

## Digestion of spermatophore contents in the female genital system of the hermaphroditic flatworm *Dugesia gonocephala* (Tricladida, Paludicola)

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**Abstract.** In *Dugesia gonocephala*, unlike other planarians, the cells of the copulatory bursa fulfill both a secretory and phagocytic function. Enzymes are synthesized in the absence of a spermatophore and are secreted into the lumen of the bursa when a spermatophore is received from a partner. During the days following copulation, seminal material is engulfed by the cells through cytoplasmic protrusions that extend into the lumen of the bursa. This material disintegrates and, as a result, the cells are filled with phagosomes of different sizes 3–4 days after copulation. Sperm cells are also digested in phagocytic cells that line the proximal side of the vitellarian follicles. Upon arrival in the lumen of the sperm receptacles, sperms penetrate the surrounding epithelial cells.

*Additional key words:* copulatory bursa, resorptive vesicle, sperm receptacle

Ejaculates usually contain many more sperms than the few necessary for the fertilization of the eggs. They also contain secretions from accessory glands known to be of considerable nutritional value (Mann 1984). Many invertebrate taxa have therefore become adapted to use these ejaculates as an additional source of food. In bushcrickets and grasshoppers, females actually feed upon parts of the received spermatophore during and after insemination (Wedell 1994). This may lead to an increase in female fecundity and offspring survival, when the nutrients thus obtained are used by the female for the production of the eggs (Bowen et al. 1984; Gwynne 1984, 1988; Boggs 1990; Simmons 1990; Reinhold & Heller 1993). In other taxa, ejaculates may be digested in special organs of the female genital system, e.g., the copulatory bursa (bursa copulatrix) of pulmonate snails (Tompa 1984).

In flatworms, little is known about the physiology and fine structure of the different sperm resorptive organs, despite the possible importance of sperm digestion from an evolutionary point of view. Light microscopical studies have shown that excesses of sperms and prostatic secretions are mainly digested in the copulatory bursa, while further resorption of sperms takes place in resorptive vesicles near the vitellarian follicles and in the sperm receptacles near the ovaries, where sperms are stored for fertilization (Cernosvitov 1931, 1932; Sluys 1989a).

Ultrastructural studies on the subject are scarce and

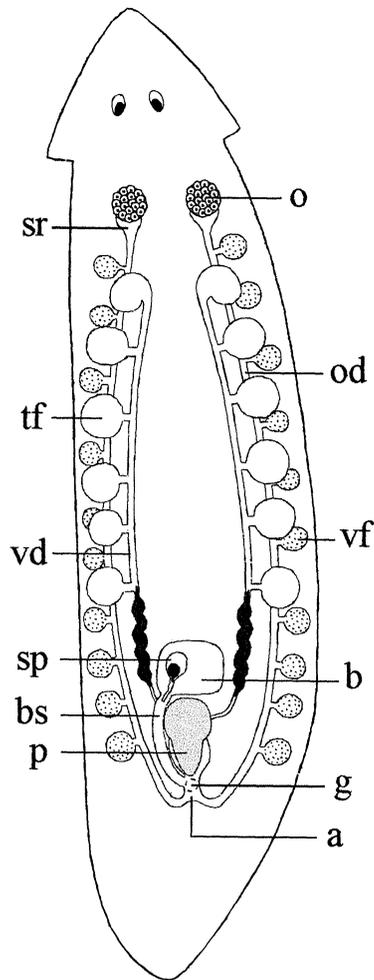
restricted to the copulatory bursa (Farnesi et al. 1979; Fischlschweiger & Clausnitzer 1984). According to Farnesi et al. (1979) the bursal epithelium of *Dugesia lugubris* (SCHMIDT 1861) consists of two different types of cells, secretory and phagocytic cells, both of which show a positive reaction for acid phosphatase. The latter is in agreement with the histochemical study of Jennings (1968) on two temnocephalid rhabdocoels. Fischlschweiger & Clausnitzer (1984) also recognized a layer of phagocytic cells in the copulatory bursa of *D. tigrina* (GIRARD 1850). The second type of cell, however, was considered to be a layer of replacement cells.

