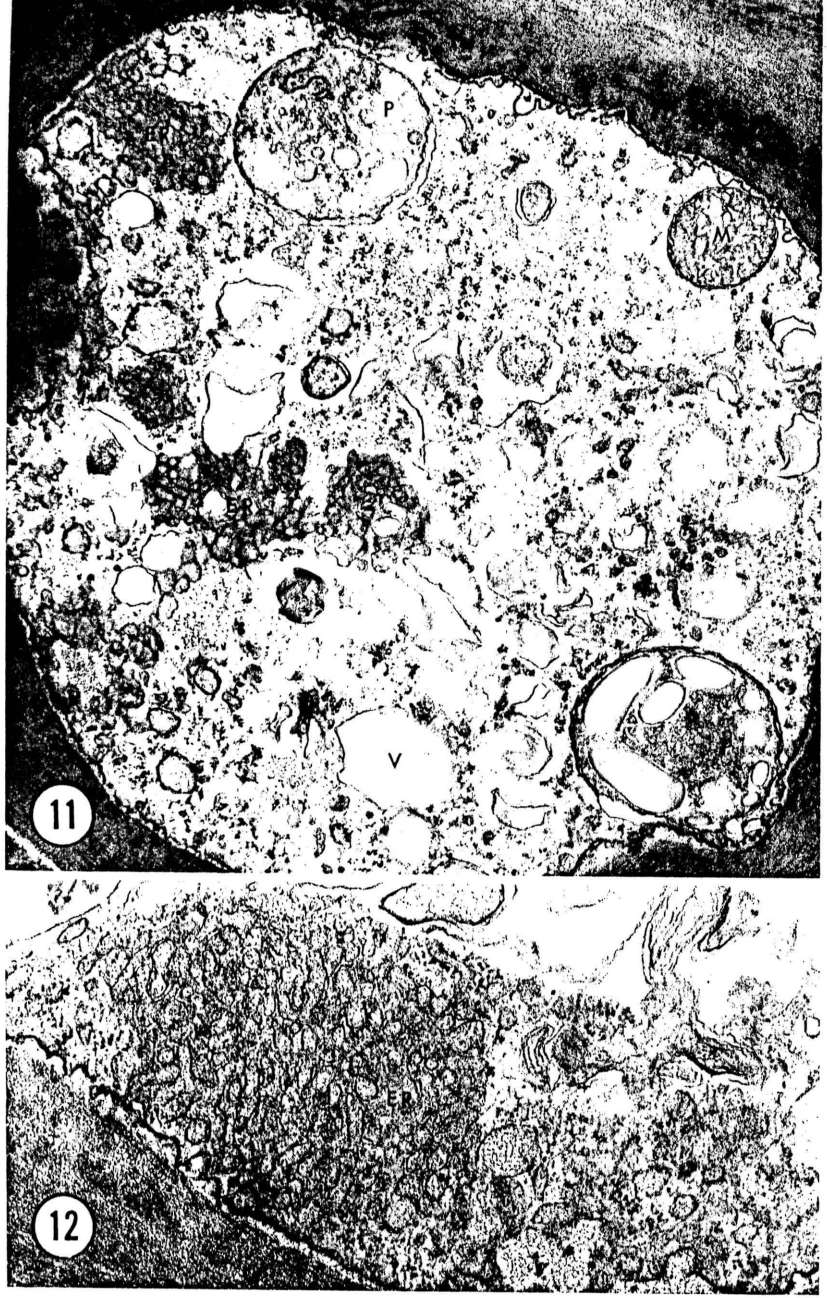


Fig. 11. Transection of immature sieve cell with recently-formed aggregates of smooth ER. Each plastid (*P*) contains several starch grains. *M* = mitochondrion, *V* = vacuole. ×20,000
Fig. 12. Aggregate of smooth ER in immature sieve cell. ×40,000

