MORPHOLOGY, SYSTEMATICS, EVOLUTION

Ultrastructure of the Salivary Glands in *Cimex hemipterus* (Hemiptera: Cimicidae)

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ABSTRACT *Cimex hemipterus* (Fabricius) is a hematophagous insect that can be an experimental host of *Trypanosoma cruzi* and may play a role as vector of Chagas’ disease. This work analyzed the structure of the salivary glands of *C. hemipterus*. The secretory portion of main salivary glands has a single oval lobe that is translucent and is formed from a simple columnar epithelium lined by muscle cells. The gland cells are high, with one or two spherical nuclei, nucleolus, and some condensed chromatin. The cell cytoplasm has a well-developed rough endoplasmic reticulum, electron lucent vesicles, lysosomes, and glycogen deposits. The apical plasma membrane has microvilli, zonula adherens, and desmosomes, whereas the basal plasma membrane has some infoldings associated with mitochondria. The duct of the main salivary glands has flattened cells. The secretory portion of the accessory salivary glands is a single vesicular lobe that is translucent and is formed from a single layer of cells that varies from flattened to cubical onto muscle cells. The cytoplasm contains a well-developed smooth endoplasmic reticulum, vacuoles of different sizes containing secretions, electron lucent, and abundant mitochondria. The baso-lateral plasma membrane of adjacent cells shows septate junctions. The duct is formed from a flattened epithelium like the duct of the principal salivary gland. The secretory cells of the main salivary glands are related to protein synthesis and transport of ions. However, the secretory cells of the accessory salivary glands are related mainly to transport of ions and water from the hemolymph to glandular lumen.

KEY WORDS morphology, insects, gland, saliva, zoology

The bed bug *Cimex hemipterus* (Fabricius) (Hemiptera: Cimicidae) is an hematophagous insect found in houses, feeding on human blood; it has a high infestation rates in developing countries (Nagem 1985, Temu et al. 1999, Boase 2001, Marcondes 2001, Ter Poorten and Prose 2005, Reinhardt and Siva-Jothy 2007).

The bed bug may be a vector of diseases, because they are obligate hematophagous, feeding several times during the immature stages and repeatedly during adult life. They can feed on several transitory hosts and become infected by a high number of pathogens in laboratory conditions (Reinhardt and Siva-Jothy 2007). These insects are suspected of transmitting a variety of etiological agents such as bacteria, viruses, and protozoa of 41 human diseases (Lent 1939, Burton 1963, Pipkin 1969, Jupp and McElligott 1979, Ogston et al. 1979, Lyons et al. 1986, Webb et al. 1989, Sousa 1999). Although Cimicidae species do not have a significant role as vectors of human diseases (Rey 1991, World Health Organization 2006), the possible transmission of pathogenic agents by these insects cannot be discarded (Jörg and Natula 1982, Forattini 1990, Amato Neto et al. 2000, Dias et al. 2005).

The possibility of pathogen transmission by *C. hemipterus* during feeding makes it necessary to understand the structural and physiologic aspects of the salivary glands of these insects.

The salivary glands of Cimicidae are described based on the internal anatomy of *Cimex lectularius* (Usinger 1966). The main salivary glands of this insect are pearshaped, and their duct opening is in the mouth parts. The accessory salivary glands are spherical, with a long duct that opens in the hylus of the main salivary gland. The main salivary glands of *C. lectularius* have only one lobe, whereas the accessory salivary glands are alveolar (Baptist 1941, Usinger 1966).

*Cimex hemipterus* represents the most common species of bed bugs in Brazil, and this study describes the structure and ultrastructure of the main and accessory salivary glands of *C. hemipterus*.

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Materials and Methods

Adults of *C. hemipterus* were obtained from colonies of the Morphology, Ultrastructure and Biochemistry of Arthropods and Parasites Section, Department of Entomology, Oswaldo Cruz Institute (IOC/FIOCRUZ), Rio de Janeiro, and were transferred to the Laboratory of Cell Biology of the Department of General Biology of the Federal University of Lush (UFV), Viçosa, state of Minas Gerais, Brazil.