In this study we provide new insight into the activity of the different sperm resorptive organs of the hermaphroditic planarian *D. gonocephala* (DUGÈS 1830). As in other turbellarians, *D. gonocephala* shows internal fertilization following reciprocal copulation (Vreys et al. 1997a). Self-fertilization does not occur (Vreys et al. 1994). During copulation, spermatophores are transferred to the copulatory bursa of the partner and sperms migrate to the sperm receptacles within 5 d after copulation (Vreys et al. 1997b).

### Methods

**Study animals and maintenance.** Specimens of *Dugesia gonocephala* were obtained from a small, shallow stream (Cottesserbeek) near Cottessen (SE Netherlands, 50°9'N, 5°9'E) and cultured in the laboratory under constant conditions of 12 h light/12 h dark (day starting at 0700) and 14 ± 1°C. Animals

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**Fig. 1.** Organization of the genital system of the hermaphroditic planarian *D. gonocephala*. Genital atrium (a); copulatory bursa (b); bursal stalk (bs); common ventral gonopore (g); ovary (o); oviduct (od); penis (p); spermatophore (sp); sperm receptacle (sr); testicular follicle (tf); vas deferens (vd); vitellarian follicle (vf).

were kept in constantly filtered and aerated stream-water and fed twice weekly with punctured fresh fly larvae (*Chironomus*).

The female reproductive system of *D. gonocephala* consists of a pair of ovaries just behind the brain, anterior to the vitellarian follicles (Fig. 1). Near each ovary, the oviduct widens, forming a small chamber, the sperm receptacle, in which viable allosperm can be stored for up to 6 months after copulation (Vreys et al. 1994). In these chambers, ripe eggs are fertilized (Gremigni 1983). The oviducts open separately into the terminal part of the bursal stalk, a strongly muscular canal that forms the connection between the atrium and the copulatory bursa. Testicular follicles are scattered throughout the body. Sperms collect in the vasa deferentia, which enlarge as they approach the

copulatory organ. Further details on the morphology are given by De Vries & Ball (1980) and De Vries (1984).

**Light microscopy (LM).** Unmated individuals isolated for 1–14 d (n=12) and individuals taken at different times during (n=14) and after (n=8) copulation were fixed with Steinmann's fluid (1 part concentrated nitric acid, 1 part saturated solution of mercuric chloride in 5% sodium chloride, 1 part distilled water), stored in 70% ethanol, and embedded in paraffin. Serial 8- $\mu$ m saggital or frontal sections were stained with Haematoxylin Erlich or Heidenhain, using eosine as counterstain (Romeis 1968).

