

## HISTOLOGICAL AND ULTRASTRUCTURAL ASPECTS OF THE BRINDLEY'S GLANDS OF *PANSTRONGYLUS MEGISTUS* (BURMEISTER, 1835) (HEMIPTERA: REDUVIIDAE)

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*The Brindley's glands of Panstrongylus megistus were studied under the anatomic, histologic and ultrastructural point of view. These glands located in the insect's methatorax are paired and have an opening near the third pair of the feet. Beside this aperture, there are evaporation areas. Shape, size and aspect of the gland vary according to the feeding status. The glands are composed by a tubular part corresponding to the duct and a sack-like portion corresponding to the secretory part. By electron microscopy we observed that the basal part of the epithelium has many interdigitations associated with mitochondria. On the apical surface where epicuticular foldings are located an electronlucent space is often seen. The glands are composed of the following elements: 1) superficial epithelial cells, located just below the apical surface foldings; 2) secretory cells, which are long and have an intracellular canalicule which changes according to the functional state of the cell; 3) a collecting duct connected to the secretory cells and covered with an epicuticle, reaching up to the gland's lumen; and 4) cells around the duct.*

Key words: Brindley's glands – *Panstrongylus megistus* – Hemiptera – histology – ultrastructure

A pair of ventral scent-glands is usually found in the metathoracic region of Hemiptera. The Reduviidae family possesses in this region another pair of glands (Brindley, 1930) that release a defensive secretion with a characteristic odour whose major component was identified as iso-butyric acid (Pattenden & Staddon, 1972). These glands dorsally located open into the superior lateral region of the metathoracic epimeron near the third pair of legs. Their localization is in the metathoracic region although they may extend up to the abdominal cavity. Brindley (1930) and Kalin & Barret (1975) described those glands located in the second abdominal segment.

Previous works described histological and some ultrastructural aspects of the Brindley's glands. Kalin & Barret (1975) found in *Rhodnius prolixus* a glandular unit composed of a saccule, secretory apparatus and a duct similar

to that in the type "B" dermal gland described by Wigglesworth (1933). Schofield & Upton (1978) in *Panstrongylus megistus* showed that the gland is composed of two types of cells: the secretory and the epithelial cells. Lattely, Barret et al. (1979) also in *R. prolixus* confirmed the earlier data and reported the existence of another type of glandular unit named type "A", composed by the same elements of type "B" but differing in the structural aspect as in the shape of the secretory apparatus and in the curved structure of the saccule in contrasting with the round structure found in the type "B".

The results presented in this paper confirm some of the earlier observations and will give further ultrastructural details of the Brindley's gland.

### MATERIALS AND METHODS

Adults of *P. megistus* (Burm., 1835) were obtained from a colony maintained in the Anatomy and Histology Laboratory, Department of Entomology of the Instituto Oswaldo Cruz, Rio de Janeiro, RJ, Brazil.

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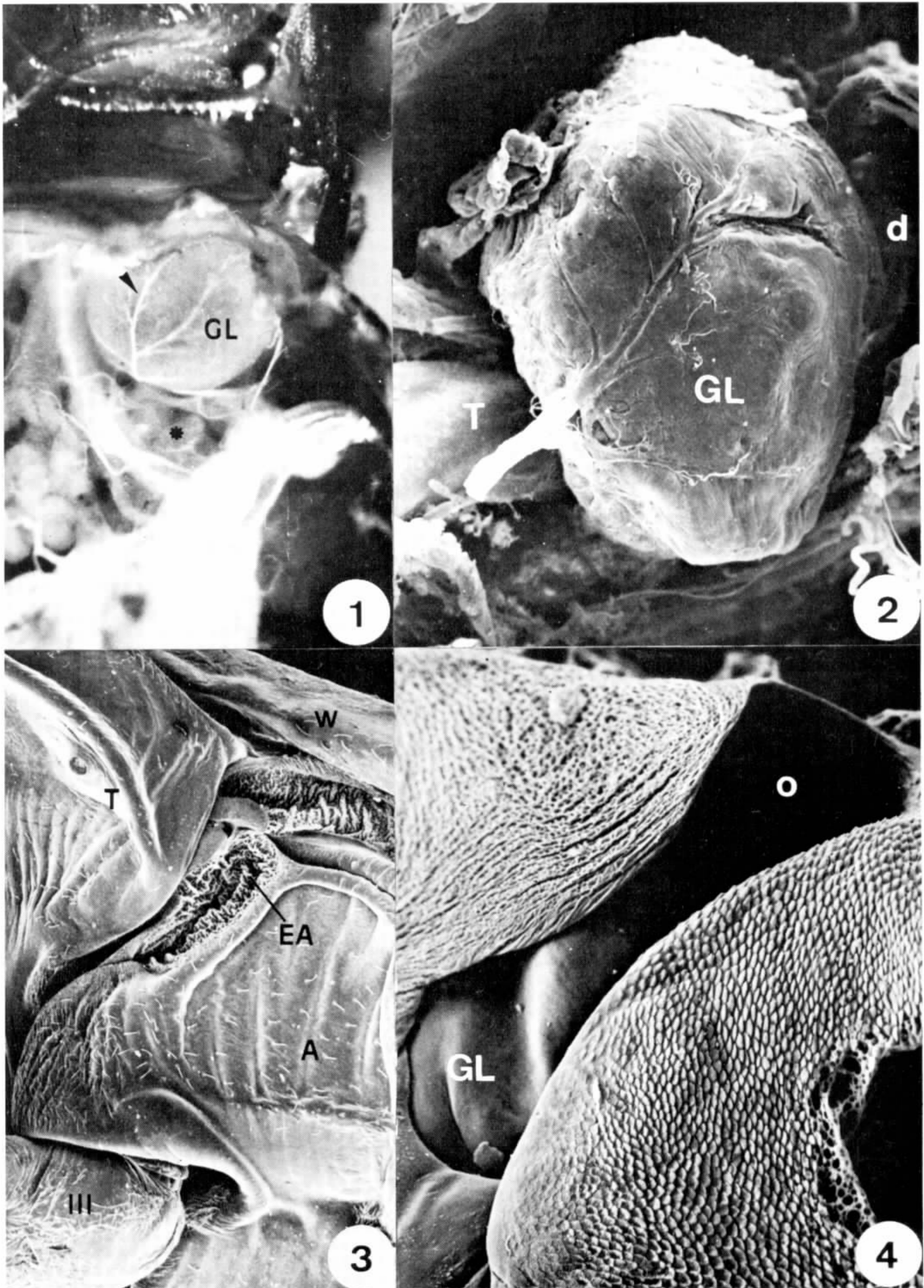


Fig. 1: after remotion of the tergites we can see the gland (GL) surrounded by tracheoles (arrowhead). X 12. Fig. 2: scanning electron micrograph showing the external part of the Brindley's gland (GL). We can observe the round proximal part and the short duct (d). The second thoracic branch of tracheole (T) spread out and divide in some tracheoles. X 100. Fig. 3: lateral view of the insect, in the region between the thorax (T) and abdomen (A). Near the intersegmental membrane (arrow) we can see the region of the opening of the gland (O) and the evaporation areas (EA). III – third pair of legs. X 20. Fig. 4: detail of the opening of the gland (O) that have a hole lock appearance. GL = Brindley's gland. X 200.

