Cytodifferentiation in the Accessory Glands of *Tenebrio molitor*

I. ULTRASTRUCTURE OF THE TUBULAR GLAND IN THE POST-ECDYSIAL ADULT MALE

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ABSTRACT The tubular accessory reproductive glands of the male mealworm beetle consist of a secretory epithelium surrounded by a thin muscular sheath. Each columnar secretory cell is divisible into three zones: *basal* which is adjacent to the muscle layer and contains rough endoplasmic reticulum and Golgi, *intermediate*, which contains endoplasmic reticulum and Golgi zones in the immature gland and is filled with secretory vesicles in the mature gland, and *apical*. Maturation also involves proliferation and organization of the rough endoplasmic reticulum in the basal and intermediate zone. The process appears to be complete at four days after ecdysis. Parallels with other insect glands and with the mammalian prostate are striking.

Semen consists of sperm, produced in the testes, and seminal fluid, secreted by the male accessory glands. In vertebrates, endocrine signals regulate both spermatogenesis and the secretory activity of the accessory glands (see Brandes, '66, '74b for references). Among insects, most studies on reproductive maturation have concentrated upon the primary reproductive organs, and especially on the hormonal control of oocyte growth and vitellogenesis in the female (Davey, '65; Englemann, '70; deWilde and DeLoof, '73). Relatively little research has concerned the process of maturation or its hormonal regulation in accessory reproductive glands which produce the spermatophore and seminal fluid of male insects. Notable exceptions include studies of *Rhodnius* (Wigglesworth, '36), *Melanoplus* (Weed-Pfeiffer, '36; Gillot and Friedel, '76), *Schistocerca* (Loher, '60; Cantacuzène, '67; Ohdiambo, '66, '71) *Gomphocercus* (Hartmann, '71) *Periplaneta* (Blaine and Dixon, '73), *Leptinotarsa* (DeLoof and Lagasse, '72), *Periplaneta* (Blaine and Dixon, '73) *Danaus* (Herman, '75a,b) and *Nymphalis* (Herman and Bennett, '75); in all of these cases, the corpora allata influenced the accessory glands.

It is our intent to analyze the maturation and regulation of the reproductive accessory glands in a holometabolous male insect, the mealworm beetle, *Tenebrio molitor*. Our aim will be to exploit the mealworm model as a general example of endocrine regulation of insect accessory glands and as a general paradigm for the study of processes of cytodifferentiation.

The form, number and disposition of male accessory glands vary greatly among the insect groups. In *Tenebrio*, the male has two distinct pairs, which have been classically designated as ectadenia and mesadenia, because of their presumed different embryological origins (Bordas, 1900). Singh-Pruthi ('24) argues for an ectodermal origin of both pairs. However, Huet ('66) has recently shown that both pairs of accessory glands are derived from two mesodermal ampullae in the ventral anterior portion of the ninth abdominal segment of the larva. In the present paper, we describe the ultrastructure of one pair of the accessory glands, classically called ectadenia but herein referred to as tubular accessory glands, in the post-ecdysial adult.

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In a companion paper (Happ et al., '77), we will describe the patterns of protein synthesis over the same period.

MATERIALS AND METHODS

Pupae of *Tenebrio molitor* L. were picked from stock cultures and the sexes were segregated. The light and electron microscopy were performed by standard techniques (Happ and Happ, '70; Lower, '55; Pearse, '68). In brief, tissues for transmission electron microscopy were fixed successively in glutaraldehyde and in osmium (after Locke, '66), were embedded in Epon 812, and the thin sections were stained with uranyl acetate and lead citrate (Reynolds, '63). Sections were photographed in an RCA-2E or an RCA 3-D electron microscope.

In male *Tenebrio*, the two seminal vesicles and the two pairs of reproductive accessory glands open into the proximal end of the ejaculatory duct (figs. 1–3). The tubular accessory glands are much coiled; when stretched to full length, each measures ca. 10 × 0.2 mm. Upon dissection of fresh tissue in Ringer's, the tubular gland appears translucent and makes slight rhythmic movements, three to four times each minute. Both pairs of accessory glands consist of a simple secretory epithelium surrounded by a thin muscular coat. The present paper will deal with the structure of the tubular gland; the bean-shaped accessory gland will be considered in a later paper.

The secretory epithelium

The secretory cells are tall and columnar, measuring 40–60 μ × 3–5 μ in a mature gland. Their nuclei are in the basal quarter of the cell, close to the surrounding muscle layer. Perinuclear and basal cytoplasm is intensely basophilic (Azure A, toluidine blue), and the cytoplasmic basophilia is abolished by pretreatment with RNAse (figs. 5, 6).

The intermediate regions of the secretory cells are packed with vesicles, which are not readily stained with Azure B or toluidine blue (figs. 5, 7). Lipid stains had no affinity for the vesicular contents, while PAS and protein stains (bromphenol blue, dinitrofluorobenzene) gave a moderate reaction in both the vesicles and the product in the lumen.

In low-power electron micrographs, each of the secretory cells exhibits regional differentiation. There are three distinct zones: basal, intermediate and apical (fig. 8).

