

# Structure and Development of Male Accessory Glands in Insects

GEORGE M. HAPP

## *1. Introduction*

Reproductive accessory glands of male insects facilitate transfer of sperm to the females. The heterogeneous products of these glands include both the seminal fluids and paraseminal structures. The secretions vary widely in physical properties, biochemical composition, and physiological function. Secretions of low viscosity bathe the sperm. Within that fluid mixture are components which promote sperm maturation, which provide nourishment for stored sperm, which contribute nutrients for investment into egg yolk, or which modulate the behavior or the physiology of the female (Leopold, 1976). Secretions of higher viscosity solidify to become copulatory plugs (Bishop, 1920; Bairate and Perotti, 1970) or complex spermatophores (Tuzet, 1977). These organized secretory masses are composed of several distinct zones that form as the glandular products flow over one another in extracellular space.

In the past few decades, most research on the reproductive physiology of insects has concentrated on the phenomenology of reproductive maturation and the endocrine control of that maturation in the female sex (e.g., Engelmann, 1970; Kafatos *et al.*, 1977; Hagedorn and Kunkel, 1979; Telfer *et al.*, 1982). Although much less studied, male reproductive physiology is no less complex (e.g., Davey, 1965, 1967). At the ultrastructural level, we have many stunning descriptions of insect spermatozoa (Baccetti, 1972). The processes of spermiogenesis and spermatogenesis provide superb examples of the profound morphological changes that can occur during terminal differentiation of specialized cells (Phillips, 1974). This differentiative sequence takes place in preadult

stages for most insects. Spermatogenesis is modulated by the rise and fall of the concentrations of juvenile hormones and ecdysteroids that simultaneously regulate metamorphic growth (Dumser, 1980).

By the time an insect has reached the adult stage, there are so many spermatozoa present that numbers alone rarely constitute limiting factors for population growth. Delivery is another matter. Delivery of sperm to the female requires a vehicle which is produced by the accessory glands. The nature of that vehicle and its elaboration from the glands are poorly understood in most insect species. For males which mate repeatedly, the glands must go through recurrent secretory cycles to produce the charge of semen and paraseminal material for each copulation. The maturation of accessory glands is regulated by endocrine and neuroendocrine factors in almost all groups. These glands offer attractive models in which to study hormone action and secretory kinetics.

## 2. Accessory Glands and Their Secretions

In insects as in mammals (Mann, 1964), semen and its accompaniments are an aggregation of biochemical constituents derived from morphologically diverse and complex glands. Some of these glandular products are proteins; included among them are structural components of the wall of the spermophore, enzymes of uncertain function such as esterase 6 (Gilbert, 1982), and molecules of known physiological significance such as mating refusal substances of *Drosophila* (Garcia-Bellido, 1964). Smaller molecules, including sugars and lipids, are reported in the semen of many phyla (Mann, 1964), including such insects as honeybees (Blum *et al.*, 1962, 1967). The enormous diversity of reproductive strategies among species and the biochemical heterogeneity within the semen of every species are widely acknowledged. We are quite ignorant of the biochemical nature and functional significance of most seminal constituents.

The male reproductive tract is a muscular tube which runs from the testes to the gonopore. In insects, the proximal portion of the tract is mesodermal in origin while the distal portion is ectodermal. In all species, some secretions are produced by cells along the wall of the tract. In many groups, there are also distinct glands attached to the reproductive tract. These accessory glands differ in size, shape, number, anatomical placement, and embryological origin (Adiyodi and Adiyodi, 1975; Grassé, 1977).

The accessory secretions may mingle with the sperm, may precede the sperm mass, may enclose it, or may follow along afterward. Seminal vesicles, phallic glands, mushroom glands, conglobate glands, accessory glands, secretory segments of ejaculatory ducts, paragonia, ectadenia, mesadenia, simplex, and duplex are just a few of the terms which describe the organs that produce seminal or paraseminal secretions. I will employ the common terminology for each group, with the general caveat that the use of a similar trivial name does not necessarily connote homology. I will not attempt to review the morphological peculiarities of various groups nor to consider in detail the evolutionary or embryological origins of accessory glands. Rather, I will emphasize common

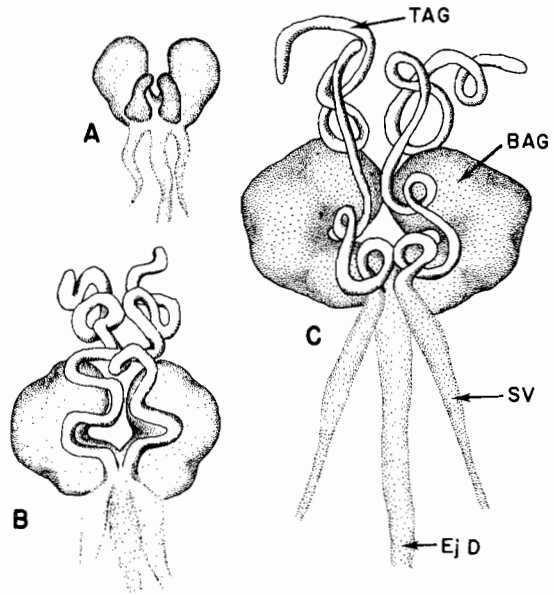


Figure 1. The accessory glands of *Tenebrio molitor* in the newly ecdysed pupa (A), the newly enclosed adult (B), and the reproductively mature adult (C). BAG, bean-shaped accessory gland; Ej D, ejaculatory duct; SV, seminal vesicle; TAG, tubular accessory gland.

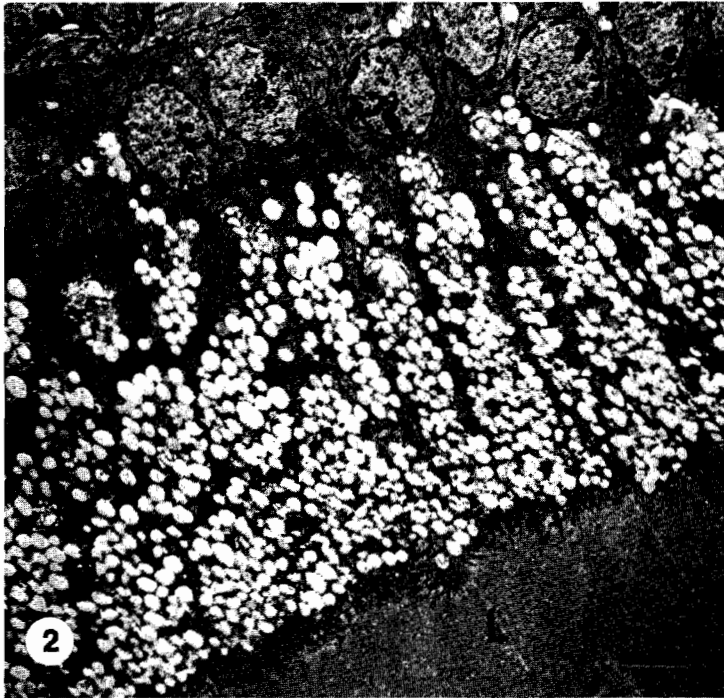
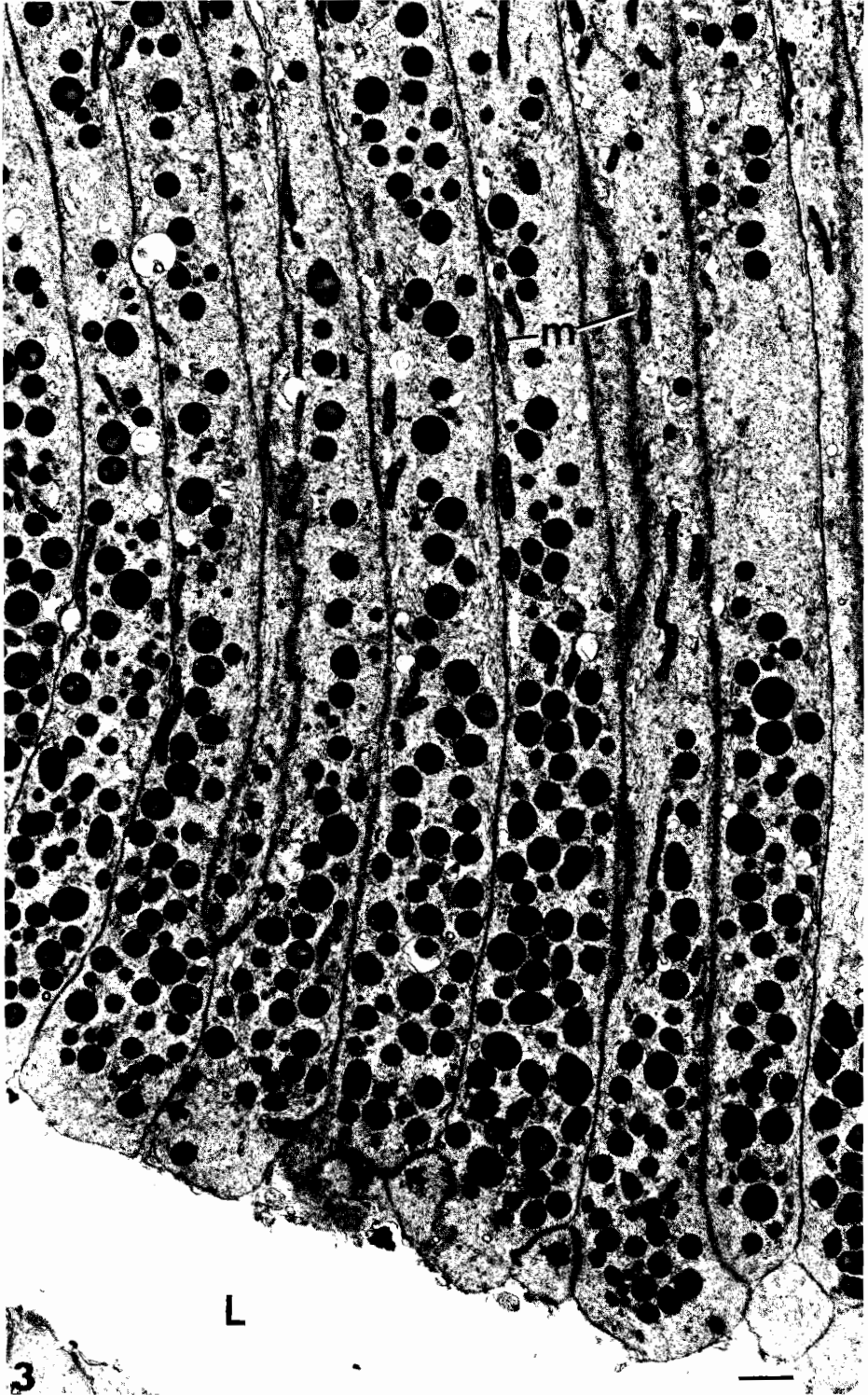


Figure 2. The secretory epithelium in TAG of a mature male. The muscle layer (M) is at the upper left and the lumen (L) of the gland at lower right. Note the basally situated nuclei and the many large secretory vesicles. (Bar = 5  $\mu$ m.)



structural features of secretory epithelia, some characteristics of their products, and the process of their differentiation.

The morphology of accessory glands will be illustrated by micrographs of the tubular accessory glands (TAGs) (Figure 2) and of the bean-shaped accessory glands (BAGs) (Figure 3) of mealworm beetles (*Tenebrio molitor* L.) which have been studied over the past decade in my laboratory. For reference, the shapes and positions of these glands in the male reproductive tracts in pupae and adults are shown in Figure 1.

### 3. Muscular Coats and Basement Membranes

Most accessory glands consist of a secretory epithelium surrounded by a muscular sheath. The exceptions are certain ectodermal accessory glands, such as the phallic gland of cockroaches, which export their products via cuticular plumbing systems and lack any clear muscle coat. When present, the muscles typically have long sarcomeres (6–10  $\mu\text{m}$ ) and a poorly developed T system. The sarcoplasm contains a few free ribosomes and scattered profiles of ER. Mitochondria are usually tubular and often aligned in parallel with the muscle filaments. Within any one layer, the muscle fibers are tightly attached to each other (end-to-end) by the intercellular matrix. For most glands, there are two or more layers of muscle cells which spiral obliquely to the right or to the left, around the generally cylindrical gland. The thickness of the muscle coat seems to be correlated with the consistency of the product of each gland. In *Tenebrio*, the tubular gland secretes a fluid product and is surrounded by two or three layers of muscle cells (Figure 4) while the bean-shaped gland produces a semisolid plastic plug which is forced out by the contraction of six to eight layers of muscle cells (Figure 5).

