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# Electron Dense Particles Appeared in the Microvilli Zone of the Cuboidal Cells of the Ventral Receptacle in *Drosophila melanogaster* Mated Female

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**ABSTRACT**—It has been previously known that the secretions of the male accessory glands of *Drosophila melanogaster* stimulate female reproductive behaviors such as oviposition. Their transfer to and fates in mated females , however, is only poorly known. We have examined some morphological changes in the female reproductive organs, suggesting relation to reproductive behaviors specifically manifested in mated female. The common oviduct, the uterus and the vagina did not show any morphological changes after mating. In the capsule lumen of the spermatheca, quantity of the secretions discharged from the glandular cells seemed not to be changed after mating. No morphological change in the epithelial cells of this organ was observed. In the ventral receptacle, however, many electron dense particles appeared after mating in the microvilli zone of a layer of the cuboidal epithelial cells. These particles were inferred to be formed by the substances absorbed through the pore canals of the chitinous intima of the ventral receptacle. In the cytoplasm of the cuboidal cells, however, typical features of micropinocytosis such as formation of the coated vesicles were not found.

## INTRODUCTION

The male accessory glands of *Drosophila melanogaster* secrete proteins that are transferred along with sperm into female reproductive organs during copulation [8]. The secretions of the male accessory glands stimulate oviposition and egg production, and decrease female receptivity to courtship [8, 13, 14]. It has been shown that the secretions include several kinds of proteins and peptides [18, 19] or transcripts expressed specifically in the accessory gland [2, 7, 10, 17]. Among them, a peptide composed with 36 amino acid residues has been purified by Chen *et al.* [9]. This peptide elicits a transient (<24 hr) increase in oviposition and reduction in female receptivity to male courtship.

Monsma and Wolfner [17] have found two tightly linked accessory gland transcripts. One of which has an amino acid sequence similarly to the egg laying hormone of *Aplysia*. Both proteins translated from the transcripts are transferred to the female during mating, and rapidly enter the female hemolymph [16]. Thus, specific proteins of the accessory glands enter the female hemolymph through the female reproductive organs.

In this article, we report on a morphological change in the mated female reproductive organs, suggesting a possible incorporation of the secretes of the male accessory glands.

## MATERIALS AND METHODS

Flies were reared on a standard cornmeal, yeast and agar medium at 25°C under 14 : 10 light-dark cycle.

Strains of *Drosophila melanogaster* used for observation were *ebony* and *Oregon-R*. The former strain showed, in the preliminary experiments, very clear behavioral changes in mated females for oviposition and receptivity to male courtship. The latter wild type strain, *Oregon-R*, was used for comparison with observations of the *ebony* strain. As the results of observation in the both strains were not different at all, strain name was not specified both in the text and each figure's legend of the present paper. Usually, 2 to 3 days

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old virgin females and mated females which were ranged from 3 hr to 9 days after copulation were used for observation. Five replicates of the glass vials each containing 10 virgin females and 15 males were set up for copulation. After 10 minutes, unmated females and males were discarded and mated pairs were left to finish copulation.

Anesthetized virgin or mated females were dissected using the tungsten needles in chilled 2% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4). The inner reproductive organs which were excised the ovaries at the lateral oviducts were transferred to the fresh 2% glutaraldehyde fixative solution and fixed for 2 hr at 4°C.

Then, the reproductive organs were post-fixed in 2% $OsO_4$ in 0.1 M phosphate buffer (pH 7.4) for 2 hr at 4°C. After dehydration in a series of graded concentrations of ethanol, they were embedded in TAAB 812. The Reichert Om U2 ultramicrotome was used for sectioning. Sections which were doubly stained with uranyl acetate and Reynold's lead acetate were examined in a HITACHI HU-12 or HU-12A electron microscope.