The *basal* plasma membrane lacks significant infoldings, such as those seen in the accessory glands of male *Periplaneta* (Adiyodi and Adiyodi, '74), or the spermathecal accessory gland of female *Tenebrio* (Happ and Happ, '70) (figs. 8–10, 12). The nuclei are 5–10 μ down from the basal surface, and the intervening basal cytoplasm contains mitochondria, rough endoplasmic reticulum, and prominent Golgi zones. The mitochondria are small (usually 1–2 μ in length) and have a dense matrix (figs. 9, 10, 12).

The endoplasmic reticulum and the Golgi regions change dramatically over the four to six days just after ecdysis. In the first two days, free ribosomes abound, and the membranes of the rough endoplasmic reticulum are locally-inflated into spheroid cisternae connected by flattened isthmi creating a beaded appearance (fig. 9). The cisternal contents are homogeneous and rather electron-dense (fig. 9). The Golgi

Fig. 1 Dorsal aspect of the reproductive tract of the male mealworm beetle. TAG, tubular accessory gland; BAG, bean-shaped accessory gland; SV, seminal vesicle; Tes, testis; EjD, ejaculatory duct. Fresh preparation × 6.

Fig. 2 Ventral aspect of the convergence (arrow) of seminal vesicles, and accessory glands into the ejaculatory duct. Abbreviations as in figure 1. SEM × 24.

Fig. 3 Medial aspect of the junction (arrow) between the tubular gland and the ejaculatory duct. The right tubular and bean-shaped glands and seminal vesicle have been removed. Abbreviations as in figure 1. Fresh preparation × 26.

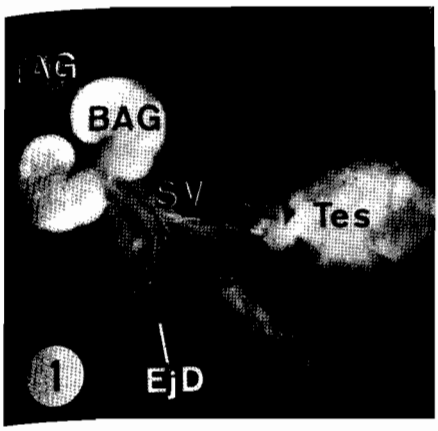
Fig. 4 The epithelium of the tubular gland at 2 hours after ecdysis. Compare with figure 7. M, muscle layer; B, basal zone; I, intermediate zone; L, lumen. Glutaraldehyde-osmium-Epon-toluidine blue × 536.

Fig. 5 Cross-section of mature TAG, stained with Azure B for nucleic acids. Cytoplasmic staining is heaviest in the basal zone. Abbreviations as in figure 4. Carnoy-Paraffin-Azure A × 187.

Fig. 6 Cross-section of mature TAG, exposed to RNAse before staining with Azure B. Staining is restricted to the nuclei in the secretory epithelium and in the muscle layers (arrows). Carnoy-Paraffin-Azure A × 220.

Fig. 7 The epithelium of the tubular gland at 130 hours after ecdysis. Compare with figures 4 and 8. Abbreviations as in figure 4. Glutaraldehyde-osmium-Epon-toluidine blue × 536..

