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FINE STRUCTURE OF THE PYGIDIAL GLANDS OF *BLEDIUS MANDIBULARIS* (COLEOPTERA : STAPHYLINIDAE)

ABSTRACT. The pygidial glands of *B. mandibularis* produce a mixture of terpenes, fatty acid derivatives, and a benzoquinone. The morphology of these glands is described with particular attention to the ultrastructure of the secretory cells and their efferent ductules. Each functional secretory unit consists of two secretory cells (cortical and medullary) both of which are associated with a common extracellular cuticular ductule. The fenestrated tip of the ductule lies in a cavity bounded by the invaginated plasma membrane of the cortical cell; within the cavity surrounded by the medullary cell, the ductule is divided into a bulb region (where a spherical mass of fine cylinders surrounds the ductule itself) and an unfenestrated switchback region. Inflated cisternae of rough endoplasmic reticulum, filled with flocculent material of low electron density, are abundant in the cortical cytoplasm, and presumably represent primary secretory product *en route* to the cavity of this cell. The plasma membrane bounding this cavity is much infolded, and the inner surface of this membrane is studded with fine particles. In contrast, few cisternae are inflated in the medullary cell and the corresponding infolded plasma membrane is smooth. The manner in which both cells may cooperate to produce the heterogeneous secretory product is discussed.

Introduction

THE insect integument is both a functional skeleton and a barrier to free exchange of molecules between internal and external milieux. Yet this integument is heterogeneous, and among the more interesting cuticular specializations are those which facilitate entry or egress of particular molecules and thus allow the permeability barriers to be selectively breached. The best described modifications for entry of molecules are those found in insect chemoreceptors, for example the elaborate 'kettles' described by Ernst (1969) in the antennal receptors of *Necrophorus*. Adaptations for egress of glandular products are diverse, ranging from percolation through hypertrophied

pore canals in the wax glands of the honeybee (Locke, 1961) to flow via 'special plumbing'—fine ductules which begin near a secretory cell and traverse the overlying cuticular sheet (see Lai-Fook (1970) and Happ *et al.* (1971) for reference to some of the many examples.) The present paper describes a particularly complex integumental secretory system in the pygidial gland of a staphylinid beetle (*Bledius mandibularis*).

The genus *Bledius* includes many species of sub-social beetles which live in dense colonies in salty areas (and even within the tidal zone) along the European (BroLarsen, 1952) and American coasts of the North Atlantic. When disturbed, the adults bend the tips of their abdomens forward and emit the pygidial gland secretion. The pygidial secretion of *B. mandibularis* (U.S.) and *B. spectabilis* (European) contains five components: two terpenes (geranial and neral), methyl-*p*-benzoquinone, γ -dodecalactone, and 1-undecene (Wheeler *et al.*, 1972). Terpenes have been reported in the pygidial secretions of other staphylinids. In *Stenus*

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the secretion contains 1-8 cineole, *isopiperitenol*, and methyl-heptenone (Schildknecht, 1970) while in *Staphylinus*, the cyclopentanoid monoterpene iridodial is a principal product (Abou-Donia *et al.*, 1971). In all three genera the secretions appear to facilitate escape from predators. In *Stenus*, the pygidial product promotes rapid movement across the surface of a pond (*Entspannungschwimmen*, see Linsenmair, 1963) and the reactive natures of the products in *Staphylinus* and *Bledius* argue strongly for repellent roles.

In order to reach the body surface (or a reservoir), the products of the secretory cells must traverse a cuticular barrier. In those staphylinid glands which have been described previously (for example, see Leydig, 1859; Dierckx, 1899, 1901; Jenkins, 1957; Pasteels, 1968; Pasteels and Kistner, 1971), the overlying cuticle is appropriately specialized. By far the most common specialization is an efferent cuticular ductule which arises within an enclosed cavity, formed by invagination of the plasma membrane of a single secretory cell. Since there is a one-to-one correspondence between secretory cells and ductules, the products of all such cells are exported in parallel. In the present paper, we describe the ultrastructure of a staphylinid integumentary gland. In this pygidial gland of *Bledius*, the secretory system is rather complex; each independent secretory unit consists of two secretory cells, arranged in series along a common efferent ductule. With the notable exception of the defensive glands of a tenebrionid beetle (Roth, 1943; Palm, 1946; Eisner *et al.*, 1964), such cytoarchitectural complexity is rare in insect glands.

Materials and Methods

Adults of *Bledius mandibularis* were excavated from their burrows in the sandy salt flats south of Dewey Beach, Delaware. For light histology, the abdomens were removed, fixed in alcoholic Bouin's, embedded in paraffin, and stained with Heidenhain's iron haematoxylin or Mallory's trichrome.

For electron microscopy, the abdomens were immersed in cold fixative and the individual glands dissected free. Fixation was in glutaraldehyde (5% in 0.1 M phosphate buffer) pH 7.4 at 0-4°C for 2 to 6 hr,

followed by washing in the same buffer with 10% sucrose added for 1 hr (Locke, 1966) with post-fixation for 1 hr in 1% osmium, buffered with phosphate (0.1 M, 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 min with saturated uranyl acetate in ethanol-methanol (equal parts 70% ethanol and absolute methanol), followed by 5 min in lead citrate (Reynolds, 1963). The electron micrographs were taken on an Akashi Tronscope, an RCA EMU-2E and an AEI EM6B.

Observations

Each of the paired pygidial glands of *Bledius mandibularis* consists of three distinct parts: a *secretory cell mass* linked by an efferent cuticular duct to an *intermediate tubular chamber* from which a duct runs to the lightly muscled *storage reservoir* (Figs. 1, 2). When the muscles of the reservoir contract, the product is expelled through a thick exit duct that opens dorso-laterally near the tip of the abdomen. Unlike the pygidial glands of many other staphylinids (e.g. *Stenus* as described by Linsenmair (1963) and *Dianous* as described by Jenkins (1957)), the reservoir itself is not evaginated when secretion is released. The present report concerns primarily the cytoarchitecture of the cells in the secretory mass.

The secretory cell mass contains several hundred secretory units; each unit consists of two secretory cells drained by a common fine cuticular ductule. Within each unit the secretory cells are of two types, differing in position, nuclear morphology, and the enclosed central cavity. The nucleus of the more cortical cell type is rounded and usually contains a single prominent nucleolus, while the oval nucleus of the more medullary cell type is characterized by small scattered masses of chromatin, many of which are near the nuclear margin (Fig. 3). The cavity surrounded by the cortical cell is elongate, stains only lightly with toluidine blue (Fig. 3), and does not persist through the manipulations necessary for fresh squash preparations (Figs. 4, 5). In contrast, the cavity enclosed by the medullary cell is ovoid, has a high

affinity for toluidine blue (Fig. 3), and is quite prominent in fresh squash preparations (Figs. 4, 5).

The fine cuticular ductule which drains each unit arises in the cavity of the cortical cell. Within this cavity, the cylindrical tip of the ductule is either straight or gently curved, but as it runs into the cavity of the medullary cell, the ductule appears to thicken, forming a sort of bulb, and then in the proximal portion of the medullary cavity, the ductule loops back and forth in switch-back fashion (Fig. 5). The latter two regions have differing affinities for stains: the bulb stains red with Mallory's and dense black with Heidenhain's while the switchback is either blue (Mallory's) or a pale gray (Heidenhain's). As it leaves the cavity surrounded by the medullary cell, the ductule appears 'beaded' in both fresh phase and toluidine blue preparations (Figs. 3, 5). Ten to twenty such ductules converge on a secondary branch of the main efferent duct which drains the entire mass of secretory units (Figs. 4, 6, 7).

Lower power electron micrographs (Fig. 8) confirm the interpretation of cellular relationships derived from the light micrographs. In addition, they show that the secretory cell mass is invested with a basement membrane which also separates the cortical cells from one another. Tracheoles are embedded in this basement membrane. The major features of the secretory units are indicated diagrammatically in Fig. 24.

The cortical cell

The cytoplasm of the cortical cell is packed with irregular cisternae which enclose loose flocculent material of quite low electron density (Figs. 8, 9, 10). Ribosomes are attached to the cisternae at various points, and some ribosomes are scattered in the cytoplasmic matrix (Fig. 10). A variety of dense bodies (some showing internal structure) and a few microtubules are present. Each cell contains several Golgi zones with stacked cisternae and numerous dense microvesicles (Fig. 10). Mitochondria are elongate and small ($1 \mu \times 200 \text{ m}\mu$) and these organelles tend to be clustered near the invaginated plasma membrane which defines the central cavity (Fig. 11).