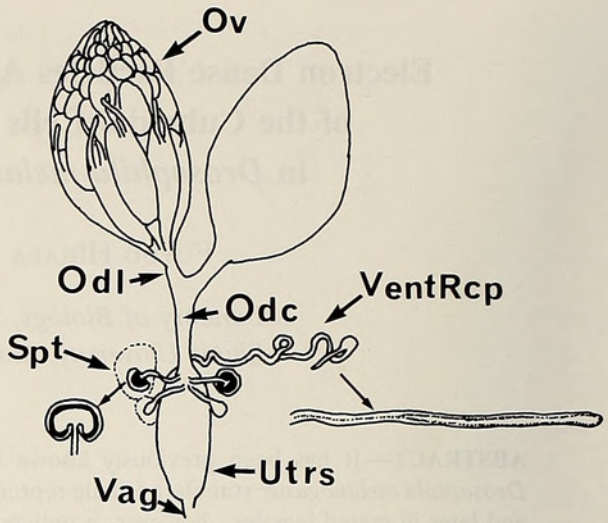



FIG. 1. Dorsal aspect of the female inner reproductive organs of *Drosophila melanogaster*. Both the ventral receptacle and the spermatheca are redrawn to show these inner structures. Ov, ovary; Odl, lateral oviduct; Odc, common oviduct; Spt, spermatheca; VentRcp, ventral receptacle; Utrs, uterus; Vag, vagina.