Fig. 8 The epithelium 96 hours after ecdysis. A, apical zone; other abbreviations as in figure 4. TEM × 1,573.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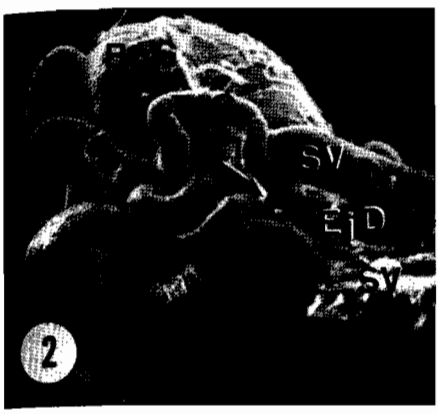
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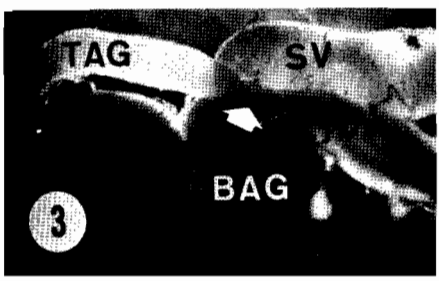
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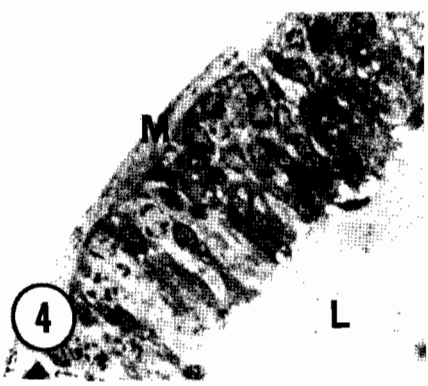
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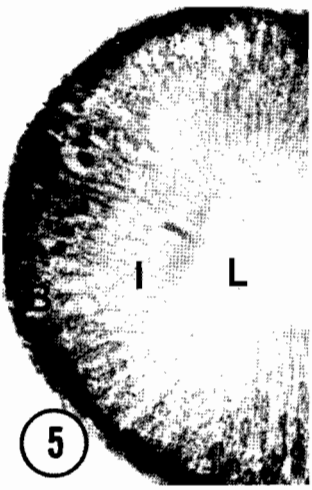
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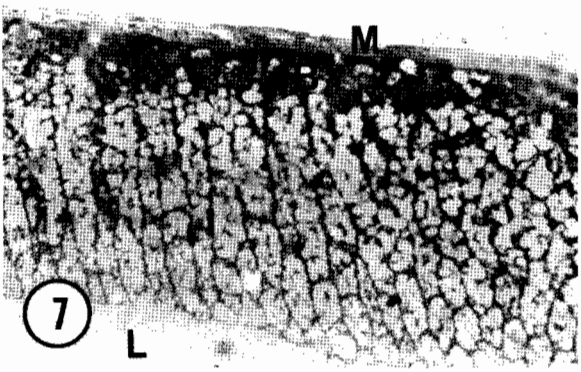
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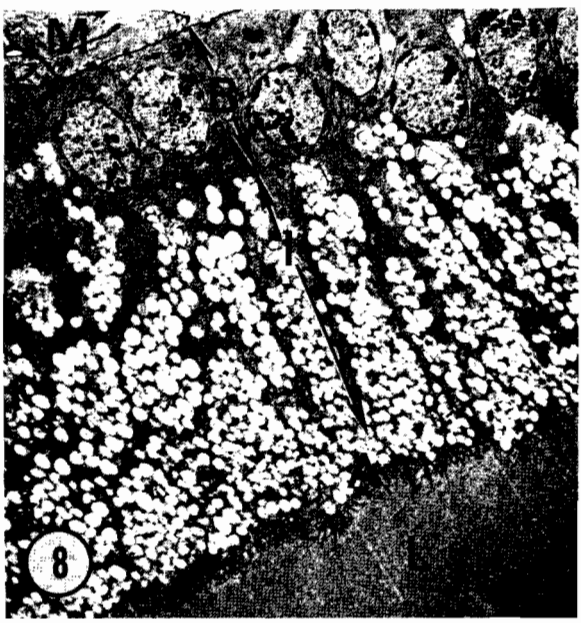
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regions consist of scattered,small vesicles (50 mμ in diameter) set in a granular matrix, and irregular flattened saccules with electron-transparent contents (fig. 11).

By three to four days after ecdysis, the intra-cisternal spaces in the basal cytoplasm have become enlarged and they seem to form a more-or-less continuous compartment, so that the true "cytoplasmic" space is confined to the narrow flattened channels between the membranes of the endoplasmic reticulum (figs. 10, 12). The membranes of the reticulum run parallel to the nearby cell surface. The Golgi zones are enlarged, and are surrounded by a granular matrix which obscures much of the detail (fig. 14). The intra-cisternal space appears to be continuous with the Golgi zones (fig. 14).

Large secretory vesicles, apparently derived from the Golgi, are prominent on the fourth day and thereafter. Dense, smaller bodies, suggestive of secondary lysosomes are less common (fig. 12). We failed to detect acid phosphatase activity in these granules.

The texture of the material within the membrane-bound spaces changes markedly over the first four days after ecdysis. At first, the cisternae of the endoplasmic reticulum contain homogeneous materials of moderate electron density, while the contents of Golgi saccules is electron-transparent. By three days after ecdysis, the membranes have become ordered into parallel cisternae with less dense content than before. Golgi saccules enclose wisps of electron-dense material. On day four and thereafter, the vesicles apparently derived from the Golgi contain a loose irregular network of fibers.

The nuclei of the secretory cells are ovoid. Peripheral patches of heterochromatin are closely associated with the inner leaflet of the nuclear envelope. Between the dense heterochromatin patches are the nuclear pores. The regions of the nuclear envelope with pores contain a central dense line and are associated with microtubular structures which apparently transverse the nuclear envelope (fig. 13). Immediately after ecdysis, the large nucleolus is often near the nuclear pores, and within the nucleolus are many particles of a size similar to that of ribosomes. At four days, the nucleolar granules are much less common and the nucleolus appears more compact and more centrally located.

At the time of ecdysis, the *intermediate zone* is similar to the previously-described basal zone. A prominent Golgi area is just apical to the nucleus, free ribosomes abound, and the cytomembrane system is not extensive. Within the first two days, a few large secretory vacuoles appear at the most apical end of the cell, and then progressively accumulate toward the nucleus until more and more of the cytoplasm is occupied by these vacuoles. The secretory vacuoles are first filled with a compact fibrous network (fig. 16) which progressively becomes more diffuse (fig. 15) until the mature vacuole, with diffuse fibers, is formed (fig. 17). The process appears to be a decondensation. By three days, almost three-quarters of the apical region is occupied by vacuoles, and by four days, the apical zone is packed with secretory vacuoles to the exclusion of most other organelles (figs. 3, 17). In mature glands the large secretory vesicles are densely packed in this region; often the vesicles seem to fuse with one another (figs. 15–17). Mitochondria, rough endoplasmic reticulum, and small Golgi zones persist in the interstices (fig. 17).

