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Morphology of the Aedeagal Gland of the Male Mealworm Beetle (*Tenebrio molitor* L.)

PATRICK J. DAILEY AND GEORGE M. HAPP
Department of Zoology, University of Vermont, Burlington, Vermont 05405

ABSTRACT The aedeagal gland of male *Tenebrio molitor* consists of numerous acini containing several secretory units (organules) of three epithelial cells in series. The distal cortical cell and intermediate cell are secretory cells. Secretory products are passed into microvilli-lined extracellular reservoirs. From these storage areas products flow through minute canaliculi and into the efferent ductule. Canaliculi, cuticular trabeculae, and fibrillar material are characteristic features of the efferent ductules within the extracellular reservoirs of secretory cells. After passing from the secretory cells, the efferent ductule penetrates the basal ductule cell. The thin epicuticle that comprises the wall of the ductule is confluent with the epicuticle of the cuticular sheath forming the wall of the genital pocket. Secretory products flow from the cortical cell ductule into the intermediate cell and eventually empty into the genital pocket. A chemical reaction apparently takes place in the intermediate cell ductule, resulting in a frothy secretion product. When released from the ductule, this frothy product forms a foam-like layer that coats the inner wall of the genital pocket. Ultrastructural and probable functional aspects of this gland are described and discussed.

Insect pheromones act to attract members of the opposite sex, to release mating behavior, to mark territories and oviposition sites, to alarm other members of a colony, to recruit nestmates to a food source, and to regulate physiologic functions (see reviews by Wilson and Bossert, '63; Happ, '73; Birch, '74; Shorey, '76). As Percy and Weatherston ('74) emphasize, the morphology and location of the glands responsible for producing pheromones are as diverse as the roles pheromones play. For the most part, the glandular epithelium is of ectodermal origin and is associated with cuticle-lined ductules that channel secretory products to their point of release.

The pheromone system of *Tenebrio molitor* is complex. Female scent attracts males and releases male mating behavior (Valentine, '31; Tschinkel et al., '67; Happ and Wheeler, '69). Females respond to male scent, often with oviposition behavior (Valentine, '31; Happ, '69; August, '71). When stimulated by female scent, males also release an antiaphrodisiac scent that inhibits the response of other males to the female attractant (Happ, '69). Mature males and females emit primer pheromones that act on immature females to increase the rate of growth of their terminal oocytes and to enhance the level of sex pheromone emission (Happ et al., '70).

We have recently discovered two exocrine glands associated with the terminal abdominal segments of *T. molitor* that may be responsible for the production of at least one of these male pheromones. One gland is associated with the aedeagal sheath and the other opens into the spicular pocket. The gross morphology of both and the ultrastructure of the aedeagal gland are presented in this paper.

MATERIALS AND METHODS

Aedeagal glands were removed from adult virgin *Tenebrio molitor* males less than 1 day, 3 days, 6 days, and greater than 8 days old. Colonies were kept at room temperature and maintained on potato and Purina Chick Startena (Ralston Purina).

P.J. Dailey's current address is University of Southern Illinois, School of Dentistry, Alton, IL 62002.

Address reprint requests to George M. Happ, Department of Zoology, University of Vermont, Burlington, VT 05405.

Light microscopy

Removal of the aedeagal apparatus and the spicular pocket, as illustrated in Figure 1A,B, C, was performed by immersing decapitated beetles in either phosphate-buffered saline, pH 7.9 (Dulbecco and Vogt, '54), or *Tenebrio* saline, pH 7.3 (Butz, '57), grasping the abdomen with forceps at the 7th abdominal segment and slowly pulling away from the remainder of the abdomen held in position by another pair of forceps. Once removed, the structures were immersed in 3% glutaraldehyde or alcoholic Bouin's fixatives for 1–2 h. Whole-gland staining was done on glutaraldehyde-fixed tissues or fresh unfixed tissue. In either case the aedeagal apparatus was stained for 1 h in 0.5% safranin 0 in 70% ETOH, 0.1% aqueous toluidine blue-methylene blue, or 0.1% aqueous brilliant cresyl blue (Barbosa, '74). Destaining was done in 30% alcohol for selected time periods. Glands for wax histology were dehydrated in a graded ethanol series, embedded in Paraplast Plus embedding medium (Lancer), and stained with Heidenhain's iron haematoxylin or Lower's Trichrome Stain (Barbosa, '74).

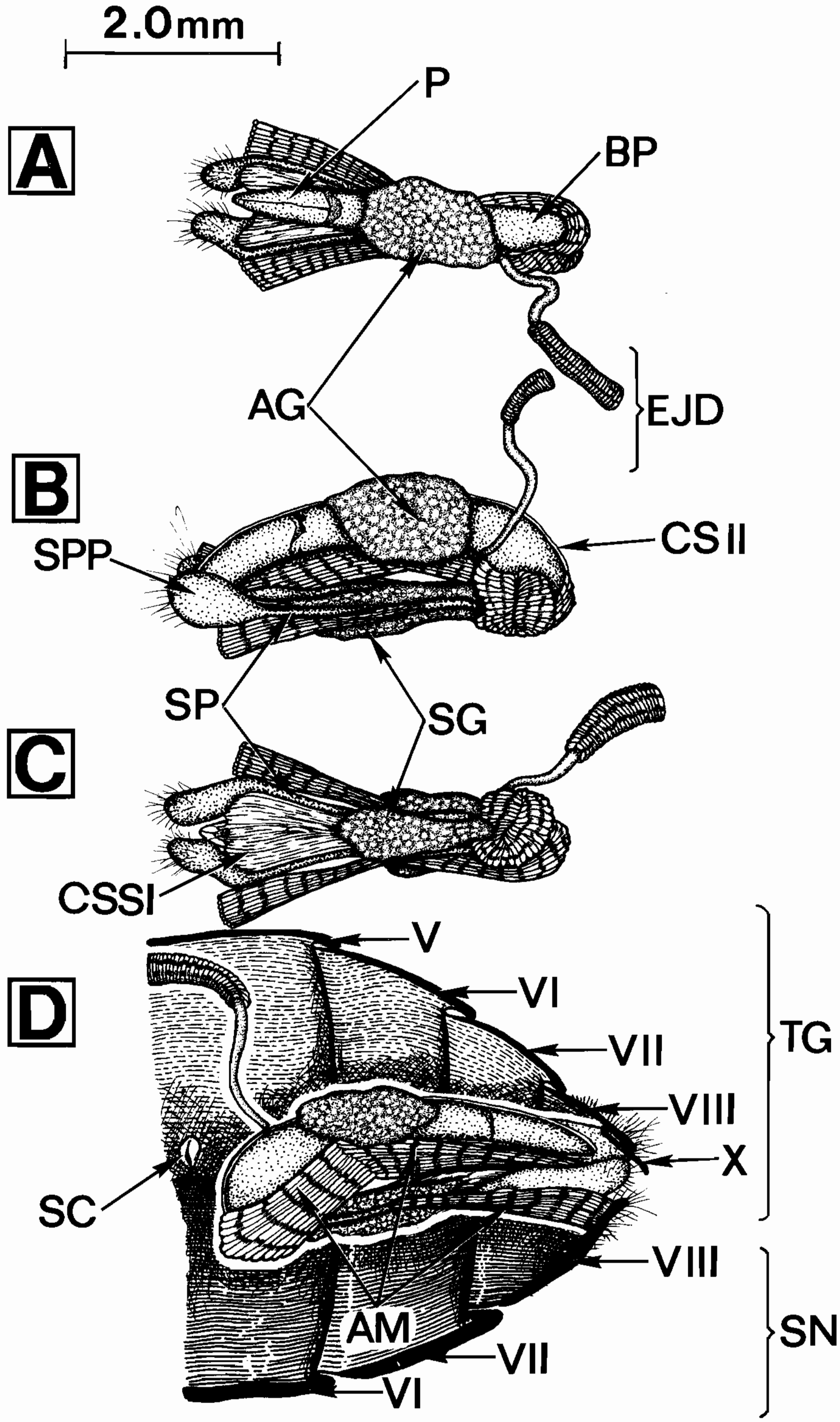
Electron microscopy

Aedeagal structures were removed while decapitated males were immersed in 3% glutaraldehyde (0.1 M phosphate buffer, pH 7.4) by the method described above. Once removed, the aedeagal apparatus was transferred immediately to fresh glutaraldehyde for 1 h. The aedeagal gland and the associated cuticular sheath of the basal piece were then removed from the aedeagus by slowly sliding the sheath posteriorly. Further preparation of aedeagal glands followed techniques outlined in Dailey et al. ('80) for the bean-shaped accessory glands of *T. molitor*. Thin sections were stained for 20 min each in 2.5% aqueous uranyl acetate and Reynolds' lead citrate (Reynolds, '63). Sections were examined on a Philips EM 200 transmission electron microscope.

Abbreviations

AC, acinus
AG, aedeagal gland
AM, aedeagal muscles
BL, basal lamina
BP, basal piece
C, cuticle
CC, cortical cell(s)
CCN, cortical cell nucleus
CM, connecting membrane
CMV, cortical cell microvilli
CN, centriole
CP, coated pit(s)
CSI,II, cuticular sheaths (of aedeagus)
CSSI, cuticular sheath of spicular invagination
CT, cuticular trabeculae
CV, coated vesicle
DC, ductule cell
DL, ductule lumen
E, epidermis
EJD, ejaculatory duct
ER, extracellular reservoir of cortical cell
F, funnel
FC, fibrillar canaliculi
FM, fibrillar material
G, gonopore
GC, Golgi complex(es)
GP, genital pocket
H, haemocoel
IC, intermediate cell
ICN, intermediate cell nucleus
IE, inner epicuticle
IF, intermediate filaments
IMV, intermediate cell microvilli
IR, extracellular reservoir of intermediate cell
IS, inner subcuticle
L, lumen of aedeagus
LB, lamellar body
LD, lipid droplet(s)
LP, lateral penis rod(s)
LY, lysosome(s)
M, mitochondria
MB, microbody
MF, myelin figures
MSG, membrane-containing secretory granule
MT, microtubules
MVB, multivesicular body
OE, outer epicuticle
OS, outer subcuticle
P, paramere
PC, procuticle
PE, penis
PV, pinocytotic vesicles
RB, residual body
RC, reservoir canaliculi
RER, rough endoplasmic reticulum
S, secretion
SC, spiracle
SD, septate desmosome
SEP, setae of paramere
SER, smooth endoplasmic reticulum
SG, spicular gland
SI, spicular invagination
SN, sternites
SP, spicule
SPP, spicule plate
T, tracheoles
TG, tergites
TZ, transition zone

Fig. 1. Views of the aedeagal apparatus of *T. molitor*. A: Dorsal view; the aedeagal gland (AG) is situated between the paramere (P) and basal piece (BP) of the aedeagus. B: Right lateral view; note that the spicular gland (SG) lies below the cuticular sheath (CSSI) and between the cuticular spicules (SP) (see Fig. 2A). C: Ventral view. D: Left lateral view of the aedeagal apparatus as it appears in the abdomen. CSSI, cuticular sheath of spicular invagination; TG, tergites; SN, sternites; AM, aedeagal muscles; SPP, spicule plates; SC, spiracle; EJD, ejaculatory duct.


2.0mm
A
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BP
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EJD
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CS II
SPP
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CSSI
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VIII
X
TG
SC
VIII
AM
VII
VI
SN

OBSERVATIONS AND RESULTS

Gross morphology

At the posterior tip of the abdomen of male *Tenebrio molitor*, the hindgut and the reproductive tract open to the exterior in a space between the tergite of the tenth abdominal segment and the sternite of the eighth (Fig. 2). Three cuticular inpocketings lead anteriorly from this space. The most dorsal is the hindgut, the middle one is the aedeagal apparatus, and the most ventral is the spicular apparatus.

Except during sexual activity, the aedeagus (intromittent organ) is retracted anteriorly into the abdomen. The aedeagus proper consists of a penis surrounded by stiff cuticular structure, the tegmen. The tegmen includes two distal fused parameres (which are covered with setae) and a single basal piece (Figs. 1,2). The tegmen is connected to the penis by a flexible cuticular sheath. The tegmen is surrounded by yet another cuticular sheath, the flexible cuticle (CS II, Fig. 2) that comprises the inpocketing. This second sheath is attached anteriorly to the basal piece. When the aedeagus is retracted, the penis is telescoped anteriorly into the tegmen.

The spicular inpocketing ends blindly. Its shape is maintained by two strong cuticular struts, the spicules (SI, Fig. 2A). The spicules may be derived from the ninth sternite, and provide sites for the attachment of the genitalic muscles (AM, Fig. 1D) and also act as a guide along which the tegmen moves in and out. A short retractor muscle connects the spicules to the tegmen anteriorly, and paired slender protractors provide the posterior attachment (Doyen, '66).