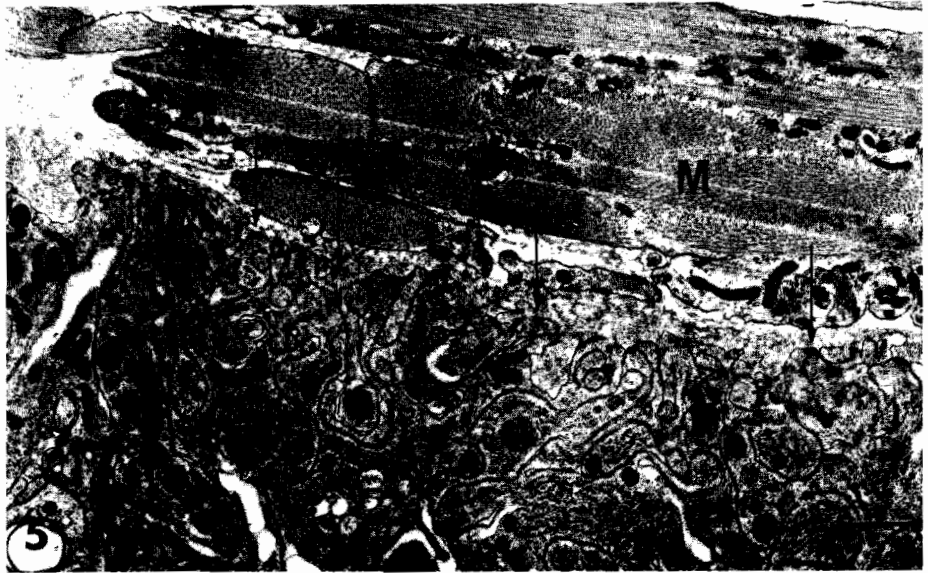
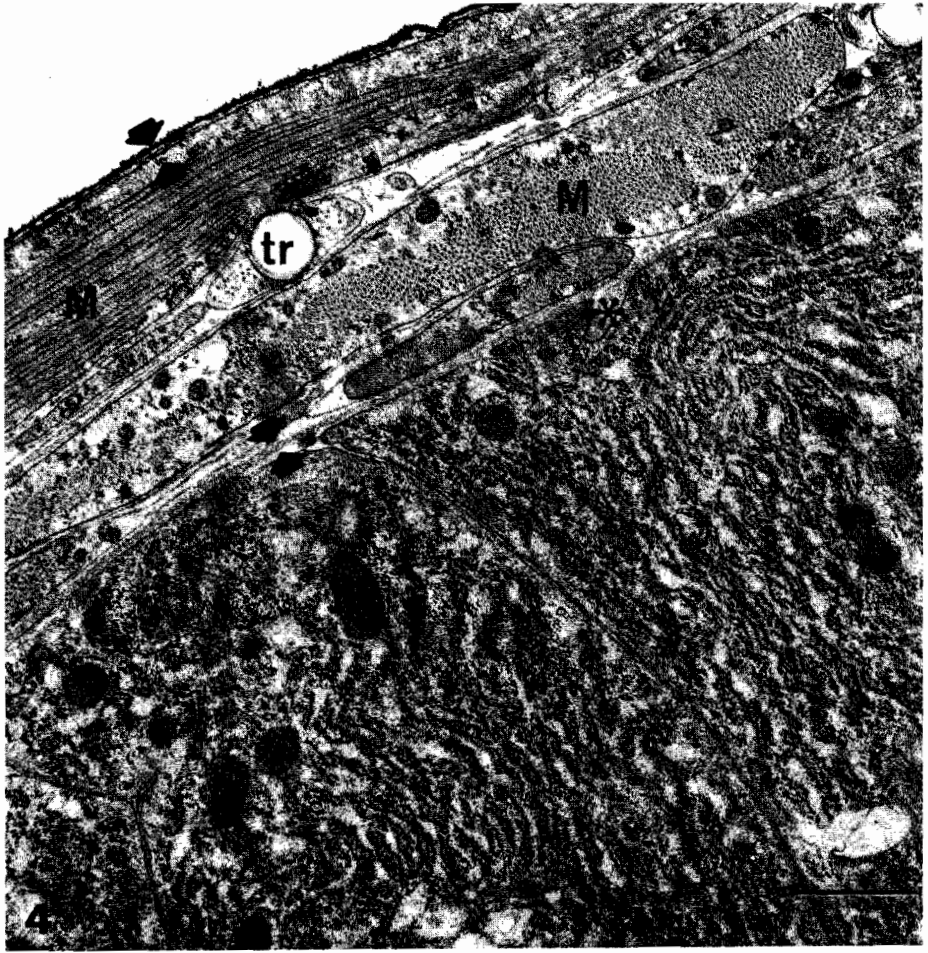
Basement membranes surround the muscle layer and also coat the basal surface of the secretory cells (Figure 4). These extracellular matrices appear as a loose fibrous network which fills in many of the interstices between muscle cells (Figure 4). Tracheoles, enclosed in their cellular coats, run between the muscle cells and deep into the secretory epithelium. The nuclei of the tracheolar end cells may sometimes be found squeezed between the secretory cells.

### 4. The Secretory Epithelia

The secretory epithelia elaborate a battery of seminal and paraseminal components. In female insects, massive amounts of vitellogenic proteins are manufactured by the fat body, carried by the hemolymph, and transported by follicular cells to form the yolk (Telfer, *et al.*, 1982). With rare exceptions (Friedel and Gillot, 1976), secretory proteins are not made in the fat body of the male and

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**Figure 3.** The apical zone of several cells of the secretory epithelium in the BAG. The many secretory vesicles (type 5) are packed above the zone of apical desmosomes (arrows). m, mitochondrion; L, lumen. (Bar = 1  $\mu\text{m}$ .)



taken up by accessory glands. Secreted proteins appear to be manufactured *de novo* within the epithelium. The relative unimportance of other organs is illustrated by the fact that the accessory glands of *Tenebrio* (Happ *et al.*, 1977) and *Rhodnius prolixus* (Barker and Davey, 1982) continue to make cell-specific proteins during organ culture *in vitro*.

Secretory epithelia of accessory glands, like most insect epithelia, are histologically simple, i.e., they are monolayers (Figure 2). There have been reports of stratification in some epithelia of accessory glands (Ohdiambo, 1969), but we suspect that the multilayered appearance is an artifact due to the plane of section and interdigitation of the cells. The individual cells may be quite long; in the bean-shaped gland of *Tenebrio*, single cells reach 300–500  $\mu\text{m}$  from the basement membrane to the lumen of the gland (Dailey *et al.*, 1980).

Each secretory cell is an independent factory for manufacture, storage, and export of secretory products. At its basal surface, precursors are absorbed from the hemolymph. Throughout most of the length of the cell, rough or smooth ER and Golgi zones are engaged in manufacture, packaging, and transport of products in membrane-bound vesicles. At the apex of each cell, secretions are expelled into the lumen.

As the male becomes reproductively mature, the lumina of the ducts and the accessory glands fill with products. During copulation, a large portion of these secreted materials is lost in the ejaculate, and subsequently they must be replenished from the secretory cells. Within a short period after mating (usually 10 hr at the most), the lumen is recharged with new products. Thus, the secretory process is discontinuous; it proceeds in episodes which are triggered by the occasional emptying of the lumina of the tract and the glands.

#### 4.1. Intercellular Junctions

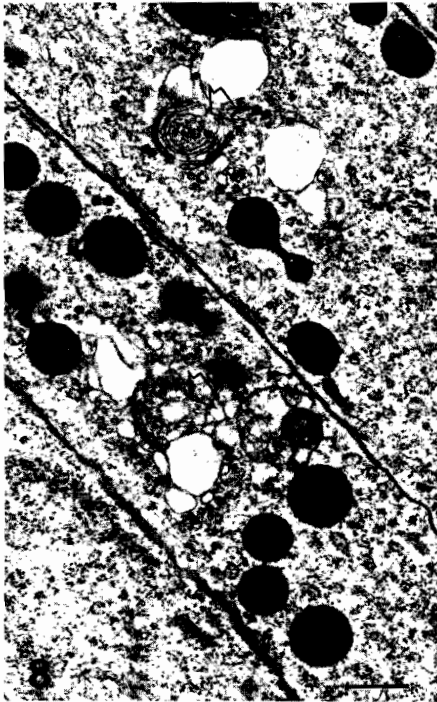
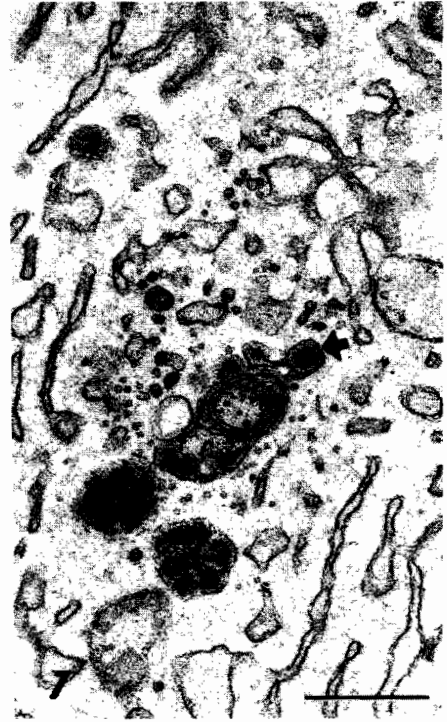
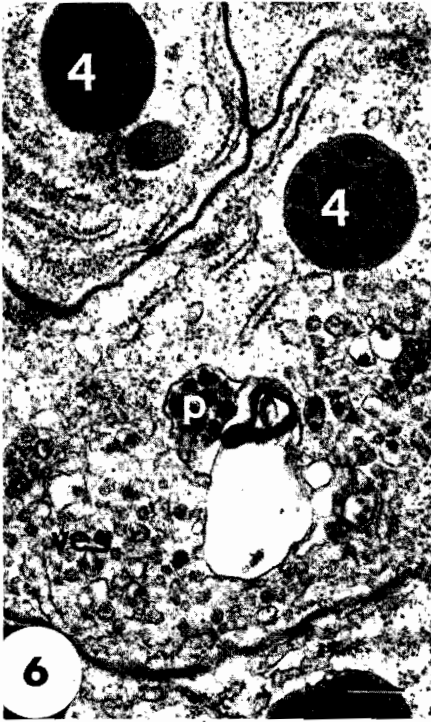
The cells of the secretory epithelium are appressed tightly to one another along their lateral surfaces. Two intercellular links, septate junctions and apical desmosomes, are major factors in maintaining the integrity of the epithelial sheet in most accessory glands.

In the bean-shaped glands of *Tenebrio*, the apical desmosomes form a belt, 0.6–1  $\mu\text{m}$  in width, around each secretory cell (Figures 4, 17–19). Adjacent cells are separated by a 23- to 25-nm zone which contains flocculent extracellular material that is not well resolved in our transmission micrographs (Figure 12). Dense plaques, 30–40 nm in thickness, lie on the inner cytoplasmic surface (Figure 12). As in other insect desmosomes (Lane and Skaer, 1980), these plaques lack tonofilaments which run toward the center of the cell. Microtubules, which

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**Figure 4.** The muscle coat and basement membranes (between arrows) surround the secretory epithelium of the TAG. The filaments in the two muscle cells (M) run at right angles to each other. A process of the inner basement membrane (asterisk) indents the basal edge of one secretory cell. The secretory cells contain scattered mitochondria (m) and much granular ER. tr, tracheole. (Bar = 1  $\mu\text{m}$ .)

**Figure 5.** A portion of the muscle coat and the basal zone of secretory cells of the mature BAG. Note the infoldings of the basal plasma membrane and the hemidesmosome plaques (arrows) which anchor the cells to the inner basement membrane. (Bar = 1  $\mu\text{m}$ .)



**Figure 6.** The Golgi region of cell type 4 of the mature BAG. Small vesicles (ves) are found throughout the Golgi zone. A precursor (p) of the definitive secretory vesicle (4) is seen at the edge of the Golgi. (Bar = 0.2  $\mu\text{m}$ .)

**Figure 7.** Golgi zone of a secretory cell of the mature BAG. A precursor granule is indicated by the arrow. Potassium permanganate fixation. (Bar = 1  $\mu\text{m}$ .)

run parallel to the long axis of the secretory cell, generally end at the level of the apical band (Figure 20).

Above the apical band, cells are held together by septate junctions. The septa are more difficult to resolve (Figure 12) as is typical of the smooth septate or continuous junctions (Lane and Skaer, 1980). The junctions usually extend 50  $\mu\text{m}$  or so above the apical desmosomes. In the tall cells within thick epithelia, there is a zone above the desmosomes where the membranes of adjacent cells are separated into no more than 40–50  $\mu\text{m}$ , and wisps of material (extracellular matrix?) can be seen in the gap. Finally, the cells are anchored to the basement membrane by hemidesmosome plaques (Figure 5). These structures keep the fabric of the epithelium intact during powerful contractions that occur during ejaculation.

#### 4.2 Absorption of Precursors

Precursor molecules must percolate through basement membranes and the interstices between the muscles to reach the secretory cells. The movement and incorporation are rapid. Within 10 min of injection of tritiated amino acids into the hemocoel, we have detected radioactive secretory proteins in the accessory glands of *Tenebrio*. In the basal regions of secretory cells in the utriculi majores of the mushroom-shaped gland of male *Periplaneta americana* (Adiyodi and Adiyodi, 1974), in the simplex of *Calopodes ethlius* (Lai Fook, 1982b), in the ejaculatory duct of *Stomoxys calcitrans* (Meola, 1982), and sometimes in the basal edges of the bean-shaped gland of *Tenebrio* (Figure 5), the plasma membrane may be deeply infolded, presumably to increase the absorptive surface. No coated pits or coated vesicles have been reported at the basal surfaces of accessory glands. Coated pits usually indicate receptor-mediated endocytosis of large molecules (Steinman *et al.*, 1983). The absence of coated pits or vesicles is consistent with absorption of small molecules either by active transport, facilitated diffusion, or passive diffusion or by fluid-phase endocytosis (pinocytosis). Fluid-phase endocytosis is difficult to capture in an electron micrograph since the half-life of endocytotic vesicles is probably no more than a few seconds (Steinman *et al.*, 1983).

