MORPHOLOGICAL AND FUNCTIONAL ASPECTS OF AN INSECT EPIDERMAL GLAND

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ABSTRACT

The sternal gland of primitive termites of the genus *Zootermopsis* (*Z. nevadensis* or *Z. angusticollus*) (Hagen) seems more organized than that of higher termites, in being comprised of three cell layers. It is also studded with about 200 campaniform sensilla. Below the meshwork cuticle of the gland lies a layer of columnar epithelial cells whose apical surfaces form a brush border, and whose basal surfaces are sculptured into a basketwork into which the second layer fits. Below the brush border are small microtubule-associated pits and coated vesicles. No channels can be seen either within or, except for the sensilla, between the cells. The second cell layer probably secretes the trail-following pheromone. Numerous electron-lucent droplets and large channels containing lipid micelles are found in the cytoplasm here, but the channels cannot be traced out of the secretory layer. The third layer consists of large pyriform cells. The campaniform sensilla are composed of three cells: the sensory cell proper whose dendrite carries a modified 9 + 0 sensory process, an accessory supporting cell that secretes an electron-opaque sheath, and an enveloping cell. At the cell borders of the sensillum, regions of septate and tight junction appear. There are also septate junctions between columnar cells and possibly tight junctions between columnar and secretory cells that would open an intracellular and molecular pathway to the endocuticle. The campaniform sensilla may be part of a feedback control system that determines the amount of pheromone deposited during trail laying.

INTRODUCTION

The sternal gland of the primitive Californian termite of the genus *Zootermopsis* is now known to secrete a pheromone that induces trail following (Stuart, 1960, 1961, 1963; Lüscher and Müller, 1960). The pheromone secretion is apparently a continuous process within the gland, but the actual act of trail laying is not obligatory (Stuart, 1964). In normal activity (Fig. 1 *a*), the animal walks with its abdomen so held that the undersurface only occasionally touches the ground. When an alarmed nymph runs off, it does so with a characteristic "zigzag" movement; at the same time it presses its abdomen down against the substratum (Fig. 1 *b*). In *Zootermopsis*, the fourth abdominal sternite is especially long and overlaps the fifth (see Fig. 2 of Stuart, 1964); this produces a space that Stuart has postulated is a reservoir for the storage of the pheromone. Evidently, pheromone is expelled from the reservoir to form the trail when the abdomen is pressed against the substratum. The sternal gland is equipped with a distinct pattern of proprioceptors, the campaniform sensilla. Stuart (1964) has suggested that these sense organs are part of a feedback control

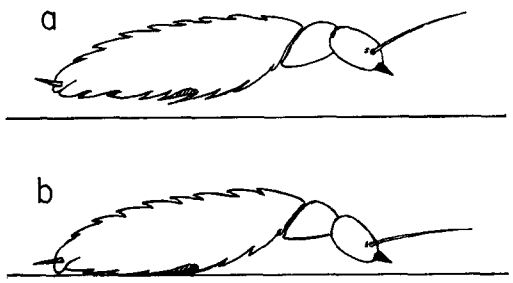


FIGURE 1 Diagrammatic representation of posture of *Zootermopsis* nymphs in (*a*), normal activity and (*b*) when trail laying. The approximate position of the sternal gland is shown by the hatched region of the abdomen.

system that determines the amount of pheromone deposited as a trail.

The sternal gland, then, appears to be an unusual organ because an elaborate proprioceptive function is imposed on significant secretory processes. This paper describes in some detail the fine structure and relationships of certain of the main cellular components of the gland, including the sensilla. It is a continuation of our earlier studies (Stuart 1964; Satir and Stuart, 1965 *a*, *b*). A detailed description of Materials and Methods is to be found in the first two of these papers. Unless otherwise stated, the glands used in the present study were those from 12-mm nymphs or nymphs with wing pads in a mid-intermoult condition. Both *Z. nevadensis* (Hagen) and *Z. angusticollus* (Hagen) nymphs have been examined. There appear to be no significant interspecific differences in the structures to be described. This is in accord with the finding that pheromone secreted by the sternal gland of *Z. nevadensis* induces trail following by *Z. angusticollus*, and with the generally supposed close taxonomic relationship between these animals. The material was routinely fixed in osmium tetroxide and embedded in Epon for light (phase contrast) and electron microscope examination.

Since we have initiated our studies, investigations have been started by other workers on the sternal glands of other genera of termites (Noirot and Noirot-Timothée, 1965 *a*, *b*; Pasteels, 1965; Mosconi-Bernardini and Vecchi, 1964; Smythe and Coppel, 1966).

OBSERVATIONS

General Survey

Below the cuticle of the crescent-shaped sternal gland lies a compact layer of columnar epithelial cells (Figs. 2–4) studded with sensilla (Fig. 3) of the campaniform type (Pringle, 1938; Hsü, 1938). In potash preparations, the sensilla appear as circular profiles embedded in homogeneous cuticular matrix (Fig. 5). The sensilla are particularly dense over the center of the gland and occur in rough rows at intervals as close as 12 μ; they are sparser and less regular in arrangement at the edges of the gland. A total of about 200 sensilla are present.

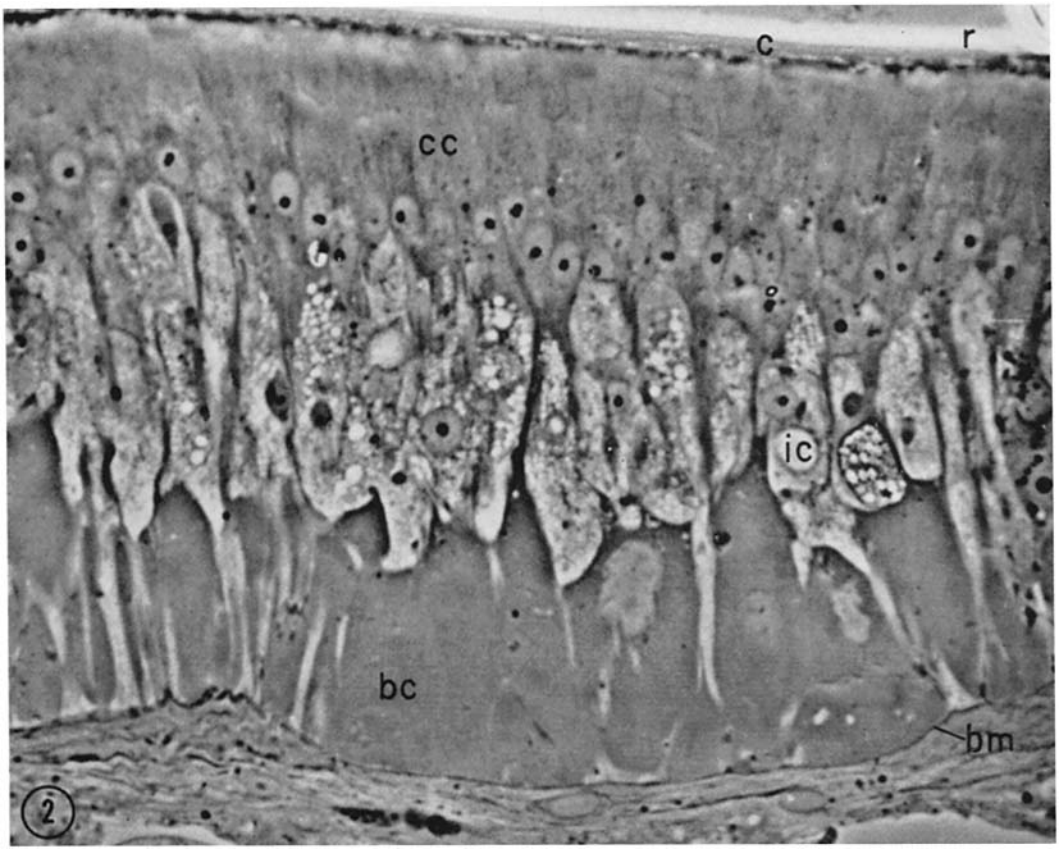
In the columnar cell region, the apical cytoplasm is divided into distinct zones, and the nuclei, containing prominent nucleoli, lie in a row near the basal ends of the cells (Figs. 2, 4). As is obvious in Figs. 2–4, large intercellular spaces are not present between the cells, and, except that it is studded with the sensilla, the epithelium is homogeneous.

Immediately below their nuclear zone, the columnar cells form baskets into which the clubbed ends of the large, highly vacuolated cells of the second layer of the gland fit (Figs. 2, 4). The overall cytological appearance of this second layer indicates that it is secretory, and small clear droplets are apparent throughout the cytoplasm of the cells (Fig. 2). In addition, larger channels are sometimes seen.

The third and most basal layer of the gland is composed of very large cells with a dense cytoplasm and large nuclei. These pyriform cells have extensions into the more apical regions of the gland, and they rest on the basement membrane. The gland is innervated and supplied with tracheoles. Infection with intracellular bacteroids and rickettsiae is common.

The Cuticle

The cuticle overlying the central portion of the gland is approximately 2.6 μ thick. A thin, osmiophilic epicuticle is present. A longitudinal section (reservoir to epithelium) through the endocuticle shows a conspicuous lamination (about five lamellae) which is formed by successive rows of bundles of fine, long, closely packed parabolic fibrillae that are probably chitin or a chitin-protein component of the cuticle (Locke, 1961; Neville, 1967). The bundles are not very wide and are



Technical note on electron micrographs: all material Epon embedded. Normally, gold sections cut with Huxley ultramicrotome were stained in uranyl acetate (saturation solution in 50% ethanol) for 60 min. Micrographs taken with Siemens Elmiskop I.

Key to Symbols

a, axon
ao, apical organelles of columnar cells
asc, accessory supporting cell
bb, basal body of sensory process
bc, basal cell
bm, basement membrane
bw, *bp*, basketwork process of columnar cell
c, cuticle
cc, columnar cell
ch, channel of enveloping cell
d, dendrite
ec, enveloping cell
etd, electron-lucent droplet
g, Golgi apparatus
gl, glycogen
ic, intracellular channel of secretory cell
mt, microtubules
mv, microvilli
n, nucleus
r, reservoir of gland
s, secretion surrounding sensory process
sc, sensory cell of campaniform sensillum
scn, sensory cell nucleus
sd, septate junction
sp, sensory process
ssc, sense system cell
tb, terminal bar

Figs. 2–5 are phase-contrast micrographs.

FIGURE 2 Transverse section through the sternal gland of *Z. angusticollus* showing main cellular components. A portion of this section is diagrammatically represented in Fig. 21. × 770.