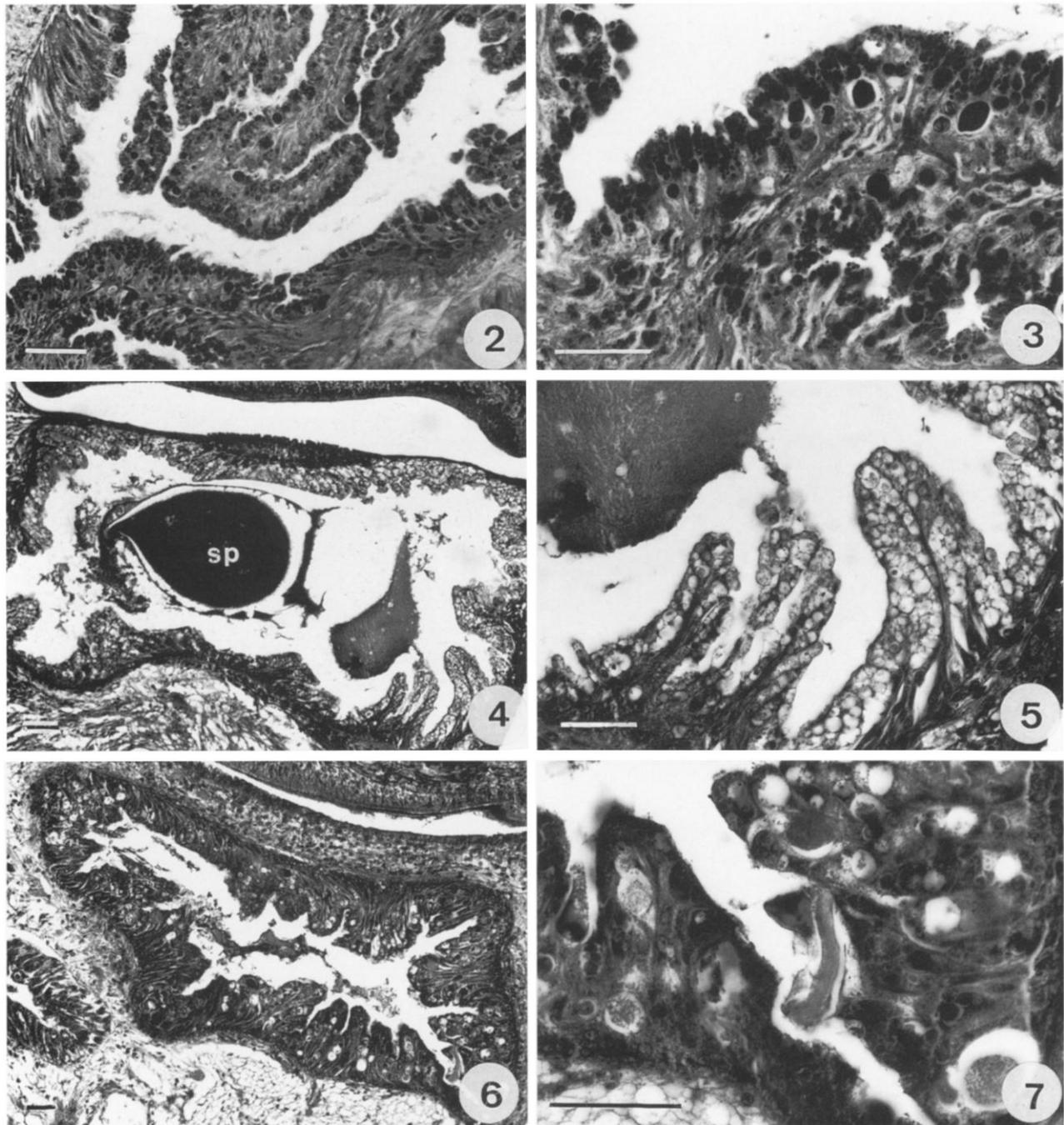
**Transmission electron microscopy (TEM).** Unmated individuals (n=2) and individuals taken 3 h (n=2) and 3 d (n=2) after copulation were fixed in 2.5% glutaraldehyde in 0.1 M Na-cacodylate buffer (pH 7.2; 350 mOsm), postfixed in cacodylate-buffered 2% osmium tetroxide, dehydrated in an acetone series, and embedded in araldite. Specimens of *D. gonocephala* are too large (up to 25  $\times$  4 mm) to be fixed in pairs *in copula* for ultrastructural studies. Both semithin and ultrathin sections were made with a Reichert ultramicrotome. Semithin sections (0.3  $\mu$ m) were stained with thionine methylene blue. Ultrathin sections (50–70 nm) were stained with uranyl acetate and lead citrate for TEM (Phillips 400).

## Results

### Copulatory bursa

The wall of the bursa is composed of a single-layered epithelium that rests on a basal lamina and a thin layer of connective tissue. The latter is discontinuously surrounded by several layers of muscle fibers. The columnar epithelial cells are joined by apical septate desmosomes. The epithelium of the bursa exhibits different morphological and physiological states at different stages in the mating sequence.