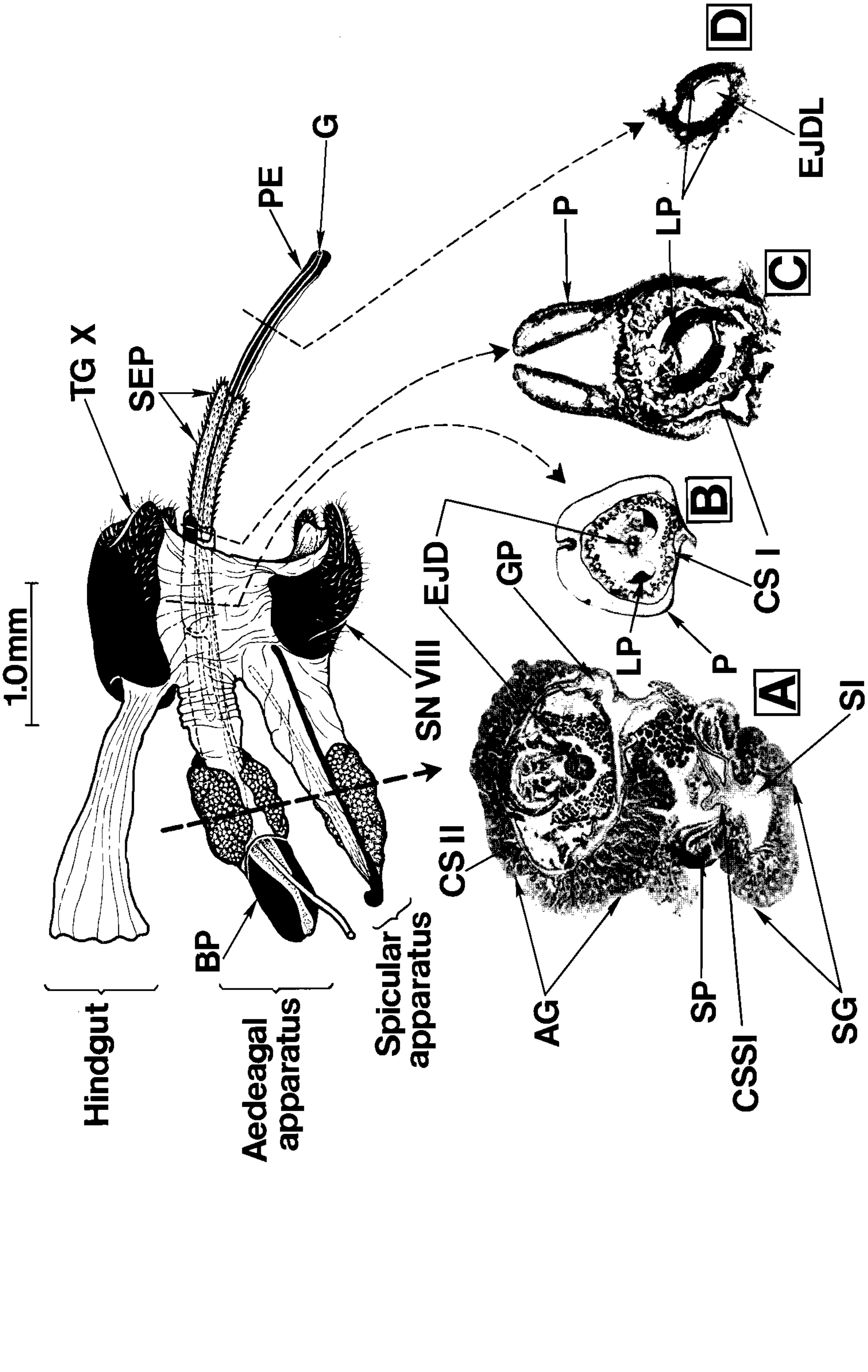
The aedeagal gland is a flat oval pad that wraps around the dorsal surface of the second cuticular sheath of the aedeagus. In fresh preparations, the gland measures 1.6 mm × 2.2 mm; its total volume is approximately 2 μl. The glandular mass does not fuse ventrally (Figs. 1C, 2). The gland appears to be a derivative of the intersegmental membrane between the tenth and eleventh tergite. When the aedeagus is retracted (as in a typical dissection), the aedeagal gland is just anterior to the parameres and midway along the basal piece (Fig. 1A,B). When the aedeagus is fully everted, the basal piece slides posteriorly and the second cuticular sheath and aedeagal gland are pulled along so that the cuticle just posterior to the aedeagal gland is folded in an accordion-like manner. The gland passes its products through numerous fine cuticular ductules and thence into the space between the genital pocket and the basal piece and eventually to the exterior (Fig. 2).

A second glandular mass, here termed the spicular gland, is wrapped around the ventral surface of the spicular pocket. The spicular gland appears to be derived from the intersegmental membrane between the eighth and ninth sternites. Like the aedeagal gland, it empties into its cuticular pocket by fine efferent ductules. In a dissection of the unfixed aedeagal apparatus, the aedeagal and spicular glands are similar in color to the adjacent muscles and connective tissue; this similarity may account for their being overlooked previously.

The aedeagal gland

The aedeagal gland is composed of numerous acini. Individual acini consists of 5–9 secretory units or organules (Fig. 3). Each organule contains three cells in series: a distal cortical secretory cell, an intermediate secretory cell, and a basal ductule cell (Fig. 30). Cortical and intermediate cells are readily distinguished by the fact that cortical cell nuclei are slightly larger (7.0 μm diameter) and more lightly staining than intermediate cell nuclei (5.0 μm diameter) (Figs. 3–6). The epicuticular lining of the ductules of the aedeagal gland is continuous with that of the second cuticular sheath (Figs. 3, 29), and thus products of the gland pass into the genital pocket. Ultrastructurally the aedeagal gland and the spicular gland show no obvious morphologic differences. This paper will concentrate solely on the detailed morphology of the aedeagal gland.

Fig. 2. Left lateral view of an extended aedeagus as it appears during copulation (posterior to the right). All muscles and soft tissues have been removed by KOH treatment. Aedeagal and spicular glands (AG, SG) have been stippled in to show their location with respect to the cuticular sheaths (CSI,II, CSSI). A: Transverse section of anterior aspect of aedeagus. The bracelet-like aedeagal gland (AG) partially surrounds the basal piece (BP) and empties its secretory product into the genital pocket (GP). The spicular gland (SG) is located on the ventral aspect of the cuticular sheath (CSSI) and empties its secretory product into the pocket of the spicular invagination (SI). Gland secretions collect in the cuticular-lined genital pocket and spicular invagination. B: Transverse section through anterior region of the paramere (P). The ejaculatory duct (EJD) enters the anterior region of the penis (PE) and travels posteriorly, eventually opening at the gonopore (G). C: Transverse section through posterior region of paramere. D: Transverse section through penis. EJDL, ejaculatory duct lumen; LP, lateral penis rod(s); TGX, tenth tergite; SN VIII, eighth sternite; SEP, setae of paramere; SP, spicule. A, × 360; B, × 365; C, × 720; D, × 730.


1.0mm
TG X
SEP
PE
G
Hindgut
Aedeagal apparatus
BP
Spicular apparatus
SN VIII
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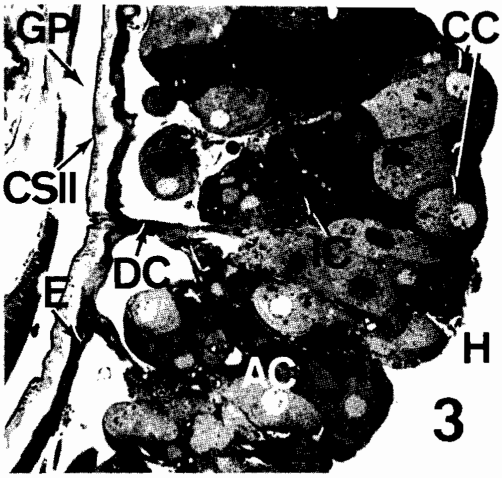


Fig. 3. Thick section (0.75 μm) of the aedeagal gland from an adult male over 8 days post-ecdysis. Note how clusters of cells comprising several secretory units are segregated in acini. IC, intermediate cell; CC, cortical cells; AC, acinus; DC, ductule cell; CSII, cuticular sheath; GP, genital pocket; E, epidermis. Toluidine blue, × 1,000.

The cortical secretory cell

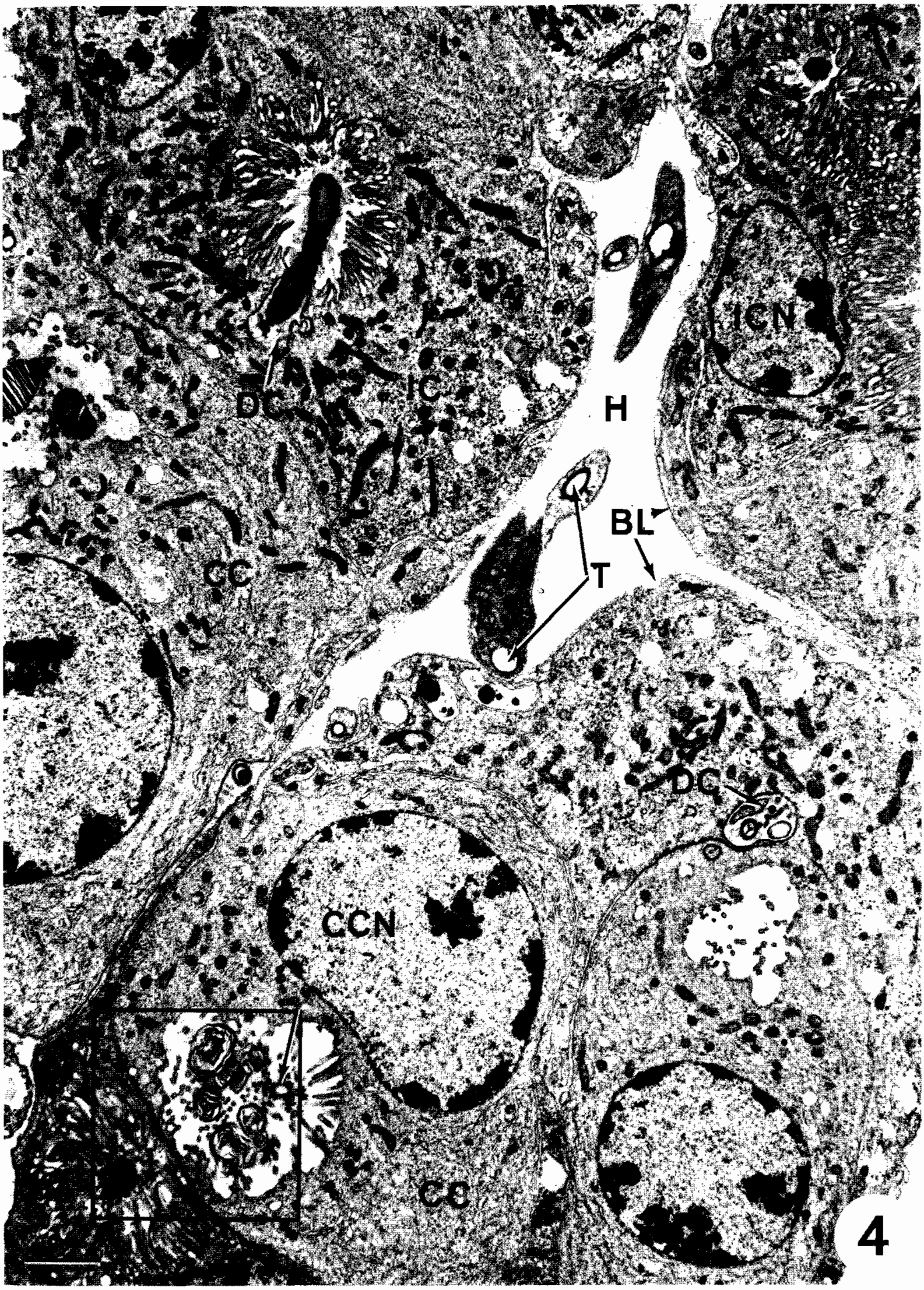
A thin basal lamina surrounds each acinar cluster of organules (Fig. 4, 14). The basal surfaces of the cortical secretory cells and those of the intermediate secretory cell are extensively infolded and intertwined in the zone immediately inside the basal lamina (Fig. 7A). The interweaving of these processes may reflect an adaptation for exchange of materials within the intercellular spaces and may also help to maintain the structural integrity of each acinus.