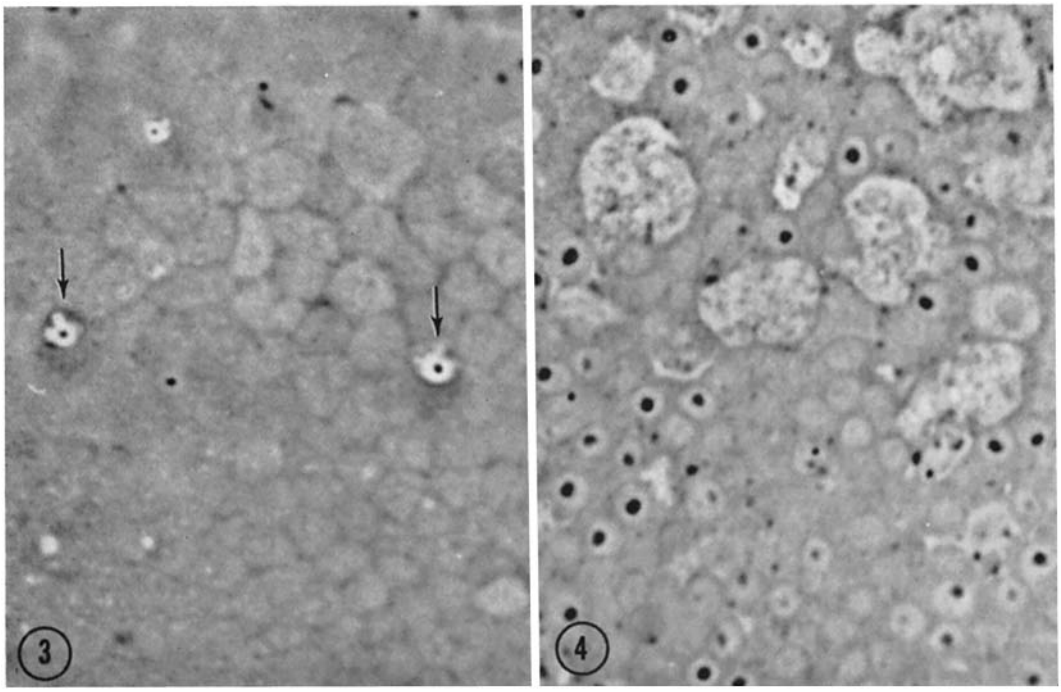


FIGURE 3 Frontal section of gland showing campaniform sensilla (arrows) and columnar cells. *Z. angusticollus*. × 1040.

FIGURE 4 Frontal section at junction of columnar and secretory cells. *Z. angusticollus*. × 960.

not packed together solidly; thus large spaces often appear between them. These spaces may correspond to greatly enlarged pore canals similar to the enlarged pore canals found by Locke (1961) in the honeybee wax-secreting cuticle. In any event, in transverse section (Figs. 6, 7) the endocuticle resembles a grid whose irregular mesh is the fibrillae bundles and whose interstices are filled with a network of tubules, 175 A in diameter, that resemble the wax canal filaments described by Locke. Electron-opaque, short spicules of amorphous, ground material often partially enclose the filament network. The wax canal filaments are thought to be complex lipid micelles involved in the channeling of wax or wax precursors through the cuticle. The diameter of the wax canal filaments of sternal gland endocuticle is somewhat larger in *Zootermopsis* than that in other insects. We have not been able to ascertain the exact relationship of the canals to the epicuticle, but again the situation appears similar to that in the honeybee. Around the campaniform sensillae the cuticle is quite different in appearance; it is relatively electron-lucent, but amorphous (Fig. 7) although fibrillae apparently enter the region.

Columnar Cells

We have already demonstrated some of the main features of the apical cytoplasm of the columnar cells (Satir and Stuart, 1965 *a*). These features are readily confirmed by our more recent work (Figs. 8–10). In addition to the conspicuous, small microtubule-associated pits (junctional organelles), numerous coated vesicles of the type described by Roth and Porter (1964), Brightman and Palay (1963), Bowers (1964), and others are present (Fig. 9). Both these elements originate as extensions of the crevasses below the microvillous border of the cells.

When viewed in cross-section, the columnar cell borders fit together like jigsaw puzzle pieces (Figs. 3, 11), interrupted at intervals only by the campaniform sensilla. No channels can be seen

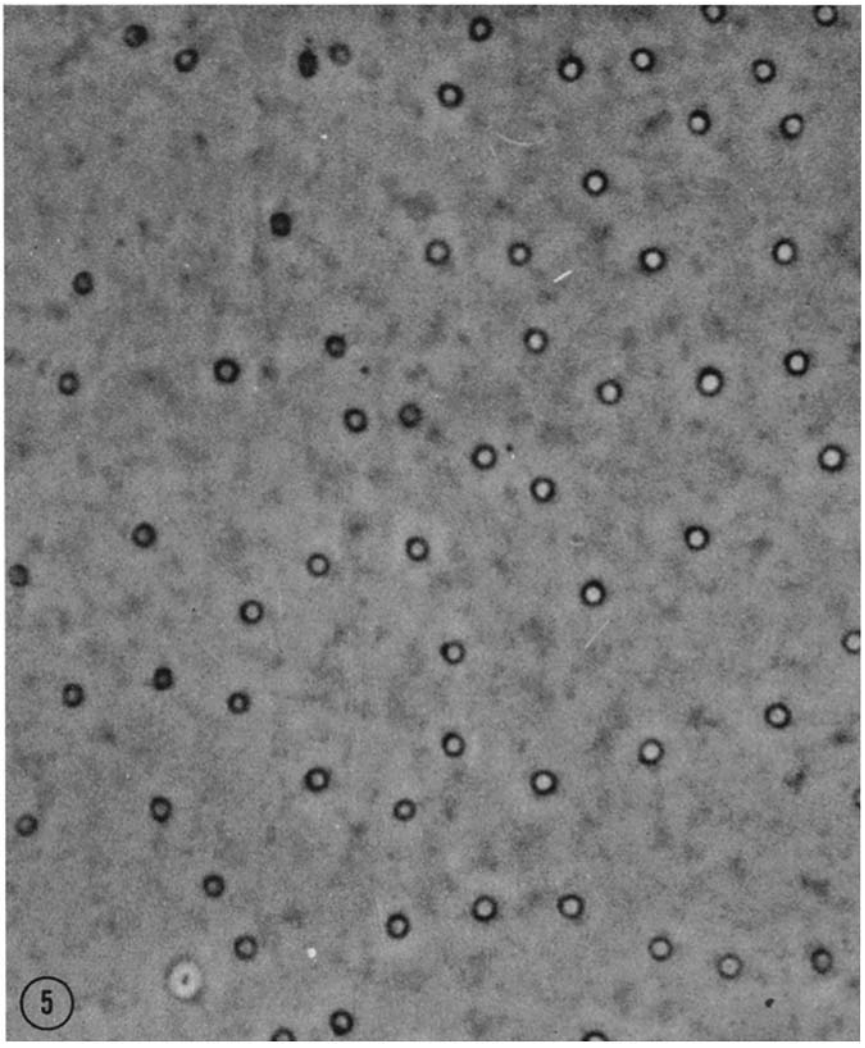


FIGURE 5 Potash preparation of portion of cuticle of fifth abdominal sternite of *Z. nevadensis* showing campaniform sensilla (circular profiles). × 690.

either within or between the columnar cells in this region.

The columnar cells are joined by a typical invertebrate-type cell junction. This includes an apical desmosome region (terminal bar, Fig. 8) followed by a short, normal contact zone and then by a septate junction (Figs. 12, 13). The cell membrane in the region of the septate junction, which extends much of the way towards the basal surface of the cell, consists of a closely packed array of hexagonal units.

The bulk cytoplasm is characterized by the presence of small, smooth, membrane-bounded vesicles and many microtubules. The microtubules run parallel to the long axes of the cells and extend to the cell borders in many cases. Some microtubules disappear into the hexagonal complex at the septate junction.

At their bases, the cells contain numerous glycogen deposits and remaining bundles of microtubules (Fig. 14). Typically, below their nuclear zone the cells become very narrow and form many interdigitating projections. Even there the epithelial character of the columnar cells still remains,

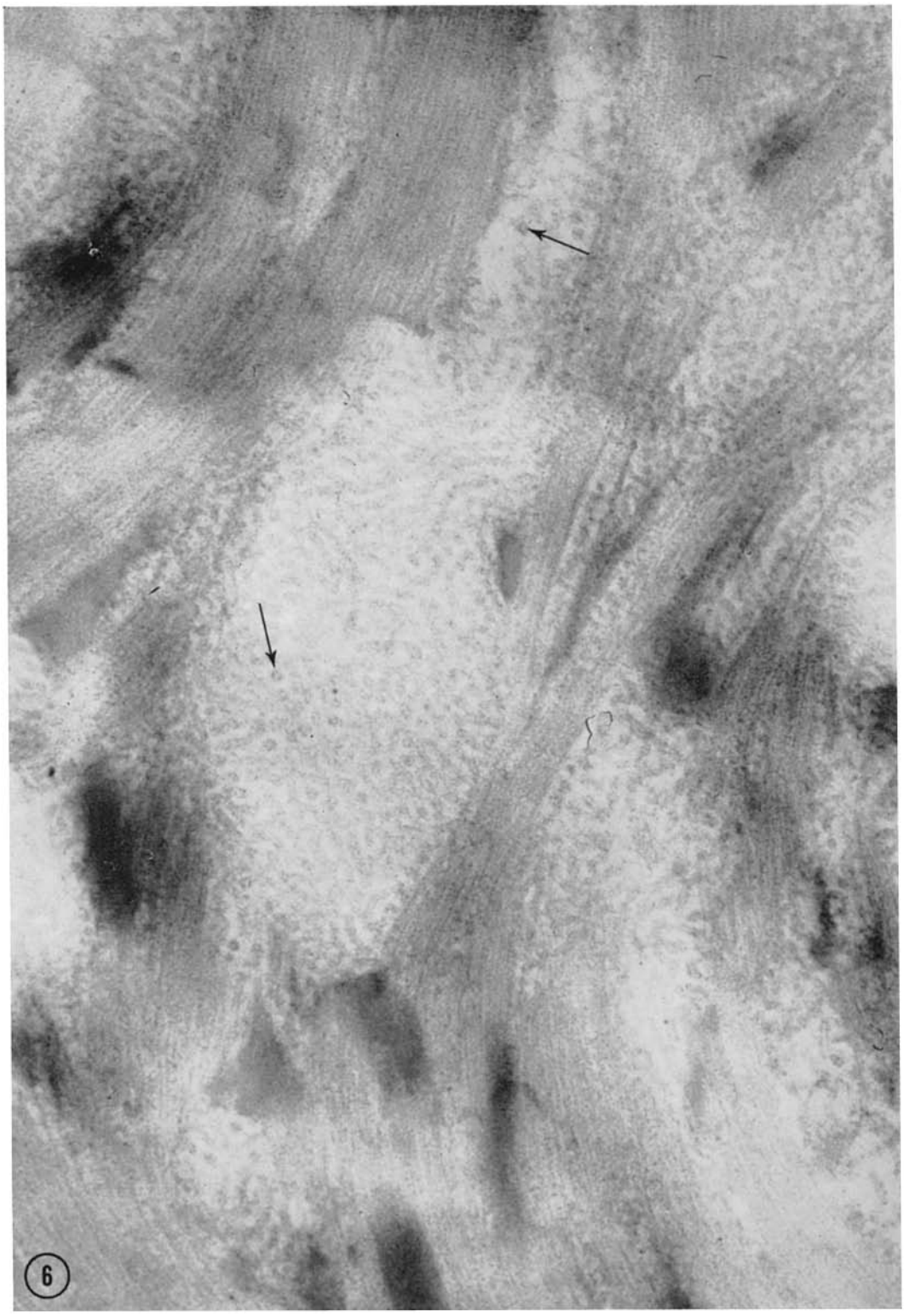


FIGURE 6 Portion of the endocuticle. Frontal-oblique section. Note tubular appearance of wax canal filaments (arrows). *Z. angusticollus*. × 98,000.

