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## FINE STRUCTURE OF THE PROTHORACIC MYCANGIUM, A CHAMBER FOR THE CULTURE OF SYMBIOTIC FUNGI, IN THE SOUTHERN PINE BEETLE, *DENDROCTONUS FRONTALIS*

**ABSTRACT.** The ultrastructure of the prothoracic mycangium of female *Dendroctonus frontalis* is examined. The mycangium consists of a cuticular invagination within which symbiotic fungi are cultured by the pine beetle and transported to new host trees. Secretions from two types of gland cells pass into the mycangial lumen. The plasma membrane of type-1 cells is invaginated to form an enclosed extracellular cavity. The secretory product passes into the cavity, then through fine cylindrical channels into an end apparatus and finally via an efferent cuticular ductule to the lumen of the mycangium. Secretion of the type-2 cells is released into a cavity just beneath the mycangial cuticle. The cuticle over this cavity is quite thin (1-2 $\mu$ ), consisting mostly of inner epicuticle riddled with irregular canals through which the secretion reaches the lumen. Beneath the patches of porous cuticle are ribs (up to 10 $\mu$  in thickness) which flank the cavities and presumably provide structural support for the porous secretory zones.

### Introduction

MANY insect species which inhabit wood exploit their habitat as a food source. Although some cellulose may be degraded by enzymes of these insects (Wigglesworth, 1965), in most cases cellulose and pectin are digested by symbiotic microorganisms (Buchner, 1930; Buchner, 1953; Graham, 1967). The microorganisms may be sequestered in special regions of the insect gut, such as the flagellates which digest cellulose in lower termites and related cockroaches (Honigberg, 1967). Alternatively, the symbiotic microbes (usually fungi) may proliferate in the wood itself and the resulting hyphal mass can serve as food and provide essential nutrients for the insects (Francke-

Grosmann, 1963, 1967; Norris, Baker and Chu, 1969; Kok, Norris and Chu, 1970). In the latter ectosymbiotic associations, emigration of the insect macrosymbiont disperses the fungal microsymbiont. In the present paper, we wish to describe the ultrastructure of a specialized chamber for fungus transport and propagation in the southern pine beetle, *Dendroctonus frontalis*.

Many Scolytidae, including several species of *Dendroctonus*, possess a cuticular invagination called the mycangium, in which fungi are transported from one host tree to another. Shortly after imaginal ecdysis of the beetle, fungi from the surrounding host tissue enter the mycangium and under the influence of secretions from its gland cells, they proliferate to fill the chamber with budding yeast-like spores or other repeatedly germinating ambrosia propagules. Thus, the mycangium serves not merely for transport, but it also provides a culture medium for the fungi. Perhaps the most intriguing characteristic of the mycangium is its ability to

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favour the growth of specific fungi through the apparent selective action of its glandular secretions (Barras and Perry, 1971a).

Within a few days after ecdysis, the beetle emigrates to a new host where the symbiotic fungi are liberated from the mycangium into an egg gallery in the tree. The fungi proliferate and spread into the surrounding phloem; both phloem and fungi are consumed by the beetle larvae. Development of the beetle and associated fungi cause rapid death of the tree. The success of this association is responsible for a major portion of tree mortality in the southern and western United States.

The gross morphology and histology of prothoracic mycangia have been described for several species of *Dendroctonus* (Francke-Grosmann, 1965 and 1967; Barras and Perry, 1971b). The prothoracic fungal chamber is well-developed only in the female of this genus. It consists of an elongate groove in the anterior entothoracic fold (Hopkins, 1909) on the anterior margin of the expanded pronotum. Around this groove, which appears tubular in cross-section, there are many secretory cells; it has been suggested that the secretion from these cells provides nutrients, determines the form of fungal growth (Abrahamson, Chu and Norris, 1967), and regulates the species composition of the fungi (Barras and Perry, 1971a, 1971b and references cited therein). Other ectodermal pockets or tubes of *Dendroctonus* which contain fungi have been termed mycangia, but these were not shown to possess glandular cells (Farris, 1965; Whitney and Farris, 1970). In no species of scolytid beetle has the ultrastructure of the mycangium and associated cells been examined. Such information is a pre-requisite to a more complete understanding of interactions between symbiotic partners.

#### Materials and Methods

Sections from loblolly pine trees (*Pinus taeda* L.) which had been attacked by *Dendroctonus frontalis* Zimm. were collected in the field near Olla, Louisiana and were taken to the Southern Forest Experiment Station at Pineville. After the beetle pupae in these pine bolts had undergone adult ecdysis, they were removed, sexed, individually isolated, and shipped in refrigerated con-

tainers to New York University. Upon arrival in New York their cuticle had only begun to darken. The teneral beetles were immediately fixed for histological and electron microscopical examination. Although we have briefly examined the fine structure of the mycangia in older *Dendroctonus*, the following description of mycangial structure will be restricted to that of teneral female adults at the stage when fungal proliferation is just beginning.

The anterior projection of the prothorax which contains the mycangium was removed and fixed for electron microscopy. Fixation was in glutaraldehyde (5% in 0.1M phosphate buffer, pH 7.4) at 0-4°C for 2 to 6 hours, followed by washing in the same buffer with 10% sucrose added for 1 hour (Locke, 1966) with post-fixation for 1 hour in 1% osmium buffered with phosphate (0.1M, pH 7.4) containing 4% sucrose. The glutaraldehyde was prepurified by repeated washings through Norit EX charcoal. Tissues were dehydrated in graded alcohols and embedded in Epon 812. Thin sections were stained routinely for 20 minutes with saturated uranyl acetate in ethanol-methanol (equal parts 70% ethanol and absolute methanol), followed by 5 minutes in lead citrate (Reynolds, 1963). The electron micrographs were taken on an RCA EMU 2E microscope at instrument magnifications between 1,500 and 17,000 times.

#### Observations

The mycangium of *D. frontalis* is an elongate groove, shaped rather like an inverted horse-shoe, which runs along the inner surface of the anterior pronotal projection (Figs. 1, 2). In whole mounts, viewed in transmitted light, the cuticular wall of the mycangial invagination appears to have a complex surface pattern, consisting of a network of interconnected ridges running between irregular lozenge-shaped depressions (Fig. 2). In *Trypodendron*, a similar condition was described as 'thin polygonal membranous windows or pits' (Abrahamson, Chu and Norris, 1967). However, examination of 'thick' Epon sections through the mycangium of *D. frontalis* (Fig. 3) reveals that the outer surface of the mycangial cuticle is quite smooth; the apparent patterning reflects differences in the thickness of the under-



Fig. 1. The prothorax and head of a female *D. frontalis*. The callus (asterisks) marks the position of the mycangium within the anterior projection of the pronotum. Lactophenol cleared. approx.  $\times 70$ .



Fig. 2. A whole mount of the anterior prothorax. The hairs to the right project from its leading edge. The mycangial tube (*m*) runs vertically through the photograph. In such a preparation, the surface of the mycangial cuticle appears to consist of thin polygonal zones separated by thicker struts (arrows). Phase contrast,  $\times 120$ .

lying cuticular layers. Patches of thin cuticle (ca.  $2\mu$  in thickness) are flanked by cuticular ribs, up to  $10\mu$  in thickness and  $4-10\mu$  in width. The substance of the thin cuticle is not interrupted above the ribs. When sections are stained with toluidine blue, the ribs appear as dense blocks below the lighter overlying layers (Fig. 3). Beneath each patch of thin cuticle is a cavity, flanked by the ribs, containing the flocculent product of several columnar secretory cells (Fig. 3, 12).