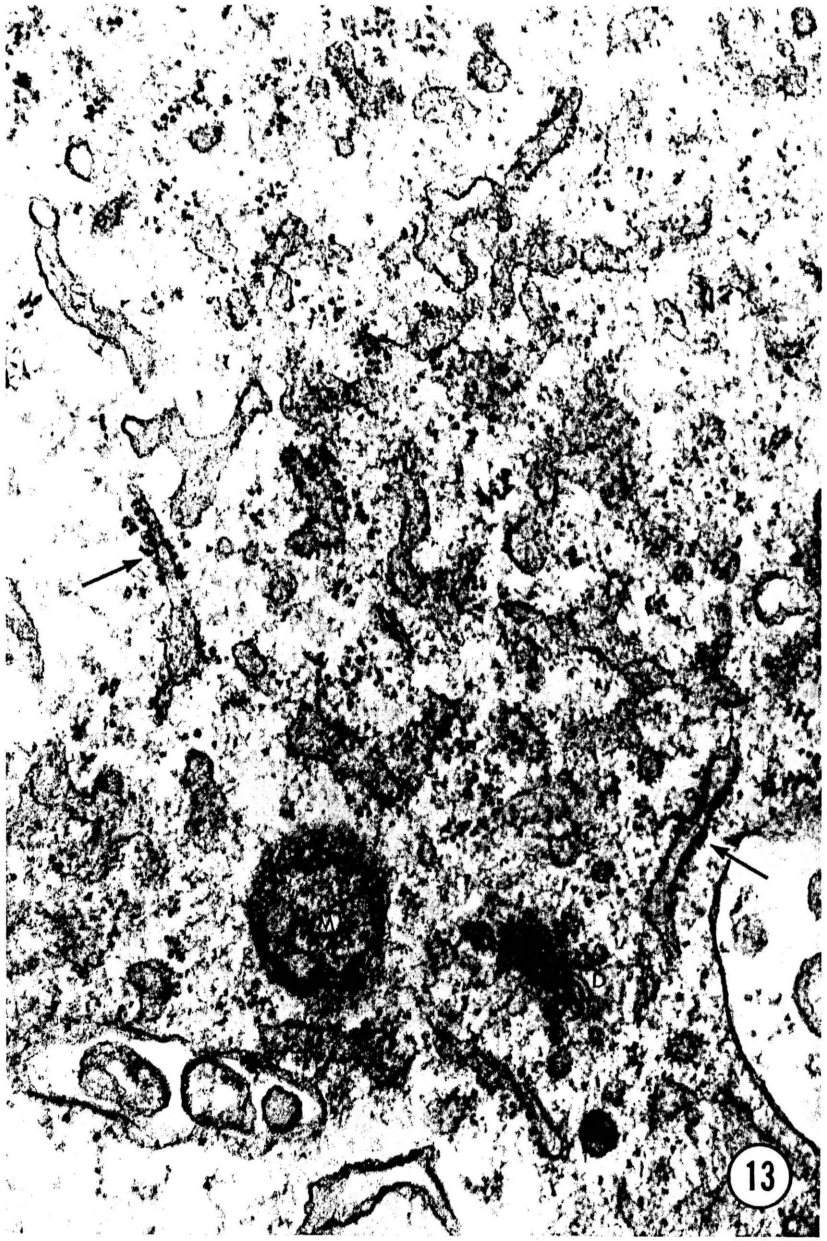


Fig. 13. View of smooth ER in immature sieve cell. The branched and tubular nature of this ER is apparent. Some rough ER (arrows) is still present. *D* = dictyosome, *M* = mitochondrion. ×60,000

