Ultrastructure of organic acid secreting trichomes of chickpea (*Cicer arietinum*)

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Received January 3, 1989

LAZZARO, M. D., and THOMSON, W. W. 1989. Ultrastructure of organic acid secreting trichomes of chickpea (*Cicer arietinum*). Can. J. Bot. **67**: 2669–2677.

The acid-secreting trichomes of chickpea (*Cicer arietinum* L.) were composed of 18 cells, including 1 basal cell, 3 elongate stalk cells, and 14 head cells. A subcuticular secretion chamber with cuticular pores was present above the head cells at the trichome tip. The basal and stalk cells had large central vacuoles, endoplasmic reticulum, mitochondria, and small vacuoles. In the stalk cells, these small vacuoles were aligned along microtubles extending from the bottom to the top of the cells. Head cells had more dense cytoplasm than stalk cells and also had numerous mitochondria and small vacuoles. A labyrinth of tubules and vesicles at the edges of the head cells contained granular material similar to that observed in the extraplasmic space of the head cell and in the secretion chamber. In older head cells, the tubules were thinner and lacked granular material, the cells contained sequestering membranes and vacuoles, and calcium oxalate crystals were observed in the extraplasmic space. Plasmodesmata were not observed between the basal cell and the surrounding mesophyll cells, although numerous plasmodesmata with associated desmotubules and endoplasmic reticulum connected the trichome cells. Chloroplasts were not observed in the head or stalk cells, whereas the basal cell had small chloroplasts with reduced thylakoid networks and the mesophyll cells had large chloroplasts with well-developed thylakoids that may provide the fixed carbon for organic-acid secretion.

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Les poils sécréteurs d'acide du pois chiche (*Cicer arietinum* L.) sont constituées de 18 cellules dont 1 cellule basale, 3 cellules allongées de pédicelle et 14 cellules de tête. Une chambre de sécrétion subcuticulaire avec des pores cuticulaires est présente au-dessus des cellules de tête à l'extrémité du poil. La cellule basale et celles du pédicelle sont pourvues d'une grande vacuole centrale, de réticulum endoplasmique, de mitochondries et de petites vacuoles. Dans les cellules du pédicelle, ces petites vacuoles étaient disposées le long de microtubules qui se retrouvaient de la base au sommet des cellules. Le cytoplasme des cellules de tête était plus dense que celui des cellules du pédicelle et renfermait aussi de nombreuses mitochondries et de petites vacuoles. Un labyrinthe de tubules et de vésicules sur les bords des cellules de tête contenait une substance granuleuse semblable à celle observée dans l'espace extraplasmique de la cellule de tête et dans la chambre de sécrétion. Dans les plus vieilles cellules de tête les tubules étaient plus minces et n'avaient pas de matière granuleuse et les cellules avaient des membranes d'isolement et des vacuoles tandis qu'on pouvait observer des cristaux d'oxalate de calcium dans l'espace ectoplasmique de la cellule. On n'a pas observé de plasmodesmes entre la cellule basale et les cellules de mésophylle qui l'entourent, quoique de nombreux plasmodesmes avec leur desmotubules et leur réticulum endoplasmique reliaient les cellules du poil entre elles. On n'a pas identifié de chloroplastes dans les cellules de la tête ni dans celles du pédicelle, tandis que dans la cellule basale on a observé de petits chloroplastes avec un réseau réduit de thylakoïdes et dans les cellules du mésophylle on a noté de gros chloroplastes avec des thylakoïdes bien différenciés qui fournissent peut-être le carbone fixé nécessaire pour la sécrétion d'acide organique.

[Traduit par la revue]

Introduction

Chickpea (*Cicer arietinum* L.) is a major food crop in India, Pakistan, the Middle East, Africa, and Central America (Van Dermaesen 1972). Trichomes on the stems, leaves, and seed pods secrete organic acids, primarily malate, with traces of citrate, oxalate, and acetate (De Candolle 1832, cited in Uphof 1962; Koundal and Sinha 1981; Rembold 1981; Lauter and Munns 1986). In fact, the secretions are quite acidic, with a pH of 1.0 or less (Lauter and Munns 1986).

Plant trichomes have been extensively studied (see Fahn 1979; Uphof 1962; Haberlandt 1914). Trichomes can be unicellular or multicellular and have been shown to function in the secretion of a range of compounds, including inorganic salts (Thomson *et al.* 1988), sugars (Findlay 1988), oils, and proteins (Lüttge and Schnepf 1976). However, chickpea is the only known example of trichomes in which the primary function is the secretion of organic acids.

The anatomy of chickpea trichomes was first described by Sahasrabuddhe (1914), who noted the occurrence of both unicellular, simple hairs and multicellular, glandular hairs. Schnepf (1965) reported that the glandular trichome was made up of 11 or 12 cells: 1 basal cell, 3 elongate stalk cells, and a head of 7–8 cells in four tiers of 2 cells each, with the lowest tier containing 1 but sometimes 2 cells. The ultrastructure of the head cells and the uppermost stalk cell was also described by Schnepf (1965). In particular, he reported that the head cells occasionally contained amyloplasts but did not contain chloroplasts. Thus a carbon source must move from underlying cells, presumably the leaf mesophyll, to the head cells via the symplast, the apoplast, or both. In this paper, we outline the possible pathways for carbon transport by examining the ultrastructure of the basal, stalk, and head cells using scanning (SEM) and transmission (TEM) electron microscopy.