In light microscopical sections of individuals without a spermatophore (unmated individuals and individuals fixed in copula before spermatophore transfer), the wall of the bursa is intensely colored due to a high number of small, eosinophilic granules that fill the apical cytoplasm of most of the epithelial cells. In some cells, the granules are large and mainly stored in vacuoles (Figs. 2, 3). In individuals that have received a spermatophore from a partner (individuals fixed in copula after spermatophore transfer and individuals fixed immediately after the end of the copulation), the lumen of the bursa is enlarged due to the presence of a spermatophore and additional secretions. Few cells still contain the small, eosinophilic granules, while



**Figs. 2–7.** Bursal wall of *D. gonocephala*. LM. Scale bars, 50  $\mu$ m.

**Figs. 2, 3.** Sections of individuals without a spermatophore, showing numerous small, eosinophilic granules in the apical region of most of the epithelial cells. In some cells the granules are large and stored in vacuoles.

**Figs. 4, 5.** Sections of individuals with a spermatophore (sp), showing numerous vacuoles in most of the epithelial cells and an enlargement of the lumen of the bursa. Only a few cells contain eosinophilic granules.

**Figs. 6, 7.** Sections of individuals fixed 3 d after copulation, showing phagosomes of different sizes containing dense material in most of the epithelial cells. The lumen of the bursa has decreased in size and the spermatophore is disintegrating.

most of the cells are filled with vacuoles (Figs. 4, 5). At 3–4 d after copulation, the epithelial cells contain phagosomes of different sizes containing dense material. Small, eosinophilic granules may be present in the

cytoplasm. The spermatophore disintegrates and the lumen of the bursa decreases in size (Figs. 6, 7).

On an ultrastructural level, bursal cells show a secretory and phagocytic phase that may be observed

together depending on the reproductive state of a single individual. In unmated individuals, endoplasmic vesicles and Golgi complexes are present in the central region of the cells (Fig. 8), whereas many electron-dense granules accumulate in the apical region of the cells (Fig. 9). Cytoplasmic protrusions extend into the lumen of the bursa and in some cells residual bodies can be observed. At 3 h after copulation, no granules are present in the cells and the cytoplasmic protrusions are elongated (Fig. 10). Secretions and entangled sperm cells become enclosed by these protrusions and are engulfed by the cells (Figs. 11, 12). In a few cells, this engulfed material was found stored in vacuoles and the cytoplasmic protrusions became smaller (Fig. 13). At 3 d after copulation, the cells are filled with phagosomes containing homogeneous, electron-dense material (Fig. 14) and the apical surface of the cells bears only a few cytoplasmic protrusions. In some cells, the phagosomes decrease in size and number and become residual bodies. Lipid droplets observed in bursal cells of post-copula individuals (Fig. 15) may represent storage of absorbed material.

### Resorptive vesicles

At regular intervals, the oviduct communicates with several phagocytic cells that line the proximal side of the vitellarian follicles. Clusters of sperms that enter the vitelloglands are captured by these cells (Fig. 16), which contain phagosomes in different phases of intracellular sperm digestion (Figs. 17, 18). Sperms are never found inside the vitellarian follicles.

### Sperm receptacles

Sperm cells accumulate in the lumen of the sperm receptacles (Fig. 19) and are also found in the surrounding epithelial cells (Figs. 20, 21), even in individuals that have not been involved in copulation for at least 14 d. These sperm cells undergo no morphological changes. Sperm cells are resorbed only in the vitellarian follicles. No sperm cells are found in the ovary itself.

