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Ultrastructure of the Extrafloral Nectaries of *Pteridium aquilinum*

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The presence of nectaries on the stipe and frond distinguishes *Pteridium aquilinum* (L.) Kuhn from most other vascular cryptogams. The largest and most obvious nectaries are located on the stipe at the base of the lower pinnae. Nectaries on the stipe decrease in size toward the apex of the frond. The smallest nectaries are located on the abaxial surface of the frond, scattered on pinnae axes.

Described first in 1877 by Darwin, the nectaries of *Pteridium* have undergone further microscopic examination (e.g., Lloyd, 1901; Lüttge, 1961; Schremmer, 1969; Page, 1982). Lloyd (1901) noted that cells in the glandular tissue are smaller and contain more protoplasm than adjacent ground parenchyma tissue. These cells have vacuolated cytoplasm and thin cell walls. The anatomical differences noted by Lloyd have provided the basis for subsequent reviews without further elucidation. Fahn (1979a), when reviewing differences between structured and non-structured nectaries, cited the nectaries of *Pteridium* as non-structured. Structured nectaries can be identified macroscopically and their secretory cells differentiated microscopically whereas non-structured nectaries are basically unmodified tissue that secretes nectar through stomates. We find that nectaries of *Pteridium* which exude nectar through stomates can be distinguished both macroscopically and microscopically. Macroscopically, they are distinct protuberances, differing in color from the rest of the stipe and lacking trichomes. Microscopically, these nectaries are composed of layers of nectariferous tissue, distinctly specialized compared to ground parenchyma. Thus they can be defined as structured nectaries.

MATERIALS AND METHODS

Two populations of *Pteridium aquilinum* yielded the specimens in this study: one from Reston, Virginia (*Power 7734* in George Mason University Herbarium) and one from Mountain Lake Biological Field Station of the University of Virginia in Pembroke, Virginia (vouchers in Mountain Lake Herbarium). Secreting and non-secreting nectaries from stipes were excised and fixed in FAA (formalin, acetic acid, alcohol), or glutaraldehyde according to procedures outlined by Warmbrodt and Evert (1974a). Directions for dehydrating and embedding followed those in Mokotoff (1978). Tissue was embedded in Spurr's medium (Spurr, 1969). Thick (5–10 μm) sections for light microscopy were mounted on glass slides and stained with either safranin-fast green or toluidine blue; thin

sections (0.6–0.7 μm) for transmission electron microscopy (TEM) were placed on grids and viewed with a JEOL 100C microscope.

Secreting and nonsecreting nectaries were prepared for scanning electron microscopy (SEM) by fixing 5 mm sections in FAA or glutaraldehyde and dehydrating in an absolute alcohol series. Prior to fixation some nectaries were cut in two with a razor blade. They were then dried in a critical point drier. All nectaries were mounted on stubs and coated with gold-palladium, prior to viewing with either a JEOL 35C microscope or a Hitachi S530 microscope.

Results

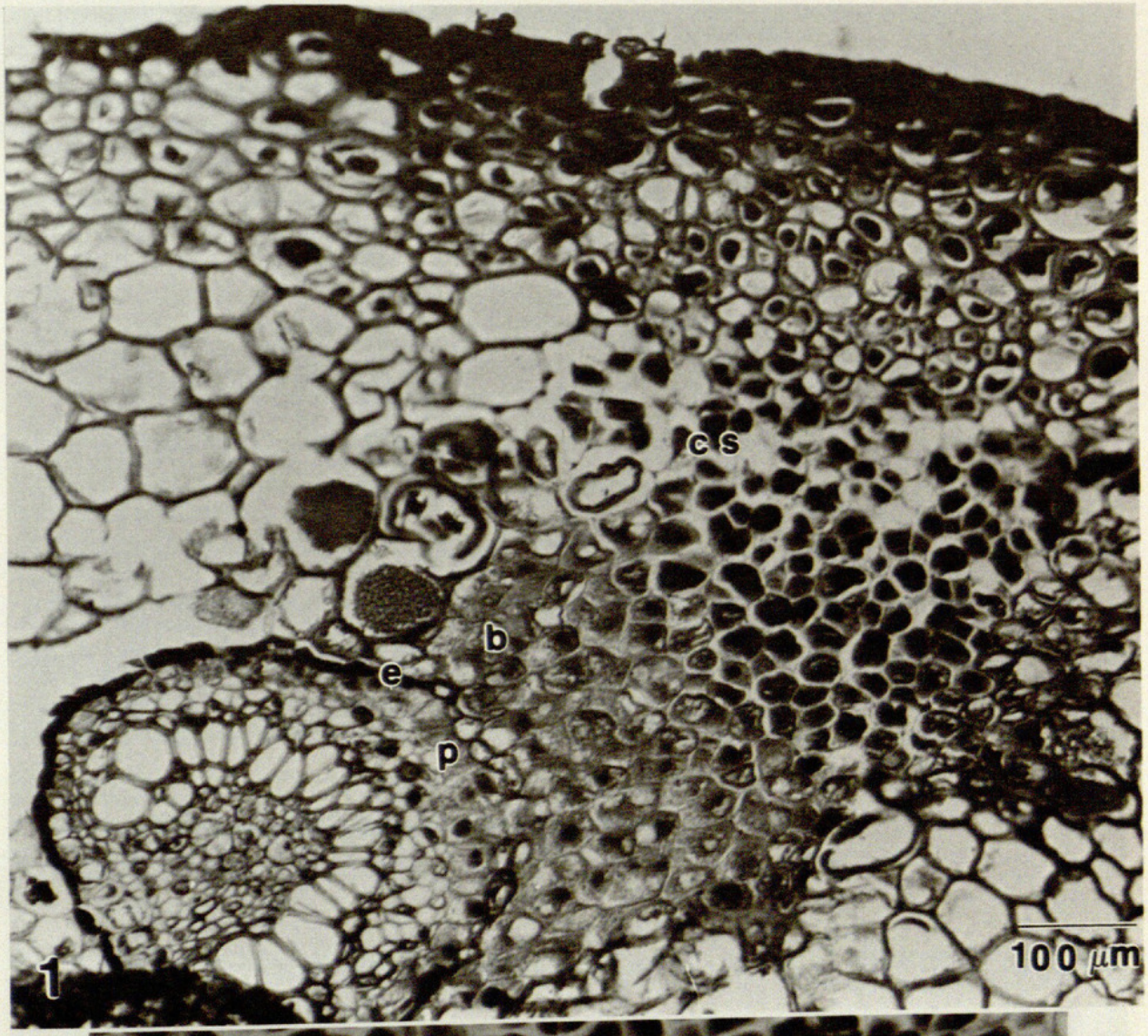
Morphologically, nectaries appear as smooth protuberances, raised 0.1–1.0 mm above the surface of the stipe. Located on the abaxial surface of the stipe at the base of each pinna or pinna pair, they are approximately 1–4 mm in diameter and vary in color from dark green (secretory) to brown (nonsecretory).

