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Morphological Regional Differences of Epithelial Cells along the Midgut in *Diatraea saccharalis* Fabricius (Lepidoptera: Crambidae) Larvae

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Diferenças Morfológicas Regionais das Células Epiteliais ao Longo do Intestino Médio de Larvas de *Diatraea saccharalis* Fabricius (Lepidoptera: Crambidae)

RESUMO - A broca da cana, *Diatraea saccharalis* Fabricius, é uma praga da cana-de-açúcar e outras plantações. O objetivo deste trabalho foi caracterizar variações morfológicas nas células epiteliais (colunares, calciformes e regenerativas) ao longo do intestino médio de larvas de *D. saccharalis*. Fragmentos do intestino médio (anterior, mediano e posterior) foram fixados e processados para microscopia de luz e eletrônica de varredura. Existem diferenças morfológicas citoquímicas e ultra-estruturais nas células epiteliais, dependendo da sua localização no intestino médio. A superfície apical de algumas células colunares exibe projeções citoplasmáticas que aumentam em número e volume da região anterior para a posterior do intestino médio. Existe aumento dos grânulos PAS-positivos (Reação de Schiff) no citoplasma apical das células colunares e regenerativas, da região anterior para a posterior. A câmara das células calciformes, na região anterior do intestino médio, mostra seu ápice estreito, enquanto que na posterior essa porção da câmara é alargada; as evaginações citoplasmáticas da câmara são pequenas e finas no ápice, sendo numerosas, longas e mais espessas na porção basal. Os resultados sugerem que o intestino médio da broca da cana apresenta duas regiões morfológicamente distintas, a anterior e posterior; a região mediana é uma região de transição.

PALAVRAS-CHAVE: Morfologia, broca da cana, histoquímica, microscopia eletrônica de varredura, microscopia de luz

ABSTRACT - The sugarcane borer, *Diatraea saccharalis* Fabricius, is a pest to sugarcane and many other crops. This work aims to characterize morphological variability in the epithelial cells (columnar, goblet and regenerative) along the midgut of *D. saccharalis* larvae. Fragments of the midgut (anterior, middle and posterior regions) were fixed and processed by light and scanning electron microscopy. There are both cytochemical and ultrastructural differences in the morphology of the epithelial cells, depending on their localization along the midgut. The apical surface of columnar cells shows an increase in both number and size of the apical protrusions from the anterior to the posterior midgut regions. There is an increase in the amount of PAS-positive (Periodic Acid-Schiff Reaction) granules detected in the cytoplasm of both the columnar and regenerative cells, from the anterior to the posterior region. The goblet cell apical surface is narrow in the anterior region, and enlarged in the posterior midgut; the chamber's cytoplasm extrusion are small and thin at the apical cavity surface, being thicker, longer and more numerous at the basal portion of the cavity. Our results suggest that the sugarcane borer midgut has two morphologically different regions, the anterior and the posterior; the middle region is a transitional region.

KEY WORDS: Morphology, sugarcane borer, histochemistry, scanning electron microscopy, light microscopy

Studies, in diverse Orders of insects, show the importance of midgut in food absorption, ion exchange, the entry of insecticides, viruses and toxins, and the release of neurohormones that regulate the activity of various other physiological processes (Dow 1986, Lehane & Billingsley 1996).

The insect midgut consists of a columnar epithelium underlayered by a more internal layer of circular striated muscular fibers and another more external layer of longitudinal fibers (Chapman 1998). The epithelium presents, in the majority of insects, four cellular types: columnar, goblet, regenerative and endocrine (Lehane &

Billingsley 1996). Many of the works referencing epithelial cells correlate their morphology with possible functions (Cioffi 1979, Santos *et al.* 1984, Pinheiro & Gregório 2003).