The insects were cryoanesthetized and dissected in saline solution for insects (0.7% KCl + 0.3% NaCl), and their main and accessory salivary glands were isolated.

The glands were transferred to Zamboni solution (Stefanini et al. 1967) for 4 h at room temperature for histological analyses. They were dehydrated in a graded ethanol series and embedded in historesin JB4. Sections (3 µm thick) were stained with hematoxylin and eosin, analyzed, and photographed using a light microscope.

The glands were transferred to 2.5% glutaraldehyde in sodium cacodylate buffer 0.1 M and postfixed in 1% osmium tetroxide in the same buffer for 2 h at room temperature for ultrastructural studies. Later, these pieces were washed in the same buffer and stained en bloc with 1% aqueous uranyl acetate overnight. After dehydration in a graded acetone series, the samples were embedded in Epon-Araldite resin. The ultra-thin sections were stained with 1% uranyl acetate and lead citrate (Reynolds 1963) and analyzed using a transmission electronic microscope (Zeiss EM 109; Carl Zeiss EM 109, Jena, Germany) in the Nucleus of Microscopy and Microanalyses-UFV.

Results

The salivary system of *C. hemipterus* is formed by a pair of main salivary glands and a pair of accessory salivary glands located in the prothorax (Fig. 1). The main salivary glands are oval, and the accessory salivary glands are spherical and smaller than the main ones, characterizing vesicular glands. Both glands have only one lobe and exhibit translucent-white color in physiologic solution for insects. Each gland have only one lobe and exhibit translucent-white color in physiologic solution for insects. Each gland

**Figure 1.** Schematic drawing of the salivary system in *C. hemipterus*. Dorsal view. a, antennae; ad, duct of accessory gland; cl, clypeus; cp, cybarial pump; e, compound eye; md, duct of main salivary gland; msg, main salivary gland; oe, esophagus; pt, prothorax; vas, vesicular accessory salivary gland; arrows, distal end of accessory salivary gland. Not drawn to scale. Modified from Puri (1924).

are flattened, lined with a thin cuticle and with a nucleus containing condensed chromatin (Fig. 4).

The basal plasma membrane of the secretory cells has well-developed infoldings (Fig. 5).

The cytoplasm of the secretory cells contains droplets of electron-lucent secretion, electron-dense secretory granules, and well-developed rough endoplasmic reticulum (Figs. 6 and 7). Clusters of small electron-dense granules, likely glycogen deposits, and electron-dense vesicles of heterogeneous content lysosome-like are found closely to apical microvilli (Fig. 8). The cell–cell contacts are maintained by adherens junctions, desmosome-like junctions, and interdigitations (Fig. 6).

Secretion stored in the gland lumen has a heterogeneous aspect, with electron-lucid content in the central area and electron-dense ones close to the microvilli (Fig. 7).

Accessory Salivary Glands. The secretory parts of the accessory salivary glands of *C. hemipterus* have single-layered epithelium varying from squamous to cubic lining a widened lumen (Figs. 9 and 10). The secretory cells of the gland have an acidophil cytoplasm and oval nucleus with condensed chromatin (Fig. 10).

A small area with cells that gradually increase in size and result in a narrow lumen is found in the distal portion of the gland (Fig. 11). This cell cluster abruptly distends, resulting in a widened lumen in the vesicular portion of the accessory salivary gland (Fig. 12). The lumen of the distal portions is filled with an acidophil and homogeneous content, whereas the secretion in the lumen of the vesicular portion is diluted and heterogeneous (Figs. 9 and 11).
A folded duct emerges from the anterior end of the vesicular portion of the gland and with a narrow lumen covered by a thin cuticle onto flattened cells (Fig. 12). These cells present homogeneous cytoplasm and flattened nucleus (Fig. 13). The duct is unfolded near the insect head (Fig. 14).