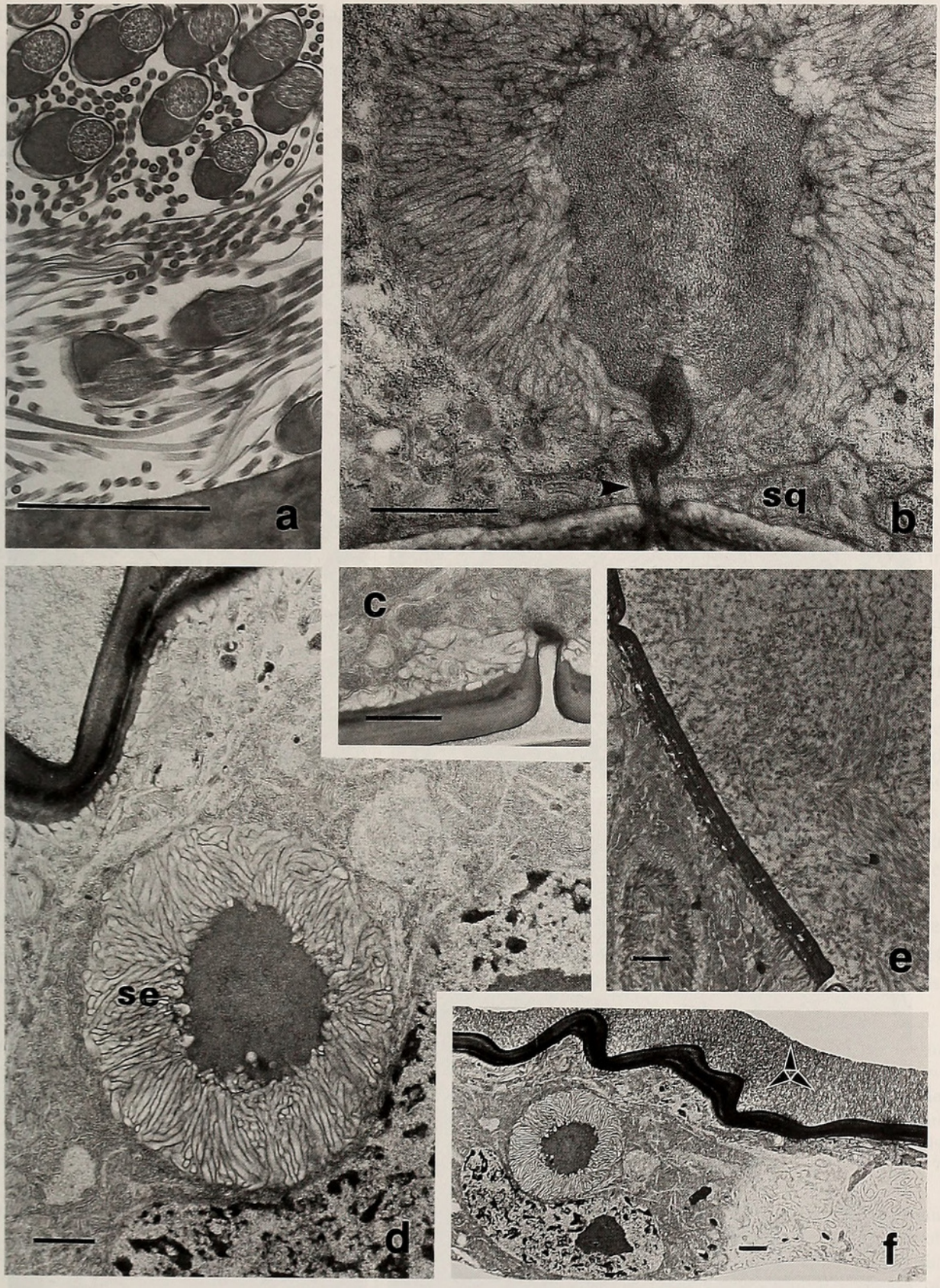
## RESULTS

In *Drosophila,* the female internal reproductive system consists of a pair of ovaries, the lateral oviducts which unite medially to form the common oviduct, the genital chamber or vagina, anterior part of which is termed the uterus. Besides these efferent ducts, the female reproductive system includes three sperm storing organs; a pair of the spermathecae and the ventral receptacle. The spermathecae are a pair of mushroom shaped organs which connected close together to the uterus by slender ducts. The ventral receptacle is a compactly coiled tube applied to the anterior end of the uterus. The diameter of the lumen of the ventral receptacle is wider in the distal part than the proximal region [15] (Fig. 1).

No structural change was observed before and after copulation in the surface layer including the epithelial cells with a chitinous intima and surrounded by circular muscles of the common oviduct, the uterus and the vagina. In the lumen of the capsule of the spermathecae which are a pair of sperm storage organs, numerous tubular elements tightly intermingled with the sperm were found (Fig. 2a). These tubular elements were found also in the lumen of the ventral receptacle (Fig. 5b) and were thought to be derived from the male accessory gland as suggested by Bairati [3] and Acton [1].

Each capsule of the spermathecae was covered externally by two kinds of cells: a layer of the squamous epithelial cells and a layer of the glandular cells located outside of the squamous cells. Each of the glandular cells has a secretory structure which discharges the secretion into the capsule lumen through the ductule piercing the cuticular intima (Figs. 2b, 2c). This fusiform secretory structure was defined with very numerous long microvilli of the invaginated plasma membrane of the glandular cell (Figs. 2b, 2d). Many electron

FIG. 2. Electron micrographs of the spermatheca. (a) numerous tubular elements intermingled with the sperm in the lumen (3 hr after copulation). (b) the secretory structure of the glandular cell, with the ductule piercing the cuticular intima (lower). sq, squamous epithelial cell; arrow head, ductule (c) the ductule of the secretory structure opening to the capsule lumen. (d) transverse section of the secretory structure and the amorphous




substance deposited in the capsule lumen (upper left) of the 3 days old virgin female. se, secretory structure (e) amorphous or granular substance in the capsule lumen of 3 days old virgin female. (f) lower magnification figure of d. Triangular markers shows substance stored in the capsule lumen. Bars, 1 $\mu$m.

