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GEORGE M. HAPP and CHRISTINE M. HAPP

CYTODIFFERENTIATION IN THE ACCESSORY GLANDS OF *TENEBRIO MOLITOR*. III. FINE STRUCTURE OF THE SPERMATHECAL ACCESSORY GLAND IN THE PUPA

ABSTRACT. To establish indices for studying the hormonal control of differentiation of the accessory reproductive glands of insects, the ultrastructural development of the spermathecal accessory gland (SAG) of female mealworm beetles has been analyzed. Over the 9 days between adult and pupal ecdysis, the SAG transforms from a stubby sac of columnar epithelium into an elongate cylindrical gland, lined with cuticle, and containing several distinct types of differentiated cells. The first phase of pupal differentiation is one of cell division and overall gland morphogenesis which lasts 3–4 days; at its close, two populations of cells can be distinguished. One of these populations will produce the cuticular ductules while the other will yield the three-cell secretory units or organules. In the second phase which lasts 2 days, the three cells of each organule become wrapped around one another and then the innermost puts out a pseudocilium and retracts within the next ensheathing cell. In the third phase which lasts 4 days, the cuticles of the axial duct, of the efferent ductule, of the vestibule upon which the ductules converge, and of the end apparatus, are deposited. The ciliary process degenerates, and after ecdysis, the secretory cells undergo peak differentiation.

Introduction

INSECT epidermal cells elaborate a great variety of secretory products, the most prominent of which form the cuticle. The diversity, complexity, and reproducibility of cuticular microsculpturing attest to the precise control which each cuticulogenic cell exerts over the ordered assembly of its products. Especially intriguing morphogenetic capacities are shown by certain small clusters of epidermal cells which act in concert to form a cuticular structure associated with secretory or sensory cells. The general term 'organule' applies to all such structures, be they dermal glands or butterfly scales (Lawrence, 1966; Kuhn, 1971; Kafatos, 1972).

In most instances, organules are formed by small isogenic clusters of four to eight cells, all of which are derived from a common epidermal cell. Divergent specializations of the daughter-cells and morphogenetic movements accompany deposition of the specialized cuticle (e.g. Wigglesworth, 1953a, b; Sreng and Quennedey, 1976; Selman and Kafatos, 1975). Many organules are isolated from one another and are surrounded by epidermal cells which form the continuous body cuticle. Examples include the tactile hairs and dermal glands of *Rhodnius* which have been so carefully studied by Wigglesworth (1953a, b). Other organules may be packed together in clusters as are the sensillae in a moth antenna (Sanes and Hildebrand, 1976) and the secretory units in the spermathecal accessory gland of the mealworm beetle, *Tenebrio molitor* (Happ and Happ, 1970). The present paper concerns the development of the latter.

Cuticle presents a variety of permeability barriers and thus, ingress or egress across cuticle is facilitated by localized cuticular

Department of Zoology and Entomology and Graduate Faculty of Cellular and Molecular Biology, Colorado State University, Fort Collins, Colorado 80523.

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specializations. Olfactory sensillae are riddled with fine channels that allow molecules to breach the cuticular barrier and to reach the dendrites at the receptor cells (Ernst, 1969). Complementary cuticular adaptations for export of exocrine secretions may involve a fine efferent cuticular ductule which runs to the surface from an extracellular cavity bounded by secretory cells (see Noirot and Quennedey, 1974, for examples). The ductule cells and secretory cells comprise an organule. As a general rule, the ductule has at least two structurally and functionally different segments: the *ductule* proper which acts as a conduit to the exterior, and the *end apparatus*, a mass of filaments or a region of fenestrated cuticle which allows secretory products to flow from the enclosed extracellular cavity into the lumen of the ductule.

During the differentiation of such an exocrine organule, the cells in the isogenic cluster must undergo morphogenetic movements and the ductule and end apparatus must be formed. As noted by Kafatos (1972) and others (Barbier, 1974; Lai-Fook, 1973; Percy, 1972; Berry and Johnsen, 1975), the deposition of ductule cuticle follows the formation of a temporary ciliary structure. As will be discussed below, an homologous ciliary structure is present in developing spermathecal accessory glands of *Tenebrio*.

Reproductive maturation in holometabolous insects requires not only the definitive maturation of the primary gonads, but also the terminal differentiation of accessory glands concerned with sperm transfer, sperm storage, paralhormonal signals, and even transfer of nutrients (Hinton, 1974; Friedel and Gillot, 1977). The major differentiative events in these accessory glands usually occur in the pupal stage, with extensive cell division, morphogenetic movements, and appearance of the adult cell types. The final stages of peak differentiation may occur before adult eclosion in insects which mate very shortly thereafter or may be delayed until after eclosion in insects with longer adult lives. In *Tenebrio*, the pupal stage is one for morphogenesis, cell division, and appearance of alternative phenotypes, while the peak differentiation of the reproductive accessory glands occurs after ecdysis (Poels, 1972a, b; Gadzama *et al.*, 1977; Happ *et al.*, 1977; Happ and Happ, 1975; Gerber, 1976). The progress of peak differentiation of the spermathecal accessory gland in the adult has been studied at the ultrastructural and biochemical levels (Happ and Happ, 1970; Happ and Yuncker, 1977). We herein describe the major morphological events which occur in the pupa, with a view to defining specific morphological criteria for the stages in the progressive differentiation. In future studies, we will analyze these steps to determine which are autonomous and which are dependent upon hormonal signals.

Materials and Methods

As last instar mealworms (*Tenebrio molitor* L.) eclosed to pupae, they were picked from stock cultures and the sexes were segregated. After allowing up to 9 days at 26°C for further development, the spermathecal accessory glands were removed for study. The developmental age of the pupae was confirmed by the morphological criteria of Delachambre (1973). For wax histology, the glands were fixed in alcoholic Bouin's and stained with either Delafield's haematoxylin or Lower's trichrome (Lower, 1957). For mitotic arrests, animals were injected with 2 μl of 1 mg/ml colchicine in *Tenebrio* saline (Butz, 1957).

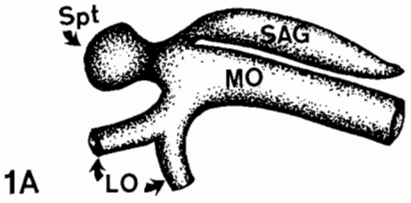
For electron microscopy abdomens were opened in fixative at room temperature and the glands were placed in a fresh aliquot of fixative. Fixation was in fresh Ladd glutaraldehyde (3% in 0·1 M phosphate buffer) pH 7·3 for 2 hr at room temperature. After a rinse in buffer, the tissues were transferred to 1% osmium tetroxide in the same buffer for 1 hr at room temperature. Following dehydration in graded acetones, the glands were embedded in Epon 812 (1:1). Thick sections were stained with toluidine blue. Thin sections were routinely stained for 20 min in saturated uranyl acetate in 50% ethanol followed by 5 min in lead citrate (Reynolds, 1963). Micrographs were taken on a RCA-EMU 3D or on a Philips 300 (Fig. 32).

Observations and Results

The median oviduct, bursa copulatrix, spermatheca and spermathecal accessory gland (SAG) of *Tenebrio* originate from an invagination of the hypodermis of the eighth and ninth abdominal sternites (Huet, 1974). At ecdysis to the pupa, the anlagen of these adult structures have already undergone pri-

mary organogenesis and are readily distinguishable upon dissection (Fig. 1a). The spermatheca appears as a small spherical projection at the anterior tip of the bursa copulatrix and the SAG is cigar-shaped (0·2 × 1·0 mm), tapering at both ends and running backward from its attachment to the bursa. In the light micrographs, both the spermatheca and the SAG are seen to be constructed of simple epithelia. In the spermatheca, the cells are cuboidal and arranged in hollow branching cords (not shown here) while the tall columnar cells of the SAG (30–50 × 3–5 μm) run from the basement membrane to the large lumen of the sac (Fig. 2). Neither the SAG nor the spermatheca are lined with cuticle at this stage.

Over the 9 days of the pupal stage, the SAG shows profound changes in size and organization. Within the first 36–48 hr of the pupal stage, the SAG undergoes torsion such that by the end of 2 days, the gland runs forward from its attachment to the bursa. Over the first 4–5 days after ecdysis, mitotic activity is high; cells characteristically become rounded and undergo division in the apical zone of the epithelium where interphase nuclei are absent (Figs. 2, 4, 5). At 5 days, vestibules (distinct inpocketings) which contain ciliary processes appear (Figs. 6, 7, 11, 12). By 7 days, the cuticle of the axial duct is being deposited and it becomes progressively thicker for the next 2 days. The ciliary processes then degenerate, leaving behind numerous fine efferent ductules which radiate out from the vestibule into the epithelium. The mature secretory units, or organules, consist of a ductule-carrying cell and a secretory cell. Each ductule terminates in an end apparatus which lies in a cavity surrounded by the apical surface of the secretory cell. The end apparatus has many fine perforations which apparently allow products from the secretory cell to traverse its wall (Fig. 3). The process of cytodifferentiation in the SAG involves three major phases: a phase of cell division and overall gland morphogenesis (3–4 days), a phase of cellular morphogenesis (2 days), and a phase of cuticle deposition (4 days).



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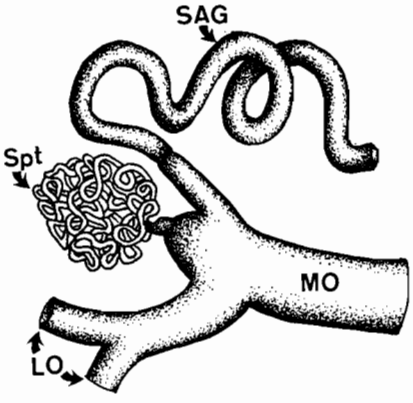

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Fig. 1a. The spermatheca and spermathecal accessory gland at ecdysis to the pupa.

Fig. 1b. The spermatheca and spermathecal gland at ecdysis to the adult. SAG, spermathecal accessory gland; Spt, spermatheca; MO, median oviduct; LO, lateral oviduct; BC, bursa copulatrix.

Phase of cell division

Throughout the first 96–100 hr of the pupal stage, the rate of cell division is quite high. In any section through the gland, several mitotic figures can be seen, and the extent of cell division can be directly assessed by injection of colchicine to produce metaphase arrest for estimation of mitotic indices. Data from such experiments are summarized in Fig. 12. As can be seen, the rate of cell division remains constant over the first 100 hr. The interphase cells remain fairly tall and narrow throughout this phase, and different morphological cell types are not readily distinguishable; cytoplasmic microtubules run along the long axes of the cells.