Materials and methods

Seeds of *C. arietinum* L. cv. UC5 were germinated in vermiculite-filled pots in a growth chamber, and plants were transferred to the greenhouse 2-3 weeks after sowing. Mature leaves were cut into 1-mm^2 pieces in one of four fixatives: (A) half strength Karnovsky's (2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M phosphate

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Printed in Canada / Imprimé au Canada

buffer) (Karnovsky 1965); (B) 5% glutaraldehyde in 0.1 M phosphate buffer; (C) 2.5% glutaraldehyde in 0.1 M phosphate buffer; or (D) 0.5% glutaraldehyde in 0.05 M phosphate buffer. Material was then transferred into vials containing the same fixative and fixed for 2 h at room temperature, rinsed for 15 min in 0.1 M phosphate buffer at pH 6.8, then postfixed in 1% osmium tetroxide in 0.1 M phosphate buffer overnight at 4°C; material fixed in (D) was postfixed in 1% osmium tetroxide in 0.05 M phosphate buffer overnight at 4°C. The quality of fixation was similar with all procedures. The next day, material was dehydrated through a series of 30, 50, 70, 95, and 100% acetone solutions with hourly changes, then stored in 100% acetone overnight at room temperature. Material was put into fresh 100% acetone the next day and infiltrated with Spurr's resin (Spurr 1969) by dropwise addition over 12 h, then stored uncovered overnight at 4°C. The next day material was brought to room temperature, put in blocks of fresh Spurr's resin, allowed to settle for 1 h, then polymerized at 70°C for 48 h. Gold sections (90 μm thick) were cut with a Dupont diamond knife on an RMC ultratome. Sections were collected on 100-mesh Formvar-coated copper grids, stained with 1% uranyl acetate for 10 min, and poststained with Reynolds' lead citrate (Reynolds 1963) for 1.5 min. Sections were examined with a Philips 400 transmission electron microscope at 80 kV.

Tissues from leaves, stems, and pods were prepared for SEM. Material was cut into 10-mm^2 pieces in fixative (D), fixed for 2 h at room temperature, and postfixed in 1% osmium tetroxide in 0.1 M phosphate buffer overnight at 4°C. The next day, material was dehydrated through a graded acetone series with hourly changes, then in 100% acetone overnight at room temperature. Material was then transferred to fresh 100% acetone and critical-point dried with liquid carbon dioxide using a Balzers/Union CPD 020 critical point dryer. Material was mounted onto metal stubs with silver paint, sputter coated with 20 nm of gold and palladium using an Emscope SC500 sputter coater, and examined with a Philips 515 scanning electron microscope at 10 or 20 kV.

Results

Both secretory (Fig. 1) and nonsecretory (Figs. 1, 2) trichomes were present on the leaves, stems, and pods. Secretory trichomes were most numerous on the pods (Fig. 1), with fewer on the stems and fewer still on the leaves. All growth stages of the secretory trichomes were present on mature tissue. The number of nonsecretory trichomes per unit area appeared to be the same between leaves, stems, and pods.

Mature secretory trichomes were composed of 18 cells, including 1 basal cell, 3 stalk cells arranged in three tiers, and 14 head cells arranged in four tiers (Fig. 3). Each tier of stalk cells had one cell (Fig. 4), whereas the lowest tier of head cells had two cells (Fig. 5) and the upper three tiers of head cells had four cells each (Figs. 6 and 7). Each tier of head cells was rotated approximately 45° with respect to the previous tier (Fig. 8). In addition, the upper stalk cell occasionally divided, producing a fourth stalk cell; an additional tier of four head cells was sometimes observed.

The basal cell was 5–6 times larger in cross section than the surrounding mesophyll cells and had a large central vacuole through which cytoplasmic strands passed, a nucleus, mitochondria, chloroplasts, small vacuoles, smooth and rough endoplasmic reticulum (ER), polyribosomes, and dictyosomes (Figs. 9–12). Since the basal cell plasma membrane was often invaginated near the stalk cell (Fig. 11) and the basal cell primary wall was thinner than that of the stalk cell, the middle lamella was closer to the basal cell (Fig. 12). The basal cell chloroplasts were approximately one-tenth the size of the chloroplasts in the neighboring mesophyll cells and had a reduced thylakoid network compared with the granal–fretwork system in the mesophyll chloroplasts (Fig. 10).

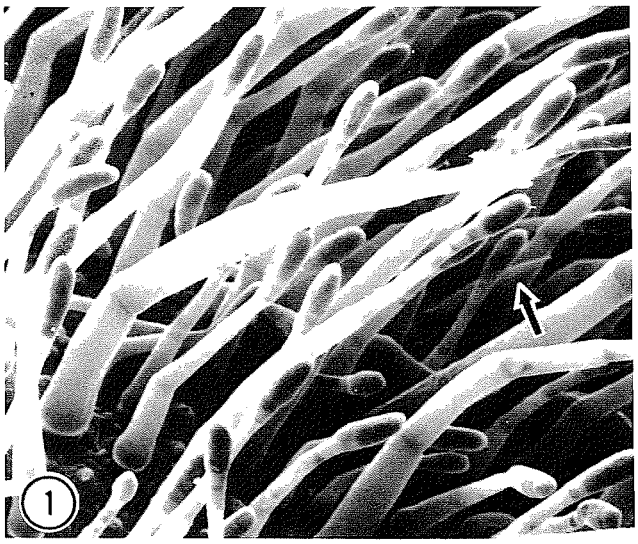
The stalk cells contained a large central vacuole, with the other organelles confined to the peripheral cytoplasm (Fig. 13) and cytoplasmic strands (Fig. 14). ER was arranged in loose, coil-like aggregates (Fig. 15) or loose, tubular aggregates at the ends of the cell (Fig. 12) and in straight, vertical strands in the peripheral cytoplasm (Fig. 13). ER was also arranged in rings with swollen, ribosome-free regions and thinner, ribosome-studded regions (Fig. 16) in the stalk cells and occasionally in the basal cell. Also, the stalk cells had small vacuoles and microtubules aligned parallel with the long axis of the cell (Fig. 18). In addition, proplastids (Fig. 16) and dictyosomes with multifaceted edges and coated vesicles (Fig. 17) occurred in the stalk cells but were more frequent in the basal cell.