The insects were decapitated and dissected in insect's saline (0.7% NaCl + 0.3% KCl).

For light microscopy the glands were fixed in Bouin's solution or 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) for 1 h, dehydrated in graded ethanol and embedded either in paraffin or in glycol methacrylate (JB-4) (Bennett et al., 1976). Paraffin sections (3-5  $\mu$ m thick) were prepared with a Leitz microtome and JB-4 sections (1-2  $\mu$ m thick) with a Sorval JB-4A Porter Blum microtome and stained with haematoxylin-eosin or 1% Toluidine blue.

For scanning electron microscopy the thoracic region was dissected, the tergites removed and the glands were fixed *in situ* for 1 h with 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2. After washing in the same buffer the glands were post-fixed with 1% osmium tetroxide in 0.1 M phosphate buffer, dehydrated in graded ethanol, critical point dried using CO<sub>2</sub>, and coated with gold. The observations were made in a Jeol 25S II scanning electron microscope.

For transmission electron microscopy the glands were dissected and fixed for 1 h in a solution containing 2.5% glutaraldehyde, 4% paraformaldehyde and 0.5 mM CaCl<sub>2</sub> in 0.1 M cacodylate buffer, with 5% sucrose, pH 7.2, at room temperature. After fixation, they were washed in buffer, post fixed in 1% osmium tetroxide, 0.8% potassium ferricyanide, 0.5 mM CaCl<sub>2</sub> in 0.1 M cacodylate buffer, pH 7.2, for 1 h at room temperature, dehydrated in ethanol and embedded in Epon. Ultrathin sections were examined in a Jeol 100 CX electron microscope after staining with uranyl acetate and lead citrate (Reynolds, 1963).

## RESULTS

The Brindley's glands found in the metathorax of the reduviid insect *P. megistus* (Figs 1, 2) have their opening at the lateral region of metathoracic epimeron, near the third pair of legs (Fig. 3). Beside this aperture, which has the appearance of a lock (Fig. 4), there are rough areas on the tegumentar surface, with a spongy aspect, which corresponds to the evaporation areas (Fig. 5).

When the insects were frightened or manipulated during dissection the release of a volatile

secretion by only one of the glands was observed. After the dissection of the insect generally only one of the glands had been emptied. The insects do not present any smell when they are free in the breeding bottle. In insects observed 1 to 30 days after ecdyse no external morphological modifications of the glands were seen. On the other hand, shape, size and aspect of the glands vary according to the feeding status. In well-fed animals, they were of a hyaline aspect and had a round shape, extending into the abdominal cavity up to the second segment, whereas in starved animals the aspect was milky and shriveled and they were located just in the thorax.

The glands are composed of two parts, a tubular portion covered with a thick internal surface cuticle, corresponding to the duct, and a sack-like portion the internal surface of which is composed of several foldings covered with an epicuticle, corresponding to the secretory portion (Fig. 9). They are situated beside the postmesenteron, and their tubular part is covered by thoracic muscles.

Scanning electron microscopy showed that the gland is surrounded by a connective membrane which is penetrated by tracheoles from the second thoracic branch up to the epithelial cells (Fig. 6).

The examination of 1 to 5  $\mu$ m thick sections by light microscopy showed the presence of rounded condensed nuclei mainly located in the basal region of the cells, and two types of circular structures near by. One having a smooth surface was strongly stained by anionic dyes. In the other one the surface showed projections into the lumen. In the apical region, many infoldings were seen in contact with the glandular lumen (Figs 7, 8).

Electron microscopy allowed the observation of more details. The basal part of the epithelium had many interdigitations associated with mitochondria. Just below the epithelium there was a thin basal lamina (Figs 10, 11). The glands are composed of the following elements: (a) superficial epithelial cells; (b) secretory cell; (c) a collecting duct and (d) cells around the duct. The superficial epithelial cells were located in the apical region, giving a waved aspect to the lumen's surface. They were small cells with a voluminous nucleus and scarce cytoplasm, in which some secretory granules