Two types of secretory units pour their products into the mycangial lumen, and they differ considerably in morphology. Each type-1 unit consists of two closely associated cells: a secretory cell and an accessory cell which ensheathes the efferent cuticular ductule. Each type-2 unit is a columnar cell which releases its product into a cavity beneath the cuticle of the mycangial wall. The major structural features of the mycangium and related secretory cells are shown in Figs. 3 and 4.

The secretory cells of the type-1 units are not a part of the mycangial epithelium proper; rather they lie in the space between this epithelium and the epidermal cells of the body cuticle (Fig. 3). These two-cell units correspond to the G-1 cells described by Barras and Perry (1971b) in their study of the mycangium of *D. adjunctus*. Within the cytoplasm of the secretory cells are numerous small mitochondria, many Golgi zones, and considerable amounts of ribosome studded endoplasmic reticulum (Figs. 5, 7). The plasma membrane of each secretory cell is invaginated to form an enclosed extracellular central cavity (Fig. 5). In most cells, the finely particulate electron-dense secretory product fills this central cavity (Figs. 6, 7). The plasma membrane bounding this cavity is deeply infolded. Small vesicles, ca.  $100m\mu$  in diameter and packed with electron-dense material, are often encountered in the apical cytoplasm near the margins of this central cavity (Figs. 6, 7). In many sections, the

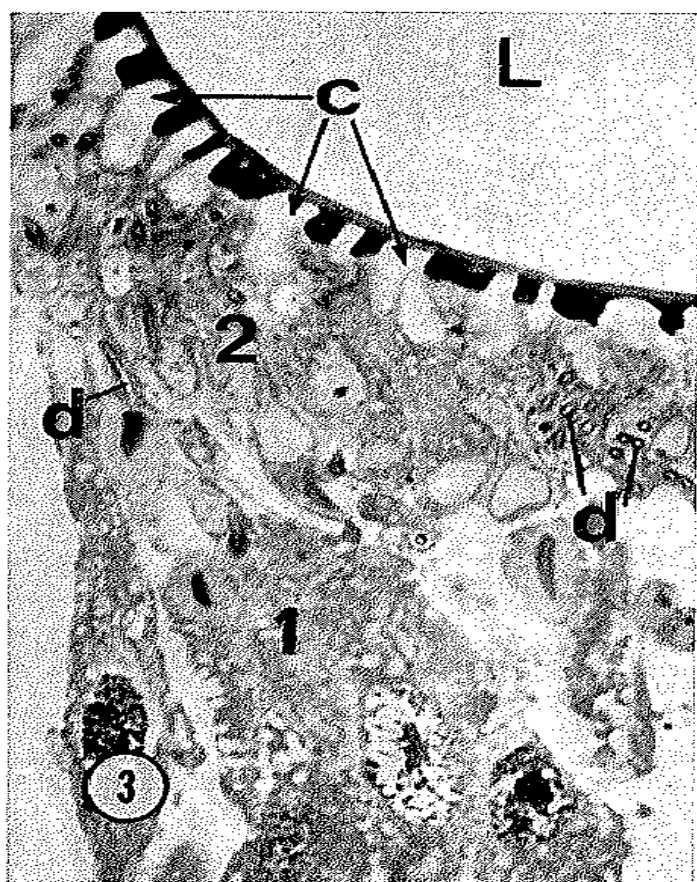


Fig. 3. A section through the mycangial cuticle with its associated secretory cells. The mycangial lumen (*L*) is at the upper right. Beneath the upper layer of the cuticle are more densely staining ribs which flank the secretory cavities (*c*) of the type-2 units (2). The type-1 units (1) pass their products to the lumen via efferent ductules (*d*). Toluidine blue,  $\times 1300$ .

vesicles closely approach the plasma membrane and apparently fuse with it (Fig. 6); it is our belief that these densely-staining vesicles contain secretory product *en route* to the central cavity.

Within the central cavity lies a hollow cylindrical end apparatus (Figs. 5–9). It appears to be cuticular, and like the efferent ductule and the mycangial cuticle, it is PAS positive. The wall of this end apparatus is ca.  $170\text{m}\mu$  in thickness and perforated by many fine regular channels, each of which is about  $150\text{Å}$  in diameter (Figs. 5–9). Similar regular channels have been reported in the 'head' of the cuticular end apparatus of the type-1 cells of the defensive glands of *Eleodes longicollis* (Eisner, McHenry, and Salpeter, 1964). The basal portion of the end apparatus lacks the fine perforations and as the perfora-

tions disappear, a cuticulin layer can be observed (Fig. 8). The wall of the end apparatus is continuous with the inner epicuticle of the efferent ductule (Fig. 9). The secretory cell and the accessory ductule-carrying cell are tightly linked by desmosomes (Fig. 9). Within its cellular sheath, the efferent ductule runs through the mycangial epithelium proper (Figs. 10, 12–14) and through the cuticle which forms the wall of the mycangium (Figs. 11, 14).

Type-2 units lie within the epithelium beneath the mycangial wall (Fig. 3). These units correspond to the G-2 cells described by Barras and Perry (1971b). From the apical surfaces of the type-2 cells, elongate, irregular loosely-packed microvilli project into the secretory cavity beneath the mycangial cuticle. These microvilli begin as broad out-pocketings of the plasma membrane and taper to a diameter of approximately  $100\text{m}\mu$  (Figs. 12, 14, 15). Fine filaments lie within each villus; in tangential sections they appear to be arranged in a cylinder (Fig. 14) which may provide internal support for the elongate projection. On their outer surfaces, the microvilli are coated with a filamentous fuzz, of about  $150\text{Å}$  in thickness (Figs. 12, 14, 15). Similar glycocalyx-like coats have been reported in other insect secretory systems, for example in the midgut of *Ephesia* (Smith, 1968; Fig. 74) and in the salivary glands of *Calliphora* (Oschman and Berridge, 1970). At the tip of each villus is a deposit of dense material, much like the plasma membrane plaques described by Locke (1969). Microtubules, oriented predominantly along the long axis of the cell, are scattered throughout the cytoplasm but are especially common in the broad projections which give rise to the microvilli (Figs. 12, 15) and are often found near the Golgi zones (Fig. 15). The latter are especially well-developed in the apical cytoplasm, where they consist of a stack of agranular membranes between which are patches of electron-dense material. Along the outer surface of the Golgi are small dense vesicles of about  $50\text{m}\mu$  in diameter. Often the Golgi cisternae are inflated, and on the inner surface of the membrane-complex the cisternae may form more-or-less spherical vesicles of low electron density which possess a cap of much denser material (Fig. 15). Similar vesicles are frequently seen near the apical surface of the cell (Fig. 15) and