At the peak of secretory activity, 8 days after adult ecdysis, the cortical secretory cells contain an extensive rough endoplasmic reticulum, many mitochondria, and several Golgi complexes (Figs. 4–6). There are numerous membrane-bound organelles, including multivesiculate bodies, lysosome-like bodies, coated vesicles, and putative peroxisomes (Figs. 6, 8, 12, 13). Coated pits were seen on the plasma membrane toward the hemolymph, as well as toward the secretory reservoir (Figs. 12, 13), and coated vesicles were most common near the Golgi complex (Fig. 8). The cells contain a large number of membrane-bound bodies with membranous inclusions and a finely granular matrix (Figs. 8, 12, 13). We believe these bodies to be peroxisomes, on the basis of their morphologic and histochemical similarities to secretion granules of the spermathecal gland of the beetle *Dytiscus marginalis* (Autuori et al., '71), and granules present in the sternal defensive glands of the cockroach *Eurycotis floridana* and those associated with the tracheal glands of the cockroach *Leucophaea maderae* (Brossut and Sreng, '80). The maturation of the cytomembrane system in these cells, and its reorganization after secretory climax will be described in a subsequent paper (Dailey and Happ, unpublished observation).

Secretory products of the cortical cell exit into an enclosed extracellular reservoir, which is surrounded by the cell (Figs. 4–8, 12–14). Irregularly spaced microvilli, containing a rather ordered ring of intermediate-sized filaments (each about 88 Å in diameter), project into the reservoir, and the microvilli apparently anchor the tip or end apparatus of the efferent ductule (Figs. 7d, 9). Around the margins of the reservoir are microtubules (240–350 Å diameter) and intermediate filaments (88 Å diameter) (Figs. 12, 14), which may help to maintain the shape of this distinct enclosed space. Mitochondria are most concentrated in the vicinity of the reservoir (Figs. 4–7, 14), as are membrane-bound organelles that contain stocks of membranous lamellae (Figs. 8, 13). In their morphology, these organelles resemble peroxisomes. We believe that they contribute their contents to the secretory product. In newly eclosed animals, the reservoir contents come to resemble the contents of the putative secretory granules. In parallel with the increased density of granular materials, membranous whorls or myelin figures and lamellate bodies accumulate progressively until they almost fill the reservoir (Figs. 7–9, 12).

The reservoir is drained by an efferent cuticular ductule. The end apparatus of this ductule, which lies within the reservoir, is riddled with minute canaliculi (219–302 Å diameter) through which secretions can pass into the lumen (Figs. 9–11, 14). The irregular blocks of

Fig. 4. Section through several acini of the aedeagal gland showing several secretory organules in close proximity of each other. Area within square illustrates the distal region of the extracellular reservoirs of a cortical and an intermediate cell. Note the presence of myelin figures in the extracellular reservoir of the cortical cell (CC) and the transverse section through the distal aspect of the end apparatus (arrow at upper right of square enclosure) (compare with Figs. 5,6). DC, ductule cell; IC, intermediate cell; T, tracheoles; H, haemocoel; BL, basal lamina; CCN, cortical cell nucleus; ICN, intermediate cell nucleus. Day 0 adult; bar equals 2 μm.


ICN
DC
IC
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CC
4

cuticle between the canaliculi are quite electron-dense in thin sections (Fig. 11). The cuticle of the end apparatus can be traced through confluent layers to the inner epicuticle of the body surface, and we believe these layers are homologous.

The reservoir of the cortical cell is sealed off from that of the intermediate cell by a narrow isthmus of cytoplasm, composed of a sandwich of projections from both the cortical and intermediate cells (Figs. 9, 15). The closely apposed plasma membranes are joined by septate desmosomes (Fig. 15). The efferent ductule leads from the end apparatus in the cortical cell reservoir to the intermediate cell reservoir. In contrast to the end apparatus, the cuticle in this zone of transition is less dense, lacks canaliculi, and has a distinct trilaminate outer epicuticle (Figs. 15–17).

The intermediate secretory cells

Although the intermediate cells lie toward the center of the acinar cluster, they are not isolated from the hemocoel and its nutrient contents. As noted above, basal projections of these cells are intertwined with similar ones from the cortical cells, and in addition, narrow channels of hemocoelic space actually penetrate between the organules of an acinus. In newly eclosed animals, the cytoplasm of the intermediate cell contains numerous, evenly distributed mitochondria, various dense bodies that are difficult to characterize, predominant smooth endoplasmic reticulum, scattered profiles of rough endoplasmic reticulum, Golgi zones, spherical bodies, and a few putative secondary lysosomes (Figs. 4–6, 18, 22). Several days later the basal cytoplasm is packed with spherical bodies with a homogenous content that we assume are lipid droplets (Figs. 7, 22). Golgi zones bud off electron-lucent vesicles (Figs. 19, 21). Dark bodies, presumably microbodies, were observed (Fig. 20). Coated pits were frequently seen near the secretory reservoir (Fig. 19). As seen in the light micrographs, the nuclei are small and contain much condensed chromatin. Their envelopes contain many nuclear pore complexes (Fig. 18) that appear typically octagonal with a central granule in face view (Fig. 18a) and appear to be pierced by a regularly spaced set of filaments in side view (Fig. 18b).

The secretory reservoir of the intermediate cell, like that of the cortical cell, is surrounded by microvilli and encloses the efferent ductule, but the microvilli, the reservoir contents, and the ductule itself are quite different in detailed structure from their cortical counterparts. Between the bases of the microvilli is an irregular labyrinth of electron-lucent spaces, some of which are secretory vesicles and others of which are continuous with the lumen. Each microvillus contains a central tubule of smooth endoplasmic reticulum (Fig. 18). Midway between this tubule and the plasma membrane are regularly spaced filaments (92 Å diameter) (Dailey and Happ, unpublished observation). The tips of the microvilli approach the outermost zone of the efferent ductule. From the outside inwards, the ductule consists of a fringe of electron-dense fibrils (180–240 Å diameter) that lead successively into a zone of high electron density, and apparently through it, to enter and traverse the inner epicuticle (Figs. 17, 18). The fibrils end in the outer epicuticle, suggesting that they are "epicuticular filaments (fibrils)" in the sense of Locke ('74).

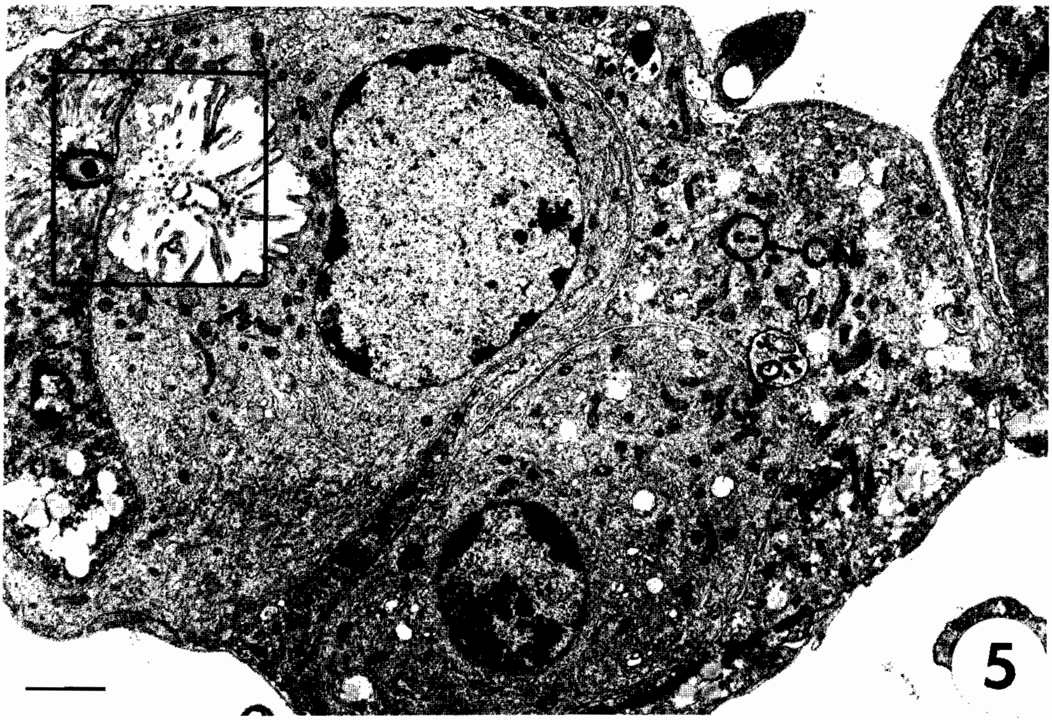
Centrioles were observed in thin sections of cortical, intermediate, and ductule cells (Figs. 5, 14, 22, 23, 28).

The ductule cell

As is common in insect integumentary glands, the function of the ductule cell is not secretory but rather to support and maintain the cuticular conduit by which secretion moves toward the surface of the animal. The ductule cell is linked to the corresponding intermediate cell by septate desmosomes. As it leaves the intermediate reservoir, the ductule loses its filamentous investitute (Fig. 18) and runs toward the aedeagus as simply two concentric layers of inner and outer epicuticle (Fig. 29). As the secretions move through the ductule, they change in staining properties and presumably in consistency. In the cortical reservoir and to a lesser degree in the intermediate reservoir,

Fig. 5. Section through the cells described in Figure 4 is taken at a point where the ductule is about to run into the intermediate cell. Note the absence of myelin figures in the reservoir of the cortical cell. An oblique section through a centriole is also visible. CN, centriole. Day 0 adult; bar equals 2 μm.

Fig. 6. Section through the basal region of the intracellular reservoirs of the cortical and intermediate cells of Figures 4 and 5. Note that the ductule is continuous between cells and that the outer epicuticle is found within the ductule shortly before it enters the intermediate cell reservoir (see Figure 17 for further detail). Rough endoplasmic reticulum (RER) and Golgi complexes (GC) are numerous in the cortical cell cytoplasm, and secondary lysosomes with cellular debris (LY) are common. M, mitochondria. Day 0 adult; bar equals 2 μm.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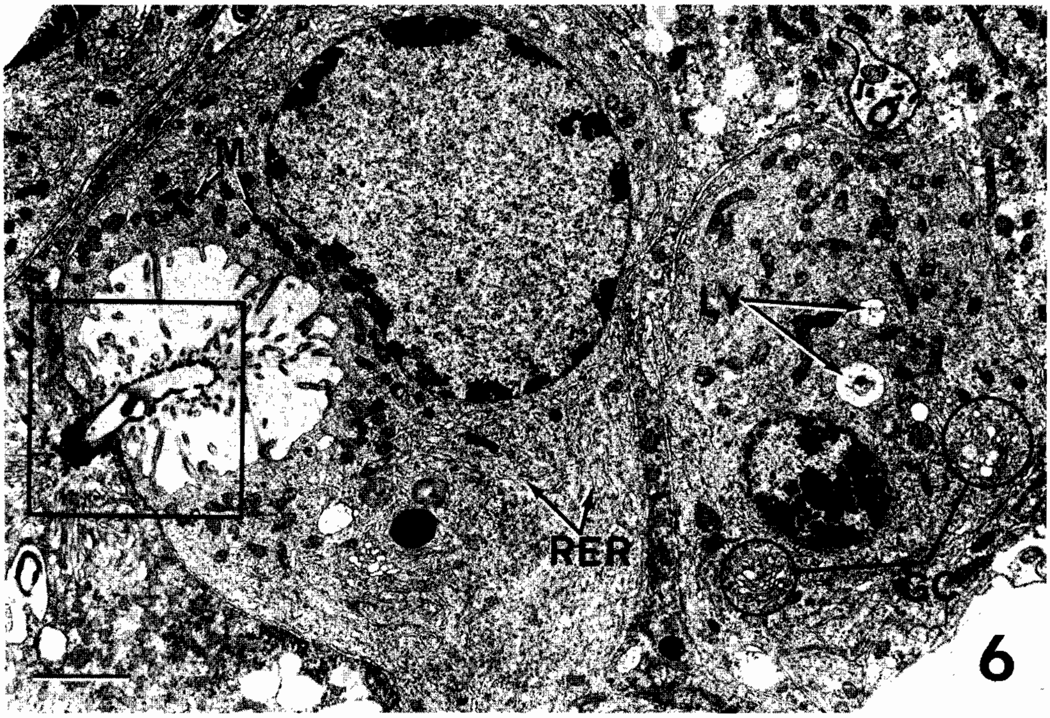
M
RER
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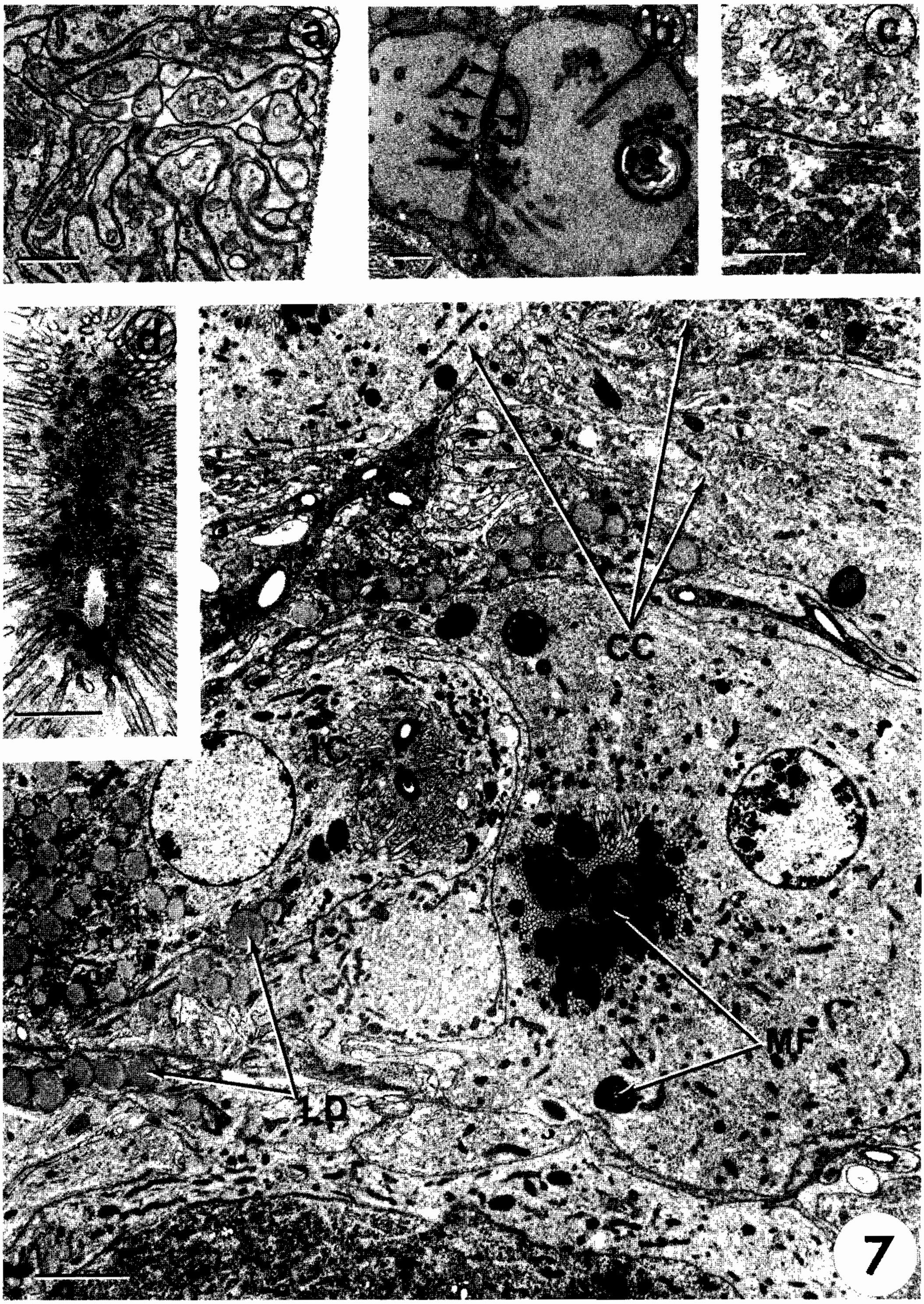
a
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7

