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Histology and Ultrastructure of the Fat Body of *Anticarsia gemmatalis* (HÜBNER, 1818) (LEPIDOPTERA: NOCTUIDAE)

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ABSTRACT

The aim of this study was to analyze the morphologically the fatty body of fourth-instar Anticarsia gemmatalis *larvae under light, transmission and scanning electron microscopy. Two distinct portions of the fat body were detected: the parietal (PA) and perivisceral (PV). The PA, the parietal portion, presented a long-stripped shape located below the tegument and lateral to the digestive tube. The PV, rarely observed, was in dorsal region, adhered to digestive wall. Both the portions were constituted of only one cellular type, the trophocytes. These cells in the PA were organized in one layer of thickness showing cylindrical contiguously morphology, whereas the PV was comprised by a mass of small cells, superposed as clusters. Both the portions were covered by a layer of connective tissue, grouping the trophocytes and keeping them separated from the hemolymph. The cytoplasm of the trophocytes from the PA presented acidophilic stain, while the basophilic cytoplasmic of the trophocytes from the PV was due to the large amount of rough endoplasmic reticulum. From the results, it could be concluded that the fat body presented morphological and ultrastructural differences according to the portion and that these features could characterize distinct functions.*

Key words: Velvetbean, Caterpillar, Soybean

INTRODUCTION

Analogous to the liver of vertebrates and to the hepatopancreas of crustaceans, the insect fat body (FB) is an organ of multiple metabolic functions and takes parts into the metabolisms of lipids, carbohydrates and proteins (Chapman, 1998; Eldridge and Edman, 2004). It is responsible for the trehalose synthesis, the desamination and transamination of amino acids as well as the removal of salts of calcium, urates and other nitrogenated products from the hemolymph, (Wigglesworth, 1984; Kritsky, 2002). In Lepidoptera, the FB cells are able to perform phagocytosis and participate in the tissue remodelation during the metamorphosis (Wigglesworth, 1984).

The ability to maintain a balance between the food resource and the energy demands during the insect development also is observed in FB. During the periods in which the insect is actively feeding, this organ accumulates molecules, which could be used in the periods of food scarcity for developmental demands. The FB cells may change their activity in response to the nutritional and hormonal signals to supply the insect growth needs, metamorphosis

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and reproduction, but may vary also under several environmental conditions and in pathological (Cunha and Cruz-Landim, 1983; Gullan and Cranston, 2005).

The *Anticarsia gemmatalis*, velvet bean caterpillar and its biology has been widely studied; however, there is no information regarding the morphology of the insect. This work aimed to study the morphology of *A. gemmatalis* for its biological control.

MATERIAL AND METHODS

The larvae of *A. gemmatalis*, aged 10 and 12 days (4^{th} instar) were obtained from the Entomology Laboratory of EMBRAPA Soja, Londrina, Parana, Brazil and maintained at 25±27°C, photoperiod of 14 h of light and 10 h of darkness and 80% of humidity on an artificial diet (Hoffmann-Campo et al.. 1985). After cleaning, the larvae were anaesthetized through cooling and dissected under stereomicroscope with saline solution (1.80g NaCl; 1.88g KCl; 0.16g CaCl; 0.004g $NaHCO_3$ and distilled water – q.s.p 100 mL). A longitudinal incision was made along the ventral region, from the first until the penultimate abdominal segment, exposing the FB. A light green solution (0.5%) was used to make easy, through better contrast, the location of the organ. For the analysis under the light microscope, the FB was removed and fixed for 4 h in glutaraldehyde (2.5%) + paraformadehyde (4.0%) in phoshate buffer solution (0.1M; pH 7.2) according to the protocol proposed by Cerri and Sasso-Cerri (2003), dehydrated in ethanol-increasing solutions and included in acrylic resin glycolmethacrylate (GMA). The material was cut in 7μm wide sections using tungsten razor and stained with hematoxylin and alcohol eosin, then analysed.

For the analysis under transmission electron microscope, the FB was fixed in glutaraldehyde (2.5%) in phosphate buffer solution (0.1M, pH 7.2) for 8 h, post-fixed in osmium tetroxide (1%) in the same buffering solution (2 h), contrasted in block with 0.5% uranyl acetate (2 h), dehydrated and included in Araldite® resin. The ultra-thin cuts (50nm) were contrasted in alcoholic solution saturated with uranyl acetate (20 min) and lead citrate (20 min) and then analyzed in MET TECNAI 12 (FEI) of (LMEM/UEL).