The central cavity is surrounded by tightly packed microvilli, about 1μ in length

and tapering to *ca.* 0.15μ in diameter at their apices. The plasma membranes bounding adjacent microvilli are separated by no more than 200 \AA except at the bases of the villi, where the extracellular space between them is inflated into a small sac (Fig. 11). The interior of each microvillus is packed with microfilaments, and on the cytoplasmic surface of the plasma membrane is a coat of small particles (Fig. 12). Each particle is about 90 \AA in diameter and is apparently joined to the membrane by a fine stalk, about 150 \AA in length. Adjacent particles are 200 \AA apart. A dense fibrous material, presumably glycocalyx, is found at the apices of the microvilli and within the narrow extracellular channels between them (Figs. 12, 13).

The tip of the efferent ductule lies within the central cavity. In its more distal regions, this ductule tip is not a simple, regular, hollow cylinder, but is rather like a shaggy finger of coarsely porous cuticle. Irregular spike-like projections coat the surface, and between fenestrations in the wall, broader, roughly spherical projections intrude into the lumen (Figs. 11, 13, 14, 24).

An evagination of the cortical cell surrounds the ductule as it runs toward the cavity of the medullary cell, and at this level, the lumen of the ductule is clear of internal projections. Within this evagination, the microvilli are more widely spaced (Fig. 15). The two cells are linked by septate desmosomes.

The medullary cell

The cytomembrane system of the medullary cell is quite different from that of the cortical cell. Small, almost tubular, profiles of the endoplasmic reticulum are sparsely distributed in the peripheral cytoplasm and are rather more numerous near the margins of the central cavity, often impinging on the infoldings of the plasma membrane at the bases of the microvilli (Fig. 8, 16, 18). Although the villi do contain fine filaments, a portion of endoplasmic reticulum often runs down the axis of a villus. Unlike the analogous membrane of the cortical cell, which is studded with particles, the inner surface of the plasma membrane bounding the cavity is smooth (Fig. 17).

Within the cytoplasm are Golgi regions, a variety of dense bodies, scattered micro-

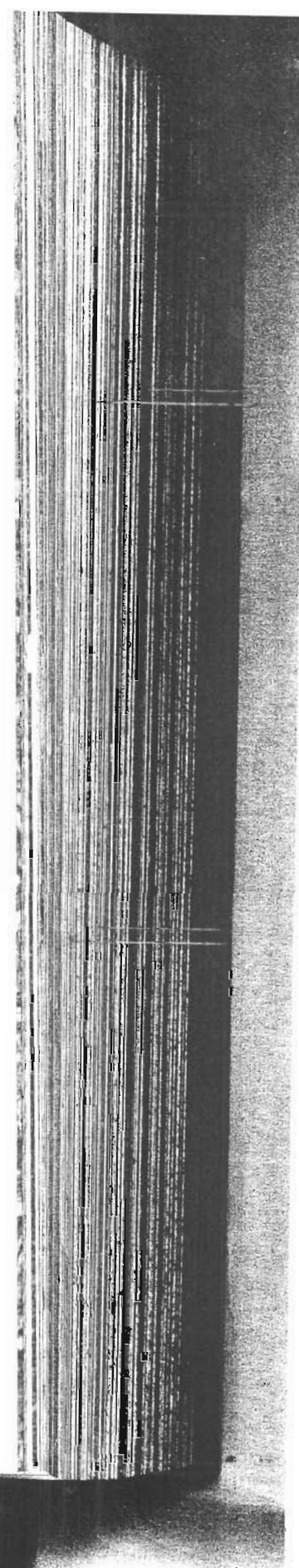


Fig. 1. The abdomen of *B. mandibularis*. Terga and most of the viscera have been removed to expose the two pygidial glands. Sm, secretory mass; R, reservoir. $\times 25$.

Fig. 2. A single pygidial gland viewed in darkfield. SM, secretory mass; I, intermediate tubular mass; R, reservoir. $\times 80$.

Fig. 3. A section through the secretory cell mass. The layer of cortical cells, with their characteristic nuclei (N_c) and cavities (C_c) are seen at the top of the photograph. A number of nuclei (N_m) and cavities (C_m) of medullary cells are visible around the cluster of efferent ductules (d) seen in the lower center. Glutaraldehyde-osmium, toluidine blue. $\times 1000$.

Fig. 4. A fresh squash preparation of the secretory mass viewed in phase contrast. Cavities of the medullary cells (C_m) are abundant. The efferent ductules (d) from these cavities converge at the lower right. $\times 500$.

Fig. 5. The apical regions of the efferent ductule are divisible into a switchback (s) and bulb (b) which lie within the cavity of the medullary cell and the tip (t) which projects into the cortical cell layer. Fresh squash preparation, phase contrast. $\times 1800$.

Fig. 6. A portion of the secretory mass viewed in darkfield. The fine cuticular ductules which drain the secretory units converge into a secondary duct (D_2) and the secondary ducts join the primary duct (D_1). Fresh squash preparation. $\times 400$.

Fig. 7. A phase-contrast view of the ductules (d) converging upon a secondary duct (D_2). Note the 'beaded' appearance of the ductules. Fresh squash preparation. $\times 900$.

Fig. 8. A low power electron micrograph showing both types of secretory cells. A portion of the basement membrane (bm) is seen between the two cortical cells present at the top of the field; both cells contain the converging microvilli which bound their central cavities (C_c). The medullary cell filling most of the field has a prominent central cavity (C_m) and contains relatively sparse endoplasmic reticulum. A variety of dense bodies (db) and mitochondria (m) are present in this cell. $\times 10,200$.

Fig. 9. A cortical cell occupies most of the field and the edge of a medullary cell is seen at the lower left. The two cells are separated by a basement membrane in which lies a tracheole (tr). The nucleus of the cortical cell (N_c) contains a prominent central nucleolus. In the surrounding cytoplasm are Golgi zones (G) and many inflated cisternae containing flocculent material. $\times 11,200$.

Fig. 10. The cortical cytoplasm. Two sections through a Golgi zone show a number of small dense vesicles (arrows) and inflated cisternae of very low electron density (small asterisks). In comparison, the contents of the inflated cisternae of the rough endoplasmic reticulum (large asterisk) are of moderate density. $\times 29,000$.

Fig. 11. A transverse section through the tip of the efferent ductule (t) and the surrounding cortical cell. The inflated cisternae of the endoplasmic reticulum (asterisks) are common in this area, and certain of these cisternae appear to contact directly the infolded plasma membranes (vertical arrow). At their origins, the infoldings between the irregular microvilli are often slightly inflated (horizontal arrowheads). $\times 24,600$.

Fig. 12. An enlargement of the outlined portion of Fig. 11. Periodicity of opposed plasma membranes (hollow arrow) apparently stems from the presence of attached particles, roughly spherical in cross-section (solid arrow). $\times 81,400$.

Fig. 13. A longitudinal section through the tip of the ductule. Toward its apex (left) the dense deposits almost occlude the lumen whereas toward the medullary cell, there is more space within the ductule. $\times 24,300$.

Fig. 14. An oblique section through the tip of the efferent ductule showing the irregular outline of the wall, the irregular projections into the lumen (asterisks), and the fenestrations in the wall (arrow). No cuticulin layer is present in the ductule. $\times 72,200$.

Fig. 15. Enclosed within a tongue of cortical cytoplasm (C_c), the tip of the ductule approaches the cavity bounded by the medullary cell. Projections in the lumen of the ductule are less prominent than in Figs. 11, 13, 14, and the apparent free space within the surrounding cavity is greater. Medullary and cortical cells are linked by septate desmosomes (arrow). $\times 35,000$.