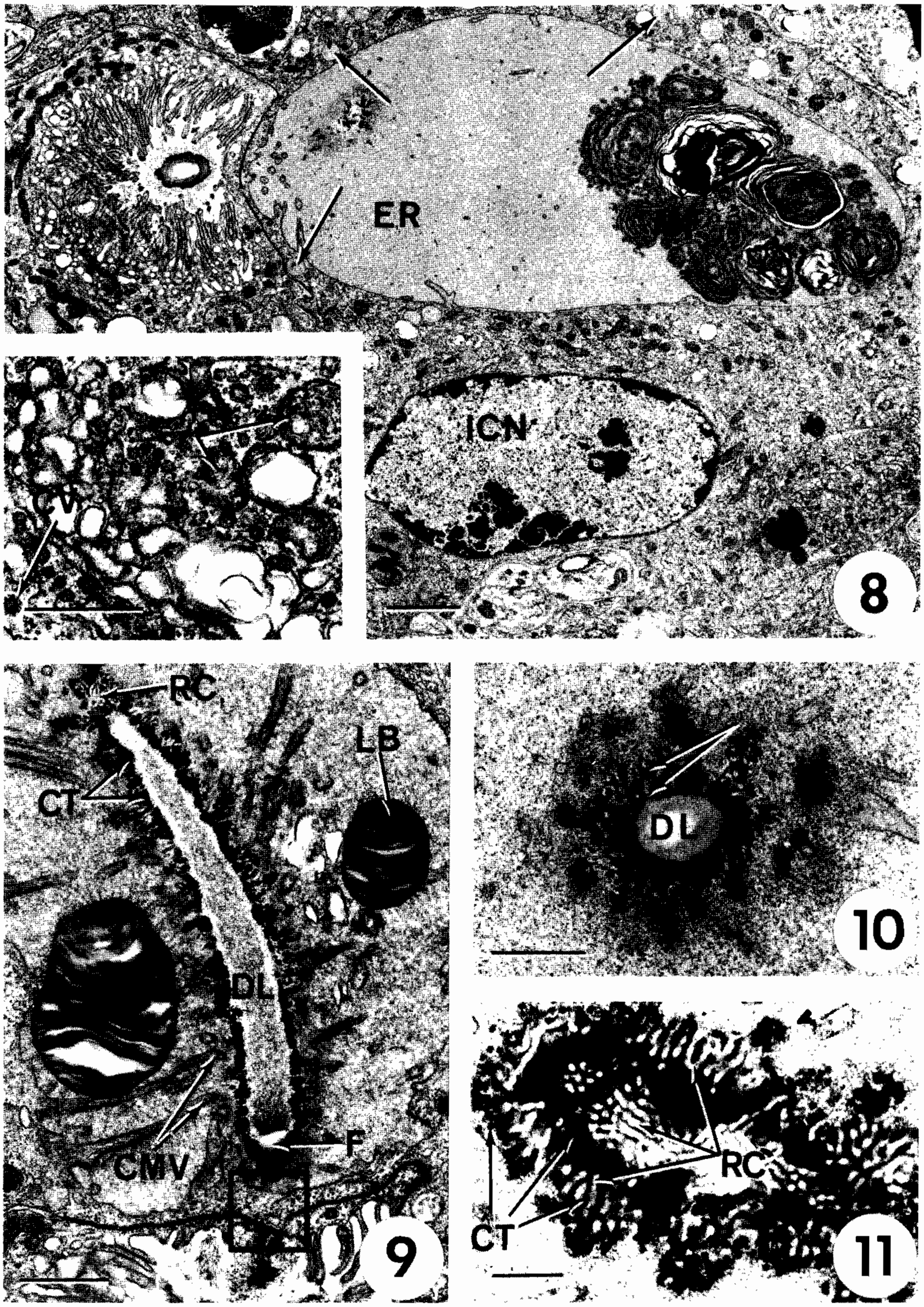
the secretory product appears finely granular (Figs. 12, 13, 18). As the ductule leaves the cortical cell reservoir, the products begin to condense and the progressive condensation yields a frothy mass in the efferent ductules of the intermediate secretory cells and ductule cells (Figs. 17, 22, 25, 26). As this frothy product is released into the genital pocket it forms a foam-like layer on the surface of the cuticle. In electron micrographs, a striking resemblance is seen between this layer and the outer layer of the spermatophore (Figs. 25, 27). However, no direct connection exists between the genital pocket and the ejaculatory duct, which leads through the penis to the gonopore.

DISCUSSION

The aedeagal gland is composed of acinar clusters of three-cell secretory organules (Fig. 30). They are of the type 3 gland cell classification of Noirot and Quennedey ('74) in which a cuticular (efferent) ductule penetrates the gland cell(s). The inner cuticular layer of the efferent ductule is usually structurally modified. This is especially true when the ductule penetrates different types of secretory cells. The trabeculae of the cortical cell end apparatus and the fibrillar material coating the extracellular ductule of the intermediate cells in the aedeagal gland are examples of these modifications. Ductules within ductule cells lack cuticular specialization and are structurally consistent with the epicuticular layer of the cuticular sheath. A similar situation is observed in the defensive glands of the tenebrionid *Eleodes longicollis* (Eisner et al., '64) and in various glands of other insects (Noirot and Quennedey, '74, and references therein). As suggested by Eisner et al. ('64), these cuticular modifications may function in several ways. For example, the cortical cell canaliculi could act to extract selectively the reaction products from the extracellular reservoir. The fibrillar material and fibrillar canaliculi of the intermediate cell could act in the same manner. Similarly, it is also quite possible that cortical cell products passing through the intermediate cell ductule are selectively sequestered from the ductule. These products could then pass into the intermediate cell reservoir and consequently through endocytosis (coated vesicles or pinocytotic vesicles), be transported and released at the basal plasma membrane, or be acted upon by lysosomes within the cell cytoplasm. As shown by Happ ('68), the extracellular spaces within the ductules and reservoirs of a tenebrionid defensive gland can act as reaction chambers in the production of secretory product.

A striking morphologic resemblance occurs between the type A dermal gland cells of *Tenebrio* described by Delachambre ('73) and the cells that compose the secretory unit of the aedeagal gland. As a general rule, insect dermal glands are thought to secrete the cement layer over the epicuticular surface within the first few hours following ecdysis (Wigglesworth, '48; Kramer and Wigglesworth, '50; Way, '50; Malek, '58). It was generally assumed that after secreting the cement layer, the glands became inactive and subsequently degenerated. In support of this assumption, Kendall ('72) found that in *Tenebrio* the reaction of the dermal gland secretion (cement layer) with ammoniated silver nitrate stopped several hours after adult ecdysis. However, Delachambre's ultrastructural study ('73) of dermal glands in pupae and adults showed that the glands were active over these developmental stages. Moreover, in older adults (following hardening of the cuticle), the dermal glands possessed a well-developed endoplasmic reticulum and extensive Golgi complexes not observed in newly emerged adults. Delachambre ('73) believes that Kendall's results are indicative of a change in the nature of the secretion and do not reflect cessation of secretory activity. Changes observed in the endoplasmic reticulum of the dermal gland and also of the aedeagal gland following adult ecdysis suggest a shift in the biochemistry of the secretory product during adult life.

Fig. 7. In animals of greater than 8 days of age the cortical cells (CC) show variations in the morphology of the endoplasmic reticulum. These differences in the endoplasmic reticulum are reflected by the darker staining properties of certain transitional cells. In these cells the rough cisternal endoplasmic reticulum (inset *c*, upper cell) is replaced by vesiculate smooth endopolasmic reticulum (characterized by the presence of numerous vesicles in the ribosome-free, swollen cisternae) (insert *c*, lower cell). Note that the intermediate cell (IC) cytoplasm appears unchanged (compare with Figs. 4–6) and that the extracellular reservoirs of the cortical cells are densely packed with myelin figures (MF). Inset *a* illustrates the convoluted nature of the basal plasma membrane of the secretory cells. Inset *b* shows the looping or folding-over of the distal end of the cortical cell extracellular reservoir; arrows indicate the septum that divides one lobe of the reservoir from the other. Inset *d* demonstrates the apparent anchoring of the ductule by microvilli that traverse the extracellular reservoir and closely associate with the cuticular matrix (trabeculae) of the ductule. All micrographs are from glands of adults more than 8 days old. LD, lipid droplets. Bar equals 4 μm; inset *a* bar equals 0.5 μm; inset *b* bar equals 1 μm; inset *c* bar equals 0.5 μm; inset *d* bar equals 1.0 μm.


ER
CV
ICN
8
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11

Ultrastructural examination of aedeagal gland cell-mediated endocytosis, autophagocytosis, and developmental changes of organelles involved in the synthesis of secretory products will be discussed in Dailey and Happ (unpublished observation).

The dermal gland and the aedeagal gland are of ectodermal origin, like the many glandular epithelia responsible for secreting pheromones or allomones, for example, in fruit flies (Lhoste and Roche, '61; Pritchard, '67; Flethcher, '69), in the mecopteran *Harpobittacus* (Bornemissza, '64), in cockroaches (Roth, '52, '69; Roth and Barth, '64; Engelmann, '65; Dimeo et al., '78; Persoons and Ritter, '79), in lepidopterans (Brower et al., '65; Alpin and Birch, '68; Pliske and Eisner, '69), and in beetles (Eisner et al., '64; Yinon and Shulov, '67; Tumlinson et al., '69; Noirot and Quennedey, '74). Characteristic of these secretory systems are the cuticle-lined ductules that channel secretory products to the exterior.

