A NEW APICAL MICROTUBULE-ASSOCIATED ORGANELLE IN THE STERNAL GLAND OF *ZOOTERMOPSIS NEVADENSIS* (HAGEN), ISOPTERA

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ABSTRACT

The apical portion of the columnar cells of the sternal gland of the termite *Zootermopsis nevadensis* (Hagen) has been examined with the electron microscope. The cell surface abutting the cuticle is thrown into ridges upon which stand microvilli. Sections show a network of smooth membrane-bound cisternae penetrating the interior of the microvilli. At the bottom of the crevasses between the ridges, an inpocketing of the cell membrane is often found. This is surrounded by a 40-mμ electron-opaque zone that is the insertion of a 22-mμ microtubular component of the cell cytoplasm. The pouch-like structures and their associated microtubules are considered to represent a new cell organelle.

The sternal gland of termites has been described in some detail by Stuart (1964). The gland is a swelling of the epidermis in the region of the fifth abdominal sternite and is of particular interest because it secretes a trail-following pheromone (Stuart, 1960, 1961, 1963; Lüscher and Müller, 1960) as well as the normal secretions of the epidermis. The gland is three layered. The cells immediately abutting the cuticle are columnar in appearance with brush borders at their apical ends (Stuart, 1964). Striations running in the long axes of the cells can be seen in 6-μ sections of Bouin's fixed material with a Zeiss phase contrast microscope. The striations run to the apical end of the cells and end at the brush border.

An electron microscope study was initiated with a view to ascertaining the nature of the brush border, the striations, and the cuticle of the gland. A hitherto unknown organelle was discovered during this study. The morphological associations of this organelle with the brush border and the striations form the basis of the present report.