At ecdysis, the *apical* portion of the cell supports a number of microvilli and a variety of other irregular protrusions of the plasma membrane. The secretory bodies are small, 0.2–1.5 μ in diameter, and contain granular electron-dense material (figs. 18, 19). In mature glands the content of the vesicles seems to be expelled by apocrine secretion (figs. 20, 22) and, as shown by the heterogeneity of the contents in the lumen (fig. 21), merocrine secretion apparently also takes place. When fresh glands are detached from their junction with the ejaculatory duct, the secretory exudate often appears heterogeneous in phase contrast.

In freshly-ecdysed adults, the tubular accessory gland is ca. 80 μ in diameter and 5 mm in length. At six days it is twice the diameter and length. Increases in gland volume were determined by water displacement (fig. 23). The 8-fold volume increase occurs mostly in the fourth, fifth, and sixth day after ecdysis. Since no mitotic figures

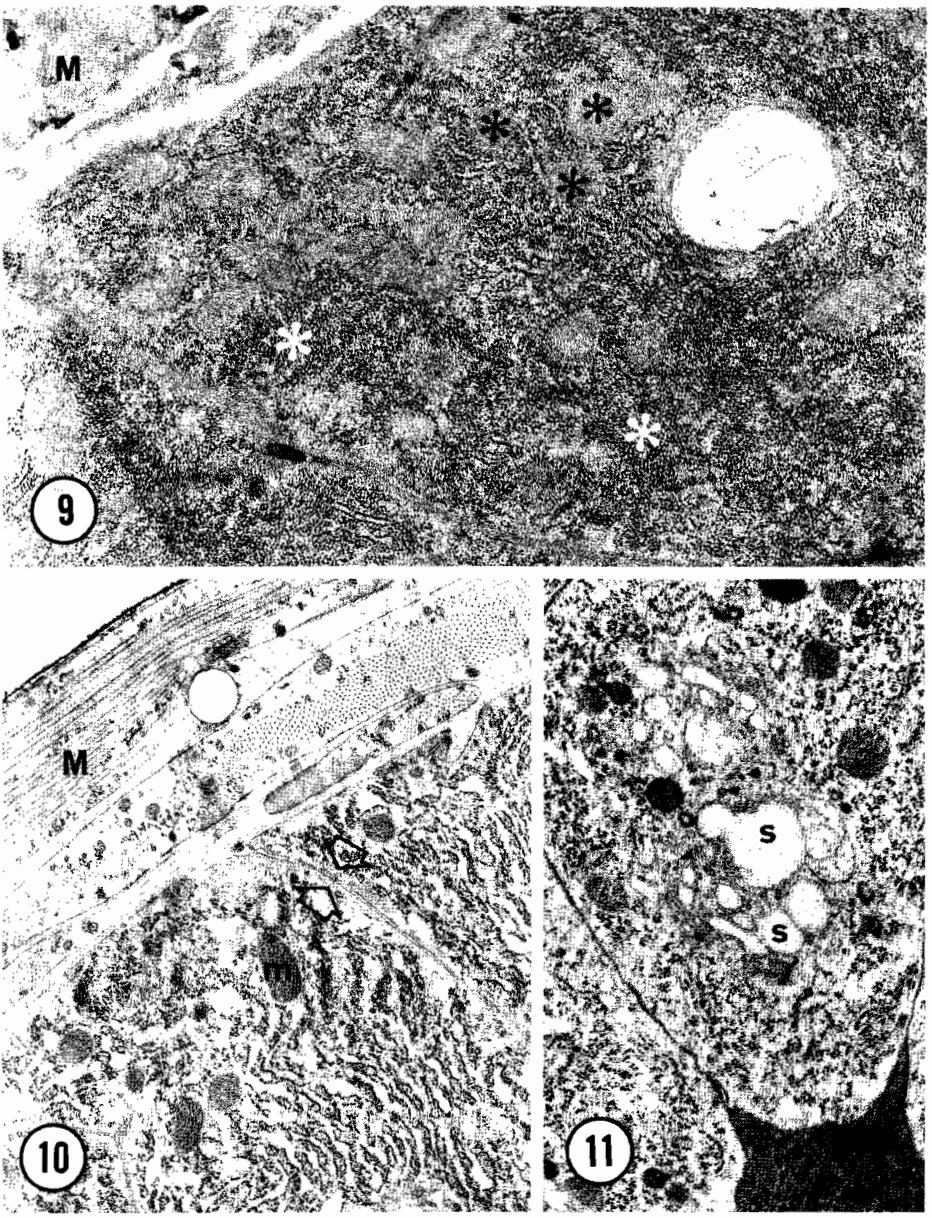
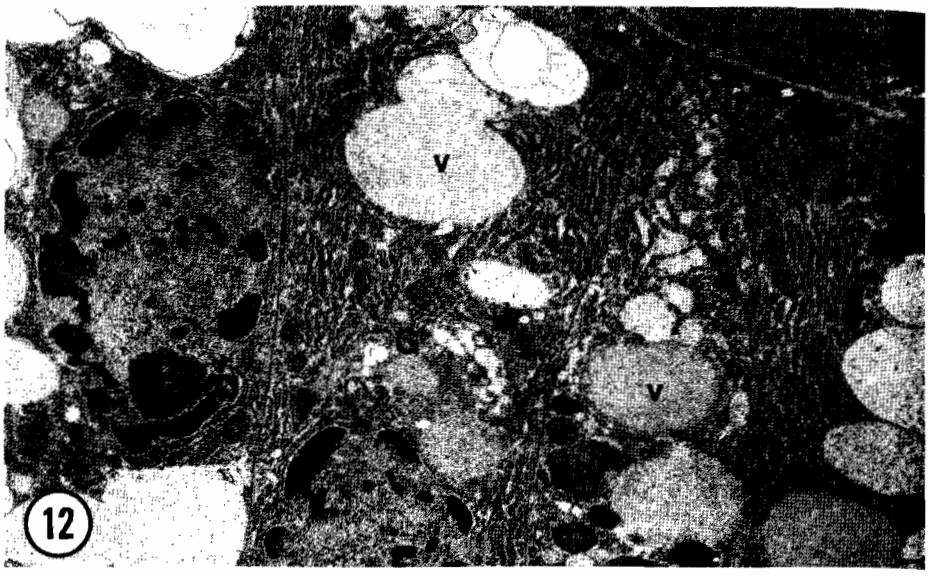
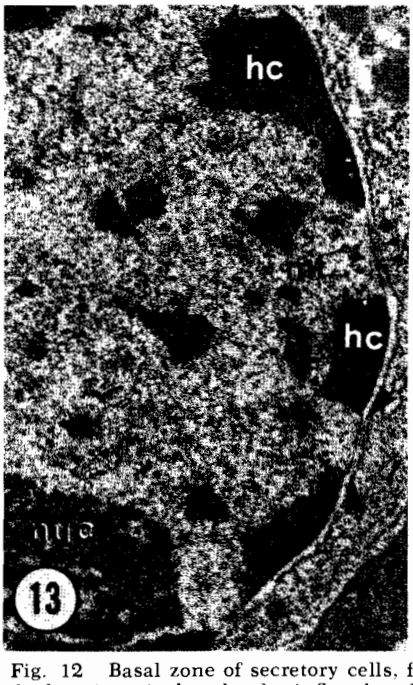