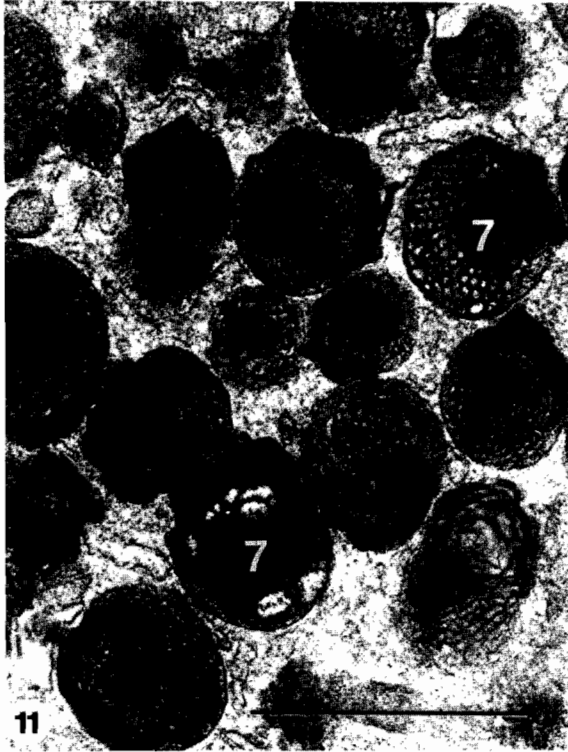
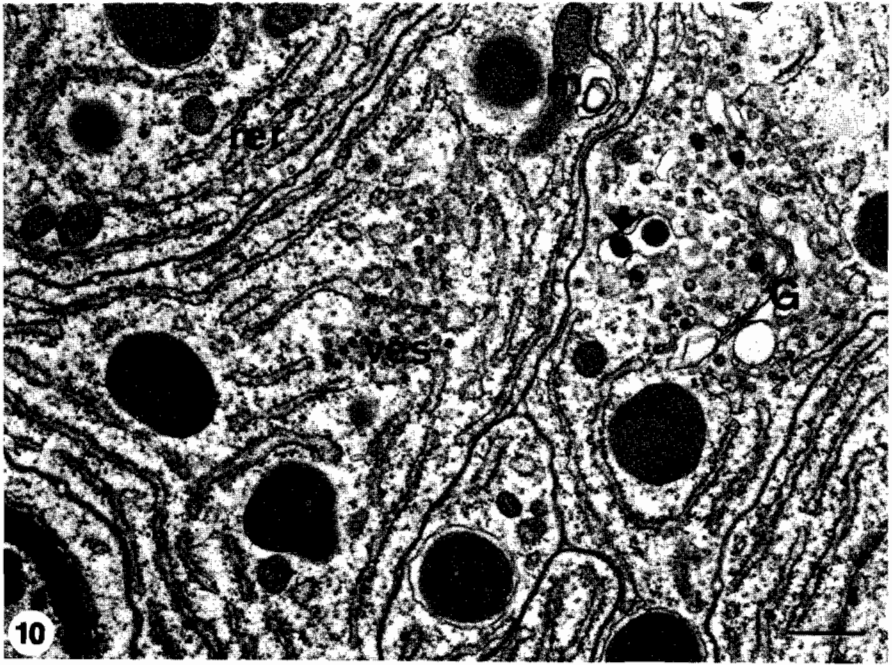
#### 4.3 Biosynthetic Machinery

In active secretory cells, the cytoplasm is packed with profiles of ER, Golgi complexes, microtubules, and secretory vesicles. The membranes of the ER may be smooth (Figure 13) or ribosome-studded (Figure 10). Secretory granules accumulate in the apical half of the cell, sometimes to the exclusion of other organelles, with the result that most of the cytomembranes become confined to the basal portion of the cell (Figures 2, 4).

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**Figure 8.** Golgi zones of a secretory cell in a BAG, 39 hr after adult ecdysis. Secretory vesicles are beginning to form. Note the central precursor granule (hollow arrow) in each Golgi zone. (Bar = 1  $\mu\text{m}$ .)

**Figure 9.** Golgi zones of a secretory cell of a mature BAG. Note the three flattened saccules (G) and the inflations with dense central condensations (arrows). Potassium permanganate fixation. (Bar = 1  $\mu\text{m}$ .)



Golgi complexes lie among the cisternae. In these glands as in many other insect tissues, it is often difficult to resolve the individual components of the Golgi apparatus. Smooth-surfaced irregular vesicles (100–200  $\mu\text{m}$  in diameter) appear to be budding off the membranes of the ER (Figure 10) in the vicinity of large numbers of small (50–70  $\mu\text{m}$  in diameter) fuzzy vesicles which may be coated with clathrin (Pearse and Bretscher, 1981). These coated vesicles are present in large numbers throughout the Golgi zones (Figure 10). Such coated vesicles have been reported near the Golgi of the vas deferens of a rat (Friend and Fahrquhar, 1967). We presume that in the BAG, one or both of these classes of membranous sacs are transition vesicles, moving proteinaceous products from the RER to the forming face (the convex surface) of the Golgi. In favorable sections, the Golgi apparatus has at its center three (occasionally four) saccules (Figure 9) of smooth membranes which curve around sharply to form a deep cut at the maturing face (Figure 8).

The edges of the Golgi saccules are inflated. Within swollen tips of many saccules are electron-dense granules surrounded by an electron-transparent zone (Figures 7, 9, 10). We presume that these dense-core granules are secretory precursors. Often, the precursor granule lies at the center of the concave face of the membrane stack (Figure 8). A similar concentric architecture of the Golgi has been reported in the seminal vesicle of *Locusta migratoria migratorioides* (Cantacuzène, 1972). The central precursor granule(s) apparently grows in size, as if it were a nucleating site, until the electron-lucent cortex is filled with dense product. The secretory vesicles of the cells vary from homogeneous masses to multiparticle bodies. When the definitive secretory vesicle is of a complex type (see Figure 22), a cluster of presecretory granules may collect together in the membrane-bound cavity (Figure 6).

The membranes of the secretory vesicles may have surface specializations. In the tubular gland of *Acanthoscelides obtectus* (a bruchid beetle), there is a fringe of short filaments around the newly formed secretory granule (Cassier and Huignard, 1979). Surface specializations are also found in the BAG of *Tenebrio*, and the membrane may be modified as the granule matures. When a type 3 vesicle is first formed, it has scattered plaques of electron-dense material in its cortical zone (Figure 13), but by the time it moves away from the Golgi zone, it has acquired an unusual and characteristic cortex (Figure 14). In type 5 vesicles of the BAG, the newly formed vesicles have a surface fringe and approach no closer than about 100 nm to each other (Figure 15), but as these type 5 vesicles move toward the apical cell surface, they become more tightly packed together (Figure 16), perhaps due to modification of that surface coat.

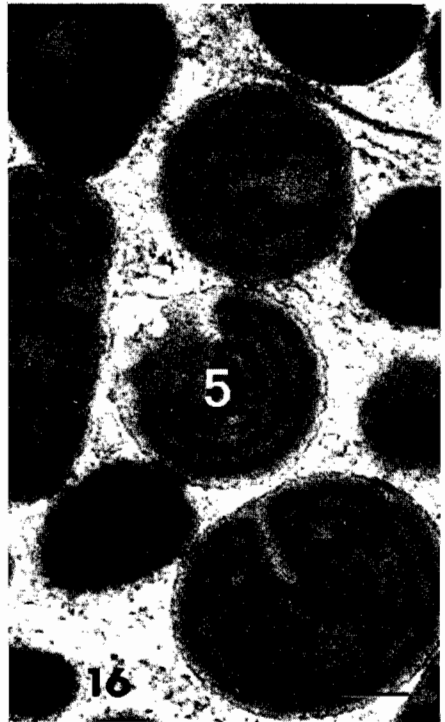
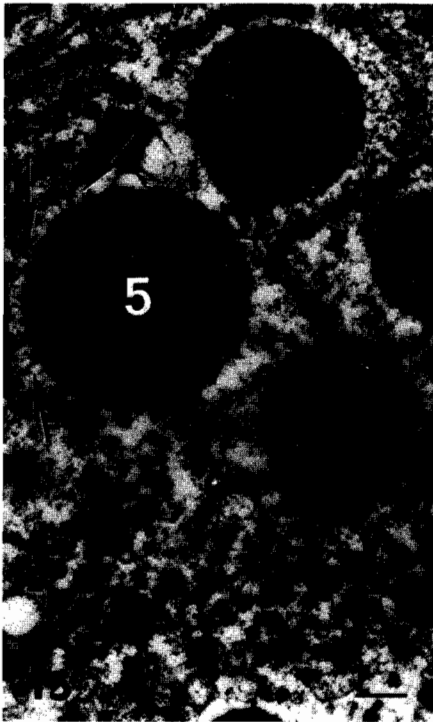
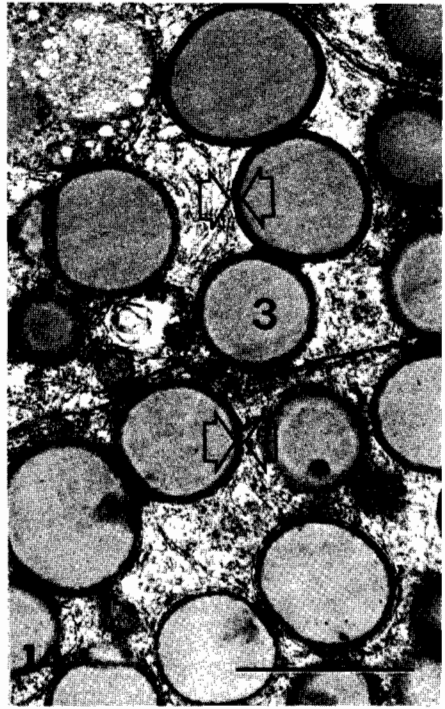
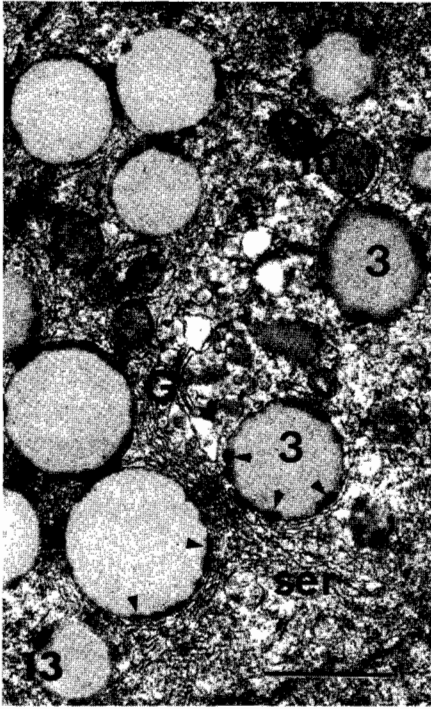
Large quantities of product are present within secretory cells of active

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**Figure 10.** Type 2 cells of mature BAG. Note the saccules of the Golgi zone and the precursor granules (arrow). The dense cluster of small coated vesicles (ves) appears to be associated with inflations of cisternae of the RER. m, mitochondrion; 2, type 2 secretory vesicle. (Bar = 1  $\mu\text{m}$ .)

**Figure 11.** Type 7 secretory vesicles of the BAG. (Bar = 1  $\mu\text{m}$ .)

**Figure 12.** Smooth septate junction (SJ) and belt desmosome (BD) near the apical edge of the secretory epithelium of the BAG. Note the fibrous mat on the cytoplasmic face of the desmosome and the coarse granular material in the intercellular space. (Bar = 0.2  $\mu\text{m}$ .)



accessory glands. In cell types 1 and 2 of *Schistocerca gregaria*, Ohdiambo (1969) reports that the cisternae of the RER are much dilated but there are relatively few Golgi-derived vesicles. In these cells, storage appears to be in the cisternae, upstream from the Golgi. In most other secretory cells, storage is downstream from the Golgi. The individual secretory vesicles may remain discrete and collect in large numbers, as in the granular cells of *Anagasta kuhniella* (formerly *Ephestia*) (Reimann and Thorson, 1979b) and in the BAG of *Tenebrio* (Figure 4) (Dailey *et al.*, 1980). In the TAG, the smooth-surfaced vesicles derived from the Golgi do not remain distinct. They pack tightly together and appear to fuse forming large vesicles in the apical cytoplasm (Figure 17) (Gadzama *et al.*, 1977). Such large fusion vesicles are also seen in the middle segment of the seminal vesicle of *Locusta* (Cantacuzène, 1972).

#### 4.4. Export of Product

As the glands mature, their lumina become filled with product. During copulation, most of the products are lost in the ejaculate, and subsequently they must be replenished from the secretory cells. After mating, the lumen is rapidly recharged with new products.

All variety of secretory devices—merocrine, holocrine, and apocrine—are found in accessory glands and other secretory tissues of the male tract. The paragonia (accessory glands) of *Drosophila melanogaster* liberate their product by holocrine secretion and dramatic cell death (Perotti, 1971). Holocrine secretion has also been reported in the secretory cells of the ejaculatory duct of *Musca domestica* (Reimann, 1973). But in most glands the mechanisms are either merocrine or apocrine.

Merocrine secretion is seen in the vasa deferentia of *Calpodes* (Lai-Fook, 1982a) and in the TAGs of *Tenebrio* (Figure 17). The apical surfaces of these cells support irregular folds and microvilli which we presume are generated by the continual addition of membrane from the vesicles.