The secretory cells are cubic with a flattened nucleus containing a small nucleolus and descondensed chromatin (Fig. 15). Muscle fibers are found near the gland lumen, as well as myelin figures (Figs. 15 and 19). The adjacent cells have interdigitations, septate junctions, and mitochondria associated with the baso-lateral plasma membrane.

**Discussion**

The anatomy of the salivary system of *C. hemipterus* with a pair of main and a pair of accessory salivary glands is similar to those found in other Heteroptera; however, it differed from the pear-shaped salivary glands of *C. lectularius*, that is, they are oval and spherical, respectively (Puri 1924, Baptist 1941, Usinger 1966, Forattini 1990).

The main salivary glands of *C. lectularius* have different aspects, varying from short (Baptist 1941), to long (Usinger 1966), to very long (Forattini 1990). These differences may be because of feeding and the age of this insect. The secretory cells of the main salivary glands of *Rhodnius*, *Triatoma*, and *Cimex* do not present structural differences before and immediately after feeding (Baptist 1941). However, the main salivary glands of starved *Triatoma infestans* are filled with secretions, with flattened and inactive gland epithelium, but after a bloodmeal, many intermediary types of secretory cells between the beginning of the regeneration and the stage of maximum secretion are found (Barth 1954). There are differences in the gland swollen and in the general aspect of the salivary gland according to physiological stage of the secretory cells, what may be an explanation for the different anatomic descriptions found in the pioneering studies.

The distal end of the accessory salivary glands of *C. hemipterus* have a cell cluster lining a narrow lumen, with an enlarged ending in the posterior region of the vesicular portion of the gland. A similar region was found in the posterior lobe of the main salivary gland of the predator *Brotocoris tabidus* (Azevedo et al. 2007). However, it is uncertain if this cell cluster synthesizes different substances, because the results presented here did not show ultrastructural differences in the secretory cells, although differences on the aspect of the lumen content were found. Because the accessory salivary glands play a role in increasing the water in the saliva (Baptist 1941; Miles 1960, 1972; Miles and Slowiak 1976), differences in its luminal content may be caused by the dilution of the secretion in the vesicular portion of the gland.

The main salivary glands play a role in the synthesis of proteins (Baptist 1941; Miles 1960, 1972; Reis et al. 2008).
2003; Swart and Felgenhauer 2003; Swart et al. 2006; Azevedo et al. 2007); however, the translucent aspect of the main salivary glands content of *C. hemipterus* may be caused by the great amount of water in the saliva resulting from the water transport from the hemolymph to the gland lumen, which is supported by…

Figs. 5–8. Transmission electron micrographs of the main salivary gland of *C. hemipterus*. (5) Basal region of the secretory cell with plasma membrane infoldings (arrows). BM, basal membrane. (6) Median apical cell region showing electron lucent granule (EG), electron dense granules (SG), desmosome-like (arrow), and adherens junction (arrowhead). Mv, microvilli. (7) Secretory cell with rough endoplasmic reticulum (RER). Notice electron dense secretion (Sc) into de lumen near the microvilli (Mv). (8) Apical region of the secretory cell showing electron lucent granule (EG), lysosomes-like (Ly), and glycogen-like deposits (G). Arrow, desmosome-like; arrowhead, adherens junction; Mv, microvilli. Bars = 0.5 μm.
the presence of basal plasma membrane infoldings in
the secretory cells. These plasma membrane infoldings increase the surface for ion exchange and transport of water from the hemolymph through the cell, modifying the gland content (Serra-˜o and Cruz-Landim 1996a, 2000; Abdalla and Cruz-Landim 2005).