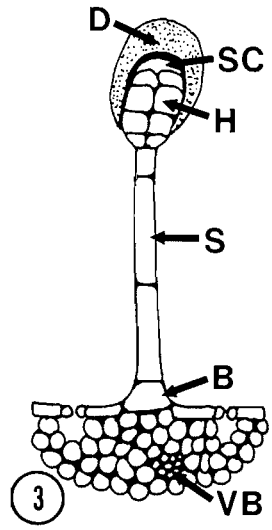
Head cells were smaller than stalk cells, with denser cytoplasm (Fig. 19) and more densely packed mitochondria (Fig. 20). Also, small plastids containing lipid droplets (Fig. 20) and starch grains were present in the head cells. The subcuticular secretion chamber was formed by a separation between the cell wall and cuticle of the upper head cells (Fig. 21), and the cuticle was perforated at the trichome tip (Figs. 21 and 22).

Granular material was observed between the plasma membrane and the cell wall of the head cells (the extraplasmic space), and the plasma membrane was associated with an extensive membrane network of tubules and vesicles (Figs. 23 and 24). These vesicles were filled with a granular material similar to the material between the plasma membrane and the cell wall (Figs. 20, 21, and 24) and in the secretion chamber

NOTE: All TEM micrographs are oriented so that the end closer to the trichome tip is at the top in each of the figures.

FIG. 1. Secretory trichomes from pod tissue. A large number of secretory trichomes and a few nonsecretory trichomes (arrow) were present on mature pod tissue, with all growth stages of secretory trichomes present on the same tissue. Also, nonsecretory trichomes were approximately one-third the size of mature, secretory trichomes. ×110. FIG. 2. Nonsecretory trichome from leaf tissue. Nonsecretory trichomes were one-celled, slightly recurved structures. Fewer (none in this case) secretory trichomes were present on leaf tissue compared with pod tissue. ×600. FIG. 3. Diagram of secretory trichome. This diagram represents a median longitudinal section through a secretory trichome and should be used as a map when examining the following micrographs. Shown are a vascular bundle (VB), mesophyll cells, stomata, the basal cell (B), three stalk cells (S), four tiers of head cells (H), the secretion chamber (SC), and a secretion droplet (D). FIG. 4. Stalk cell in cross section. There was only one cell in each of the three tiers of stalk cells. The magnification of this section is about twice that of Fig. 5, so the stalk cell was actually about half the size of the first tier of two head cells in cross section. Fixation A. ×4500. FIG. 5. Lowest tier of head cells in cross section. The first tier of head cells contained two cells. Fixation A. ×2200. FIG. 6. Upper tier of head cells in cross section. The upper tiers of head cells contained four cells. Fixation A. ×2200. FIG. 7. External view of trichome tip. The four head cells (H) in the upper tier and the secretion chamber (SC) were visible from outside the trichome. ×1800. FIG. 8. External, side view of trichome head. Each tier of head cells was rotated approximately 45° with respect to the previous tier. The cuticular lines on the trichome outline the head cells; if the tiers were not rotated, these lines (arrows) would be contiguous, running from the bottom to the top of the trichome's head. These lines stood out well because the head cells collapsed from either the vacuum of the SEM or from senescence. ×1700.

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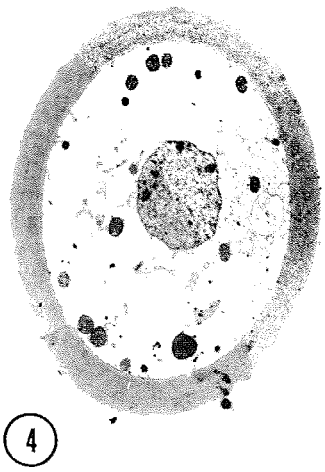