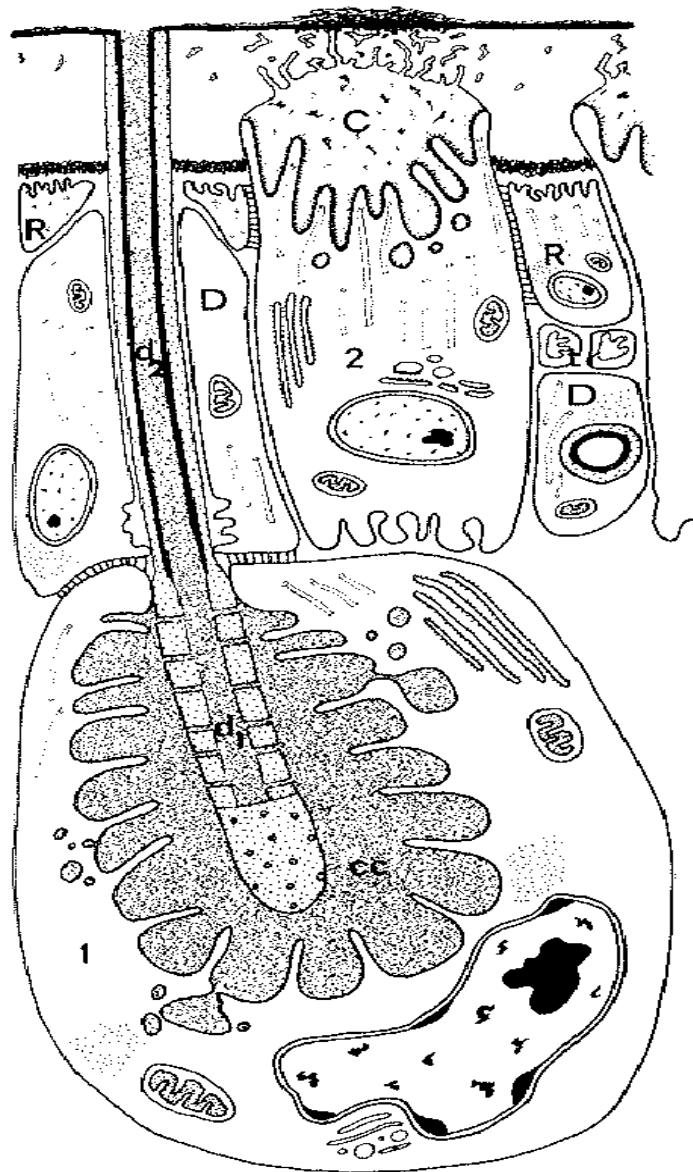


Fig. 4. Diagram showing a portion of the secretory epithelium of the mycangium. The lumen is at the top of the drawing. Two types of secretory cells are present. The product of type-1 cells (1) passes into a central cavity (*cc*) through fine perforations into a cuticular end apparatus (*d<sub>1</sub>*) which is continuous with an efferent cuticular ductule (*d<sub>2</sub>*). Enclosed in a ductule-carrying cell (*D*), the ductule runs to the mycangial wall and through it to the lumen at the top of the diagram. Type-2 cells (2) lie against the wall of the mycangium. Their secretion passes into a cavity (*C*) and through the overlying porous cuticle to reach the lumen. Within both types of secretory cells are membrane-bound vesicles, Golgi zones, microtubules, mitochondria and ribosome-studded endoplasmic reticulum. Thicker ribs of cuticle overlie the rib cells (*R*). Tracheoles (*tr*) are frequently encountered beneath the cuticular ribs. For clarity, the cellular organelles have not been drawn to scale.

may represent cellular product passing to the cavity.

Several type-2 units pour their products into each cavity beneath the mycangial cuticle (Figs. 3, 12, 14). No efferent ductules drain these cavities. Apparently the flocculent product passes through the numerous irregular canals which penetrate the overlying homogenous cuticle (Figs. 12, 13). These canals do not continue through the superficial layers of the epicuticle, which appear to consist of a cuticulin layer (in the sense of Locke, 1966) and an outer epicuticle (in the sense of Filshie, 1970). Rather, the irregular canals terminate in a diffuse lacunar system beneath the cuticulin (Fig. 13). We are not sure exactly how the flocculent product passes through the cuticulin and outer epicuticle.

In contrast to the porous cuticle over the secretory cavities, the homogenous cuticle over the ribs contains rather fewer of the irregular canals (Figs. 12, 13), has an underlying fibrous layer (Fig. 14), and at its lower margin possesses an electron-dense subcuticle (Figs. 12, 14). The surface of the adjacent rib cells may be thrown up into short microvilli with plasma-membrane plaques at their apices (Fig. 14). Microtubules are densely packed in the rib cells (Fig. 15).

### Discussion

In his comprehensive treatment of the integumentary glands of termitophilous staphylinid beetles, Pasteels (1968) has divided the diverse array of secretory cells into two general classes: the palisade glandular cells (*cellules glandulaires palissadiques*) and the ductule-associated glandular cells (*cellules glandulaires à canicule*). These classes are distinguished by the cuticular modifications for export of their secretions. The product of the palisade class passes directly into a subcuticular space and thence to the exterior through wax or pore canals in the overlying cuticle—for examples see the wax glands of the worker honeybee (Locke, 1964) and the pheromone glands of the oriental silkworm (Steinbrecht, 1964). The product of the ductule-associated class moves first into a central cavity and then passes to the exterior within an elongated tubular cuticular invagination, the efferent ductule—for examples see the defensive glands of *Eleodes*

(Eisner, McHenry and Salpeter, 1964) or the spermathecal glands of *Periplaneta* (Gupta and Smith, 1969). In both classes the epicuticle is usually fenestrated to permit export of the secretory product. The two types of secretory units which pour their products into the mycangium nicely illustrate these two classes: type-1 units are ductule-associated while type-2 units are of the palisade class.

The epicuticle associated with each unit is appropriately modified. The product of the type-2 units passes through hypertrophied irregular pore canals in the homogenous inner epicuticle, next into the lacunar system, and by some means this product moves through the cuticulin layer (Figs. 12, 13). A more elaborate lacunar system, including a large number of fine tubular structures, has been reported in the Gilson glands of a larval trichopteran (Quennedey, 1969). In the case of *D. frontalis*, the route through the cuticulin was not clearly seen in the present study: perhaps the flocculent product of the type-1 cells 'dissolves' through the cuticulin or perhaps it passes through very narrow channels, such as those described in tracheoles by Locke (1966). No distinct epicuticular slits, such as those in the osmeteria of papilionid caterpillars (Crossley and Waterhouse, 1969) were seen in the porous cuticle of the mycangial wall. However, very narrow pores could have been missed in our sections which were usually over 800 Å in thickness.

The efferent ductule which transports the product of the type-1 units begins in an end apparatus, consisting of inner epicuticle (in the sense of Locke, 1969 and Filshie, 1970) that is riddled with fine regular channels. Most ductule-associated cells possess an end apparatus; however, the details of these structures vary widely. The end apparatus may be a fan-like array of filaments (Mercer and Brunet, 1959; Lai-Fook, 1970) or it may be a hollow 'finger' of cuticle. The wall of the blind cylinder may be a loose fibrous network (Gupta and Smith, 1969), may be inner epi-

cuticle penetrated by quite irregular channels (Happ and Happ, 1970), or may contain what corresponds to a thin electron-dense cuticulin layer. However, the cuticulin layer either terminates before the fenestrations begin (as in the type-1 units of the mycangium) or is itself fenestrated (cell 2a, Eisner, McHenry and Salpeter, 1964). As suggested in an earlier paper (Happ and Happ, 1970) it seems most unlikely that the 'inner epicuticle', defined primarily by its position, is chemically identical in all insectan cuticles. In integumentary glands, the inner epicuticle often changes in density as the efferent ductule joins the end apparatus (Figs. 6, 9 of the present study; see also Happ and Happ, 1970 and the type-1 units of Eisner, McHenry and Salpeter, 1964). These differences in density may reflect varying proportions of cuticulin, the lipid polymer (in the sense of Wigglesworth, 1933 and 1970), within the inner epicuticle.