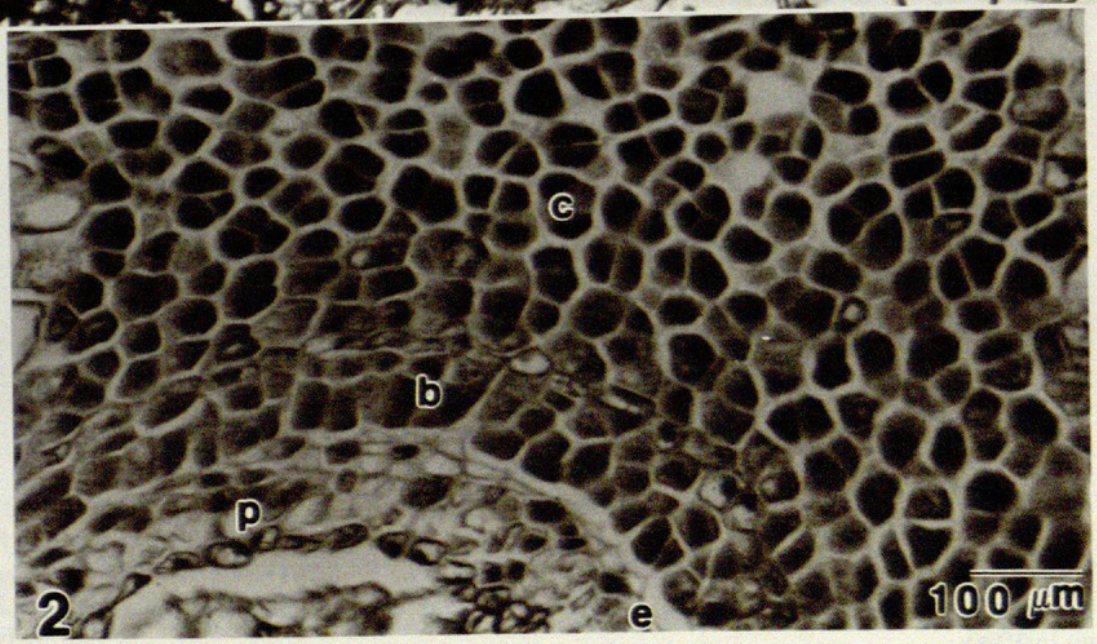
Transverse sections of the stipe show that the nectaries are distinguished from other tissues by specialized secretory parenchyma cells between the endodermis and the epidermis. The nectariferous tissue appears to be divided into three regions: a basal layer adjacent to a meristele, a broader cortical region, and an epidermal region. These combined regions measure approximately 0.3 mm from meristele to nectary surface. The tissue is 0.4 mm wide and is bordered on either side by cortical ground parenchyma cells which are large, irregular in outline and highly vacuolated. Figure 1 shows an overview of a nectary shortly after cessation of secretion; Figure 2 depicts a secretory nectary.

The basal region next to the meristele ranges from 3–7 cells wide and abuts the outer walls of the endodermis (Figs. 1 and 2). The cells are smaller than the surrounding cortical parenchyma cells and appear cuboidal. The granular cytoplasm is filled with organelles and several large vacuoles (Fig. 3).

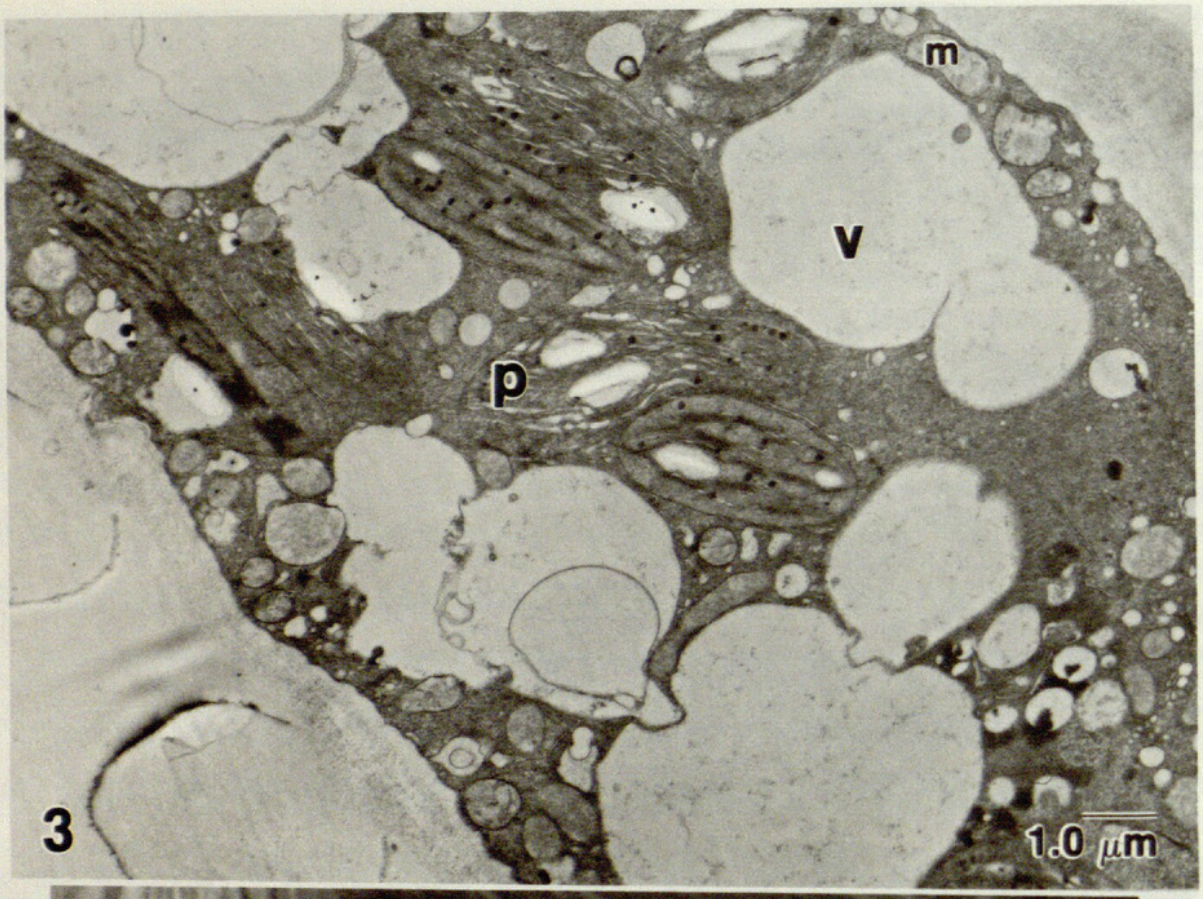
Adjacent to the basal region is an area composed of specialized parenchyma cells, the cortical secretory parenchyma. These cells are small and isodiametric, compared to the larger elongated ground parenchyma cells which compose most of the cortex and pith (Figs. 1 and 2), and contain densely staining cytoplasm filled with small vesicles but no large vacuoles. Nuclei appear large compared to the volume of the cell; each nucleus contains a nucleolus. In contrast, cytoplasm in ground parenchyma cells is more diffuse, often parietal, and hence stains very little. Nuclei occupy a smaller volume in these larger cells and are thus often excluded from the plane of section.