The apical plasma membranes (toward the lumen) have a variety of irregular projections (Figs. 36, 37) and small vesicles with electron transparent centers are close to and appear to fuse with the plasma membrane (Fig. 37). Near the lumen, the lateral margins of the cells are tightly linked by adhering zonules. In general, these zonules begin about 400 nm

from the lumen and are themselves *ca.* 200 nm in length. The cytoplasmic side of the zonule membrane is usually associated with a plaque of electron-dense material (Figs. 36, 37). Above the junctions (just basal to them) closely appressed membranes suggest the presence of gap junctions (not shown) and more basally, indistinctly septate junctions are present (Fig. 37).

Over the first 3 days, the plane of the

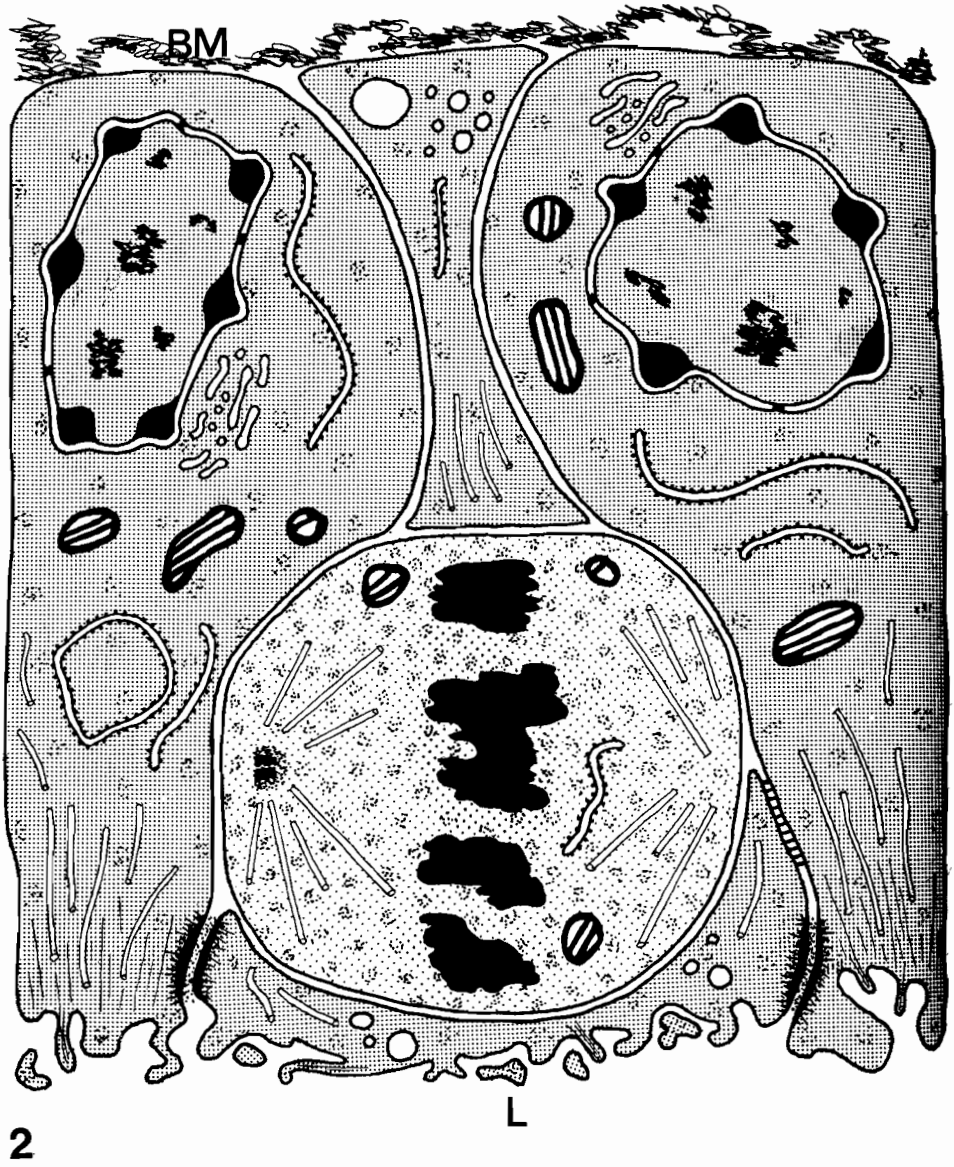

Fig. 2. Diagram of the SAG epithelium at 1 day after pupal ecdysis. The interphase columnar cells span the height of the epithelium. These cells have scattered profiles of rough endoplasmic reticulum, Golgi zones, and a concentration of microtubules and microfilaments in their apical regions where adjacent cells are linked by adhering zonules. BM, basement membrane; L, lumen; for clarity, organelles have not been drawn to scale.

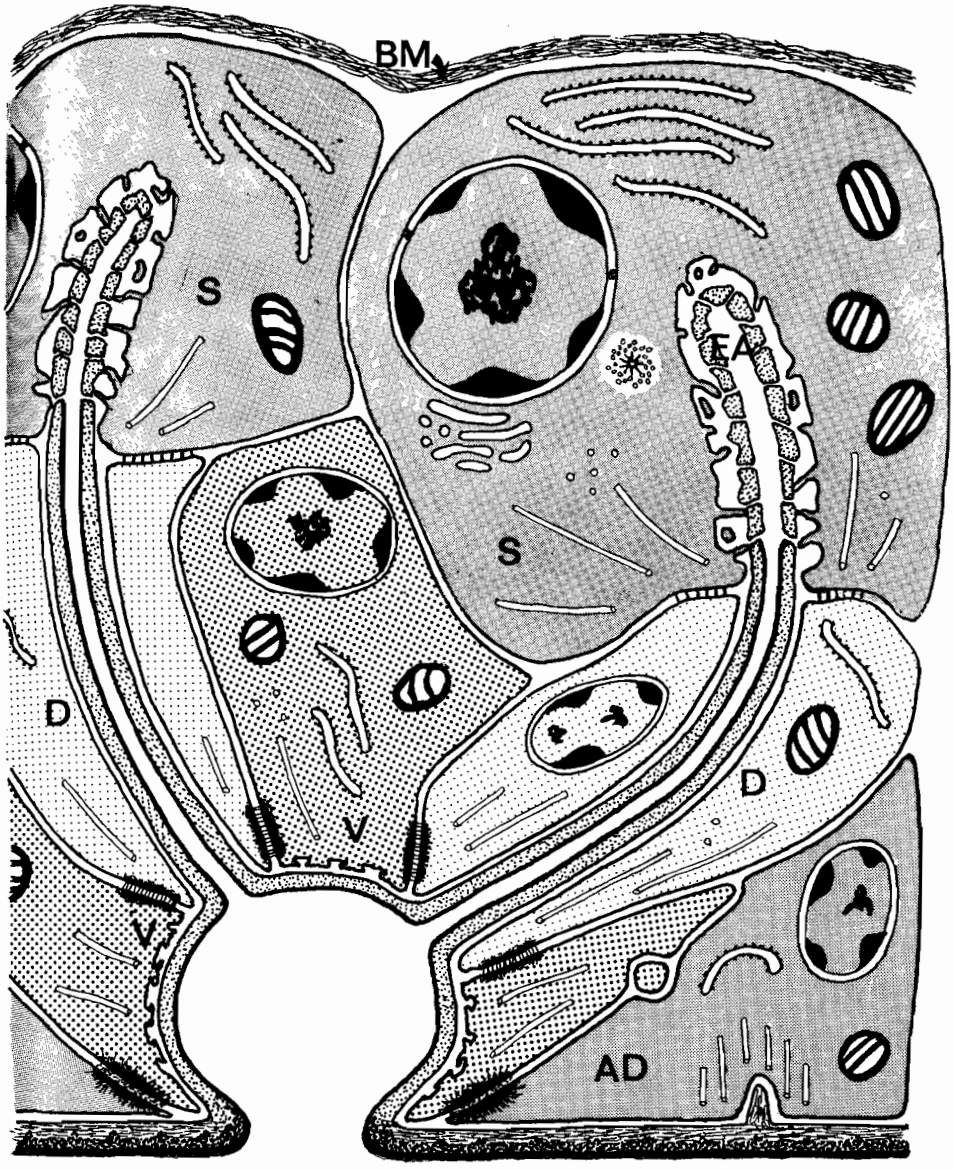

Fig. 3. Diagram of the SAG epithelium at adult ecdysis. Four types of cells are readily distinguishable: (1) cells lining the axial duct (AD), (2) cells lining the vestibule (V), (3) cells surrounding the efferent ductules (D), and (4) the secretory cells (S). The end apparatus (EA) lies in a cavity bounded by the plasma membrane of the secretory cell, which contains scattered elements of rough endoplasmic reticulum, Golgi zones, mitochondria and microtubules. The secretory and ductule-enclosing cells are linked by separate junctions, while adhering zonules link adjacent cells along the vestibule and the axial duct. Cuticular projections of the axial duct indent the cells lining that duct and are surrounded by microtubules. BM, basement membrane; L, lumen of the axial duct. For clarity, the organelles have not been drawn to scale.