Male *Tenebrio molitor* produce sex pheromones, as described in the introduction. The production of pheromones by male insects is not restricted to *Tenebrio*. Other male beetles also produce attractants. The male boll weevil, *Anthonomus grandis*, for example, releases a pheromone that attracts only females in the laboratory (Tumlinson et al., '69). However, both sexes are attracted in the field. Pheromone production by male cockroaches has been demonstrated in several species. In *Supella longipalpa*, *Blattella germanica*, and *Leucophaea maderae* these pheromones are produced by distinct tergal glands (Roth, '52, '69; Roth and Barth, '64; Engelman, '65; Persoons and Ritter, '79). In one species, *Nauphoeta cinerea*, the male secretes a pheromone called "seducin." This substance, produced by glands in the integument of the abdominal sternites and tergites, functions to attract the female and as an arrestant keeping her in position long enough to insure connection with the male aedeagus (Dimeo et al., '78). Similarly, certain male noctuid moths produce pheromones with several possible functions: antiaphrodisiacs to deter other males, isolating mechanisms between species, or aphrodisiacs to stimulate the female to mate (Alpin and Birch, '68). Danaid butterflies require aphrodisiac pheromones for successful mating. Generally, the pheromone produced by the male hair pencils is dusted onto the female antennae. This behavior is generally followed by copulation (Brower et al., '65; Pliske and Eisner, '69). Fletcher ('69) has found that male Queensland fruit flies, *Dacus tryoni*, release a volatile sex pheromone that elicits characteristic mating behavior patterns in sexually receptive females.

We believe that the aedeagal and spicular glands may produce one or several pheromones. The secretions could be emitted as the male genitalia are extruded following the reception of female scent as discussed above. These secretions could be antiaphrodisiacs, thus reducing the attractiveness of females for other males, or aphrodisiacs luring the female towards the receptive male. Or they could be continuously emitted and be the primer pheromones that act on the maturation of the terminal oocytes and/or increase maturation of other tissues in the immature female. Alternatively, it is possible that the secretions act as genital lubricants during copulation.

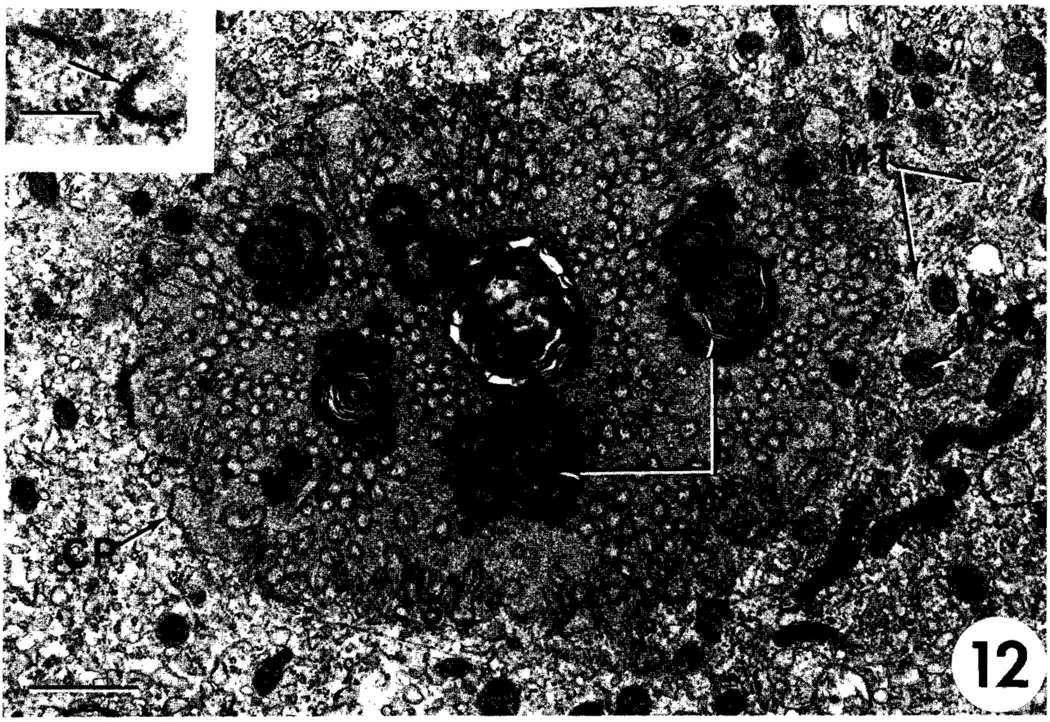
Although the aedeagal and spicular glands are similar ultrastructurally they may differ in the biochemistry of their secretion products. Histochemical investigations of the aedeagal glands are now under way in our laboratory to answer these questions. In addition, a pheromone bioassay is being used to examine the effects of the secretions on adult behavior.

Fig. 8. Oblique section through a cortical cell illustrating the accumulation of myelin figures (MF) at the distal end of the extracellular reservoir (ER). Arrows indicate peroxisome-like secretory granules near the periphery of the reservoir. Coated vesicles (CV) and immature secretory granules (arrows in inset) observed in the cortical cell Golgi complex (inset) are usually present in glands of all ages; however, their numbers may vary with age or with the cell secretory cycle. ICN, intermediate cell nucleus. Day 0 adults; bar equals 2 μm, inset bar equals 0.5 μm.

Fig. 9. Longitudinal section through the end apparatus within the cortical cell reservoir. The cuticular matrix of the ductule is irregular and is perforated by tiny channels or canaliculi. Toward the basal end of the reservoir the ductule lumen (DL) appears constricted. In other sections (Fig. 16) this neck of the funnel (F) marks the distal limit of the transition zone, where a definite epicuticular layer is formed. Area within the square is shown in more detail in Figure 15. LB, lamellar body; RC, reservoir canaliculi; CT, cuticular trabeculae; CMV, cortical cell microvilli. Day 0 adult; bar equals 1 μm.

Fig. 10. Section through the tip of the end apparatus. Poststaining of this particular section demonstrates the staining specificity of the secretory product present in the cortical cell reservoir. This is characterized by minute electron-dense particles evenly distributed in the secretion and in the canaliculi leading into the ductule lumen (DL) (arrows). Adult more than 8 days old; bar equals 0.5 μm.

Fig. 11. Small, tubular reservoir canaliculi (RC) penetrates the cuticular layer of the cortical cell ductule giving it a perforated appearance. As a result, the cuticular projection of trabeculae (CT) may themselves be mistaken for tubules. Day 6 adult; bar equals 0.2 μm.



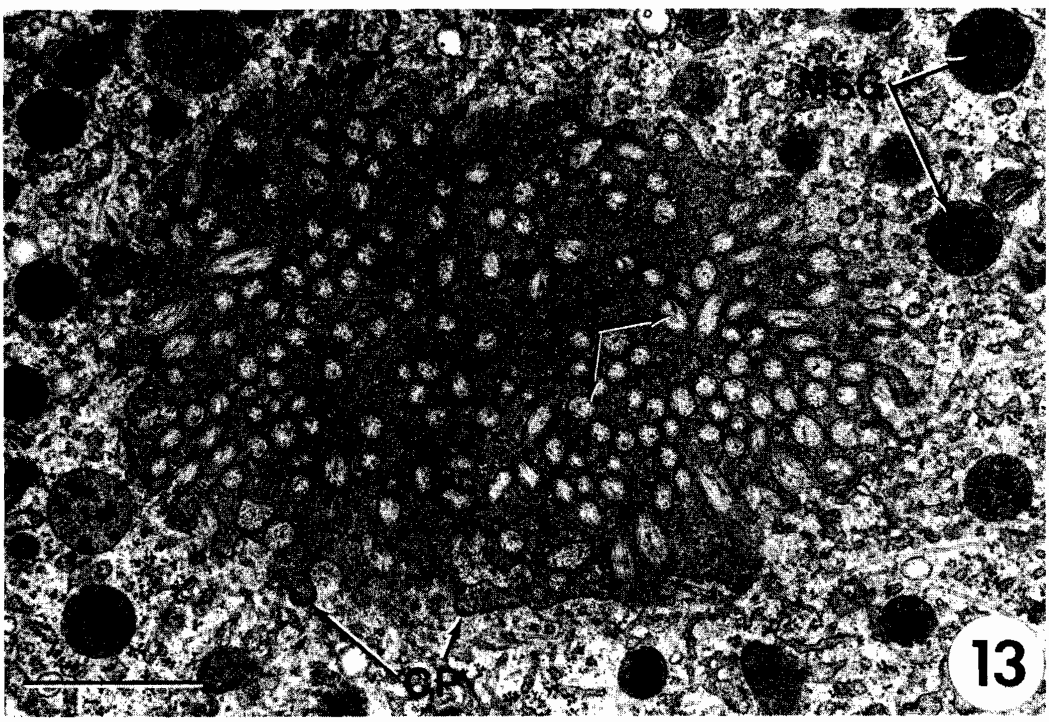


Fig. 12. The distal region of a cortical cell extracellular reservoir from a newly emerged adult contains myelin figures (MF) and is bound on all sides by numerous microtubules (MT). Membrane-containing secretory granules (MSG) are not very numerous in this area. Coated pits (CP) are found in the crypts between microvilli. A higher-magnification view of one of these structures (arrows in inset) illustrates the morphologically distinct coat. Day 0 adults; bar equals 1 μm, inset bar equals 0.2 μm.

Fig. 13. Numerous secretory granules (MSG) lie in close proximity to the reservoir in this micrograph from a gland more than 8 days old. As in other sections, coated pits (CP) are common. A characteristic feature of the secretory granule is the presence of small patches of electron-dense lamellae scattered throughout the matrix. IF, intermediate filaments. Bar equals 1 μm.

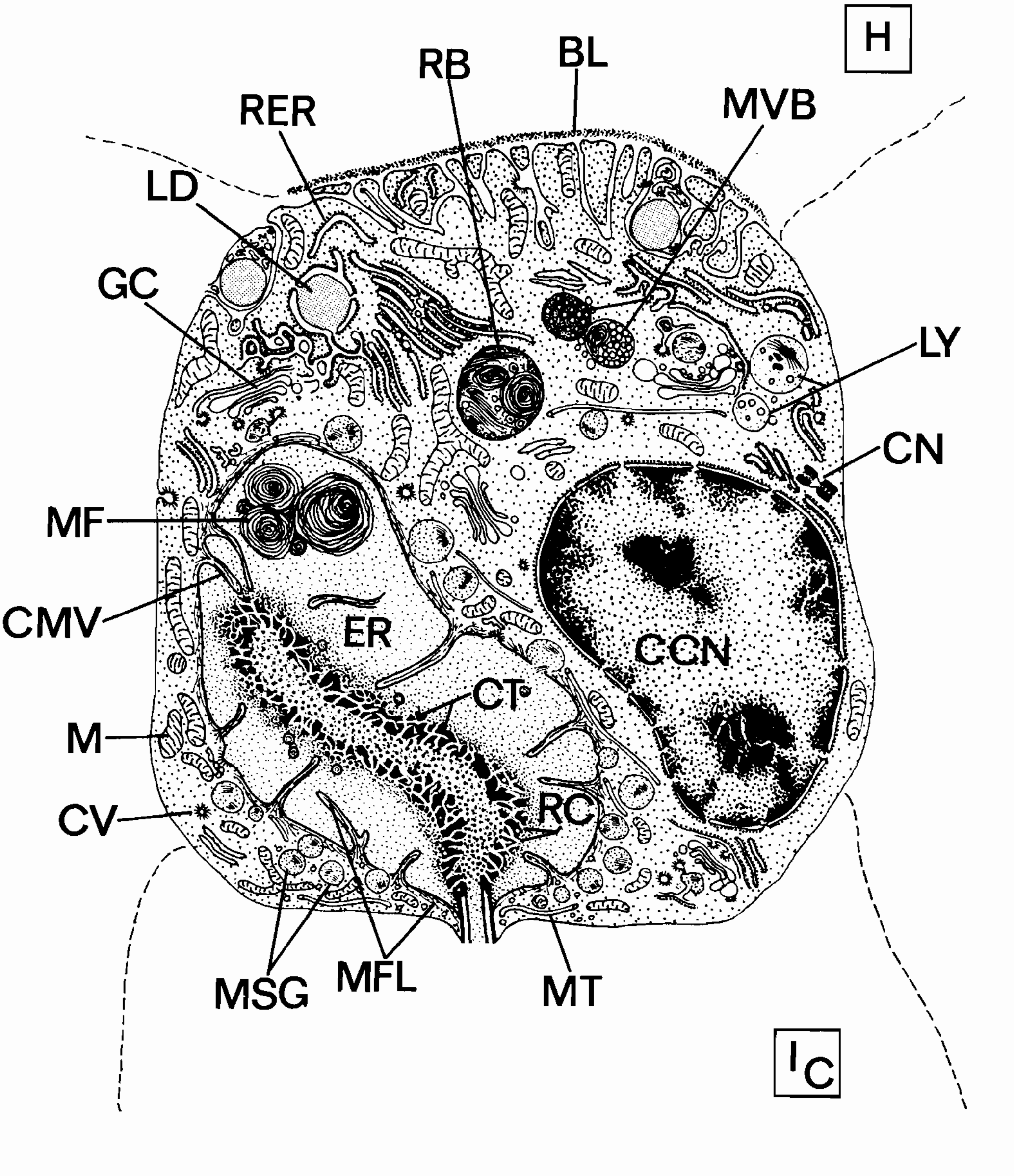
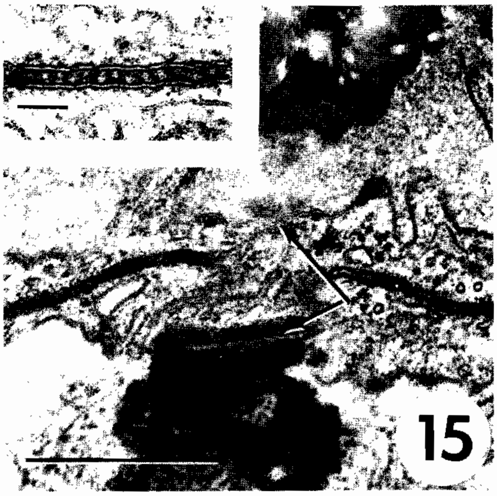
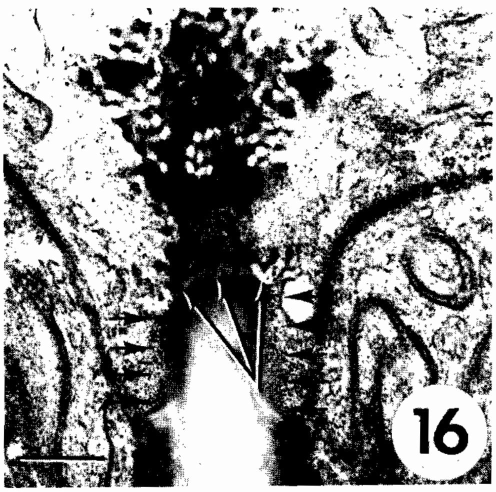