In some sections through the mycangium of *D. frontalis* the efferent ductule contains little or no secretory product (Figs. 5, 12), yet in every section which we examined, the lumen of the end apparatus was filled with material of high electron density, even when none was present in the central cavity or the efferent ductule (Fig. 5). Furthermore in cases where the central cavity was packed with secretion, the density of the material in the end apparatus was greater than that within the cavity. It may be that the product only spills from the end apparatus into the efferent ductule when the lumen of the hollow finger is filled past capacity, or alternatively, the consistently high density may indicate that a permanent electron-dense core, perhaps involved in the final manufacture of the product, is built into the end apparatus. The latter function has been suggested by the work of Eisner, McHenry, and Salpeter (1964), Happ (1968) and Lai-Fook (1970) on other insect integumentary glands.

Working in concert, the secretions from the two types of secretory units modulate the

Fig. 5. The type-1 secretory cell with its central cavity (*cc*) surrounded by microvilli and containing the perforated end apparatus (*d<sub>1</sub>*) which is drained by an efferent ductule (*d<sub>2</sub>*). In this micrograph the secretory product is absent from both the efferent ductule and the cavity. The irregular nucleus (*N*) of the cell is in the upper centre and rough endoplasmic reticulum (*ER*) is in the upper left. The cytoplasm adjacent to the central cavity is rich in mitochondria (*mi*). A Golgi region (*G*) is present near the nucleus and another, at the lower right. The edge of another type-1 secretory cell is seen at the lower left.  $\times 19,000$ .



N

C

co

d

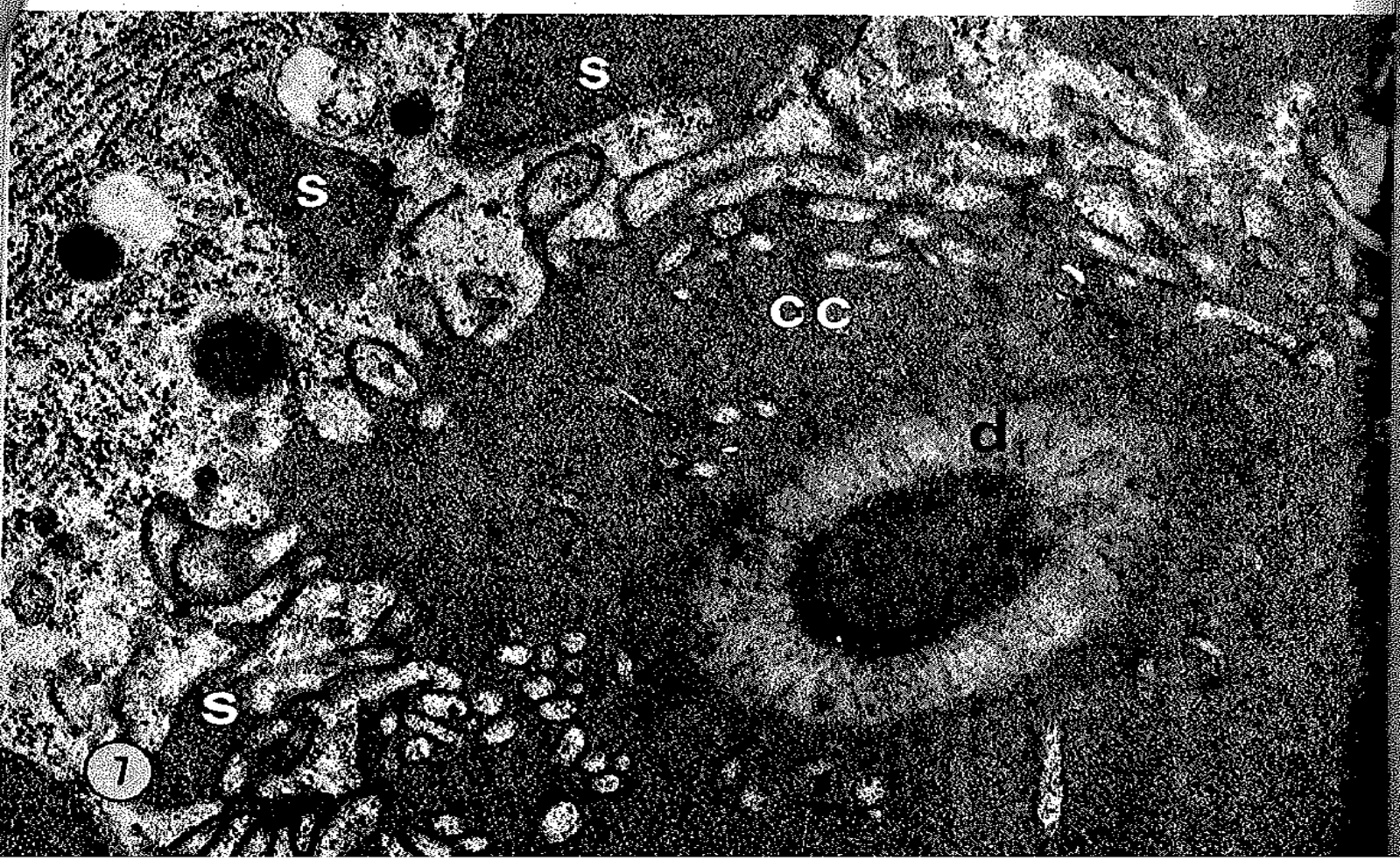
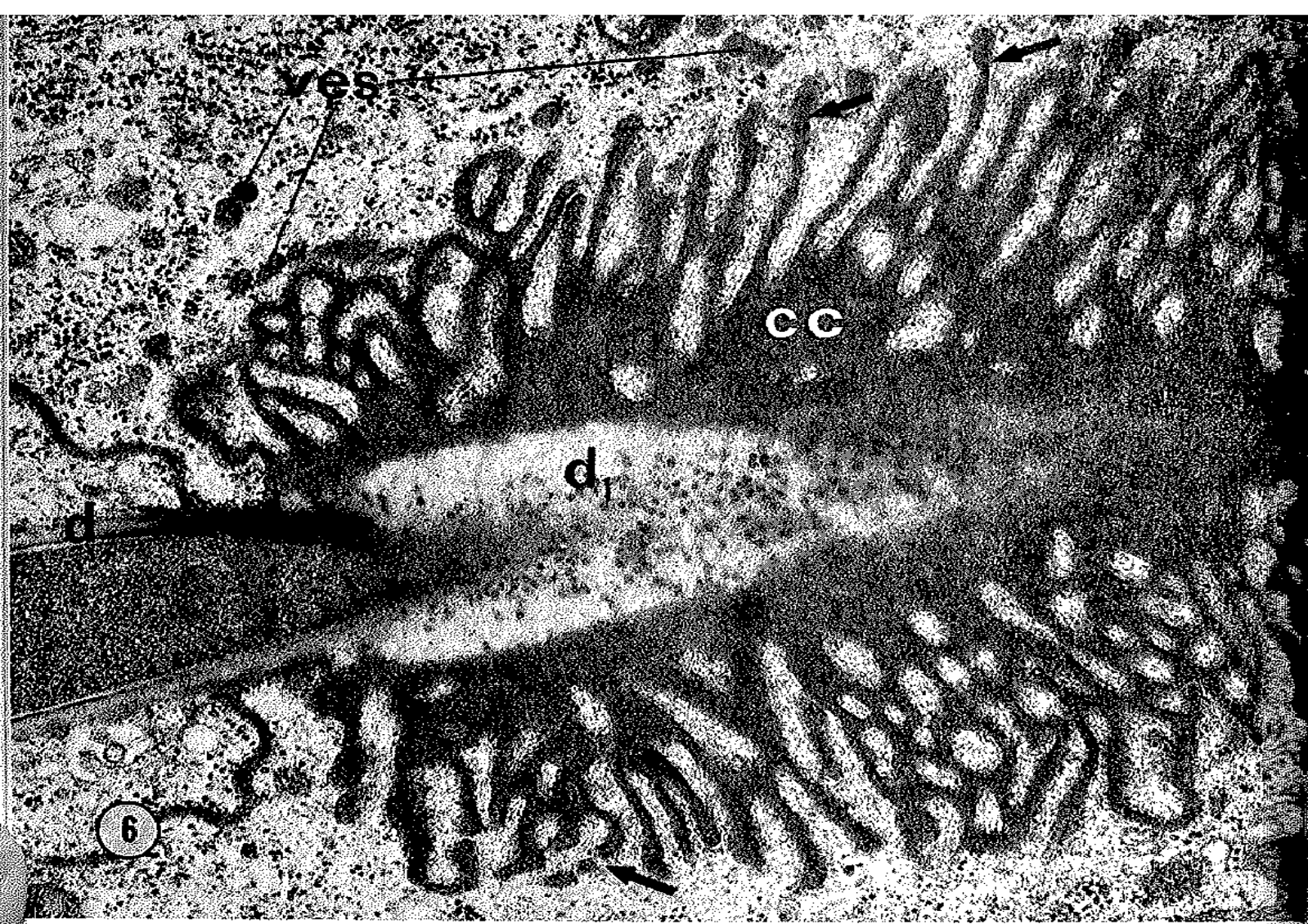