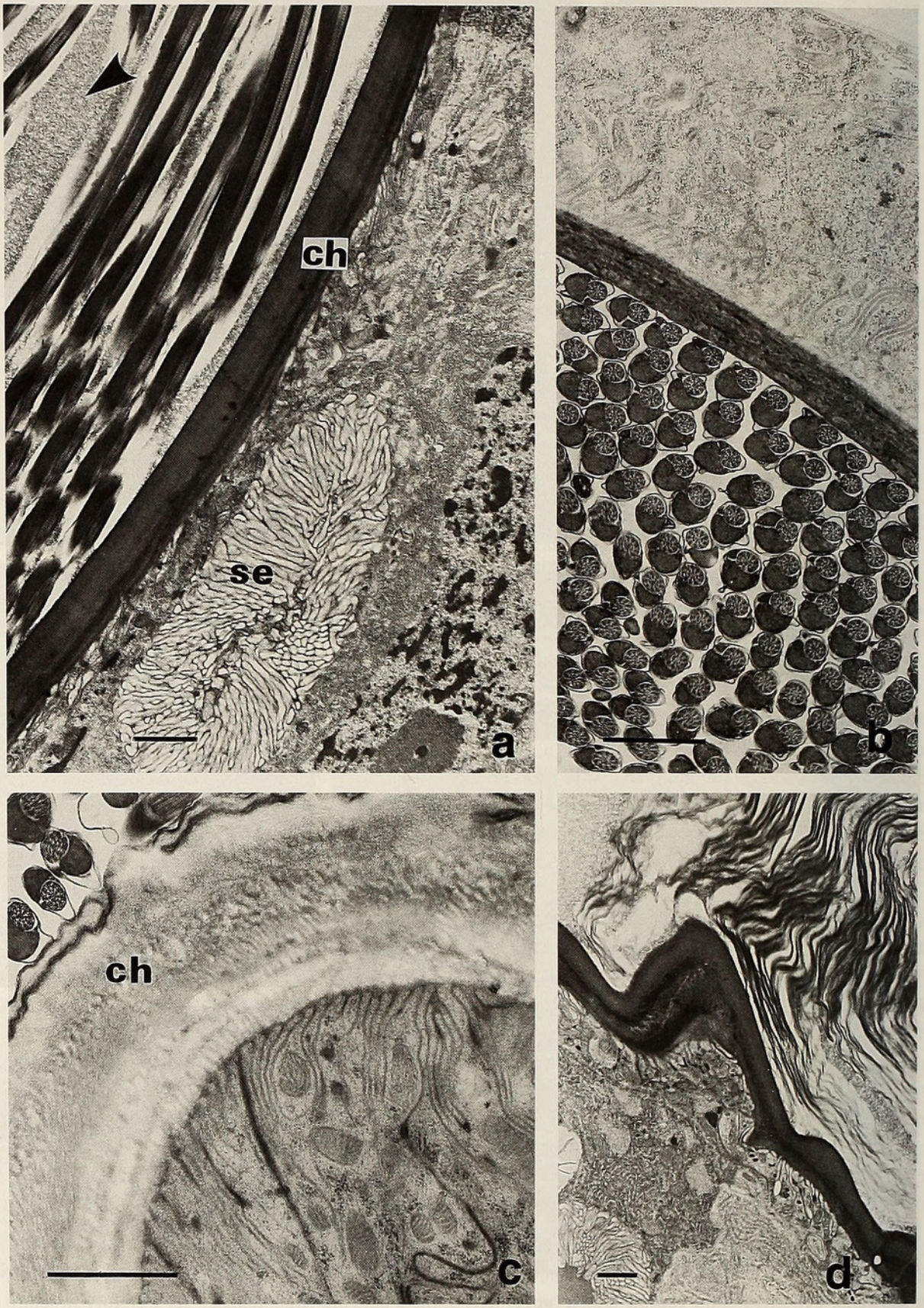



Fig. 3.

dense small granules were accumulated in the secretory cavity of the secretory structure. These observations on the structure of the spermatheca were fundamentally identical with those reported by Filosi and Perotti [12].