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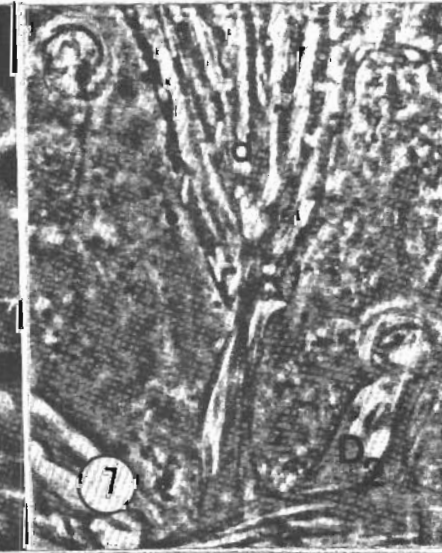
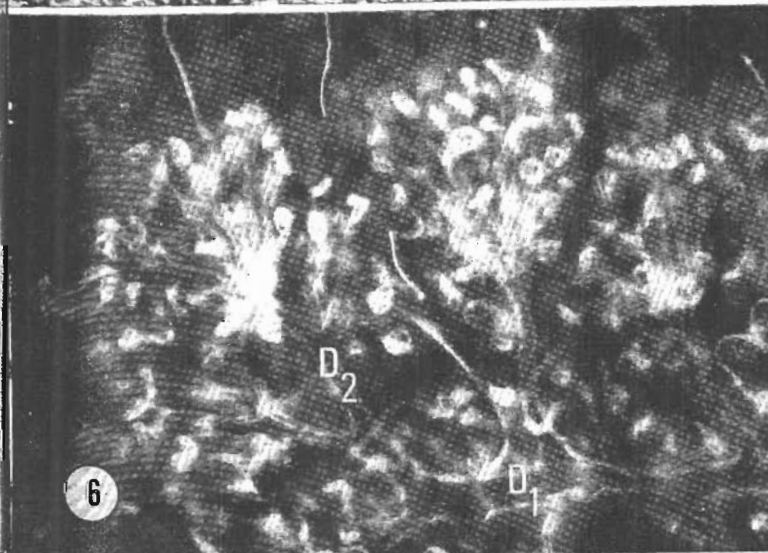
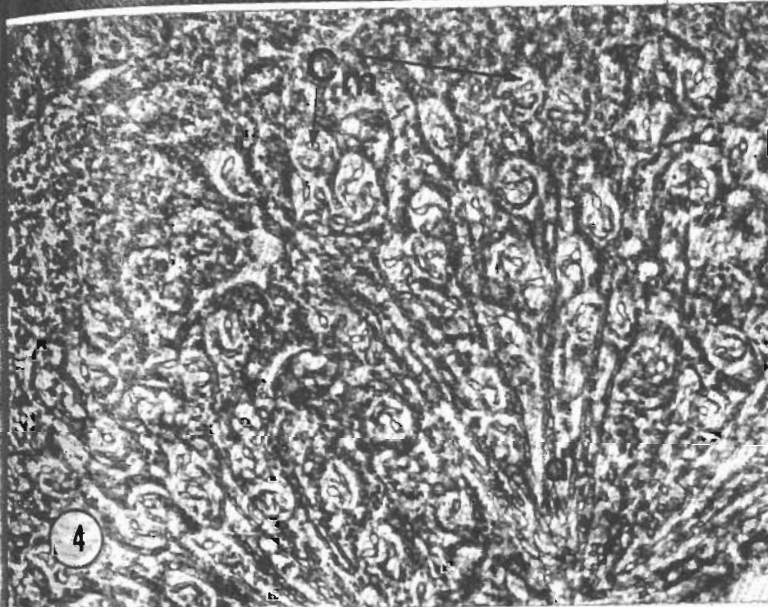
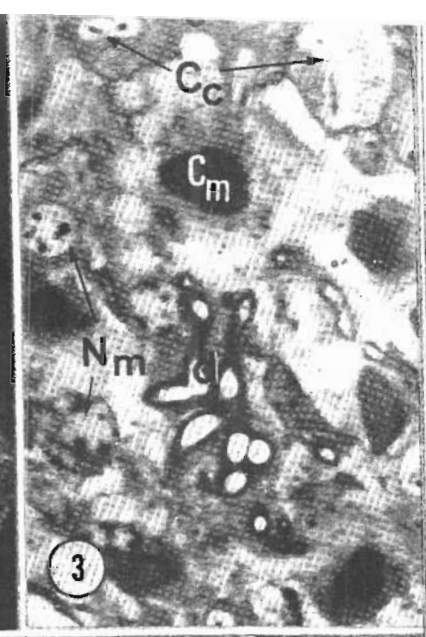
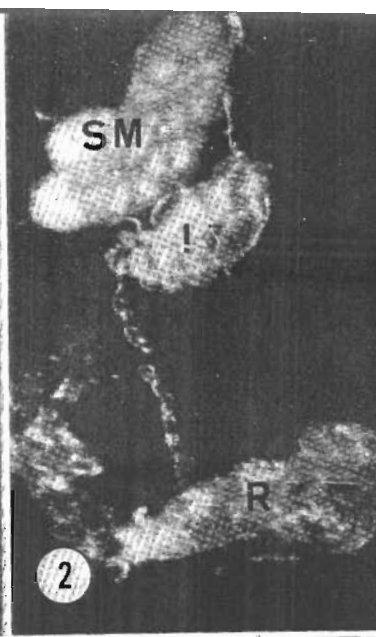
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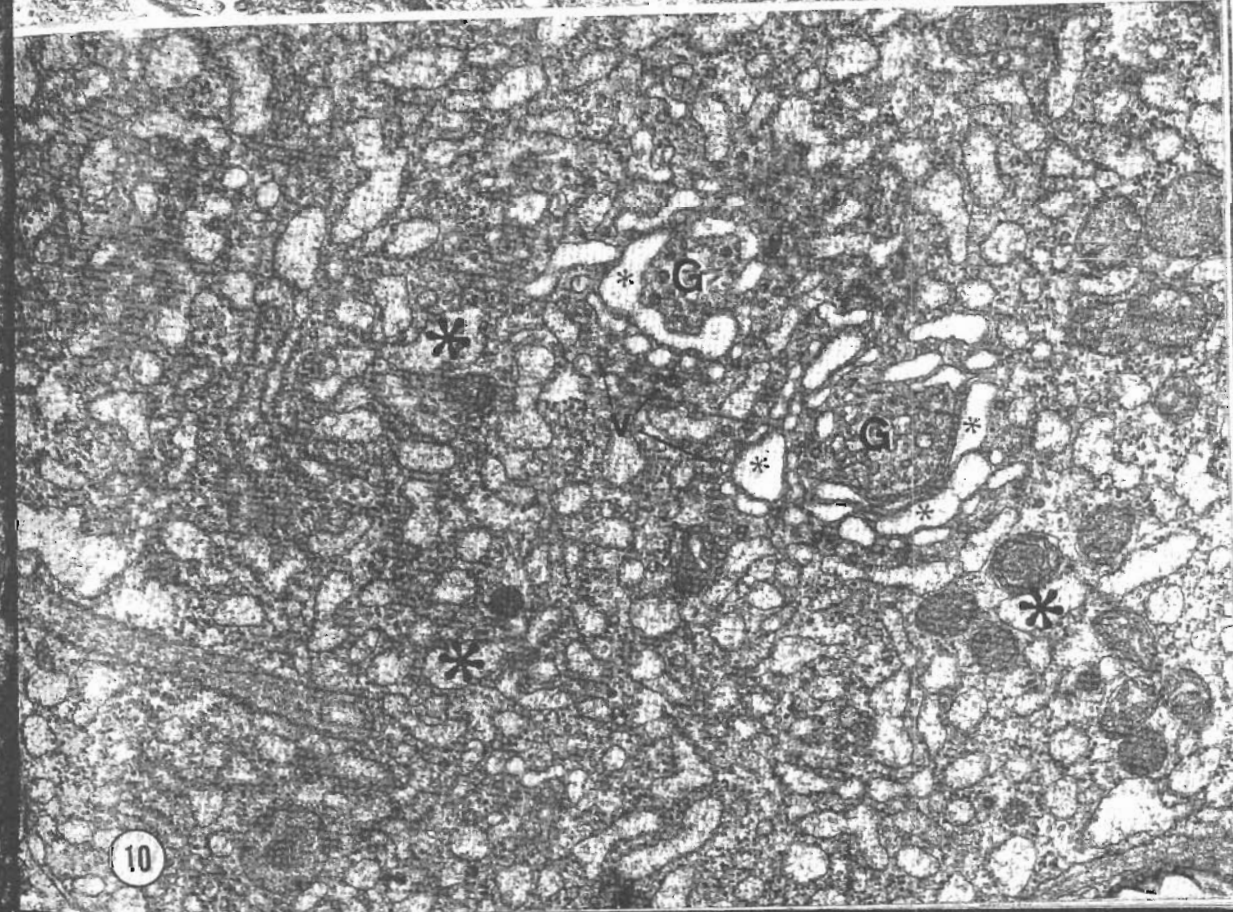
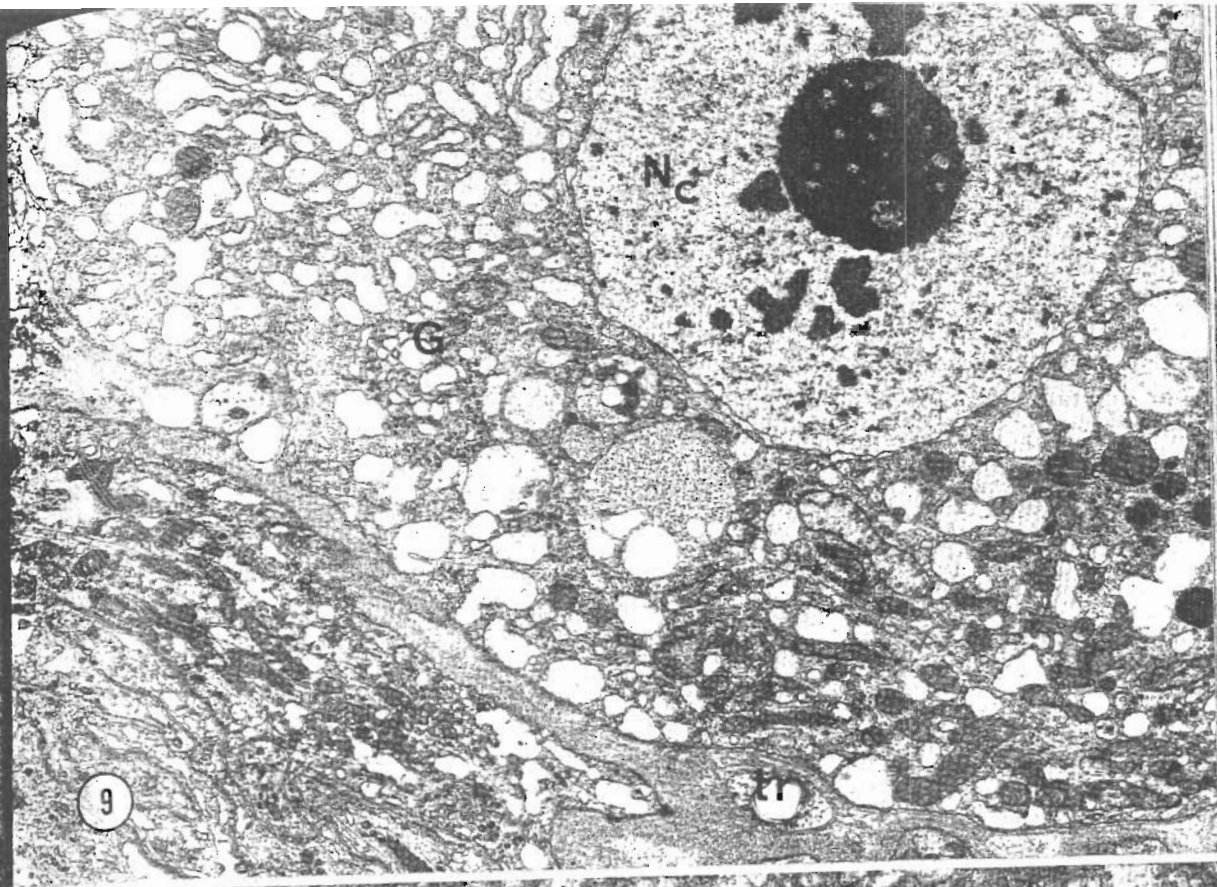
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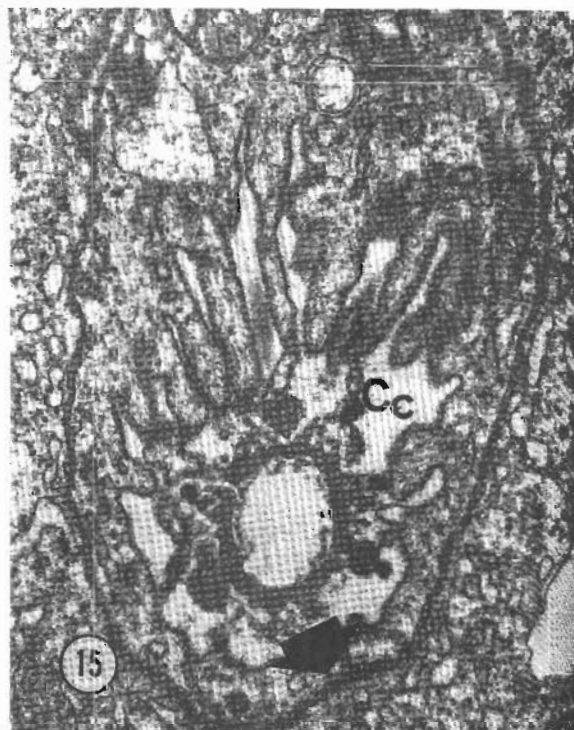
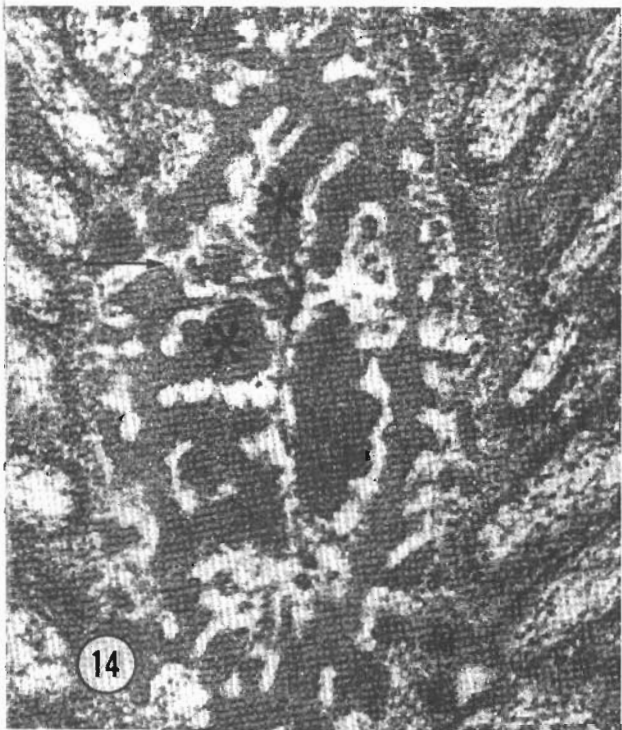
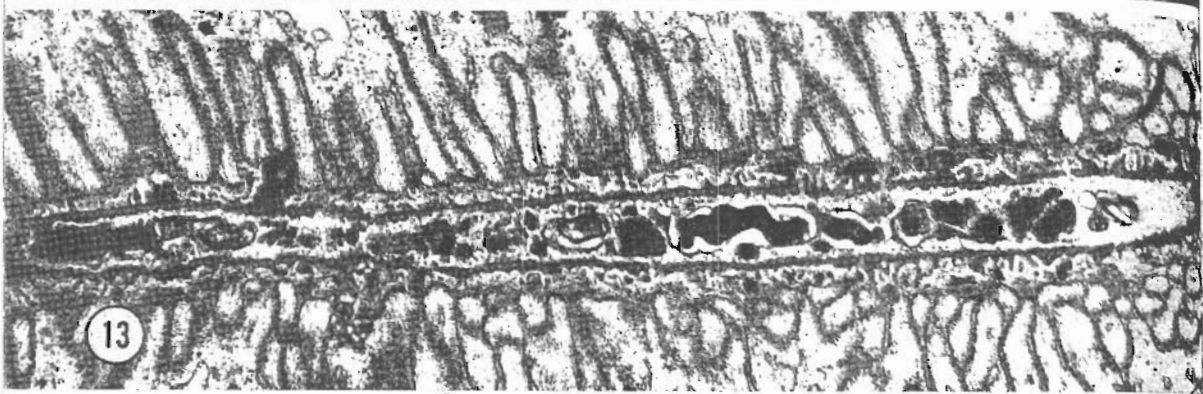