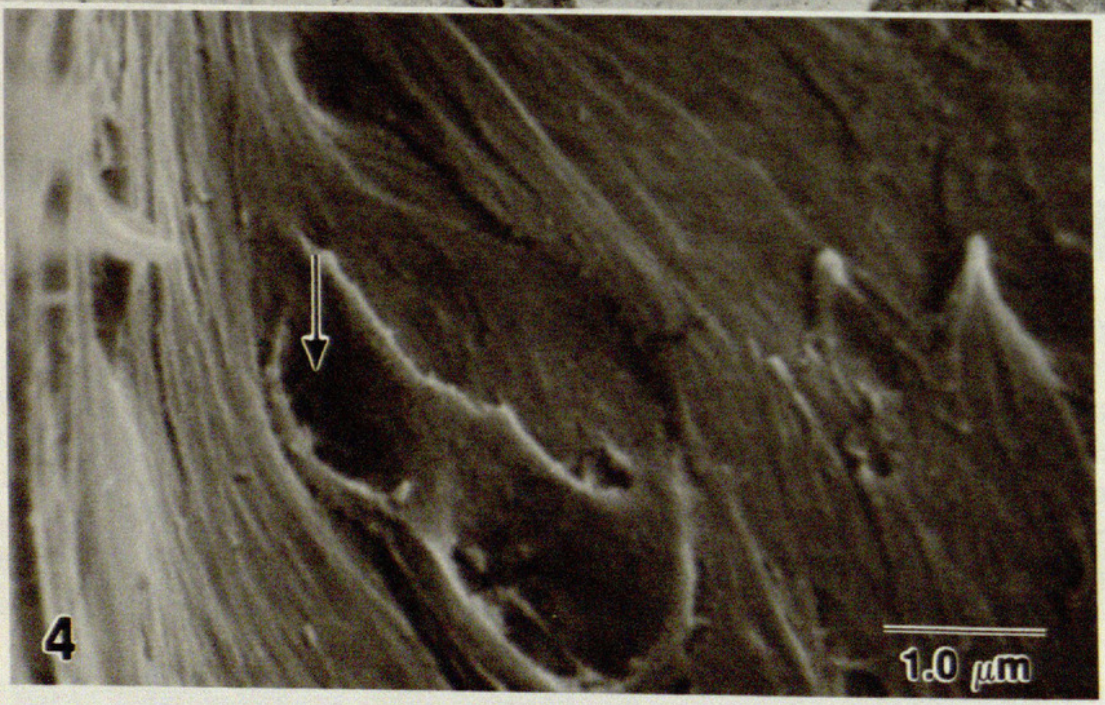
The cortical secretory parenchyma cells are connected by plasmodesmata which are often found within primary pit fields and simple pits if any secondary cell wall has been laid down (Fig. 4). The cells contain much endoplasmic reticulum (ER), some with enlarged cisternae, and dictyosomes. Fibrillar inclusions similar to fibrous material described by Fahn (1979b, fig. 11) can be seen outside the membrane and within the enlarged cisternae (Fig. 5). Figure 6 shows a plasmodesma between two cortical secretory parenchyma cells; this plasmodesma contains a relatively large (0.27 μm) multivesicular body and endoplasmic reticulum. A small segment of a nucleus is visible in one cell. Near the nuclear envelope is





FIGS. 1 and 2. *Pteridium* nectary anatomy. FIG. 1. Cross-section of stipe at cessation of secretion. Stomate is open, but epidermal cell walls have been thickened. Cortical secretory parenchyma (cs), basal region of parenchyma (b), the endodermis (e), and cells in the pericycle region (p) can be easily distinguished. FIG. 2. Cross-section of a secretory nectary. The cortical secretory cells (c) are distinct.





FIGS. 3 and 4. Ultrastructure of secretory nectary cells. FIG. 3. Transmission electron microscope (TEM) view of a cell characteristic of the basal region showing vacuoles (v) with some fibrillar material, plastids (p) with starch, and mitochondria (m). FIG. 4. SEM view of a cortical secretory parenchyma cell in split section showing a primary pit field. Several openings for plasmodesma can be seen (arrow).

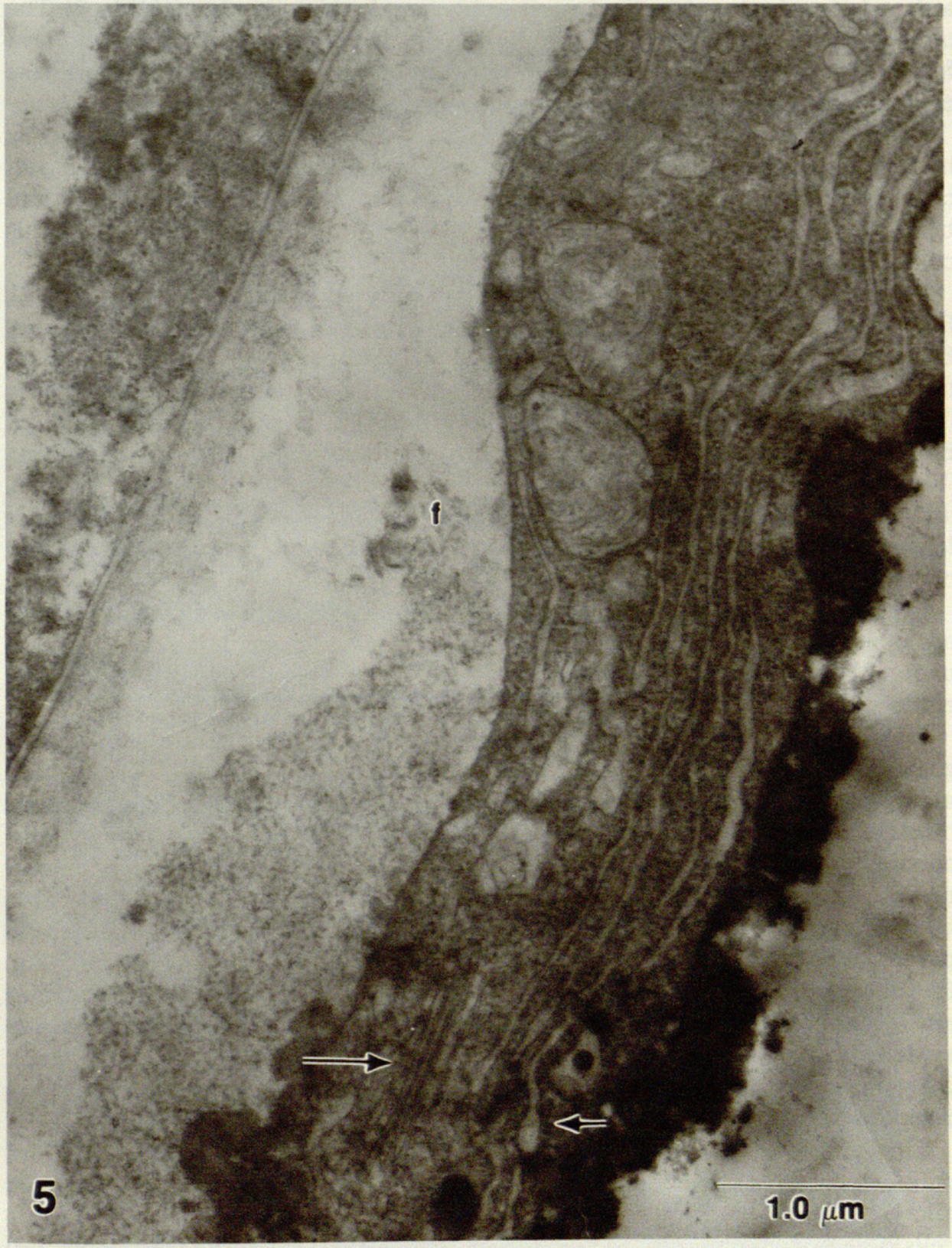


FIG. 5. TEM of a portion of a cortical secretory cell. The vacuole is at the right. Cytoplasm contains much ER with enlarged cisternae (right arrow). Dictyosomes are near the outer membrane (left arrow) and some fibrous material (f) is outside the membrane. Some cisternae contain possible fibrous material.

a strand of endoplasmic reticulum, the cisterna of which is enlarged (Fig. 6). The other cell contains several mitochondria near the plasmodesma. Vesicles have formed adjacent to the endoplasmic reticulum. No plastids are visible in these cells.