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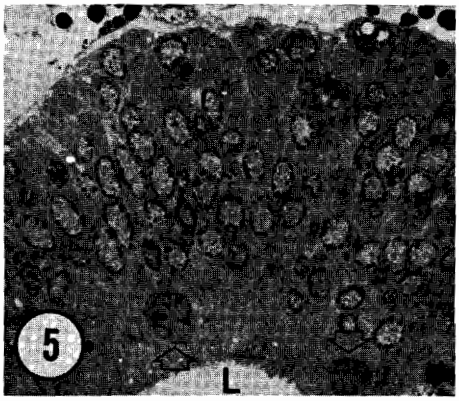
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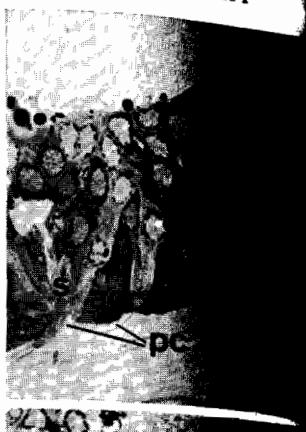
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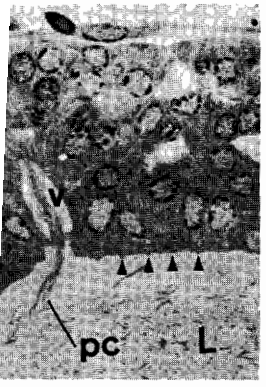
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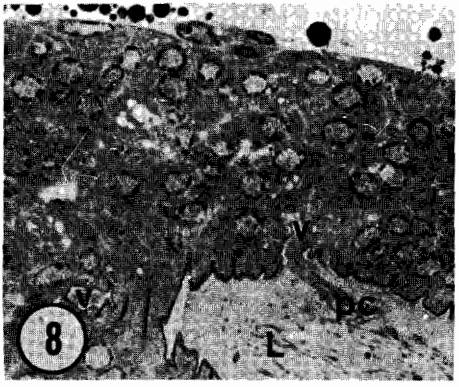
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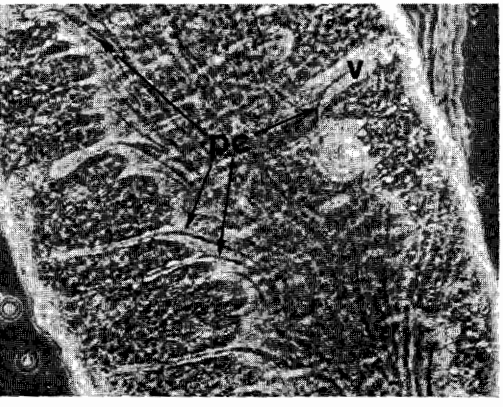
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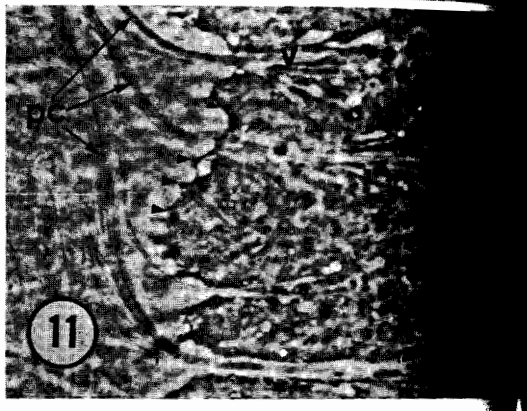
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mitotic spindle almost always is parallel to the long axis of the SAG (Figs. 4, 5). During the fourth and fifth days, it is common to see division with a radial spindle orientation (i.e. metaphase plate parallel to the surface of the lumen). The two distinct spindle orientations, i.e. first parallel and then perpendicular with respect to the lumen may reflect respectively, early proliferative divisions, and later differentiative ones in the sense of Lawrence (1966) and Kafatos (1972). Throughout this period of cell division, pyknotic nuclei can frequently be seen beneath the basement membrane. Due to the close packing of the columnar cells, we have been unable to determine with confidence the exact cell lineages. A study of the birth and death of cells within the lineages is now in progress and will be reported in a later paper.

Phase of cellular morphogenesis

In the fourth and fifth days, two populations of cells can be distinguished from one another in the SAG epithelium. Cells of any one population are arranged in clumps of 50 to several hundred. The two populations are distributed in patches, rather like an irregular checkerboard as viewed from the apical surface.

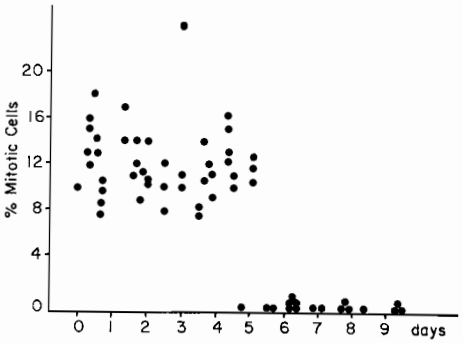

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Fig. 12. Mitotic indices of the SAG after 4 hr colchicine arrest.

Fig. 4. Longitudinal section through the SAG epithelium 1 day after pupal ecdysis. The interphase nuclei are absent from the apical zone which contains a spherical mitotic cell (hollow arrow). The metaphase plate is perpendicular to the plane of section and to the lumen; thus, the spindle is parallel to the long axis of the gland. L, lumen. Toluidine blue. ×630.

Fig. 5. Cross-section through epithelium 3 days after pupal ecdysis. The two mitotic cells (hollow arrows) are in anaphase; their spindles are perpendicular to the plane of section and parallel to the long axis of the gland. L, lumen. Toluidine blue. ×630.

Fig. 6. Longitudinal section 5 days after pupal ecdysis. Clusters of lightly staining secretory organules (s) are interspersed between the more darkly staining cells which will lay down the axial duct (ad). Clumped pseudocilia (pc) run from secretory cell clusters into the lumen (L). Toluidine blue. ×630.

Fig. 7. Longitudinal section 6 days after ecdysis. The pseudocilia (pc) converge into the vestibule (v) and run together into the lumen (L). At sites of apical intercellular contacts, 'ridges' (small arrowheads) project outward toward the lumen. Toluidine blue. ×630.

Fig. 8. Oblique section 7 days after ecdysis. The vestibules (v) and the lumen (L) are lined with a thin cuticle. Pseudocilia (pc) persist. Toluidine blue. ×630.

Fig. 9. Oblique section 9 days after ecdysis. Pseudocilia are absent. Toluidine blue. ×630.

Fig. 10. Whole mount of the SAG 6 days after ecdysis. Clumps of pseudocilia (pc) run from the vestibule (v). Phase contrast, fresh preparation. ×260.

Fig. 11. Epithelium 6 days after ecdysis. When the clumps of pseudocilia (pc) run into the lumen, they tend to splay out into individual thread-like processes. Intercellular ridges are indicated by the small arrowheads. Phase contrast, fresh preparation. ×770.

Fig. 13. Five days after ecdysis. A section through the apical processes of many secretory organules as they approach and impinge upon the edge of a vestibule (v). The three cells of each organule are wrapped around one another. The innermost (cell 1) has a pair of centrioles (paired upward arrows), which are associated with a pseudocilium (pc). Arrows indicate other centrioles. The distal centriole (d) tends to be of smaller diameter than the proximal (p). Adhering zonules (az) link the lateral membranes of the cells between and within the organules. × 14,000.

The first of the cell populations will lay down the thick cuticle of the axial duct. Over days 4 and 5, these cells remain firmly linked at their apical margins by the sub-terminal zonules. The apical margins are relatively smooth and lack the many microvilli and the irregular projections which were characteristic of the younger glands (Fig. 38).

The second cell population gives rise to the secretory organules. These cells become associated in clusters of three, the apical processes of which are arranged concentrically and contain the paired centrioles (Fig. 13). The innermost cell of the cluster has been arbitrarily designated, cell 1; the intermediate, cell 2; and the outermost, cell 3 (Figs. 13, 14). The entire epithelial patch invaginates so that the many organules tend to lie around an outpocketing of the lumen, termed the vestibule. In favorable sections, the two types of cells appear as alternate wedges, with the broad ends of the axial duct wedges projecting toward the lumen and with the narrow end of the organule wedge lying between these (Fig. 6).

At least for the innermost cell, the centrioles are characteristically in pairs, linked in series by intercentriolar fibers and with their long axes perpendicular to the lumen (Figs. 13–15). The proximal centriole contains a central cartwheel and the usual nine triplets embedded in a rather dense matrix. In addition, there are two alternating sets of peripheral fibers (Fig. 16). The larger set projects outward *ca.* 20 nm while the smaller set projects out only 10 nm. The proximal centriole as a whole is *ca.* 220 nm in diameter. Fine intercentriolar fibers converge from the outer margins of the proximal centriole towards the lumen of the distal centriole (Fig. 15). The distal centriole also has nine triplets and a central cartwheel but lacks the sets of peripheral fibers (Fig. 17). The distal centriole gives rise to the pseudocilium. Immediately distal to the centriole proper is the neck of the pseudocilium (Figs. 14, 15). In this region, the pseudociliary microtubules are arranged in nine doublets and are surrounded by a granular matrix which seems to include radial projections to the plasma membrane. No central microtubules are present (Fig. 18). The plasma membrane bounding the neck is smooth (not at all wavy in sections) and appears to show repeating structures with a periodicity of 260–280 Å. These structures reflect the presence of a ciliary 'necklace', a membrane specialization reported in molluscs, vertebrates, and protozoans (Gilula and Satir, 1972). The neck is usually 360–380 nm in diameter. A similar neck is present in the colleterial glands of *Galleria* (Barbier, 1975). The distal pseudocilium is packed with less ordered single microtubules (Figs. 14, 20, 21, 26), and the ciliary process as a whole is larger and more variable in diameter. Fig. 19 shows a transition region between the neck and the distal process.

Over the fifth and sixth days, the innermost cell retracts, leaving its pseudocilium in a cylindrical cavity bounded by cell 2 (Figs. 21, 22). A halo of fibers tends to surround the pseudocilium within the cylindrical cavity and also in the more proximal regions of the vestibule (Fig. 21). Along the margins of the proximal vestibule and along those of the cylindrical cavity, the plasma membrane appears gently rippled with dense fibrous plaques at the tip of each ripple. Similar dense plaques are seen at the tips of narrow microvilli in the type 3 cells which line the distal vestibules, and also in the axial duct cells which line the lumen (Fig. 23). These plaques probably represent an early stage in cuticle deposition.

Phase of cuticulogenesis

From the sixth to the ninth day, the cuticles of the end apparatus, the efferent ductule, the vestibule and the axial duct are laid down. Except for the end apparatus, all of the other cuticular regions have a distinct laminate outer epicuticle and an underlying dense homogeneous inner epicuticle (Figs. 26, 28, 33, 40–43). The outer epicuticle is formed over the latter portion of the sixth day and the inner epicuticle deposition follows immediately. Along the axial duct, the zones of cell contact support elongated microvilli (Fig. 39), which presumably allow increased surface of the outer epicuticle in order to permit the characteristic folding of the definitive axial duct cuticle. The efferent ductule does not form directly along the plasma membrane of the pseudocilium—rather it forms some distance away, at the tips of the shallow scallops in the plasma membrane (Fig. 21). When inner epicuticle deposition in the efferent ductule and vestibule is complete, the plasma membrane of the cell is closely ap-

Figs. 14–20. Five days after ecdysis.

Fig. 14. The three cells of an organule (1, 2, 3) are attached to one another by adherin zonules (az). The axes of the paired centrioles (cen) of cell 1 are aligned in series wi the pseudocilium near the distal centriole; the pseudocilium is bounded by a thicken straight plasma membrane in the neck region (n); the distal pseudocilium (dpc) bounded by a more undulating membrane. The hollow arrows indicate the transiti between neck and distal pseudocilium. v, vestibule. × 18,000.

Fig. 15. A pair of centrioles in cell 1. The proximal centriole (p) is attached to t distal one (d) by intercentriolar fibers. The vertical lines suggest a periodicity (300 perpendicular to the long axis of the neck (n). An adhering zonule is at the top of t micrograph. × 56,000.

Fig. 16. A slightly oblique section through a proximal centriole showing the car wheel in the center and the nine large and nine small peripheral fibers (indicated b large and small arrowheads, respectively), which are closely appressed to the out surface of the cylinder. The cylinder itself is *ca.* 2100 Å in diameter. × 80,000.