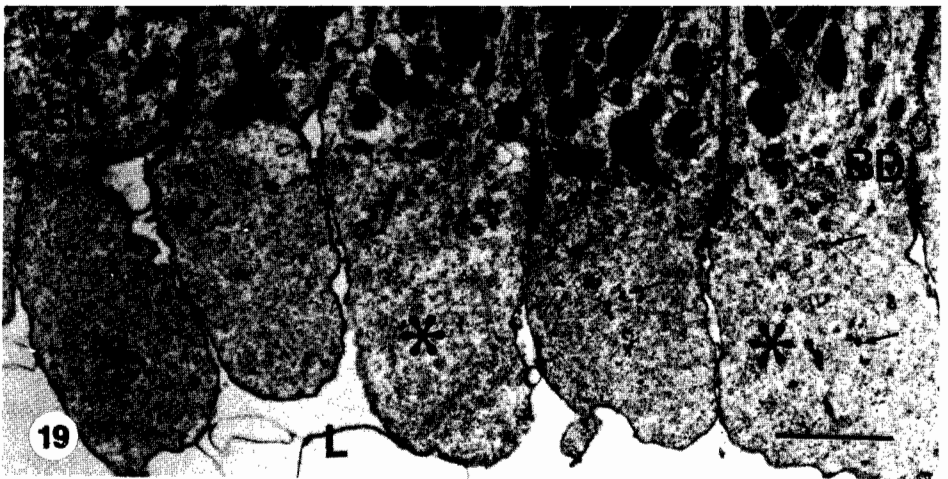
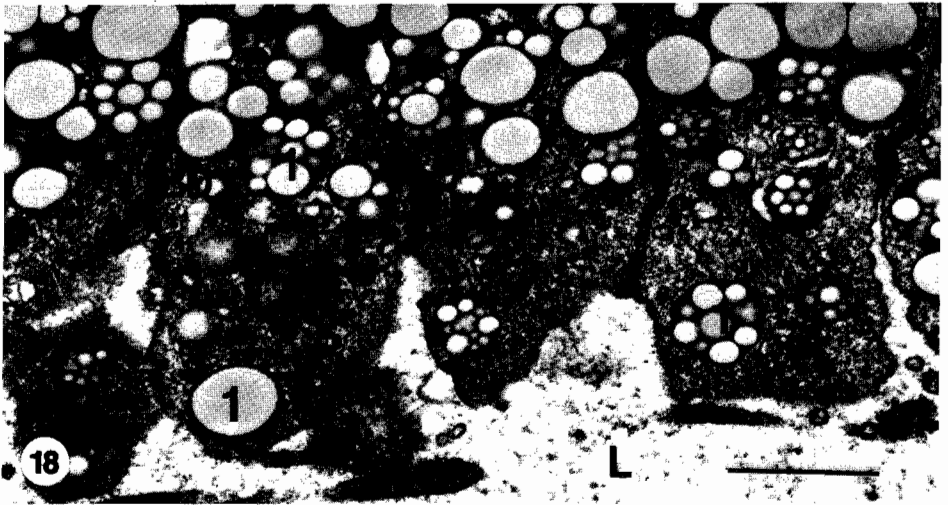
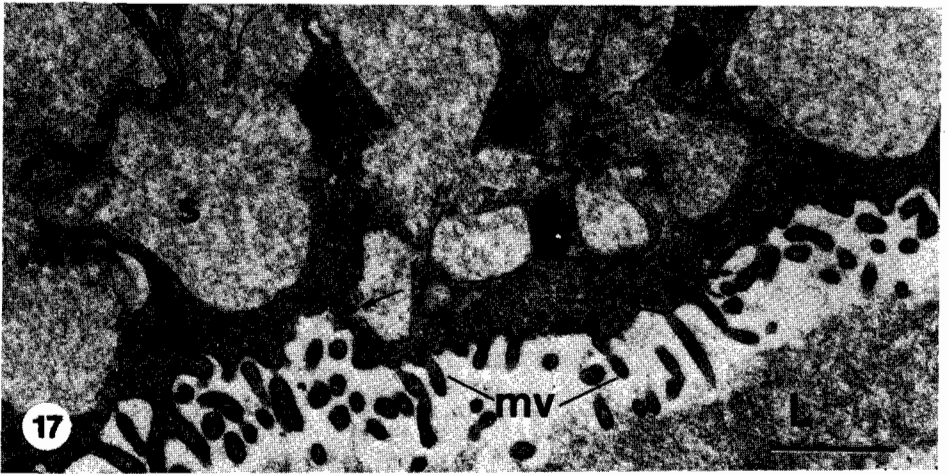
In the BAGs, clusters of microtubules run longitudinally along the outer zones of the cell and surround masses of secretory vesicles which become tightly packed just above the level of the desmosomal belt (Figure 20). There is a network of intracellular filaments at that level, which often seem to run across the cell, and the desmosomal membrane is frequently infolded (Figures 20, 21). A broad apical cellular process, which is poor in mitochondria and other

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**Figure 13.** Newly formed secretory vesicles (3) near the Golgi (G) of a type 3 secretory cell of the BAG. Dense irregular plaques (arrowheads) are scattered about at the surface of the vesicle. m, mitochondrion. (Bar = 1  $\mu\text{m}$ .)

**Figure 14.** Mature secretory vesicles (3) of type 3 cells of the BAG. Note the thick peripheral structure, between the hollow arrows. (Bar = 1  $\mu\text{m}$ .)

**Figure 15.** Newly formed secretory vesicles (5) near the Golgi (G) of a type 5 secretory cell of the BAG. The arrows indicate a surface coat on each vesicle. The print has been overexposed to make the surface coat more obvious. (Bar = 0.2  $\mu\text{m}$ .)

**Figure 16.** Mature secretory vesicles of type 5 cells contain whorls or concentric spheres of secretion of moderate electron density. The surface coat has been lost, and the vesicles are packed tightly together. (Bar = 0.2  $\mu\text{m}$ .)



organelles, projects beyond the desmosomal belt (Figures 19, 20). In immature glands where secretion is at a low level, microtubules can be seen in that process. In this young material, the apical plasma membrane may invaginate to form a sort of funnel which reaches back up to the desmosomal level and apparently carries products to the lumen (Figure 21). In older tissues, one or more secretory vesicles can be seen in the apical process (Figures 3, 18), and in favorable sections we have seen the vesicles approaching the membrane as if about to empty their contents by exocytosis (Figure 18).

I suspect that traffic in secretory vesicles is regulated at the level of the desmosomal belt. Microtubules stream down to that belt and secretory vesicles accumulate above it. The infolded plasma membrane and network of transverse filaments might be viewed as a gate which limits the access of secretory vesicles to the apical tip.

One frequently sees coated vesicles in these apical zones (Ohdiambo, 1969; Lai-Fook, 1982a; Figures 19, 21). These vesicles may reflect recovery of membrane for transport upstream to the Golgi zones for subsequent recycling (Oliver, 1982).

In apocrine secretion, cells shed their apical process and thus export secretory vesicles. Such a mode of secretion has been reported in accessory glands of many species, e.g., gland 2 of *Schistocerca* (Ohdiambo, 1969), the seminal vesicle of castrate *Locusta* (Cantacuzène, 1972), the accessory glands of *Leptinotarsa decemlineata* (De Loof and Lagasse, 1972), and the vasa deferentia of *Anagasta* (Reimann and Thorson, 1976a).

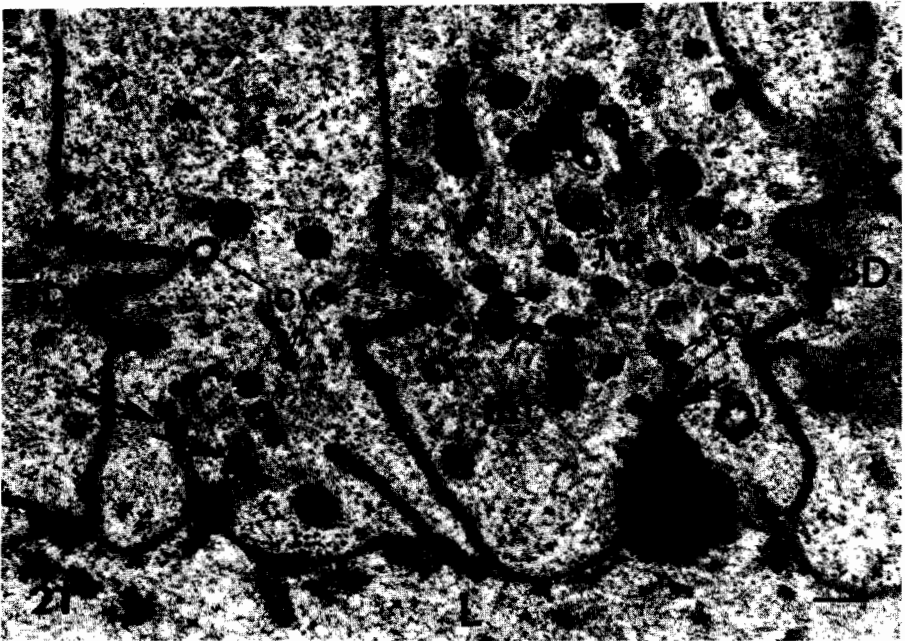
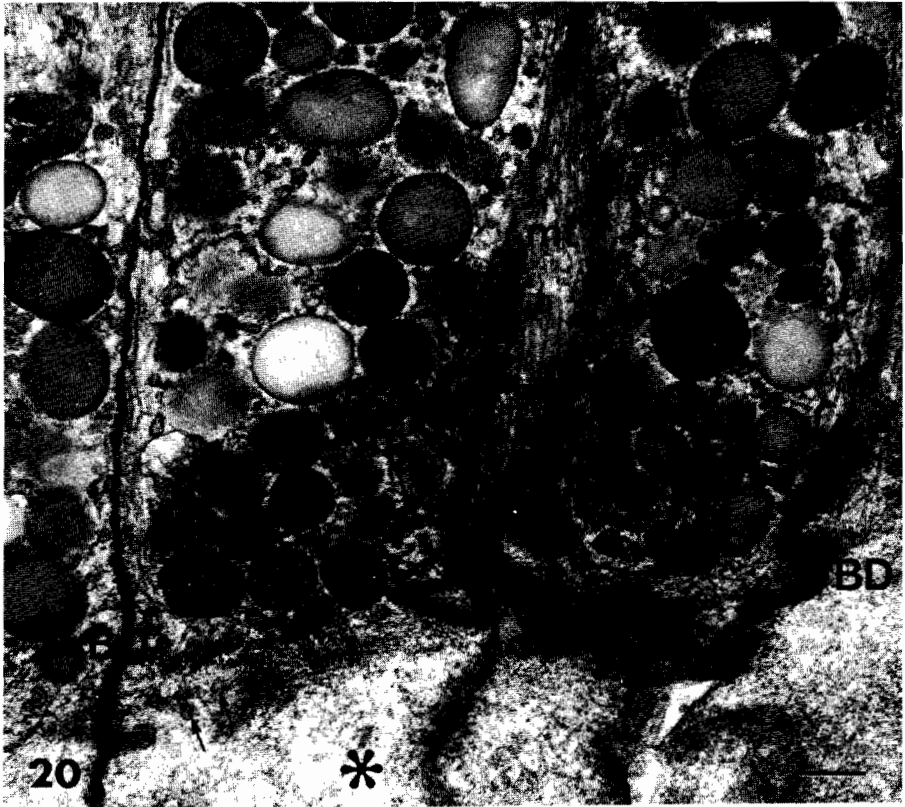
An extreme modification for apocrine secretion is seen in the foliate cells of the simplex (ejaculatory duct) and accessory glands of *Anagasta* (Reimann and Thorson, 1979a,b) and in similar cells of *Calpodes* (Lai-Fook, 1982b). The foliate cells have elongate apical extensions, which extend well out into the lumen of the gland. These extensions arise at a narrow neck or petiole. Large numbers of microtubules run from the basal portion of the cell to end at the petiole. Distal to the petiole, the apical process lacks the ER and Golgi zones, but contains many whorls of membranes and very few microtubules and mitochondria. The apical processes are shed into the lumen and become a part of the ejaculate, and then they are rapidly regenerated within the next 7–8 hr (Reimann and Thorson, 1979a,b). Even in this special case of apocrine secretion, the desmosomal belt is the demarcation between the basal portion of the cell and the disposable apical zone.

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**Figure 17.** The apical edges of two secretory cells of the TAG. The large irregular secretory vesicles (s) readily fuse with one another. The small arrow may indicate a vesicle approaching an invagination of the plasma membrane. mv, microvilli; L, lumen; BD, belt desmosome. (Bar = 1  $\mu\text{m}$ .)

**Figure 18.** The apical edge of type 1 secretory cells of the BAG. Apical processes (asterisk) extend below the zone of belt desmosomes (BD). Type 1 secretory granules are packed tightly above the zone of desmosomes, and a few have entered the apical processes. These vesicles contain one or more electron-transparent "bubbles." (Bar = 1  $\mu\text{m}$ .)

**Figure 19.** The apical zone of the secretory epithelium of type 6 cells of the BAG. The apical processes (asterisks) are devoid of the secretory vesicles (6) but contain small dense membrane profiles that may be coated vesicles (arrows). (Bar = 1  $\mu\text{m}$ .)