A significant quantity of the amorphous or granular substance was deposited in the lumen of the capsule before transfer of the sperm and the male accessory gland secretion into the capsule lumen by copulation (Figs. 2d, 2e, 2f). The amount of the granules stored in the lumen, however, did not seem to be obviously changed after copulation (Fig. 3a). Thus, the quantity of the granular substance secreted by the glandular cells of the spermatheca remained unchanged after copulation. In the capsule lumen of mated female which was aged more than three days after copulation, both the tubular elements derived from the male accessory gland and substance secreted by the glandular cells disappeared although many sperm were yet stored in it (Fig. 3b). The spermatheca at the level of junction with the duct which arises from the inside of the capsule has a different type of the epithelium cells from the cuboidal or the squamous cells (Fig. 3c). In this region, the chitinous intima is thicker than those of the other parts and the epithelial cells have many basal compartments in the cytoplasm contiguous to the chitinous intima. In our observations, the laminae structure reported by Filosi and Perotti [12] in the lumen of the capsule or the secretory cavity was observed only in aged females and the quantity of the laminae did not increase markedly after mating (Fig. 3d). Structurally, neither the squamous epithelial cells nor the glandular cells of the spermatheca showed the figures absorbing the male accessory gland secretion stored in the lumen of the capsule after copulation.

The ventral receptacle is known to be another one of the sperm storage organs, with a compactly coiled tubular structure. This organ is structurally divided into two distinct, the proximal and distal, regions. In the proximal region, the chitinous intima is very thin and the lumen of this region is usually quite narrow even after the end of copulation (Fig. 4b). The number of the sperm found in this narrow region is very few. In the distal half of the ventral receptacle, thick chitinous intima which comprises many pore canals ensures wide space for sperm storage (Fig. 4c).

A single layer of the cuboidal epithelial cells which surfaces protrude many microvilli is arranged outside the intima, both in the proximal and distal regions of the ventral receptacle (Figs. 4a, 4c). Many electron dense particles appeared in the region between the chitinous intima and microvilli of the epithelial cells about one hour after copulation (Figs. 4b, 4c). These particles remained in the same region for nine days after copulation, though the density had become a little low. These particles have the stratified structure which is similar to the finger print when observed at higher magnification (Fig. 4d). Smaller particles with same stratified structure were observed between the pore canals of the thick chitinous intima (Fig. 4c). The diameter of the particles become larger as they get nearer to the microvilli zone.

The particles were already formed when the tubular elements secreted by the secondary cells (named by Bairati [4]) of the male accessory glands

FIG. 3. Electron micrographs of the spermatheca. (a) amorphous or granular substance intermingled with the sperm in the capsule lumen of mated female (3 hr after copulation). ch, chitinous intima; se, secretory structure; arrow head, amorphous substance (b) the sperm stored in the capsule lumen. Both tubular elements derived from the male accessory gland and amorphous substance discharged from the grandular cell disappeared from the lumen in female of 3 days after copulation. (c) the "inside" part of the capsule with thicker chitinous intima and the cells with many basal compartments. ch, chitinous intima (d) the laminae structure observed in the capsule lumen of 7 days old virgin female. Bars, 1 $\mu$m.

FIG. 4. Electron micrographs of the ventral receptacle. (a) the proximal region of the ventral receptacle of virgin female. ch, chitinous intima; mv, microvilli zone; lu, lumen (b) the proximal region of the ventral receptacle of mated female (2 days after the copulation). black triangles, electron dense substance in the pits at the basal region of microvilli; arrows, electron dense particles; sp, sperm. (c) the distal region of the ventral receptacle of mated female (2 days after the copulation). arrows, electron dense particles; sp, sperm; ch, chitinous intima; lu, lumen. (d) the stratified structure of the electron dense particles observed at higher magnification. ch, chitinous intima. Bars of (a), (b), (c), 1 $\mu$m, Bar of (d), 0.5 $\mu$m.