Fig. 14. Composite illustration of a cortical cell showing placement and types of organelles present in the cytoplasm. Position of this cell in relation to the intermediate and ductule cells is seen in Figure 30. Illustration not drawn to scale.





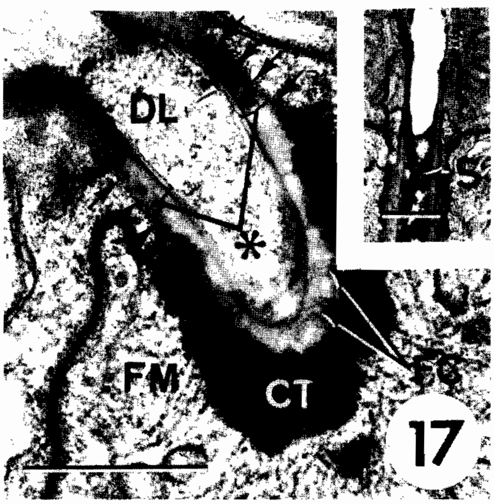


Fig. 15. The zone of junction between cortical and intermediate cell reservoirs of the ductule can be seen in the area between the two arrows. Contact between the intermediate cell and cortical cell plasma membranes is usually strengthened by the formation of septate desmosomes (inset). Day 0 adults; bar equals 0.5 μm, inset bar equals 0.1 μm.

Fig. 16. Below the cytoplasm the beginning of the zone of transition is distinguished by a row of terminal reservoir canaliculi embedded in the cuticular matrix (black and white arrows). The density of the cuticular matrix lessens at the transition zone and appears to be ensheathed by the plasma membrane of the cortical cell surrounding this region (short black arrows). Adult more than 8 days old; bar equals 0.5 μm.

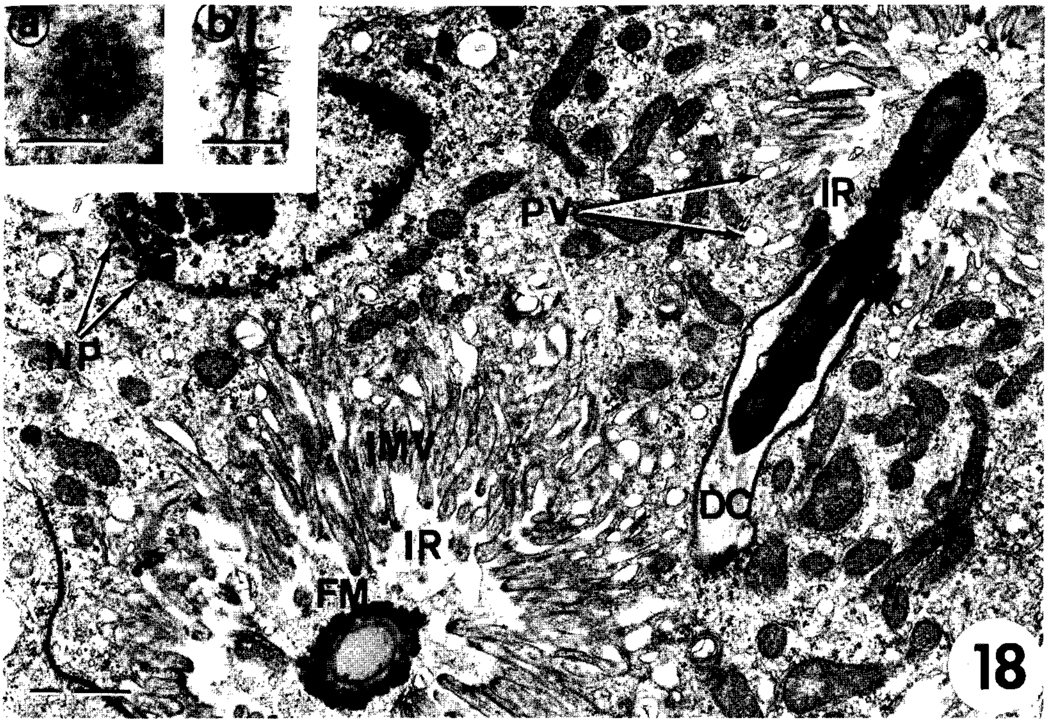
Fig. 17. The outer epicuticle begins to form in the transition zone below the lip of cuticular matrix described above (asterisk; area between brackets in the inset). The inward extension of the epicuticle is ensheathed by the cortical cell plasma membrane (arrows at top). The cuticular matrix of the cortical cell ductule becomes confluent with the inner epicuticle, and the outer epicuticle remains intact from this point to its union with the cuticular sheath of the aedeagus. DL, ductule lumen; FM, fibrillar material; FC, fibrillar canaliculi; CT, cuticular trabeculae. Day 0 adult; bar equals 0.5 μm, inset bar equals 0.5 μm.

Fig. 18. This section through an intermediate cell illustrates the connection between the intermediate cell ductule and that of the ductule cell (DC) (area between thick arrows). The extracellular reservoir (IR) of the intermediate cell is convoluted and it is for this reason that two appararently distinct reservoirs appear in the micrograph. Many of the intermediate cell microvilli (IMV) contain a tubule of smooth endoplasmic reticulum. Once the ductule cell leaves the intermediate cell it joins with other ductule cells to form a multiductular array that passes through the epidermis of the cuticular sheath. The ductule epicuticle then becomes confluent with the cuticular sheath epicuticle (see Fig. 26). Nuclear pores (NP) were clearly observed in this section. Inset *a* shows a section through the face of a pore complex. Inset *b* shows a cross section through the nuclear pore; the small arrows indicate equally spaced electron-lucent zones bordered on either side by electron-dense zones that are probably indicative of the filamentous nature of the complex. PV, pinocytotic vesicle; FM, fibrillar material. Day 0 adult, bar equals 0.1 μm; inset *a*, day 0 adult, bar equals 0.1 μm; inset *b*, day 6 adult, bar equals 0.2 μm.

Fig. 19. Intermediate cell cytoplasm containing Golgi complexes (GC), smooth endoplasmic reticulum (SER), and microtubules (MT). Developing lipid-like droplets surrounded by SER (arrows) are commonly observed in the cytoplasm. As these droplets mature their periphery becomes less fuzzy, resulting in a more compact structure (droplet at upper left). Inset illustrates the formation of a coated vesicle (arrow) from the wall of a microvillus, and the central core of smooth endoplasmic reticulum (asterisk). Day 6 adult, bar equals 0.2 μm; inset, day 0 adult, bar equals 0.2 μm.

Fig. 20. A loosely bound microbody (left) containing rod-like structures (arrows) lies adjacent to a maturing lipid-like droplet (LD). Day 0 adult; bar equals 0.5 μm.

Fig. 21. Intermediate cell Golgi complex. Adult more than 8 days old; bar equals 0.5 μm.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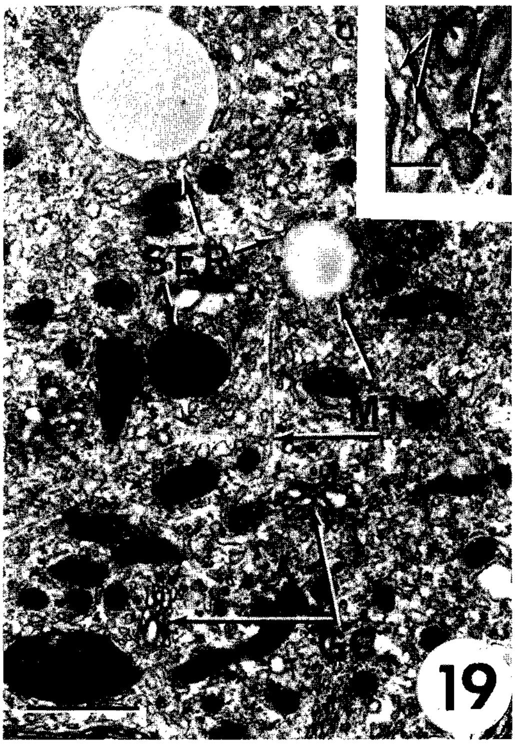
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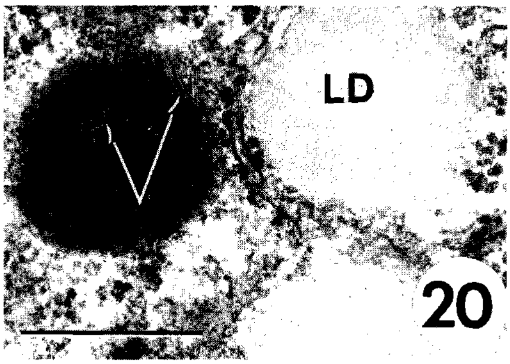
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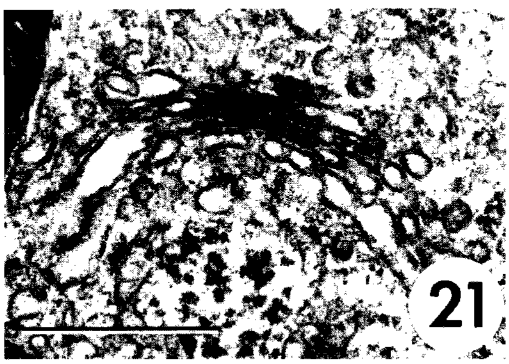
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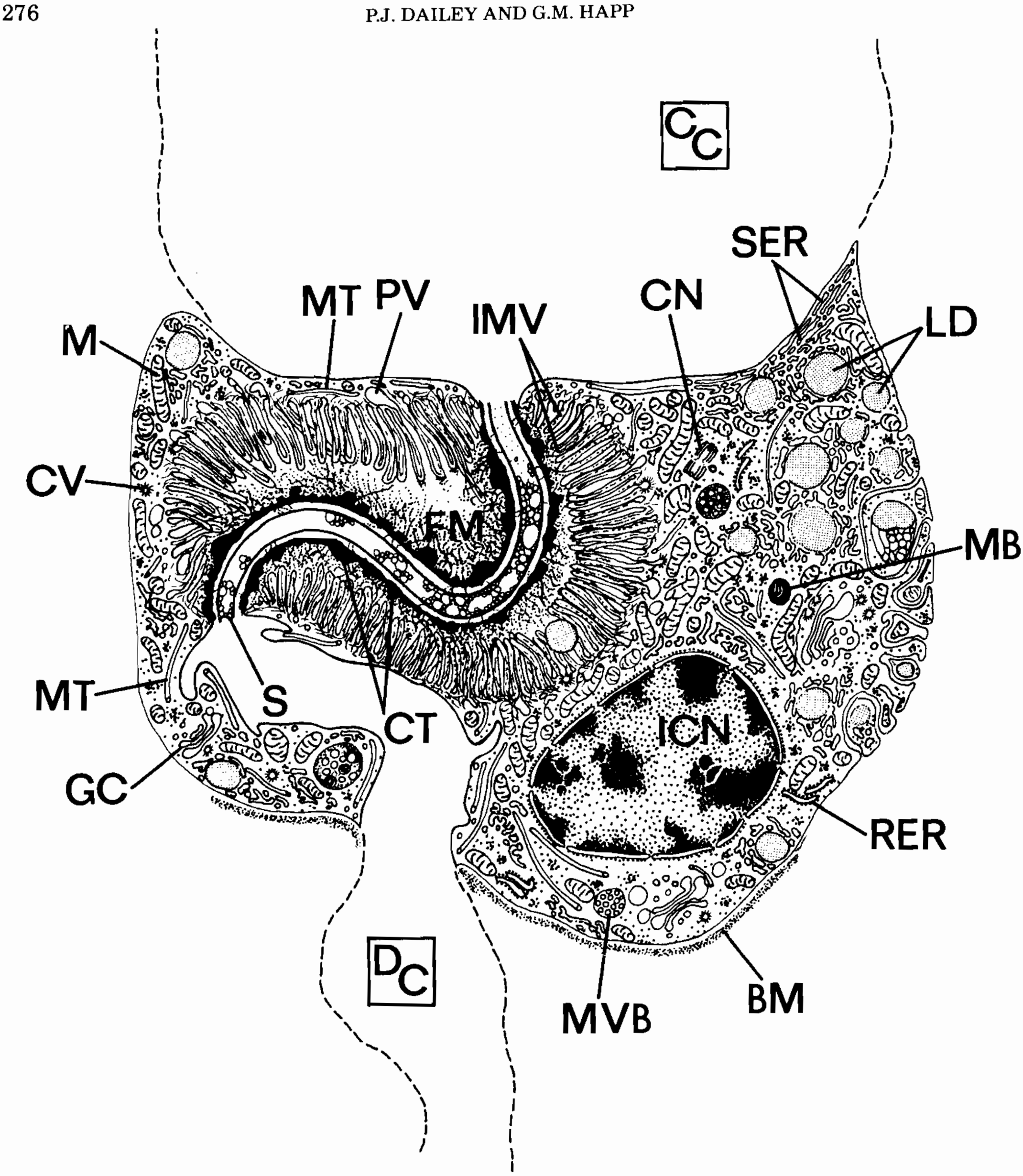