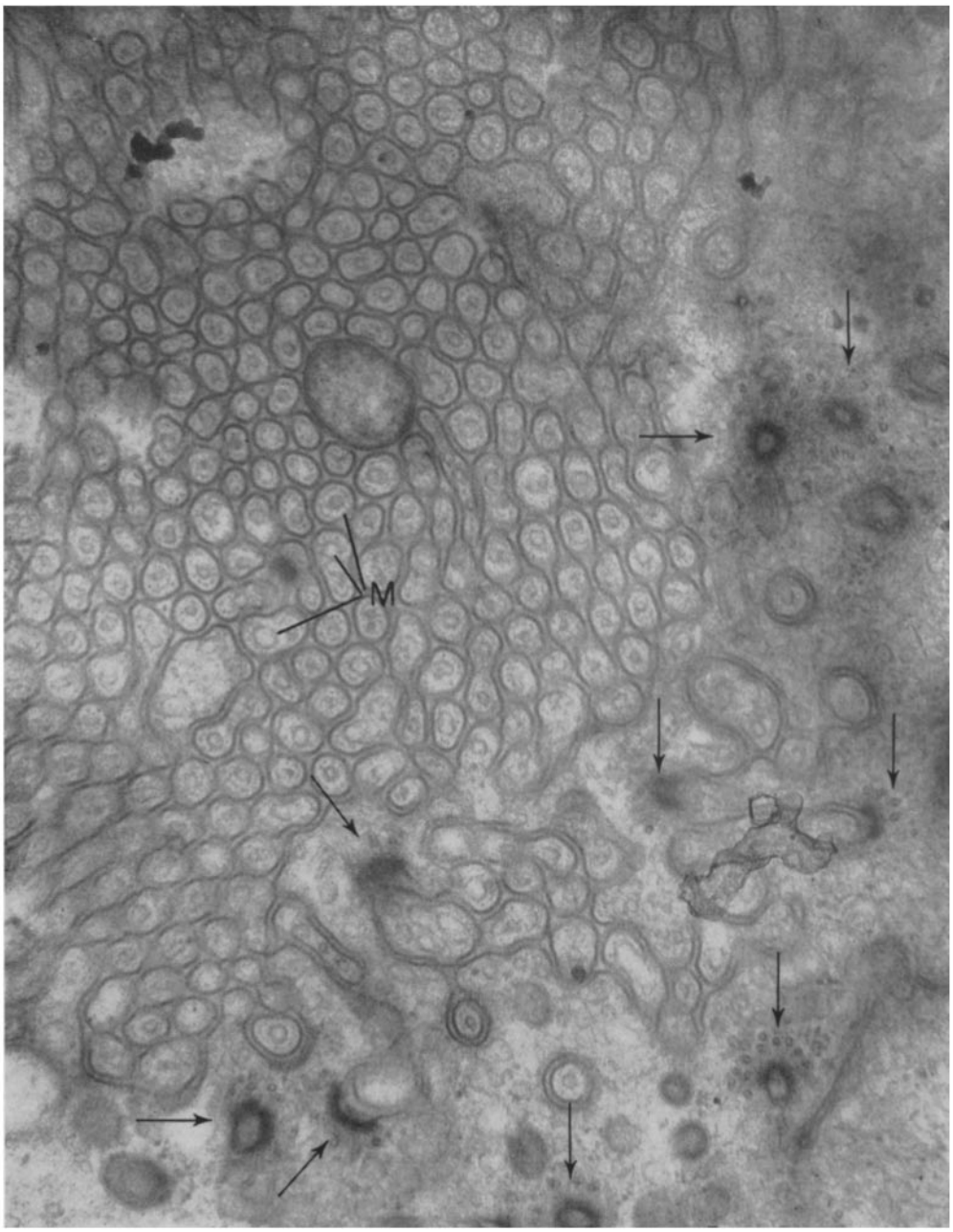
MATERIALS AND METHODS

Throughout the study, *Zootermopsis nevadensis* (Hagen) nymphs of approximately 12-mm length were used as the source of material. The nymphs were obtained from a laboratory colony originally collected from Del Norte County, California. Each insect was dissected under a solution of Ringer's fluid. The gland was extirpated and placed immediately into a solution of 1 per cent osmium tetroxide in veronal acetate buffer, pH 7.3. The usual dehydration procedures for electron microscopy were employed. The glands were embedded in Epon, stained with uranyl acetate for 90 minutes (Gibbons and Grimstone, 1960), and viewed with either an RCA EMU 3B or a Siemens Elmiskop I.

OBSERVATIONS

1. *Brush Border and Apical Cytoplasm*

With the electron microscope, the brush border of a columnar cell of the gland is seen to be composed of numerous microvilli (Fig. 1) of circular, elliptical, or dumbbell-shaped cross-section. The circular cross-sectional diameter is about 125 mμ.



Abbreviations

C, crevasse
CE, centriole
CU, cuticle
E, extracellular space within organelle
M, microvillus
MT, mitochondrion
P, plasma membrane
S, smooth membrane-bound cisternae or vesicles
T, microtubule
W, outer osmiophilic wall of apical organelle

FIGURE 1 Apical cytoplasm of sternal gland columnar cell. Arrows indicate organelles. × 49,000.