Fig. 6. An oblique section through the junction of the end apparatus ( $d_1$ ) and the efferent ductule ( $d_2$ ) which drains it. The finely-particulate electron-dense product of the type-1 unit lies within the central cavity ( $cc$ ) and the efferent ductule. Small membrane-bound vesicles ( $ves$ ), containing secretory product, are seen near the infolding of the plasma membrane which delimits the central cavity. Often the vesicles appear to be fusing with these infoldings (arrows). The wall of the end apparatus is perforated by fine cylindrical channels, each about 150 Å in diameter.  $\times 30,000$ .

Fig. 7. A transverse section through the central cavity ( $cc$ ) and end apparatus ( $d_1$ ). This central cavity contains more secretory product than that shown in Fig. 6. Many of the infoldings of the plasma membrane have become inflated with the particulate secretion ( $s$ ).  $\times 26,000$ .

Fig. 8. The base of the end apparatus ( $d_1$ ) in the type-1 unit. The dense inner layer of epicuticle, presumably cuticulin, terminates (arrows) before the cylindrical channels appear.  $\times 73,000$ .

Fig. 9. A section of the junction between the end apparatus ( $d_1$ ) and the efferent cuticular ductule ( $d_2$ ). Although the cuticle of the end apparatus is continuous with that of the efferent ductule, that of the end apparatus is thicker, of lower electron density, and perforated. Both an intermediate junction ( $ij$ ) and a septate desmosome ( $sd$ ) are seen between the secretory cell and the ductule-carrying cell of this type-1 unit. The narrow zone of extracellular space (asterisk) into which project the short microvilli of the ductule-carrying cell shows clearly that this ductule is extracellular.  $\times 34,000$ .

Fig. 10. The efferent ductule ( $d_2$ ) and the nucleus of a ductule-carrying cell ( $N$ ).  $\times 48,000$ .