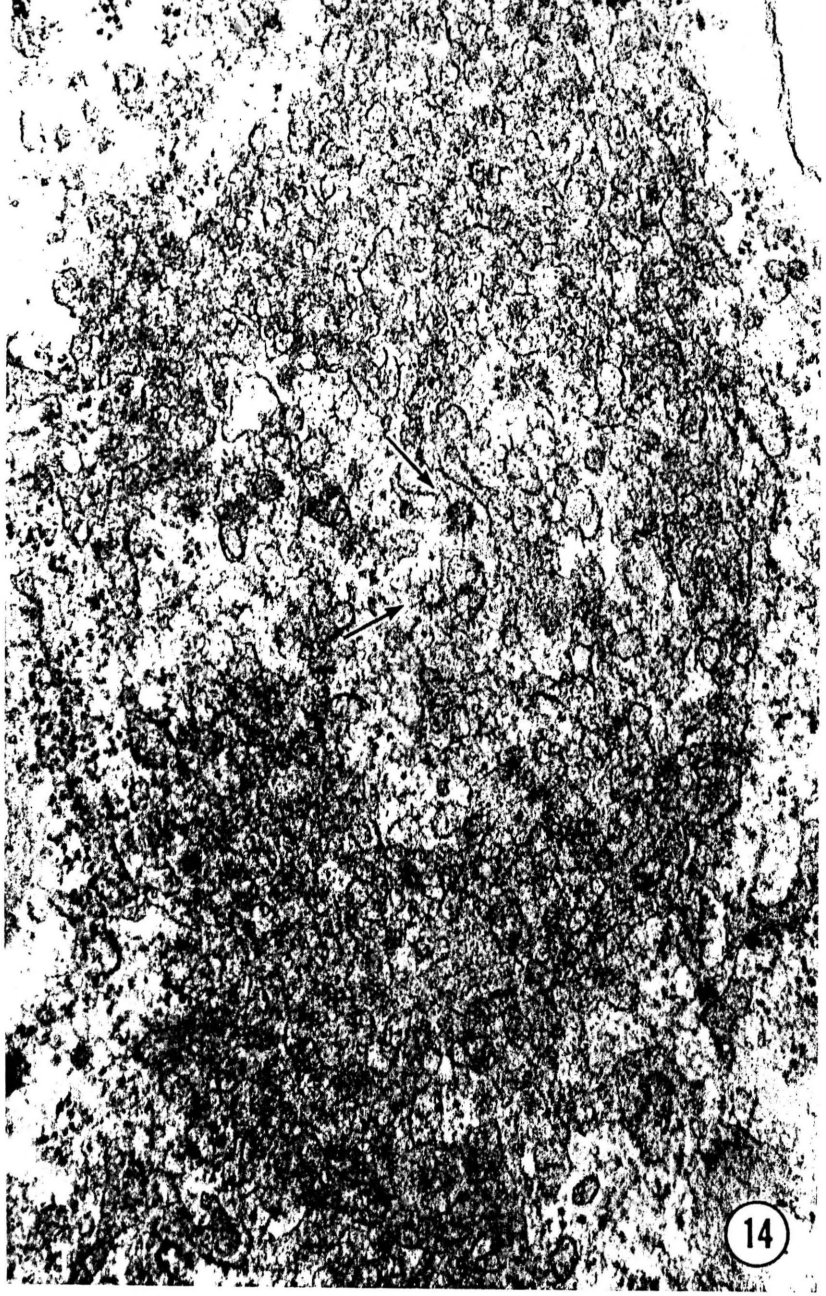
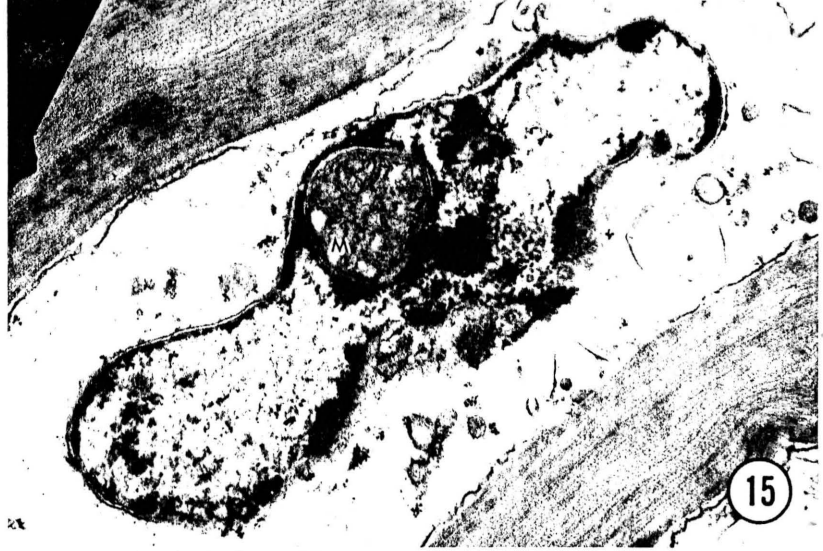