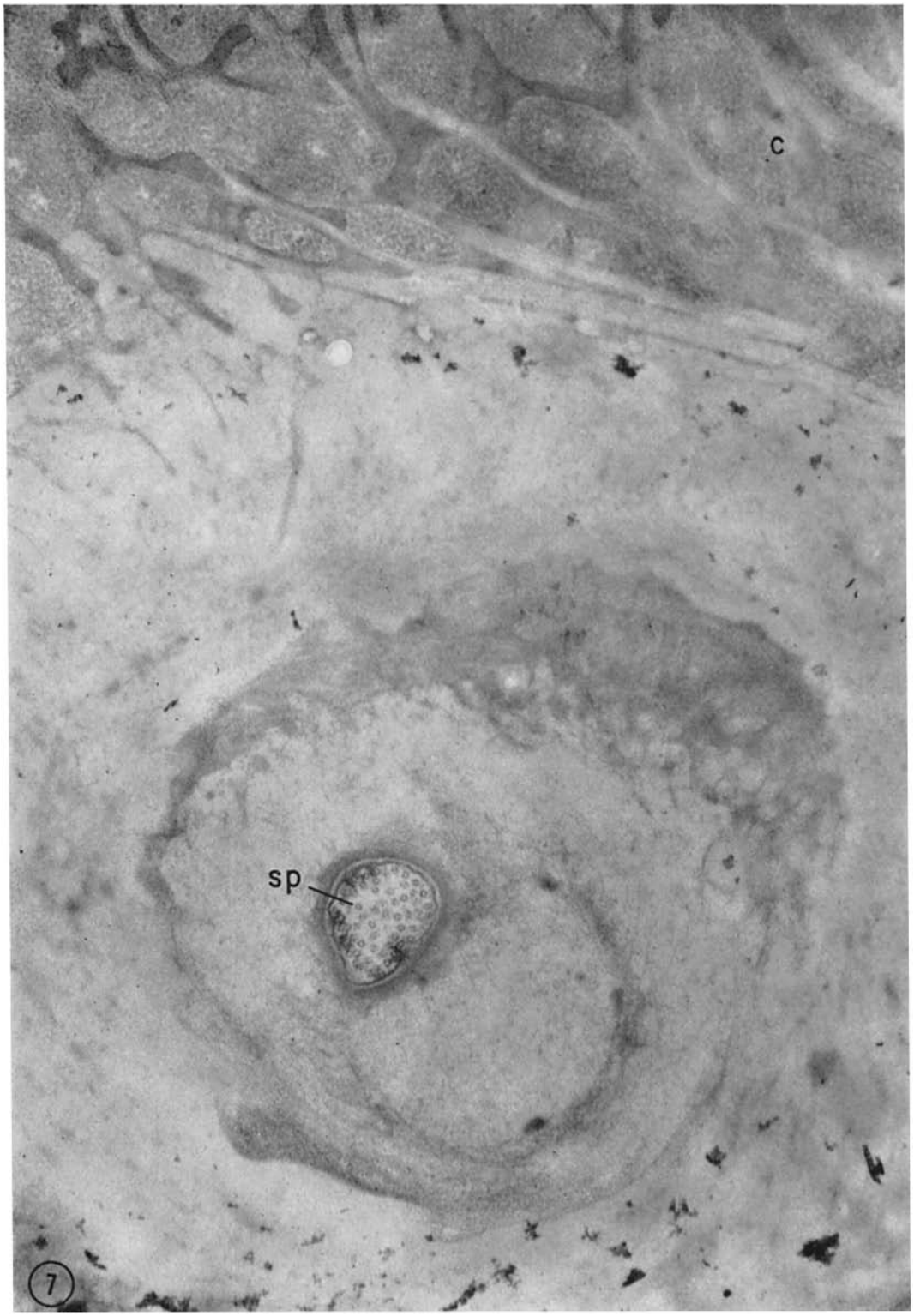
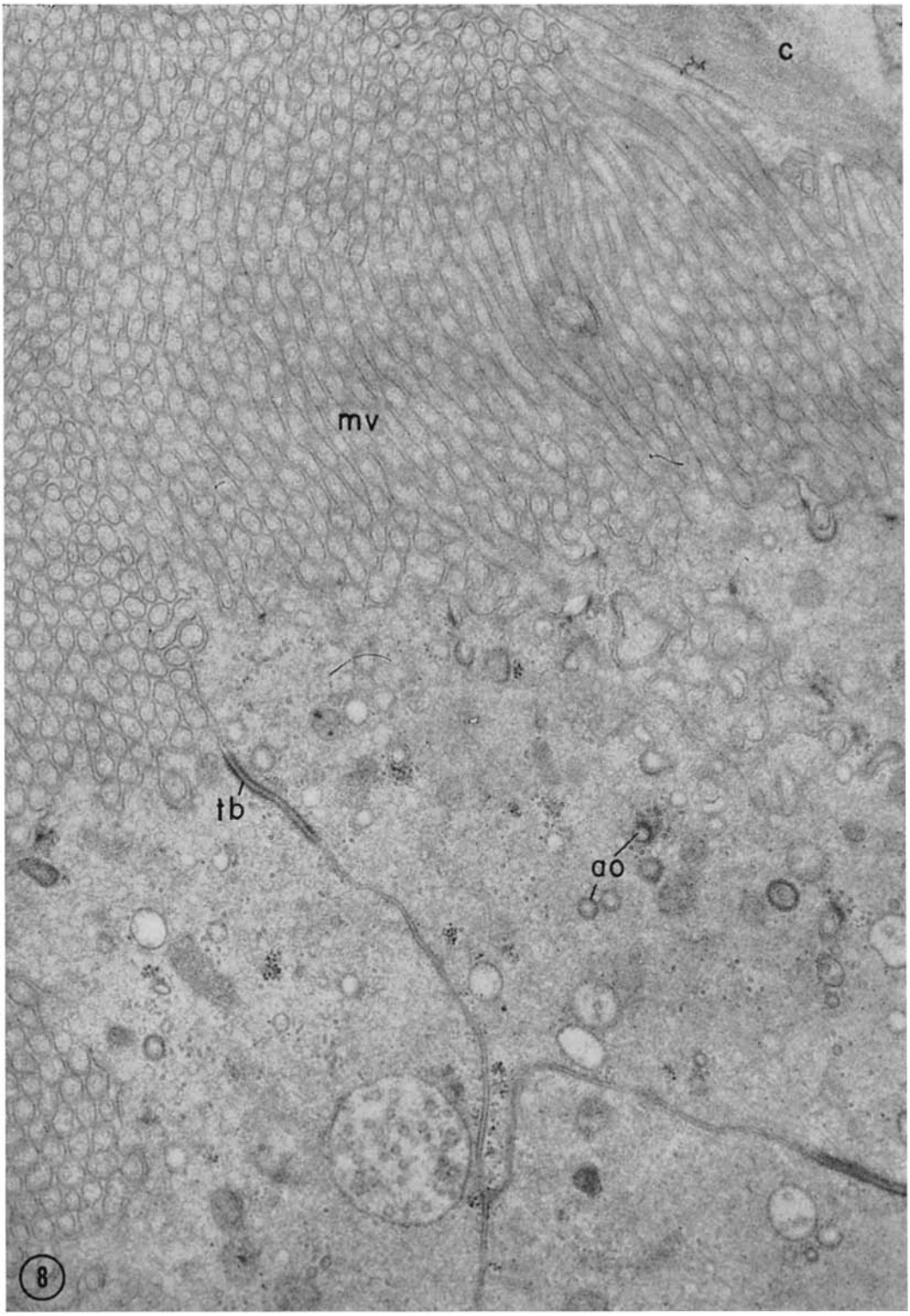


FIGURE 7 Frontal-oblique cut of endocuticle and bulbous portion of a campaniform sensillum sensory process. *Z. angusticollus.* × 45,500.



Figs. 8–10 Apical border of columnar cells. Note junctional organelles (*j*) and coated vesicles (*cv*). *Z. angusticollus*. Similar features have been shown for *Z. nevadensis* in Satir and Stuart (1965 *a*).

FIGURE 8 Overview showing portion of cell borders. × 31,500.