Fig. 22. Composite illustration of an intermediate secretory cell demonstrating approximate location and type of organelles present in the cell cytoplasm. See Figure 30 for composite picture of organule of which this cell is a part. Figure not drawn to scale.

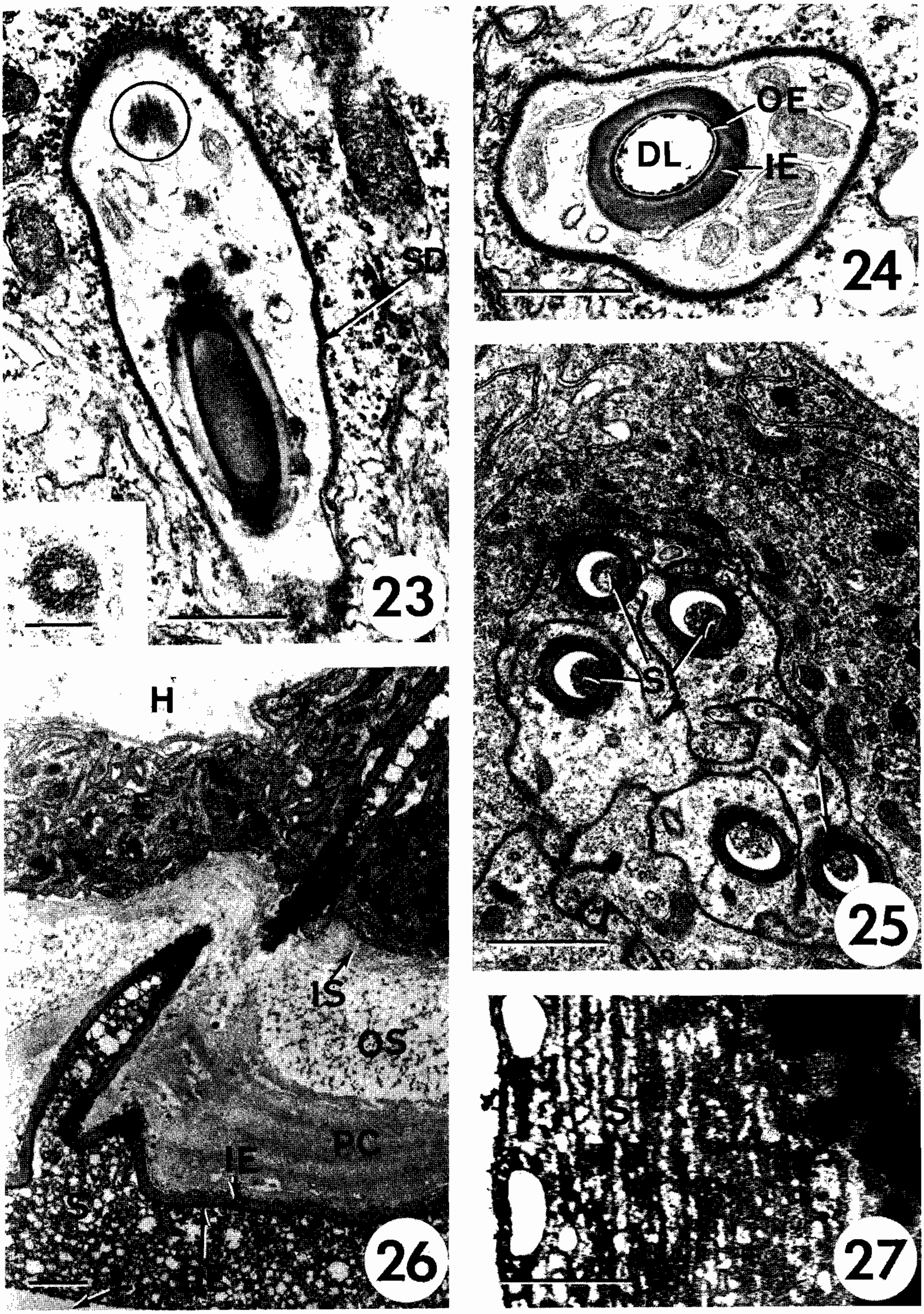
Fig. 23. Oblique section through part of a ductule cell within the intermediate cell cytoplasm. Septate desmosomes (SD) form between opposing cell membranes. Centrioles (structure within circle and inset at lower left) were frequently observed in oblique sections of the ductule cell. Day 0 adult, bar equals 0.5 μm; inset, adult more than 8 days old, bar equals 0.2 μm.

Fig. 24. Transverse section through ductule cell (DC) illustrating the epicuticle and cytoplasmic contents. OE, outer epicuticle; IE, inner epicuticle. Day 0 adult, bar equals 0.5 μm.

Fig. 25. A group of duct cells (multiductular complex) is shown penetrating the epidermis (E) of the cuticular sheath. Secretion product (S) is visible within the ductules. Ductule cell microvilli are frequent along the inner margin of the epicuticle (arrow).

Fig. 26. As the ductules penetrate the epidermis (E) the epicuticle becomes confluent with the epicuticular layer of the cuticular sheath. Note in particular the frothy secretion (S) within the ductules and lining the surface of the cuticle within the lumen (L) of the aedeagus. PC, procuticle; OS, outer subcuticle; IS, inner subcuticle; IE, inner epicuticle; OE, outer epicuticle, H, haemocoel. Adult more than 8 days old; bar equals 0.1 μm.

Fig. 27. Section through a spermatophore demonstrating the similarity between the outer spermatophore coat and the frothy secretion (S) produced by the aedeagal gland. Bar equals 0.5 μm.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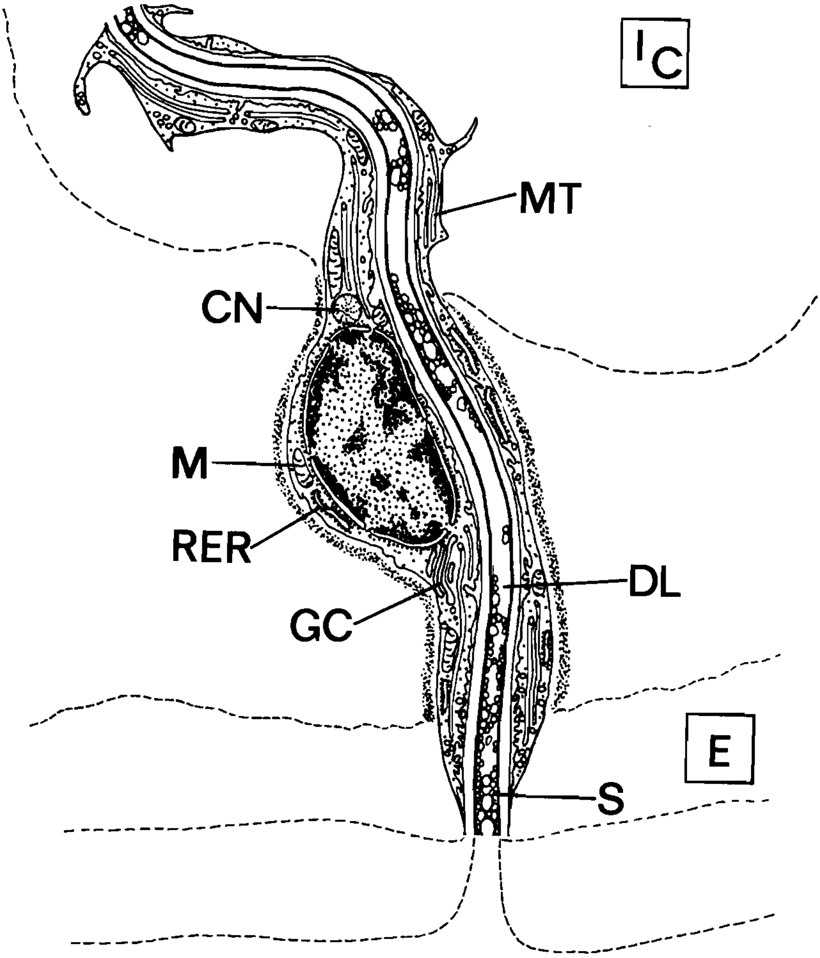

Fig. 28. Composite illustration of a ductule cell demonstrating organelle location and type. The relationship of this cell with the cortical and intermediate cell is depicted in Figure 30. Figure not drawn to scale.

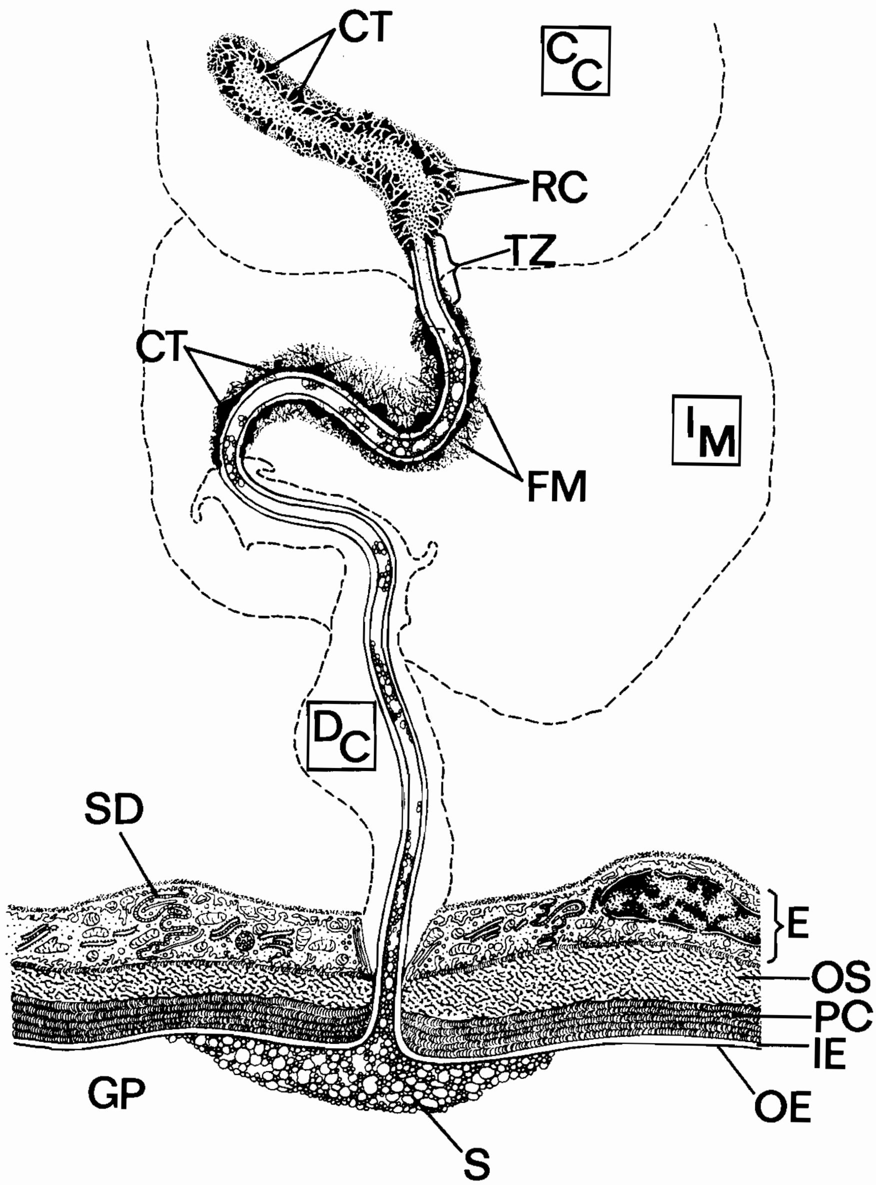

Fig. 29. Composite illustration of the efferent ductule. Morphologically, the outer cuticular components of the ductule are distinct in the extracellular reservoirs of the cortical and intermediate cells. However, the inner and outer epicuticles of the ductule cell ductule are confluent with similar components of the cuticular sheath. Figure not drawn to scale.