Fig. 14. Aggregate of ER in immature sieve cell. Some segments of ER are associated with spinelike processes and in transection resemble spiny vesicles (arrows). ×60,000



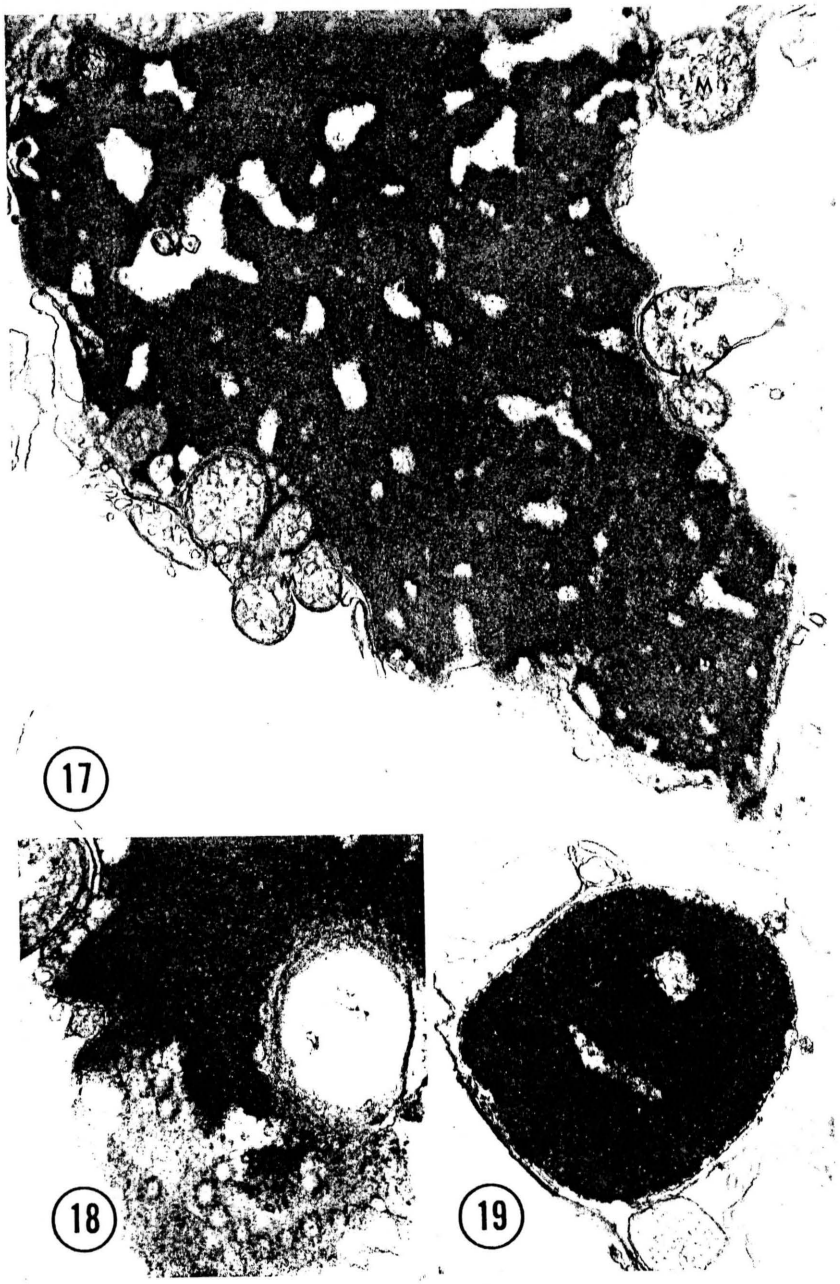
Figs. 15 and 16. Different stages in nuclear development. Fig. 15 shows nucleus in sieve cell approaching maturity. Chromatin material is aggregating near the nuclear envelope. A mitochondrion appears surrounded by the nucleus. Fig. 16 shows nucleus in mature sieve cell. The nuclear envelope remains intact. *M* = mitochondrion. Fig. 15 ×24,800, Fig. 16 ×21,800