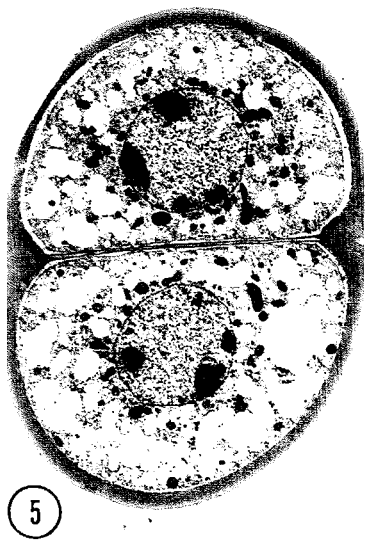

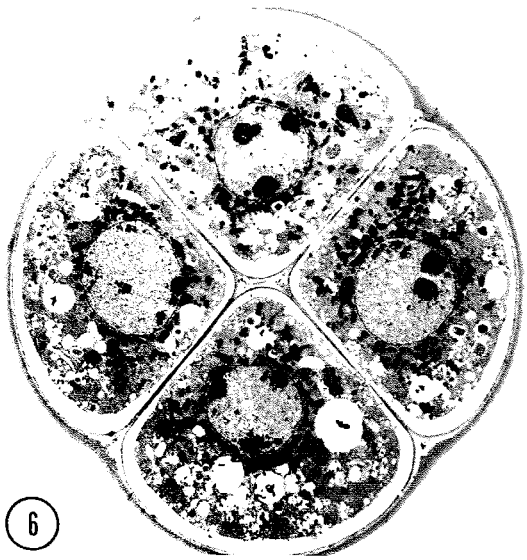

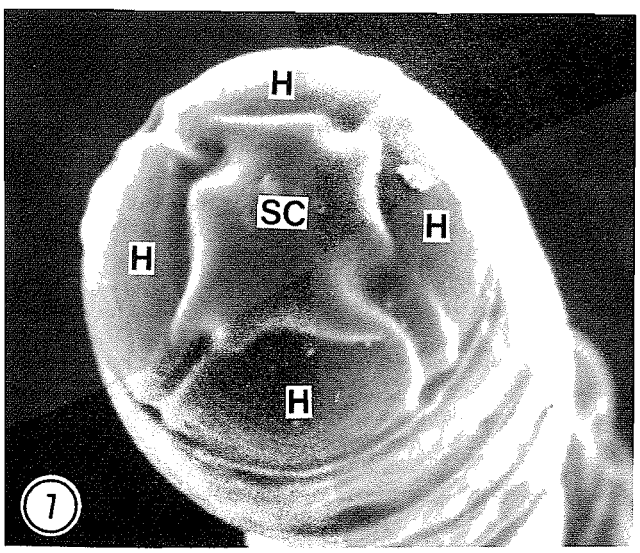
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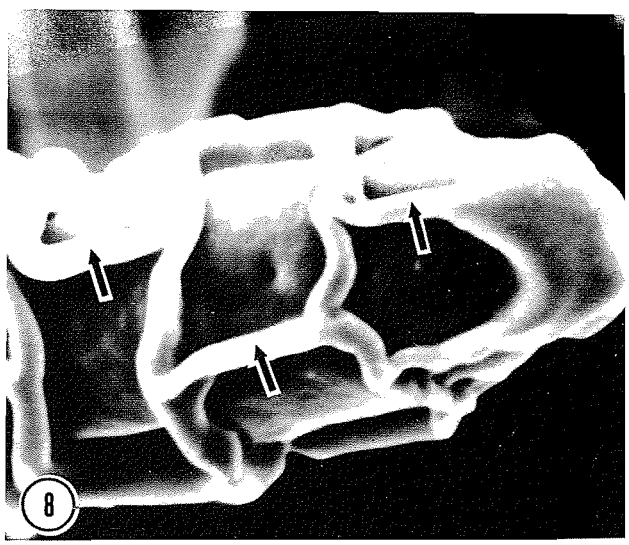


(Fig. 25). In addition, membranes accumulated in the extraplasmic space at the periphery of the membrane network (Fig. 24).

Small vacuoles and sequestering membranes were present in older head cells (Fig. 26). Also, sequestering membranes sometimes surrounded the granular-filled vesicles at the cell periphery (Fig. 26). In older cells, calcium oxalate crystals formed in the cell-wall space (Fig. 27), and the tubules of the peripheral membrane network were thinner than the tubules in younger cells (Fig. 28).

Plasmodesmata interconnected the basal, stalk, and head cells, and those plasmodesmata connecting between basal and stalk cells and between the stalk cells had slightly expanded median nodules at the middle lamella and were often branched (Fig. 29). The plasmodesmata between the upper stalk and head cells and between the head cells were usually not branched nor did they have a thickened region at the middle lamella, but ER was associated with them (Fig. 19). No plasmodesmatal connections between the basal cell and the surrounding mesophyll cells (Figs. 9 and 10) were observed in any trichome.