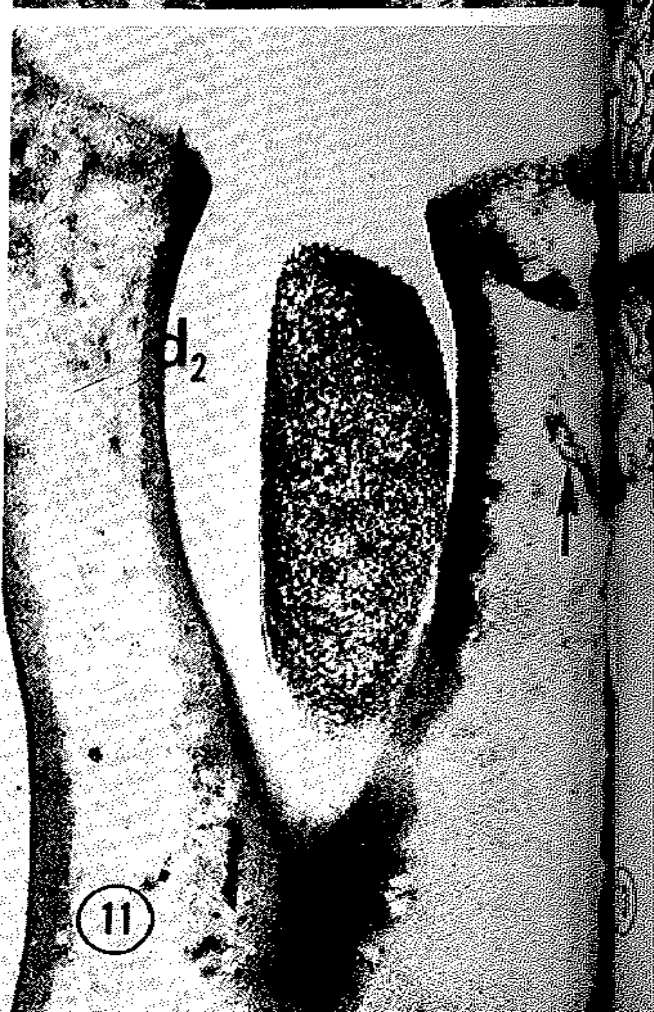
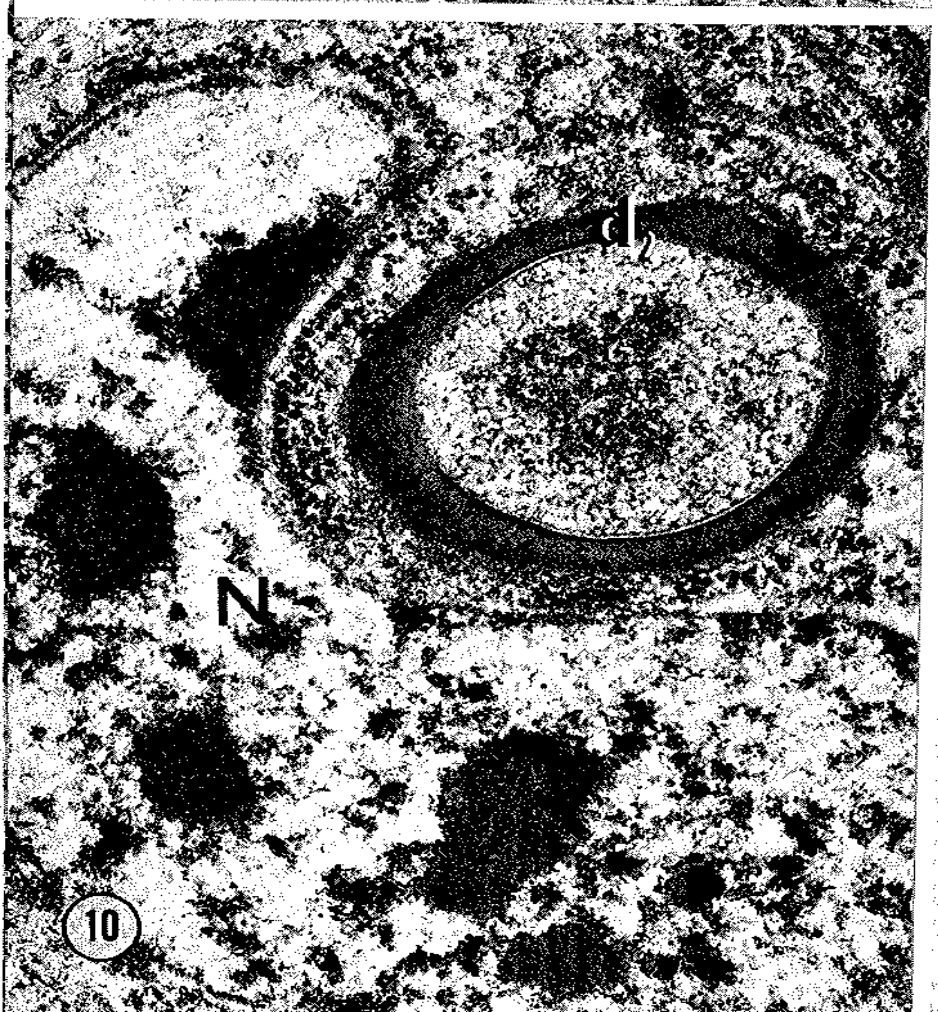
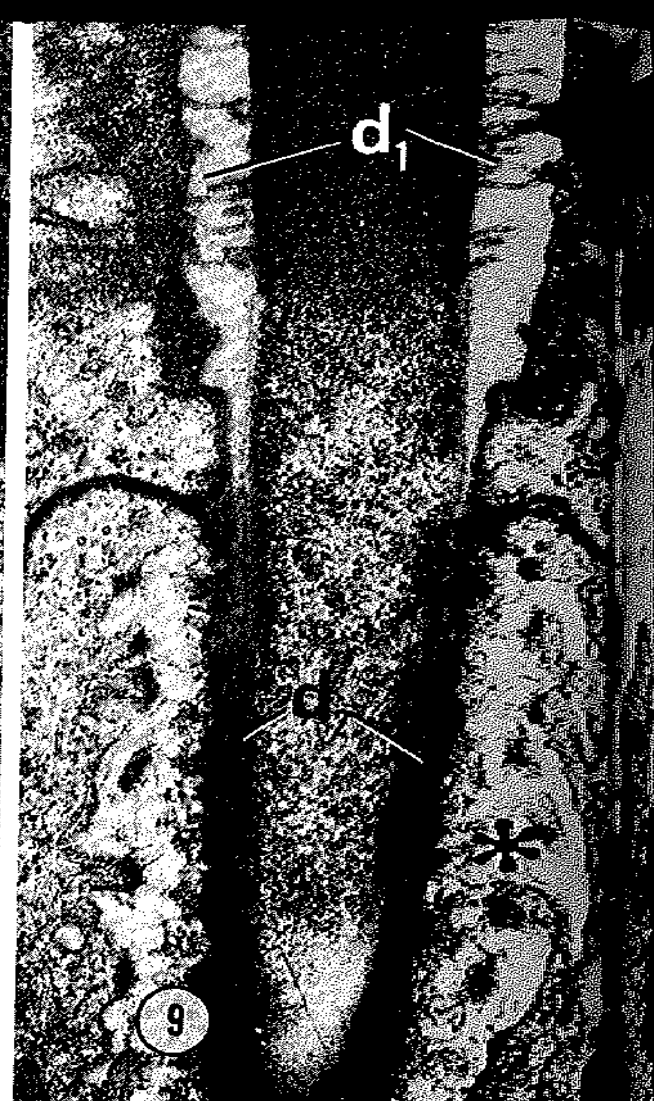
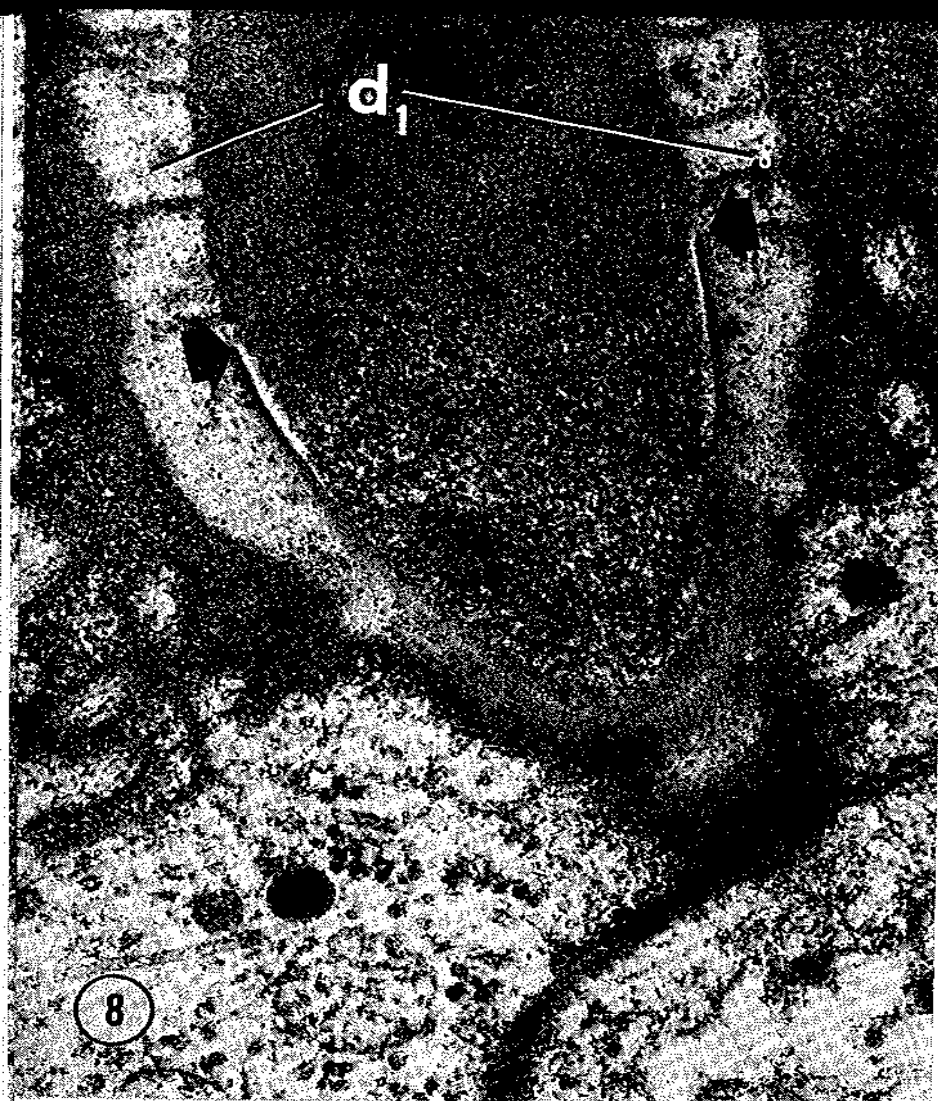
Fig. 11. The efferent ductule ( $d_2$ ) as it runs through the mycangial wall. The edge of another efferent ductule is seen at the lower left. Several of the irregular canals (arrows) can be seen in the mycangial cuticle.  $\times 36,200$ .