Midgut epithelium is coated internally with an acellular membrane (peritrophic membrane) in most insects (Lehane & Billingsley 1996, Terra 2001). In Lepidoptera larvae, this membrane is secreted along the midgut by columnar cells (De Priestler 1971, Terra 1988, Hopkins & Harper 2001). Although it impedes direct contact of food with the striated border of columnar cells, this membrane permits the passage of digestive enzymes in the direction of the midgut lumen and the absorption of products resultant from digestion that are subsequently eliminated with the feces (Lehane & Billingsley 1996), preventing mechanical injuries and hindering or impeding the entry of pathogens (Terra 1988, 2001).

The more abundant columnar cells are responsible for the production of enzymes and the absorption of digestive products (Lehane & Billingsley 1996). Goblet cells receive their denomination by possessing invagination of apical plasma membrane, forming a cavity similar to a calyx or goblet; these cells are involved in the process of ionic homeostasis and absorption of metabolites, a function performed in cooperation with the columnar cells (Chapman 1998, Lehane & Billingsley 1996, Terra *et al.* 2006). The regenerative cells are observed isolated, in pairs or forming groups (nidi) of regeneration, localized at the epithelial base (Dow 1986, Cruz-Landim *et al.* 1996, Cavalcante & Cruz-Landim 1999). There is a consensus that regenerative cells have a role in the reposition of epithelial cells (Endo *et al.* 1983, Lehane & Billingsley 1996). Endocrine cells are localized in the epithelial base, and are characterized by the presence of a large quantity of cytoplasmic granules (Sehnal & Zitnan 1996, Serrão & Cruz-Landim 1996). There are various roles attributed to this cell: hormonal control of peristaltic movements and of digestion, diuresis, development and reproduction, among other functions (for a review see Sehnal & Zitnan 1996).

The sugarcane borer, *Diatraea saccharalis* Fabricius, is a pest that significantly affects agricultural and industrial productivity of sugarcane cultivation, and is found in the Antilles and in Central and South America. Their worm-like larvae also attack other grasses, and are considered prejudicial to corn, rice and sorghum, wheat and some species of grass. Despite the importance of this insect, there are few works on its internal morphology, especially in reference to the midgut. The existent descriptions reference the ultrastructural differences of columnar cells (Pinheiro & Gregório 2003), and morphometric distinctions in different cellular types (Pinheiro *et al.* 2003, 2006), besides biochemical differences of enzymatic activity (Azevedo *et al.* 2003). However, none of these studies characterize morphological variations in the different cellular types along the midgut of the sugarcane borer. Thus, the objectives of these study are describe and characterize morphological variations in the different epithelial cells along the midgut of the *D. saccharalis* by scanning electron microscopy and histochemical technique.

Material and Methods

Larvae of *D. saccharalis* were maintained on artificial diet, with controlled temperature (25-27°C) and humidity (70%). The insects, in the final larval instar, were dissected under a stereoscopic microscope and the midgut immediately fixed according to the technique utilized.