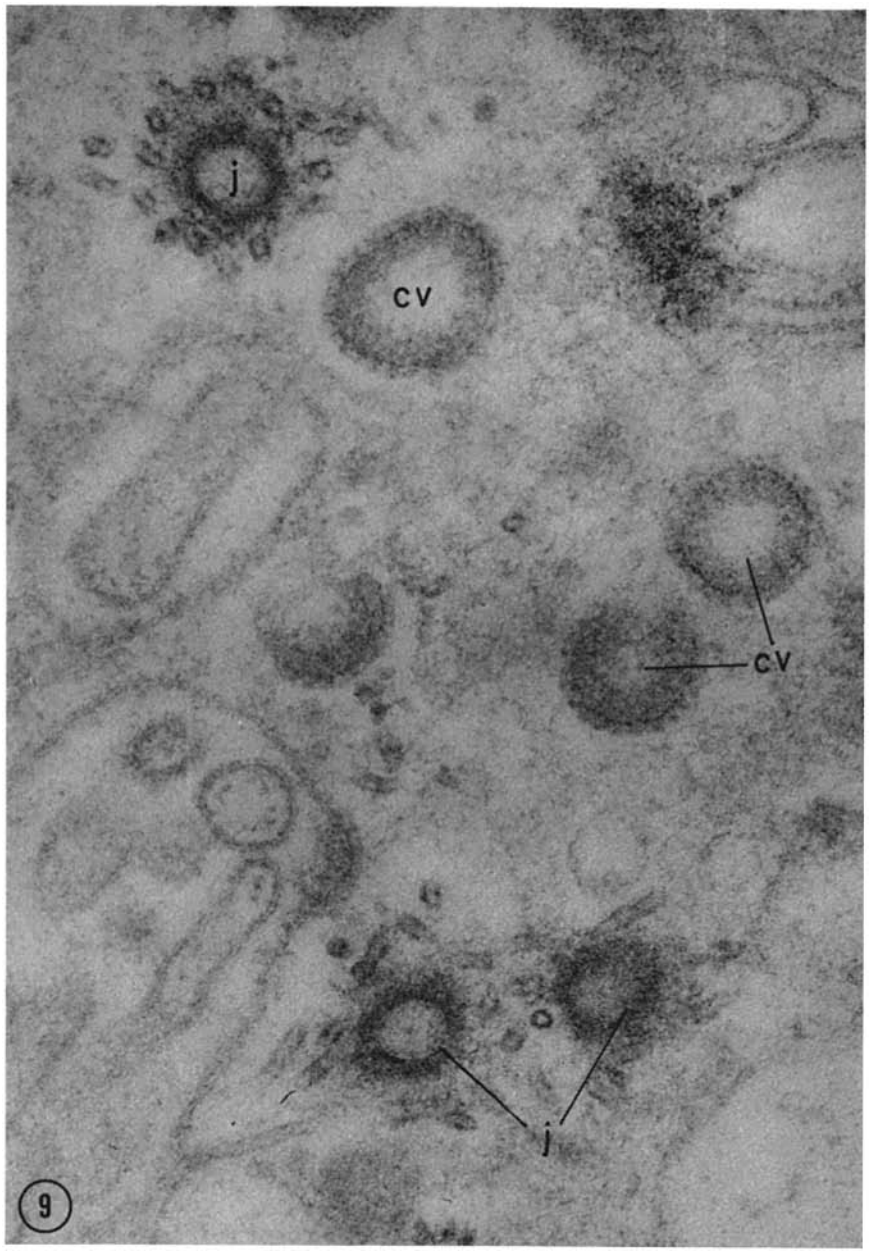


FIGURE 9 Detail of apical organelles. × 112,000.

and the cell extensions persist as thin, laminated ridges separating the secretory cells below. The cell borders in this region are not usually connected by regions of septate junction; certain of our images suggest that true, tight junctions may be present both between two columnar cells and between columnar and secretory cells.

Secretory Cells

The second layer of cells extends from just below the nuclear zone of the columnar epithelium to intertwine with processes from the basal region. The cells of this layer are quite large (Figs. 2, 4, 14–16) (two to three times the diameter of a

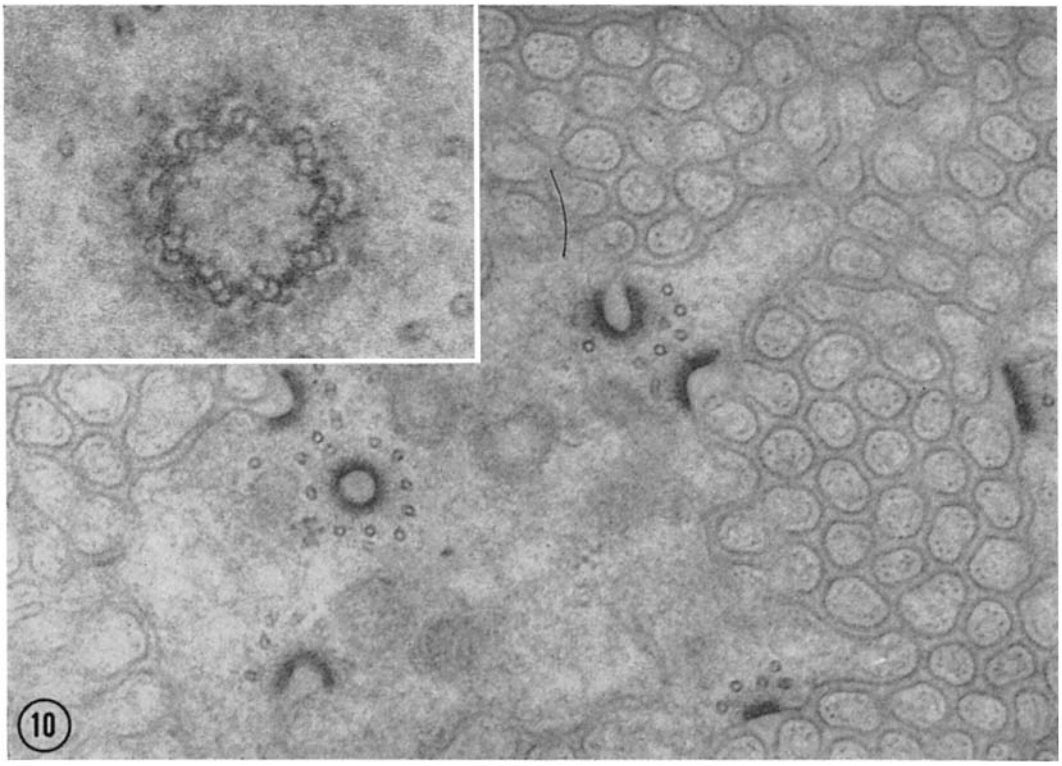


FIGURE 10 Junctional organelles. × 56,000. Insert, columnar cell centriole. × 118,000.

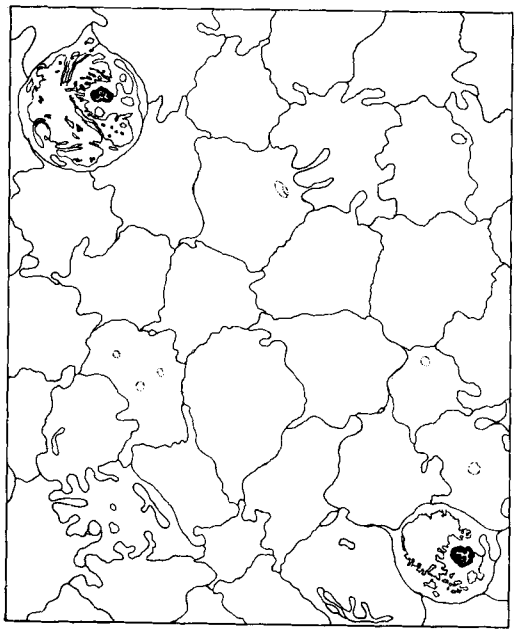
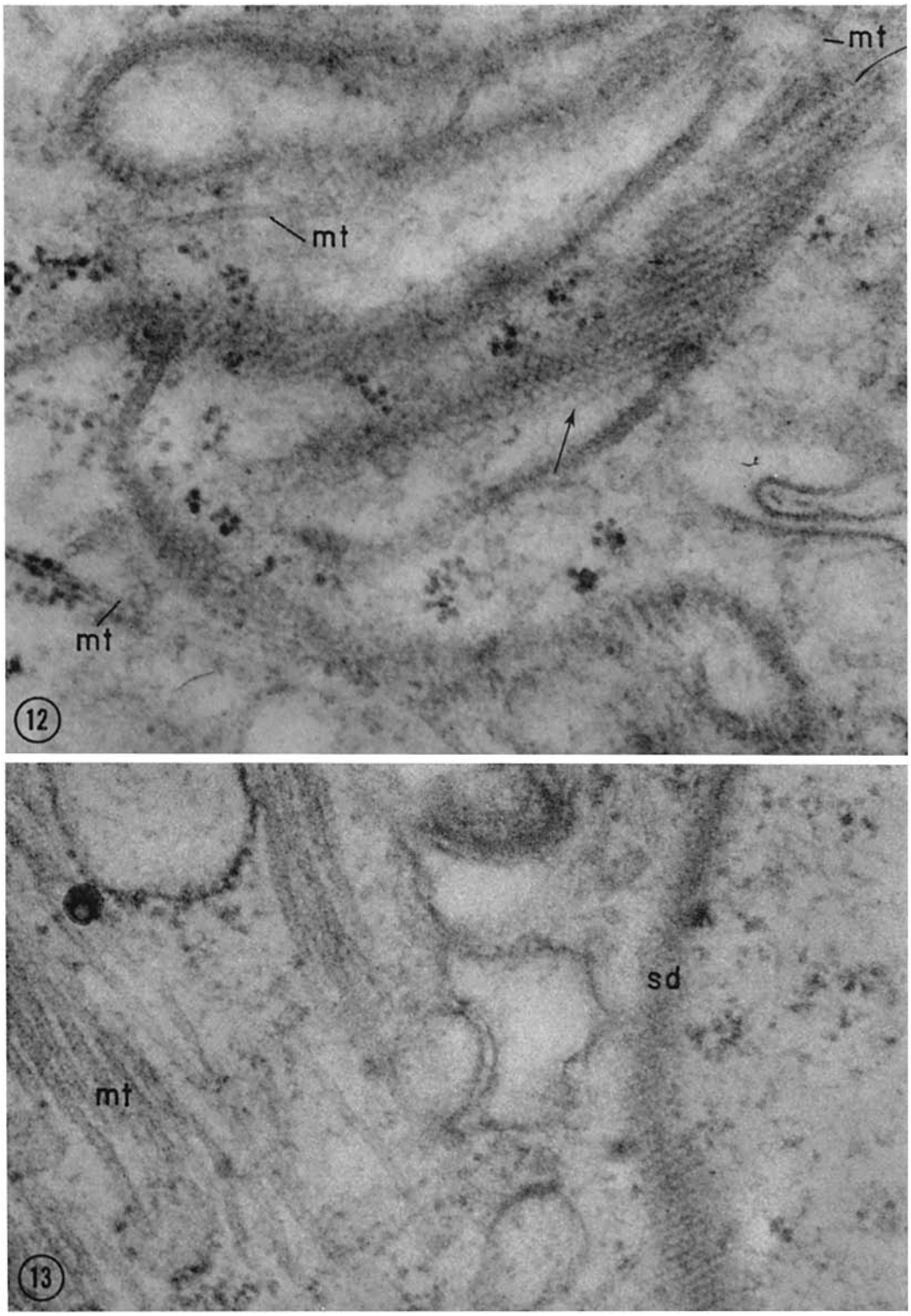


FIGURE 11 Tracing of apical cell borders and campaniform sensilla. Frontal section of gland. *Z. angusticollus*. × 2500.

columnar cell) and somewhat heterogeneous in appearance. This heterogeneity is probably due to functional variation, perhaps linked to different stages in the secretory cycle.

The cytoplasm of the secretory cells contains abundant electron-lucent membrane-bounded droplets (Fig. 16) as well as some denser granules that are probably lysosomes. The nuclear membrane of the secretory cell is unusually well provided with pores (Fig. 15). Mitochondria, some microtubules, profiles of the granular endoplasmic reticulum arranged in small groups of concentric lamellae, and typical Golgi regions are present around the nucleus.