## 5. Semisolid Secretory Products

In addition to soluble secretions, accessory glands make spermatophores. Like the cuticle and the chorion, the wall of the spermatophore is an ordered structure formed in the extracellular space. The cuticle is a multilayered structure which is laid down by epidermal cells. As the epidermal cell adjusts the secretory blend over time, the various layers of the cuticle are deposited in succession. For production of the chorion, the follicle cells play the analogous part, temporally shifting their patterns of biosynthesis to lay down the components of the eggshell in serial order. But for a spermatophore, the various layers and zones are produced by several cell types, arranged in a parallel array of cell types which simultaneously secrete a corresponding array of products. Each of the cell types contributes to a particular layer or zone of the spermatophore.

### 5.1. Organized Secretion Masses

Formation of the spermatophore requires that the various secretory products be delivered or mixed in a fixed temporal order. The general strategy is as follows. Several cell types produce the heterogeneous secretory products. Each set of products is stored in a discrete site in the male tract. At mating, several precursors are mixed together in a fixed order. The order of export of the components is partly dictated by the anatomy of the male tract. During and after incorporation into the spermatophore, the components may undergo changes in physical state and arrangement.

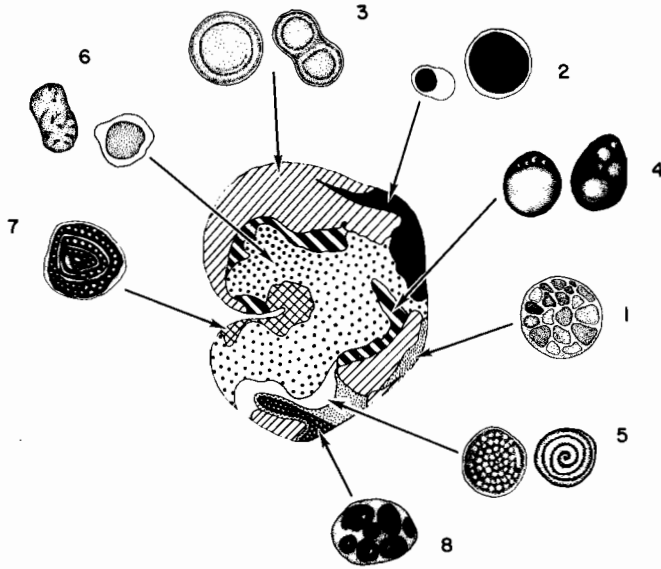
The secretory masses within accessory glands often include insoluble structures. In the paragonia of *Drosophila*, the secretion contains many hollow filaments, with dimensions rather like microtubules (Tandler *et al.*, 1968; Perotti, 1971; Beaulaton and Perrin-Waldemer, 1975). Histochemical stains and enzyme digests indicate that these arrays of tubules have protein and glycoprotein subunits (Beaulaton and Perrin-Waldemer, 1975). The dimensions of the tubules and their ordering relative to each other change after they are expelled into the lumen. These changes may reflect reorganization of the subunits in the extracellular cavity (Perotti, 1971).

On histochemical evidence, Huignard (1975) reported that the secretion of the external median accessory gland of *Acanthoscelides* consists of a central

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**Figure 20.** The apical zone of the secretory epithelium of type 6 cells of the BAG. Note the sinuous infolding of some of the belt desmosomes (BD). Intracellular material, both irregular membranous tubes (arrows) and filaments (f), run across the cell and appear to impede the passage of the secretory vesicles into the apical projection (asterisk). mt, microtubules; m, mitochondrion. (Bar = 0.2  $\mu\text{m}$ .)

**Figure 21.** The apical zone of secretory cells in a maturing BAG just after adult ecdysis. As in Figure 20, the belt desmosomes (BD) are infolded. Microtubules (mt) run through the zone of belt desmosomes and into the apical processes. Invaginations of the apical plasma membrane (arrows) appear to be associated with coated vesicles (cv). The invagination on the right is filled with secretory products en route to the lumen (L). (Bar = 0.2  $\mu\text{m}$ .)



**Figure 22.** A diagram of the pattern of secretory cell types in the BAG and drawings of representative vesicles of each cell type.

protein mass surrounded by an acid mucopolysaccharide cortex. In the lumen of the accessory glands of *Calpodes*, there are a variety of single fibers, parallel bundles of fibers, glycogen, cellular debris, diverse electron-dense granules, and a multitude of small particles (Lai-Fook, 1982c). The contents of the accessory glands of *Anagasta* are heterogeneous, and elastic. Reimann and Thorson (1979b) have observed that the secretion of the apical section of the accessory gland will snap back to its original length even after it has "been drawn out several fold." In the BAG of *Tenebrio*, the secretory mass is semisolid, springy, and composed of several distinct zones (Figure 24). In electron micrographs, the complexity of each layer and the heterogeneity among them are quite marked (Figure 23).

### 5.2. Secretion into Separate Compartments Arranged in Series

In many species, the primary secretory products are kept separate from one another. In the accessory glands of *Schistocerca*, Ohdiambo (1969) recognized eight or nine different cell types based on ultrastructural and histochemical criteria: these cell types are segregated from one another so that each is confined to one or the other of the 16 tubular accessory glands. Such regional specialization also exists in the tubules of the adult and late-nymphal accessory glands of *Acheta domesticus* (Kaulenas, 1976; Kaulenas *et al.*, 1979).

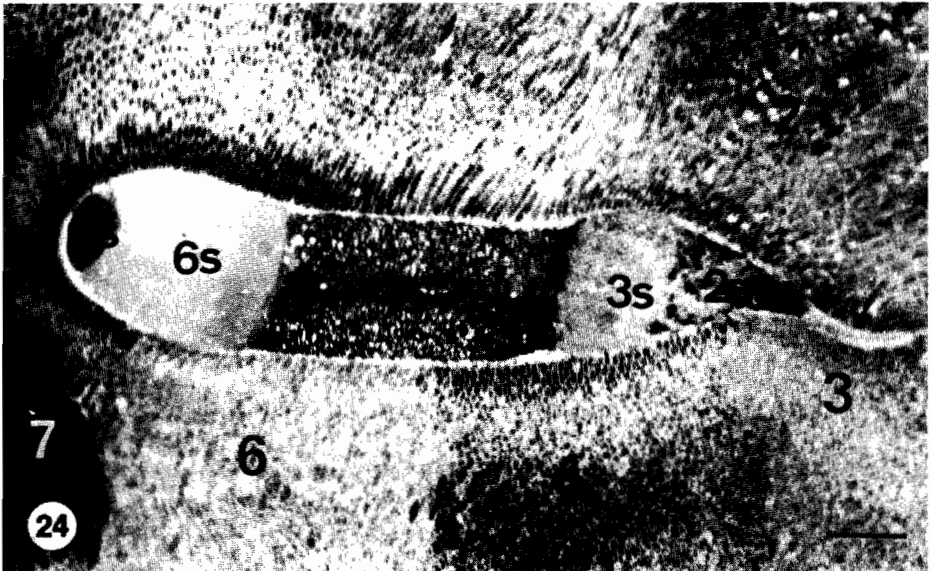
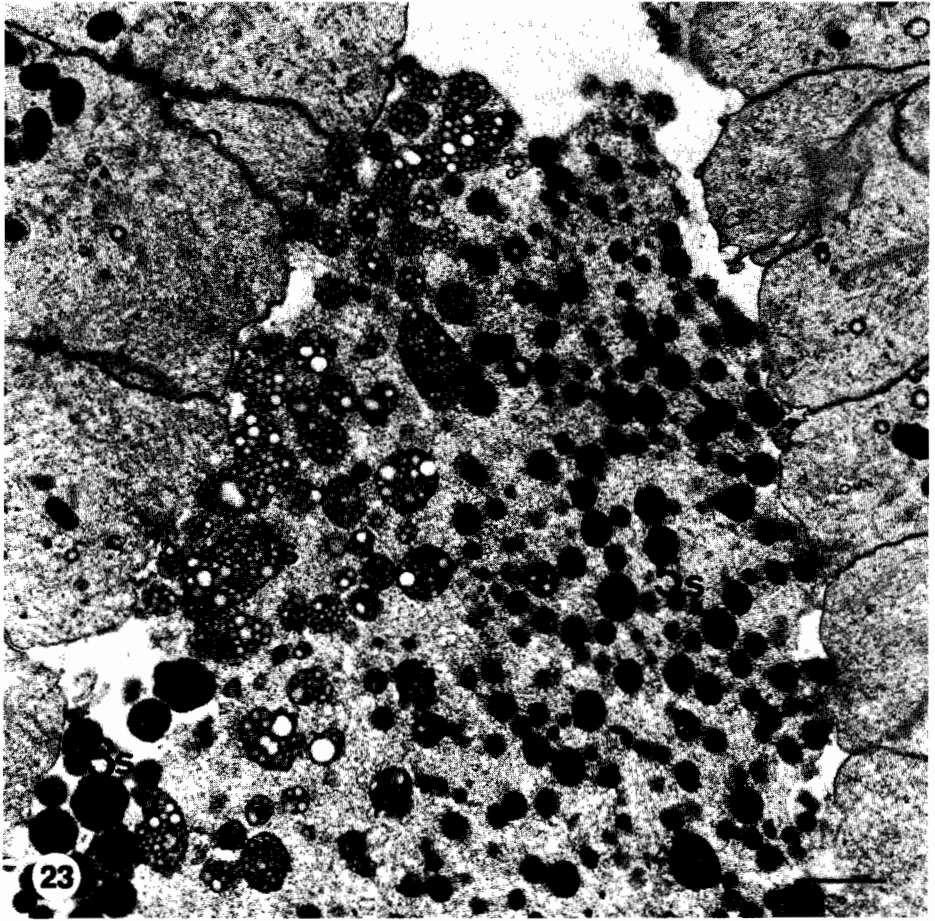
In the reproductive tract of male Lepidoptera, secretions are produced by the vas deferens, accessory glands, and ejaculatory duct (composed of duplex and simplex segments). Because of differences in the morphology of the cells and their secretions, this tract can be divided into distinct regions, each composed

of a band of secretory cells. In the accessory glands of *Anagasta*, there are five regions, many of which contain two cell types (foliate cells and granular cells) (Reimann and Thorson, 1979b). In *Calpodes*, there are at least 10 different regions in the tract as a whole (Lai-Fook, 1982b). Between many of the secretory regions are boundary zones which are constricted in such a way that inward extensions of the epithelial cells completely occlude the lumen. Similar constrictions were reported in *Anagasta* by Reimann and Thorson (1976b). These constrictions effectively divide the reproductive tract into many compartments arranged in series, each of which is sealed away from the others unless the circular muscle sheath relaxes. As a result, the various products are segregated from one another. Shortly after copulation begins, peristalsis squeezes out the posterior secretions. In its later stages, all the longitudinal muscles of the surrounding tract act together to shorten its length and to inject all the luminal contents including the seminal fluids (Lai-Fook, 1982d).

### 5.3. Secretion in Parallel to a Common Lumen

In mosquitoes and tsetse flies, there are several cell types which pass their secretory granules to a single or bipartite lumen of the accessory gland (Tongu *et al.*, 1972; Ramalingam and Craig, 1978; Kokwaro, 1982). In the BAG of *Tenebrio*, eight cell types secrete in parallel to produce the layered secretory plug. The materials exported from any one cell type form one layer of the ordered plug. To establish the distribution of the secretory cell types over the epithelium, we combined the information from stained whole glands, light histology, and transmission electron microscopy (Dailey *et al.*, 1980). The pattern is shown in Figure 22. The secretory vesicles of cell type 1 are globular, with one or more electron-transparent "bubbles" in an electron-dense cortex (Figure 18). Once the secretion from type 1 cells is expelled, it appears frothy (Figure 23). A similar secretory granule in the tubular gland of *Acanthoscelides* appears to be composed of glycoprotein (Cassier and Huignard, 1979). Secretory vesicles of cell type 3 have a complex peripheral structure (Figure 14). Secretory vesicles of cell types 4 and 8 appear faceted in cross-section (Figure 6). Those of cell types 5 (Figure 16) and 7 (Figure 11) enclose ordered networks of electron-dense granules or membranes, whereas those of cell type 6 are fairly homogeneous (Figure 20). See Dailey *et al.* (1980) for further descriptions. All cells pass their products onto the lumen of the BAG. The single secretory mass in the lumen is the ordered aggregate of the contribution from each cell type (Figure 24). Except for rudimentary histochemistry (indicating proteins, lipids, etc.) we know nothing about the biochemical composition products of these eight cell types.

Each of the eight cell types of the BAG is confined to a particular patch of the epithelium and each patch contains only one cell type. The shapes of these patches are irregular and appear random at first glance. However, the pattern is the same in every individual, and left and right glands have mirror-image patterns. In fact, the pattern appears to be an adaptation to allow the secretory products to be precisely positioned in the organized plug. Each layer of the aggregate mass is the product of cells nearby (Figure 24). The pattern of the cells



is mapped into the pattern of the plug. Not all cell types are equally common; for example, cell type 3 and cell type 6 each occupy about one-third of the total surface area, while type 5 occupies only a few percent of the total epithelium (Figure 22). The area of each patch of cells is reflected in the size of the corresponding layer in the plug. Thus, the predominant cell types (3 and 6) give rise to the thickest layers of the plug. It seems likely that in this gland, as in many others, all cell types in the epithelium are triggered to secrete after the lumen has been emptied by ejaculation. If such is the case, morphology alone, i.e., placement and size of the patch of each cell type, can explain placement and thickness of the layers in the plug. In addition, it is possible that some cell patches might secrete before others, but we have no evidence for such temporal patterning.