The FB for the scanning electron microscopic analysis was fixed in 2.5% glutaraldehyde in phosphate buffer solution (0.1M, pH 7.2) for 4 h, post-fixed in osmium tetroxide at 1% in phosphate buffer (1 h), dried in critical point with liquid CO_2 and metalized with a 10nm coating of gold in MEV QUANTA 200 (FEI) (LMEM/UEL).

RESULTS

The FB (Fig. 1A) of larvae of *A. gemmatalis* was a whitish, spongy mass, suspended in the hemocoel and supported by the tracheae and involved the organs of body cavity. Two distinct portions were presented: the parietal portion (PA) (fig. 1B) and the visceral portion (PV) (fig. 1C). The PA was observed occupying a peripheral portion, right below the tegument, laterally to the digestive tube (Figs. 1A, 1B, 1D), which presented an anatomical relation, showing a delicate, granular, long-stripped shape, irregular distribution reaching the anterior portion of the prothorax until the last abdominal segment. The PV (Figs. 1C, 1E) were scarce, at the dorsal region adhered in little amounts to the digestive tube walls.

The PA consisted of a single layer of cells cylindrical, contiguous, and covered on both sides by a layer of connective tissue, which kept the grouping of cells profiled (Fig. 1D). This layer (membranous structures) was very thick under the electron microscope, isolating the cells from the hemolymph, besides keeping the cell grouped (Figs. 2C and 2D). A lower electron-dense space was observed between the basal lamina and the plasmic membranes (Fig. 2D). The plasmic membrane presented the folds (Fig. 2A), which were accompanied by the basal lamina, forming the channels that invaded the cytoplasm of trophocytes (Fig. 2C). The spaces formed contained little lamellar bodies of myelin aspect (Fig. 3A).

The trophocyte of PA was acidophilous, with intense vacuolization with nucleus of varied location and shape containing chromatin in small lumps (Fig. 1 D). The cytoplasm presented varied electron density, increased at the regions close to the cell membrane and nucleus (figs. 2D and 3B), due to the high concentration of organelles (Figs. 2C and 3D); rough endoplasmic reticulum (RER), forming dilated cisterns (Figs. 3A and 3C), dense bodies of several shapes (Figs. 2C and 3A) and round and elongated mitochondria (Figs. 2C, 3A). In the less electron-dense region, lipid droplets of

several sizes and shapes (Fig. 3B) and islands of glycogen were observed (Figs. 2D, 3C and 3D).