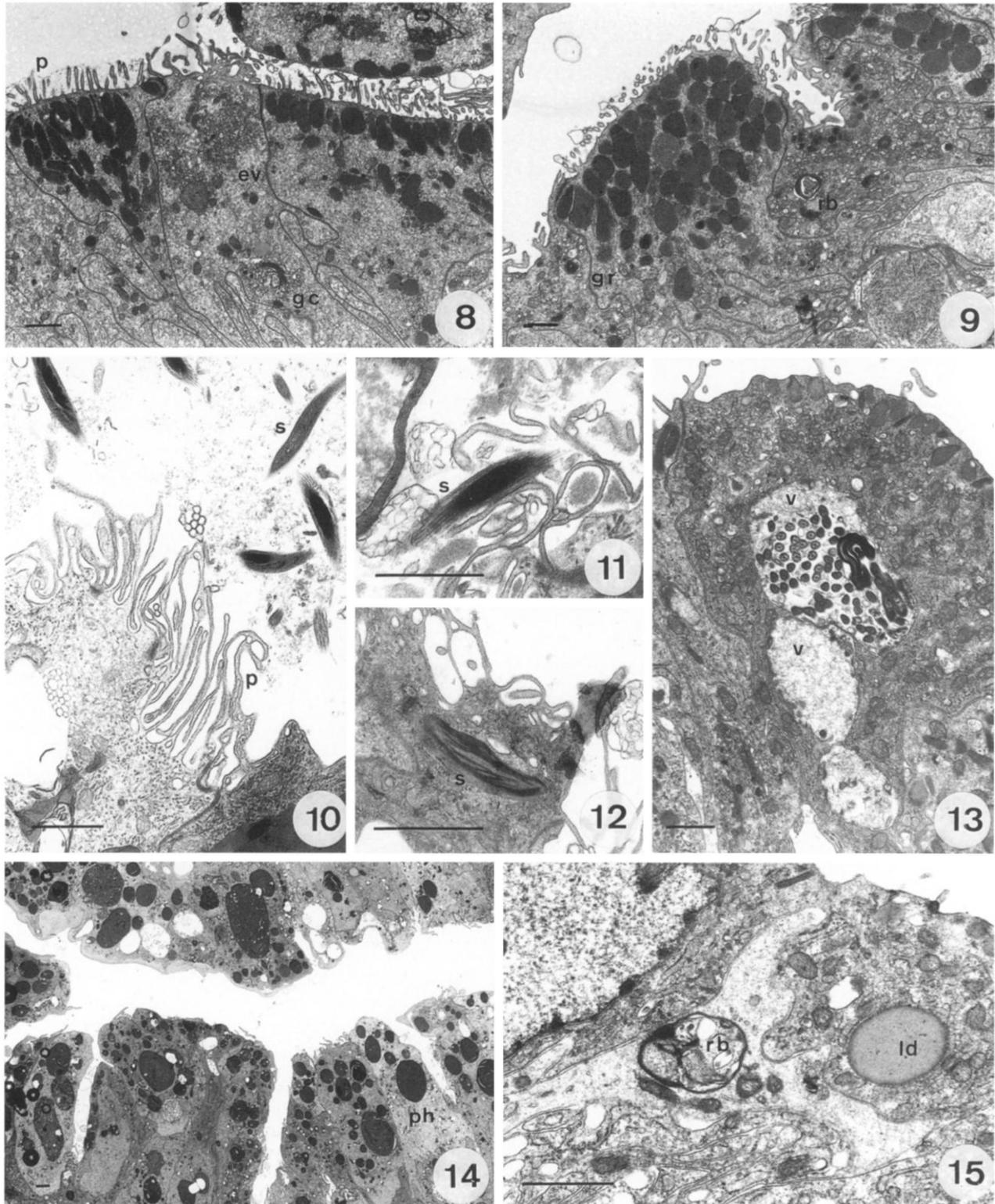
## Discussion

During copulation in *Dugesia gonocephala*, a spermatophore is transferred to the bursa of the partner. The number of sperms contained in the spermatophore, however, far exceeds the number of sperms that is usually present in the sperm receptacles near the ovaries. This difference results from sperm digestion taking place in the copulatory bursa and in resorptive vesicles near the vitellarian follicles during the days following copulation. However, the stalk of the spermatophore

(see Fig. 1) usually points in the direction of the bursal canal; moreover, the wall of the spermatophore remains intact until no migrating sperms are left inside (Vreys et al. 1997b). These findings indicate that the spermatophore of *D. gonocephala* might protect (some) of the migrating sperms from being digested in the copulatory bursa (Vreys et al. 1997b).