but upon closer inspection they can be seen to consist of elaborate, three-dimensional networks of smooth ER (Fig. 12), most of which is tubular. The tubular and branching nature of the new ER is apparent in Fig. 13, a region in which the ER is not compactly arranged. Rough ER is still present and at places appears to be continuous with the newly-formed, smooth ER. Some membranes of the more compact aggregates of ER possess numerous radiating projections similar in appearance to those of spiny vesicles (Newcomb 1967). Indeed, cross-sectional profiles of such membranes closely resemble spiny vesicles (Fig. 14).

As the sieve cell approaches maturity the chromatin material of the nucleus becomes mostly peripheral in distribution, lying just inside the nuclear envelope (Fig. 15). Except in sieve cells with poorly preserved protoplasts, the chromatin material maintains its coarse, granular appearance. The nucleus persists in the mature sieve cell (Figs. 16–19). Commonly it occurs in a median position in the cell, either lying along one wall or extending from wall to wall across the lumen of the cell (Fig. 16). In non-plasmolyzed cells the sieve cell nuclei are relatively large, about the same size as those of immature cells, while in plasmolyzed cells the nuclei are much smaller and generally round or oval in outline, as seen in transverse sections. At maturity many mitochondria are closely associated with the nucleus (Figs. 16–18), but are separated from the dense chromatin material by the nuclear envelope, which remains intact and contains distinct pores (Fig. 18).

As the sieve cell approaches maturity most of the ER becomes associated with the sieve areas, where it is found in massive aggregates, portions of which often extend across the lumen of the cell from wall to wall (Figs. 20 and 21). Much of the wall of the mature sieve cell is bordered by one or two layers of similar, smooth-surfaced ER closely appressed to the plasmalemma. It is probable that the massive ER aggregates associated with the sieve areas are connected with one another by this ER. Stacks of ER cisternae, similar to those of mature angiospermous sieve elements (Evert and Deshpande 1969, and literature cited therein), were only occasionally encountered in mature *Welwitschia* sieve cells.

In addition to a nucleus, mitochondria, and ER, the mature, plasmalemma-lined sieve cell contains plastids with starch granules. In contrast to plastids of immature sieve cells, those of mature ones lack internal membranes and their matrices are very clear in appearance (compare plastids in Figs. 6, 7, 11, and 22). The mitochondria undergo no apparent structural modifications.



Figs. 17–19

Figs. 17–19. Nuclei in mature sieve cells. Several mitochondria are closely associated with the nucleus in Fig. 17. Fig. 18 shows a surface view of the nuclear membrane, with pores, of that nucleus. Fig. 19 shows apparently shrunken nucleus in plasmolyzed sieve cell. Fig. 17 × 19,000, Fig. 18 × 40,000, Fig. 19 × 30,300

At maturity, the sieve cells lack ribosomes, dictyosomes, and microtubules. The vacuoles of young sieve cells and the lumina of mature ones sometimes contain a coarse fibrous substance (*e.g.*, Fig. 16), similar in appearance to that often found in vacuoles of parenchymatous elements of the leaf (Fig. 5). The nature of this substance has not been determined, but it should not be confused with the proteinaceous substance called slime or P-protein. P-protein does not occur in the sieve cells of *Welwitschia* at any time. During maturation the tonoplasts, which delimit vacuolar contents from cytoplasm in young cells, cease to be identifiable and the sieve cell then appears to contain one large, central cavity.

As the sieve cells undergo senescence, massive quantities of definitive callose are deposited at the sieve areas. In many cells the entire inner surface of the wall is lined with definitive callose (Fig. 3, below). All of the protophloem sieve cells are stretched and eventually obliterated; in bundles with secondary growth, many of the metaphloem sieve cells may eventually collapse. Much ER and starch are often found in partially collapsed cells.