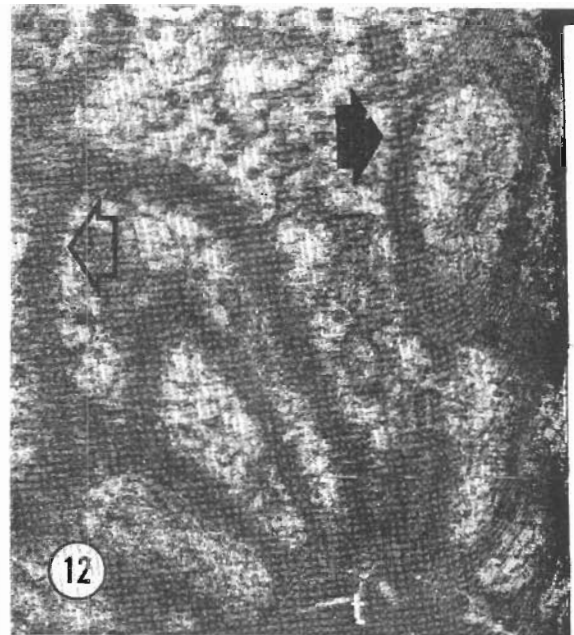
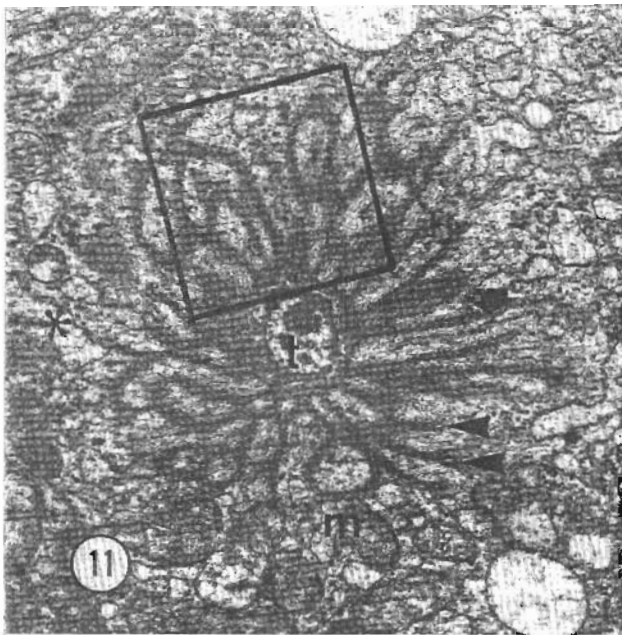
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tubules, and mitochondria. The Golgi regions usually lack the numerous fine vesicles found in those of the cortical cell. The interior of the dense bodies may be amorphous, may contain membrane inclusions, or may appear much like autophagic vacuoles, such as those described by Locke (1969). The mitochondria are elongate ($3 \mu \times 300 \text{ m}\mu$) and are packed rather closely near the margins of the central cavity (Figs. 8, 16, 18).