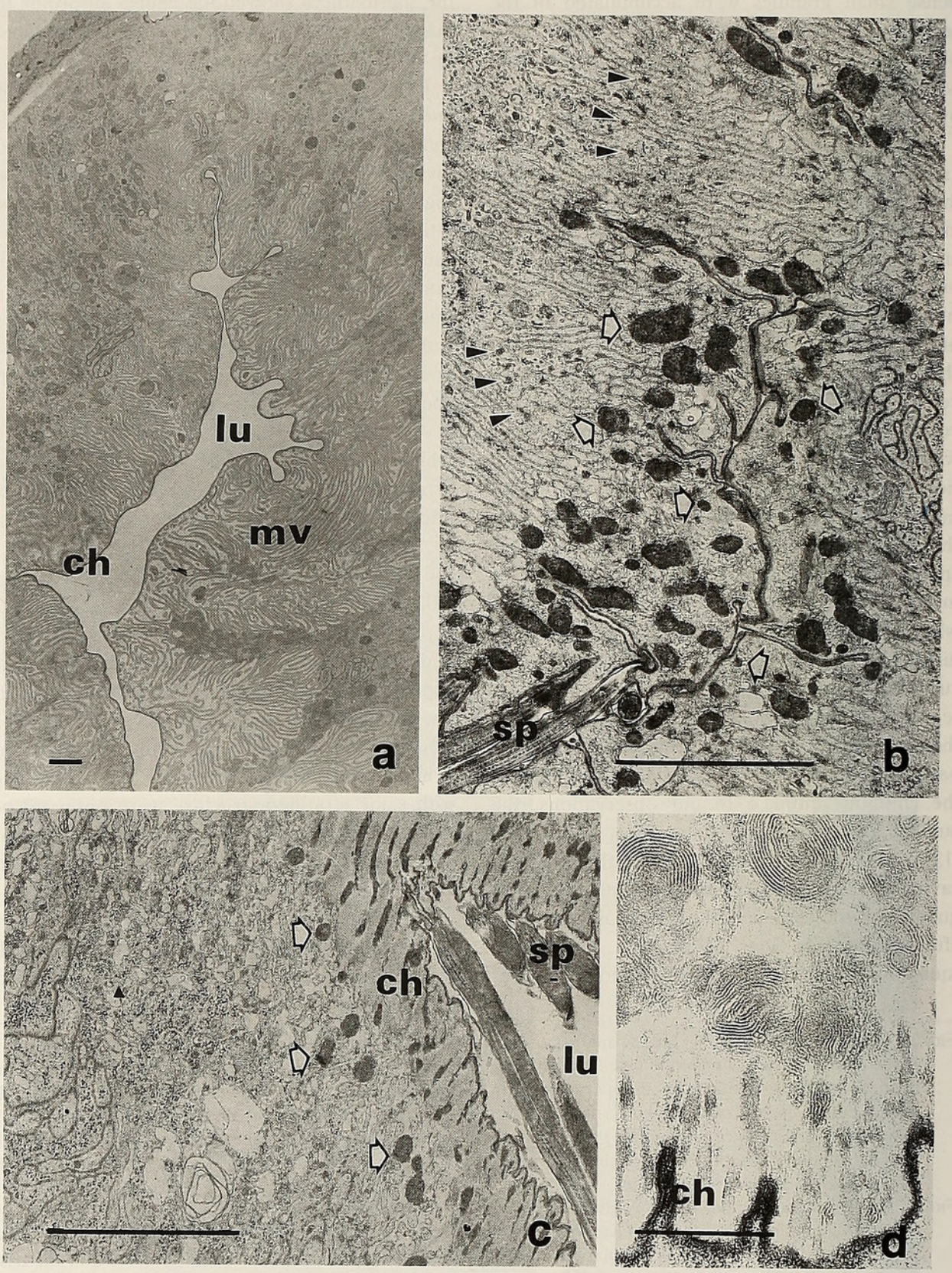



FIG. 4.