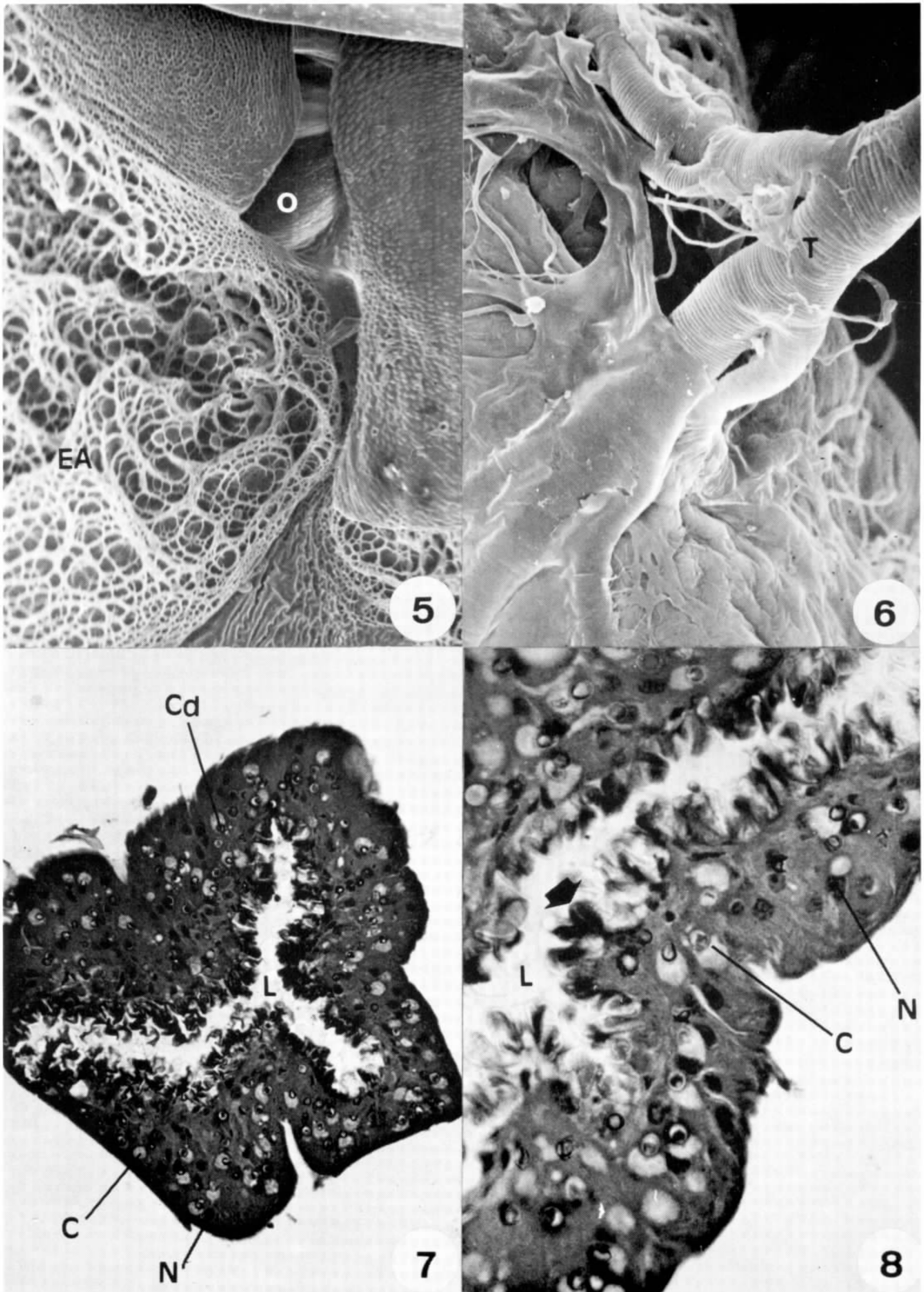


Fig. 5: evaporation areas (EA) with a spongy aspect located near the gland's opening (O). X 140. Fig. 6: second thoracic branch of tracheole (T) penetrating under the connective membrane that involves the gland. X 600. Fig. 7: transversal histological section of the Brindley's gland. N = nucleus; Cd = collecting duct; C = canalicule; L = glands' lumen. X 35. Fig. 8: detail of the glandular epithelium showing the epicuticular infoldings (arrow). N = nucleus; C = canalicule; L = lumen. X 140.

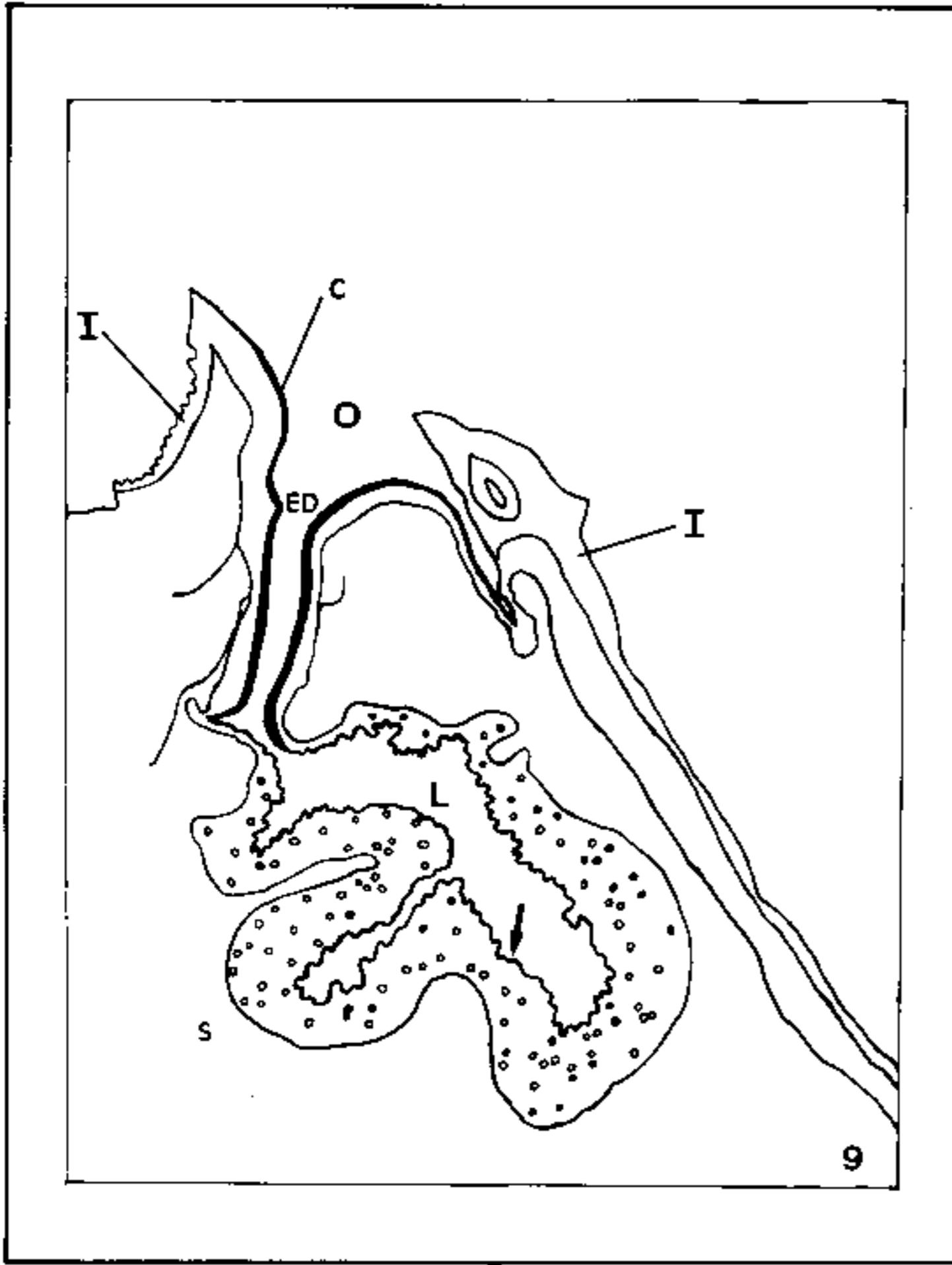


Fig. 9: scheme showing the secretory portion (S) and the excretory duct (ED). I = integument; C = cuticle; L = gland's lumen; O = gland's opening;  $\rightarrow$   $\hat{=}$  epicuticular infoldings.

and some mitochondria could be seen. They sometimes accompany the luminal infoldings. They are covered by an about  $1 \mu\text{m}$  thick epicuticle with a lamellar aspect, where at least four layers could be seen. Under the superficial epithelial cells there was an electronlucent space (subepithelial space) (Fig. 12). In some cases this space was not observed and the epicuticular surface was less folded (Fig. 13).