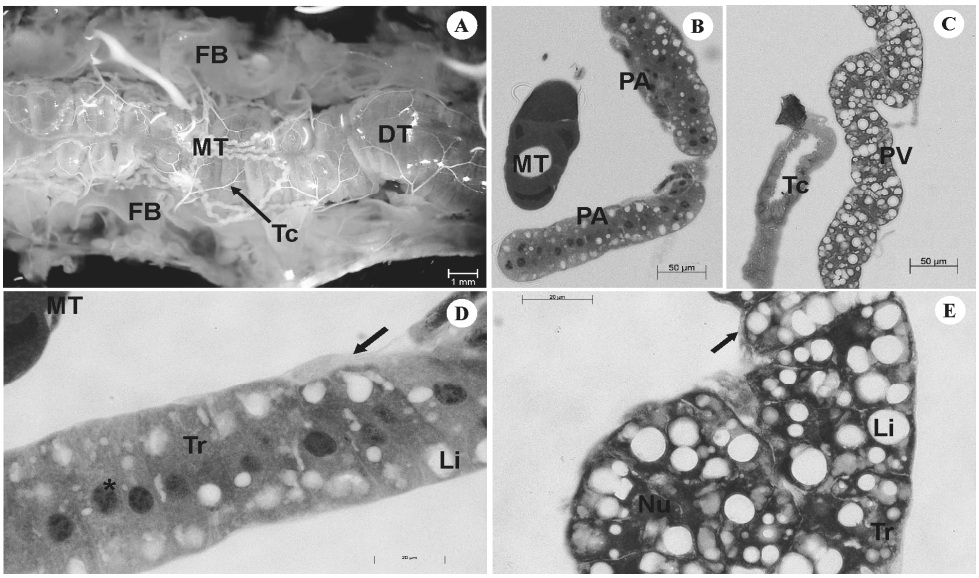

Figure 1 - A) Location and distribution of the fat body (FB) of fourth-instar larvae of *A. gemmatalis*; note the presence of the digestive tube (DT), Malphigian tubules (MT) and tracheas (Tc), light green staining. Bar = 1 mm. **B)** General aspect of the parietal portion (PA) of fat body, Malpighian tubule (MT) Bar = 50 µm. **C)** General aspect of the perivisceral portion (PV), tracheole (Tc) Bar = 50 µm. **D)** PA portion, showing the acidophilus columnar trophocytes (Tr) with lipid droplets (Li). Note the nucleus (*) in several positions. Layer of connective tissue (→). Bar = 20 µm. **E)** Fat body, perivisceral portion: trophocytes (Tr), layer of connective tissue (→), nucleus (Nu), lipid droplets (Li). Bar = 20 µm.

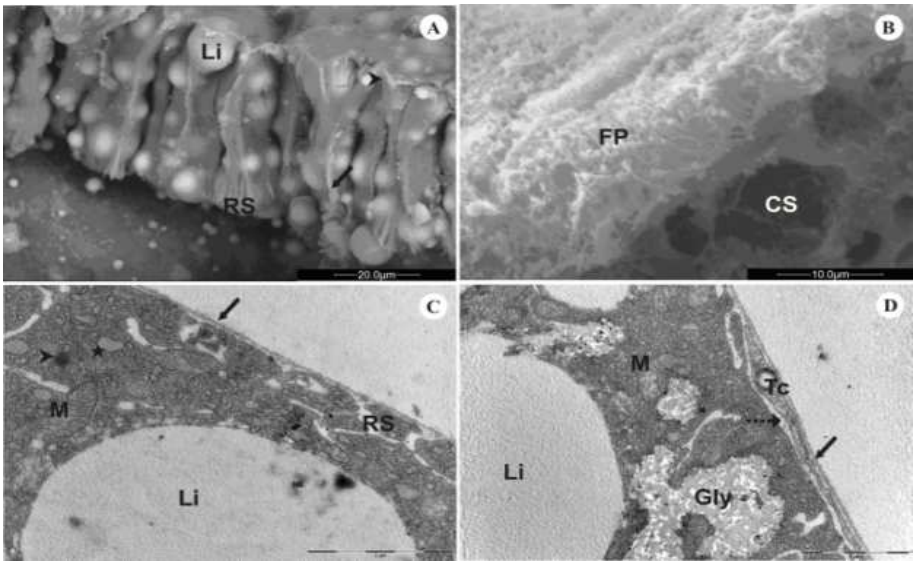

Figure 2 – A-B) Scanning electronic micrographs of the parietal fat body (PA) of fourth-instar larvae of *A. gemmatalis*. Fixation in 2.5% GA in phosphate buffer (0.1 M, pH 7.2) for 2 h. **A)** Aspect of a surface-breaking region of PA, note the lipid droplets (Li), cell membrane (→) and reticular systems (RS). Bar = 20 µm. **B)** Detail of the layer of connective tissue with filiform projections (FP), (CS) cytoplasmic spaces previously occupied by lipid. Bar = 10 µm. **C-D)** Transmission electronic micrographs of the parietal fat body of fourth-instar larvae of *A. gemmatalis*. Fixation in 2.5% GA in phosphate buffer (0.1 M, pH 7.2) for 8 h. **C)** Detail of the cell showing the basal lamina (→), the reticular systems (RS), dilated cisterns of RER (★), dense bodies (➤), mitochondria (M), lipid droplets (Li). Bar = 2 µm. **D)** General aspect: note the presence of a less electron dense space (⇢) between the basal lamina (→) and the trophocyte membrane, the islands of glycogen (Gly), lipid droplets (Li), mitochondria (M), tracheole (Tc) covered by membrane. Bar = 2 µm.