Fig. 17. A cross-section through a distal centriole. The central cartwheel is prese and the triplets of microtubules are discernible. The diameter of the cylinder is a 1900 Å. × 80,000.

Fig. 18. A cross-section through the neck of a pseudocilium showing the nine doubl microtubules but *no* central singlets. The doublets appear to be attached to the plas membrane by strands of fibrous material (arrowheads). × 68,000.

Fig. 19. A cross-section through a pseudocilium near the transition between t neck and the distal process. Many of the peripheral doublets are incomplete and n regularly spaced. The central doublets are present. × 70,000.

Fig. 20. Cross-section of pseudocilia in the vestibule. Both numbers of microtubul and diameter of the pseudocilia are highly variable. × 63,000.

Figs. 21–23. Six days after ecdysis.

Fig. 21. A vestibule containing many pseudocilia (pc) each of which is surrounde by a halo of filamentous material. The pseudocilium at the upper left of the micrograp lies within a smaller cavity, probably bounded by the plasma membrane of cell Short cellular outfoldings, associated with plaques of electron-dense materials (arro heads), are found only along the margins of the vestibules and that of the smal cavity at the upper left. A centriole is above the asterisk. × 20,000.

Fig. 22. The outer zone of the secretory epithelium. The large ovoid nuclei in t upper portion of the photograph are apparently those of secretory cells (cell 1) wh the smaller nuclei seen in the center are probably from ductule cells (cell 2). pc, pseud cilium. × 7260.

Fig. 23. A cluster of pseudocilia runs from the vestibule into the lumen (L) of t axial duct. At the boundaries between cells, the microvilli tend to form ridges (arrow × 7900.

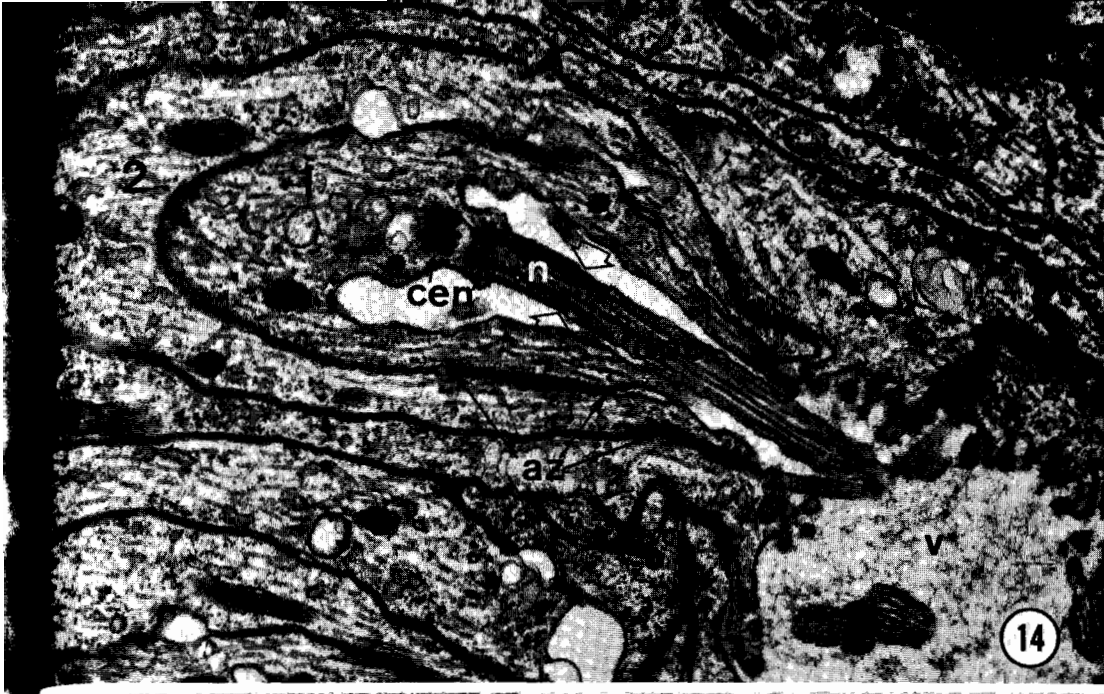
Fig. 24. The vestibule at 7 days. A thin outer epicuticle layer (between arrows) a an underlying inner epicuticle have been laid down. The microtubules in the pseud cilia (pc) are less distinct than at earlier ages. Adhering zonules (az) link the cuticul genic cells. × 21,000.

Fig. 25. An oblique section through a degenerating pseudocilium at the level whe the end apparatus is being deposited at 7 days. The wall forms at the tips of the micro villi, *ca.* 80–100 nm from the outer membrane of the pseudocilium and directly again the filamentous halo of the pseudocilium. × 37,000.

Fig. 26. A section through the ductule (d) and pseudocilium (pc) at 8 days. × 37,00

Fig. 27. At 8 days the microtubules within the distal pseudocilia have degenerat and filamentous material remains. v, vestibule, d, ductule. × 27,500.