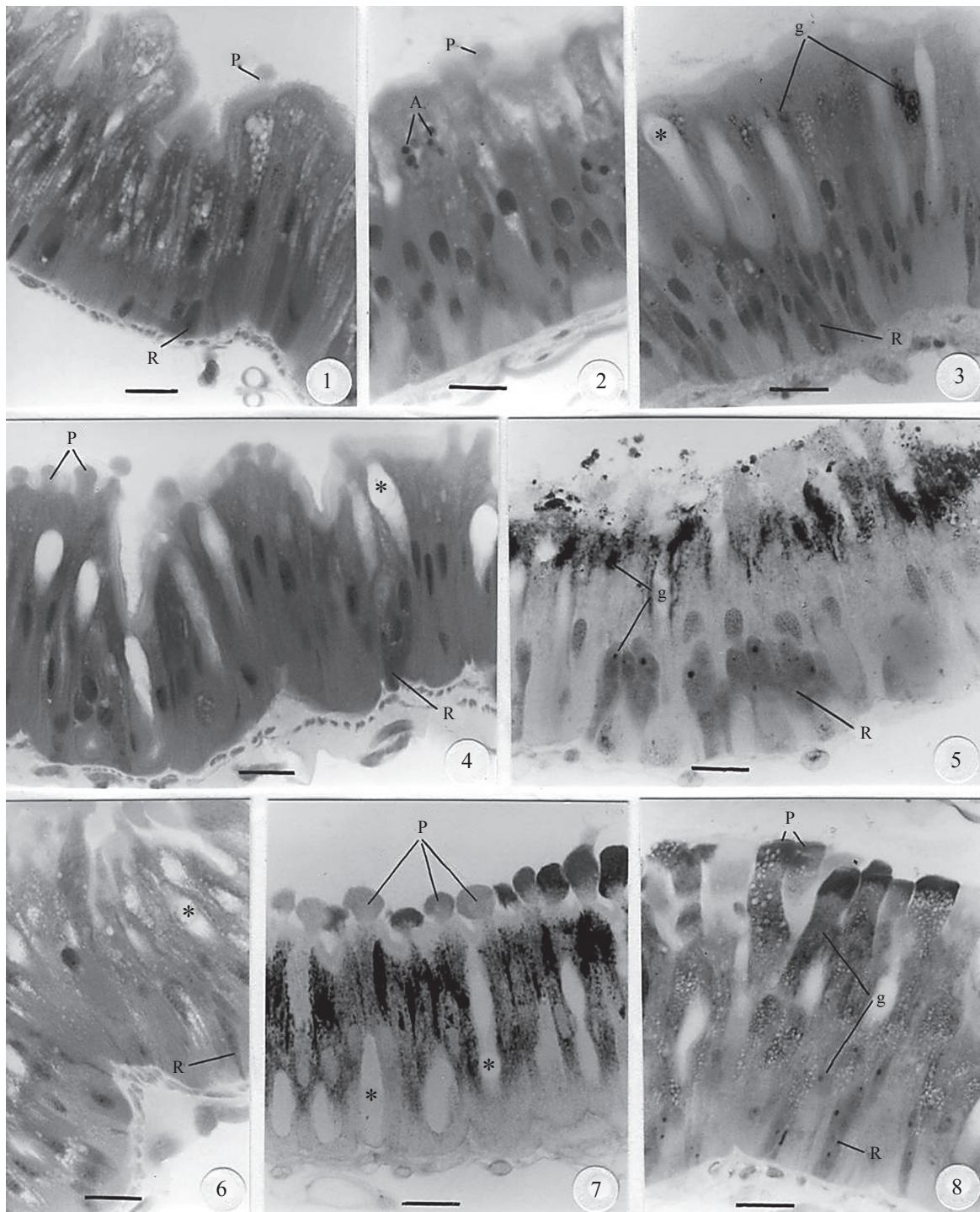
Light microscope (LM). The midguts collected were subdivided into three regions: anterior, middle and posterior; the materials were fixed in glutaraldehyde solution 2.5% - paraformaldehyde 4% in phosphate buffer 0.1M, pH 7.3 for 6h, and dehydrated in an increasing sequence of ethyl alcohol solutions. The inclusion was accomplished in JB4 resin from Polysciences, and polymerized in an incubator at 37°C for 24h. Cross sections of 3-7 µm were submitted to hematoxylin-eosin and Periodic Acid-Schiff (PAS) coloration techniques (Pearse 1972). The control of PAS technique was performed with pretreatment of the tissue sections with amylase.

Scanning electron microscope (SEM). The midgut was not removed from the insect, and instead only longitudinal incision was done to expose the luminal surface, as well as the lateral surface of the epithelium. The material was fixed in glutaraldehyde 2.5% in phosphate buffer 0.1M, pH 7.3 for 24h and post-fixed in osmium tetroxide 1% for 2h in the same buffer. Dehydration was accomplished with an increasing sequence of ethyl alcohol solutions, and drying done by means of critical point in CPD 020 (Balzer Union), with liquid CO₂. The specimens were pasted up in adequate support and covered with a 20 nm layer of gold in the apparatus MED 010 (Balzer Union). The analysis was completed under Scanning Electron Microscope SEM 515 from Philips.