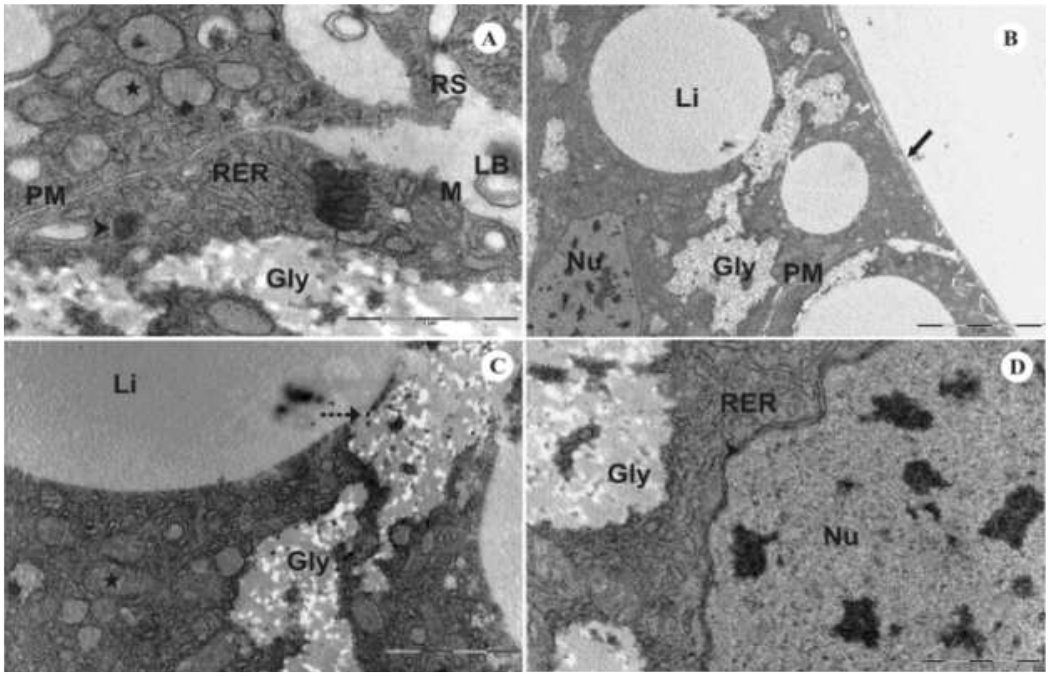

Figure 3 - Transmission electronic micrographs of the parietal fat body of fourth-instar larvae of *A. gemmatalis*. Fixation in 2.5% GA in phosphate buffer (0.1 M, pH 7.2) for 8 h. **A)** Detail of the peripheral region of trophocyte, showing the presence of lamellar bodies (LB) in the space of the reticular systems (RS). RER with large amount of dilated vesicles (★), mitochondria (M), islands of glycogen (Gly); cellular membrane (PM). Bar = 1 µm. **B)** General aspect of trophocyte showing the distribution of the lipid droplets (Li), islands of glycogen (Gly) and nucleus (Nu), presence of tracheole (Tc) and basal lamina (→). Bar = 5 µm. **C)** Detail evidencing the communication relation (⇢) with the glycogen island (Gly), the lipid (Li), and the dilated cisterns of RER (★). Bar = 2 µm. **D)** Detail of the perinuclear region: note the richness of rough endoplasmic reticulum (RER) without dilation, glycogen islands (Gly), Nucleus (Nu) and nuclear envelope (➤). Bar = 1 µm.

The visceral portion PV presented an alveolar aspect with little and round trophocytes forming agglomerates recovered by the layer of connective tissue (Figs. 1C, 1E). Ultrastructurally, the PV has presented a double layer of cell agglomerates covered by a layer amorphous material membranous structures (Figs. 4B and 4C). On the outer surface of these double layers, several galleries were observed, forming the reticular system by the plasmic membrane folding with the basal lamina in the space between the cells (Figs. 4C and 4D). The nucleus was central and the basophilic cytoplasm with large amount of round lipid-like droplets.

Some organelles were observed close to cellular and nucleus boundaries. Mitochondria of round and elongated shape, RER sometimes distended forming cisterns containing granular and electron-dense material, lamellar bodies of myelinic aspect and developed Golgi complex. (Fig. 4D). Peripherally, lipid droplets were found in contact with the islands of glycogen (Fig. 4C). In the PV, the basal lamina was continuous, presenting smooth surface and involved externally the trophocytes agglomerates (Fig. 4B).