Fig. 28. A secretory cell (1) at 9 days. Golgi zones (G), a centriole (arrow), mitocho dria, and scattered cisternae of endoplasmic reticulum are present. A process of t ductule cell (2) is also seen. BM, basement membrane. × 15,200.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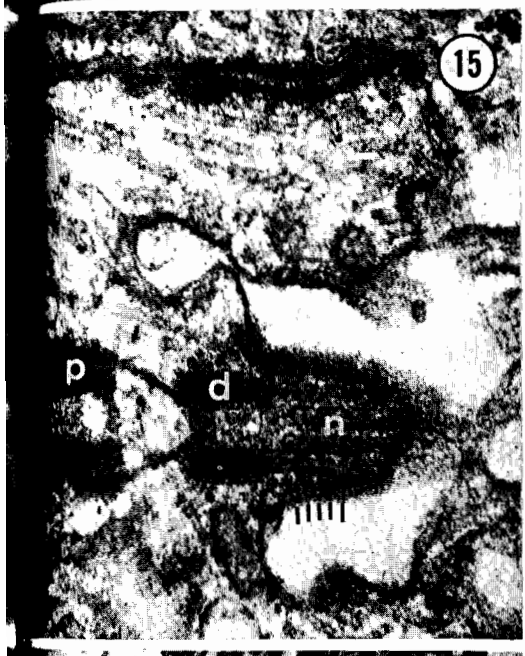
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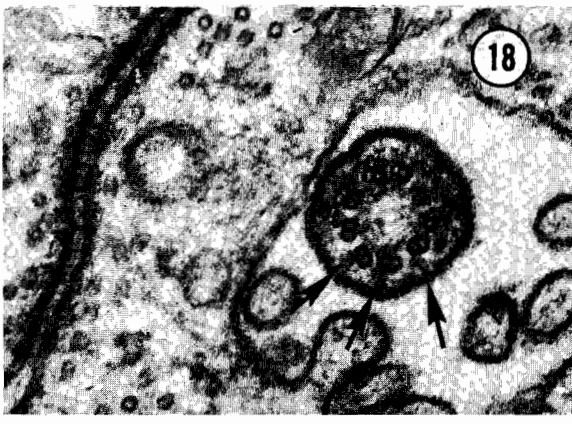
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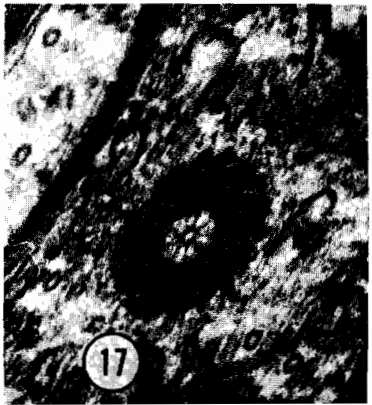
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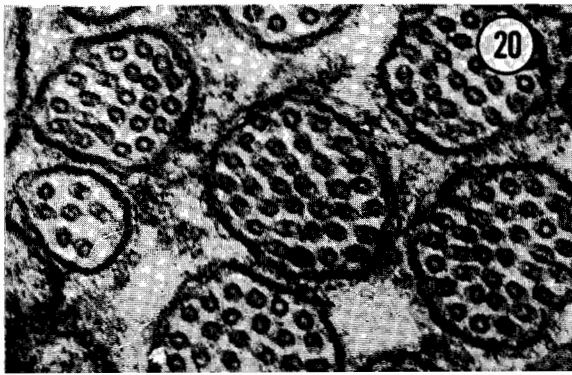
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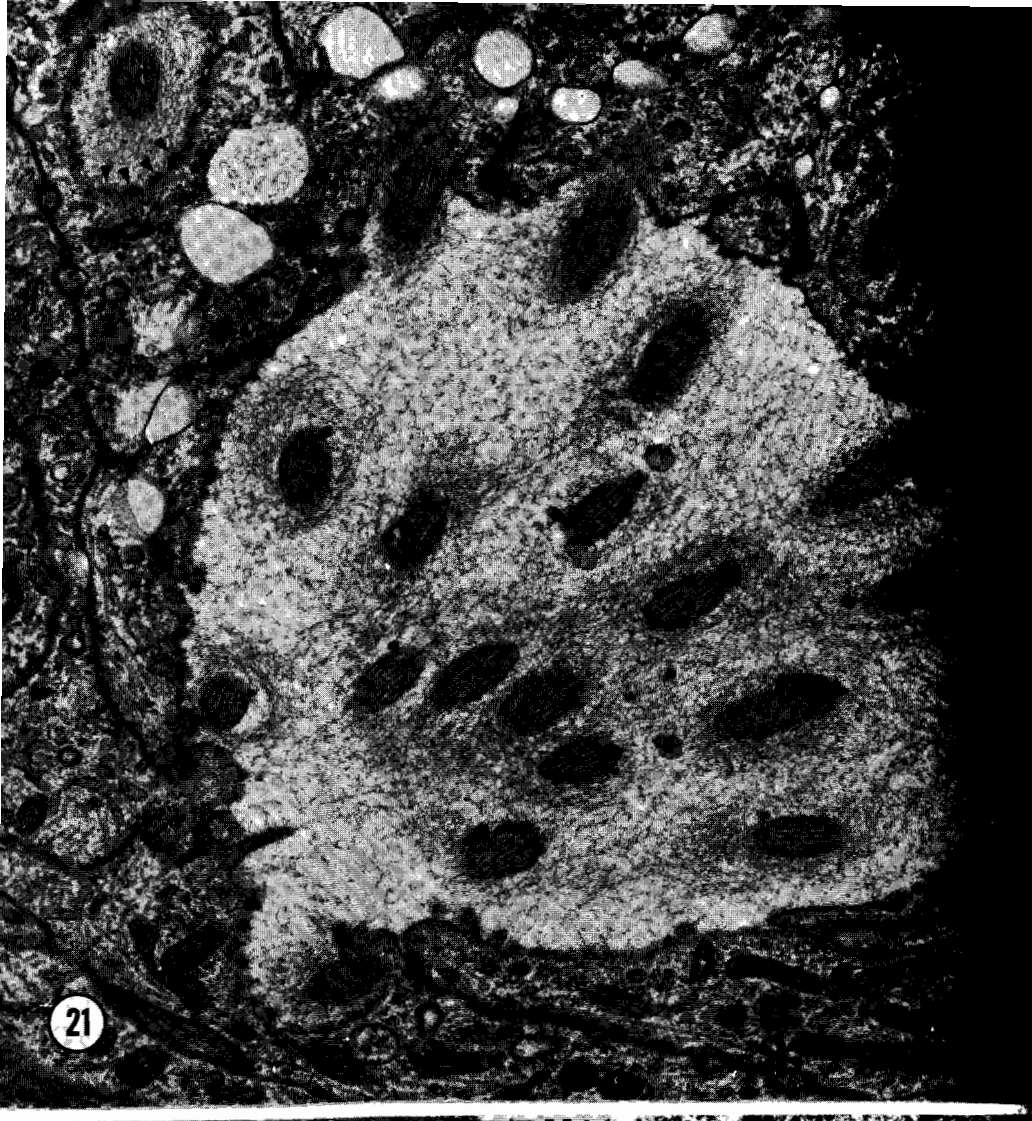
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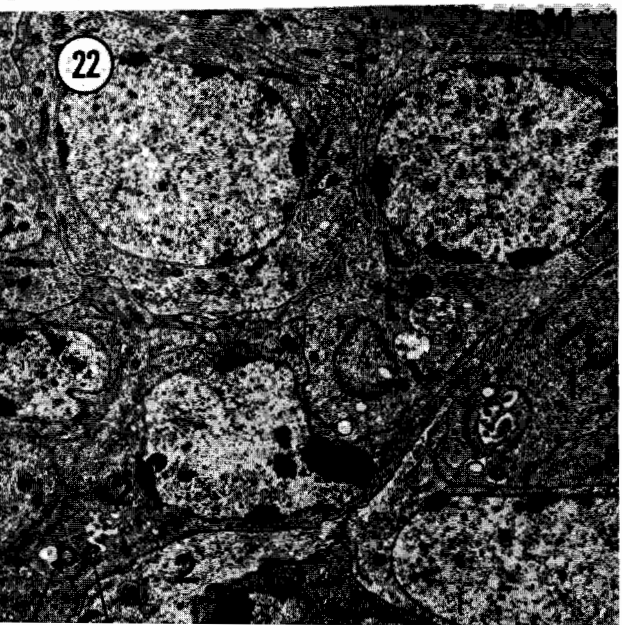
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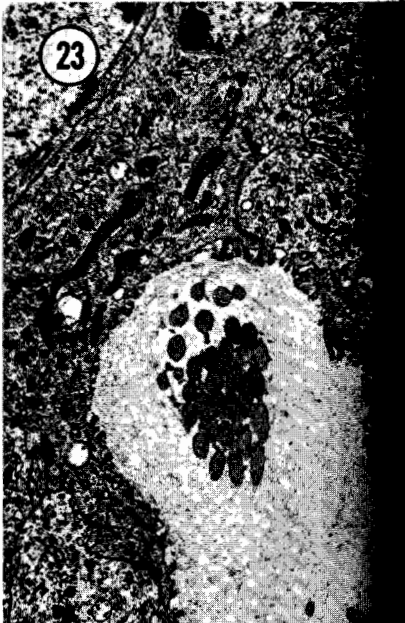
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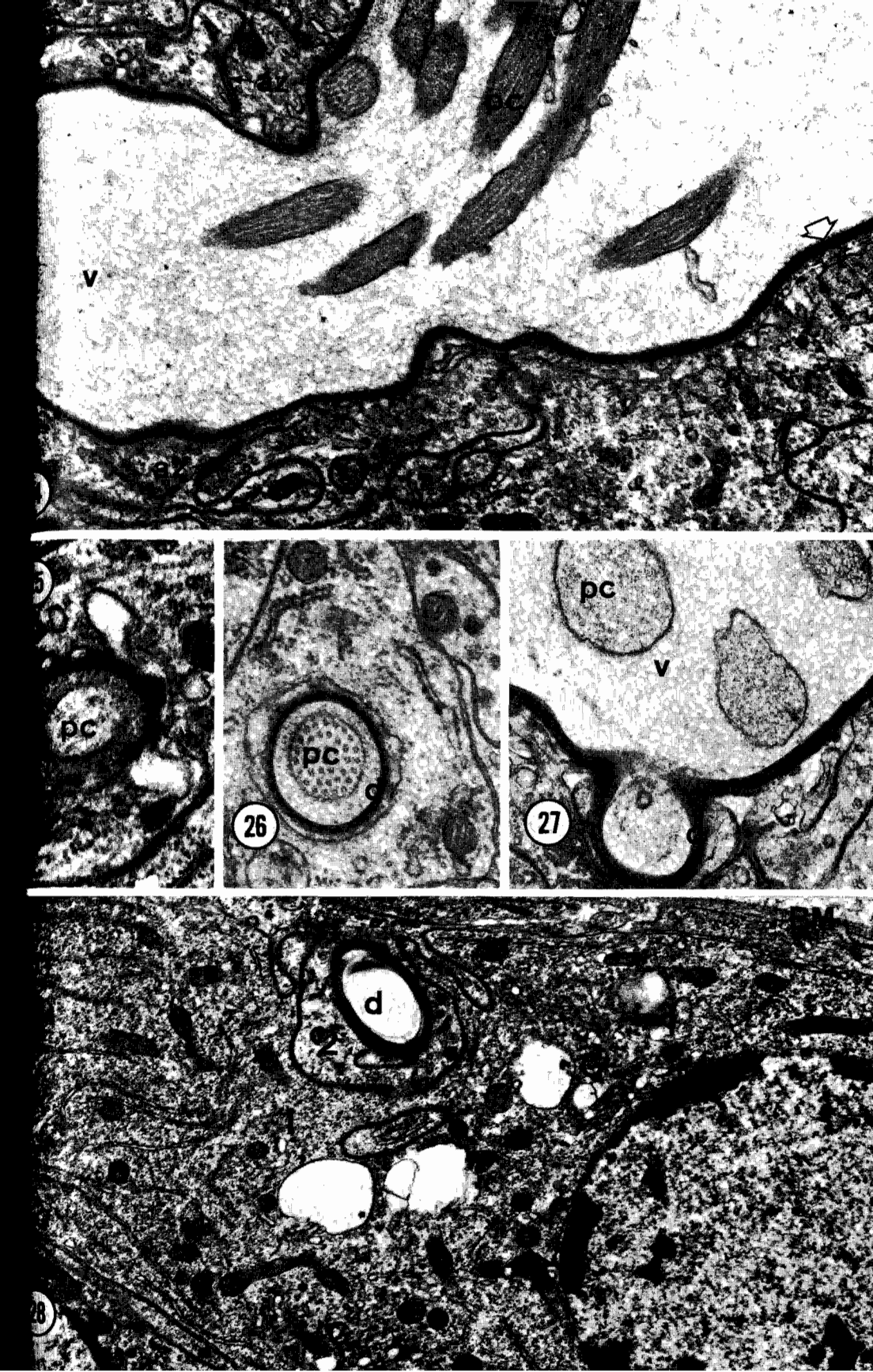
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pressed to the inner surface of the cuticle (Figs. 28, 33). The microtubules of the pseudocilium become increasingly fuzzy (compare Figs. 14, 21, 24) and then disappear altogether (Fig. 27). The process of ductule formation is summarized in Fig. 34a–c.

The wall of the end apparatus is continuous with the inner epicuticle (Happ and Happ, 1970) and the end apparatus is formed at approximately the same time as the inner epicuticle. Large dense plaques (Fig. 25) apparently coalesce to yield the wall, into which electron-dense filaments project; the filaments apparently arise as tufts from the surrounding microvilli (Figs. 29, 30). At 9 days, the dense filaments have disappeared, leaving the fine perforations characteristic of the end apparatus (Figs. 31, 32). This process is summarized in Fig. 35a–c.

The cuticle of the axial duct contains an outer epicuticle, a thick dense layer that is continuous with the inner epicuticle of the vestibule and a fibrous inner layer that is not present in the vestibule. The uneven surface of the dense layer is unusual (Fig. 42). By 7 days, the dense layer is 300 nm in thickness and at its inner surface supports a zone of irregular fibrous material that might have been derived from degenerated distal portions of microvilli (Fig. 41). Electron-dense vesicles frequently approach the apical plasma membrane which has short microvilli. Over the eighth day, more electron-dense material is contributed to the dense layer until its surface appears almost shaggy, and the fibro innermost elements become increasingly di cult to distinguish (Fig. 42). By 9 days, t plasma membrane no longer has microvi and it is closely appressed to the cuticl Tonofibrillae-like processes are surrounded by invaginations of the plasma membran and cytoplasmic microtubules (Fig. 43), and axial duct deposition is complete.

The major events in the cytodifferentiation of the SAG are summarized in Fig. 44.

Discussion

The spermathecal accessory gland of femal *Tenebrio molitor* originates as an epidermal inpocketing from a ventral sternite of the larva. The process of determination to yield the SAG and/or the spermatheca includes a period of developmental regulation (Huet 1974) After pupal ecdysis, the rudimentar inpocketing differentiates into an elongate cuticle-lined gland whose epithelium co tains patches (clones?) of cells which produc the axial duct, and patches of secreto organules. The differentiation in the pup stage is conveniently divided into thr phases: cell division and organogenes organule morphogenesis, and cuticle depo tion.