The digestive process in the bursa of *D. gonocephala* is somewhat different from that observed in other planarians (Farnesi et al. 1979; Fischlschweiger & Clausnitzer 1984). In *D. lugubris*, enzymes produced by secretory cells are secreted into the lumen of the bursa just after copulation. Sperm cells are then phagocytized by a second type of cell and the engulfed material is digested intracellularly during the 4 d following copulation (Farnesi et al. 1979). Fischlschweiger & Clausnitzer (1984) also described the presence of phagocytic cells in the copulatory bursa of *Dugesia tigrina*, but they did not mention the presence of secretory cells.

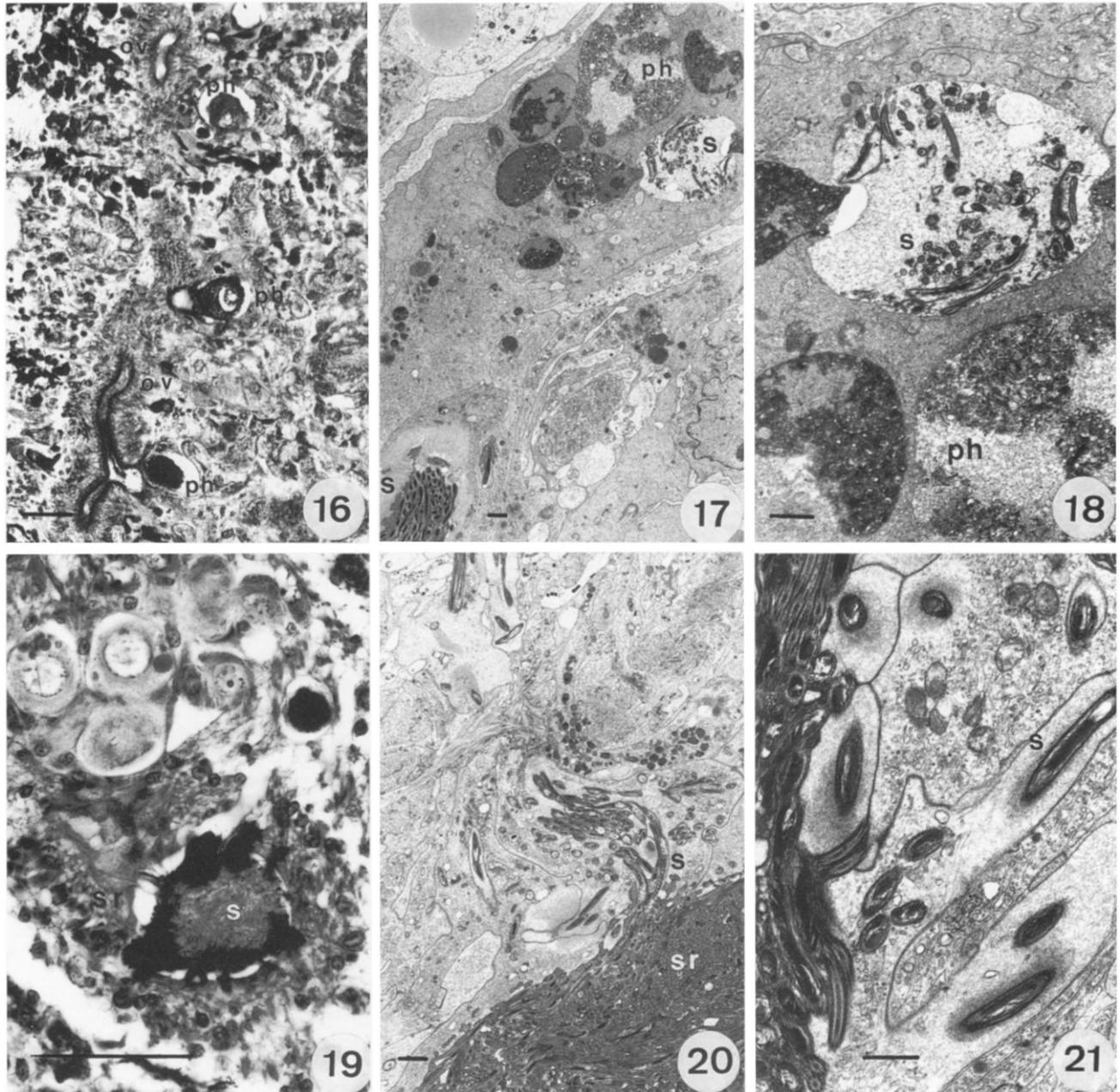
In *D. gonocephala* most or all of the bursal cells contain numerous small eosinophilic granules when no spermatophore is present, large vacuoles just after spermatophore transfer, and phagosomes of different sizes with dense material 3 d after copulation. These data suggest that in *D. gonocephala*, unlike *D. lugubris* and *D. tigrina*, the wall of the bursa is composed of only one type of cell, fulfilling both a secretory and phagocytic function (Fig. 22). In the absence of a spermatophore, the cell contains endoplasmic vesicles and Golgi complexes, indicating that enzymes are being synthesized (Fig. 22A). Secretory granules accumulate in the apical zone of the cell (Fig. 22B,C). When a spermatophore is received from a partner, the eosinophilic granules disappear, indicating that enzymes have been secreted into the lumen of the bursa. As in *D. lugubris* (Farnesi et al. 1979), these enzymes are probably responsible for some extracellular digestion. When enzymes are secreted by the cell into the lumen of the bursa, the membranes of the secretory vacuoles fuse with that of the apical surface of the cell. This could provide increased membrane material for the increase in size and number of the cytoplasmic protrusions. The phagocytic phase starts when secretions and entangled sperm cells become enclosed by these protrusions and are engulfed by the cell (Fig. 22D). The content of the resulting vacuoles (Fig. 22E) disintegrates and, 3 d after copulation, the cell is filled with homogeneous, dark-staining phagosomes (Fig. 22F). Next, the phagosomes (as residual bodies) clearly decrease in size and number. The presence of endoplasmic vesicles and a few electron-dense granules (Fig. 22G) indicates that the cell is returning to a secretory phase (Fig. 22A).