DISCUSSION

Anatomically, the FB of the *A. gemmatalis* larvae was very similar to what Keeley (1985) and Richards and Davies (1994) described for other Lepidoptera such as *Diatraea saccharalis* (Fabricius, 1794) *Calpodes ethilus* (Stoll, 1780) and *Manduca sexta* (Cramer, 1779). The description of the FB layer in the hemocoel followed the pattern found in other Lepidoptera (Keeley 1985; Snodgrass 1993). According to Wigglesworth (1965) and Dean et al. (1985), the disposition could vary among the orders, but was constant in the same species. Keeley (1985) mentioned that the FB in the Hemiptera occurred in lamellar formations; in Diptera such lamellas were fenestrate. In Orthoptera, they were found as

a thin ropes bound to the tegument. A PA layer was present right below the tegument and one PV layer was around the intestine – a distribution pattern also found for larvae of *A. gemmatalis*. The present results were different from those described for *Pachycondyla villosa* (Fabricius, 1804) (Formicidae) by Zara and Caetano (2004) who reported that the FB was distributed as a single layer between the cuticle and the digestive tract, forming a group of cells covered by a thin membrane.

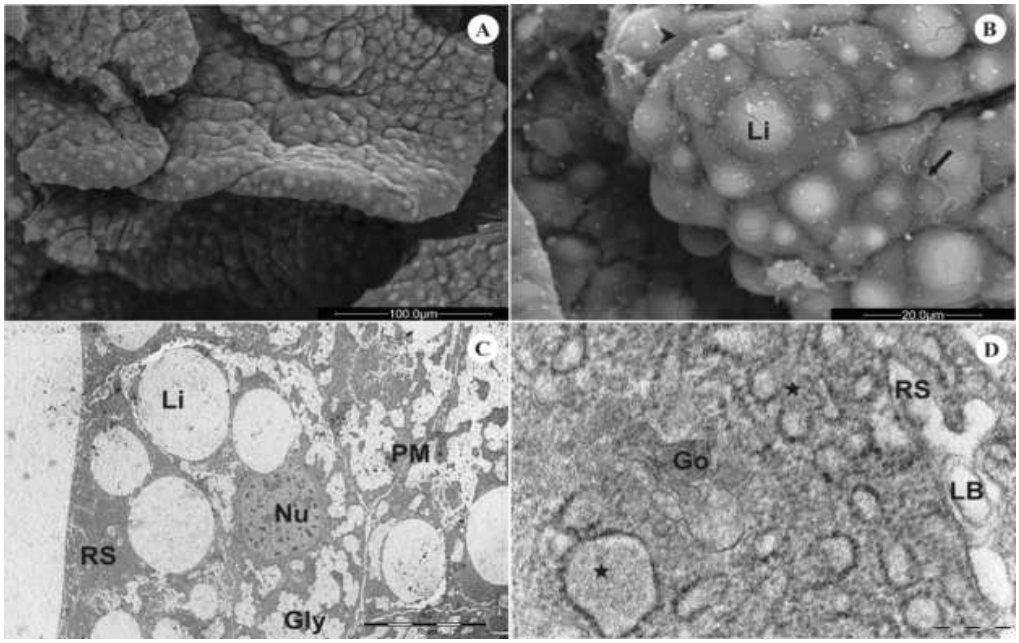

Figure 4 – A-B) Scanning electronic micrographs of the perivisceral fat body of fourth-instar larvae of *A. gemmatalis*. Fixation using 2.5% GA in phosphate buffer for 8 h. **A)** General aspect of the perivisceral portion (PV), showing its organization in lobules. Bar = 100 µm. **B)** Detail of the trophocytes covered by the smooth membrane(➤), lipid droplets (Li), and tracheoles (→). Bar = 20 µm. **C-D)** Transmission electronic micrographs of fat body of fourth-instar larvae of *A. gemmatalis*. Fixation in 2.5% GA in phosphate buffer for 8 h. **C)** Detail showing the trophocytes and their surrounding relations. Observe the presence of reticular systems (RS) at the free periphery of cell; lipid droplets (Li) and islands of glycogen (Gly) around the nucleus (Nu), Cell membrane (PM). Bar = 10 µm. **D)** Detail showing the presence of Golgi (Go) little developed, cisterns with RER dilations (★) and lamellar bodies (LB) in the reticular systems (RS). Bar = 0,2 µm.