The medullary cell surrounds a large central cavity (ca. $4 \mu \times 8 \mu$). As it traverses this cavity, the efferent ductule possesses discrete layers: a superficial electron-dense layer, a tri-partite cuticulin layer, and an inner epicuticle (Figs. 19, 21). The tip of the ductule which lies in the cortical cavity is

continuous with the inner epicuticle in the medullary cavity. Within the latter cavity, light microscopy had shown the ductule to be divisible into bulb and switchback regions. Electron micrographs demonstrate that the 'bulb' is not an inflated segment of the ductule, but rather a segment where the ductule runs through a spherical mass of fine cylinders, which wind about in a manner reminiscent of a ball of micro-spaghetti (Figs. 19, 20). These fine cylinders, $150\text{--}180 \text{ \AA}$ in diameter, consist of a wall, ca. 30 \AA in thickness, which surrounds an electron-transparent (hollow?) center. Irregular electron-transparent patches, up to $200 \text{ m}\mu$ in diameter, are seen in the central portions of this mass, and often are adjacent to the ductule itself (Figs. 19-21). Some of these

Fig. 16. The central cavity of a medullary cell (C_m) and the end of the cavity of a cortical cell (C_c). The ductule first traverses the bulb region (b) and then loops back and forth in the switchback region (s). Dense material, presumably secretion, is seen in the most distal section of the switchback (asterisk). $\times 12,600$.

Fig. 17. A higher power view of the border of the central cavity of the medullary cell (C_m). In contrast to the cortical cell, the bounding plasma membrane is smooth (arrow). Small vesicles (asterisks) are seen within the irregular microvilli. $\times 36,600$.

Fig. 18. Nucleus (N_m) and cytoplasm of a medullary cell. Mitochondria (m), dense bodies (db), and the edge of the medullary cavity (C_m) are seen. $\times 13,200$.

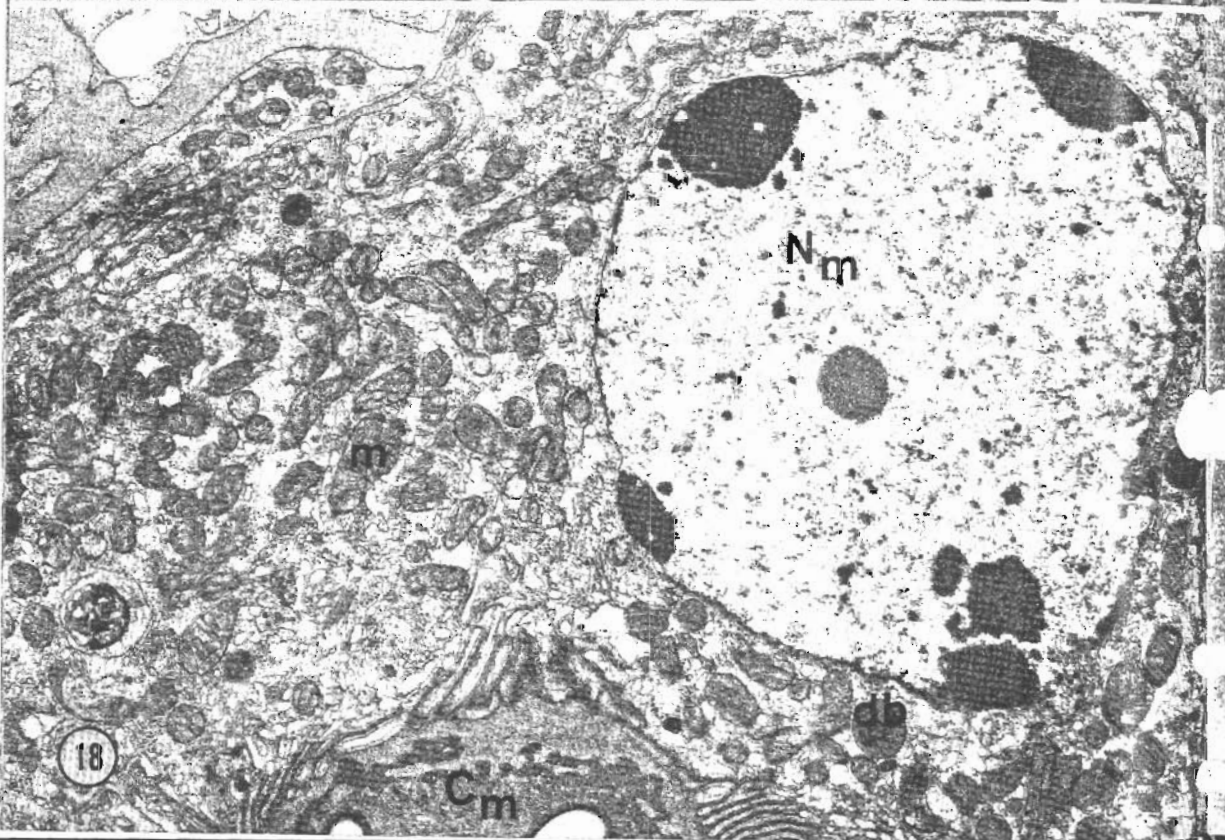
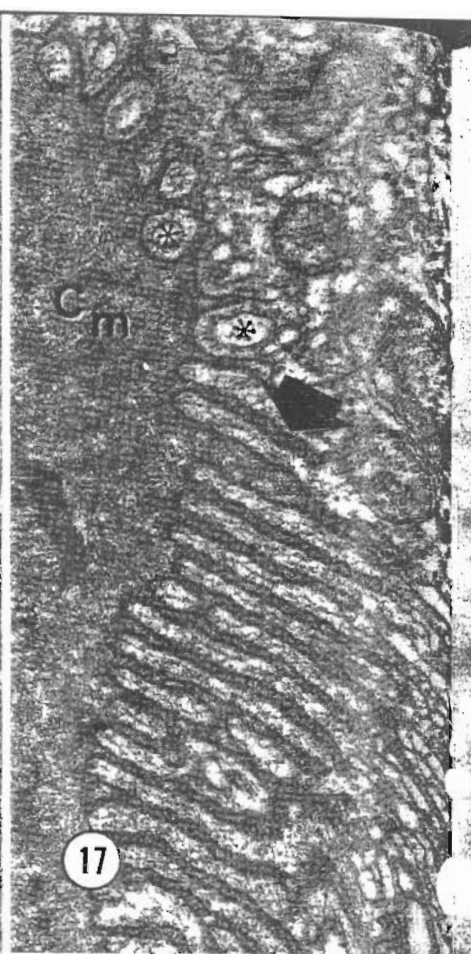
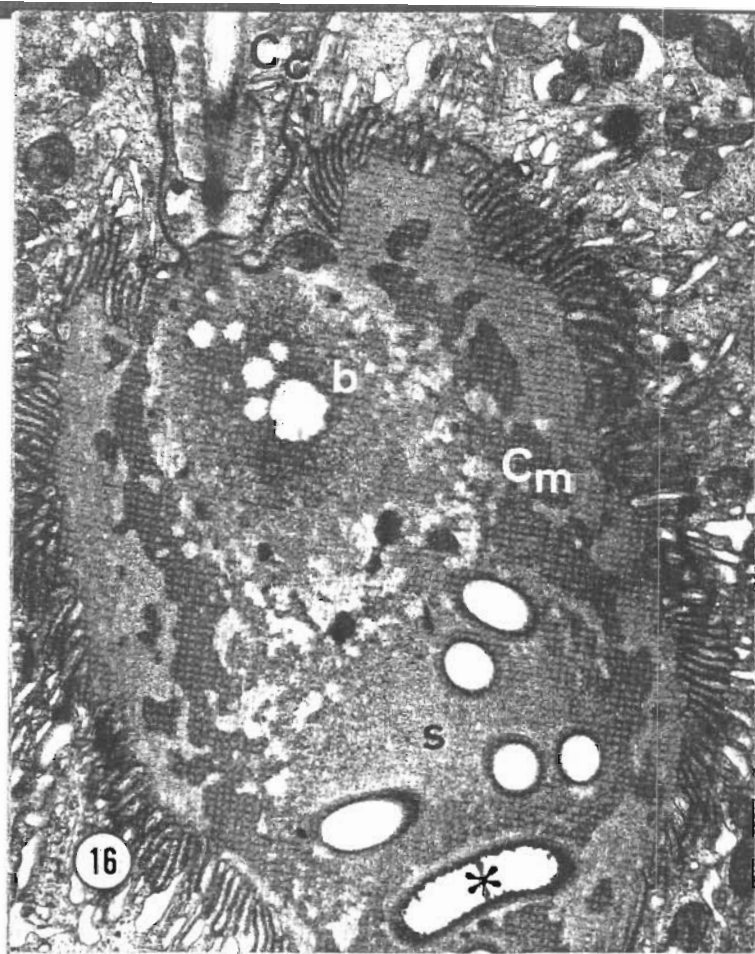
Fig. 19. A section through the bulb region of the medullary cell. Microvilli formed by the plasma membrane of this cell, are seen at the upper right. The ductule (d) lies at the center of a mass of fine cylinders. Within this mass are irregular patches (asterisks) lacking the cylinders, but containing a few fine fibers. Around this mass concentric zones of amorphous material (1, 2, 3); compare with Fig. 20. $\times 27,000$.