## 6. Formation of the Spermatophore

Spermatophores are constructed within a brief period which ranges from a few minutes to several hours (Tuzet, 1977). The spermatophore may be formed outside the animal and deposited on a substrate for a female to retrieve (see Schaller, 1971), it may be laid down, layer by layer, within the bursa of the female (e.g., Gerber *et al.*, 1971), or it may be assembled during the exit of the various secretions from the male tract (e.g., Linley, 1981). Each spermatophore is composed of jellylike and waxy components which vary in viscosity, opacity, and elasticity. Some layers are visible in brightfield microscopy of unfixed spermatophores. Others can be detected by interference contrast (Linley, 1981, for *Culicoides*), by histochemistry (Gadzama and Happ, 1974, for *Tenebrio*; Gerber *et al.*, 1971, for *Lytta*), or by transmission electron microscopy (Gadzama and Happ, 1974; Kokwaro and Ohdiambo, 1981, for *Glossina*). The complexity of the wall structure in the spermatophore of *Tenebrio* is shown in Figure 25. Comparison with the secretory plug of the BAG (Figure 23) demonstrates the extensive alterations which occur during spermatophore formation.

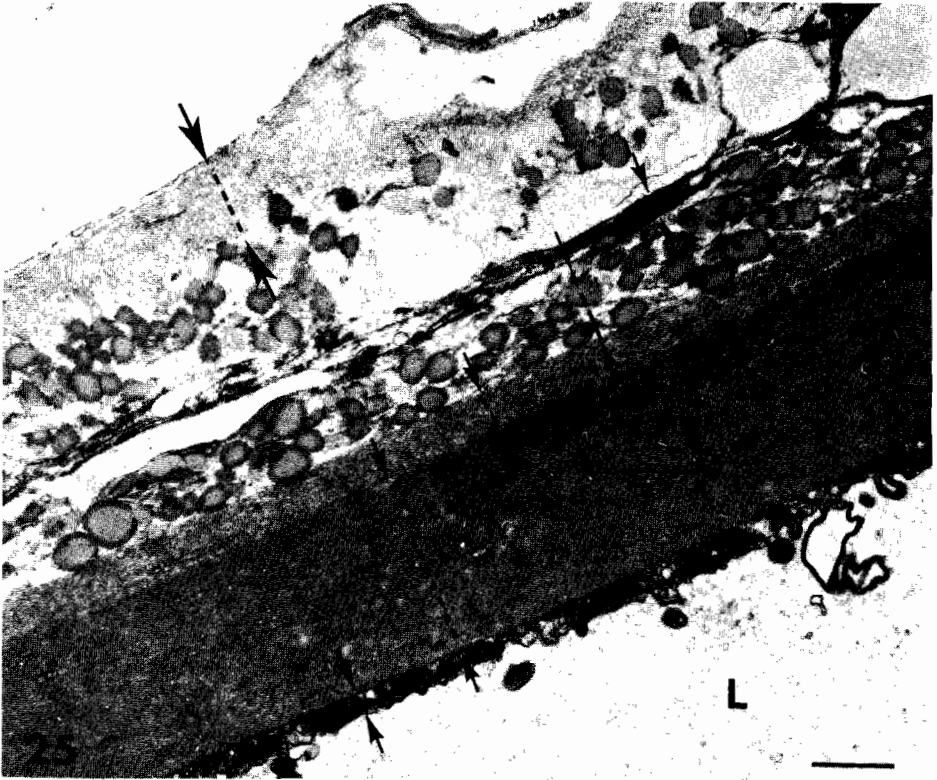
The organization of the spermatophore depends on the architecture of the male tract. Secretory masses are passed into the distal ejaculatory duct and then through the aedeagus in a fixed order. Thus, for example, the foliate cells of *Anagasta*, which are found toward the anterior tip of the accessory gland, contribute their product to the last-formed (outermost) layer of the spermatophore (Reimann and Thorson, 1979b).

The various components must be present in balanced quantities. The amount of each is partly defined by the volume of the lumina within the male tract, and perhaps also the female bursa. The aliquots of secretion must be delivered in an appropriate order. Some of that order is inherent in the

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← **Figure 23.** The edge of the secretory plug of the BAG. Secretions from cell type 1 (1s), cell type 3 (3s), and cell type 5 (5s) are shown. (Bar = 1  $\mu$ m.)

**Figure 24.** A light micrograph of the secretory plug of the BAG *in situ* stained with toluidine blue. The numbers indicate the cell types (2, 3, 4, 6, 7) and their respective secretions in the plug (2s, 3s, 4s, 6s, 7s). (Bar = 50  $\mu$ m.)



**Figure 25.** A section through the wall of the spermatophore of *Tenebrio*. The lumen, which contains the sperm (not shown), is at the lower right, and the outer surface is toward the upper left. The arrows bracket the layers in this wall. Comparison with Figure 23 shows that profound transformation of the secretion takes place when the spermatophore is formed. (Bar = 1  $\mu$ m.)

sequential arrangement of the secretory lumina as in *Calpodex*—or in the architecture of the secretory plug as in *Tenebrio*. But nervous coordination is also important; thus, induction of ejaculation by a massive eserine injection in *Tenebrio* produces a malformed spermatophore (Happ, unpublished). For the accessory secretions from the dozen or more converging accessory glands of a cricket (*Teleogryllus commodus*) to be properly mixed together, the brain appears to be required for the coordination of the secretory sequence (Loher, 1974).

### 7. Evacuation of the Spermatophore

The sperm must somehow be delivered from the spermatophore to the spermatheca of the female. In some staphlinid beetles, the male aedeagus has a narrow chitinous process which forms a "guide rail" which causes the tip of the spermatophore to slip into the spermatheca (Peschke, 1978). Even when

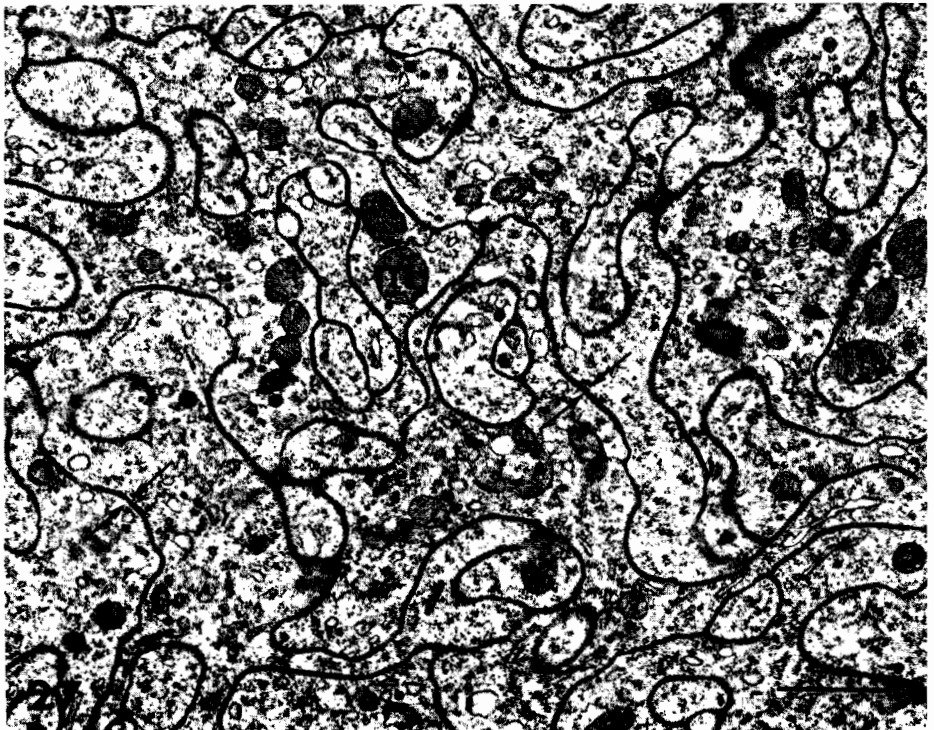
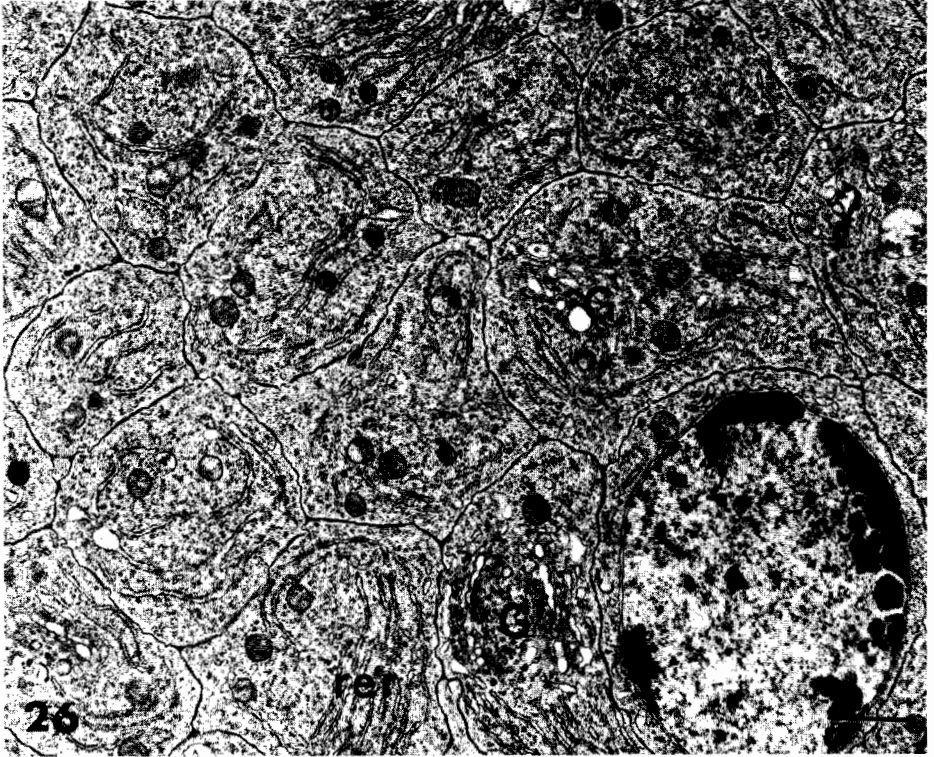
placement is not so precise, the sperm tend to leave the spermatophore near the spermathecal opening. In order for the sperm to escape from the multilayered secretory mass, the spermatophore undergoes dramatic rearrangements and contortions. The driving forces underlying spermatophore expansion and sperm expulsion are not well understood in any species. Osmotic flow of water has been invoked to explain sperm expulsion in *Acheta domesticus* (Khalifa, 1949). Gaseous CO<sub>2</sub> production apparently accounts for movement of sperm into the bursa of a tick (Feldman-Muhsam *et al.*, 1973). Neither explanation seems sufficient to explain the programmed sequence of evaginations which leads to the rupture of the spermatophore of *Tenebrio* (Gadzama and Happ, 1974). In this spermatophore, it appears that the outpocketings swell and then snap out, as if the walls are recoiling from previous strain. The compression and the shear forces which act on the viscous materials as they are forcibly ejected through the long narrow ejaculatory duct and the aedeagus must be of considerable magnitude. Expansion of the spermatophore of *Tenebrio* might be due in part to relaxation from shear-induced deformations during formation of the overall structure. A similar suggestion has been implied for *Culicoides melleus* by Linley (1981), who suggests "that the spermatophore behaves so as to minimize an intrinsic elastic energy."

If the molecular components of the spermatophore are actively involved in the expansion and rupture, then one might expect adaptations to prevent premature mixing or to avoid spoilage. As Lai-Fook (1982d) has suggested, the constrictions in the male tract of Lepidoptera should prevent such mixing. In *Tenebrio*, where there is considerable potential for mixing in the lumen of the BAG, the secretion is not liquid but semisolid, and the plug is regularly discarded, even if no females are encountered. Isolated males ejaculate a spermatophore onto the substrate each day (P. J. Dailey, personal communication). It may be that this "casting of seed upon the ground" is an adaptation to avoid deterioration of the wall components during protracted storage in the gland lumen. These and other intriguing possibilities can be investigated only when we know much more of the biochemistry of the secretions from the accessory glands, the rheology of the viscous materials as they surge through the male tract, and the molecular architecture of the spermatophore itself.

## 8. Development of Accessory Glands

Primary organogenesis of accessory reproductive organs occurs in preadult stages. Significant growth and early differentiation occur late in the preimaginal instar, either in the last larval stage of Hemimetabola or in the pupal stage of Holometabola. In those insects which mate shortly after ecdysis, the glands are fully differentiated at eclosion. For species which mate some days thereafter, there is a postecdysial period of peak differentiation.

In *Tenebrio*, the two pairs of male accessory glands are derived from a common mesodermal pouch (Huet, 1966; Poels, 1972), and the four glands are anatomically distinct at pupation (Figure 1A). At that stage, both gland pairs consist of an epithelium surrounded by a poorly differentiated muscle coat.



The tubular gland is like a small thumb on the surface of the mitten-shaped BAG. The closely packed columnar cells of the secretory epithelium contain sparse membranes of the ER and scattered mitochondria.