Histologically, the FB of larvae of *A. gemmatalis* presented similarity to those described by several authors in different insect such as Wigglesworth (1965); Richards and Davies (1994); Oliveira and Cruz-Landim (2003); Fontanetti et al. (2004) and Zara and Caetano (2004). These authors reported that the FB was formed by strip-shaped loose cell aggregates or in compact mass with one or two cells in width, covered by an acellular layer.

Among the denominations found for the FB cells described by several authors, in this work the term trophocyte was opted to identify the FB-comprising cells, similar to the denominations made by Rosel and Wheeler (1995), Oliveira and Cruz-Landim (2003), Zara and Caetano (2004), Sarmento et al. (2004) and Roma et al. (2005). Fontanetti et al. (2004) and Gullan and Cranston (2005), working with different orders of arthropod, used the term adipocyte for these cells since they were not only the fat deposits.

The FB of larvae of *A. gemmatalis* presented structural and anatomical differences. Such differences allowed the division of the FB into distinct portions – the PA and the PV, as described by other authors who worked with lepidopteran (Coupland 1957; Dean et al. 1985; Wang and Haunerland 1993; Fontanetti et al. 2004).

In general, the PA was the largest part of the organ and was formed by the cylindrical cells contiguously lined, presenting one layer of cell in thickness, whereas the PV was comprised by a mass of small cells, superposed as clusters. On

both the portions, a layer of connective tissue covered the cells, grouping them and keeping them separated from the hemolymph.

The cytoplasm of the trophocytes from the PA presented acidophilic stain, while the basophilic cytoplasmic of the trophocytes from the PV was due to the large amount of RER. In PA, the cytoplasm coexisted with little acidophilia and those with slight basophilia, called attention, what could indicate a transition between the FB of PA and that of PV. However, no reports corroborating these finds were found in the literature. It was not possible to evidence using the methodology proposed whether there was transformation of a type of cell into another or not. In an affirmative case, the portion that the FB initiated this process and how it occurred were still unknown.

The intense vacuolization observed in the cytoplasm of the PA and PV trophocytes was due to the presence of lipid droplets. As the deposition of lipid increased in PA, the size of droplets increased and fused forming great drops of lipid, changing the shape and the position of the nucleus. These results corroborated the descriptions of Oliveira and Cruz-Landim (2003). In the PV, the drops of lipid seemed as empty round spaces of several sizes all over the cytoplasm. However, the nucleus was located centrally and its shape was defined by the amount of lipid present in its vacuoles. Besides the lipids, the presence of large reserves of glycogen was found in both the portions (PA and PV). Such substances could be either produced or obtained from other sources such as the hemolymph by the trophocytes, indicating the intense biosynthetic activity besides energy storage for starved pupal period.

The acellular layer (membranous structures) covered the FB was thicker, with irregular surface and several projections in the PA, the PV presented a smooth and thin laminar surface. This showed that both the portions could present functional differences.

The presence of basal lamina in the PA and PV showed that it promoted the filtration of particles without hindering the substance exchanges between the two ambiences – the inner (cytoplasm of the trophocytes) and the outer (the hemolymph). This selective role has already been described by Harrison and Locke (1998) and according to them, such selection occurred because the basal lamina was negatively charged, separating and agglutinating the cations and excluding the particles with 15 nm or more in diameter.

The projections towards several directions in the acellular layer (membranous structures) of the PA could be involved in the retention of particles in this portion besides acting in the filtration, supporting and keeping the organ morphology more rigid as the strip formed by the cells placed contiguously. Chapman (1998) reported that in some rare cases, gap junctions and adhesive junctions could be seen between the cells. In the material of this study, the cell union was not observed in either PA or PV; however, the membranous structures as well as basal lamina that allowed the adhesion were observed. Corroborating such ideas, Harrison and Locke (1998) reported that due to its morphology, there were evidences that this membrane was related with the adhesion and permeability of substances present in the hemolymph and inside the cells, promoting ion and macromolecule exchange.

Underneath the basal lamina both in the PA and PV, a region of low density was noticed and it presented a more or less uniform width, which kept it separated from the plasmic membrane. Such structure disposition was probably related with the formation of a place which favoured the exchanges between the ambiences.

In the inner part of the basal lamina in both the PA and PV, plasmic membrane folds were observed forming the chambers continuous with intercellular space denominated basal labyrinth. Their basal labyrinth increased the surface of exchange between the hemolymph and the cytoplasm important while the larva was feeding, not during pupation, but already necessary in adult.