Fig. 9 Basal zone of secretory cell, two days after ecdysis. Local inflations of the rough endoplasmic reticulum (black asterisks) are filled with homogeneous material of moderate electron density. Many of the ribosomes do not appear to be membrane-bound (white asterisks). M, muscle. × 15,130.

Fig. 10 Basal zone of secretory cell, three days after ecdysis. Most of the ribosomes are membrane-bound and cisternae contain material of rather low electron density. Adjacent to the lateral plasma membranes is a thin layer of fine filaments (between hollow arrows). M, muscle; m, mitochondrion. × 11,645.

Fig. 11 Basal zone of secretory cell on day of ecdysis. The saccules (s) of the Golgi have electron-transparent contents. Small Golgi vesicles are also present (arrowheads). × 14,450.





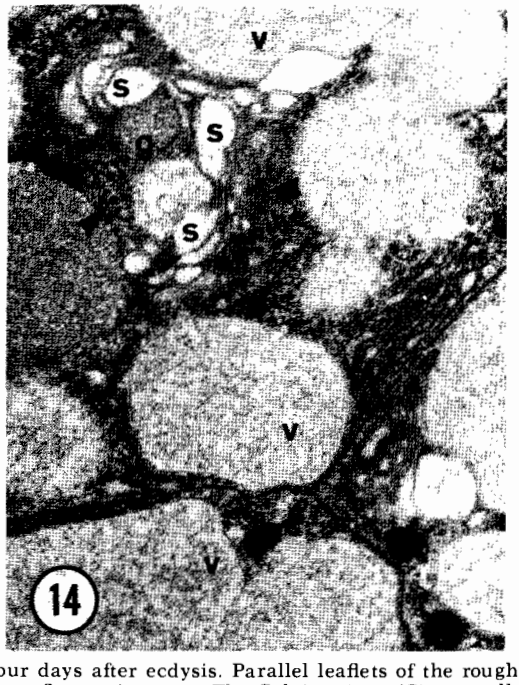
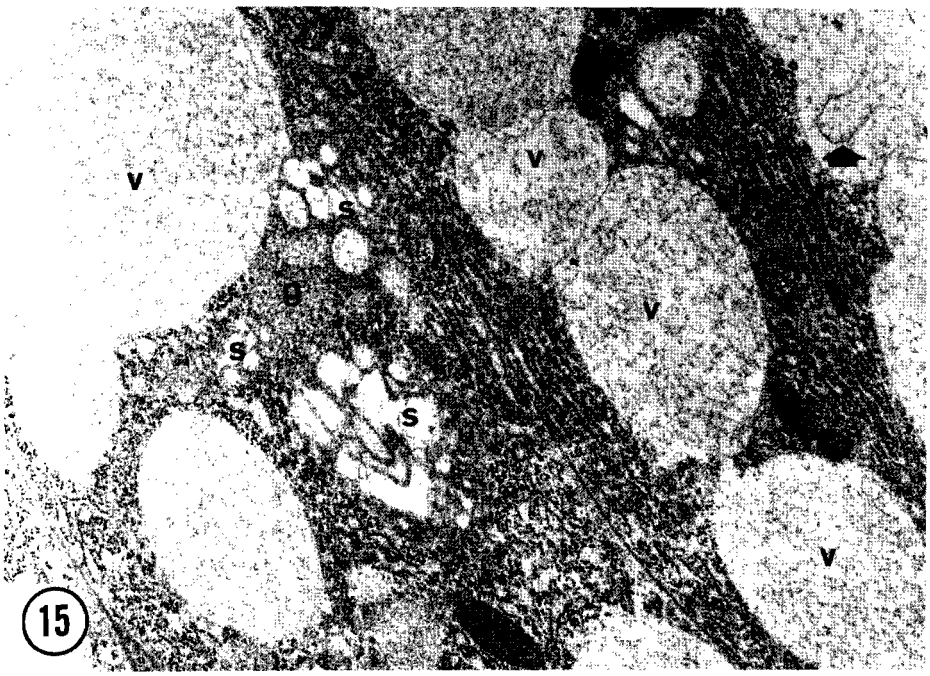
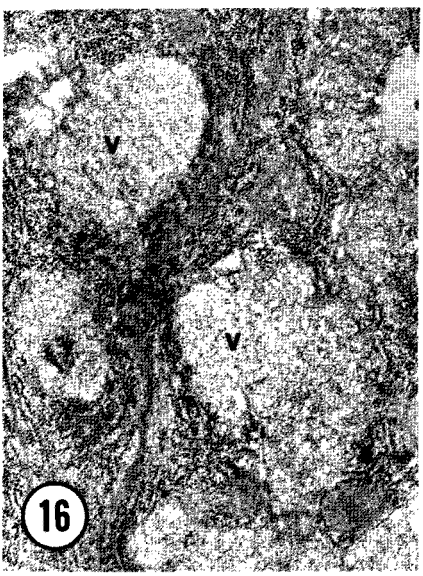