Throughout the phase of cell divisio metaphase cells accumulate at a constant r and then abruptly, at approximately 110 after ecdysis, mitosis ceases. In contrast

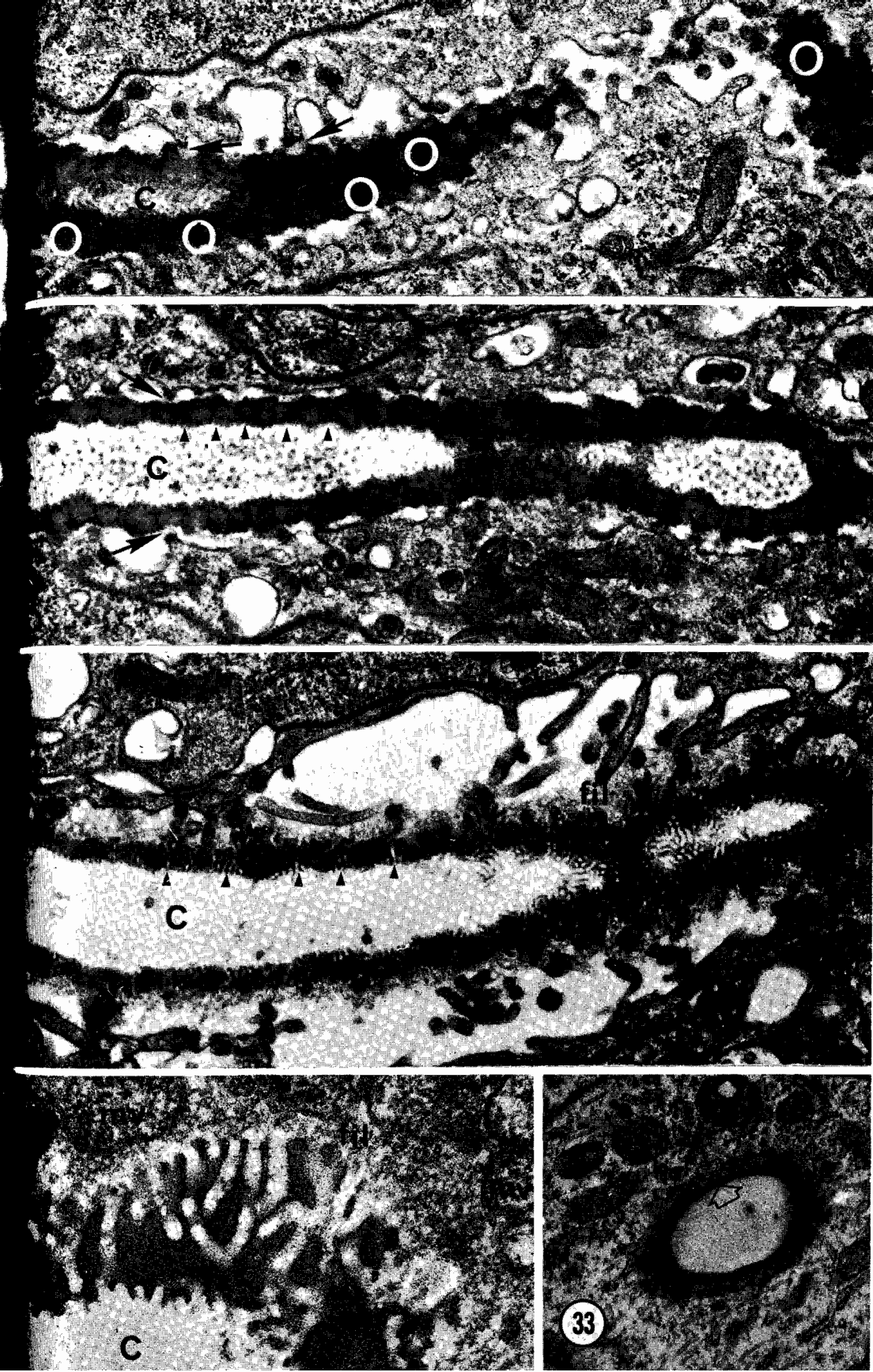
Fig. 29. The end apparatus at 8 days (oblique section). Filaments from the microvi (arrows) project into the homogeneous wall of the end apparatus where they are seen clusters of electron-dense materials (within circles). × 21,000.

Fig. 30. The end apparatus at 8 days (longitudinal section). Electron-dense filame from the microvilli (large arrows) project into the wall of the ductule (small arrowhead Diffuse clusters of fine fibers lie in the central cavity (c). × 26,000.

Fig. 31. The end apparatus at 9 days (oblique section). The central cavity (C) is f of fine fibers, the fenestrations through the wall of the apparatus are electron-transpar (arrowheads) and a fuzzy filamentous layer coats the outer surface of the end appara (fil). × 28,000.

Fig. 32. Wall of the end apparatus, showing the irregular fenestrations. C, cent cavity; fil, filamentous layer; mv, microvilli. × 70,000.

Fig. 33. The efferent ductule at 9 days. Both homogeneous inner epicuticle trilaminate outer epicuticle (between arrows) are present. × 28,000.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other glands, for example the tergal gland of *Blatella* (Sreng and Quennedey, 1976), the daughter-cell isogenic clusters are not easily seen. Elongation of the SAG as a whole may be accomplished primarily by these mitoses: over the first 60–70 hr, the mitotic spindles are oriented with the long axis of the gland and thus the process of division would promote growth in that axis.

Migration of pre-mitotic epithelial cells toward the apical surface of the epithelium prior to division has been reported in a variety of epithelia (see Hardings *et al.*, 1971). Apparently this change in cell shape is a *sine qua non* for mitosis and/or cytokinesis. The rounding of the pre-mitotic cell, cytokinesis itself, development of new adherens junctions between daughter-cells and the assumption of columnar shape by the daughter-cells must require repeated readjustments in intercellular relationships. Microtubules and microfilaments may both account for the shape changes. The fact that the metaphase nuclei invariably accumulate in the apical zones after colchicine injection argues against a role for microtubules in that aspect of the sequence. Perhaps even more intriguing is the resumption of columnar shape by the two daughter-cells. We can only speculate about the mechanisms which account for these changes.

The phase of cellular morphogenesis involves wrapping and ciliogenesis. Ciliogenesis accompanies morphogenetic movements and

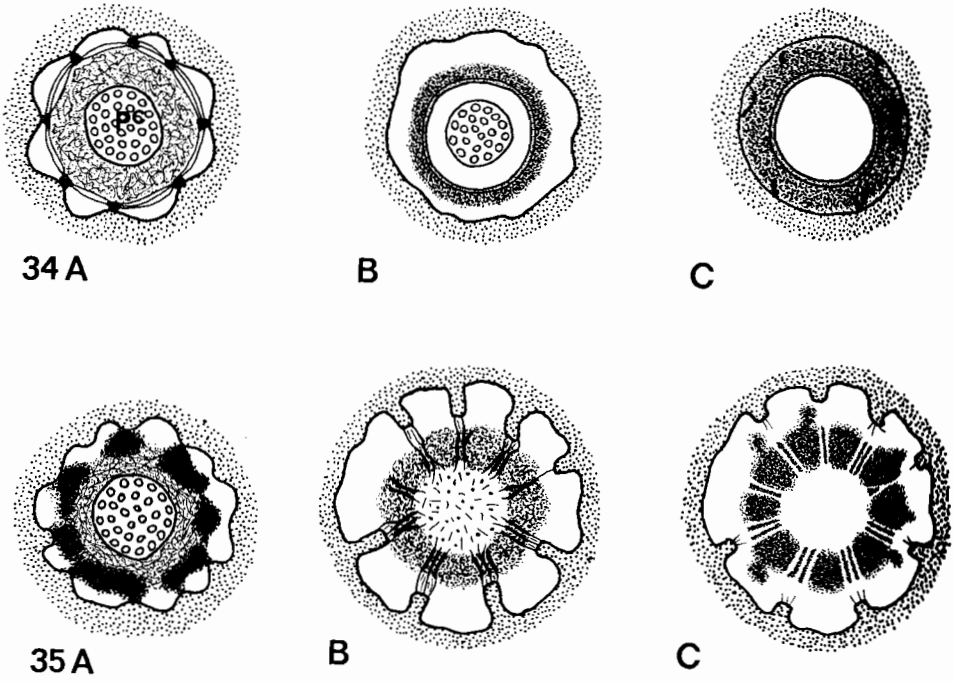


Fig. 34. A diagram of the deposition of the efferent ductule. (a) At 6 days the pseudocilium (pc) lies in a cavity bounded by cell 2. The bounding plasma membrane is gently scalloped and has plaques of dense material at its indentations. (b) At 7 days, the laminate outer epicuticle has been completed and the inner epicuticle is being deposited. (c) At 8 days, the ductule is complete and the plasma membrane is closely appressed to it.

Fig. 35. A diagram of the formation of the end apparatus. (a) At 7 days, large masses of amorphous material form at the invaginations from the surrounding plasma membrane. (b) At 8 days, the amorphous material has coalesced into the wall of the end apparatus. Dense filaments, derived from the surrounding microvilli, run through the wall. (c) At 9 days, the dense filaments have disappeared leaving perforations in the wall.

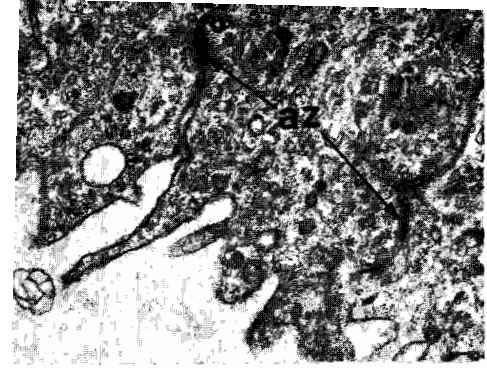
growth of cellular processes in a variety of insect secretory and sensory systems. Within sense organs, the ciliary structures persist in the mature sensillum (Slifer, 1970; McIver 1975; Sanes and Hildebrand, 1976) while in secretory systems with efferent cuticular ductules, the ciliary process characteristically degenerates after formation of the ductule (Sreng and Quennedey, 1975; Selman and Kafatos, 1975; Berry and Johnsen, 1975). The pseudocilium of the SAG is rather like the rudimentary cilium in rat lung tissue (Sorokin, 1968), where in a transitory ciliary structure lacking the central singlets, microtubules appear and then regress.

As is typical of 9+0 cilia, two basal bodies are associated with the cilium (Barnes, 1960; Wolfe, 1972). However, in rat lung the axes of the basal bodies are at right angles to one another (Sorokin, 1968), whereas in the SAG and also in other developing insect epidermal glands (e.g. Selman and Kafatos, 1975; Barbier, 1975; Sreng and Quennedey, 1976), the diplosomes have their long axes in series with those of the ciliary process. We will examine the details of the formation of the basal bodies and the pseudocilium in the future.

The pattern of microsculpture and localized specialization in cuticle reflects the patterning in the underlying epidermal cells. The cellular epithelium and its product, the cuticle, can be effectively represented in two-dimensional models (e.g. Lawrence *et al.*, 1972; French *et al.*, 1976). The primary surface features of the cuticle are defined by the outer epicuticle or cuticulin (Locke, 1966). In a certain sense, the cuticulin is laid down in a two-dimensional subcuticular space. However, the fine details of the surface structure of cuticle require local discontinuities (wrinkles, valleys, hills, interruptions) in the sheet of cuticulin. These localized intrusions are most often formed upon a template of localized outpocketings and inpocketings of the plasma membranes of the cuticulogenic epidermal cells. The importance of epidermal cell surfaces as templates for the outer epicuticle is well illustrated by the process of cuticle deposition in the spermathecal accessory gland of *Tenebrio*.