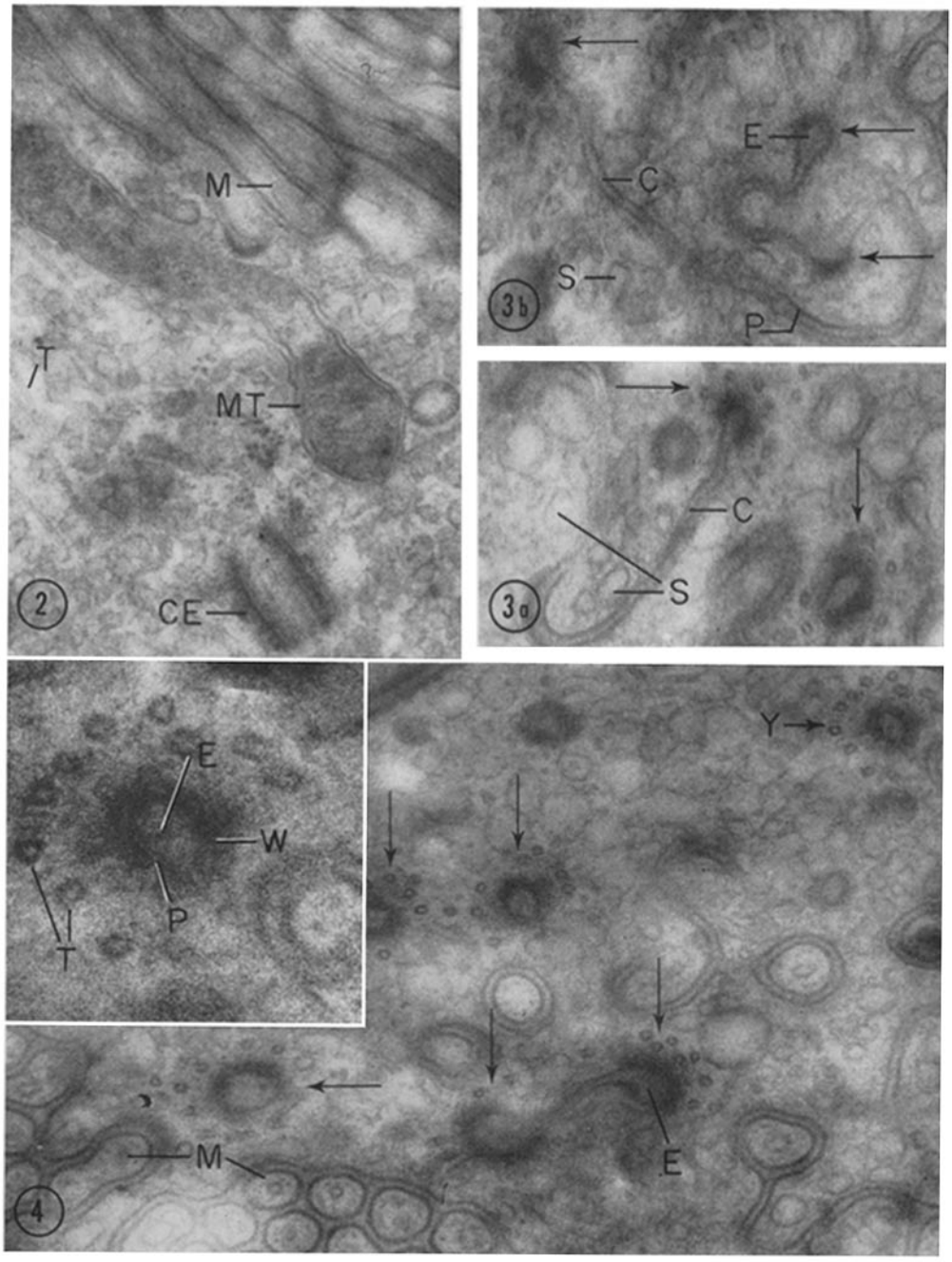


FIGURE 2 Apical cytoplasm of columnar cell showing centriole. × 42,000.

FIGURE 3*a* and *b* Crevasses in the cytoplasm and attached organelles. Arrows indicate organelles. × 56,000.

FIGURE 4 Organelles (arrows) and associated microtubular arrays. × 56,000. Insert—Enlargement of organelle (arrow *Y*). The unit membrane comprising the inner wall of the organelle is visible. × 140,000.

The microvilli extend in blunt, sloping finger-like projections from the cortex of the columnar cells for lengths of more than 1 μ, to just below the cuticle (Fig. 5). Often several microvilli originate from a single irregular ridge or plateau. The plateau may be transected by crevasses of varying depths. The deepest penetrations progress several hundred mμ further into the cortex (Fig. 6). The microvilli have a structured interior composed of a row of membranous cisternae (Fig. 5) that in certain cases may be one continuous irregularly swollen and constricted pouch. The pouches run to the bases of the cortical ridges, where the cisternae are indistinguishable from elements of the smooth endoplasmic reticulum (Fig. 3). In cross-section (Figs. 1 and 4), the cisternae are surrounded by a number of dense dots that we interpret to be fine filaments of about 70 A diameter.

The cytoplasm immediately below the brush border contains many smooth membrane-bound elements, some glycogen deposits, and occasional mitochondria. Typical centrioles are also located in this region (Fig. 2). In addition, microtubules or microfilaments are present and can be followed for distances of several microns. They are characterized by an osmiophilic cortex surrounding an electron-transparent center region. In certain instances in longitudinal section, they display a beaded or striated appearance (Fig. 5); the striations have a periodicity of about 20 mμ. The diameter of the tubules is about 22 mμ and the distance between tubules in a parallel bundle is about 33 mμ. Similar arrays of tubules running parallel to the long axis of the cell are found throughout the cytoplasm. These arrays probably account for the striated appearance of the Bouin's-fixed cells.