4. Discussion

The protoplasts of mature sieve cells of *Welwitschia* apparently have much in common with those of conifers. Nuclei, in one form or another, have been identified with the electron microscope in mature sieve cells of *Pinus strobus* (Murmanis and Evert 1966, Srivastava and O'Brien 1966) and *Pinus pinea* (Wooding 1966, 1968), and with the light microscope in similar cells of *Pinus banksiana, Pinus resinosa, Juniperus virginiana, Larix decidua, Picea mariana* (Evert and Alfieri 1965), *Metasequoia glyptostroboides, Sequoia sempervirens*, and *Taxodium distichum* (Evert *et al.* 1970). In all but the last three species, the sieve-cell nuclei have been reported to undergo varying degrees of morphological modification as the sieve cell approaches maturity. In *Pinus strobus* (Murmanis and Evert 1966, Srivastava and O'Brien 1966) and *Pinus pinea* (Wooding 1966) the nuclei of mature sieve cells lacked any fine-structural detail and the nuclear envelope was generally missing. Murmanis and Evert (1966) reported that membranes were commonly associated with the "necrotic" nuclei in *Pinus strobus*, but were not certain whether they represented ER or nuclear envelope. In well-preserved protoplasts of mature *Welwitschia* sieve cells the chromatin material is distinctly granular and the nuclear envelopes remain intact. To our knowledge, the close association of mitochondria and nuclei, as recorded in the

Fig. 20. Portion of mature sieve cell showing massive aggregate of smooth ER associated with sieve area. The sieve area is not fully developed, for the contiguous sieve cell (lower left) is still immature. *C* — callose lining pores of sieve area, *M* — mitochondrion, *P* — plastid. ×26,200