During the first 6–7 days of the 9-day pupal stage in *Tenebrio*, the secretory cells undergo cycles of division. As is common in vertebrate epithelia, the mitotic figures occur near the apical surface of the epithelium. In both BAG and TAG, the daughter cells remain linked by spindle-remnant bridges for a day or more after karyokinesis is complete. These bridges resemble fusomes reported from insect ovaries (Mandelbaum, 1980; King *et al.*, 1982) except for the fact that they are usually “plugged” with microtubules. The spindle-remnant bridges are found only near the apical surface, at about the level where apical desmosomes will eventually arise. During the later part of the pupal stage (days 4–8), adjacent cells in the BAG communicate via fused-membrane bridges which form in the midregion of the epithelium (Grimes and Happ, 1980; Happ and Happ, 1982). Once division ceases, more cytomembrane profiles appear, Golgi zones become larger, and the cells grow in volume (Figure 26). The closely appressed membranes become infolded (Figure 27), apparently in preparation for the rapid postecdysial hypertrophy. By day 8, a few secretory vesicles are seen in the TAGs (Happ and Happ, 1982) and in some of the cell types of the BAGs (Dailey and Happ, 1983). At this stage, the glands are smaller versions of the mature adult organs (Figures 1B, C). Adult-specific secretory antigens are present in TAGs from late pupae (Black *et al.*, 1982). An analogous developmental schedule has been reported for the accessory glands of *Acheta* in which Kaulenas *et al.* (1979) find low levels of adult antigens in late nymphal glands.

Growth and peak differentiation follow soon after adult ecdysis. The growth is due to hypertrophy rather than hyperplasia: In the BAG and the TAG, cells increase in height and cross-sectional area; together, these account for a 5- to 10-fold increase in gland volume (Happ and Happ, 1982; Happ *et al.*, 1982). Similar increases in cell volume occur in three of the four accessory glands of *Acanthoscelides* (Cassier and Huignard, 1979).

By 2 days after adult ecdysis, all eight cell types of the BAG contain secretory granules of the definitive adult morphology (Dailey and Happ, 1983). During this time of granule maturation, the epithelial pattern is consolidated. Some cells on the boundary lines between dissimilar patches contain transitional granules—granules intermediate in morphology between those of their two different neighbors. These transitional granules persist for only a day or two and then are destroyed as the boundary cells conform to one or the other of the adjacent definitive phenotypes (Dailey and Happ, 1983). In the TAG, maturation follows a similar time course until secretory vesicles fill most of the cell by 5–6 days after ecdysis (Gadzama *et al.*, 1977). Rapid postecdysial maturation of the ER and Golgi zones has also been reported in the male accessory

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**Figure 26.** A cross-section through the midregion of the secretory cells of the TAG in a 7-day pupa. Parallel cisternae of rough endoplasmic reticulum (rer) and Golgi zones (G) are common. (Bar = 1  $\mu\text{m}$ .)

**Figure 27.** A cross-section through the secretory cells of 9-day pupal TAG, just above the zone of apical desmosomes. The cells are linked by smooth septate junctions (between arrows) and the membranes are extensively infolded. (Bar = 1  $\mu\text{m}$ .)

gland of *Acanthoscelides* (Cassier and Huignard, 1979) and *Acheta* (Kaulens *et al.*, 1979). In contrast, the secretory machinery of the paragonia of *Drosophila* is fully organized by the time of ecdysis (Federer and Chen, 1980).

Concomitant with the ultrastructural differentiation, the accessory glands increase their production of adult proteins. Leucine incorporation into a few adult-specific protein spots can be detected in the TAG and BAG just after eclosion (Black *et al.*, 1982; Happ *et al.*, 1982). The biosynthetic emphasis shifts rapidly until at 5 days later over 50% of the leucine incorporated by the TAG goes into four protein bands (Happ *et al.*, 1977; Black *et al.*, 1982).

## 9. Endocrine Control of Accessory Gland Development

Both ecdysteroids and juvenile hormones play roles in the development and modulation of accessory glands, but their precise actions have been defined in only a few species. Ecdysteroids are important during pupal development. In *Samia cynthia*, the spermiduct consists of secretory cells surrounded by muscle. When spermiducts from diapausing pupae are cultured *in vitro*, the secretory epithelium differentiates only when ecdysteroids are added (Szöllösi and Landureau, 1977).

In the BAG and TAG of *Tenebrio*, there are two peaks of mitotic activity in the 9-day pupal instar (Grimes and Happ, 1980; Happ and Happ, 1982). The first of these mitotic bouts (0–3 days) proceeds *in vitro* in Landureau's medium, but the second bout does not occur without addition of ecdysteroids (Happ, 1982). This second bout is correlated with a single large ecdysteroid peak in the pupa (Delbecque *et al.*, 1978). The pupal ecdysteroid peak is also correlated with a change in competence which permits the glands to synthesize adult-specific proteins. Glands from young pupae will not differentiate when implanted in adults unless they have been exposed to a large ecdysteroid concentration *in vivo* or *in vitro* (Happ, 1982). Some time ago, Ohdiambo (1966) showed that the accessory glands of late-last-instar larvae of *Schistocerca* would mature when implanted in an adult, but glands from early last-instar larvae would not do so when similarly implanted. Apparently for *Schistocerca*, some hormone (perhaps ecdysteroid) or other humoral feature of the larval environment was necessary for acquisition of competence to mature. The sequence of events which depend on the last preimaginal ecdysteroid peak deserves further study in more species.

In many hemimetabolous insects, postecdysial development of male accessory glands is dependent on juvenile hormone [e.g., *Rhodnius* (Wigglesworth, 1936), *Leucophaea maderae* (Scharrer, 1946), and *Schistocerca* (Ohdiambo, 1966)]. In intact *Schistocerca*, massive growth of the ER and Golgi begins at the fifth day when the corpora allata become active. No such expansion of the secretory organelles occurs in glands of allatectomized animals. Juvenile hormone is required for the maintenance of normal secretory morphology in the accessory glands of some beetles (*Leptinotarsa*). Within 3 days after allatectomy, the secretory cells of the accessory glands of *Leptinotarsa* begin to degenerate by sloughing off their apical halves, and in some cases the process continues to cell death (De Loof and Lagasse, 1972).

In other species, such as *Calliphora erthrocephala* (Thomsen, 1942) and *Tenebrio*, it has not been possible to show that juvenile hormones play roles in postecdysial maturation. In *Tenebrio*, I have seen normal gland development in abdomens which were isolated at adult ecdysis, but I have not been able to support vigorous development *in vitro*. The glands grow and differentiate when implanted into adults of either sex, so neither innervation nor good tracheation seems to be required. Recent experiments of Barker and Davey (1983) have shown that for *Rhodnius*, both neuroendocrine factors from the brain and juvenile hormone are necessary for full development of male accessory glands. Perhaps neuroendocrine factors are also required in other species. The regulation of peak differentiation and the recurrent secretory cycles in male accessory glands needs further study.

## 10. Summary and Prospects

In insects as in vertebrates, seminal and paraseminal secretions are derived from several glandular tissues. The anatomical details of the male tract are enormously varied but include adaptations for timely delivery of products during the short period of copulation. The literature on the ultrastructure of male accessory glands is somewhat sparse, but there are notable sets of papers on *Schistocerca* (Ohdiambo), *Locusta* (Cantacuzène), *Acheta* (Kaulenas), *Anagasta* (Reimann and Thorson), *Calpodes* (Lai-Fook), *Glossina morsitans morsitans* (Kokwaro and Ohdiambo), *Acanthoscelides* (Huignard), and *Tenebrio* (Happ and co-workers). The work on *Tenebrio* has received particular attention in this review.

Accessory glands are usually surrounded by muscles which provide the thrust for ejaculation. Desmosomes and septate junctions link the secretory cells tightly to each other and thus maintain the integrity of the epithelium during the pressure surges which accompany sperm transfer.

Precursors for manufacture of the secretion percolate through the overlying basement membranes and muscles to be absorbed by the secretory cells. The cells are rich in ER and in Golgi zones. As the animals become reproductively mature, the cells become turgid with stored secretions. The secretory vesicles are enormously diverse in fine structure. In *Schistocerca*, *Anagasta*, *Calpodes*, *Glossina*, and *Tenebrio*, published reports indicate at least 5-10 kinds of secretory cells, each of which has a product of characteristic morphology. We cannot yet associate a particular morphology with a particular class of biochemical constituents.

In most accessory glands, the secretory vesicles are transported toward the apical end of the cell and exported by apocrine or merocrine mechanisms. Secretory products fill the gland lumen and are ejaculated during copulation. Thereafter, a new charge of secretion rapidly accumulates. We know little of the ways that secretory episodes are turned on and off, about the kinetics of replenishment, or about the detailed mechanisms of transport and export.

The functions of accessory glands are diverse, and most aspects of their physiological impact on the female and their importance for sperm maturation have been reviewed elsewhere (Leopold, 1976). I have concentrated particularly

on the role of many accessory glands in the formation of a spermatophore. Spermatophore structure, assembly, and evacuation are receiving increasing attention in several laboratories. The molecular components of spermatophores are the secretory products of accessory glands. The storage of these products takes place in regionally segregated compartments or in semisolid masses, so that there seems to be limited mixing of secretions until the time of assembly of the sperm sac. Biochemical, immunochemical, and histochemical analyses of spermatophores are needed to complement the morphological investigations. Information on many more species is required in order to obtain comparative information on the molecular and microscopic architecture of spermatophores. From a descriptive base, studies can proceed to analysis of the mechanisms for stabilization of the spermatophore and for its subsequent contortions which expel the sperm.

Accessory glands mature rapidly in late preadult stages and reach full secretory activity in young adults. Cell division, morphogenesis, and differentiation take place as the glands develop. These glands, which produce a small set of secretory products and have a distinct adult morphology, are attractive models for developmental biologists. They are particularly useful for questions about hormone action, about the importance of mitoses for ongoing differentiation, about pattern formation in a secretory (often mesodermal) tissue, and about the molecular rate-controlling mechanisms in progressive growth and terminal differentiation.

The ultrastructural studies of male accessory glands which have accumulated over the last two decades are intriguing examples of elaborate specializations of secretory cells and tissues. The present morphological information is sufficient to provide a springboard for biochemical analyses in experiments on the endocrine control of reproductive physiology and maturation.

#### ACKNOWLEDGMENTS

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#### References

- Adiyodi, K. G., and Adiyodi, R. G., 1975, Morphology and cytology of the accessory sex glands in invertebrates, *Int. Rev. Cytol.* 43:353-398.
- Adiyodi, R. G., and Adiyodi, K. G., 1974, Ultrastructure of the utriculi majores in the mushroom-shaped male accessory gland of *Periplaneta americana*, *Z. Zellforsch. Mikrosk. Anat.* 147:433-440.
- Baccetti, B., 1972, Insect sperm cells, *Adv. Insect Physiol.* 9:315-397.
- Bairati, A., and Perotti, M. E., 1970, Occurrence of a compact plug in the genital duct of *Drosophila* females after mating, *Drosophila Inf. Serv.* 45:67-68.

- Barker, J. F., and Davey, K. G., 1982, Intraglandular synthesis of protein in the transparent accessory reproductive gland in the male of *Rhodnius prolixus*, *Insect Biochem.* **12**:157-159.
- Barker, J. F., and Davey, K. G., 1983, A polypeptide from the brain and corpus cardiacum of male *Rhodnius prolixus* which stimulates *in vitro* protein synthesis in the transparent accessory reproductive gland, *Insect Biochem.* **13**:7-10.
- Beaulaton, J., and Perrin-Waldemer, C., 1975, Contribution à l'étude de la sécrétion des paragonies de *Drosophila melanogaster* Meig.: Ultrastructure et cytochimie des grains a microtubules, *J. Microsc. (Paris)* **24**:91-104.
- Bishop, G. H., 1920, Fertilization in the honey-bee. I. The male sexual organs: Their histological structure and physiological functioning, *J. Exp. Zool.* **31**:225-265.
- Black, P. N., Landers, M. H., and Happ, G. M., 1982, Cytodifferentiation in the accessory glands of *Tenebrio molitor*. VII. Crossed immunoelectrophoretic analysis of terminal differentiation in the post-ecdysial tubular accessory glands, *Dev. Biol.* **94**:106-115.
- Blum, M. S., Glowska, Z., and Tauber, S., III, 1962, Chemistry of the drone honey bee reproductive system. II. Carbohydrates in the reproductive organs and semen, *Ann. Entomol. Soc. Am.* **55**:135-139.
- Blum, M. S., Bumgarner, J. E., and Tauber S., III, 1967, Composition and possible significance of fatty acids in the lipid classes in honey bee serum, *J. Insect Physiol.* **13**:1301-1308.
- Cantacuzène, A.-M., 1972, Recherches morphologiques et physiologiques sur les glandes annexes males des orthoptères. IV. Ultrastructure de la vésicule séminale de *Locusta migratoria migratorioides* L., *Ann. Sci. Nat. Zool. Biol. Anim.* **14**:389-410.
- Cassier, P., and Huignard, J., 1979, Etude ultrastructurale des glandes annexes de l'appareil genital male chez *Acanthoscelides obtectus* Say (Coleoptera: Bruchidae), *Int. J. Insect Morphol. Embryol.* **8**:183-201.
- Dailey, P. J., and Happ., G. M., 1983, Cytodifferentiation in the accessory glands of *Tenebrio molitor*. XI. Transitional cell types during establishment of patterns, *J. Morphol.* **178**:139-154.
- Dailey, P. J., Gadzama, N. M., and Happ, G. M., 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.
- Davey, K. G., 1965, *Reproduction in the Insects*, Oliver & Boyd, Edinburgh.
- Davey, K. G., 1967, The physiology of reproduction: Some lessons from insects. In *Insects and Physiology*, edited by J. W. L. Beament and J. E. Treherne, pp. 351-364, American Elsevier, New York.
- Delbecque, J.-P., Hirn, M., Delachambre, J., and De Reggi, M., 1978, Cuticular cycle and molting hormone levels during the metamorphosis of *Tenebrio molitor* (Insecta, Coleoptera), *Dev. Biol.* **64**:11-30.
- De Loof, A., and Lagasse, A., 1972, The ultrastructure of the male accessory reproductive glands of the Colorado beetle, *Z. Zellforsch. Mikrosk. Anat.* **130**:545-552.
- Dumser, J. B., 1980, The regulation of spermatogenesis in insects, *Annu. Rev. Entomol.* **25**:341-369.
- Engelmann, F., 1970, *The Physiology of Insect Reproduction*, Pergamon Press, Elmsford, N.Y.
- Federer, H., and Chen, P. S., 1980, Ultrastruktur und Funktion der Paragonien von *Drosophila funebris*, *Rev. Suisse. Zool.* **87**:875-880.
- Feldman-Muhsam, B., Borut, S., Saliternick-Givant, S., and Eden, C., 1973, On the evacuation of sperm from the spermatophore of the tick, *Onithodorus savignyi*, *J. Insect Physiol.* **19**:951-962.
- Friedel, T., and Gillot, C., 1976, Extraglandular synthesis of accessory reproductive gland components in male *Melanoplus sanguinipes*, *J. Insect Physiol.* **22**:1309-1314.
- Friend, D. S., and Farquhar, M. G., 1967, Functions of coated vesicles during protein absorption in the rat vas deferens, *J. Cell. Biol.* **35**:357-376.
- Gadzama, N. M., and Happ, G. M., 1974, The structure and evacuation of the spermatophore of *Tenebrio molitor* L. (Coleoptera: Tenebrionidae), *Tissue Cell* **6**:95-108.
- Gadzama, N. M., Happ, C. M., and Happ, G. M., 1977, Cytodifferentiation in the accessory glands of *Tenebrio molitor*. I. Ultrastructure of the tubular gland in the post-ecdysial adult male, *J. Exp. Zool.* **200**:211-222.
- Garcia-Bellido, A., 1964, Das Sekret der Paragonien als Stimulus der Fekundität bei Weibchen von *Drosophila melanogaster*, *Z. Naturforsch. Teil B* **19**:491-495.
- Gerber, G. H., Church, N. S., and Rempel, J. G., 1971, The structure, formation, histochemistry, fate, and functions of the spermatophore of *Lytta nuttalli* Say (Coleoptera: Meloidae), *Can. J. Zool.* **49**:1595-1610.