Results

The midgut epithelium of *D. saccharalis* consists of columnar, goblet and regenerative cells, distributed throughout all its extension (Figs. 1-8). The epithelial surface is coated internally by a fine peritrophic membrane (Fig. 9).

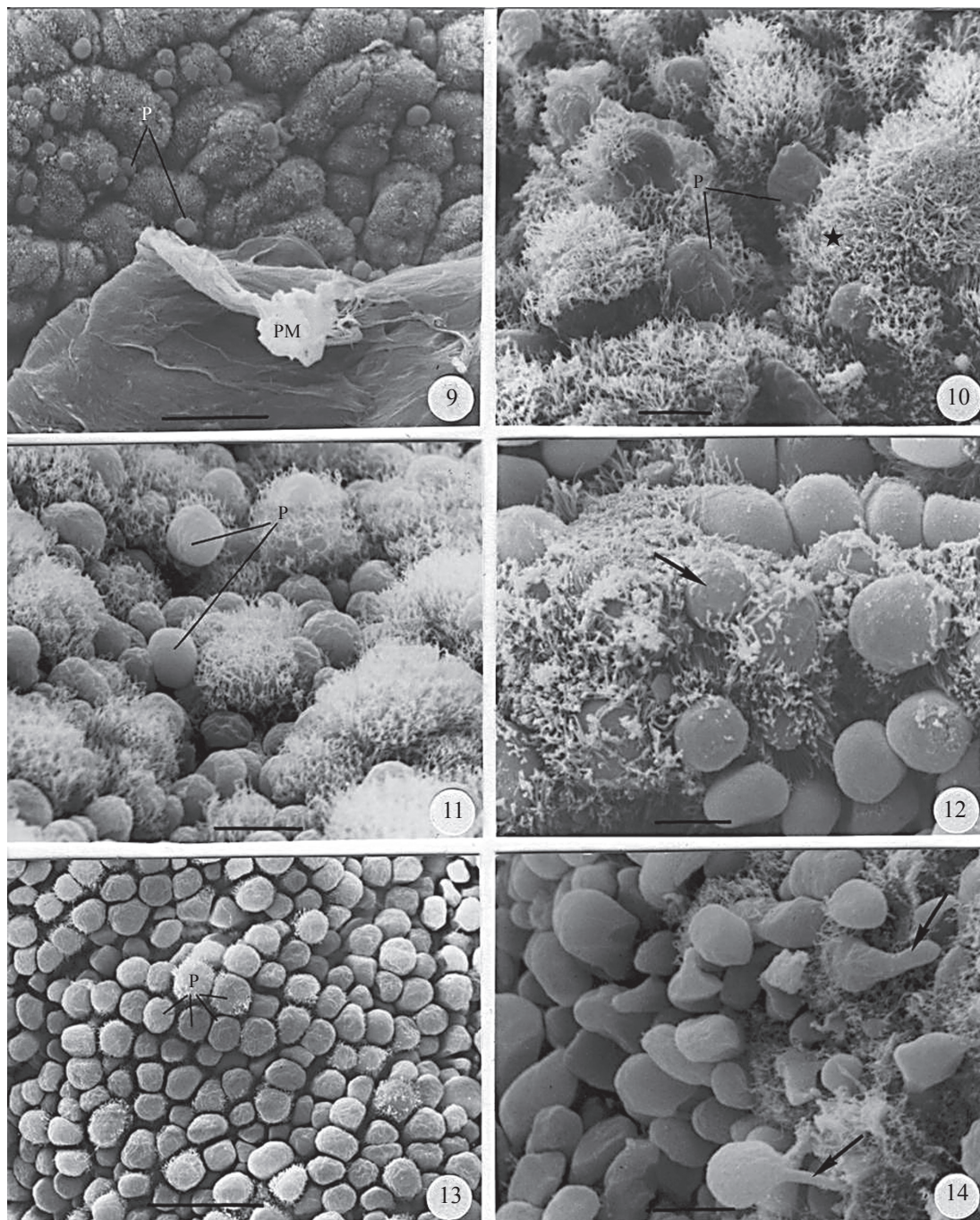
Columnar cells. Of prismatic form, they are more numerous throughout the midgut and show basophilic cytoplasm and elongated basal nucleus with dispersing chromatin (Figs. 1-8). Discrete PAS-positive granules are present in apical cytoplasm and within cytoplasmic protrusions in the anterior region (Fig. 3), presenting an increase of these granules toward the middle (Fig. 5) and posterior region (Figs. 7-8). Rare images suggestive of cellular death are visualized (Fig. 2). Cytoplasmic protrusions are observed on the apical surface among striated border; there are few cytoplasmic protrusions in the anterior midgut region (Figs. 1-3), increasing in number from the middle region (Figs. 4-5) to the posterior region, where they may be voluminous (Figs. 6-8). This finding is confirmed in SEM (Figs. 9-14). Many