Cortical secretory parenchyma is distinguished during its functional period by inclusion of many globules stained brilliantly red with safranin under light microscopy (Fig. 2). These globules are also visible in vascular parenchyma and basal parenchyma although they are smaller. The intercellular spaces in this cortical region anastomose into substomatal chambers.

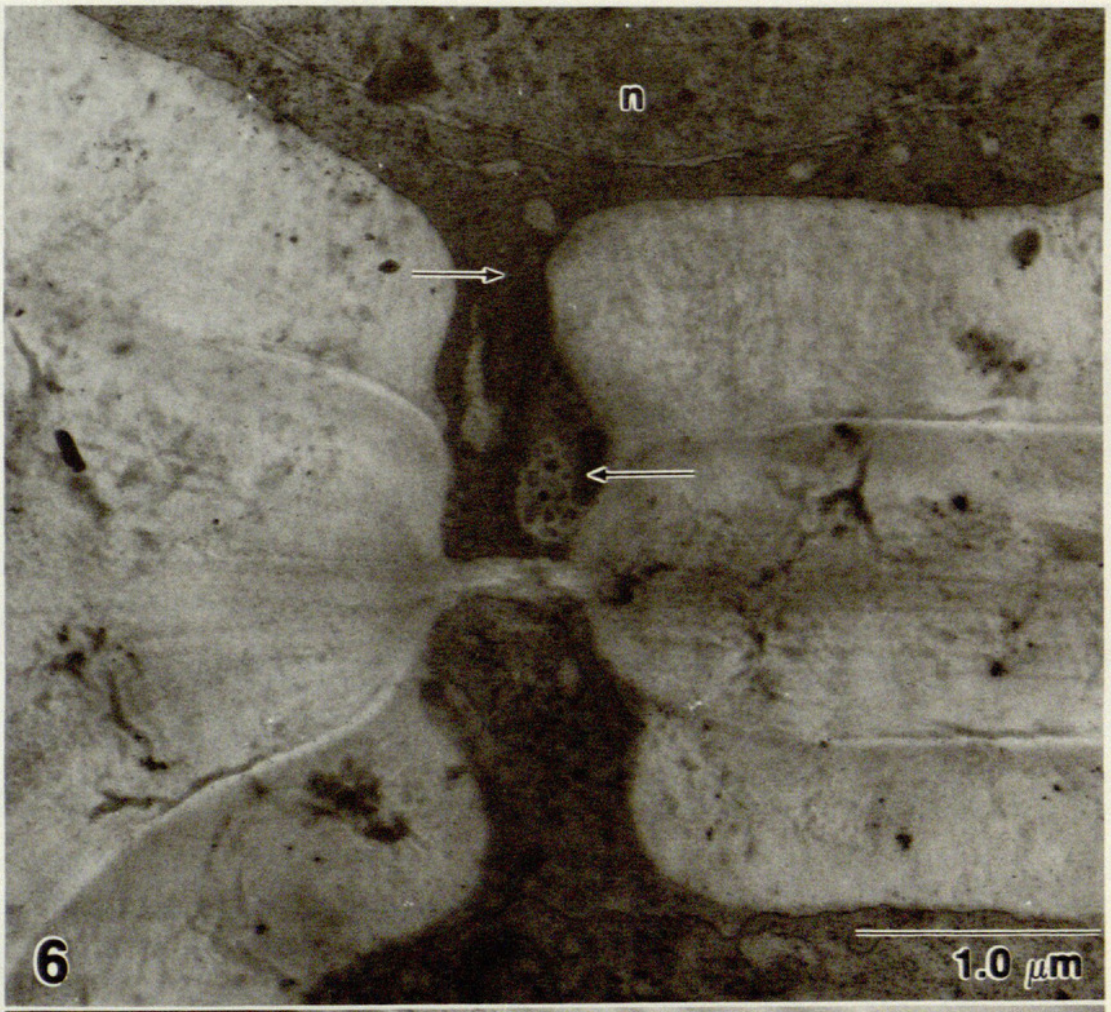
The epidermal region of the nectary is composed of 1–3 cell layers (Fig. 7). Each cell is oblong to rectangular and contains a nucleus, chloroplasts, vesicles, ER, and dictyosomes. The outer epidermal cells are covered by a thin cuticle on their outer walls (Fig. 8). The epidermal cells of the nectary are isodiametric (Fig. 9), whereas the epidermal cells of the stipe are elongated and almost linear. Stomates are much more abundant on the nectary compared to the stipe area (Fig. 9). Guard cells of the stomates are elevated above the epidermis (Figs. 1, 9, and 10). The guard cells are covered by a thin cuticle that projects into cuticular ledges (Fig. 1). The cuticle extends into the stomate and lines the substomatal chamber (Fig. 7). No subsidiary cells are present.

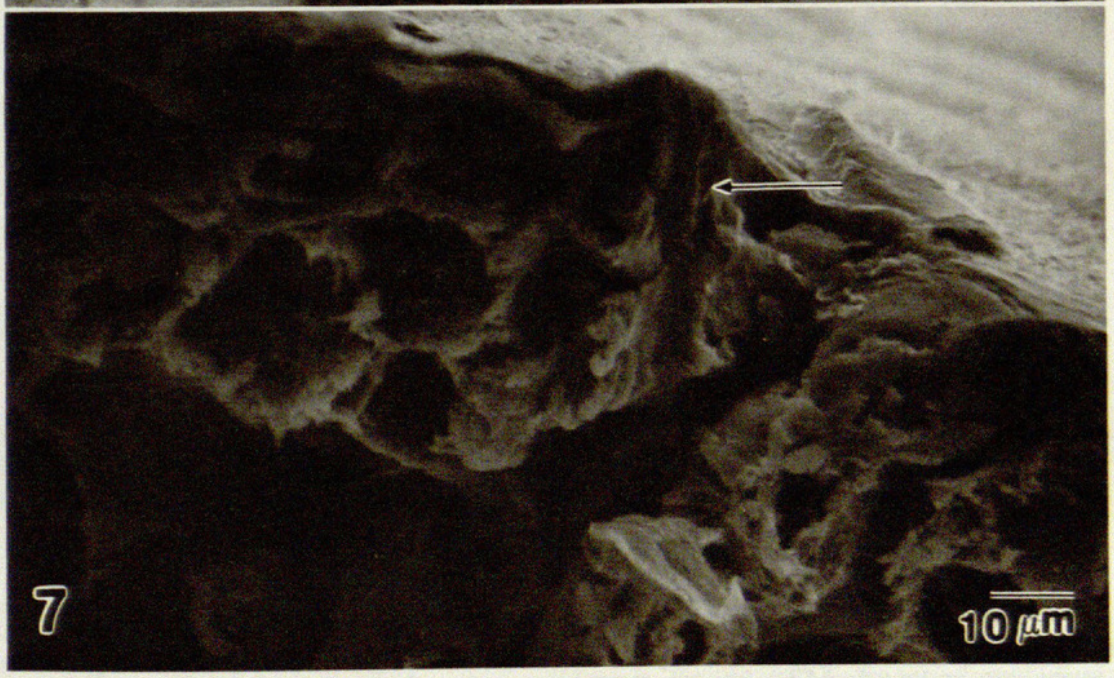
During the crozier stage when the nectaries are actively secreting, stomates are open and covered with globular secretions (Figs. 9 and 11). With cessation of function, the stomates become occluded (Fig. 10) and the cell walls of the epidermis become thickened and ridged.