- Gilbert, D. G., 1982, Ejaculate esterase-6 and initial sperm use by female *Drosophila melanogaster*, *J. Insect Physiol.* 27:641-650.
- Grassé, P.-P., 1977, Organes génitaux mâles, *Traité Zool.* 8(5A):125-137.
- Grimes, M. G., and Happ, G. M., 1980, Fine structure of the bean-shaped accessory gland in the male pupa of *Tenebrio molitor* L. (Coleoptera: Tenebrionidae), *Int. J. Insect Morphol. Embryol.* 9:281-296.
- Hagedorn, H. H., and Kunkel, J. G., 1979, Vitellogenin and vitellin in insects, *Annu. Rev. Entomol.* 24:475-505.
- Happ, G. M., 1982, Control of cell differentiation in the accessory reproductive glands of mealworm beetles. In *The Ultrastructure and Functioning of Insect Cells*, edited by H. Akai, R. C. King, and S. Morohoshi, pp. 83-86. Society for Insect Cells, Tokyo.
- Happ, G. M., and Happ, C. M., 1982, Cytodifferentiation in the accessory glands of *Tenebrio molitor*. X. Ultrastructure of the tubular gland in the male pupa, *J. Morphol.* 172:97-112.
- 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 gland of post-ecdysial adult males, *J. Exp. Zool.* 200:223-236.
- Happ, G. M., Yuncker, C., and Dailey, P. G., 1982, Cytodifferentiation in the accessory glands of *Tenebrio molitor*. VII. Patterns of leucine incorporation by the bean-shaped glands of males, *J. Exp. Zool.* 220:81-92.
- Huet, C., 1966, Etude expérimentale du développement de l'appareil génital mâle de *Tenebrio molitor* (Coléoptère: Ténébrionide), *C.R. Soc. Biol.* 160:2021-2025.
- Huignard, J., 1975, Anatomie et histologie des glandes annexes mâles au cours de la vie imaginaire chez *Acanthoscelides obtectus* Say (Coleoptera: Bruchidae), *Int. J. Insect Morphol. Embryol.* 4:77-88.
- Kafatos, F. C., Regier, J. C., Mazur, G. D., Nadel, M. R., Blau, H. M., Petri, W. H., Wyman, A. R., Gelinas, R. E., Moore, P. B., Paul, M., Efstratiadis, A., Vournakis, J. N., Goldsmith, M. R., Hunsley, J. R., Baker, B., Nardi, J., and Koehler, M., 1977, The eggshell of insects: Differentiation-specific proteins and the control of their synthesis and accumulation during development. In *Results and Problems in Cell Differentiation*, vol. 8, edited by W. Beermann, pp. 45-145., Springer-Verlag, Berlin.
- Kaulenas, M. S., 1976, Regional specialization for export protein synthesis in the male cricket accessory gland, *J. Exp. Zool.* 195:81-96.
- Kaulenas, M. S., Potswald, H. E., Burns, A. L., and Yenofsky, R. L., 1979, Development of structural and functional specializations for export protein synthesis by the accessory gland of the male cricket, *Acheta domesticus* L. (Orthoptera Gryllidae), *Int. J. Insect Morphol. Embryol.* 8:33-49.
- Khalifa, A., 1949, The mechanism of insemination and mode of action of the spermatophore in *Gryllus domesticus*, *Q. J. Microsc. Sci.* 90:281-292.
- King, R. C., Cassidy, J. D., and Rousset, A., 1982, The formation of clones of interconnected cells during gametogenesis in insects. In *Insect Ultrastructure*, edited by R. C. King and H. Akai, vol. 1, pp. 3-31, Plenum Press, New York.
- Kokwaro, E. D., 1982, Ultrastructure of the male accessory reproductive glands, spermatophore and spermatheca of the tsetse, *Glossina morsitans morsitans* Westwood. In *The Ultrastructure and Functioning of Insect Cells*, edited by H. Akai, R. C. King, and S. Morohoshi, pp. 53-56, Society for Insect Cells, Tokyo.
- Kokwaro, E. D., and Ohdiambo, T. R., 1981, Spermatophore of the tsetse, *Glossina morsitans morsitans* Westwood: An ultrastructural study, *Insect Sci. Appl.* 1:185-190.
- Lai-Fook, J., 1982a, The vasa deferentia of the male reproductive system of *Calpodus ethlius* (Hesperidae, Lepidoptera), *Can. J. Zool.* 60:1172-1183.
- Lai-Fook, J., 1982b, Structure of the noncuticular simplex of the internal male reproductive tract of *Calpodus ethlius* (Hesperidae, Lepidoptera), *Can. J. Zool.* 60:1184-1201.
- Lai-Fook, J., 1982c, Structure of the accessory glands and duplex of the internal male reproductive system of *Calpodus ethlius* (Hesperidae, Lepidoptera), *Can. J. Zool.* 60:1202-1215.
- Lai-Fook, J., 1982d, Structure, function, and possible evolutionary significance of the constrictions in the male reproductive system of *Calpodus ethlius* (Hesperidae, Lepidoptera), *Can. J. Zool.* 60:1828-1836.

- Lane, N. J., and Skaer, H. leB., 1980, Intercellular junctions in insect tissues, *Adv. Insect Physiol.* **15**:35-213.
- Leopold, R. A., 1976, The role of male accessory glands in insect reproduction, *Annu. Rev. Entomol.* **21**:199-221.
- Linley, J. R., 1981, Ejaculation and spermatophore formation in *Culicoides melleus* (Coq.) (Diptera: Ceratopogonidae), *Can. J. Zool.* **59**:332-346.
- Loher, W., 1974, Circadian control of spermatophore formation in the cricket *Teleogryllus commodus* Walker, *J. Insect Physiol.* **20**:1155-1172.
- Mandelbaum, I., 1980, Intercellular bridges and the fusome in the germ cells of the cecropia moth, *J. Morphol.* **166**:37-50.
- Mann, T., 1964, *The Biochemistry of Semen and of the Male Reproductive Tract*, Methuen, London.
- Meola, S. M., 1982, Morphology of the region of the ejaculatory duct producing the male accessory gland material in the stable fly, *Stomoxys calcitrans* L. (Diptera: Muscidae), *Int. J. Insect Morphol. Embryol.* **14**:69-77.
- Ohdiambo, T. R., 1966, Growth and the hormonal control of sexual maturation in the male desert locust, *Schistocerca gregaria* (Forsköhl), *Trans. R. Entomol. Soc. London* **118**:393-412.
- Ohdiambo, T. R., 1969, The architecture of the accessory reproductive glands of the desert locust. IV. Fine structure of the glandular epithelium, *Philos. Trans. R. Soc. London Ser. B* **256**:85-114.
- Oliver, C., 1982, Endocytotic pathways at the lateral and basal cell surfaces of exocrine acinar cells, *J. Cell. Biol.* **95**:154-161.
- Pearse, B. M. F., and Bretscher, M. S., 1981, Membrane recycling by coated vesicles, *Ann. Rev. Biochem.* **50**:85-101.
- Perotti, M. E., 1971, Microtubules as components of *Drosophila* male paragonia secretion: An electron microscopic study, with enzymatic tests, *J. Submicrosc. Cytol.* **3**:255-282.
- Peschke, K., 1978, Funktionsmorphologische Untersuchungen zur Kopulation von *Aleochara curtula* Goeze (Coleoptera, Staphylinidae), *Zoomorphologie* **89**:157-184.
- Phillips, D. M., 1974, *Spermiogenesis*, Academic Press, New York.
- Poels, A., 1972, Histophysiologie des voies génitales mâles de *Tenebrio molitor* L. (Coléoptère: Tenebrionidae), *Ann. Soc. R. Zool. Belg.* **102**:199-234.
- Ramalingam, S., and Craig, G. B., Jr., 1978, Fine structure of the male accessory glands in *Aedes triseriatus*, *J. Insect Physiol.* **24**:251-259.
- Reimann, J. G., 1973, Ultrastructure of the ejaculatory duct region producing the male housefly accessory material, *J. Insect Physiol.* **19**:213-223.
- Reimann, J. G., and Thorson, B. J., 1976a, Ultrastructure of the vasa deferentia of the Mediterranean flour moth, *J. Morphol.* **149**:483-505.
- Reimann, J. G., and Thorson, B. J., 1976b, Ultrastructure of the ductus ejaculatorius duplex of the Mediterranean flour moth, *Anagasta kuhniella* (Zeller) (Lepidoptera Pyralidae), *Int. J. Insect Morphol. Embryol.* **5**:227-240.
- Reimann, J. G., and Thorson, B. J., 1979a, Foliate and granule secreting cells in the ejaculatory duct (simplex) of the Mediterranean flour moth, *J. Ultrastruct. Res.* **66**:1-10.
- Reimann, J. G., and Thorson, B. J., 1979b, Ultrastructure of the accessory glands of the Mediterranean flour moth, *J. Morphol.* **159**:355-391.
- Schaller, F., 1971, Indirect sperm transfer by soil arthropods, *Annu. Rev. Entomol.*, **16**:407-466.
- Scharrer, B., 1946, The relationship between corpora allata and reproductive organs in adult *Leucophaea maderae* (Orthoptera), *Endocrinology* **38**:46-55.
- Steinman, R. M., Mellman, I. S., Muller, W. A., and Cohn, Z. A., 1983, Endocytosis and the recycling of plasma membrane, *J. Cell Biol.* **96**:1-27.
- Szöllösi, A., and Landureau, J.-C., 1977, Imaginal cell differentiation in the spermiduct of *Samia cynthia* (Lepidoptera): Responses *in vitro* to ecdysone and ecdysterone, *Biol. Cell.* **28**:23-36.
- Tandler, B., Williamson, D. L., and Ehrman, L., 1968, Unusual filamentous structures in the paragonia of male *Drosophila paulistorum*, *J. Cell. Biol.* **38**:329-336.
- Telfer, W. H., Huebner, E., and Smith, D. S., 1982, The cell biology of vitellogenic follicles in *Hyalophora* and *Rhodnius*. In *Insect Ultrastructure*, edited by R. C. King and H. Akai, vol. 1, pp. 118-149, Plenum Press, New York.
- Thomsen, E., 1942, An experimental and anatomical study of the corpus allatum in the blowfly

- Calliphora erythrocephala* Meig., *Vidensk. Medd. Dan. Naturhist. Foren. Kjobenhavn* **106**:319-415.
- Tongu, Y., Suguri, S., Sakumoto, D., Itano, K., and Inatomi, S., 1972, The ultrastructure of mosquitoes. 6. Male accessory gland of *Culex pipiens pallens*, *Jpn. J. Sanit. Zool.* **23**:129-139 (Japanese with English summary).
- Tuzet, O., 1977, Les spermatophores des insectes, *Traité Zool.* **8**(5A):277-330.
- Wigglesworth, V. B., 1936, The function of the corpus allatum in the growth and reproduction of *Rhodnius prolixus* (Hemiptera), *Q. J. Microsc. Sci.* **79**:91-122.