The efferent ductule is formed within a hollow cylinder of extracellular space, bounded by the pseudocilium on the inside and by the plasma membrane of the ductule-carrying cell (cell 2) on the outside. The intervening space is filled with fibrous materials and appears to be variable in width. We suspect that the role of the pseudocilium is not to serve as an organizing surface on which the efferent ductule is laid down, but that it is mainly present in order to define and stabilize the cylindrical shape of the space. Within the stable, defined space, the efferent ductule is produced entirely by the outer ductule-carrying cell, and the deposition of its outer epicuticle occurs by normal processes at the tips of shallow microvilli of the ductule-carrying cell. Once deposited, the outer epicuticle becomes laminate in a classical pattern (Locke, 1966; Filshie, 1970; Delachambre, 1970), and the ductule has a smooth inner surface. The inner epicuticle is then progressively applied asymmetrically beneath the trilaminate outer epicuticle. We did not observe a laminar structure (Delachambre, 1970) in the inner epicuticle.

The end apparatus is laid down in a slightly different manner. The corresponding cylindrical extracellular space is bounded entirely by plasma membranes of the secretory cell (cell type 1). The ciliary process, produced by this secretory cell, defines the inner boundaries of the space. The outer trilaminate epicuticle is absent; the homogeneous inner epicuticle is the only layer deposited by the secretory cell. The patches of cuticle precursors are large and irregular (Fig. 24). As the inner epicuticle is deposited, the pseudocilium breaks down and the lumen of the end apparatus seems to be filled mostly with coarse aggregations of fine filaments, presumably attached to the microvilli and running through the wall of the end apparatus. The wall of the end apparatus is variable in thickness and has many irregular projections on both outside and inside surfaces. We assume that the irregularities stem from the lack of a smooth outer epicuticle on which successive thin zones of inner epicuticle can be layered. The dense cuticular precursors coalesce in the web-work of glycocalyx-like filaments which are similar to the epicuticular filaments of the spermatheca (Happ and Happ, 1975). After these filaments retract or disintegrate, the narrow tortuous perforations which remain, allow secretions to penetrate the wall of the end apparatus and to flow down the efferent ductule. Deposition of the vestibule cuticle appears to involve the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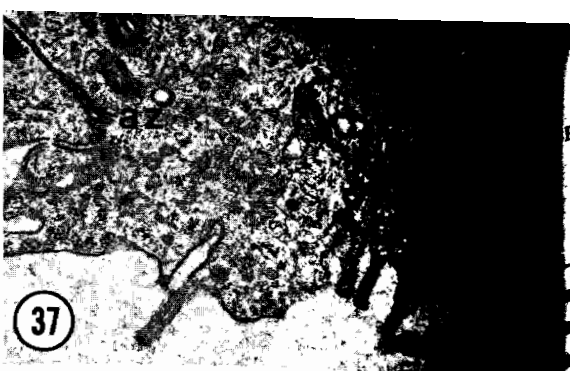
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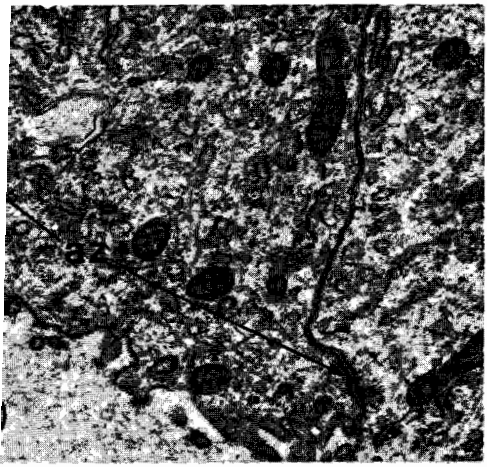

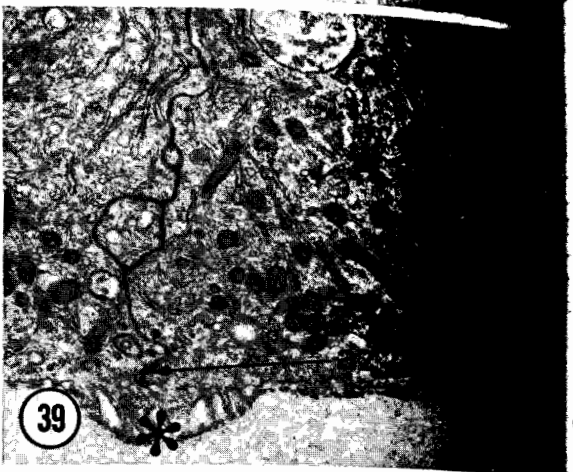
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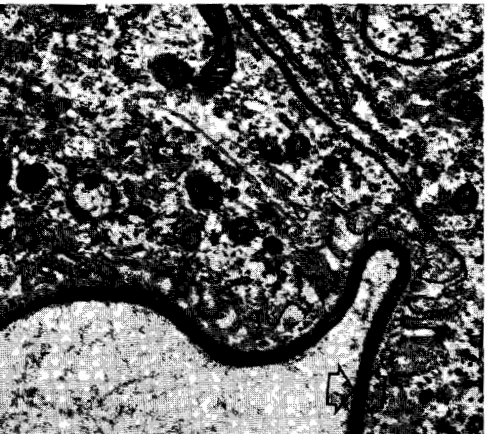

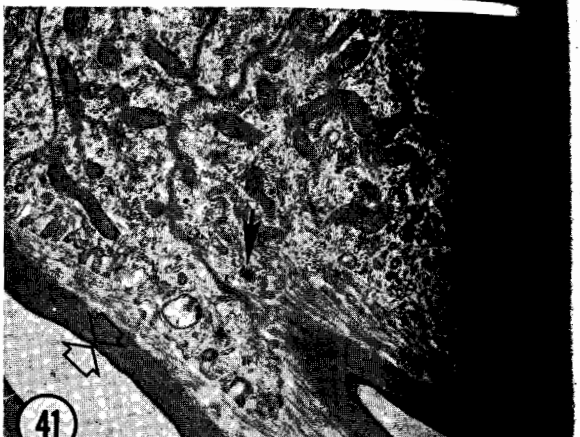
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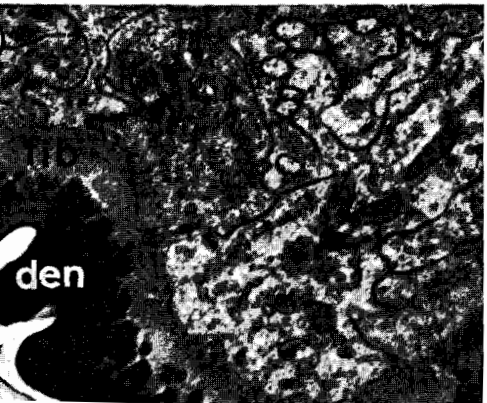
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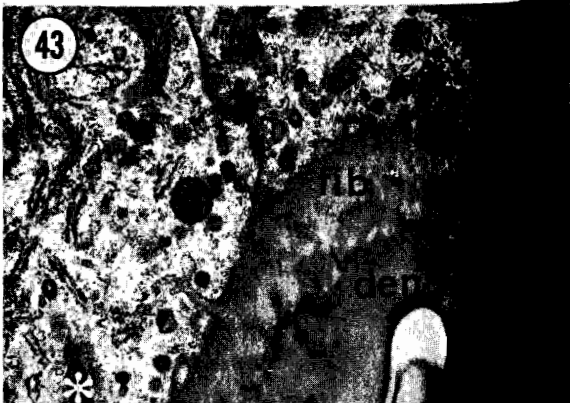
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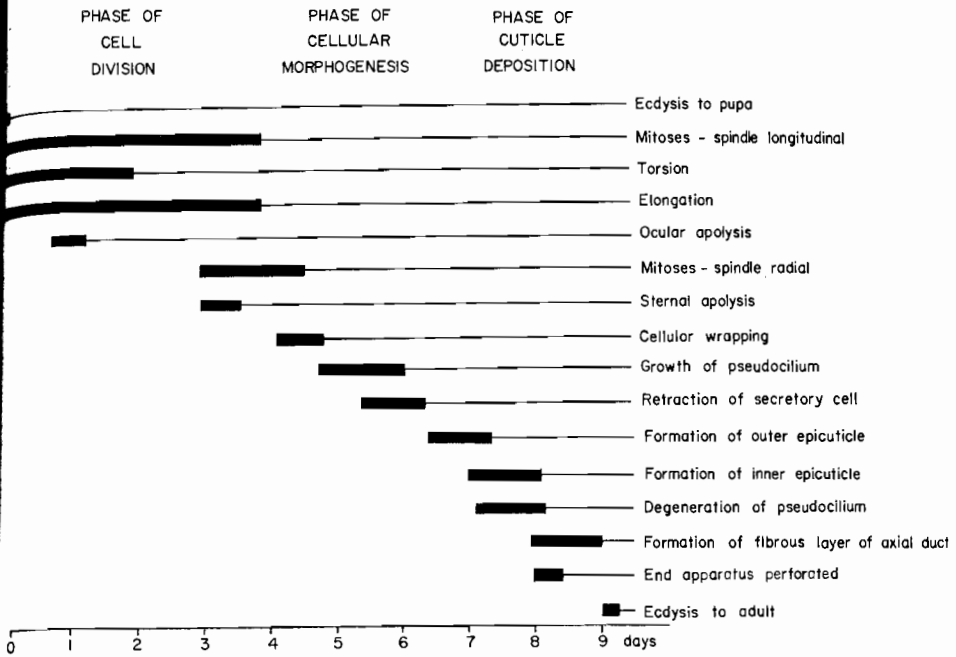

Fig. 44. Diagram showing the main events in the SAG development between larval and pupal ecdysis.

Figs. 36–43. Apical surface of epithelium. The lumen is at the bottom of the micrograph.

Fig. 36. One day. Irregular cellular processes project into the lumen. az, adhering zonule. × 31,000.

Fig. 37. Three days. Both apical adhering zonules (az) and septate junctions (sj) are present. The arrows indicate small secretory vesicles adjacent to and fusing with the apical membrane. × 24,000.

Fig. 38. Five days. The apical surface is smoother than in younger stages. az, adhering zonule. × 79,000.

Fig. 39. Six days. Short microvilli with dense plaques at their tips project into the lumen. At the margins of the cells, the microvilli are longer, creating a ridge (asterisk). × 13,600.

Fig. 40. Seven days. Both the inner epicuticle and the outer epicuticle (between arrows) have been deposited. × 19,000.

Fig. 41. Seven and a half days. Outer epicuticle is shown between the hollow arrows. The 'inner epicuticle' is apparently continuous with the dense layer (den). Beneath the dense layer are parallel fibers (fib) which stream downward toward the cells. Solid arrows indicate secretory vesicles. × 12,600.