The secretory cells are long, frequently with a racket-like aspect, presenting an intracellular canalicule in its apical portion whose appearance varies. Sometimes the canalicule was empty with cytoplasmic projections into the lumen and electrondense cytoplasm containing many secretion granules (Fig. 14). In other cases the canalicule was with the membrane foldings turned to the cytoplasm and filled with secreted material (Figs. 15, 16). Frequently, we could also see the canalicule empty with no secreted material (Fig. 17).

Among the cells, we sometimes observed electrondense material along their whole length of the cells (Fig. 18) or in small points only (Fig. 19).

Five kinds of granules of different size, shape and electron density were seen within the secretory cells: (a) round or oval granules with smooth surface and medium electron density showing a maximum diameter of  $1.40 \mu\text{m}$ , and designated as type 1 (Fig. 20); (b) round granules with smooth surface, low electron density, with convoluted membranes inside them. Their maximum diameter was  $1.2 \mu\text{m}$ . They were designated as type 2 (Fig. 20); (c) round granules with a smooth surface and medium electron density, containing several spherical particles of about  $25\text{-}30 \text{ nm}$ . These granules had a maximum diameter of  $2.0 \mu\text{m}$  and were designated as type 3 (Figs 20, 22); (d) round or oval granules, with a smooth surface and electrondense content, with a maximum diameter of  $1.2 \mu\text{m}$ . They were designated as type 4; (e) small round or oval granules with a smooth surface, presenting a moderate electron density in one part and low electron density in the other part. These granules had a maximum diameter of  $2.2 \mu\text{m}$ , and were designated as type 5 (Fig. 21).

Frequently, we observed between the secretory cells small or large tracheole (Fig. 23).

Collecting ducts are connected to the secretory cells. They are covered with an epicuticle and open inside or near the canalicular aperture of the secretory cells (Fig. 24). Under this epicuticle we have seen periodically disposed "punctiform" material, like small fibrillae in transverse sections. Near the secretory cells this duct form a saccule while in the luminal epithelial surface its diameter is smaller and the duct shows a tortuous aspect. The collecting duct ends in the lumen's gland and we can observe the interruption of the epicuticular lining, which suggests that it is the point of the duct's opening (Fig. 25).

Around the duct, we could see narrow strips of cytoplasm more easily observed on the thinner part of the duct. They are not electrondense, and their nuclei are voluminous, similar to those found in the superficial epithelial cells (Fig. 26).

Based on the observations described above we concluded that the structural organization of the Brindley's glands in *P. megistus* is as shown in the scheme of the Fig. 27.

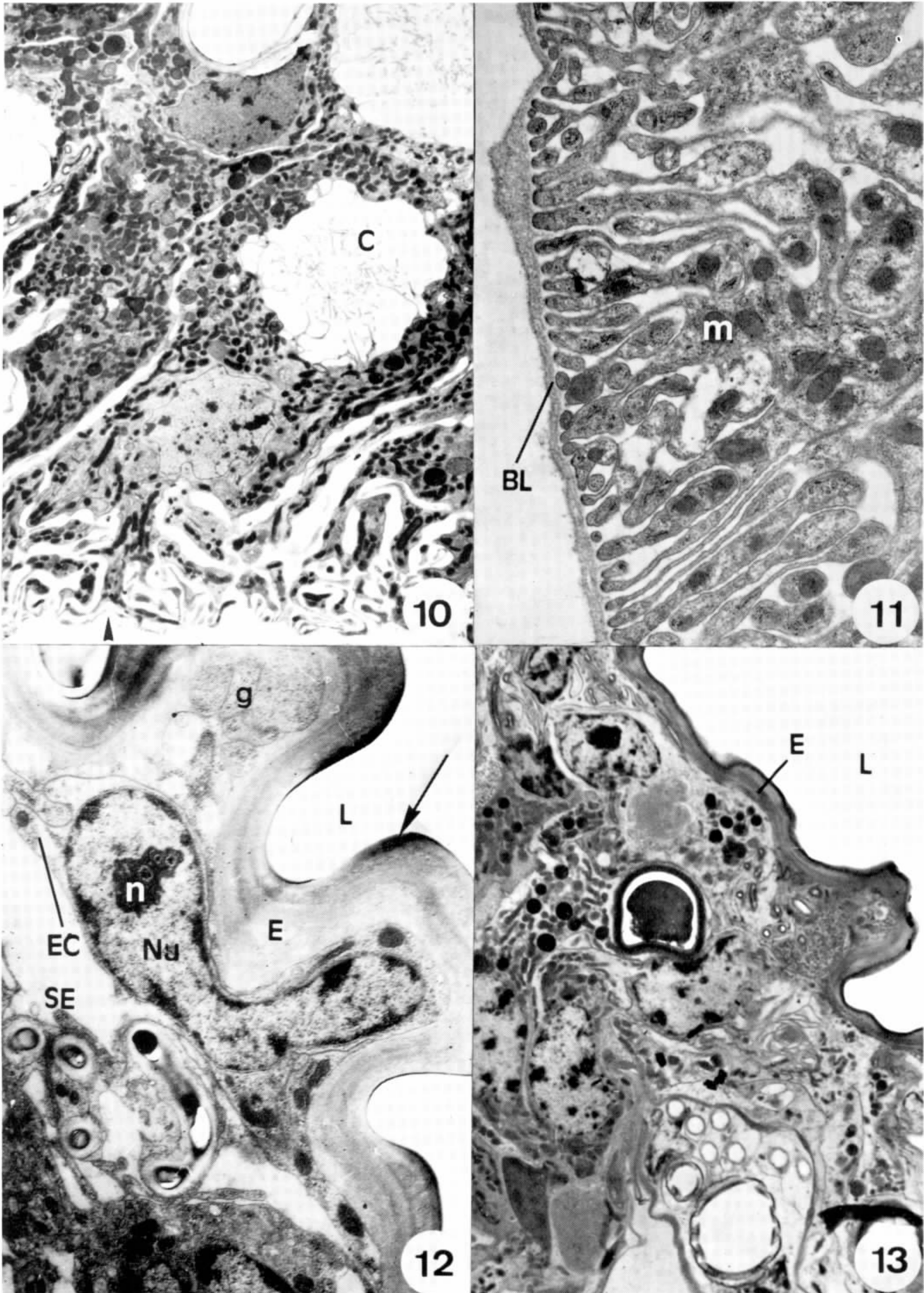


Fig. 10: general aspect of the secretory cells showing many interdigitations (arrowhead) between them. C = canalicule. X 3,500. Fig. 11: a thin basal lamina (BL) was located just below the epithelium. Mitochondria (m) could be seen in association with interdigitations. X 11,550. Fig. 12: electronmicrograph of the superficial epithelial region showing the infoldings (arrow) formed by epicuticle (E), the superficial epithelial cells (EC) and under that the subepithelial space (SE). Nu = nucleus; n = nucleole; L = lumen; g = secretion granules of the superficial subepithelial cells. X 8,800. Fig. 13: superficial region of the epithelium where we can see a less infolded epicuticle (E) and no subepithelial space. L = gland's lumen. X 3,400.