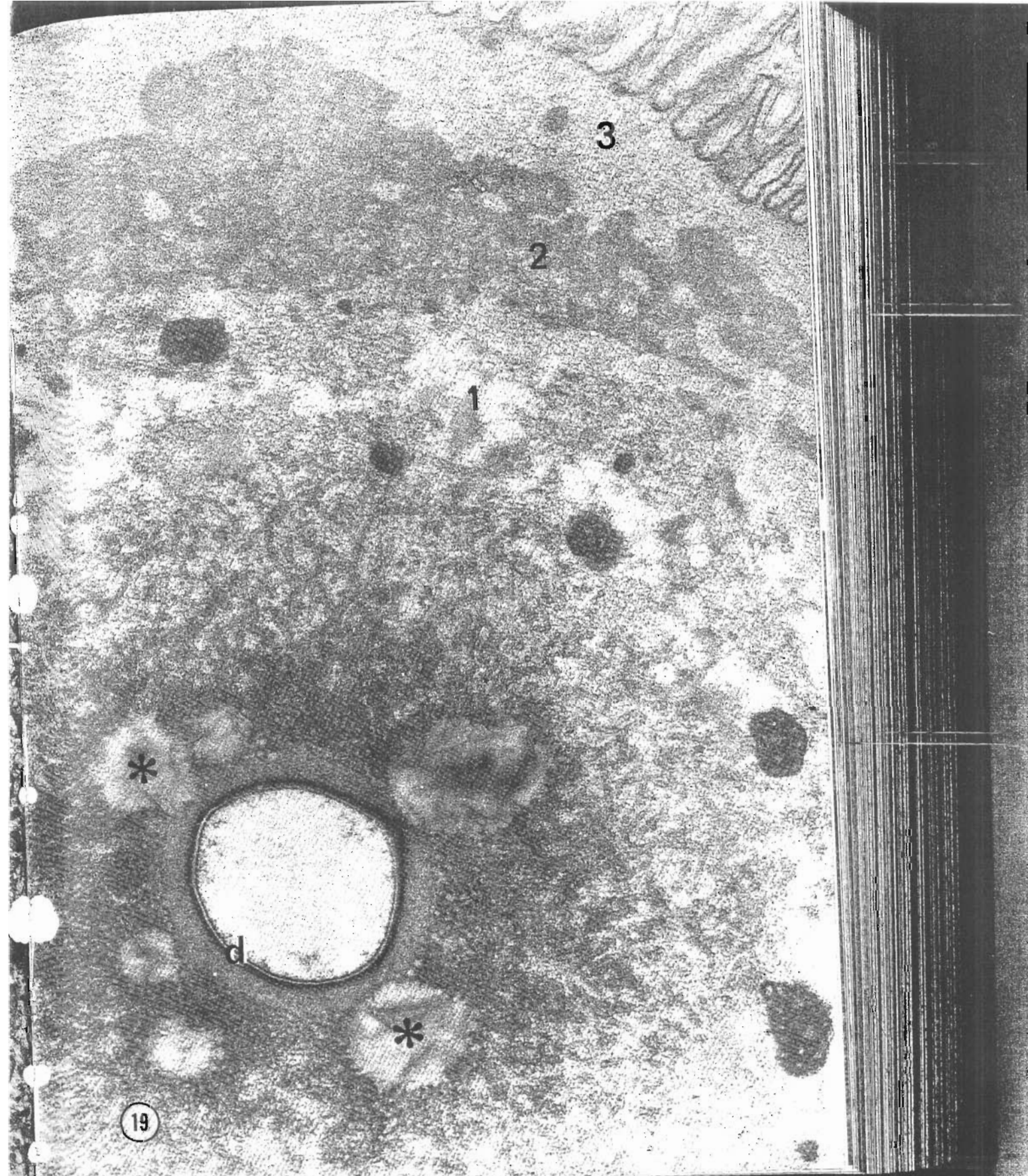
Fig. 20. A transverse section through the bulb region (b) of the medullary cell cavity. Material within this cavity is organized into concentric zones. The ductule (d) at the center is surrounded by the spherical mass of fine cylinders which characterize the bulb region. Three zones of amorphous material (1, 2, 3) can be readily distinguished. $\times 10,000$.

Fig. 21. A higher power view of the bulb region. The ductule itself clearly has a dense inner layer (cuticulin) as well as the underlying material of lower density. Within the mass of fine tubules in the surrounding cavities are electron transparent zones (asterisks) which are often associated with thin patches in the cuticular wall of the ductule (large arrow). $\times 40,000$.

Fig. 22. The ductule (d_1) as it leaves the central cavity of the medullary cell (C_m) and is enclosed in the tongue of cytoplasm of the ductule-carrying cell (d_2). At the lower right is a transverse view of a ductule (d_3), surrounded by dense deposits and ensheathed by the ductule-carrying cell. $\times 22,100$.

Fig. 23. A secondary efferent duct. Note the presence of fibrous endocuticle (end) in addition to the epicuticular layers. Flocculent material, presumably secretory product, lies in the lumen. $\times 16,500$.