Figs 1-8. Histology of midgut epithelium of *Diatraea saccharalis* larvae. Fig. 1 - Anterior midgut region. Hematoxylin-eosin techniques (HE). Fig. 2 - Anterior midgut region: image suggestive of apoptotic bodies (A). HE. Fig. 3 - Anterior midgut region. Periodic Acid-Schiff techniques + Hematoxilin (PAS+H). Fig. 4 - Middle midgut region. HE. Fig. 5 - Middle midgut region. PAS+H. Fig. 6 - Posterior midgut region: HE. Figs. 7-8 - Posterior midgut region. PAS+H. Cytoplasmic protrusions (P) of columnar cells; cavity of goblet cells (*), regenerative cells (R), PAS-positive granules (g). Bars = 20 μ m.

of these cytoplasmic protrusions, in the middle region (Figs. 11-12), are round and have a smooth surface. In the posterior region the cytoplasmic protrusions form a carpet covering all

the epithelial surface, hindering the observation of microvilli. These, when visualized, are short and restricted to the surface of cytoplasmic protrusions (Fig. 14). Some images show the



Figs 9-14. Ultrastructural aspects of *Diatraea saccharalis* epithelial midgut surface in Scanning Electron Microscopy. Fig. 9 - Anterior midgut region: ruptured peritrophic membrane (PM); a few cytoplasmic protrusions (P). Bar = 50 μ m. Fig. 10 - Anterior midgut region: cytoplasmic protrusions (P) among the prominent microvilli (\star). Bar = 10 μ m. Fig. 11 - Middle midgut region: cytoplasmic protrusions (P). Bar = 10 μ m. Fig. 12 - Middle midgut region: many round protrusions, some of them with few microvilli (arrow). Bar = 5 μ m. Fig. 13 - Posterior midgut region: many cytoplasmic protrusions (P). Bar = 25 μ m. Fig. 14 - Posterior region: abundant cytoplasmic protrusions connected to columnar cells by fine cytoplasmic peduncle (arrows). Bar = 10 μ m.

cytoplasmic protrusions linked to the cellular surface by fine cytoplasmic peduncle (Fig. 14).

Goblet cells. Present acidophilic cytoplasm, with rare PAS-positive granules, and U-shaped basal nucleus, accompanying

the base of the chamber (Figs. 3, 4, 6-8). They present a large cavity, in the form of a calyx, whose internal surface contains projections similar to microvilli; in the anterior and middle region, this cavity is located predominantly in the cellular apex (Figs. 3-4), whereas in the posterior region

enlargement of the cellular base was frequently observed (Fig. 7). The goblet chamber, in cells of the anterior midgut region, show a narrow apex (Figs. 3), while in the posterior midgut region this portion of the chamber is enlarged (Figs. 6-8). In the middle midgut region cells with two chamber formats are present (Figs. 4).