Fig. 12 Basal zone of secretory cells, four days after ecdysis. Parallel leaflets of the rough endoplasmic reticulum border inflated and confluent cisternae. The Golgi regions (G) are well-developed. Secretory vesicles (v) are large and contain granular material. Electron-dense bodies (db) are not uncommon. M, muscle. × 5,695.

Fig. 13 Nucleus of a secretory cell. Peripheral patches of heterochromatin (hc) lie between intervening clusters of nuclear pores (small arrowheads). A central dense line of the nuclear envelope is present at the pore-containing regions (large arrowheads) and a cluster of microtubules (mt) lies in the adjacent nucleoplasm. nuc, nucleolus. × 10,625.

Fig. 14 Golgi region in the basal zone of a secretory cell, seven days after ecdysis. As is characteristic of mature Golgi, a central granular zone (g) is surrounded by irregular saccules (s) which lie between loosely stacked paired membranes. A few small vesicles (arrowheads) are associated with the electron dense periphery. The secretory vesicles (v) are bounded by a single membrane. × 11,390.





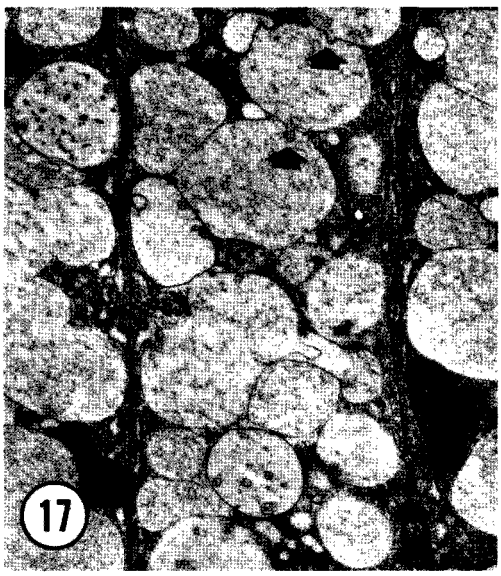


Fig. 15 Intermediate zone of secretory cell, three days after ecdysis. The granular region (g) of the Golgi lies between three clusters of saccules (s). The large secretory vesicles (v) occupy the center of the cell and the rough endoplasmic reticulum is pushed to the periphery of each cell. Outpocketings of the membranes bounding these vesicles (arrow) suggest points of fusion. × 11,645.

Fig. 16 Intermediate zone, two days after ecdysis the content of the vesicles (v) is more condensed than in older cells, shown in figures 15 and 17. × 11,050.

Fig. 17 Intermediate zone, four days after ecdysis. Secretory vesicles occupy almost all of the cytoplasmic space. Outpocketings (arrows) suggest points of fusion. × 7,395.