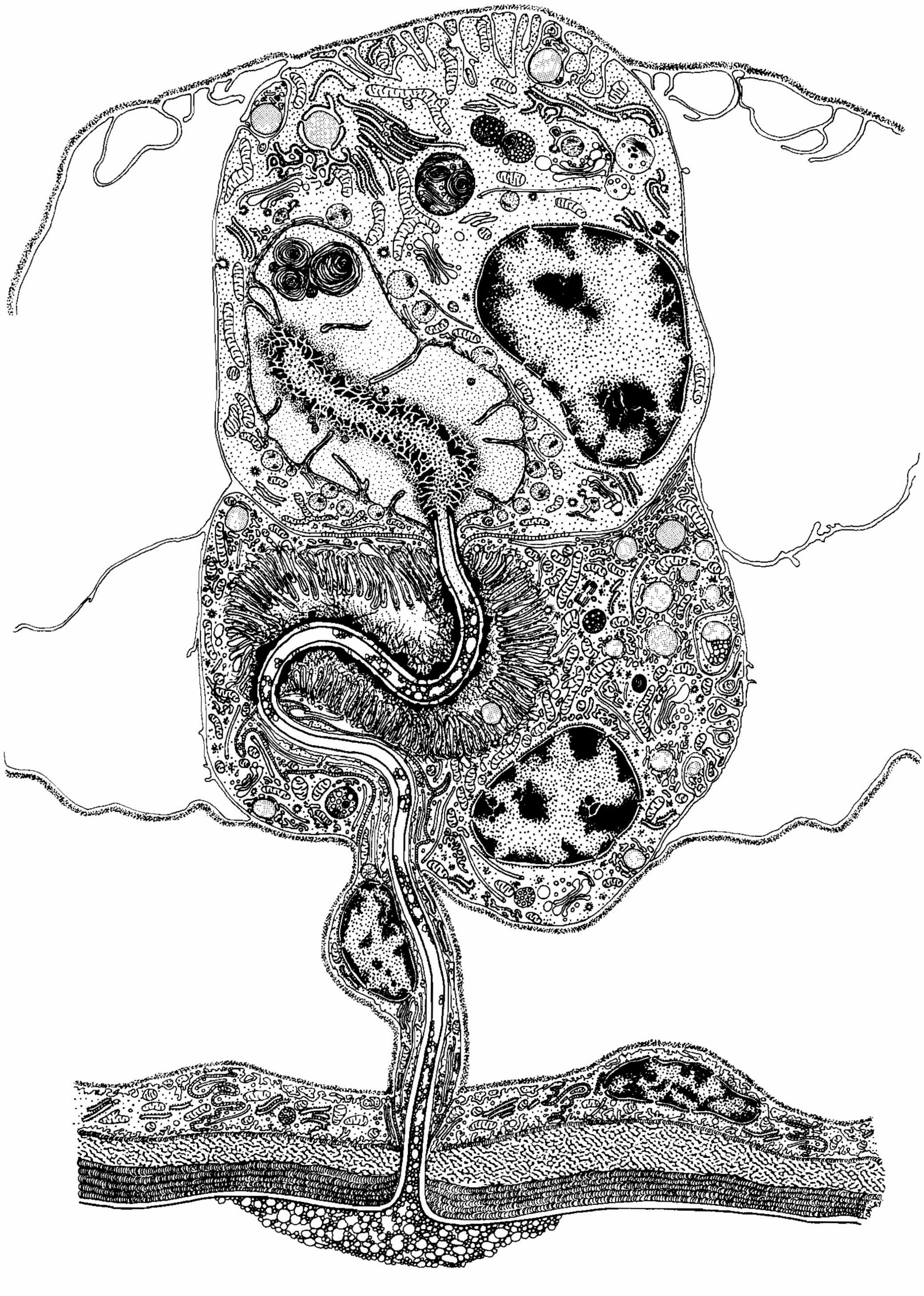

Fig. 30. Cumulative illustration of a secretory unit or organule. Hundreds of these units make up the aedeagal gland. For detailed structure of organelles see Figures 14, 22, 28, and 29. Figure not drawn to scale.

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LITERATURE CITED

Alpin, R.T., and M.C. Birch (1968) Pheromones from the abdominal brushes of male noctuid Lepidoptera. Nature (Lond.) *217:*1167–1168.

August, C.J. (1971) The role of male and female pheromones in the mating behavior of *Tenebrio molitor.* J. Insect Physiol. *17:*739–752.

Autuori, F., A. Ciofi-Luzzatto, L. Conti, and F. Leone (1971) Osservazioni morfologiche sulla ghiandola spermathecale di *Dytiscus marginalis* L. (Coleoptera). Riv. Biol. *64:*243–269.

Barbosa, P. (1974) Manual of Basic Techniques in Insect Histology. Amherst, Massachusetts: Autumn Publishers.

Birch, M.C. (ed.) (1974) Pheromones. Frontiers of Biology, Vol. 32. Amsterdam: North-Holland.

Bornemissza, G.F. (1964) Sex attractant of male scorpion flies. Nature (Lond.) *203:*786–787.

Brossut, R., and L. Sreng (1980) Ultrastructure comparée des glandes exocrines abdominales des Blattaria (Dictyoptera). Int. J. Insect Morphol. Embryol. *9:*199–213.

Brower, L.P., J.V.Z. Brower, and F.P. Cranston (1965) Courtship behavior of the queen butterfly, *Danaus gilippus berenice* (Cramer). Zoologica *50:*1–39.

Butz, A. (1957) Effects of sodium, potassium, and calcium ions on the isolated heart of the mealworm, *Tenebrio molitor* L. J.N.Y. Entomol. Soc. *65:*22–31.

Dailey, P.J., N.M. Gadzama, and G.M. Happ (1980) Cytodifferentiation in the accessory glands of *Tenebrio molitor.* VI. A congruent map of cells and their secretions in the layered elastic product of the male bean-shaped gland. J. Morphol. *166:*289–322.

Delachambre, J. (1973) L'ultrastructure des glandes dermiques de *Tenebrio molitor* L. Tissue Cell *5:*243–257.

Dimeo, A., B. Koblenz, and M. Menon (1978) Localization of the site of production of the sex pheromone 'seducin' of the male *Nauphoeta cinerea* by bioassay. Ann. Entomol. Soc. Am. *71:*111–114.

Doyen, J.T. (1966) The skeletal anatomy of *Tenebrio molitor* (Coleoptera: Tenebrionidae). Misc. Publ. Entomol. Soc. Am. *5*(3):103–150.

Dulbecco, R., and M. Vogt (1954) Plaque formation and isolation of pure lines with poliomyelitis viruses. J. Exp. Med. *99:*167–182.

Eisner, T., F. McHenry, and M.M. Salpeter (1964) Defense mechanisms of arthropods. XV. Morphology of the quinone-producing glands of a tenebrionid beetle (*Eleodes longicollis* Lec.). J. Morphol. *115:*355–400.

Engelmann, F. (1965) Pheromones and Mating Behavior. Proceedings of the XII International Congress on Entomology, London, p. 289.

Fletcher, B.S. (1969) The structure and function of the sex pheromone glands of the male Queensland fruit fly, *Dacus tryoni.* J. Insect Physiol. *15:*1309–1322.

Happ, G.M. (1968) Quinone and hydrocarbon production in the defensive glands of *Eleodes longicollis* and *Tribolium castaneum* (Coleoptera, Tenebrionidae). J. Insect Physiol. *14:*1821–1837.

Happ, G.M. (1969) Multiple sex pheromones of the mealworm beetle, *Tenebrio molitor* L. Nature *22:*180–181.

Happ, G.M. (1973) Chemical signals between animals: Allomones and pheromones. In J. LoBue and A.S. Gordon (eds): Humoral Control of Growth and Differentiation, Vol. II. New York: Academic, pp. 149–190.

Happ, G.M., and J. Wheeler (1969) Preliminary purification, and effect of age, crowding, and mating on the release of sex pheromone by female *Tenebrio molitor.* Ann Entomol. Soc. Am. *62:*846–851.

Happ, G.M., M.E. Schroeder, and J.C.H. Wang (1970) Effects of male and female scent on reproductive maturation in young female *Tenebrio molitor.* J. Insect Physiol. *16:* 1543–1548.

Kendall, D.A. (1972) The dermal glands of some adult beetles. J. Entomol. (A)*46:*153–159.

Kramer, S., and V.B. Wigglesworth (1950) The outer layers of the cuticle in the cockroach, *Periplaneta americana,* and the function of the oenocytes. Q. J. Microsc. Sci. *91:*63–72.

Lhoste, J., and A. Roche (1961) Organes odoriféranges des males de *Ceratitis capitata* (Dipt. Trypetidae). Bull Soc. Entomol. Fr. *65:*206–210.

Locke, M. (1974) The structure and formation of the integument in insects. In M. Rockstein (ed): The Physiology of Insecta, Vol. 6. New York: Academic Press, pp. 124–215.

Malek, S.R.A. (1958) The appearance and histological structure of the cuticle of the desert locust, *Schistocerca gregaria* (Forskal). Proc. R. Soc. Lond. (B) *149:*557–570.

Noirot, C., and A. Quennedey (1974) Fine structure of insect epidermal glands. Annu. Rev. Entomol. *19:*61–80.

Percy, J.E., and J. Weatherston (1974) Gland structure and pheromone production in insects. In M.C. Birch (ed): Pheromones. Frontiers of Biology, Vol. 32. Amsterdam: North-Holland, pp. 11–34.

Persoons, C.J., and F.J. Ritter (1979) Pheromones of cockroaches. In F.J. Ritter (ed): Chemical Ecology: Odour Communication in Animals. New York: Elsevier/North-Holland, pp. 225–236.

Pliske, T.E., and T. Eisner (1969) Sex pheromone of the queen butterfly: Biology. Science *164:*1170–1172.

Pritchard, G. (1967) Laboratory observations on the mating behavior of the island fruit fly, *Rioxa pornia* (Diptera: Tephritidae). J. Aust. Entomol. Soc. *6:*127–132.

Reynolds, E.S. (1963) The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell Biol. *17:*208–213.

Roth, L.M. (1952) The tergal gland of the male cockroach, *S. supellectilium.* J. Morphol. *91:*469–477.

Roth, L.M. (1969) The evolution of male tergal glands in the Blattaria. Ann. Entomol. Soc. Am. *62:*176–208.

Roth, L.M., and R.H. Barth, Jr. (1964) The control of sexual receptivity in female cockroaches. J. Insect Physiol. *10:*965–975.

Shorey, H.H. (1976) Animal Communication by Pheromones. New York: Academic.

Tschinkel, W., C. Willson, and H.A. Bern (1976) Sex pheromone of the mealworm beetle (*Tenebrio molitor*). J. Exp. Zool. *164:*81–86.

Tumlinson, J.H., D.D. Hardee, R.C. Gueldner, A.C. Thompson, P.A. Hedin, and J.P. Minyard (1969) Sex pheromones produced by male boll weevil: Isolation, identification, and synthesis. Science *166:*1010–1012.

Valentine, J.M. (1931) The olfactory sense of the adult mealworm beetle *Tenebrio molitor* (Linn.). J. Exp. Zool. *58:*165–227.

Way, M.J. (1950) The structure and development of the larval cuticle of *Diataraxia oleracea* (Lepidoptera). Q. J. Microsc. Sci. *91:*145–182.

Wigglesworth, V.B. (1948) The structure and deposition of the cuticle in an adult mealworm, *Tenebrio molitor* L. (Coleoptera). Q. J. Microsc. Sci. *89:*197–217.

Wilson, E.O., and W.H. Bossert (1963) Chemical communication among animals. Rec. Prog. Horm. Res. *19:*673–710.

Yinon, U., and A. Shulov (1967) A bioassay of the pheromone of *Trogoderma granarium* males as an attractant for both sexes of the species. Entomol. Exp. Appl. *10:*453–462.