Regenerative cells. Small cells with a round or elongated format, found isolated, in pairs or in groups at the base of the epithelium; present central nucleus and cytoplasm that is scarce and basophilic (Figs. 1, 3-6, 8). Few PAS-positive granules were observed in the supra-nuclear cytoplasm of cells situated in the anterior midgut region (Fig. 3), granules that increase in number from the middle (Fig. 5) to the posterior region (Fig. 8).

Discussion

The general morphology of midgut epithelium in larvae of *D. saccharalis* is similar to that described for many Lepidoptera such as *Manduca sexta* L. (Cioffi 1979) e *Erynnis ello* L. (Santos *et al.* 1984) (Sphingidae), *Spodoptera frugiperda* Smith (Jordão *et al.* 1999) and *Anticarsia gemmatialis* Hübner (Levy *et al.* 2004) (Noctuidae), among many other species (for a review, see Lehane & Billingsley 1996).

Our observations indicate that the epithelial cells (columnar, goblet and regenerative) present regional differences in morphology along the midgut of *D. saccharalis*. Endocrine cells were not detected in our preparations by virtue of being scarce and difficult to observe under conventional histological staining techniques, such as those already indicated in other works (Endo & Nishiitsutsuji-Uwo 1982, Endo *et al.* 1983).

The columnar cells showed an increase in the number of cytoplasmic protrusions (loosening themselves from the cellular apex in the direction of the midgut lumen) and in the quantity of PAS-positive cytoplasmic granules, from the anterior to the posterior midgut region. This large quantity of PAS-positive material in the cytoplasm of columnar cells also was observed in the midgut of *Dermatobia hominis* L. (Diptera: Cuterebridae) (Lello & Vieira 2001) and in *Triatoma infestans* Klug (Heteroptera: Reduviidae) (Burgos & Gutiérrez 1976).

The physiological significance of the release of cytoplasmic projections to the intestinal lumen has not been totally clarified. Some authors relate this process to a mechanism of cellular degeneration resultant from epithelial renovation (Anderson & Harvey 1966, De Priester 1971) or even a technical artifact (Ryerse *et al.* 1992), whereas others point to a relationship with the process of apocrine secretion for the release of digestive enzymes (Santos *et al.* 1984, Wood & Lehane 1991). Since the insects utilized in our work were not near a molting time, and due to our control of technical conditions in midgut processing, our results agree with the idea that these projections must be related to the apocrine secretion process.

Few studies describe the morphology of these projections under a light microscope (Caetano & Zara 2001, Lello & Vieira 2001, Levy *et al.* 2004, Ferreira & Cruz-Landim 2005); and although the authors did not emphasize variability in the number of cytoplasmic protrusions dependent

on the localization of columnar cells in the midgut, the morphological aspects described are similar to those observed in *D. saccharalis*. Our ultrastructural observations show that the scarce cytoplasmic protrusions of the anterior region exhibit microvilli on part of its surface and that these are absent in those of the posterior region, where its surface becomes smooth. Since the cytoplasmic protrusions are copious in the posterior midgut region, where they occupy an extensive area of the apex of columnar cells to the detriment of microvilli, it is supposed that in *D. saccharalis* the release of part of the apical cytoplasm cannot be related to the release of digestive enzymes; in other insects, it is known that the release of digestive enzymes is related to the process of microapocrine secretion, which originates from the microvilli as suggested for *E. ello* (Santos *et al.* 1984) and for *Stomoxys calcitrans* L. (Diptera: Muscidae) (Wood & Lehane 1991). Previous observations of the sub-cellular organization of columnar cells in *D. saccharalis* (Pinheiro & Gregório 2003) showed that the content of these cytoplasmic protrusions in the anterior midgut region is poor in organelles, while the voluminous ones of the posterior region contain innumerable organelles, including endoplasmic reticulum, mitochondria and digestive vacuoles, reinforcing the idea that there is a functional difference in these cytoplasmic protrusions and, consequently in the columnar cells, depending on spatial location in the midgut.