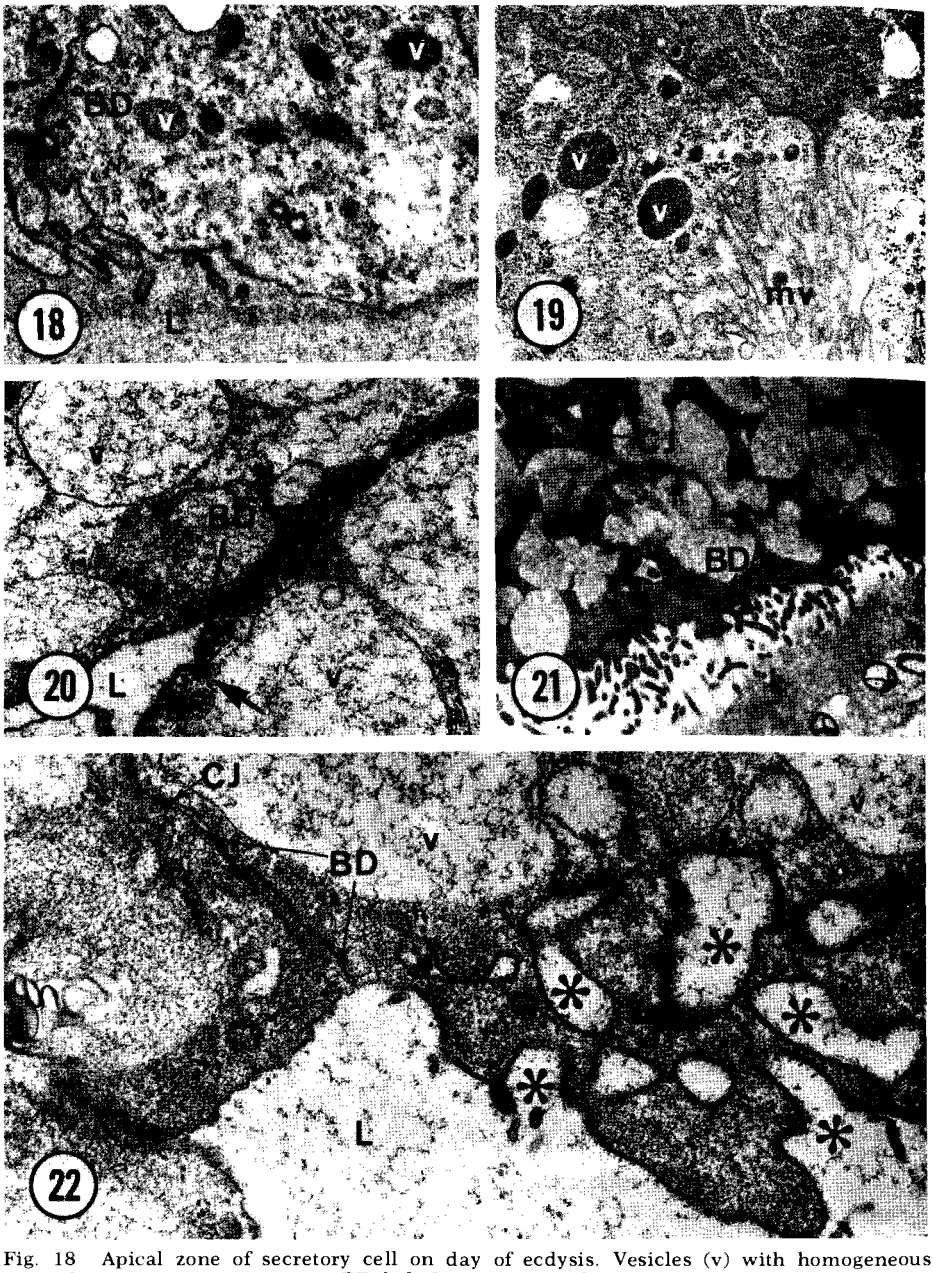


Fig. 18 Apical zone of secretory cell on day of ecdysis. Vesicles (v) with homogeneous electron-dense contents are present. BD, belt desmosome; L, lumen. × 9,605.

Fig. 19 Apical zone, one day after ecdysis. Microvilli (mv) and vesicles (v) with homogeneous electron-dense contents are present. × 11,050.

Fig. 20 Apical zone, three days after ecdysis. Large vesicles (v) with loose, flocculent content closely approach the plasma membrane and may fuse with it (arrow). BD, belt desmosome; L, lumen. × 22,100.

Fig. 21 Apical zone, three days after ecdysis. The secretion in the lumen is heterogeneous, containing both fibrous materials and cellular debris. BD, belt desmosome; CJ, continuous junction; L, lumen. × 5,780.

Fig. 22 Apical zone, three days after ecdysis. Broad inpocketings of the plasma membrane (asterisks) project toward the secretory vesicles (v). BD, belt desmosome; CJ, continuous junction. × 26,350.

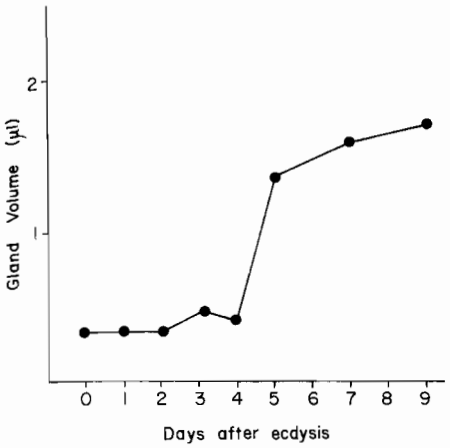


Fig. 23 Volume estimates for pairs of tubular glands, determined by counting the number of fresh glands necessary to displace fixed volumes of saline.

were observed, this volume increase must reflect primarily increase in cell or lumen size. Measurements of lumen diameter indicate a slight increase but much more significant are changes in secretory cell length (30 μ at day 0 and 50 μ at day 5) and cell diameter (3 μ at day 0 and 4–5 μ at day 5).