The reticular systems found on both the portions of the FB (PA and PV) were similar to the findings of Harrison and Locke (1998), who reported the presence of gap junctions along the plasmic membrane. The probable role of such reticular systems concerned better contact between the cells and the hemolymph to facilitate the metabolic exchanges.

The emergence of such spaces coincided with the increase of the insect metabolic activity and allowed the separation of part of the hemolymph from the rest in stock hemocoel. This lymph contained in the spaces allowed better control of the cell regarding the transportation of molecules such proteins and lipids both outward and inward the cells. Bew (1987) found that one of the roles of the reticular systems in the cells of *Manduca sexta*

(Cramer, 1779) was the absorption of proteins to the FB interior.

In trophocytes of *Calpodes ethlius* (Stoll, 1780), eleven types of different vacuoles originated from the plasmic membrane were described. However, they were not together at the same time (Harrison and Locke, 1998). In larvae of *A. gemmatalis*, only one type of vacuole was observed in the cytoplasm of the PA and PV and it presented a large amount of lipids in its interior. In the PA, such vacuoles were merged and became huge vesicles that kept contact with the electron-lucent island full of glycogen. Such sites or islands of glycogen have also been reported by Chapman (1998) and Oliveira and Cruz-Landim (2003).

The presence of any type of vacuole was not observed, as described by Mori et al. (1970), Tojo et al. (1978), Wigglesworth (1984), Locke, (1984), Dean et al. (1985), and Cruz-Landim (2000). Furthermore, the multivesicular bodies in both portions of FB of *A. gemmatalis* as reported by Dean et al. (1985) and Cruz-Landim (2000) were not observed.

The presence of lamellar bodies was observed in the spaces of reticular systems and close to the RER. This supported the studies of McDermid and Locke (1983) who reported that the lamellar bodies were involved in the membrane turnover, appearing at the fat body when a large amount of membranes was released due to the passage of tyrosine from the vacuoles to the hemolymph. The wide occurrence of RER with dilated cistern observed in this work was also described by Bhakthan and Gilbert (1972) in *Hyalophora cecropia* and by Harrison and Locke (1998) in *C. ethlius*, who found the involvement of the FB cells in the synthesis of protein, lysosomes and the secretion of hemolymph in the first developmental stages, and the accumulation of membranes or proteins destined to the formation of peroxisomes.

Among the organelles, the Golgi complex was rarely observed and the smooth endoplasmic reticulum was absent in the trophocytes of FB in studied larvae of *A. gemmatalis*. This indicated that the trophocytes apparently did not participate in the synthesis of lipid; it only stored it in its cytoplasm after being captured from the hemolymph.

The trophocytes of FB of *A. gemmatalis* presented many mitochondria of elongated and round shape as reported by Harrison and Locke (1998). The variation in the mitochondria morphology was likely related to the increase of crests, indicating that it could be due to the state of synthesis and storage to which this organ was proposed.

In fourth instar larvae of *A. gemmatalis*, the FB presented a whitish colour, delicate consistence, suspended in the hemocoel through tracheas and formed by two distinct portions: the parietal portion (PA) and the perivisceral portion (PV). Both the portions were formed by a single cell type, the trophocytes, which presented differentiated arrangement and cytoplasmic organization. The transit of molecules around the trophocytes and the hemocoel through the hemolymph was facilitated by the channels, which formed the plasmatic membrane reticular systems, which were considerably numerous in PA.

Due to the differences in the distribution and amount of organelles, it was likely possible that the cells of both portions of this organ were involved in different process during the larval development of *A. gemmatalis*.

The PA could be more related to storage processes and also of protein synthesis and polysaccharide, which would form the elements of the hemolymph and storage of lipids. It was likely that the PV was more related to lipid storage than the PA and then in this case, a reservoir of energy analogous to the fatty tissue of vertebrates.

For ultrastructural characterization, apparently the large amount of the CG of larvae of *A. gemmatalis*, both the PA and the PV, was responsible for the synthesis of various substances not just lipid levels. Apparently the PV behaved similarly as the variety of multilocular adipose tissue in the mammals, the main supplier of energy to the activities of feeding and rapid growth of this phase, with decreased or absent in adults, while the PA was responsible beyond the metabolic functions larval, the continuance of species in the adult tissue.

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