In addition to these three differentiated regions, the adjoining meristele undergoes certain modifications. The part of the meristele next to the basal region enlarges with the formation of two or three layers of cells inside the endodermis (Figs. 1 and 2). These cells are somewhat larger than surrounding pericycle cells, cuboidal, and densely staining with very granular cytoplasm, large nuclei, and no vacuoles. The neighboring pericycle cells are somewhat smaller and variable in size, vacuolated, and form only one cell layer. These modified pericycle parenchyma cells appear quite similar to the basal cells in light microscopy (Fig. 2). The endodermis also undergoes modification in this region. Large vacuoles are evident in both light microscopy (Fig. 2) and TEM. No Casparian strip is evident in this region during the crozier stage (secretory phase) of development. Only after the frond has expanded fully and nectaries have ceased functioning does the Casparian strip begin to form.

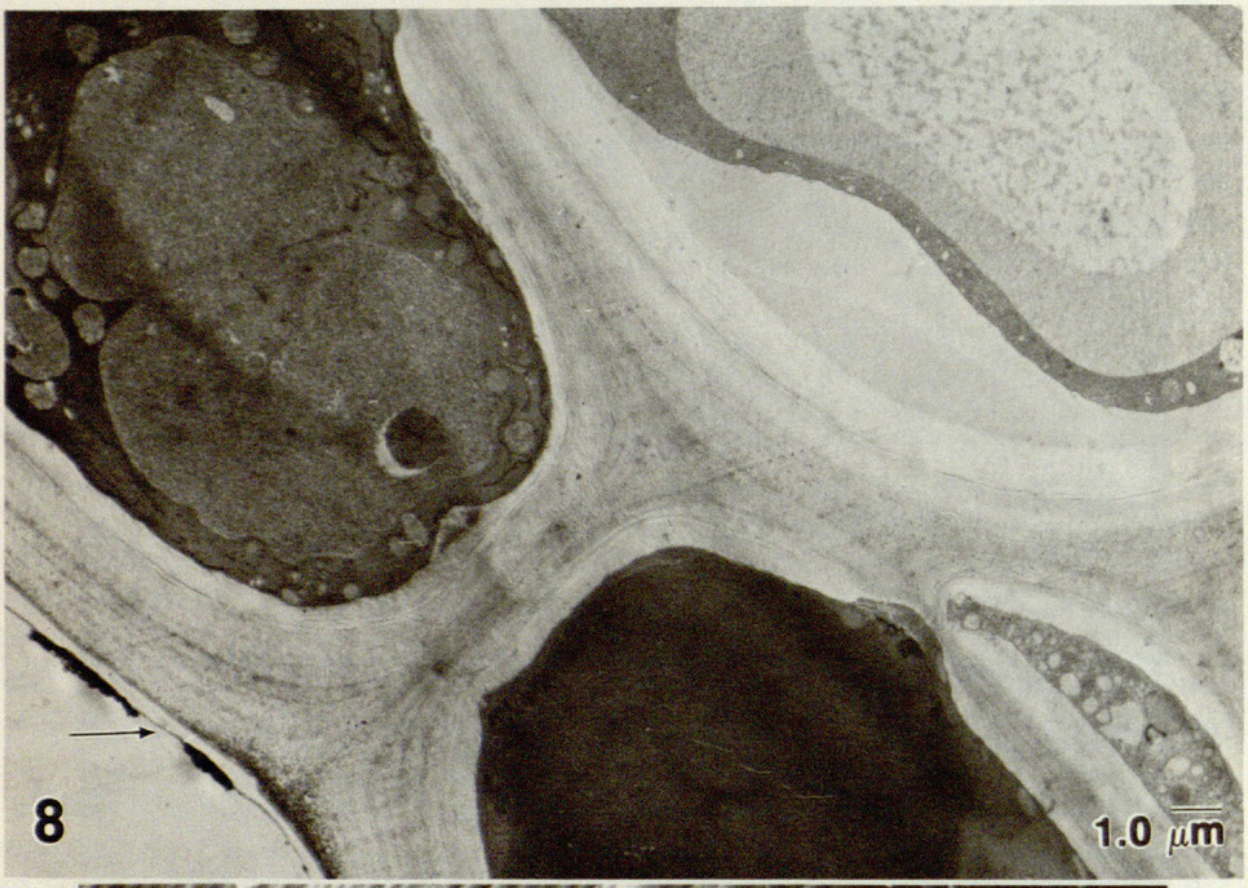
The vascular tissue, composed of sieve cells, xylem tracheids and vessels, and parenchyma cells (Figs. 1 and 2), is modified in content but not in structure. Light microscopy reveals the presence of safranin-stained globules within the sieve cells. Electron microscopy shows a large lumen characteristic of the sieve cell with remnants of cytoplasm near the periphery. A plasmalemma surrounds the lumen and abuts the primary cell wall. Some sieve cells contain an amorphous fibrillar material.

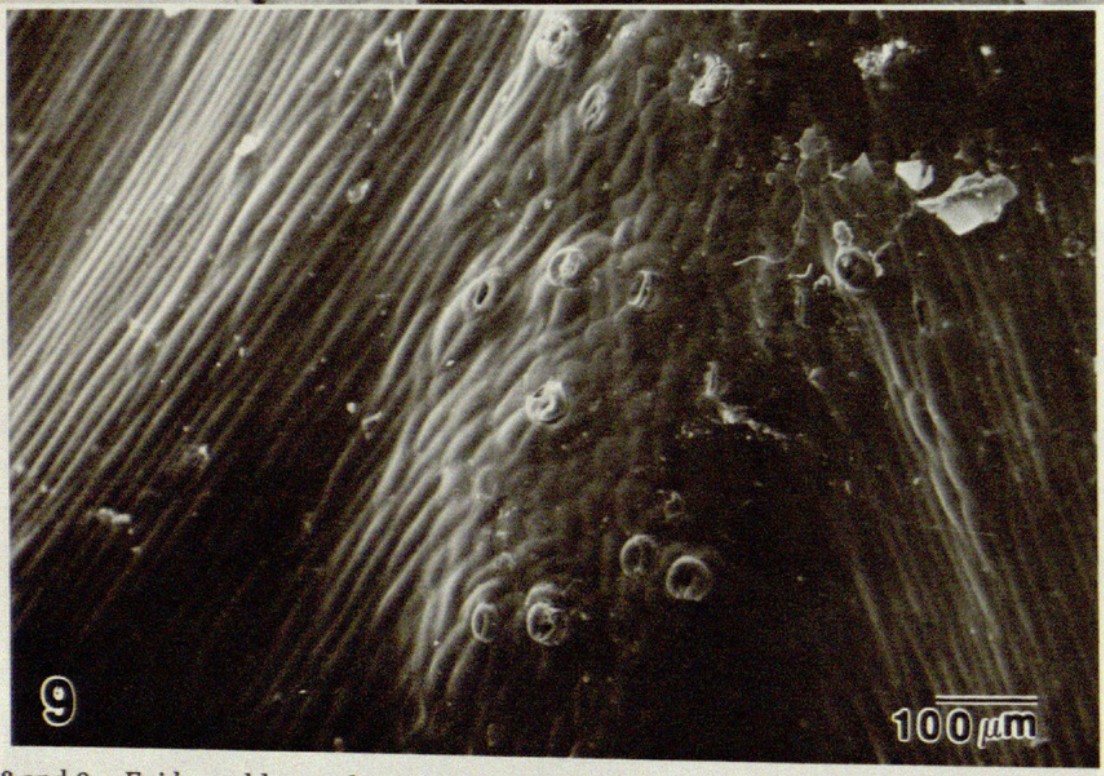
All sieve cells contain refractive spherules, located both centrally and peripherally in the lumen (Figs. 12 and 13). Bounded by single unit membranes, the spherules range in size from 0.1 to 0.7 μm. These spherules tend to aggregate