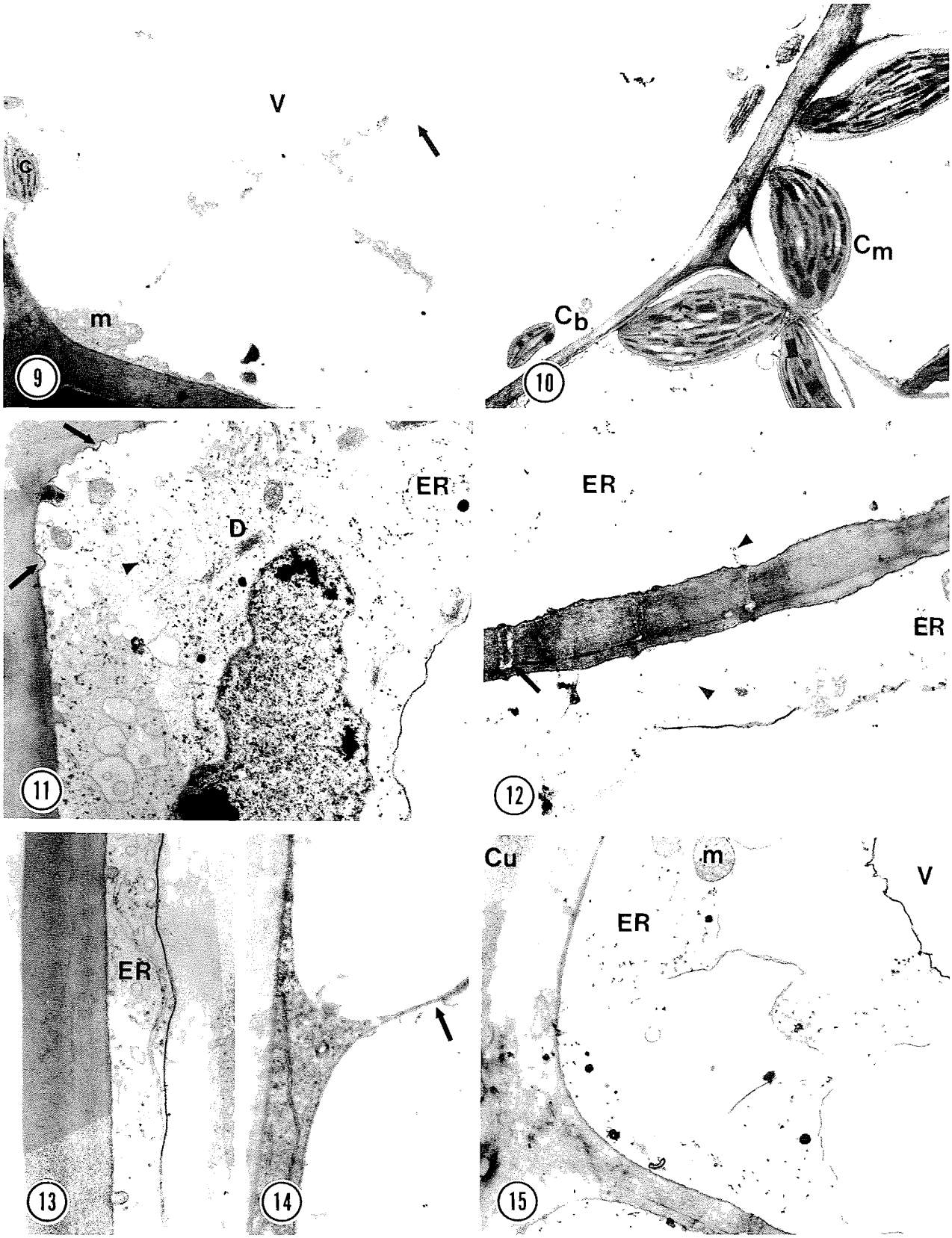
Discussion

In the head cells, the peripheral membrane network composed of tubules and vesicles and the similarity in the granular contents of this network with that of the extraplasmic space and of the secretion chamber suggest that the head cells are directly involved in the secretory process. Further, the large number of mitochondria in the head cells suggests that metabolic activity is crucial to the secretory process and that these mitochondria may be the site of the synthesis of secreted malate. Since the head cells lack chloroplasts (Schnepf 1965), a carbon substrate must be delivered to the head cells. Since the stalk cells also lack chloroplasts and the chloroplasts in the basal cell are much reduced in size, the likely source of substrate is the leaf mesophyll. It is of interest that all the trichome cells are interconnected by plasmodesmata, indicating that a symplastic pathway for solute movement is available throughout the trichome. However, plasmodesmata do not occur in the walls between the basal cell and underlying mesophyll cells, suggesting that movement to the basal cell is apoplastic. Apoplastic movement to the stalk and head cells also seems feasible, since no cuticular barrier exists at the base of the trichome, in contrast with most other secretory trichomes (Fahn 1979). The numerous invaginations of the plasma membrane and associated vesicles in the basal and stalk cells may be involved in the uptake of substrate as it diffuses along the walls.

There is a question as to how material may move from one end of the stalk to the other. With light microscopy, cytoplasmic streaming was observed in the stalk cells of living trichomes, moving only up and down the cells along fixed vertical or slightly diagonal paths. The direction of movement along each path was constant over 10 min. In addition, swirling movements were observed at the ends of the stalk cells (M. D. Lazzaro and W. W. Thomson, unpublished observations). These movements correlated with the ultrastructural organization of peripheral cytoplasm in the stalk cells. Elements of ER were aligned in the same orientation as the observed streaming, and vesicles were aligned along microtubules oriented in the same direction as the cytoplasmic streaming. This correlation of ultrastructural organization with cytoplasmic movement also holds for the swirling movements observed at the end of the cells where the ER formed coil-like and tubular aggregates.

Our observations on the head cell ultrastructure agree with those of Schnepf (1965) in that the head cells have dense cytoplasm and contain numerous mitochondria. The large number of mitochondria suggest that secretion is an active, energy-requiring process (Schnepf 1965), and one could also speculate that they have a role in the production of the secreted malate. Schnepf (1965) also found abundant ER and occasional dictyosomes and cytoplasmic vesicles at the head cell periphery with granular contents similar to that found outside the cells. Our observations confirm the abundance of ER, although dictyosomes were rarely observed in the head cells. From this, a relationship between ER-mediated vesicular transport and secretion seems probable, although the involvement of dictyosomes in this process cannot be ruled out. In addition, we observed that the plasma membrane was invaginated and the invaginations contained granular material similar to that in the vesicles and outside the cells. It is logical to conclude that the increased surface area to volume ratio provided by the labyrinthian plasma membrane, the associated vesicles, and the granular content observed in the vesicles, in the invaginations, and outside the cell are all integrally involved in the secretion of malate from the cells. The membrane observed outside the cells may be excess membrane resulting from rapid vesicular fusion, which is seen in areas of high solute flux between the