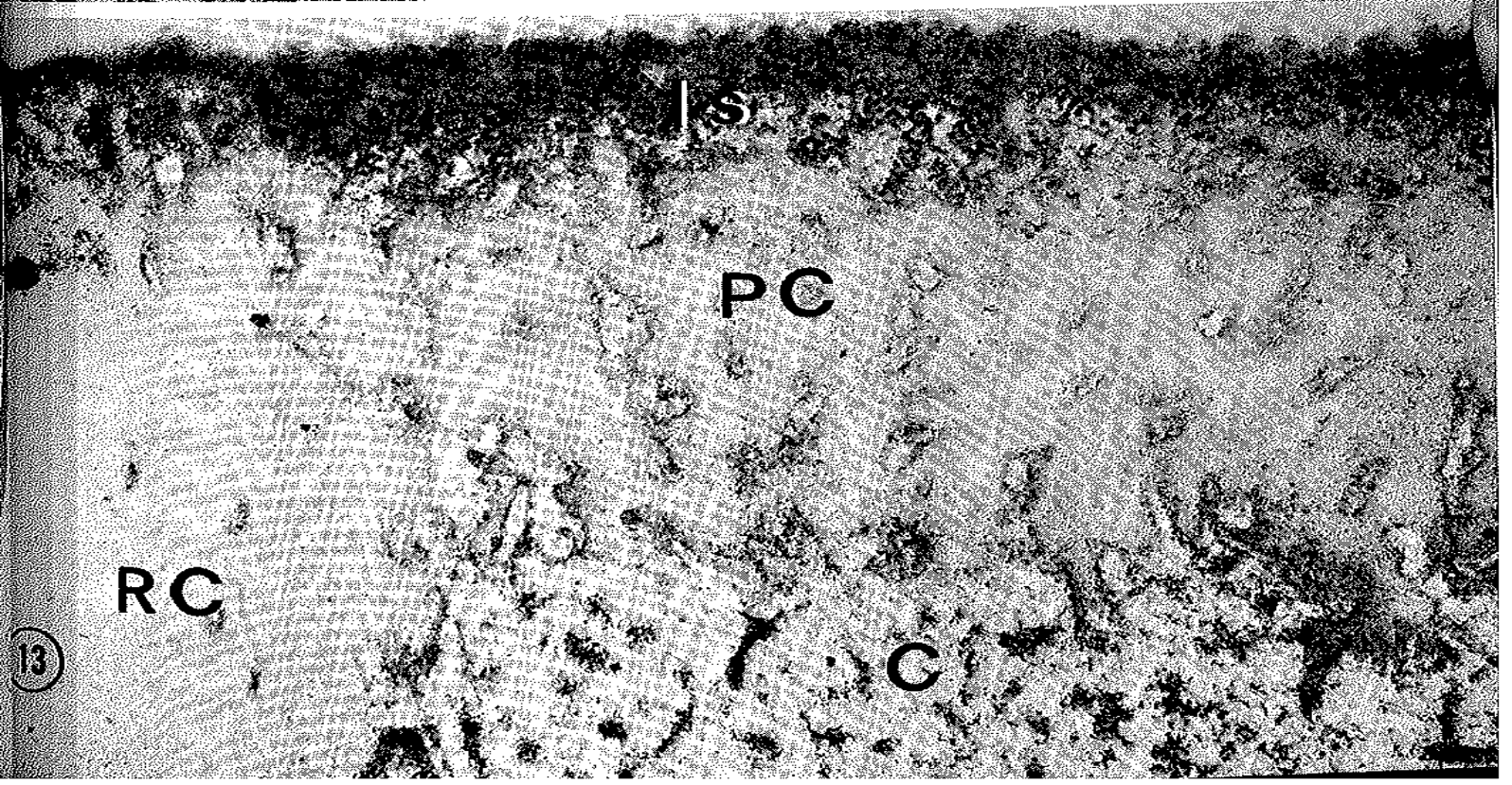
Fig. 12. A section through the mycangial wall. The porous cuticle ( $PC$ ) overlies the secretory cavities ( $C$ ). Between the two cavities is a rib ( $RC$ ) and beneath this rib an efferent ductule from the type-1 units. Microvilli from type-2 units project into the cavities which contain the product of these cells.  $\times 29,000$ .