The larger vacuoles in the Golgi region are similar in appearance to the electron-lucent droplets. Occasionally, the electron-lucent droplets apparently fuse to form a large (up to 6.5 μ in diameter) channel whose walls develop loose finger-like microvilli. Numerous droplets and large channels appear in different parts of the same cell. The channels cannot be traced out of the secretory layer. They appear to be closed and not in communication with the extracellular space. In some



FIGURES 12–13 Apical cell borders showing septate junction. Fig. 12, *Z. angusticollus*. Note close association of microtubules and cell border. Honeycomb pattern of septate junction shown at arrow. × 98,000. Fig. 13, *Z. nevadensis*. × 76,000.

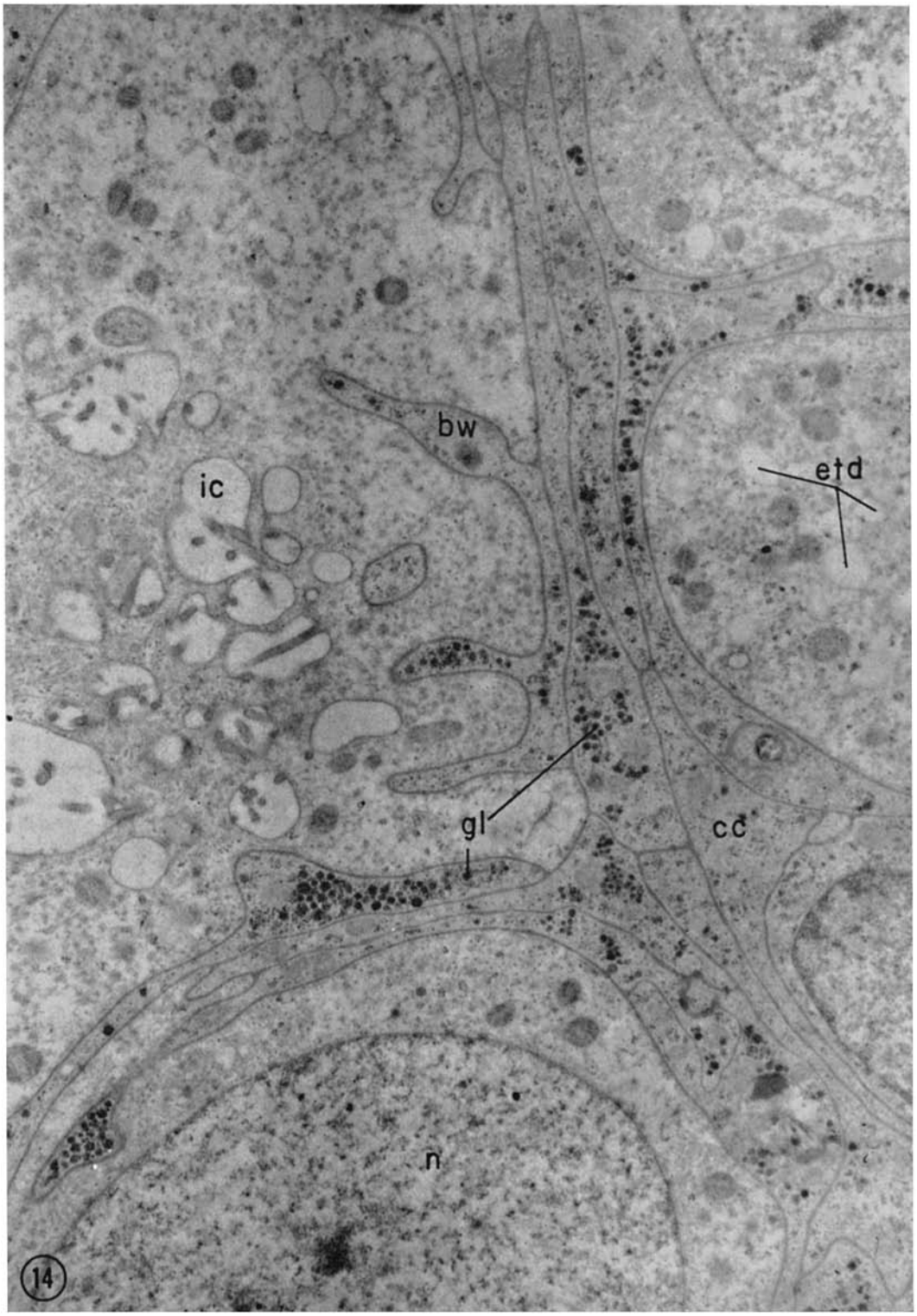


FIGURE 14 Frontal section at border of columnar cell and secretory cell layers. Note the columnar cell extensions containing glycogen and microtubules and penetrating toward the microvillus-lined intracellular channel in the secretory cell. *Z. angusticollus.* × 10,500.

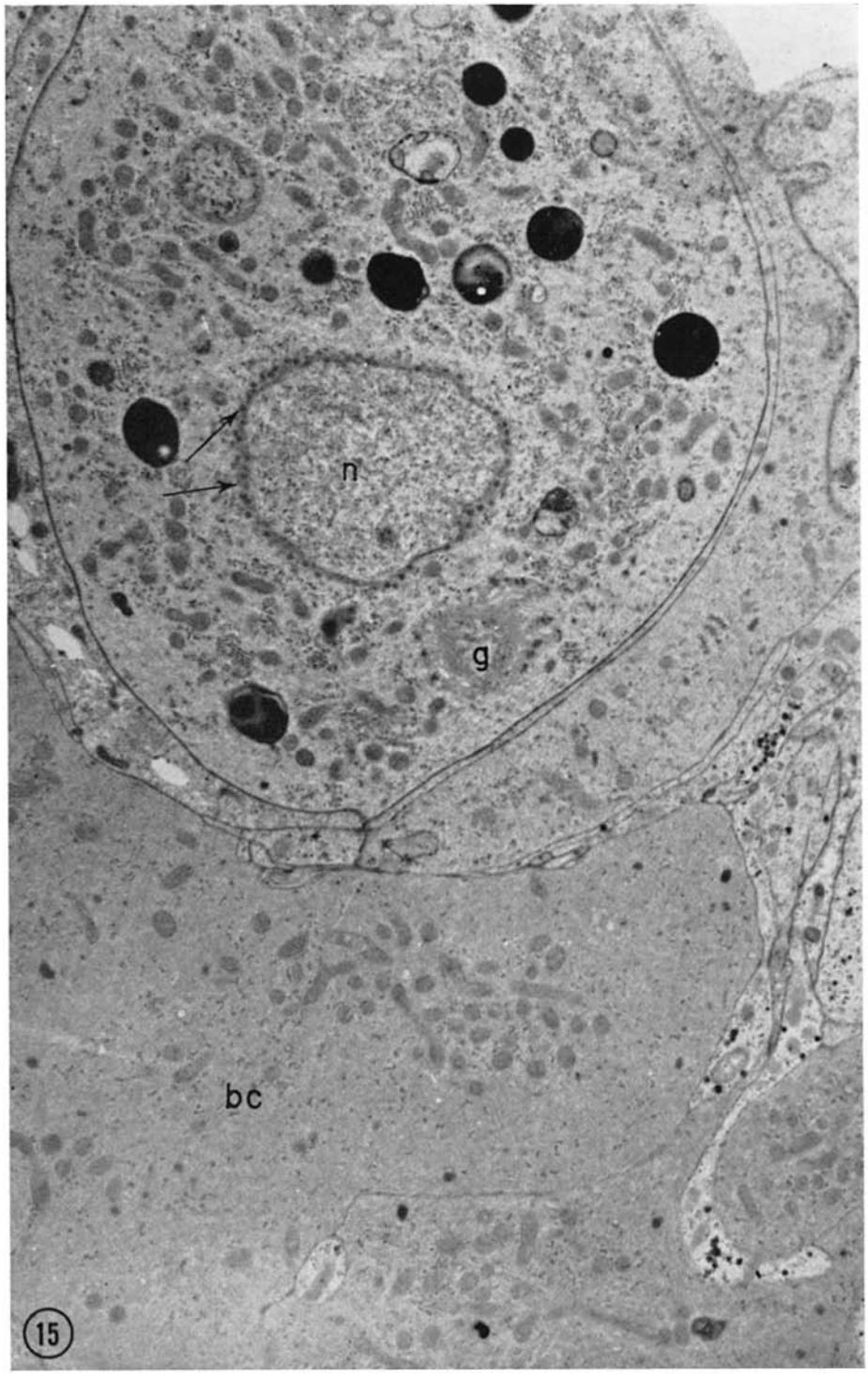


FIGURE 15 Longitudinal-oblique section of secretory and basal cell layers near border of gland. Note prominent nuclear pores (arrows), Golgi apparatus, granular endoplasmic reticulum, and lysosomes in secretory cell; and note mitochondria and polyribosomes in dense cytoplasm of basal cell. Several unidentified cell processes, possibly of sense cell system, are also present. *Z. angusticollus*. × 10,500.