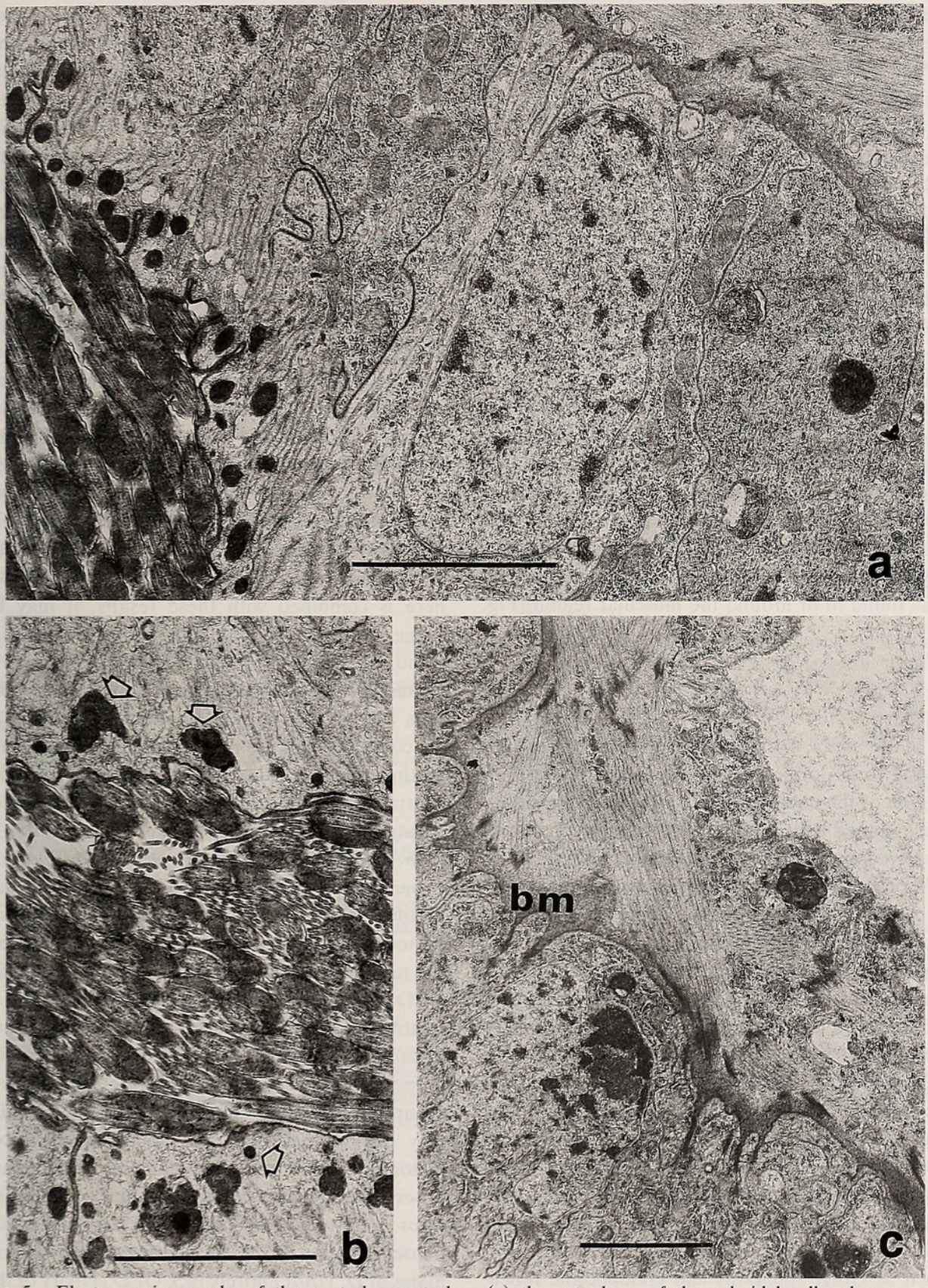



FIG. 5. Electron micrographs of the ventral receptacle. (a) the cytoplasm of the cuboidal cells where many microtubules attending perpendicular both to the inner and the outer surfaces. (b) the electron dense particles (arrows) already formed in microvilli zone when the tubular elements remained amply with the sperm in the lumen. (c) the muscles running outside of the basement membrane (bm) of the cuboidal cells. Bars, 1 $\mu$m.

remained amply in the lumen of the ventral receptacle (Fig. 5b). Morphologically, incorporation of these electron dense particles into the cuboidal cells of the ventral receptacle was obscure: typical features of micropinocytosis such as formation of the coated vesicles were not found. However, figures showing the electron dense substance in the pits at the basal region of microvilli were often seen (Figs. 4b, 4c, 5a).