2. *Junctional Organelle*

The tubular bundles approach the microvillous border, and here each bundle coalesces with the outer wall of an elongate cylindrical body (Fig. 7) whose diameter is approximately 140 mμ and whose length is 600 mμ. This body, together with its associated tubules, represents a consistent, frequently occurring morphological entity in these cells and probably has the status of an organelle. Fig. 1 shows a part of the apical region of a single sternal gland columnar cell containing at least nine such organelles. The organelle seems to be confined to the apical region of the cell. It is orientated so that its long axis is approximately parallel to the long axis of the cell. A section that is nearly parallel to the surface of a cell cuts a number of the organelles in cross-section as it penetrates the cytoplasm (Figs. 1 and 4).

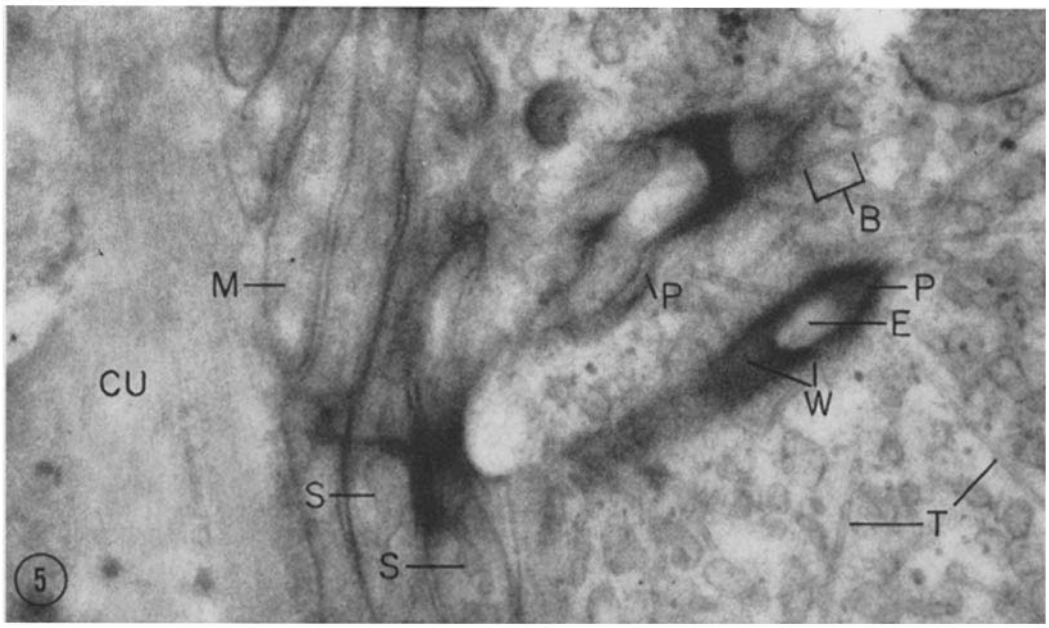
The organelle consists of two zones: (1) a 40-mμ-thick electron-opaque zone that makes up the wall of the hollow cylindrical central body, and (2) an amorphous zone containing the tubules. In the sections observed, ten or more cross-sections of tubules can clearly be counted around a single central body (Fig. 4). The boundary between the outer surface of the wall and the amorphous, tubule-containing zone is irregular and somewhat indistinct. The inner edge of the wall is more sharply defined however, and our best sections show a triple-layered unit membrane adjacent to the transparent core (Fig. 4, insert). This membrane appears to be an extension from the crevasses beneath the microvillar ridges (Figs. 1, 3, 4, and 7). Several organelles may lie in line beneath the same crevasse (Fig. 3). Since the membrane penetrates to the distal end of the organelle (Fig. 7), the hollow core of the organelle is in the extracellular space. The central body, therefore, represents a massing of osmiophilic material at the junction point between the microtubular element of the cell cytoplasm and the cell membrane. Within the wall of the central body itself, no circumferential spatial divisions are apparent. A diagrammatic representation of the relationships between components of the organelle is shown in Fig. 8.