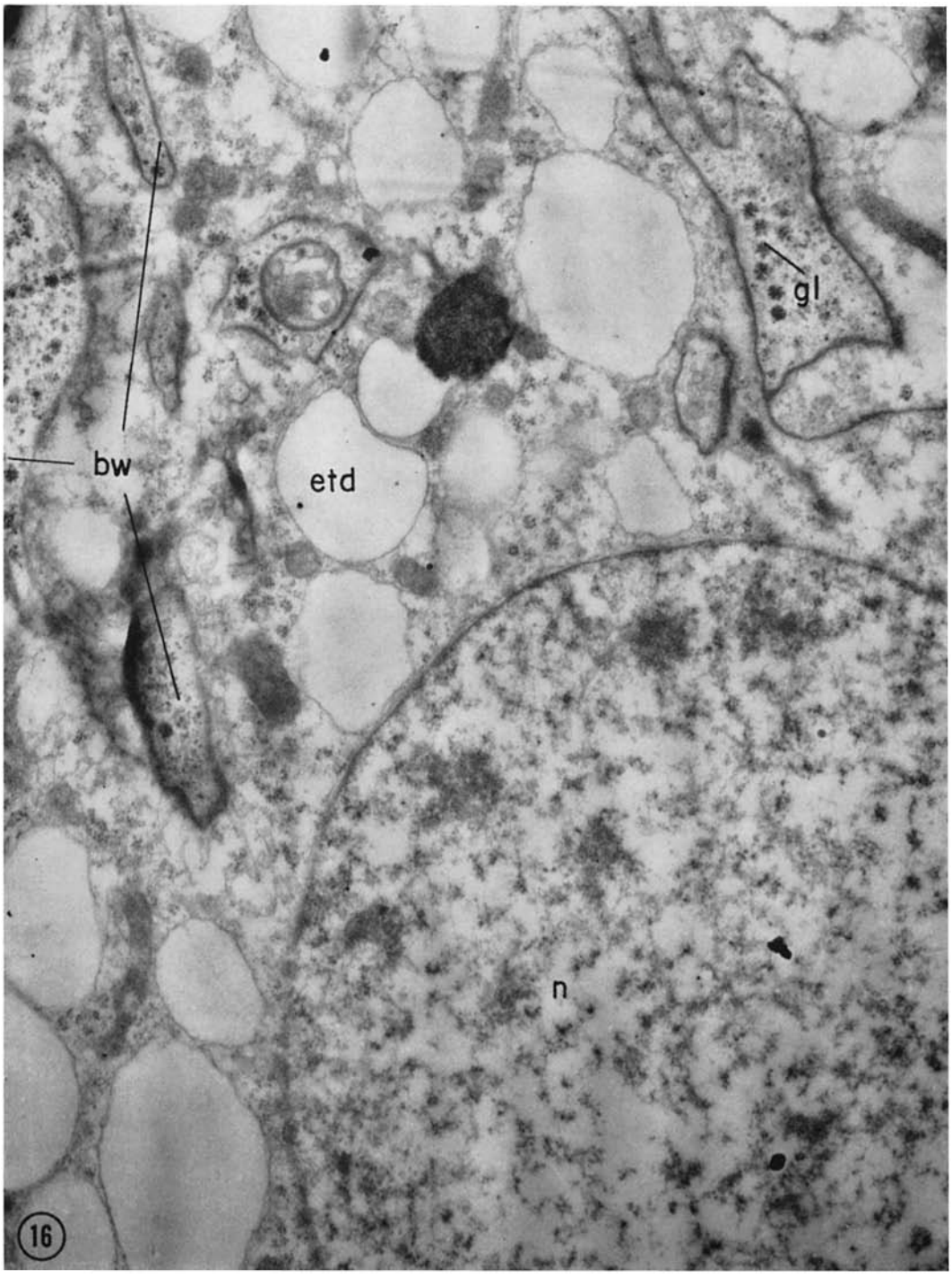


FIGURE 16 Secretory cell containing secretory droplets and glycogen-containing processes from columnar cells. *Z. nevadensis*. × 28,000.

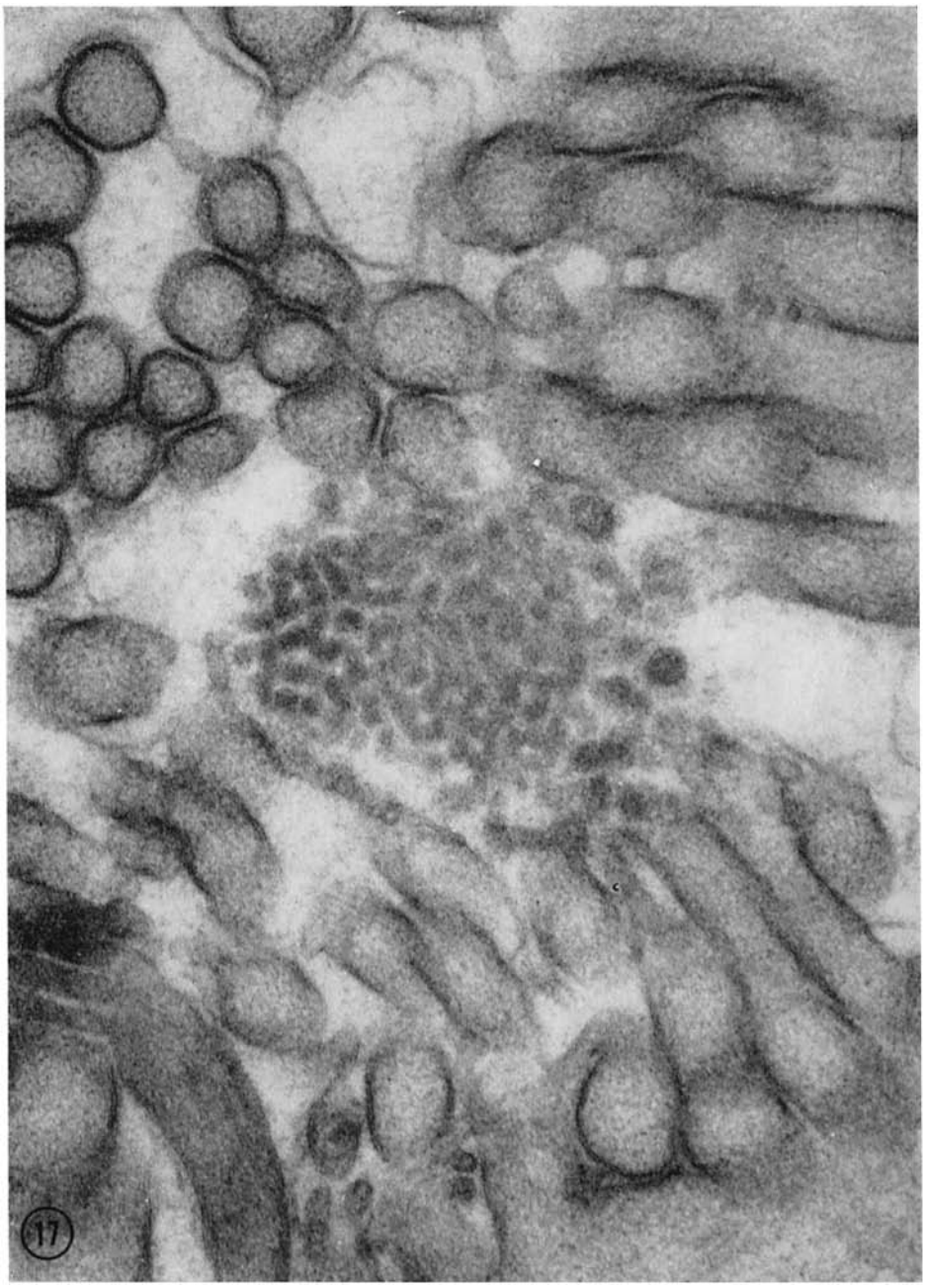


FIGURE 17 Detail of intracellular channel of secretory cell. Irregular rods composed of lipid micelles, presumed pheromonal secretion product (see text), are present within channel. *Z. angusticollus*. × 84,000.

cells an electron-opaque material that appears to be condensed lipid micelles is found in the channels. This material is not membrane-bounded, and it occurs in irregular rods ~240 A in diameter that do not have obvious center holes (Fig. 17). Sometimes, rickettsiae also inhabit the channels.

Basal Cells

The cells forming the third layer (Fig. 2) are the largest in the gland (at four times the diameter of a columnar cell). Unfortunately, fine structural details do not generally appear to be well pre-

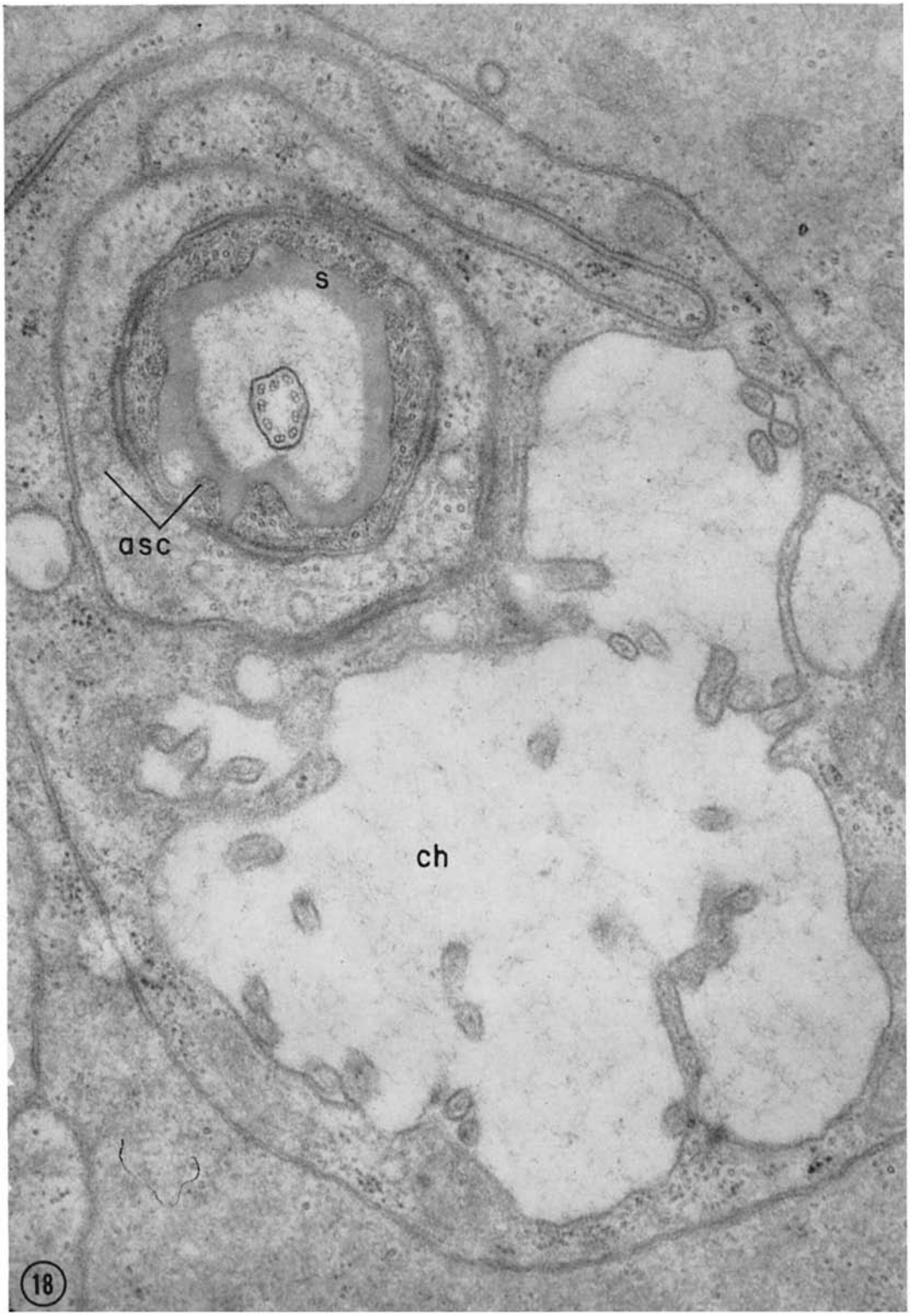


FIGURE 18 Campaniform sensillum. Cross-section of sense process showing 9 + 0 arrangement of axomenal microtubules. Note dense secretion adhering to accessory supporting cell at this level. *Z. angusticollus*. × 46,000.

served, and cytoplasmic contrast is poor in these cells when studied under our conditions. Hence, our information is correspondingly incomplete. The cells have a homogeneous electron-opaque cytoplasm containing a fibrillar matrix, numerous mitochondria, abundant polyribosomes (Fig. 15), and an irregularly shaped, centrally located nucleus. They stain with thionin in 1% phenol (Stuart, 1964). Their lateral surfaces are not extensively convoluted; they abut against other basal cells. At either end of the cell, the cell margins are extended into pseudopodia, and connections are made to long microtubule-containing processes that probably pass through the basement membrane and may be nervous elements and to glycogen-containing processes that may be sheath cells. These processes, together with the basal cell pseudopodia, form a heterogeneous layer immediately above the basement membrane.