The main salivary glands of Heteroptera commonly have two lobes (Baptist 1941); however, within Pentatomorpha, only Pentatomidae maintain the gland with one lobe, whereas the other families present a later multiplication of the number of gland lobes (Miles 1972). Phytophagous Hemiptera presents the most elaborate saliva, which may be due to the presence of different lobes in the main salivary gland to produce more elaborated saliva (Miles 1972). Hematophagous Cimicomorpha lose the anterior lobe of the main salivary gland (Miles 1972, Baptist 1941), whereas the loss of the anterior lobe in C. hemipterus may be attributed to the reduction of the number of functions of the saliva compared with the saliva of other phytophagous and predatory Cimicomorpha (Miles 1972).

The two lobes of the main salivary gland of Triatominae play a role in the synthesis of anticoagulants and hemolytic substances (Barth 1954, Lacombe 1999). Although the main salivary gland in Cimex has one lobe, it is unclear if the absence of the anterior granular lobe affects the physiological function of the saliva in Cimicidae (Miles 1972). However, the saliva of C. lectularius has proteins that are anticoagulants.
vessel dilators, and enzymes (Valenzuela et al. 1995; 1996a, b; Valenzuela and Ribeiro 1998). Therefore, like other hematophagous insects, Cimex developed similar substances for the blood, which affect the hemeostasis, inflammation, and immunity of the vertebrate host (Ribeiro and Francischetti 2003). Thus, the hypothesis of occurrence of spatial differentiation in the synthesis of proteins in the single-lobed gland of C. hemipterus cannot be discarded.

Presence of electron-lucent vacuoles and lipid-like droplets in the cytoplasm of the secretory cells of the main salivary gland of C. hemipterus suggests that its
saliva may have lipids or lipoproteins likely suggested for other insects (Sais et al. 2003, Nunes and Camargo-Mathias 2006). In this way, spherical electron-lucent droplets indicate, probably lipid (Geneser 2003, Alberts et al. 2004).


The presence of small secretory vesicles released among the microvilli of secretory cells represent a merocrine secretory pathway, whereas releasing large vesicles with secretion and cytoplasm portions indicate apocrine secretion (Barth 1954, Serrão and Cruz-Landim 1996b, Reis et al. 2003). Thus, the cells of the main salivary glands of C. hemipterus present both merocrine and apocrine secretory pathways, suggesting release of different substances.

Secretory cells of accessory salivary glands with acidophil cytoplasm containing both unstained and electron-dense vacuoles, and SER, as well as myelin figures into the gland lumen of C. hemipterus, indicate a lipidic nature of that secretion likely reported by Han and Bordereau (1982), Geneser (2003), Alberts et al. (2004).

Occurrence of many mitochondria in the cells of the salivary glands of C. hemipterus is similar to that found in other insects (Baptist 1941, Costa-Leonardo and Cruz-Landim 1990, Wayadande et al. 1997, Del Bene et al. 1999, Reis et al. 2003, Nunes and Camargo-Mathias 2006). The accessory salivary gland of C. hemipterus has mitochondria near the apical and basolateral plasma membrane and vacuoles, indicating that these cells present intense activity in water movement and lipid metabolism, with both substances involved in dilution of the saliva and in lubrication of the moth parts.

The muscle layer in both the main and accessory salivary glands suggests that the saliva is released by muscle action likely found in other Hemiptea (Reis et al. 2003).

The ultrastructural features suggest that salivary glands of C. hemipterus have two different functions: (1) ions and water transport from the hemolymph to the gland lumen and (2) the synthesis and secretion of enzymes and other substances to form saliva.

Acknowledgments

This research was supported by Brazilian research agencies CNPq and FAPEMIG. The authors thank the Nucleus of Microscopy and Microanalysis of UFV for technical assistance.

References Cited


Fig. 19. Transmission electron micrographs of the vesicular portion of the accessory salivary gland of C. hemipterus showing the cell apex releasing vacuoles (v) with different sizes and electron densities into the lumen. Some secretions are myelin figure (My). RER, rough endoplasmic reticulum. Bar = 0.1 μm.


Received 7 April 2008; accepted 11 August 2008.