The quantity of PAS-positive granules observed in the apical cytoplasm of the columnar cells increases from the anterior to the posterior midgut region. The PAS-positive material may represent deposits of glycogen and/or granules of secretion containing polysaccharides. Glycogen deposits rarely have been described in cytoplasm of columnar cells in Lepidoptera. However, Rodrigues & Cruz-Landim (1970) detected glycogen, mucopolysaccharides and protein into epithelial midgut cell of three bee's species; the authors also reported that peritrophic membrane stain indicated chitin presence. The cross sections pretreated with amylase do not eliminate the positive staining by PAS reaction in the columnar cell, suggesting exist glycogen associated with positive PAS material. It is known, that the peritrophic membrane is composed principally of chitin, a linear N-acetyl-D-glucosamine polymer (Tellam *et al.* 1999) and glycoproteins (Tellam *et al.* 1999, Terra 2001). Although it is controversial the chitin positive reaction by PAS (Pearse, 1968), chitin is susceptible to coloration by PAS, if it is associated with mucosubstance (Pearse 1968). Our findings point to the existence of precursors to chitin that can be concentrated in the apical cytoplasm for secretion (Hopkins & Harper 2001, Terra 2001). The fact that PAS-positive cytoplasmic material increases from the anterior to the posterior region suggests that columnar cells of the posterior midgut region may be secreting chitin precursors more intensely to the peritrophic membrane than the cells of the anterior region.

The format of the goblet cell chamber has been studied in other Lepidoptera, where it was indicated that the apical portion of this cavity is more enlarged in cells of the posterior midgut region (Cioffi 1979, Santos *et al.* 1984, Jordão *et al.* 1999). In *D. saccharalis* larvae differences in the morphology of this cavity were not consistent in LM; however, SEM

analyses show that, besides this enlargement of the chamber from the anterior to the posterior region, there even occurs an apparent increase in the number of chamber's cytoplasm extrusion with mitochondria throughout its extension that may be responsible for ionic transport. In *D. saccharalis* preparations it becomes clear that the chamber, in cells of the posterior region, reach the base of the cell, differently from other Lepidoptera (Cioffi 1979, Terra 1988). The major diameter of chamber's cytoplasm extrusion at the chamber base may be explained by the presence of mitochondria inside these cytoplasmic projections, as described in innumerable insects (Anderson & Harvey 1966, Lehane & Billingsley 1996, Cavalcante & Cruz-Landim 1999, Levy et al. 2004).

The regenerative cells present thick cytoplasm with few organelles and perform the function of cellular renovation (De Priester 1971, Turbeck 1974, Lehane & Billingsley 1996). Data from the literature show regenerative cell differentiation in columnar cells under Transmission Electron Microscopy (Endo et al. 1983, Lehane & Billingsley 1996). The detection of PAS-positive material in apical cytoplasm of regenerative cells similar to that observed for columnar cells, as well as the increase of these granules in the posterior midgut region in these both cells, reinforces the idea of the regenerative cells is precursor role of the columnar cells. In the renovation of intestinal epithelium, the senescent cells separate themselves from the basal membrane that would continue intact, being dislocated in the direction of the lumen by regenerative cells in division, which in this manner would have the role of cellular reposition (Endo et al. 1983, Chapman 1998). The proposition of cellular renovation of intestinal epithelium is related to the growth of the alimentary canal at each ecdysis and to the substitution of senescent cells that suffer any type of aggression (Lehane & Billingsley 1996).

Thus our results demonstrate the existence of regional morphological differences in epithelial cells of the anterior and posterior midgut regions of *D. saccharalis*. The middle region presents morphological characteristics that are intermediary between the two other regions as much for columnar cells in relation to the quantity of cytoplasmic projections and PAS-positive labeling, as for the morphology of the goblet cell chamber. These findings reinforce morphometric data indicative of the existence of a transition area between the anterior and posterior region of the midgut (Pinheiro et al. 2003).

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