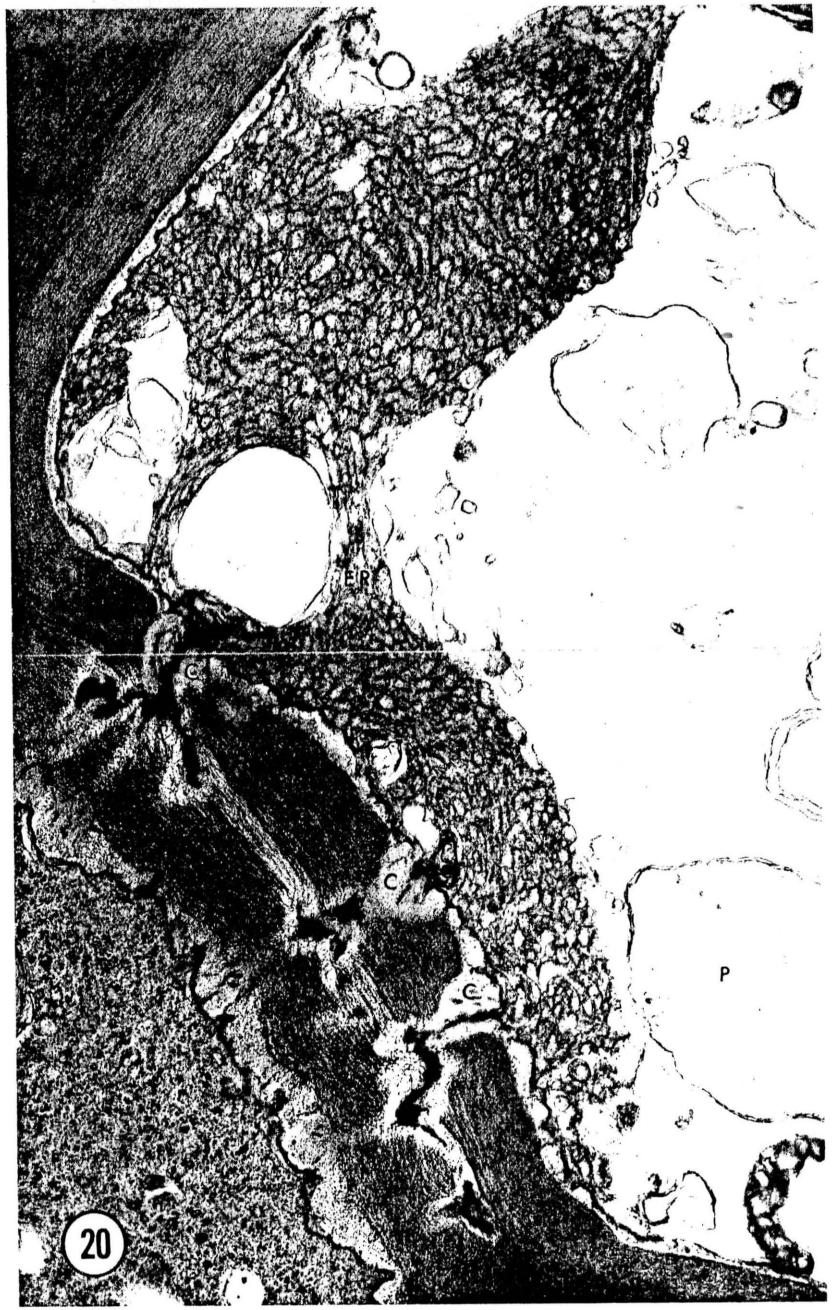


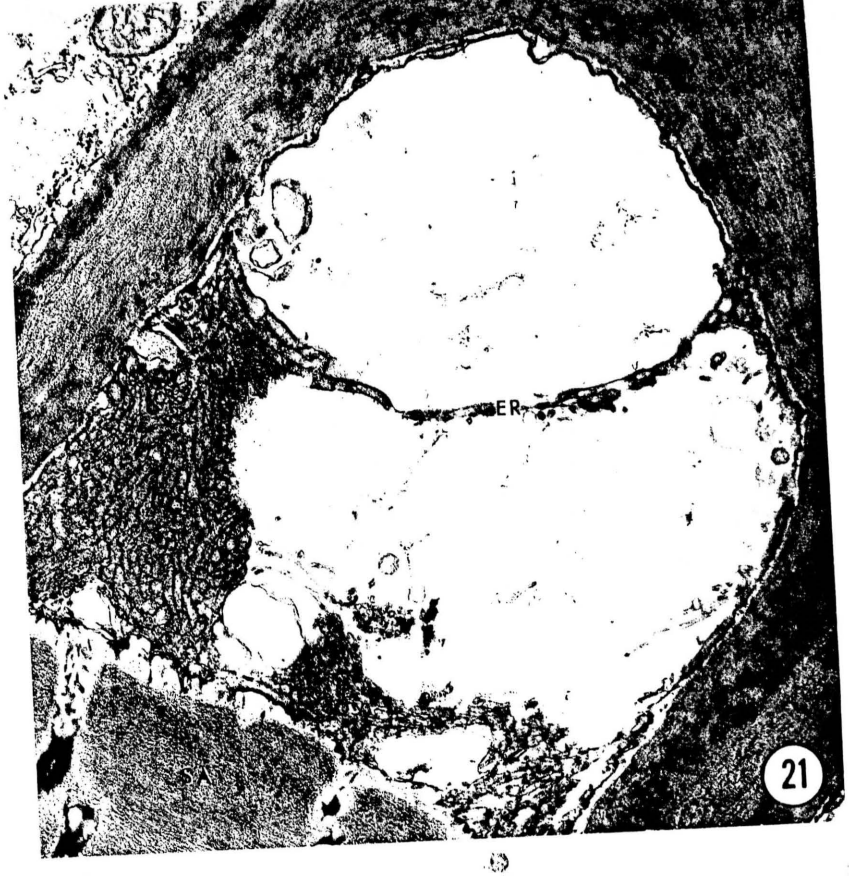
Fig. 20

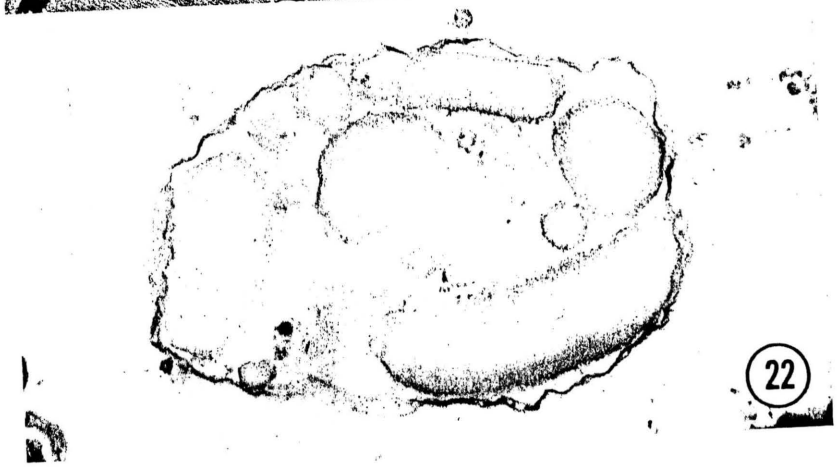
present investigation of *Welwitschia* sieve cells, has not been reported for any other species.

As the nuclei of the *Welwitschia* sieve cells undergo modification, numerous aggregates of smooth ER develop in the cytoplasm. Most of this much-branched, tubular ER becomes associated with the sieve areas. Similar ER aggregates have been demonstrated in sieve cells of *Metasequoia glyptostroboides* (KOLLMANN and SCHUMACHER 1964), *Pinus pinea* (WOODING 1966), and *Pinus silvestris* (PARAMESWARAN 1971), where they have been described as "local differentiations of a complex membrane system", "discrete vesicular masses", and "an extensive labyrinth of membranes and extra cytoplasmic spaces", respectively.

The spine-like processes associated with the ER in developing sieve cells of *Welwitschia* are similar to those of spiny vesicles reported in the phloem region of *Phaseolus vulgaris* (NEWCOMB 1967), *Cucurbita maxima* (CRONSHAW and ESAU 1968), *Coleus blumeri* (STEER and NEWCOMB 1969), and *Nicotiana tabacum* (ESAU and GILL 1970 a), and to those of spiny tubules recorded in young parenchyma cells of roots and leaves of *Beta vulgaris* (ESAU and GILL 1970 b). It has been suggested that spiny vesicles contribute to the formation of P-protein tubules in root tips of *Phaseolus* (NEWCOMB 1967) and shoot apices of *Coleus* (STEER and NEWCOMB 1969). However, spiny vesicles have been recorded in association with P-protein bodies in sieve elements of only *Cucurbita* (CRONSHAW and ESAU 1968). The spiny tubules in sugar beet were rarely encountered in the same sections as P-protein