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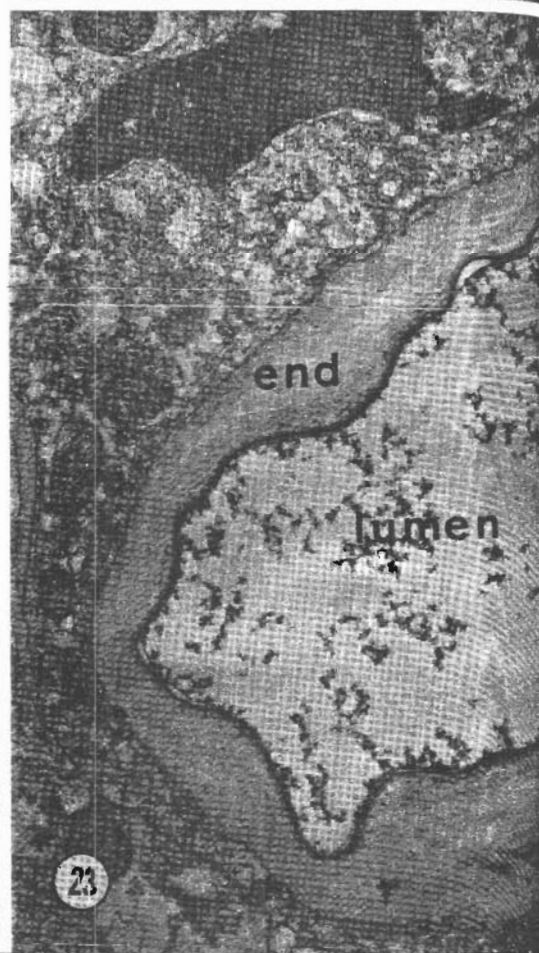
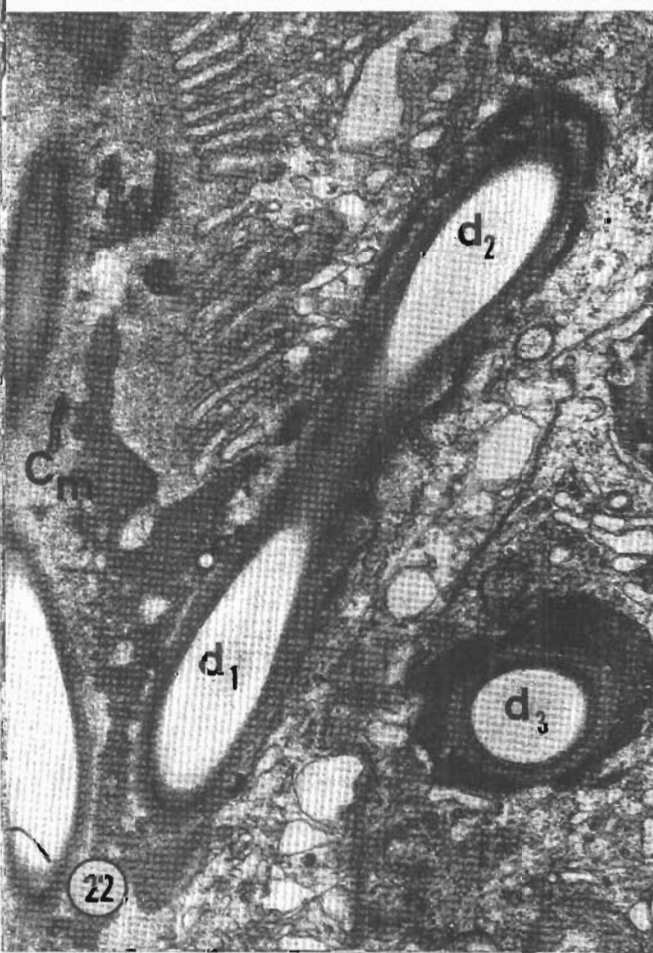
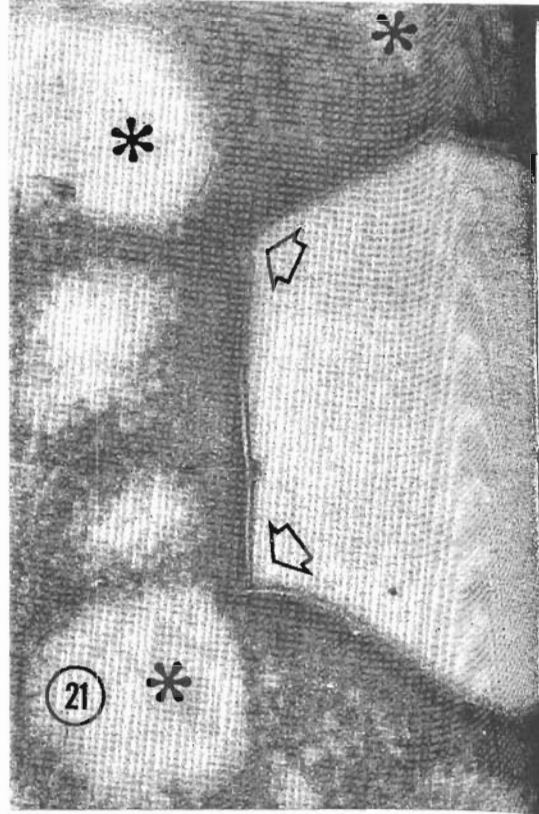
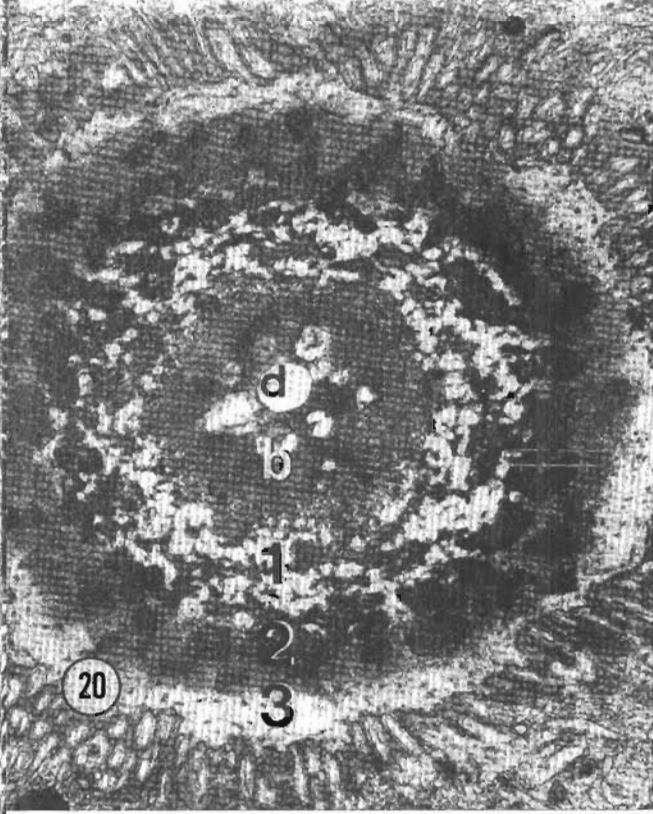
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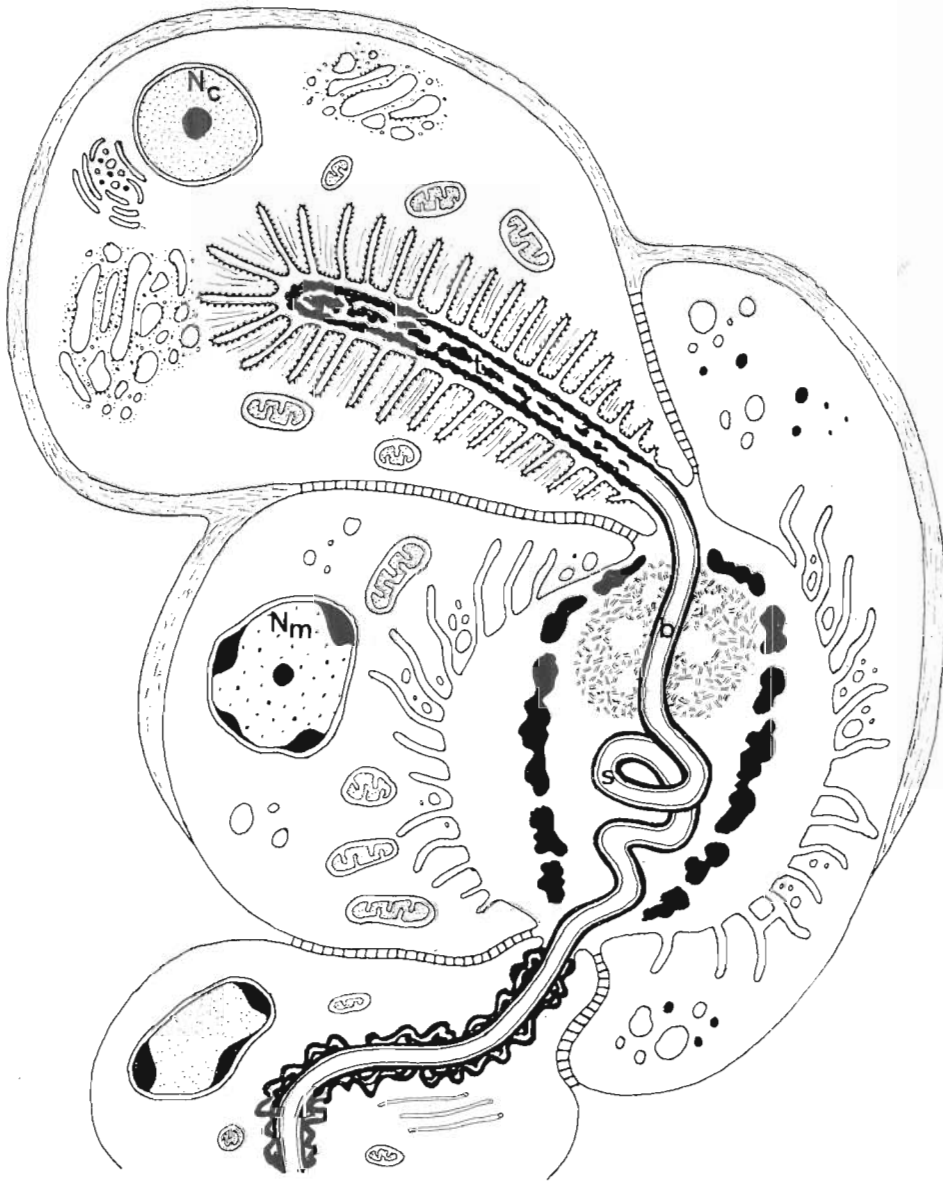