The lateral margins of the secretory cells are closely apposed to one another. The intercellular junctions appear to be of the continuous variety (Noirot and Noirot-Timothée, '67); the intercellular space (150–200 Å) is filled with electron-dense material (figs. 17, 21, 22) and septae are not evident. However, septate junctions are common components of the post-nuclear pellet. We suspect that most of the lateral junctions are septate but the septa are obscured by the electron-dense-material. Adjacent to the lumen, the lateral cell contacts are belt desmosomes (figs. 18, 19–22).

The muscle layer

Near the basal margin of the cells is a muscle layer, characteristically two cells thick. The two layers tend to be perpendicular to one another, with the inner more "circular" and the outer more "longitudinal" in relation to the gland. The sarcoplasm contains ribosomes, small mitochondria, and microtubules but very little sarcoplasmic reticulum. A T-system was not observed. A thin basement membrane lies directly over the secretory cells and between the muscle cells, and a more compact basement membrane lies outside the muscle layers (fig. 10).

DISCUSSION

In its general ultrastructural features, the tubular accessory gland of *Tenebrio* is rather like male accessory glands of other species, including those of *Schistocerca* (Ohdiambo, '69a,b, '71), *Locusta* (Cantacuzène, '72), *Periplaneta* (Beams et al., '62; Adiyodi and Adiyodi, '74), *Drosophila* (Bairati, '68; Perotti, '71), *Culex* (Tongu et al., '72), *Formica* (Jeantet, '72) and *Leptinotarsa* (DeLoof and Lagasse, '72). The secretory epithelium is surrounded by a muscular coat that is monomyofibrillar, a common situation in insect visceral muscle (Smith et al., '66). In glands of *Leptinotarsa* and *Periplaneta*, as in mature *Tenebrio* tubular glands, the secretory cells are of one morphological type. The "mixed" apocrine-merocrine mechanisms for export of secretory products are found in several other accessory glands, for example those of *Periplaneta* (Adiyodi and Adiyodi, '74) and *Leptinotarsa* (DeLoof and Lagasse, '72). In contrast to the tubular gland of *Tenebrio*, several cell types are present in the accessory gland of *Culex*, *Drosophila*, *Locusta*, and *Schistocerca*. However, such cellular heterogeneity is characteristic of the bean-shaped glands of *Tenebrio* (Gadzama, '72).

Previous histochemical studies of insect accessory glands have revealed a variety of lipoproteins, glycoproteins, and mucopolysaccharides (e.g., Ohdiambo, '69a). Our histochemical data show proteins and glycoproteins in the secretion of the tubular gland of *Tenebrio* — a result consistent with the earlier studies. The presence of a single cell type suggests that a few differentiation-specific proteins may be produced.

The rapid post-ecdysial maturation of the tubular gland involves a sequence of steps: proliferation of the rough endoplasmic reticulum, swelling of the cisternae, coincident changes in density of their contents, and formation of secretory vacuoles in the Golgi zone. The mature secretory cell is packed with secretion at four days. On morphological criteria, we cannot determine whether protein synthesis and ex-

port continue (and balance each other), or whether the gland becomes inactive at five days after ecdysis. This possibility will be considered in the companion paper (Happ et al., '77).

On the ultrastructural level, there are striking similarities between the tubular accessory gland of *Tenebrio* and accessory glands of mammals. The regional differentiation of the secretory cells is very similar in the rat ventral prostate (Dahl et al., '73; Brandes, '74a) and the *Tenebrio* tubular gland. The secretory vacuoles of the tubular gland are rather like those in human prostate (Brandes, '74a). Apocrine-merocrine secretion has been seen in the rat, mouse, and rabbit prostates (Franks and Barton, '60; Brandes and Portela, '60; Brandes, '66; Flickinger, '71; Nicander et al., '74) as well as the tubular gland of *Tenebrio* (present paper) and accessory glands of *Periplaneta* and *Leptinotarsa* (Adiyodi and Adiyodi, '74; DeLoof and Lagasse, '72).

The prostates of vertebrates are highly sensitive to changing levels of circulating testosterone (Brandes, '66, '74b). As noted in the Introduction, the accessory glands of some long-lived insects are influenced by the corpora allata. The effects of the endocrine milieu upon the morphological cytodifferentiation of the tubular gland of *Tenebrio* will be considered in future publications.

ACKNOWLEDGMENTS

Early phases of this study were conducted in the Department of Biology, New York University, Bronx, New York. The discussion of the work with Winston E. Anderson (Howard University) and Margaret Grimes is appreciated. Partial support from the National Institutes of Health GM-21711 is gratefully acknowledged. We thank Janelle Simon for typing this manuscript.

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