In the cytoplasm of the cuboidal cells, considerable numbers of the microtubules were attending perpendicular both to the microvilli zone and to the outer surface which was contiguous to the basement lamina (Fig. 5a, 5c). Function of these microtubules could not be ascertained whether they were only cytoskeletal elements responsible for maintaining the cuboidal shape of the cells or they were playing an active role in transmission of the incorporated substances. The muscles were running outside of the basement membrane of a layer of the cuboidal cells. No conspicuous nerves was found in or around the muscular region (Fig. 5c).

## DISCUSSION

In this study, many electron dense particles were found to be formed after mating in the microvilli region of the cuboidal cells of the ventral receptacle in mated female. These particles were inferred to have a significant role in releasing mated female reproductive behaviors such as oviposition or unreceptivity to male courtship: these particles could not be observed in the virgin females. The origin and subsequent role or fate of these particles, however, could not be ascertained in the present observation. The electron dense particles show stratified structure and become larger as they go off from the chitinous intima. These observations make us expect that the particles may consist of the substances absorbed from the lumen of the ventral receptacle through the pore canals of the chitinous intima. Morphologically, the particles cannot be released from the cuboidal cells of the ventral receptacle, because developed secretory structures such as Golgi apparatus and endoplasmic reticulum were not found in the cytoplasm of the cuboidal cells. Thus, one of the potent origin of the particles may be the secretions of the male reproductive organs introduced into the lumen of the ventral receptacle along with the sperm.

In *Drosophila melanogaster* male, two organs, the accessory gland and the ejaculatory duct, have been known to secrete substances emitted to female reproductive organs. The male accessory gland produces a series of species specific proteins and peptides [8]. Among these substances, Chen *et al.* [9] found a peptide consisted of 36 amino acids which induced ovulation by injection into the abdominal cavity. Monsma and Wolfner [17] and Monsma *et al.* [16] demonstrated that two kind of proteins of the male accessory gland, msP355a and msP355b, were transferred to the female inner genital tract during copulation and rapidly appeared in the hemolymph: anti-msP355a or 355b antibody recognized three male accessory gland-specific proteins among those which were extracted from mated female flies and separated by electrophoresis. When our observation shown here is combined with these results, it may be suggested that at least a part of the male accessory gland secretions incorporated at the ventral receptacle would be passed through the cuboidal cells and entered into the hemolymph, although we could not find the morphological features suggesting that the substances in the lumen of the ventral receptacle were passing through the cytoplasm of the cuboidal cells.

In the ejaculatory duct of *Drosophila melanogaster,* glucose dehydrogenase (GLD) is expressed in adult male. GLD is transferred to the female reproductive organs during copulation and inferred to stimulate female reproductive behaviors [6]. GLD activity of the ejaculatory duct, however, is relatively rare among the species of the genus *Drosophila.* Virtually, high activity of this enzyme is restricted to several species of the *melanogaster* group [5]. Thus, GLD synthesized in the ejaculatory duct cannot be regarded an indispensable factor for female reproduction. Our preliminary observation of the ventral receptacle of mated *Drosophila virilis* females showed similar electron dense granules as in *D. melanogaster,* whereas males of *D. virilis* entirely lack GLD activity of the ejaculatory duct [5].

In this article, we concluded that the electron

dense particles in the secretory cavity of the fusiform structure of the glandular cell of the spermatheca were the secretions discharged from the glandular cells. "End apparatus" reported by Filosi and Perotti [12] could not be observed except for the ductule opening to the cuticular intima. It appears that the granular substance secreted by the glandular cells of the spermatheca cannot serve as factor(s) which initiate oviposition or release unreceptive behavior to male courtship, because (1) its quantity seemed to be remained unchanged after copulation, and (2) no figure suggesting to absorb the male accessory gland secretion stored in the capsule lumen was observed.

In the efferent ducts of the female reproductive organs, any morphological change after the mating was not found at all in the present observation. This may suggest that these ducts cannot be responsible for releasing reproductive behaviors characteristic in mated female.

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