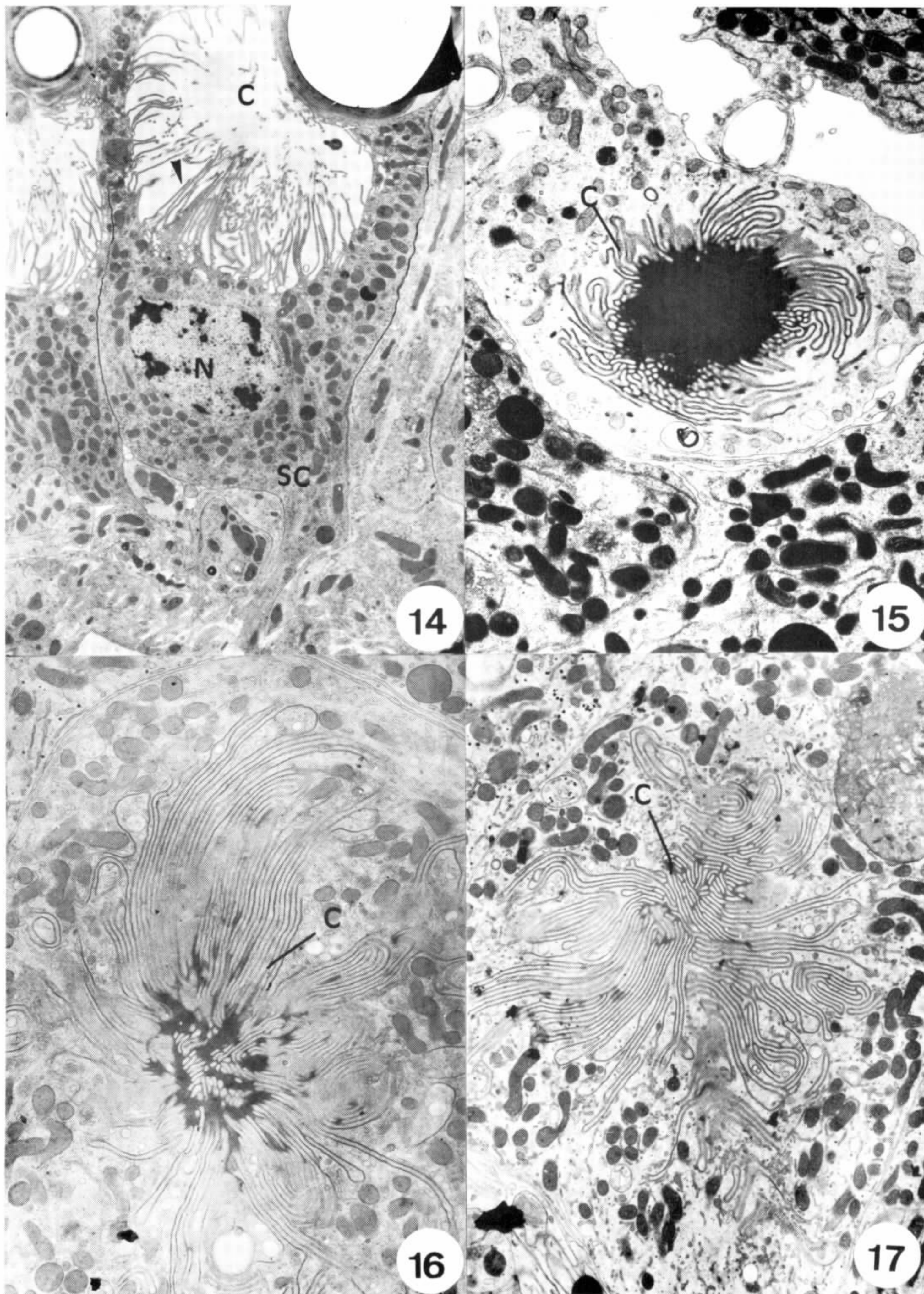


Fig. 14: the secretory cell (SC) with a racket-like aspect presenting an intracellular canalicule in its apical portion where cytoplasmic projections are seen (arrowhead). N = nucleus. X 4,300. Fig. 15: intracellular canalicule (C) showing membrane infoldings and the lumen filled with electron-dense secreted material. X 8,800. Fig. 16: canalicule (C) with few electron-dense material inside. X 9,300. Fig. 17: canalicule (C) with no secreted material. X. 7,000.