**Figs. 8–15.** Bursal wall of *D. gonocephala*. TEM. Scale bars, 1  $\mu$ m.

**Figs. 8, 9.** Sections of un-mated individuals showing cells with endoplasmic vesicles (ev) and Golgi complexes (gc) and cells with an accumulation of electron-dense granules (gr) in the apical region. Residual bodies (rb) are present and cytoplasmic protrusions (p) extend into the lumen of the bursa.

**Figs. 10–13.** Sections of individuals fixed 3 h after copulation, showing secretions and entangled sperm cells (s) enclosed by the elongated cytoplasmic protrusions (p) and engulfed by the cells. The engulfed material is stored in vacuoles (v). Note the absence of granules in the cytoplasm.



**Figs. 16–18.** Resorptive vesicles of *D. gonocephala*.

**Fig. 16.** Communication between the oviduct (ov) and several phagocytic cells (ph) that line the proximal side of the vitellarian follicles. LM. Scale bar, 50  $\mu$ m.

**Figs. 17, 18.** Sections showing clusters of migrating sperm (s) that are captured by the phagocytic cells and phagosomes (ph) at different phases of intracellular sperm digestion. TEM. Scale bars, 1  $\mu$ m.

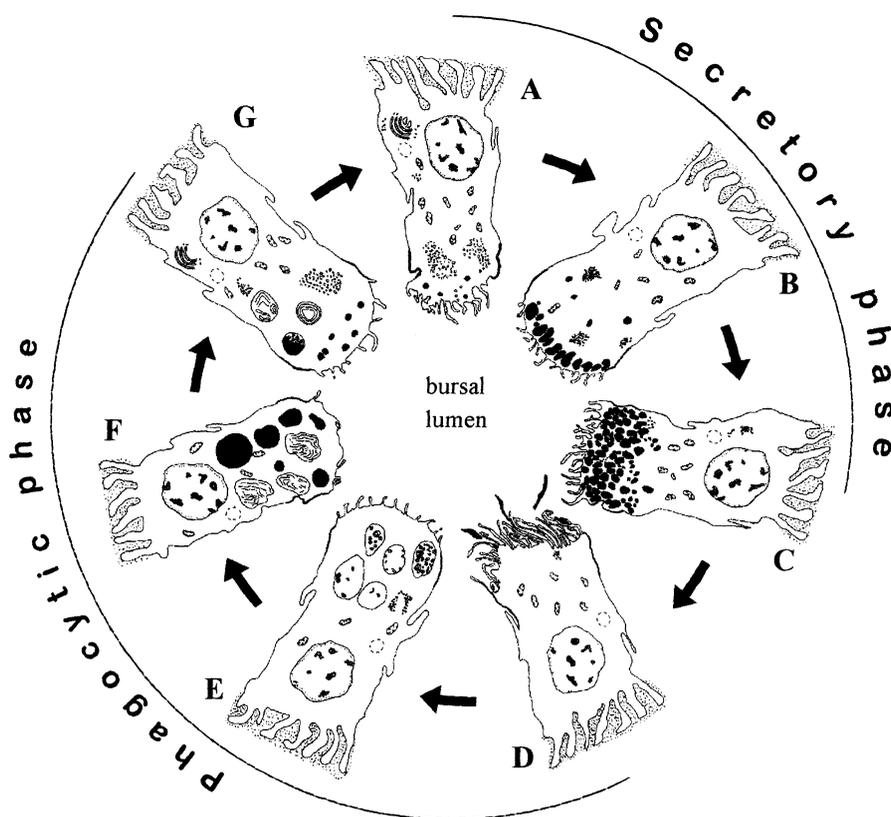
**Figs. 19–21.** Sperm receptacle of *D. gonocephala*.

**Fig. 19.** Accumulation of sperm cells (s) in the lumen of the sperm receptacle (sr). LM. Scale bar, 50  $\mu$ m.

**Figs. 20, 21.** Sections showing sperm (s) penetrating the surrounding epithelial cells of the sperm receptacle (sr). TEM. Scale bars, 1  $\mu$ m.

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**Figs. 14, 15.** Sections of individuals fixed 3 d after copulation showing homogeneous, dark-staining phagosomes (ph), residual bodies (rb), and lipid droplets (ld).



**Fig. 22.** Diagram of the secretory and phagocytic activity of the bursal cells of *Dugesia gonocephala* in the presence or absence of a spermatozoon. See discussion for details. Secretory phase in the absence of a spermatozoon: A. Cell with endoplasmic vesicles and Golgi complexes; B, C. Accumulation of electron-dense granules in the apical cytoplasm. Phagocytic phase in the presence of a spermatozoon: D. Sperm cells and secretions enclosed by long cytoplasmic protrusions; E. Cell containing vacuoles with disintegrating sperm cells and secretions; F. Cell with homogeneous, dark-staining phagosomes 3 d after copulation; G. Cell with residual bodies, endoplasmic vesicles, and a few electron-dense granules.

The proximal side of the vitellarian follicles is lined with a number of highly vacuolated cells, arranged in such a way that together they form a clear vesicle, indicated by Sluys (1989a,b) to function as a sperm resorptive vesicle. Our results confirm the observation of Cernosvitov (1931) that these cells are specialized in the phagocytosis of clusters of sperms that migrate through the oviducts during the days following copulation. Digestion of sperms takes place all over the female genital tract, even near the anteriormost vitellarian follicles, which explains why relatively few sperms finally reach the sperm receptacles.

**Acknowledgments.** We thank Dr. Nikki Watson for fruitful comments on the manuscript. Frank Van Belleghem was very helpful with the scanning procedure. C. Vreys was supported by a Research Assistantship of the Flemish Institute for Encouraging Scientific-Technological Research in Industry and by Research Project G.0094.92 sponsored by the Flemish Science Foundation.

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