FIG. 9. Basal cell near mesophyll cells. This basal cell had a large vacuole (V) with cytoplasmic strands (arrow) running through it, several mitochondria (*m*), and small chloroplasts (*c*). There were no plasmodesmata connecting the basal cell with the mesophyll cells in all trichomes observed. Fixation D. ×6200. FIG. 10. Basal cell and mesophyll cells. This basal cell had a large central vacuole with peripheral cytoplasm containing mitochondria and chloroplasts (C_b). These chloroplasts differed markedly from the mesophyll cell's chloroplasts (C_m), which were approximately 10 times larger in cross section and had a well-developed granal–fretwork system. The basal cell was 5 to 6 times larger in cross section than the mesophyll cells, and there were no plasmodesmata connecting the basal and mesophyll cells. Fixation A. ×7500. FIG. 11. Basal cell near stalk cell. The basal cell had dictyosomes (D), smooth and rough ER (ER), polyribosome clusters (arrowhead), and a nucleus. In addition, there were invaginations in the plasma membrane (arrows) near the stalk cell. Fixation A. × 12 000. FIG. 12. Basal cell and lower stalk cell. Polyribosomes (arrowhead), small and large vacuoles, and tubular ER (ER) were in the basal cell; in the stalk cell, tubular ER (ER) and polyribosomes (arrowhead) collected at the cell end. Plasmodesmata with thickened regions at the middle lamella connected the basal and stalk cells (arrow). Since the basal cell wall was thinner than that of the stalk cell, the middle lamella was closer to the basal cell. Fixation A. × 17 000. FIG. 13. Peripheral cytoplasm in stalk cell. The lower stalk cell had peripheral cytoplasm bounded by a large central vacuole that had a densely stained tonoplast and sometimes contained granular material. In the peripheral cytoplasm, the ER was oriented in vertical tubules (ER) and there were invaginations in the plasma membrane. Fixation A. × 17 000. FIG. 14. Cytoplasmic strand in stalk cell. The stalk cell's peripheral cytoplasm had a cytoplasmic strand (arrow) running out of it through the central vacuole. Fixation D. × 19 000. FIG. 15. Lower stalk cell near basal cell. Near the basal cell, tubular ER and coils of smooth and rough ER (ER) accumulated in the stalk cell. Also, the stalk cell had small vacuoles as well as a large central vacuole (V), which had a densely stained tonoplast. Although the vacuoles were irregular in shape, the ER and mitochondria (*m*) were fixed. The dark cuticle (Cu) is discernible from the cell wall. Fixation B. × 10 500.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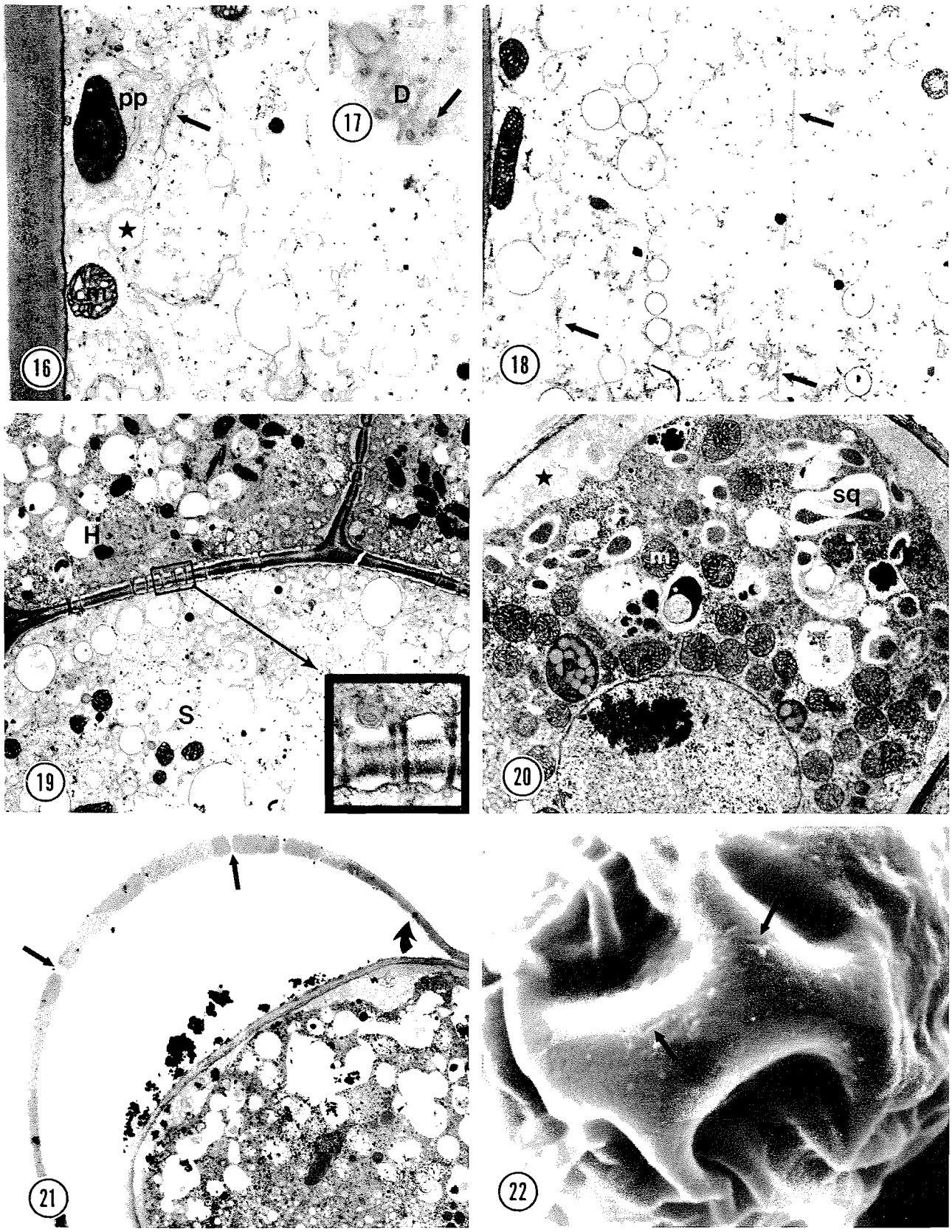


FIG. 16. Rings of endoplasmic reticulum in stalk cell. This middle stalk cell had rings of ER that were typically present in the stalk cells and occasionally present in the basal cell. These rings had swollen, ribosome-free regions (*) and thinner, ribosome-studded regions (arrow) and were not fixation artifacts since the mitochondria (*m*) and proplastid (*pp*) were both well preserved. There were also numerous polyribosome clusters. Fixation A. × 14 000. FIG. 17. Dictyosome in stalk cell. At high magnification, the fenestrated margin of a dictyosome (D) was visible in the lower stalk cell. The vesicles adjacent to the dictyosome may have been protein coated (arrow). Fixation A. × 37 000. FIG. 18. Vesicles and microtubules in stalk cells. In the peripheral cytoplasm of the upper stalk cell, large vesicles were lined up along microtubles (arrows)