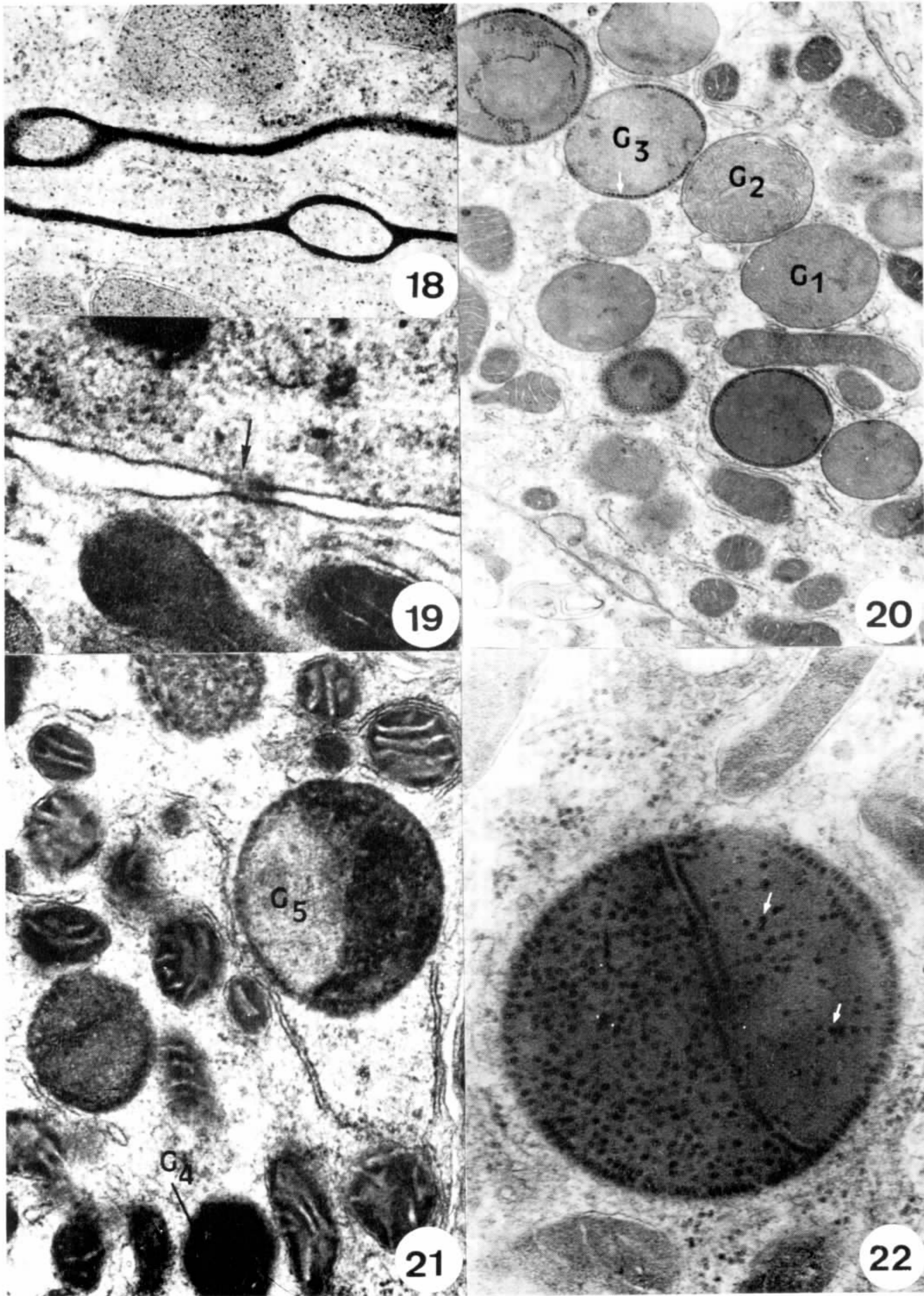


Fig. 18: electron-dense material frequently observed between the secretory cells. X 35,000. Fig. 19: area of adhesion (arrow) of two secretory cells. X 46,800. Fig. 20: different types of secretory granules observed in the secretory cells. G<sub>1</sub> = round granules with medium electron-density. G<sub>2</sub> = round granules with convoluted membranes inside. G<sub>3</sub> = round granules with medium electron-density and presenting spherical particles inside. X 18,200. Fig. 21: G<sub>4</sub> = round granules with electron-dense content. G<sub>5</sub> = mixed granules with one more electron-dense part and the other part with low electron-density. X 33,800. Fig. 22: detail of the type 3 granule (G<sub>3</sub>) showing the spherical particles inside (arrow). X 40,000.



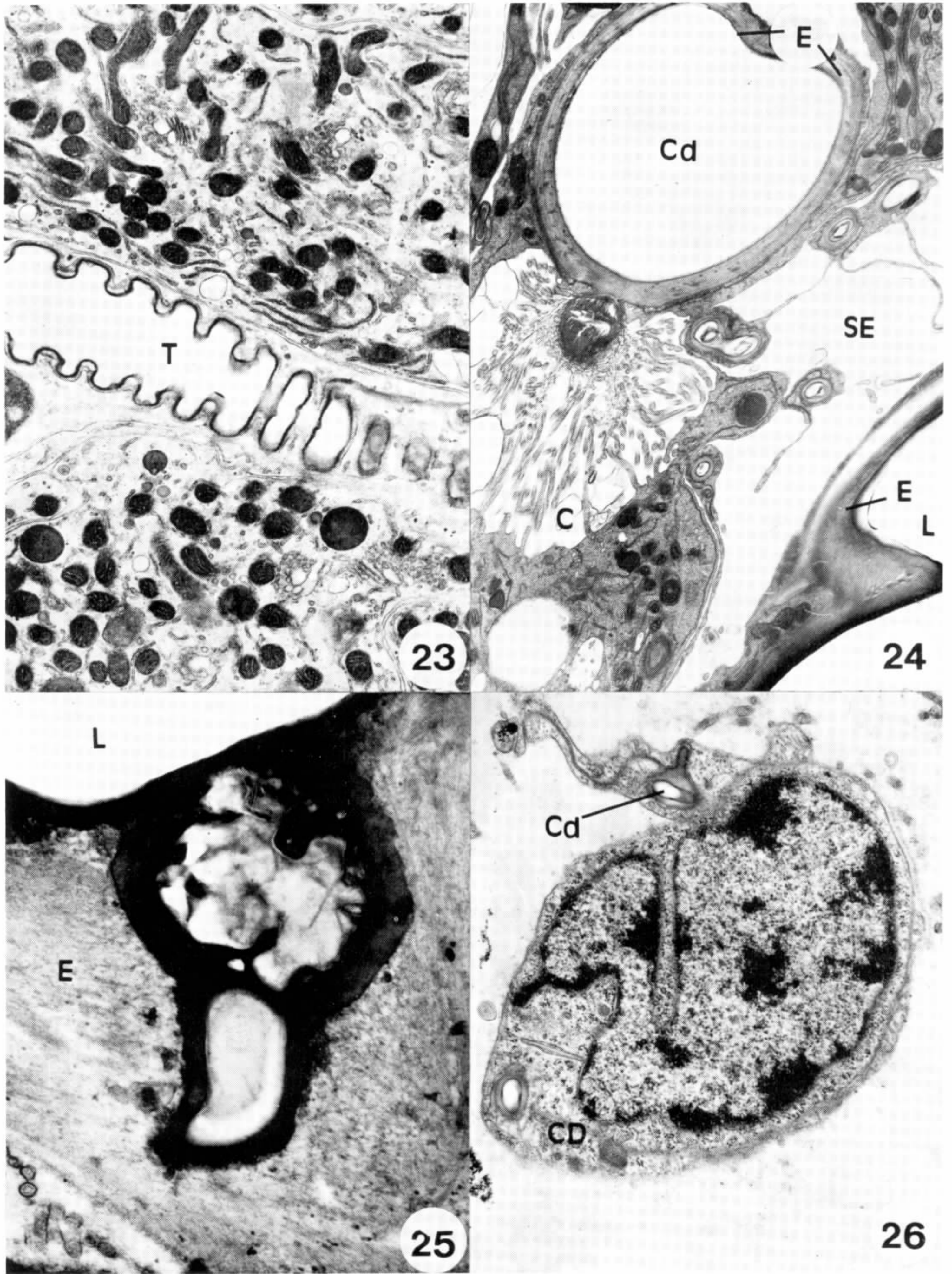


Fig. 23: tracheole (T) observed between the secretory cells. X 11,550. Fig. 24: collecting duct (Cd) covered with epicuticle (E) near the canalicule (C) of the secretory cell. SE = subepithelial space; L = gland's lumen. X 8,000. Fig. 25: opening of the collecting duct. E = epicuticle; L = lumen. X 18,200. Fig. 26: cell located around the collecting duct (CD) shows a voluminous nucleus. Cd = collecting duct. X 12,500.