Campaniform Sensilla

The campaniform sensilla of the sternal gland are quite comparable to other insect and arthropod receptors, such as those described by Slifer and Sekhon (see 1964 *a*, *b*), Thurm (1964), Gray (1960), and Whitear (1962), since they have sensory processes that are modified 9 + 0 centriolar derivatives (Fig. 18). Our interpretation of the structure of the unit is diagrammed in Fig. 19. The sensillum is composed of three cells (Figs. 18, 20): the sensory cell proper, an accessory supporting cell, and an enveloping cell. From the somewhat peripherally located cell body of the sensory cell proper, a dendrite extends apically. About 14 μ away from the cell body, the dendrite narrows and forms the sensory process. The basal body of the sensory process appears at this region and sends rootlet fibers down into the dendrite proper while its 9 + 0 derivative continues onward toward the cuticle. We have not yet observed an accessory basal body within the dendrite, although this has been seen in sensilla from other insects. No arms are present on the nine doublets of the shaft of the sensory process which are surrounded by a continuation of the unit membrane of the sense cell. The 9 + 0 arrangement is not maintained throughout the length of the structure. After a distance of approximately 8 μ, the process becomes more bulbous, and microtubular profiles replace the doublet filaments (Fig. 7). It is interesting to note that there are more microtubules than can be accounted for by simple separation of the 9 + 0 doublets. We have seen sections in which the nine doublets surround several single microtubules, and it may be that certain of the microtubular profiles in the bulbous end of the sensory process form in

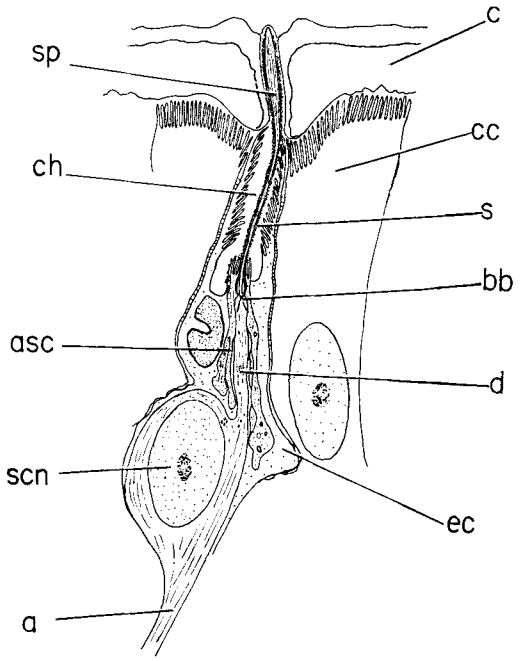


FIGURE 19 Diagrammatic interpretation of cell relationships in the campaniform sensillum.

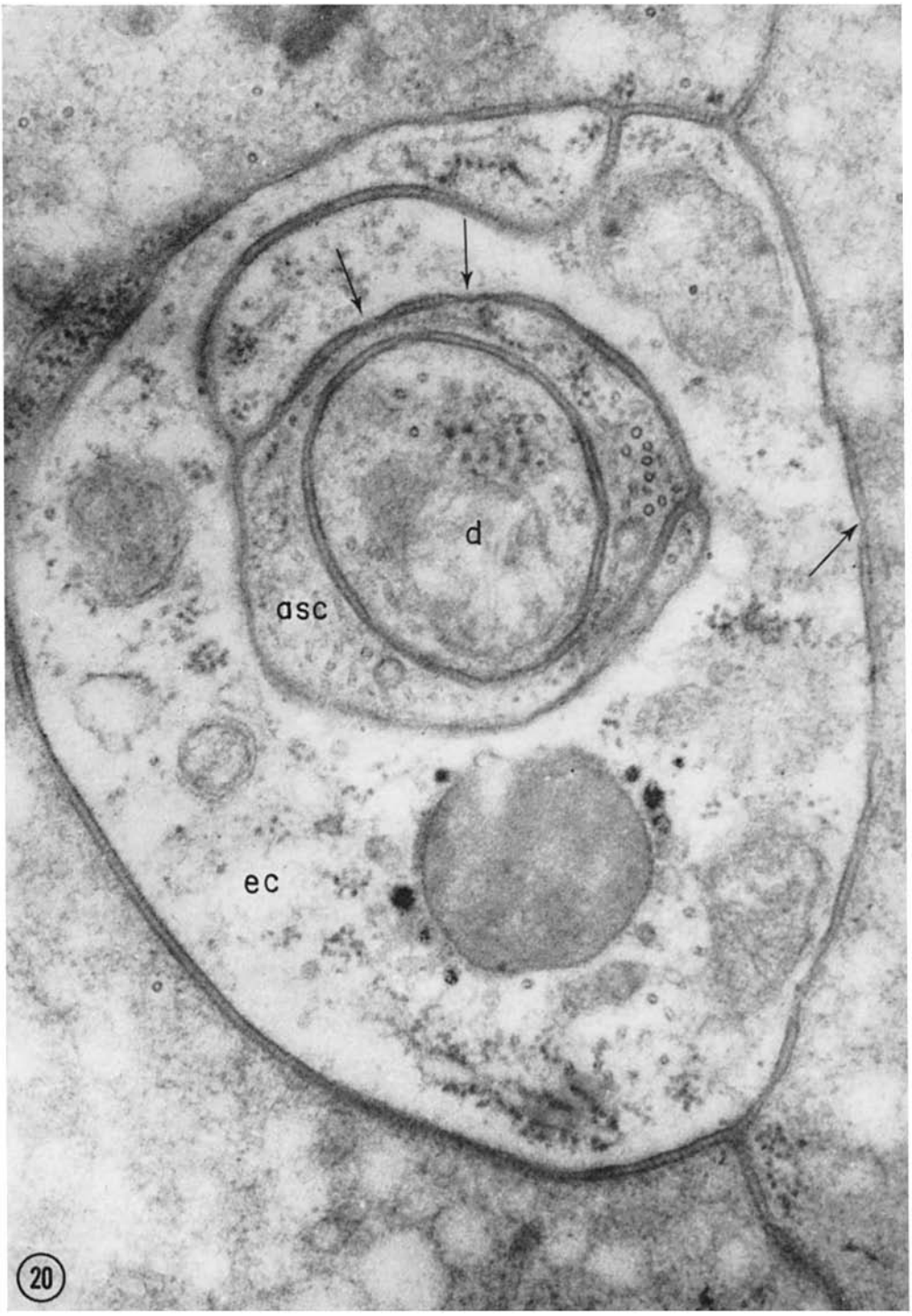


FIGURE 20 Campaniform sensillum. Cross-section at level of the sense cell dendrite. Note septate and tight (arrows) regions of cell junctions. Alate. *Z. nevadensis*. × 60,000.

the inner matrix, separately from the doublets. There is only one sensory process per dendrite and one dendrite per sensillum.

A dense material surrounds the sensory process for much of its length and eventually joins the cuticle (Fig. 7). This material is apparently secreted by the distal extensions of the accessory supporting cell, for just above the level of the basal body where it first appears, it is applied to this cell rather than to the sensory process (Fig. 18). In appearance, this extracellular product is amorphous and sometimes less condensed near its point of origin; it resembles the product comprising the tube surrounding the terminal segment of the crab chordotonal organ sensory process described by Whitear (1962) but is more closely applied to the membrane. The accessory supporting cell contains numerous microtubules, especially in the extension nearest the dendrite. It is attached to the dendrite, to itself, and to the enveloping cell in part by desmosomes and septate junctions and small patches of true tight junction where the intercellular space is obliterated (Fig. 20). It is probably a somewhat modified sheath cell. At a distance of some 5–6 μ above the basal body of the dendrite, this cell terminates in a cluster of microvilli.

The enveloping cell contains a large channel lined with microvilli and open to the cuticular surface of the sternal gland; the dendrite and the accessory supporting cell also extend into the channel. Between the channel and the cell body of the sensory cell lies the enveloping cell nucleus on the side of which the channel is somewhat deeper. The sensory process begins at the level of the centriole of the enveloping cell. The enveloping cell forms direct connections with the dendrite at the junction of dendrite and sensory cell body as well as connections to itself and the accessory cell. The sensillum is sealed from the columnar epithelium by a smooth border of the enveloping cell containing regions of septate and tight junctions.

DISCUSSION

Comparison to Other Species

The sternal glands of other termites that have recently been studied seem to be different from those of *Zootermopsis* in that the layered arrangement of the cells is absent. Thus, in *Kalotermes* (Noirot and Noirot-Timothée, 1965*a*) the secretory cells are juxtaposed with columnar cells and ordinary epidermal cells. The situation in *Nasutitermes* (Pasteels, 1965) seems somewhat similar to that in *Kalotermes*, while Mosconi-Bernardini and Vecchi (1964) claim that in *Reticulitermes lucifugus* the gland is in two parts and that small epithelial cells are present forming evacuation tubes. Smythe and Coppel (1966), studying *Reticulitermes flavipes*, have not substantiated the previous observations on *R. lucifugus*. Unfortunately, Smythe and Coppel have not compared their observations to the previous work on the morphology of this genus nor to the morphological work on *Zootermopsis* (Stuart, 1964). From the published illustrations, however, it is apparent that the gland is not so structured or layered as that of *Zootermopsis*. Mosconi-Bernardini and Vecchi do not mention the presence of campaniform sensilla in the glands studied by them. However, Bregeon (as reported by Noirot and Noirot-Timothée, 1965*b*) shows campaniform sensilla in the sternal glands of *Kalotermes*. Smythe and Coppel observe cuticular domes or caps in the gland in *Reticulitermes* which they indicate might be parts of campaniform sensilla, although they doubt that campaniform sensilla are indeed present since they find it difficult to conceive of a function necessitating large numbers of them. The probable function of the sensilla in *Zootermopsis*, in which they quite definitely occur in large numbers, has been mentioned previously by Stuart (1964) and is discussed in the present introduction. Smythe and Coppel (1966) have inferred incorrectly that we believe that *Zootermopsis* has no control over the laying of a trail. It should perhaps, therefore, again be emphasized that while pheromone may be secreted continuously by the sternal gland, trail laying need not be continuous and is probably under elaborate feedback control mediated in some measure by the campaniform sensilla. Unfortunately, we are not yet in a position to determine the pattern of synapses of the individual sensilla; it may be that a certain amount of resolution of sensory information takes place at the level of the sense organ, that is, within the sternal gland itself. In this respect, it would be interesting to examine the Australian nasutes which are believed to be obligatory trail layers (Moore, personal communication).