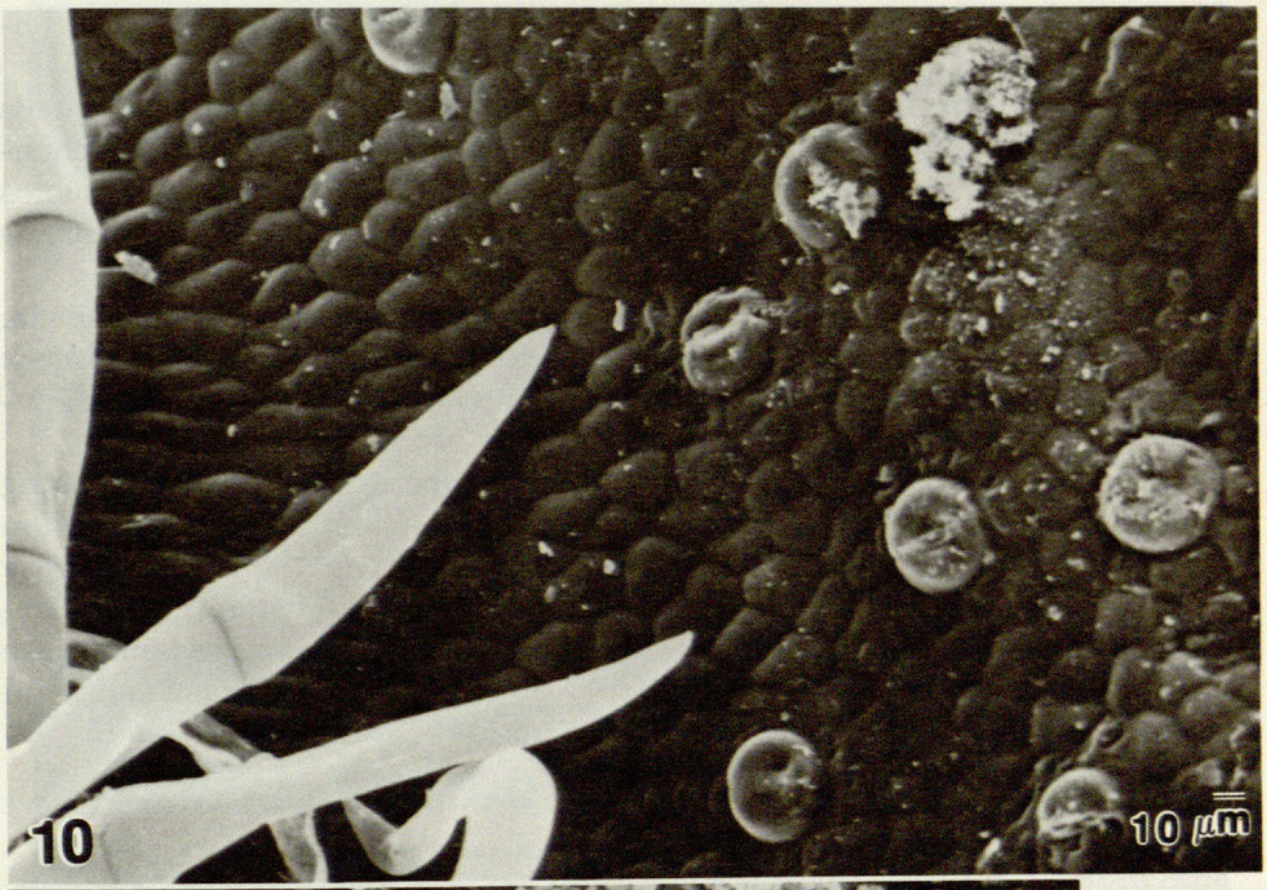


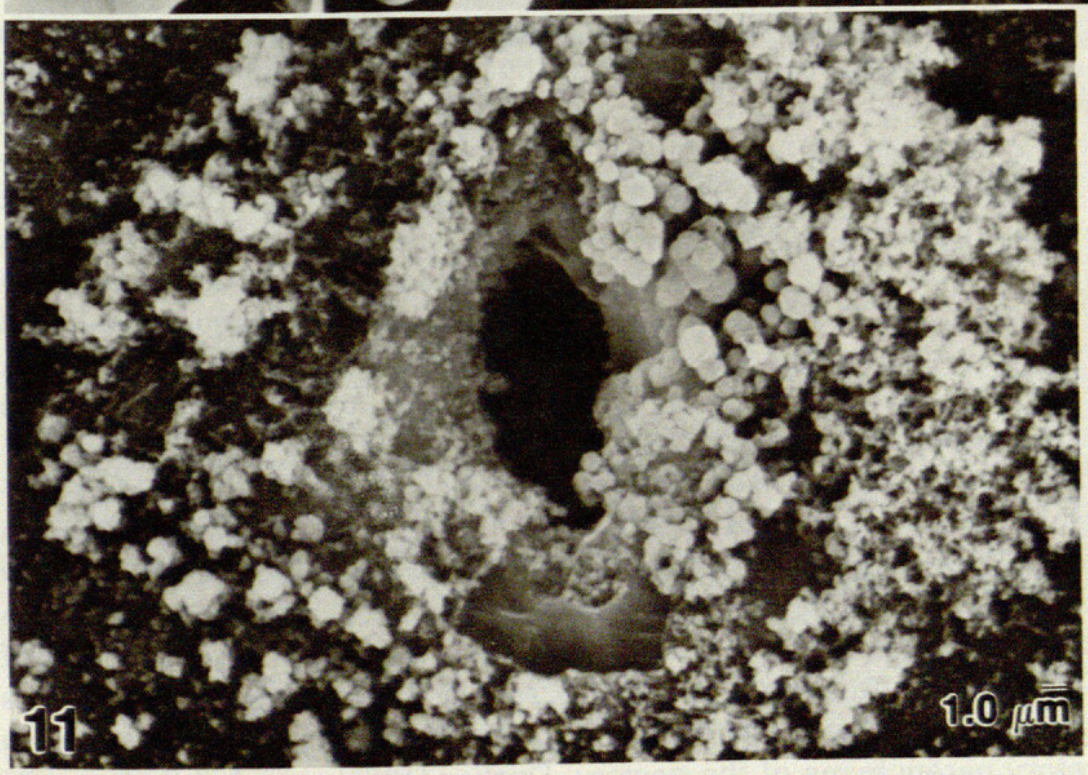
FIGS. 6 and 7. Ultrastructure of secretory nectary cells. FIG. 6. TEM of plasmodesma strand connecting cortical secretory cells. Within the plasmodesma are multivesicular bodies (right arrow) and ER (left arrow). Upper cell shows portion of nucleus (n). FIG. 7. SEM of the epidermal region around stoma. Two or three layers of cells in the epidermal region can be seen. The cuticle layer extends down into the stomate (arrow).





FIGS. 8 and 9. Epidermal layer of nectary. FIG. 8. TEM of the epidermal layer showing thin cuticle (arrow) and vacuoles with electron dense material. FIG. 9. SEM of nectary surface showing isodiametric cells and more numerous stomates as opposed to the elongated cells and very few stomates of the stipe.





FIGS. 10 and 11. Scanning views of nectary epidermis. FIG. 10. SEM of nectary epidermis at cessation of secretion with raised guard cells and trichomes of non-secretory region at left. FIG. 11. Open stomate with globular secretory material.

near the sieve pores which connect the sieve cell to the surrounding parenchyma.