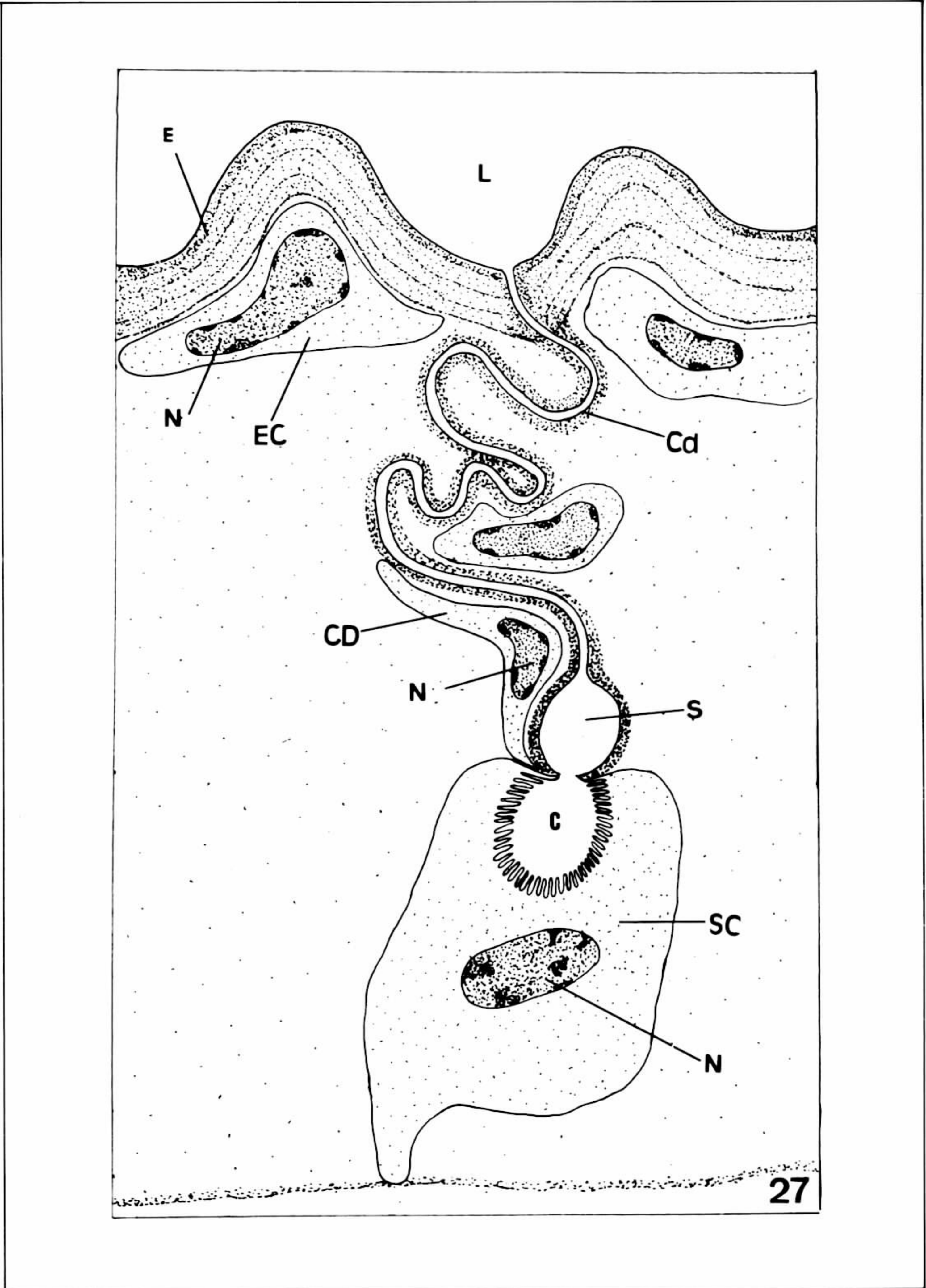


Fig. 27: general schematic view of the Brindley's gland showing its main components. E = epicuticle; L = gland's lumen; Cd = collecting duct; EC = epithelial cells; N = nucleus; S = sacule; SC = secretory cell; CD = cell located beside the collecting duct.

## DISCUSSION

The Brindley's glands are situated in the insect's metathoracic region. Brindley (1930) reported their localization in the 1<sup>st</sup> abdominal segment. Kalin & Barret (1975) described the glands dorsally located extending into the second abdominal segment. Schofield & Upton (1978) argued that the localization was in the metathorax, although they may extend up to the abdominal anterior part's cavity. Our observations are in agreement with these reported by the later authors. The fact that the gland sometime extends up to the abdominal cavity depends on the nutritional state of the insect. In well-fed insects the glands reached the second abdominal segment whereas in starved animals they were only in the thoracic region and have a shriveled aspect. However, Kalin & Barret (1975) did not observe any difference between insects unfed for 30-40 days and those fed 1 or 2 days before examination.

Puri (1924) and Weber (1930) considered that Brindley's glands were related to the sexual function because they were absent in nymphs. Carayon (1948) reported sexual dimorphism between glands of adults of certain families of the Hemiptera. No difference was observed in male and female and since the fluid is released when the insects are irritated not presenting any smell when they are free in the breeding bottle, suggests that the gland has a defensive function. In contrast to our data, Kalin & Barret (1975) reported that no release of secretion was observed during handling. The Brindley's glands in *R. prolixus* have no similar cuticular elevations observed in some Heteroptera (Remold, 1962; Filshie & Waterhouse, 1969; Carayon, 1971), but a sponge-like cuticular area found beneath the round opening of the glands on the methatoracic epimeron, a groove running parallel to the intersegmental junction and numerous slender, cuticular process (Kalin & Barret, 1975; Barret, 1981). In our observations we found a sponge-like cuticular area in *P. megistus* that after the releasing of the secretion were filled with liquid. Schofield & Upton (1978) also mentioned the existence of such areas although they did not study them in detail. Cuticular process in the intersegmental junction were no observed in *P. megistus*.

The presence of tracheoles involving the gland permits the supply of the oxygen necessary to their metabolism.

Many interdigitations were observed in the basal region of the epithelium, similar to those visualized in the salivary glands and the proximal tubules of vertebrate nephrons, as well as in salivary glands of *Calliphora* (Oschman & Berridge, 1970; Berridge & Prince, 1972; Gupta et al., 1978) in the apical membrane of globet cells of Lepidoptera (Anderson & Harvey, 1966), in the labial glands of adults of *Anthe-raea pernyi* (Kafatos, 1968), suggesting that water and ionic transport takes place in this gland.