In *Reticulitermes*, no definitive experiments have been reported showing that the sternal gland is the origin of a trail pheromone although this is very probable. It seems, therefore, that while *Zootermopsis* is a more primitive termite, its sternal

gland is more highly organized than those of the higher forms studied.

Aspects of Pheromone Secretion

Recently, Moore (1966) has characterized the trail-following pheromone of several Australian species of *Nasutitermes* as a diterpenoid hydrocarbon of empirical formula $C_{20}H_{32}$ (MW 272). In life, the pheromone is probably secreted by the sternal glands of these termites and may be associated with a lipid carrier (Moore, personal communication), as previously suggested by Stuart (1964). It is not unlikely that the trail-following pheromone in *Zootermopsis* is chemically similar to, although not identical with, that of *Nasutitermes*. Happ et al. (1966) have examined the fine structure of a terpene-producing gland of a phasmid insect. In this case, two types of cells make up the secretory epithelium: a squamous cell layer next to the cuticular reservoir, and a layer of larger secretory cells responsible for the production of the terpene. These latter cells have many electron-lucent vesicles and large central cavities with microvillous walls enclosing a secretion product which darkens on exposure to osmium tetroxide in vitro. In the cavity, this product is not membrane-bounded, and its resemblance, as well as the resemblance of the secretory apparatus proper, to our Fig. 17 are remarkable. Stein (1962) has studied a pheromone-secreting gland in the bumble bee that produces a compound that acts as a sex attractant. This compound may be a terpene, although its exact structure is still very controversial (see Butler, 1967). These secretory cells also contain central cavities open to the gland reservoir that are filled with a similar secretion product.

In the sternal gland, the cells of the second layer fit cytological criteria for secretory cells. Apparently their secretory states are not synchronized, but we have seen numerous images of cells that possess both electron-lucent droplets and the large channels containing secretion. We would suggest, on this basis, that the main function of the second layer of cells in the *Zootermopsis* gland is secretion of pheromone. At present, we have little information on the process by which the presumed pheromonal secretion product passes from the channels of the second layer through the columnar epithelium and the cuticle to the gland reservoir. In contradistinction to the *Bombus* sex attractant gland or phasmid defense glands, however, the *Zootermopsis* gland reveals no direct cuticle-enclosed tubes tapping the channels. We cannot rule out the presence of such tubes in every epidermal cell in the gland, and in fact such tubes might be expected occasionally since the gland is derived from and blends into normal insect epidermis in which cuticular tubes are occasionally associated with normal secretory cells. Neither can we rule out the possibility of functional variation that might open the channels to the outside during some stages of the life cycle. In this regard, it is important to remember that epidermal cells have a wide range of actual and potential physiological activities, many of which are connected with cuticular secretion and resorption (Wigglesworth, 1961), and that some sternal gland cells, e.g. the columnar cells with coated vesicles, must partake in these activities. Especially towards the periphery of the gland, normal epidermal cell types might be encountered.

It is possible that the mechanism for transport in the absence of tubular connections through the apical and cuticular layers is intracellular and molecular. The low molecular weight and terpenoid-lipid character of the pheromonal secretion make this an attractive hypothesis. In a series of papers, Loewenstein and his colleagues (Kanno and Loewenstein, 1964; Loewenstein and Kanno, 1964; Wiener et al., 1964; Loewenstein et al., 1965) have elegantly demonstrated that diffusion of ions and substances of low molecular weight, including dye molecules, is possible between insect gland cells that are connected by tight or septate junctions. In the sternal gland, there are septate junctions between columnar cells and possibly regions of true tight junction between columnar and secretory cells that would open a molecular pathway to the basal surface of the endocuticle. In addition, the extremely complicated basketwork pattern at the junction of the secretory layer and the columnar cells supports the idea of an intracellular transport mechanism. The columnar cell projections contain glycogen and many microtubules that might be involved in this process. Since these microtubules fix with osmium tetroxide, it is possible that they have a higher content of unsaturated lipid than is usual. The cuticle overlying the gland has been shown to be a meshwork of fibrillae surrounding lipid micelles. Locke (1961) described a somewhat similar appearance of the cuticle in the wax-secreting region in the honeybee. In that insect, the lipid micelles penetrate into the epithelial cells to some depth. Thus, it appears that in the honeybee cuticle and epithelial cell

inpocketings, in the phasmid terpene-producing cells, in bumblebee cells that may produce terpene, and in the sternal gland cuticle and secretory cells a common feature is present, namely a tubular or rodlike element composed of lipid micelles. The exact chemical nature and packing arrangement of the micelles might account for the tubular or more solid form of the element and for slight differences in dimension. For example, Locke (1965 *a*) has proposed a molecular arrangement that might fix with osmium tetroxide to give the tubular form of these micelles; he suggests that the outer osmiophilic portion of the micelle probably is polar lipid, while nonpolar lipids could be present in the core. He further suggests that newly synthesized polar lipid molecules might be added to the ends of the tubes and in this manner cause wax to move through the cuticle to the outside.

We would suggest a similar transport of the pheromone with its associated lipid carrier from the apical cell border through the cuticle to the gland reservoir. Our interpretations are summarized in Fig. 21.

Noirot-Timothée and Noirot (1966) have observed an apical attachment zone in epithelial cells of the termite (particularly, *Cephalotermes*) gut that resembles the junctional organelle at the apical border of sternal gland columnar cells of *Zootermopsis*. In both cases, microtubules insert on the cell membrane in a specialized attachment structure. Noirot-Timothée and Noirot report that these structures bear on their outer surface some slender filaments that pass through the cuticle. We have also seen images to suggest this appearance in the sternal gland, and it may be that the junctional organelle is the site of passage of lipid from the columnar cell to the cuticle in this tissue.

Sensillum Structure

Careful examination reveals no arms on the nine peripheral filaments of the 9 + 0 sensory process of the campaniform sensilla in the sternal gland. This evidence supports the hypothesis that the derivatives are nonmotile and suggests that lesions suppressing motility in centriolar derivatives may be developmental alterations not only in the presence or polymerization of the central two microtubules, but also in the presence or

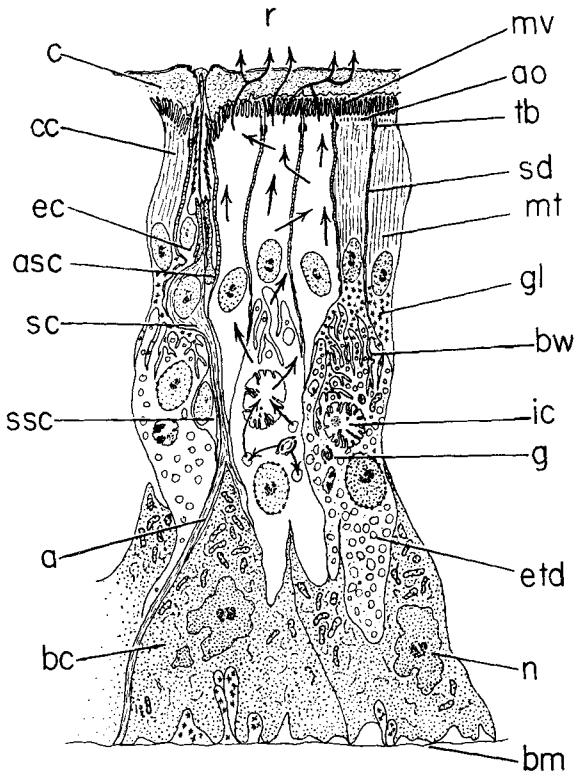


FIGURE 21 Diagrammatic representation of a portion of the *Zootermopsis* sternal gland showing prominent cellular features. In the central region of the diagram, details of cell structure are simplified to indicate (arrows) our interpretation of the probable pathway of pheromone transport from the secretory cell to the gland reservoir. Pheromone is presumably manufactured and packaged in the electron-lucent, smooth membrane-bounded droplets, originally derived from the Golgi region. These droplets fuse to form the microvillous channel. Molecules of pheromone move from the channel through the regions of tight junction in the basketwork processes into the columnar cells. Transport through the columnar cells may be free, vesicular, or as a component of the microtubule wall. At the columnar cell border, pheromone is liberated into the lipid micelle meshwork of the cuticle, and it passes through the cuticle to the gland reservoir in this manner.

absence of arms, that is, dynein ATPase (Gibbons, 1967). Slifer and Sekhon (1964 *b*) report that arms are also missing on the 9 + 0 derivatives of olfactory dendrites of grasshopper. A most noticeable feature of the sensillum is the loss of the 9 + 0 arrangement towards the proximal end of the sensory process and its replacement by numerous microtubules. These microtubules probably arise in part in the inner matrix, as does the central pair in ordinary motile cilia, and in part as the doublets end.

The dense secretion, apparently from the accessory cell, adheres quite closely to the sensory process, in contrast to that in some other insect receptors that are also centriolar derivatives such as a sensillum of the locust ear (Gray, 1960) and the olfactory dendrite of grasshopper (Slifer and Sekhon, 1964 *b*) in which the sensory process is free. However, a similar secretion is present in thinly walled pegs and pit pegs in flesh-fly antennae (Slifer and Sekhon, 1964 *a*), although there is more than one sensory process enclosed in it. In that case and in the sternal gland sensilla, the sensory processes are presumably rigidly supported through their greater length. This feature might be an important one in the functional morphology of these sense organs. The accessory cell is probably a modified sheath cell, for it encloses the nervous element, and it is wound around itself, as is the enveloping cell also. These cells exhibit most clearly the presence of both tight junctions (zonulae occludentes) and septate junctions at the cell boundaries (Fig. 20). This finding has been reported previously by Locke (1965 *b*), by Gupta and Berridge (1966), and by D. S. Smith (personal communication) in other insect cell junctions. It has proved difficult to follow with the electron microscope the sensillum as it courses through the second layer but, according to the light microscope studies of Stuart (1964), the sensory cell probably synapses in this region with a second neuron that extends to the basement membrane and thence to the nervous system. We have seen cell processes in the basal region of the gland that apparently are axons, and cell nuclei that may be from other sheath cells. We have not yet been able to determine the significance of the large basal cells.

We thank Nancy Mielinis, Virginia Berne, Doris Brown, and Robert Michalak for technical help.

This work was supported in part by grants from the NSF (GB-2557, GB-5051) and the USPHS (GM09732, GM15859).

Received for publication 2 June 1967, and in revised form 6 November 1967.

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