The vascular parenchyma cells stain more densely than the larger sieve cells (Fig. 12). The dense cytoplasm of the parenchyma contains plastids, mitochondria, endoplasmic reticulum, vesicles, and vacuoles. The vesicles and vacuoles contain a fibrillar material similar to that in the sieve cells (Fig. 13). Dictyosomes are not evident in these cells.

Vascular parenchyma and sieve cells are interconnected by plasmodesmata, which join the parenchyma to the sieve pore areas of the sieve cell (Warmbrodt & Evert, 1974a and 1974b). ER may line the interior of these cytoplasmic connections. Figure 12 indicates the location of some of the plasmodesmata.

The developmental stage of the frond determines the structure and functioning of the nectaries of *Pteridium aquilinum*. The nectaries differentiate and function only during the crozier stage. With maturation of the frond, the nectaries cease secreting and some lysigenous degradation is noted in the tissue (Fig. 1). Secondary cell walls are formed in most cells, fewer globules and more tannins are seen in the secretory cells, a Casparian strip forms in the endodermal cells, and the stomata become closed (Fig. 10).

Discussion

Studies of the ultrastructure of nectaries and the possible function of the cells have emphasized angiosperms. It is interesting to compare data derived from angiosperms with these observations of the *Pteridium* nectary. In angiosperm nectaries vascular tissue is generally separated from the secretory cells by nonglandular or subglandular parenchyma (Durkee, 1983). Characteristics of these cells include abundant mitochondria, well-developed vacuoles, large numbers of plasmodesmata, less dense cytoplasm, and less developed ER than the secretory cells. In *Pteridium* the cells of the basal region are well vacuolated, have granular cytoplasm, many mitochondria, and plastids, and are tightly packed in contrast to the surrounding parenchyma cells. Thus they possess most of the characteristics of subglandular parenchyma cells.

Although there is no specialized vascular tissue supplying the nectary in *Pteridium*, the vascular strand of the meristele is only a few cells distant from the secretory cells. Furthermore, the cells of the pericycle and endodermis are highly modified in the region of the nectary. These cells are similar to the basal parenchyma but lack the extensive development of vacuoles. The Casparian strips of the endodermal cells are not developed. Such changes in this region of the meristele may facilitate transport of the pre-nectar (phloem sap) from the phloem to the secretory parenchyma of the nectary. Durkee (1983) reported that a common component of the parenchyma cells in the phloem of *Passiflora* nectaries is membrane-bound fibrillar material (proteinaceous) and that rough ER is abundant. Vesicles containing fibrillar material and abundant ER can be seen in the vascular parenchyma cells of *Pteridium* (Fig. 13).

According to Fahn (1979b), the secretory cells of some angiosperm nectaries are characterized by increased numbers of mitochondria, increased amount of

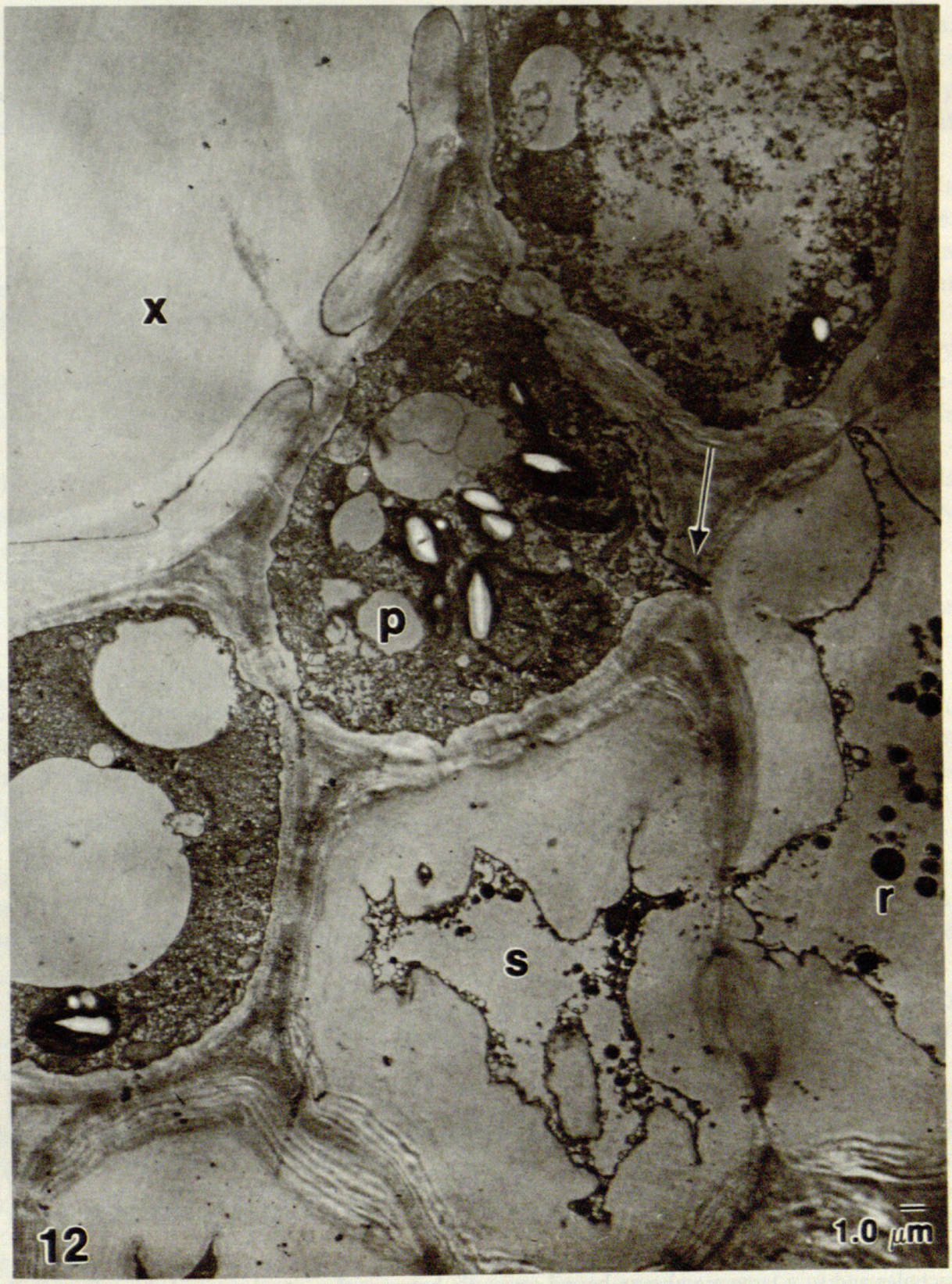


FIG. 12. TEM section of the vascular tissue with xylem element (x), vascular parenchyma (p) and sieve cells (s) containing refractive spherules (r). Plasmodesmata are seen between vascular parenchyma and sieve cells (arrow). Many small vesicles are present bordering the lumen of the sieve cell.