and none was found in sieve elements of healthy beet plants (ESAU and GILL 1970 b). The origin of spiny vesicles and spiny tubules has not been determined. Both dictyosomes and endoplasmic reticulum have been suggested as possible sources of spiny vesicles (NEWCOMB 1967). The spiny components in *Welwitschia* are clearly segments of ER and are unrelated to the formation of P-protein, which is lacking in *Welwitschia*. Structures interpreted as slime or P-protein bodies have been observed with the light microscope in several coniferous species (EVERT and ALFIERI 1965) and with the electron microscope in *Pinus strobus* (MURMANIS and EVERT 1966) and *P. silvestris* (PARAMESWARAN 1971).

The plastids of *Welwitschia* sieve cells differ from those of *Pinus*, in that they lack crystalline inclusions and fibrillar material at all stages of development (MURMANIS and EVERT 1966, SRIVASTAVA and O'BRIEN 1966, WOODING 1966, PARAMESWARAN 1971). The latter, referred to as plastid filaments





Figs. 21 and 22

Fig. 21. Transection of mature sieve cell showing massive ER aggregate associated with sieve area (*SA*). A strand of ER extends from aggregate across lumen of cell to ER along opposite wall. ×25,300

Fig. 22. Plastid in mature sieve cell lacks internal membranes. It contains several starch granules in an electron-transparent matrix. ×43,000

by PARAMESWARAN (1971), are especially well developed in *P. silvestris*. The plastids of *Metasequoia*, like those of *Welwitschia*, contain only starch granules (KOLLMANN and SCHUMACHER 1961, 1964), and apparently contain more internal membranes in younger than in older sieve cells (KOLLMANN and SCHUMACHER 1962).

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