Fig. 24. Diagram showing the relationships between cortical, medullary, and ductule-carrying cells. Cortical and medullary cells differ in nuclear morphology (N_c and N_m respectively) and in the organization of their central cavities. Within the cavity bounded by the cortical cell, the tip of the efferent ductule (t) is fenestrated, and within that surrounded by the medullary cell, the ductule is divisible into bulb (b) and switchback (s) regions. A ductule-carrying cell encloses the ductule in the lower portion of the diagram. For clarity, the cellular organelles have not been drawn to scale.

patches appear to contain a few fine filaments (Figs. 19, 21). Where the patches contact the wall of the ductule, its inner epicuticle is thinner and breaks appear in the cuticulin layer (Fig. 21). Neither thin zones of epicuticle nor the fine cylinders occur in the switchback region (Fig. 22).

The matrix within the cavity is structured. In transverse sections through the bulb region, organization of the surrounding zones of fibrous and amorphous material is particularly evident (Figs. 19, 20).

The export system

As the ductule leaves the central cavity of the medullary cell, it is ensheathed in the cytoplasm of the ductule-carrying cell (Fig. 22). Where the plasma membranes of the two cells are appressed, they are joined by desmosomes. The cytoplasm of this ductule-carrying cell is distinguished by sparse cytomembranes and scattered mitochondria. The ductule itself possesses cuticulin and dense inner epicuticle, as in the central cavity, but in addition, the outside is 'shaggy'; narrow extensions from the outer surface of the ductule form dense irregular masses (Fig. 22). These extensions most certainly account for the 'beading' seen in fresh phase preparations. The ductules appear fairly straight in light micrographs and yet in many electron micrographs, six or seven ductules may be cut transversely in one section through a single cell. It may well be that one such cell produces several radiating ductules, much like the radiating tracheoles found in tracheolar end cells.

The larger ducts (primary and secondary) are invested with several cells and their walls contain endocuticle in addition to the epicuticular layers (Fig. 23).

Discussion

The cortical and medullary cells are strikingly different in their nuclear and cytoplasmic morphology, in their central cavities, and in the ductules contained therein. Certainly the cell types differ in function. The secretory system as a whole can be visualized as an assembly line with each cell type playing a unique part in the formation of the final secretory product. The question remaining is what parts? There are two alternatives which are not necessarily mutually exclusive.

The first alternative involves segregation of the various biosynthetic pathways. The final secretory product contains three classes of compounds—terpenes, fatty acid derivatives (undecene and dodecalactone) and benzoquinone. By analogy with other insect systems, the monoterpenes are probably derived from mevalonic acid (Schmialek, 1963; Happ and Meinwald, 1966); both the dodecalactone and undecene probably stem from a C₁₂ aliphatic acid (Gilmour, 1961; Gilby, 1965; Gilbert, 1967), and the benzoquinone is produced by hydrolysis of a diphenol glucoside followed by oxidation of the resulting diphenol (see Happ, 1968, for references). It is conceivable that the two cells function in parallel, and that each cell type contributes only one or two (but not three) classes of products. All of the products are lipid and are of low molecular weight. In comparison to other insect secretory cells producing such products (for examples see Stein, 1969; Noirod and Noirod-Timothee, 1965; Eisner *et al.*, 1964; Happ *et al.*, 1966; Forsyth, 1969; Crossley and Waterhouse, 1969), one can only conclude that the ultrastructural features of the cortical and medullary cells are consistent with elaboration of lipid products. Either the cortical or the medullary cell could be involved in the production of any (or all) of the three products.

But there is a second alternative: the ductule and cavities could function as *reaction chambers* for the final steps of product formation. While the first alternative assumed spatial segregation of the three independent biosynthetic pathways between the two cell types, this second alternative assumes spatial segregation of sequential reaction steps within the ductule and cavities. According to this hypothesis, the cortical cell sequesters precursors from the haemolymph and mediates some of the intermediate biosynthetic steps before it expels the *primary* secretory product into its central cavity. As this primary product passes along through the tip, bulb, and switchback regions of the efferent ductule, the molecules are modified to yield the *final* secretory product. Such an hypothesis is not without precedent: Eisner *et al.* (1964) suggested that analogous ductules ('tubules') and cavities ('vesicles') of the defensive glands of *Eleodes* (a tenebrionid beetle) could be reaction chambers.

As in *Bledius*, the ductules are regionally specialized within the central cavities. The defensive glands of *Eleodes* produce a mixture of *p*-benzoquinones; the secretory cells responsible are arranged in two-cell units and drained by a common efferent ductule. In such a two-cell unit, relatively non-toxic diphenols are oxidized to reactive quinones within the efferent ductule (Happ, 1968) and thus the final toxic product never contacts the secretory cells.

This reaction chamber hypothesis is not only consistent with our observations on *Bledius*, but also it provides explanation for otherwise puzzling features of the secretory systems. The particles, attached to the plasma membrane around the cavity of the cortical cell, may contain enzymes which modify the primary secretion, as it passes into the cavity. Such an enzymatic role for particles attached to plasma membranes has been proposed for other systems (for example, Anderson and Harvey, 1966). The only clear-cut fenestrations in the ductule which would allow products to flow in freely are those in the tip, within this cavity. The irregular wall of the tip, and particularly the electron-dense processes which partially occlude its lumen, may be an adaptation for greater surface area to allow cuticle-bound enzymes to act on the product. Cuticle-bound enzymes are not uncommon in insect material (see Happ, 1968; Quennedey and Noirot, 1968; Locke, 1961, 1970; and Lai-Fook, 1966, for examples). Addition of a cuticulin layer to the ductule in the medullary cavity seals the secretion away from the surrounding cells. The bulb region, with its thin patches of cuticle in the ductule itself and with the surrounding mass of fine cylinders, may be a site where additional catalysts are selectively injected or perhaps a site where by-products from previous reaction steps are recovered and conserved. The switch-back can then be interpreted as an elongate reaction space, and the cell is protected by cuticular and sub-cuticular barriers from reactants and products.

Although we favor the second, reaction-chamber, hypothesis, it cannot be regarded as well established until future histochemical and autoradiographic studies allow the various stages in the biosynthetic processes to be topographically and sequentially mapped.

The complexity of the cuticular ductules raises the question of their development. Except for a very few exceptions (Lai-Fook, 1970; Plattner *et al.*, 1972), there is little published ultrastructural information on the ways in which such an elaborate morphological system originates. In the tip of the ductule of *Bledius* (and in many other insect epidermal glands) the problem is further complicated by the lack of a cuticulin layer, which generally forms the template for complex cuticular shapes and surfaces (Locke, 1966; Filshie and Waterhouse, 1969).

The cavity of the medullary cell is essentially structured subcuticle. The fine cylinders in the bulb region are reminiscent of tubules found in the Gilson gland of Trichoptera (Quennedey, 1969), and of the cylindrical structures in the osmeteria of papilionid caterpillars (Crossley and Waterhouse, 1969). The surrounding fibrous and amorphous zones are like those found beneath cuticles (for examples see Taylor and Richards, 1965). In fact, this morphologically complex secretory system provides yet another example of elaborately specialized insect epidermal cells.

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