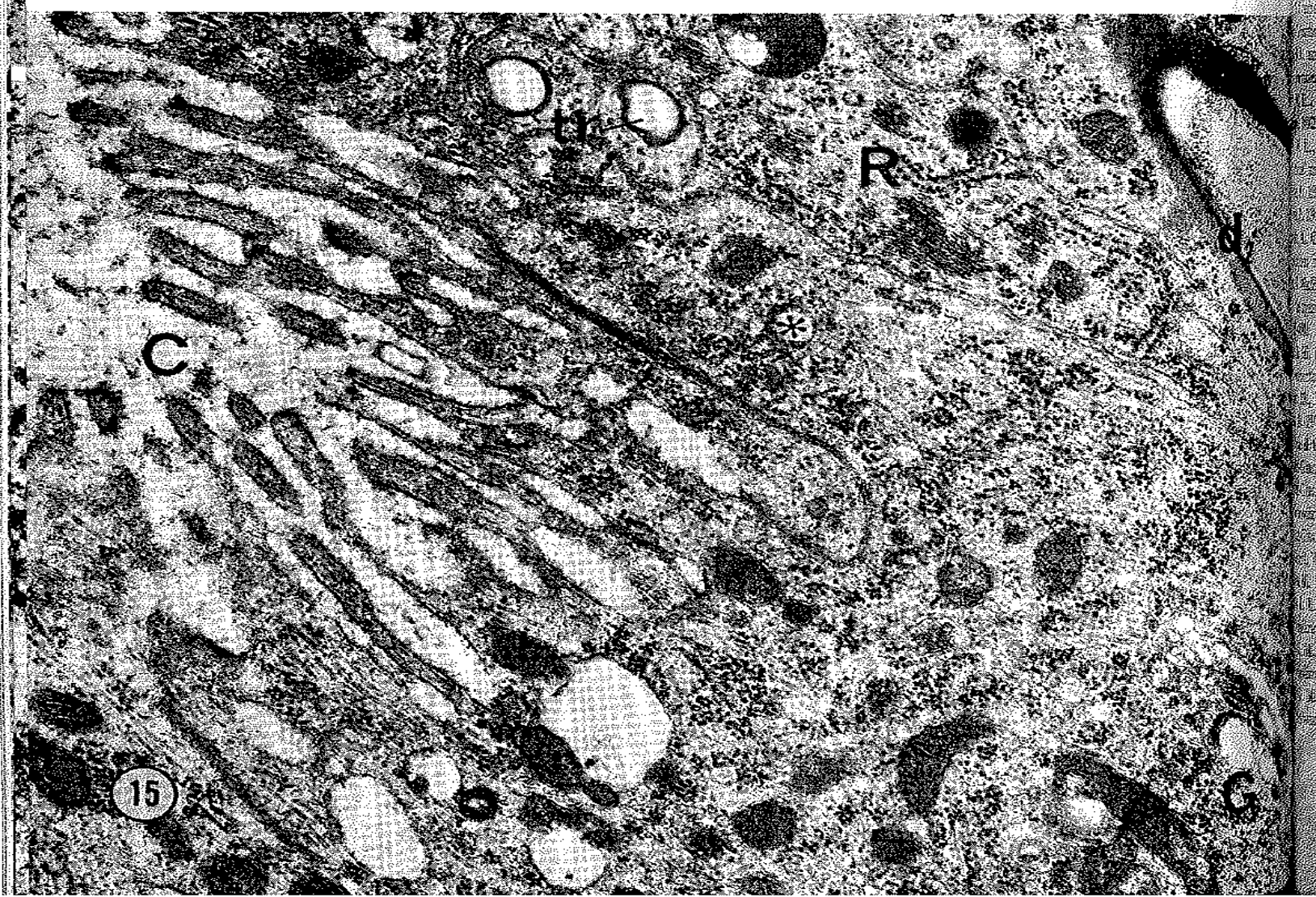
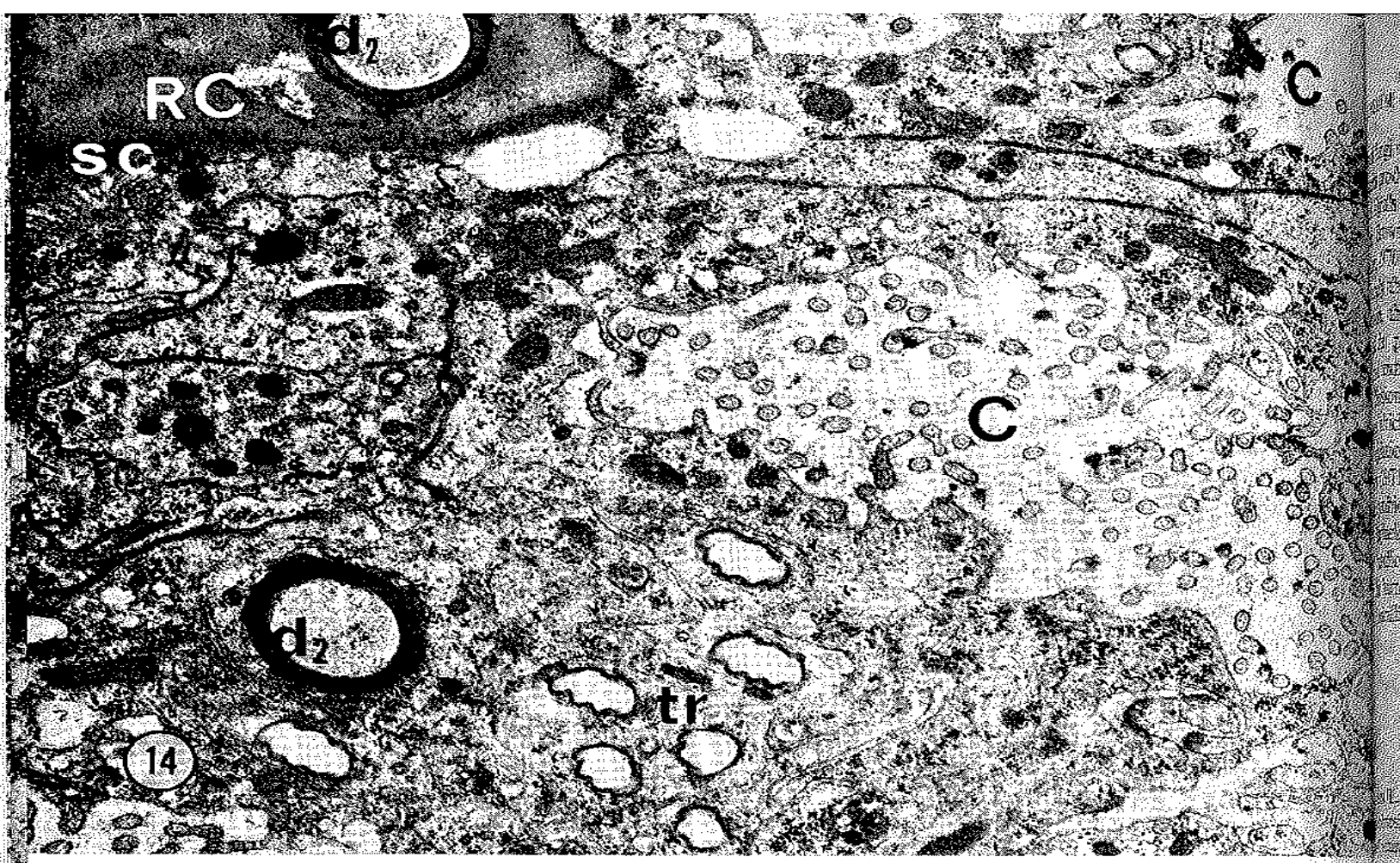
Fig. 13. An oblique section through the mycangial cuticle. A rib ( $RC$ ) is to the left and only the porous cuticle ( $PC$ ) lies over the secretory cavity. Secretion from the underlying cavity passes through the irregular canals to reach the lumen. Just beneath the surface of the cuticle, the canals appear to fuse into an irregular lacunar system ( $ls$ ) within which are deposits of electron-dense material.  $\times 36,000$ .

Fig. 14. A section through several type-2 units just beneath the mycangial wall. The lower end of a cuticular rib ( $RC$ ) can be seen at the upper left. Short microvilli from the adjacent rib cell project to the subcuticle ( $sc$ ) beneath the rib. Within the secretory cavities are elongate microvilli. The outer surface of each villus is coated with an electron-dense fuzz, and within the villus is an ordered cluster of fine filaments. Two efferent ductules ( $d_2$ ) from type-2 units and a number of tracheoles ( $tr$ ) are also present.  $\times 18,000$ .

Fig. 15. A type-2 unit and rib cell. Microvilli from the type-2 secretory cell project into the cavity ( $C$ ) at the left. At the tips of several microvilli are plasma-membrane plaques. The loose flocculent product within this cavity is quite distinct from the electron-dense secretion of the type-1 units, seen within the efferent ductule ( $d_2$ ). A Golgi zone ( $G$ ) is seen at the lower right, and a vesicle with an electron-dense cortex, similar to the Golgi vesicles, is seen in the upper centre of the micrograph (asterisk). This may represent secretory product *en route* to the cavity. The rib cell ( $R$ ) is packed with microtubules.  $tr$ , tracheoles.  $\times 28,600$ .







growth of fungi within the mycangial lumen. These units produce allomones, transspecific chemical signals which are adaptive for the producer (Brown, 1968; Brown, Eisner and Whittaker, 1970). Three morphological features, namely the differences in cytoplasmic organization, the contrasting strategies for export of the products, and the dissimilar density and texture of the products, strongly imply a division of labour, i.e. two sorts of allomones which play complementary roles in the regulation of fungal growth. Barras and Perry (1971a) have shown recently that only the symbiotic species of fungi (principally a *Raffaella* sp., SJB 133, and an unknown basidiomycete, SJB 122) flourish within the lumen. Ubiquitous weed fungi, such as *Penicillium* sp., *Trichoderma* sp., and

the blue-stain fungus *Ceratocystis minor*, are found on the body cuticle but not within the mycangium. A consideration of this selective action of the mycangial microenvironment and of our ultrastructural data suggest an intriguing possibility: one type of secretory unit could provide the basic nutrient medium while the other provides a selective agent which inhibits weed fungi and/or favours the growth of the symbiotic crop.

#### Acknowledgements

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