Fig. 42. Eight days. The inner surface of the dense layer (den) is rather irregular. Apical microvilli are fewer than in earlier ages (compare with Fig. 39). fib, fibrous layer. × 12,800.

Fig. 43. Nine days. The cells are tightly appressed to the inner surface of the fibrous layer (fib). Tonofibrillar-like projections of the fibrous layer (asterisk) are surrounded by intracellular microtubules. den, dense layer. × 18,600.

same sequence of biosynthetic events as the deposition of the efferent ductule.

The cuticle of the axial duct is somewhat thicker and is thrown up into coarse corrugations. The fibrous inner layers apparently begin as parallel arrays of fibers streaming from the microvilli, usually perpendicular to the plane of the cuticle. The dense layer appears to consist of materials rather like the inner epicuticle, first deposited in even layers (Fig. 25) and then in discrete patches. Finally, the interstices between the filaments are lightly filled in with material of somewhat lower electron density.

After ecdysis to the adult, the secretory cells show dramatic changes. Rough endoplasmic reticulum proliferates, Golgi regions enlarge, and secretions fill the cavity around the end apparatus (Happ and Happ, 1970). The increased synthesis of differentiation-specific proteins is very marked over the first 6 days after ecdysis (Happ and Yuncker, 1977).

Some of the developmental events in the SAG may reflect the expression of an autonomous program while others may be under hormonal control. The likely hormonal candidates are ecdysone and bursicon, both of which have been studied in pupal *Tenebrio* (Delbecque, 1976; Delbeque *et al.*, 1975; Grillot *et al.*, 1976). It is tempting to associate changes in these hormones with developmental events, as determined by Delbecque (1976). For example, ecdysone titre begins to rise at 3 days (when spindle orientation changes); it reaches a strong peak at 4–5 days (when cell division ceases and ciliary growth proceeds rapidly), and falls to a minimum at 7 days (when the pseudocilia degenerate and cuticle deposition accelerates).

An increase in blood-borne bursicon occurs just after the ecdysone fall (Grillot *et al.*, 1976). The roles of these and other hormonal signals in the differentiation of the spermathecal accessory gland remain to be established. But at present, we do have good morphological indices of the ongoing differentiation and thus can assess precisely the requirements of hormones for the support of these processes.

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References

BARBIER, R. 1974. Différenciation de structures ciliaires et mise en place des canaux au cours de l'organogenèse des glandes collétériques de *Galleria mellonella* L. (Lépidoptère, Pyralidae). *J. Microscopie, Paris*, **24**, 315–326.

BARNES, B. G. 1961. Ciliated secretory cells in the pars distalis of the mouse hypophysis. *J. Ultrastruct. Res.*, **5**, 453–467.

BERRY, S. J. and JOHNSEN, E. 1975. Formation of temporary flagellar structures during insect organogenesis. *J. Cell Biol.*, **65**, 489–492.

BUTZ, A. 1957. Effects of sodium, potassium, and calcium on the isolated heart of the mealworm, *Tenebrio molitor* L. *J. N.Y. ent. Soc.*, **65**, 22–31.

DELACHAMBRE, J. 1970. Etudes sur l'épicuticule des insectes. I. Le développement de l'épicuticule chez l'adulte de *Tenebrio molitor* L. *Z. Zellforsch. mikrosk. Anat.*, **108**, 380–396.

DELBECQUE, J. P., PROST, M., MAUNE, B. F., DELACHAMBRE, J., LAFONT, R. and MAUCHAMP, B. 1975. Dosage de la β-ecdysone par fragmentographie de masse au cours du dernier stade larvaire de *Tenebrio molitor* L. (Insecte, Coléopterè). *C. r. hebd. Séanc. Acad. Sci., Paris*, **281D**, 309–312.

DELBECQUE, J. P. 1976. Taux d'ecdysones et cycle de mue au cours de la metamorphose de *Tenebrio molitor* L. (Insecte, Coléopterè). Thèse de Docteur de Troisième cycle, Dijon.

ERNST, K.-D. 1969. Dei Feinstruktur von Reichsensillen auf der Antenne des Aaskäfers *Necrophorous* Coleoptera. *Z. Zellforsch. mikrosk. Anat.*, **94**, 72–102.

FILSHIE, B. K. 1970. The fine structure and deposition of the larval cuticle of the sheep blowfly (*Lucilia cuprina*). *Tissue & Cell*, **2**, 479–498.

FRIEDEL, T. and GILLOT, C. 1977. Contribution of male-produced proteins to vitellogenesis in *Melanoplus sanguinipes*. *J. Insect Physiol.*, **23**, 145–151.

FRENCH, V., BRYANT, P. J. and BRYANT, S. V. 1976. Pattern regulation in epimorphic fields. *Science*, **193**, 969–981.

GADZAMA, N. G., HAPP, C. M. and HAPP, G. M. 1977. Cytodifferentiation in the accessory glands of *Tenebrio molitor*. II. Patterns of leucine incorporation in the tubular glands of post-ecdysial adult males. *J. exp. Zool.*, **200**, 211–222.

GERBER, G. 1976. Reproductive behavior and physiology of *Tenebrio molitor* (Coleoptera: Tenebrionidae). III. Histogenetic changes in the internal genitalia mesenteron, and cuticle during sexual maturation. *Can. J. Zool.*, **54**, 990–1002.

GILULA, N. B. and SATIR, P. 1972. The ciliary necklace. A ciliary membrane specialization. *J. Cell Biol.*, **53**, 494–509.

GRILLOT, J. P., DELACHAMBRE, J. and PROVANSAL, A. 1976. Rôle des organes périsympathiques at dynamique de la sécrétion de la bursicon chez *Tenebrio molitor*. *J. Insect Physiol.*, **22**, 763–780.

HAPP, G. M. and HAPP, C. M. 1970. Fine structure and histochemistry of the spermathecal gland in the mealworm beetle, *Tenebrio molitor*. *Tissue & Cell*, **2**, 443–466.

HAPP, G. M. and HAPP, C. M. 1975. Fine structure of the spermatheca of the mealworm beetle (*Tenebrio molitor* L.). *Cell Tiss. Res.*, **162**, 253–269.

HAPP, G. M., YUNCKER, C. and HUFFMIRE, S. A. 1977. Cytodifferentiation in the accessory glands of *Tenebrio molitor*. II. Patterns of leucine incorporation in the tubular glands of post-ecdysial adult males. *J. exp. Zool.*, **200**, 223–236.

HAPP, G. M. and YUNCKER, C. 1977. Cytodifferentiation in the accessory glands of *Tenebrio molitor*. IV. Patterns of leucine incorporation in the spermathecal accessory glands of the post-ecdysial adult. *J. Insect Physiol.* (submitted).

HARDING, C. V., REDDAN, J. R., UNAKER, N. J. and BAGGCHI, M. 1971. The control of cell division in the ocular lens. *Int. Rev. Cytol.*, **31**, 215–300.

HINTON, H. E. 1974. Symposium on reproduction of arthropods of medical and veterinary importance. III. Accessory functions of seminal fluid. *J. med. Ent.* **11**, 19–25.

HUET, C. 1974. Etude expérimentale des conditions de l'organogenèse des canaux et glandes annexes de l'appareil reproducteur femelle du *Tenebrio molitor* L. *Wilhelm Roux Arch. EntwMcch. Org.* **177**, 263–278.

KAFATOS, F. C. 1972. The cocoonase zymogen cells of silk moths: a model of terminal cell differentiation for specific protein synthesis. *Curr. Top. Develop. Biol.*, **7**, 125–191.

KAHN, A. 1971. *Lectures on Developmental Physiology* (translator R. Milkman). Springer-Verlag, New York.

LAI-FOOK, J. 1973. The fine structure of Verson's glands in molting larvae of *Calpodes ethlius* (Hesperiidae, Lepidoptera). *Can. J. Zool.*, **51**, 1201–1210.

LAWRENCE, P. A., CRICK, F. H. C. and MUNRO, H. 1972. A gradient of positional information in an insect, *Rhodnius*. *J. Cell Sci.*, **11**, 815–853.

LOCKE, M. 1966. The structure and formation of the cuticulin layer in the epicuticle of an insect, *Calpodes ethlius* (Lepidoptera, Hesperiidae). *J. Morphol.*, **118**, 461–494.

LOWER, H. F. 1955. A trichrome stain for insect material. *Stain Technol.*, **30**, 209–212.

MCIVER, S. B. 1975. Structure of cuticular mechanoreceptors of arthropods. *A. Rev. Ent.*, **20**, 381–397.

NOIROT, C. and QUENNEDEY, A. 1974. Fine structure of insect epidermal glands. *A. Rev. Ent.*, **19**, 61–80.

PERCY, J. E. 1972. Ultrastructure of sex-pheromone gland cells and cuticle before and during release of pheromone in female eastern spruce budworm, *Choristoneura fumiferana* (Clem.) (Lepidoptera: Tortricidae). *Can. J. Zool.*, **52**, 695–705.

POELS, A. 1972a. Histophysiologie de voies genitale femelles de *Tenebrio molitor* L. (Coleoptère: Tenebrionidae). *Ann. Soc. R. zool. Belg.*, **102**, 143–169.

POELS, A. 1972b. Histophysiologie de voies genitale males de *Tenebrio molitor* L. (Coleoptère: Tenebrionidae). *Ann. Soc. R. zool. Belg.*, **102**, 199–234.

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.

SANES, J. R. and HILDEBRAND, J. G. 1976. Structure and development of antennae in a moth, *Manduca sexta*. *Devl Biol.*, **51**, 282–299.

SELMAN, K. and KAFATOS, F. C. 1975. Differentiation in the cocoonase producing silkmoth galea: ultrastructural studies. *Devl Biol.*, **46**, 132–150.

SLIFER, E. 1970. The structure of arthropod chemoreceptors. *A. Rev. Ent.*, **15**, 121–142.

SOROKIN, S. P. 1968. Reconstructions of centriole formation and ciliogenesis in mammalian lungs. *J. Cell Sci.*, **3**, 207–230.

SRENG, L. and QUENNEDEY, A. 1976. Role of a temporary ciliary structure in the morphogenesis of insect glands. An electron microscope study of the tergal glands of male *Blatella germanica* L. (Dictyptera, Blatellidae). *J. Ultrastruct. Res.*, **56**, 78–95.

WIGGLESWORTH, V. B. 1953a. The origin of sensory neurons in an insect, *Rhodnius prolixus* (Hemiptera). *Q. J. microsc. Sci.*, **94**, 93–112.

WIGGLESWORTH, V. B. 1953b. Determination of cell function in an insect. *J. Embryol. exp. Morph.*, **1**, 269–277.

WOLFE, J. 1972. Basal body fine structure and chemistry. *Adv. Cell molec. Biol.*, **2**, 151–192.