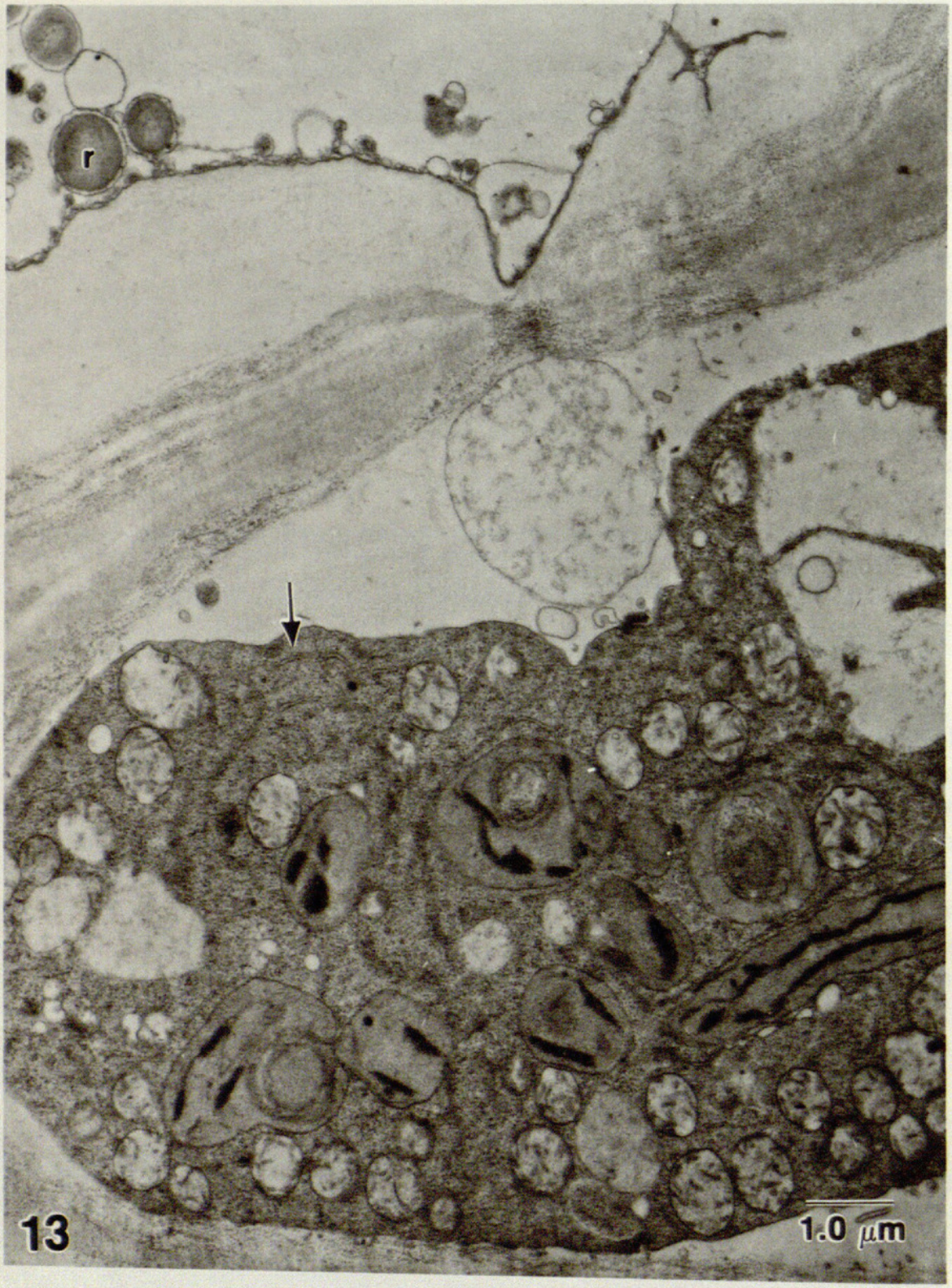


FIG. 13. TEM of sieve cell above and vascular parenchyma cell below, with fibrillar material in vacuoles and in a large membrane-bound organelle between the cytoplasm and the cell wall. Endoplasmic reticulum can be seen in parenchyma cell (arrow). Refractive spherules (r) are obvious in the sieve cell.

ER with swollen cisternae, vesicles, and also, in others, numerous Golgi bodies. These characteristics were all noted in the cortical region of the *Pteridium* nectary. In addition, the distinct differences between the cortical cells of the nectary as opposed to the surrounding cortex of the stipe (Figs. 1 and 2) indicate that they are indeed modified and are probably secretory in function.

The large nucleus which appears to fill most of each cortical secretory cell is almost obscured by the large number and size of safranin-stained red globules. These globules are similar to those seen in sieve cells and vascular parenchyma and represent an increase in size of 2–4 times, possibly indicating modification of the secretion in these cells.

Once the secretion is modified, it may be packaged and/or transported by ultrastructural organelles in the cortical secretory parenchyma before it is released into a substomatal chamber. Ultrastructural organelles involved in this transport of secretory material may include plasmodesmata, endoplasmic reticulum, and multivesicular bodies. Plasmodesmata not only link parenchyma cells to sieve cells in vascular tissue, but they also interconnect the cortical secretory parenchyma cells. They permit symplastic transport to occur throughout nectariferous tissue (Lüttge, 1971).

Durkee (1983) stated that ER is a notable feature in glandular cells, and the ER cisternae sometimes are swollen and vesicles are numerous. In *Pteridium* cortical secretory parenchyma, some ER cisternae are swollen with fibrillar inclusions and many small vesicles are present. A form of packaging and secretion, noted by Fahn and Rachmilevitz (1975), involves vesicles that may bud off from the ends of the cisternae. As vesicles approach the plasmalemma, the membranes may fuse, releasing the fibrillar contents to the outside of the cytoplasm.

Multivesicular bodies are aptly named for their structure because their function remains unclear. Noted in plasmodesmata (Fig. 6), they may function in formation or transport of pre-nectar (Rachmilevitz & Fahn, 1973). Fahn (1979a) noted that multivesicular bodies are often present in active secretory cells and summarized several functions suggested for their role in the secretory process.

Other characteristics of secretory cells also observed in the cortical secretory parenchyma of *Pteridium* include numerous mitochondria, few plastids, reduced vacuoles, and dictyosomes adjacent to the wall.

With the exception of stomates, the epidermal cells covering the surface of the nectary do not appear to be involved directly with nectar secretion. No globules appear within the cells when viewed in light microscopy. Viewed in scanning electron microscopy, however, secretory stomates that are open are covered with a globular material. Closed stomates have none of the material. Since stomates are much more abundant in the epidermis of the nectary than in the rest of the stipe, they appear to be involved with more than respiration and gas exchange. Stomates, described as modified when they are coupled with secretion, usually remain open (Fahn, 1979a). However, closed stomates do occur on nectary surfaces.

The ultrastructure of the nectaries of *Pteridium aquilinum* indicates that most of the organelles and cell types associated with vascularized angiosperm nec-

taries are present in these fern nectaries. The *Pteridium* nectaries are definitely structured nectaries, contrary to Fahn's (1979a) description of them as undifferentiated and Page's (1982) statement that they are composed only of small, isodiametric parenchymatous cells with dense protoplasmic contents.

Field observations by Page (1982) indicate a secretory period from six days to about four weeks. There are corresponding anatomical changes that occur in the nonfunctional nectary following cessation of secretion. These changes in cell structure appear to occur from the epidermal region centripetally (Fig. 1). Epidermal cell walls become thickened first and the stomata are occluded (Fig. 10). In the cortical secretory parenchyma the globules decrease and tannins become more obvious. These cells also begin to form secondary cell walls. Lignification occurs in most cells and the endodermis forms a Casparian strip in the cells within the nectary region.

The anatomy of *Pteridium* nectaries correlates with field observations and with the reported examples of other structured nectaries. Durkee (1983) noted that research studies of nectaries should be concerned with ultrastructural detail, the nature and function of subglandular tissue, and the vascular supply to nectaries in regard to phloem physiology. The presence of modified tissue in the nectary and simple sieve cells in the phloem would appear to make *Pteridium* an excellent tool for physiological studies of phloem and nectary activity.

Acknowledgments

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