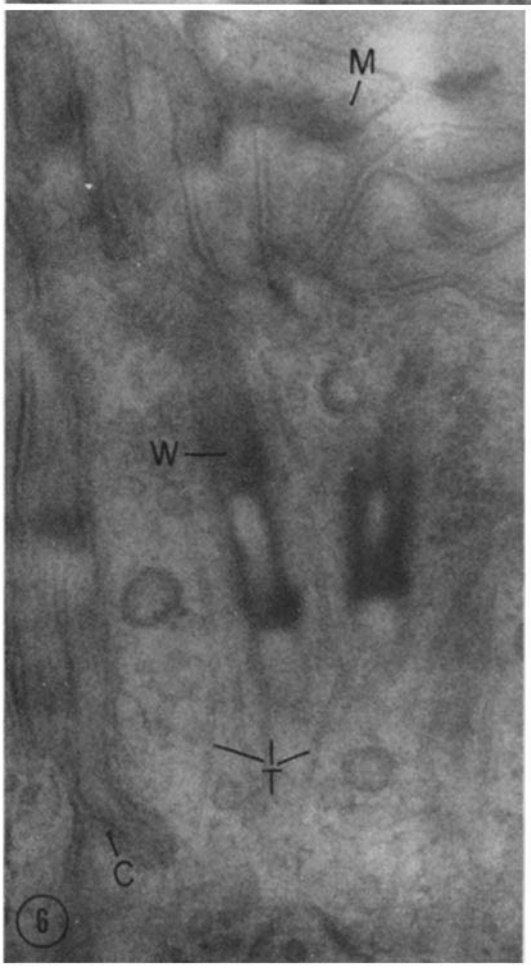
In some respects, the organelle might be thought to resemble a centriole, but comparison of Fig. 2 *vs.* Figs. 6 or 7 quickly shows certain obvious differ-

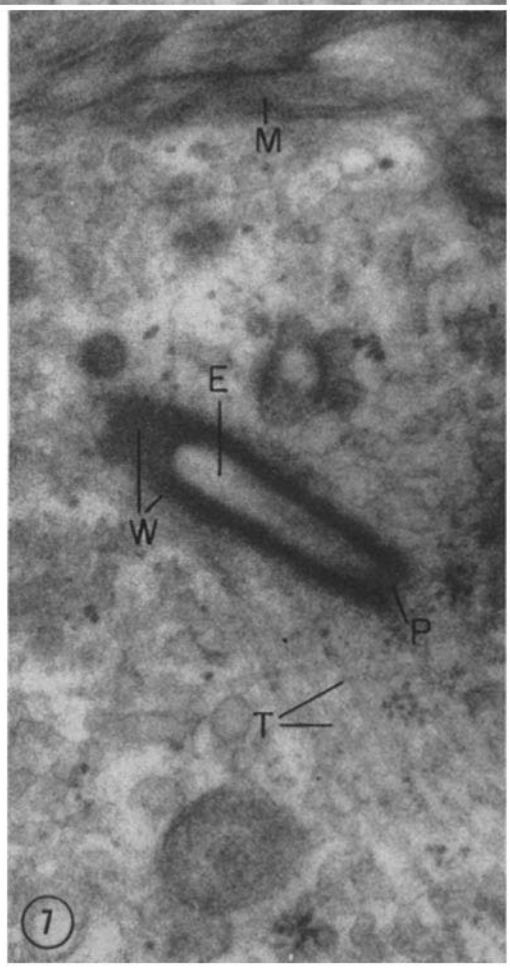
FIGURE 5 Oblique sections of organelles showing insertion of microtubules. Note beaded appearance of microtubule at *B*. × 56,000.

FIGURE 6 Organelles in cytoplasmic ridge. Note the deep crevasse bordering the ridge. × 56,000.

FIGURE 7 Near longitudinal section of organelle. × 56,000.