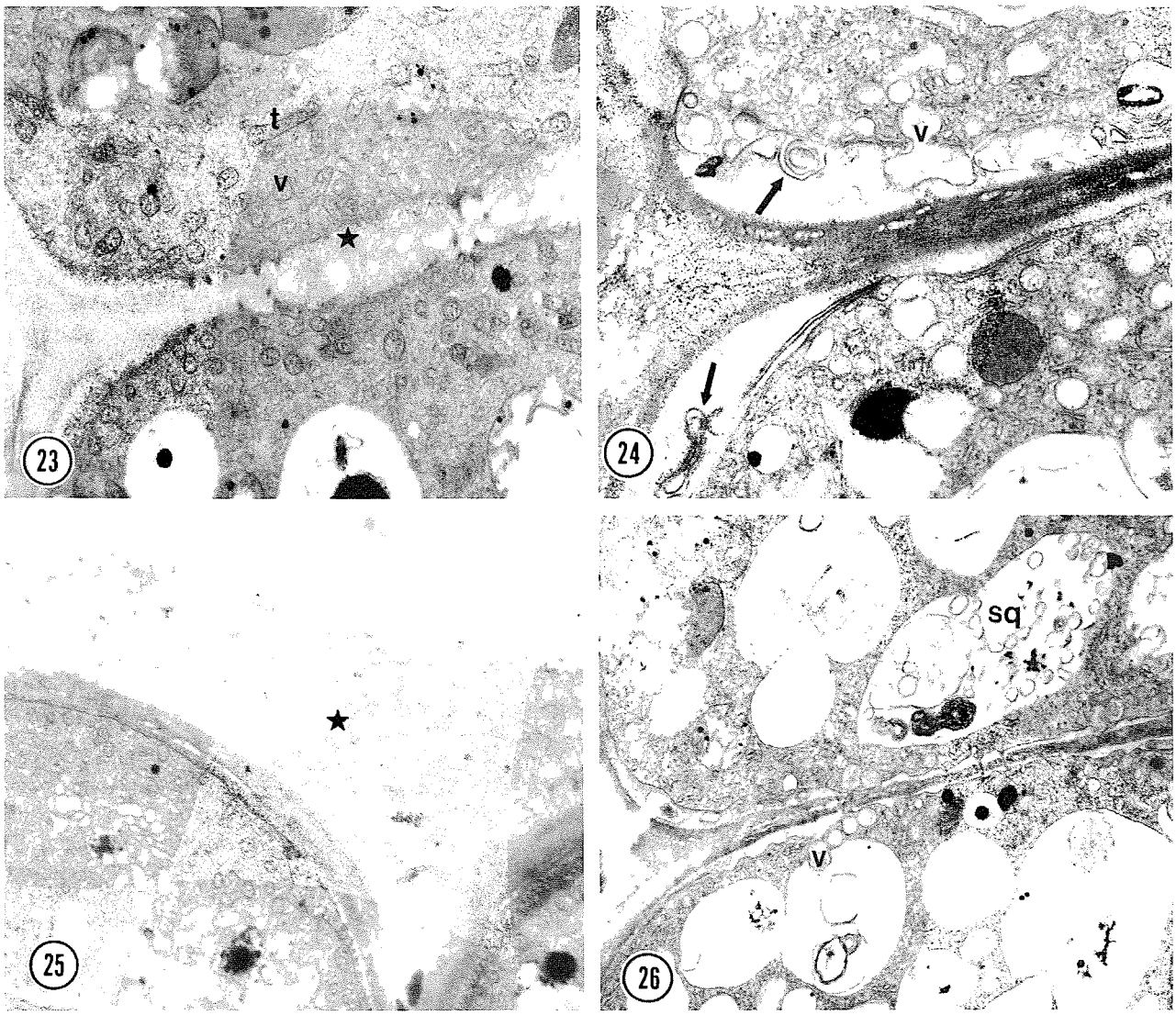
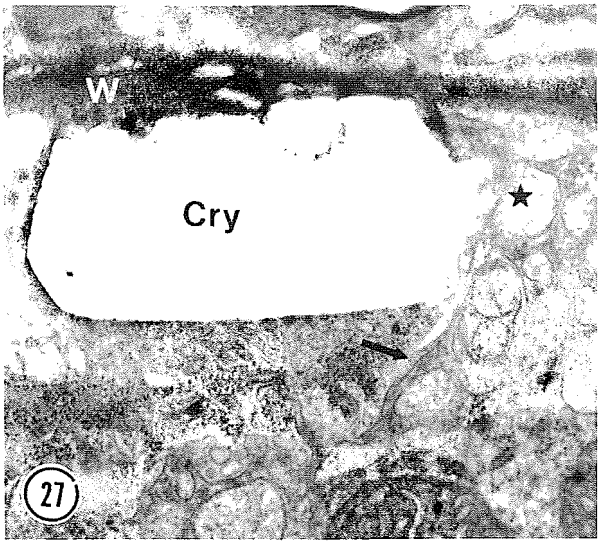
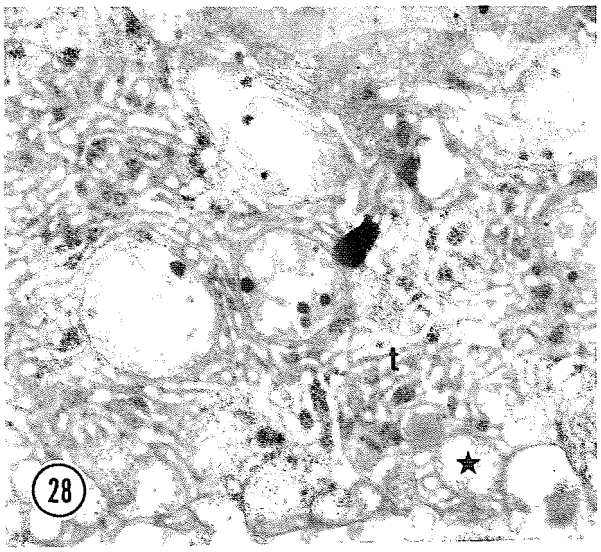
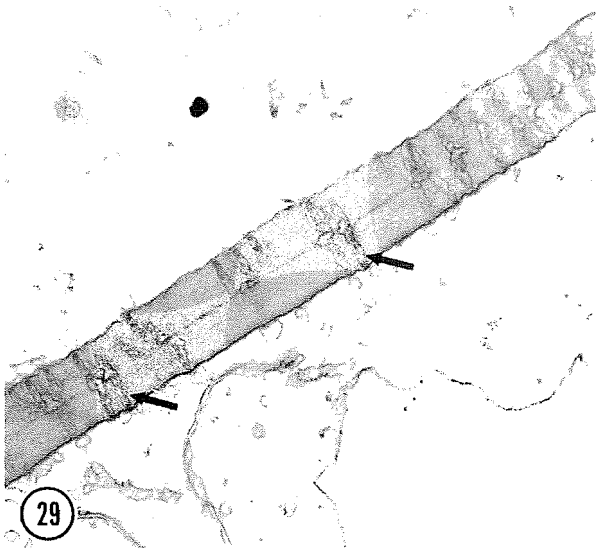