Kalin & Barret (1975) described in *R. prolixus* a glandular unit composed of a secretory apparatus, saccule and duct, each one formed by one individual cell, similar to the type B unit found by Wigglesworth (1933) and Lai-Fook (1970) in the dermal glands of the same insect. Barret et al. (1979) confirmed the data mentioned above and reported the existence of another type of glandular unit named by them "type A". This unit was composed by the same elements of the type B but differing in some ultrastructural details, mainly the intracellular canalicule found in the secretory apparatus. According to Schofield & Upton (1978) in *P. megistus* the glands were composed by one glandular unit formed by two types of cells: the secretory and the epithelial cells. Our observations show that only one type of glandular units was evident, having a secretory cell with a collecting duct, an epithelial cell under the epicuticular surface and a cell around the duct.

The presence of two types of secretory cells mentioned by the other authors was based on morphological differences between the intracellular canalicule of type A and B cells. We suggest that this depends on the functional state of the cell. When the cell is in intense secretory activity it presents a canalicule with cytoplasmic projections into the lumen. On the other hand, when the cell is not functioning the canalicule showed the membrane foldings turned to the cytoplasm. We believe that the cell is stimulated and the secretion is introduced into the canalicule and passes through the collecting duct up to the glandular lumen where it is stored for future liberation.

The denominations "duct cell" and "saccule-cell" are not proper, because the duct and the saccule aren't inside one cell. There are cells beside them with the feature of the epithelial

cells. The sacculle corresponding to the initial portion of the collecting duct functioning as a reservoir.

Summarizing we suggest that the Brindley's gland is formed by one glandular unit composed by a secretory cell and a collecting duct that has a saccular portion and a tubular portion both with a cuticular lining produced by the epithelial cells located around them.

The fourth cells were not observed by us.

The cytoplasmic round particles found in the type 3 granules resemble virus particles. Particles like this were described in the Malpighian tubes (Dolder & Mello, 1978) and in salivary glands (Mello & Dias, 1981; Mello, 1983) of *Triatoma infestans*. However, at the present moment we can not be sure about the nature of this particles. Further testing is necessary to clarify.

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#### REFERENCES

- ANDERSON, E. & HARVEY, W. R., 1966. Active transport by the *Cecropia midgut*. II. Fine structure of the midgut epithelium. *J. Cell Biol.*, 31: 107-134.
- BARRET, F. M., 1981. The effect of a juvenile hormone mimic on development of the metathoracic release site of the Brindley's glands of *Rhodnius prolixus*. *Can. J. Zool.*, 59: 850-857.
- BARRET, F. M.; MILLEN, B. F. & LAI-FOOK, J., 1979. The Brindley's glands of *Rhodnius prolixus*. I. Structure of the mature gland. *Can. J. Zool.*, 57: 1109-1119.
- BENNETT, H. S.; WYRICK, A. D.; LEE, S. W. & McNEIL, J. H., 1976. Science and art in preparing tissues embedded in plastic for light microscopy, with special reference to glycol methacrylate, glass knives and simple strains. *Stain Technol.*, 51: 71-97.
- BERRIDGE, M. J. & PRINCE, W. T., 1972. Transepithelial potential changes during stimulation of isolated salivary glands with 5-hydroxytryptamine and cyclic AMP. *J. exp. Biol.*, 56: 139-153.
- BRINDLEY, M. D. H., 1930. On the metasternal scent glands of certain Heteroptera. *Trans. R. ent. Soc. Lond.*, 2: 199-208.
- CARAYON, J., 1948. Dimorphisme sexuel des glandes odorantes métathoraciques chez quelques Hémiptères. *C. r. Acad. Sci. Paris*, 227: 303-305.
- CARAYON, J., 1971. Notes of documents sur l'appareil odorant métathoracique des hémiptères. *Annls. Soc. ent. Fr. (N. S.)*, 7(4): 737-770.
- DOLDER, H. & MELLO, M. L. S., 1978. Virus-like particles in the Malpighian tubes of blood-sucking hemipterans. *Cell Mol. Biol.*, 23: 299-310.
- FILSHIE, B. K. & WATERHOUSE, D. F., 1969. The structure and the development of a surface pattern on the cuticle of the green vegetable bug *Nezara viridula*. *Tissue Cell*, 1: 367-385.
- GUPTA, B. L.; BERRIDGE, M. J.; HALL, T. A. & MORETON, R. B., 1978. Electron microprobe and ion-selective microelectrode studies of fluid secretion in the salivary glands of *Calliphora*. *J. exp. Biol.*, 72: 261-284.
- KAFATOS, F. C., 1968. The labial gland: a salt-secreting organ of saturniid moths. *J. exp. Biol.*, 48: 435-453.
- KALIN, M. & BARRET, F. M., 1975. Observations on the anatomy, histology, release site, and function of Brindley's glands in the blood-sucking bug, *Rhodnius prolixus* (Heteroptera: Reduviidae). *Ann. ent. Soc. Am.*, 68: 126-134.
- LAI-FOOK, J., 1970. The fine structure of developing type B dermal glands in *Rhodnius prolixus*. *Tissue Cell*, 2: 119-138.
- MELLO, M. L. S., 1983. Cytoplasmic inclusions in virus-infected salivary glands cell of *Triatoma infestans*. *Rev. bras. Biol.*, 43: 251-256.
- MELLO, M. L. S. & DIAS, C. A., 1981. Detection of viral particles in the salivary glands of the Hemipteran, *Triatoma infestans* Klug. *Cell Molec. Biol.*, 23: 299-310.
- OSCHMAN, J. L. & BERRIDGE, M. J., 1970. Structural and functional aspects of salivary fluid secretion in *Calliphora*. *Tissue Cell*, 2: 281-310.
- PATTENDEN, G. & STADDON, B. W., 1972. Identification of isobutyric acid in secretion from Brindley's scent glands in *Rhodnius prolixus*. *Ann. ent. Soc. Am.*, 65: 1240-1241.
- PURI, I. M., 1924. Studies on the anatomy of *Cimex lectularius*. II. The stink organs. *Parasitology*, 16: 268-278.
- REMOLD, H., 1963. Scent-glands of land-bugs, their physiology and biological function. *Nature*, 198: 764-768.
- REYNOLDS, E. S., 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J. Cell Biol.*, 17: 208-212.
- SCHOFIELD, C. J. & UPTON, C. P., 1978. Brindley's scent-glands and the metasternal scent-glands of *Panstrongylus megistus* (Hemiptera, Reduviidae, Triatominae). *Rev. Bras. Biol.*, 38: 665-678.
- WEBER, H., 1930. *Biologie der Hemiptern*. Un vol. Springer Berlin, 543 p.
- WIGGLESWORTH, V. B., 1933. The physiology of the cuticle and of ecdysis in *Rhodnius prolixus* (Triatomidae, Hemiptera), with special reference to the function of the oenocytes and of the dermal glands. *Q. H. microsc. Sci.*, 76: 269-319.