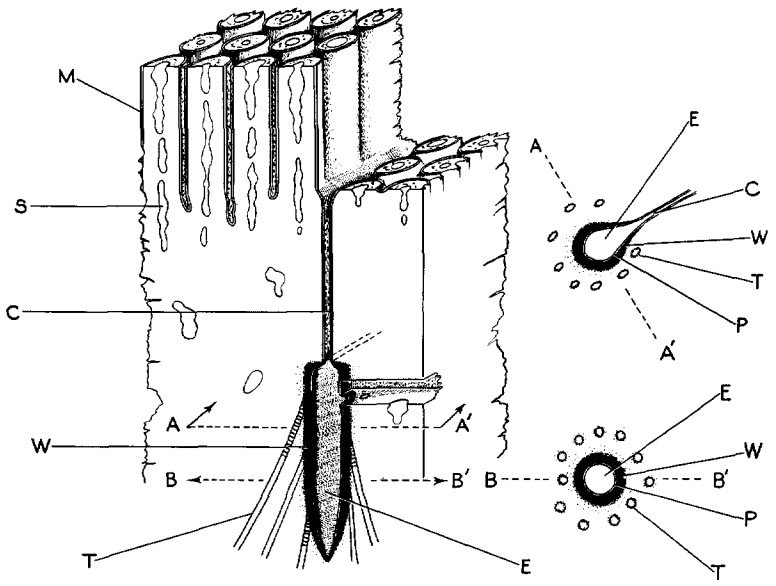


FIGURE 8 Schematic representation of relationships at the apical surface of a columnar cell.
Left—Three dimensional view and longitudinal section through organelle.
Upper right—Section in plane *A-A'*.
Lower right—Section in plane *B-B'*.

ences. The centriole in Fig. 2 is approximately 240 mμ in diameter or nearly twice as wide as the organelle in Fig. 7, but the organelle is as long, or longer than the centriole. In addition, intimate association between the centriole and the microtubules appears to be lacking, while the organelle is always accompanied by a halo of microtubules. Ninefold symmetry is missing in the organelle in cross-section, but has been seen in occasional centriolar cross-sections. There is no membranous inner wall in the centriole. Finally, no more than two centrioles appear to be present in any of the sections of cells we have examined, whereas there are numerous organelles to be seen.

The organelle has also been noticed in epidermal cells of the fourth abdominal sternite.

DISCUSSION

It is of interest in the present context that shape changes in insect epidermal cells have been correlated with changes in secretory activity (Wigglesworth, 1961; Kramer and Wigglesworth, 1950). Several structures that have previously been postulated to be involved in shape maintenance, shape change, or secretory function in a wide variety of cells have been found here in the columnar cells of the termite sternal gland. Among these structures are smooth, membrane-bound cisternae and arrays of microtubules. One special feature of the cisternae in these cells is the apparent penetration of the smooth reticulum some distance into the microvilli themselves. This would provide extensive close contact between the reticulum and the extracellular space and might facilitate bulk movements of material.

The arrays of microtubules are so numerous as to give the cells a striated appearance in light microscope preparations. Microtubules of varying sizes are a ubiquitous component of cells (*cf.* Slautterback, 1963). For example, tubules have been found in secretory cells of *Hydra* (cross-sectional diameter 18 mμ: Slautterback, 1963), in the spindle apparatus of amoeba (14 mμ: Roth and Daniels, 1961), in other animal cells (*e.g.*, 23 mμ: lamellibranch gill, Gibbons, 1961), and even in plant cells (23 to 27 mμ: Ledbetter and Porter, 1963). In two of the above cases, intimate associations between the tubules and basal bodies or centrioles have been recorded (Slautterback, 1963; Gibbons, 1961), but in the other cases, no centrioles are known in the cells. Slautterback (1963) has suggested that there are two classes of tubules based on size distinction. However, it is not yet known whether functional differences

correspond to size differences. The functions postulated for the microtubules include contraction (Ledbetter and Porter, 1963), sequestration and transport of ions or other materials, (Slautterback, 1963), or simply strengthening of the afibrillar protoplasm.

In the sternal gland cells, the size of the tubules is intermediate between Slautterback's classes, according to our calibrations. The function of the microtubular arrays is unknown, although the beaded appearance of certain tubules (Fig. 5) lends support to suggestions favoring a contractile or strengthening function. It would be of interest to study the development of the tubules, especially with regard to their numbers and disposition, during varying phases of secretory activity of the epidermis. We have focused attention on the apical insertion of the tubules, which is thus far unique to the termite; whether the organelle that we have described is, in fact, the morphogenetic origin of the tubules here is not known.

Differences in structure and distribution apparently preclude the possibility that the organelle is merely a stage in centriolar development. However, the centriole (basal body) is the only other apically located organelle that has been shown in other organisms to connect to both the microtubular element of the cytoplasm and the cell membrane.

Two other possibilities might be that (1) the organelle has a function in secretion, and (2) the organelle represents a shape-forming element of the cell membrane. The latter view requires that the crevasses be permanent pockets penetrating the apical cytoplasm. The osmiophilic outer boundary of the organelle might represent an accumulation of material such as is present on the inner surface of the cell membrane in desmosomes and similar structures.

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