FIG. 23. Peripheral membrane network in head cell. The membrane network at the head-cell periphery was composed of tubules (*t*) and vesicles (*v*). Granular material in this network was similar to material between the cell wall and the plasma membrane (*) (the extraplasmic space). Fixation D. × 20 000. FIG. 24. Membranes in head cell extraplasmic space. Excess membrane (arrows) accumulated in the extraplasmic space of the head cells, and in this case one vesicle (*v*) was either entering or leaving the tubular–vesicular membrane network. Fixation D. × 28 000. FIG. 25. Granular material in secretion chamber. Granular material (*) accumulated in the secretion chamber near the upper tier of head cells, and this material resembled that seen in the membrane network and the extraplasmic space (see Figs. 20 and 23). The tubular–vesicular membrane network was also in the upper tier of head cells. Fixation D. × 19 000. FIG. 26. Sequestering membranes in head cell. Large sequestering membranes (*sq*) in these two head cells were filled with membrane fragments and engulfed vesicles (*v*) at the cell periphery that contained the granular material seen in the membrane network, the extraplasmic space, and the secretion chamber. Fixation D. × 13 000.

running vertically from the lower to upper ends of the cell. Fixation A. ×8600. FIG. 19. Upper stalk cell and lower head cells. The cytoplasm of the head cells (H) was denser than that of the stalk cell (S), but both the head and stalk cells had numerous large vesicles, polyribosomes, and mitochondria. There were plasmodesmata connecting the stalk and head cells and between the head cells, but these plasmodesmata were not branched, nor did they have a thickened region at the middle lamella. Tubular ER was associated with the ends of these plasmodesmata (inset). Fixation A. ×7300. Inset × 31 000. FIG. 20. Head cell bordering secretion chamber. There were approximately the same number of mitochondria (*m*) in the head cells compared with the stalk cells, but they were more densely packed in the smaller head cells. In addition, sequestering membranes (*sq*) and small plastids wth lipid droplets (*p*) were in the head cells. Also, the space between the plasma membrane and the cell wall was filled with a granular material (*). Fixation A. ×8900. FIG. 21. Secretion chamber and head cell. Sequestering membranes in this head cell were large, and the granular material again filled the space between the plasma membrane and the cell wall. The cuticle separated from the cell wall as it was pushed upwards (large, curved arrow), forming the subcuticular secretion chamber, and cuticular pores (small arrows) perforated the top of this chamber. Fixation D. ×5300. FIG. 22. Cuticular pores in the cuticle above the secretion chamber. From outside the trichome, apparent pores (arrows) were evident in the cuticle. ×4400.







apoplast and symplast (Chaffey and Harris 1985).

One obvious difference between our observations and those of Schnepf (1965) is that when we examined the trichomes in cross section, we found that the head was made up of 14 cells, of which the lowest tier had two cells while the upper three tiers had four cells each. The longitudinal sections provided by Schnepf (1965) show the trichome head as having seven to eight cells.

We observed considerable variation in ultrastructure between different trichomes. Of particular importance, in some head cells we found apparent sequestering membranes, which are thought to be evidence of incipient vacuole formation (Marty *et al.* 1980). In head cells of other trichomes we observed larger vacuoles, and in these cells the tubular membrane network of the plasma membrane was thin and lacked granular material present in the extraplasmic space. Until developmental studies are done, we interpret these latter cells as being in a near-senescent stage of activity. The presence of extracellular oxalate crystals associated with the older, vacuolated cells may be an indication of cellular change in metabolism with senescence. That is, instead of secreting organic acids, the cells could be accumulating them as a calcium precipitate at this stage. Although oxalate crystals typically form in the vacuole, there is evidence that they are also present outside the plasma membrane (Franceschi and Horner 1980).

Finally, as with most secretory trichomes (Fahn 1979), a collecting compartment consisting of an expanded cuticle perforated by pores exists above the terminal head in these trichomes, as Schnepf (1965) also found.

A range of developmental stages of trichomes are present on mature tissue. Koundal and Sinha (1981) found that total acidity in secretions increases as plants develop from the early vegetative stage to the stage when pods form, and it may be that as the plant matures, and increasing percentage of the total trichomes are secreting. The large number of trichomes present on pods may be correlated with the fact that chickpea's primary insect pest is the pod-boring larval stage of the moth, *Heliothis armigera*, and that this pest is repelled by high malate levels and low pH in secretions (Rembold 1981).

Acknowledgements

We thank Dr. Kathryn A. Platt-Aloia for her continued input on research ideas, methods, and writing. This research was supported in part by a grant from the National Science Foundation (DCB-8607765) to William W. Thomson and was completed in partial fullfillment of the Master of Science degree awarded to Mark D. Lazzaro from the University of California, Riverside.

FIG. 27. Calcium oxalate crystal in head cell. In this head cell from a senescent trichome, calcium oxalate crystals (Cry) accumulated in the extraplasmic space between the plasma membrane (arrow) and the cell wall (W). The tubular–vesicular membrane network (*) opened into the hole that contained a calcium oxalate crystal. Fixation A. × 30 000. FIG. 28. Tubular membrane network in older head cell. This head cell was also from a senescent trichome. The tubular–vesicular membrane network was altered; the tubules (*t*) were thinner and lacked the granular material that was present in vesicles at the cell periphery and in the extraplasmic space (*). Fixation C. × 34 000. FIG. 29. Transverse wall between stalk cells. Numerous plasmodesmata (arrows) connected the stalk cells and had thickened regions at the middle lamella and were often branched. Fixation A. × 21 000.

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