

Fine structural analysis of the epithelial cells in the hepatopancreas of *Palaemonetes argentinus* (Crustacea, Decapoda, Caridea) in intermoult

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ABSTRACT: The aim of this study is to describe the ultrastructure of the hepatopancreas of *P. argentinus* in intermoult. *P. argentinus* hepatopancreas was studied using standard TEM techniques. Each tubule consists of four cellular types: E (embryonic), F (fibrillar), R (resorptive) and B (blister like). E-cells have embryonic features and some of them were found in mitosis. F, R and B cells possess an apical brush border. F-cells have a central or basal nucleus, a conspicuous RER, and dilated Golgi cisternae. R cells show a polar organization of organelles in three areas: apical, with numerous mitochondria and sER tubules, a central area with the nucleus and RER, and a basal area containing a sER-like tubule system and mitochondria. B-cells were observed at different stages of their life cycle. In an early differentiation stage they comprise an apical endocytotic complex and Golgi vesicles. The fusion of endocytotic and Golgi vesicles originates subapical vacuoles. During maturation, a big central vacuole is formed by coalescence of subapical vacuoles. The central vacuole is eliminated by holocrine secretion. The ultrastructure suggests that F-cells synthesize proteins, R-cells storage nutrients and B-cells have a secretory or excretory function, and confirms the independent origin of F, B and R cells from the embryonic cells.

Introduction

In crustacean, the hepatopancreas is the primary organ responsible of absorption and storage of ingested materials (Loizzi, 1971; Storch and Welsch, 1977; Vogt *et al.*, 1989; Johnston *et al.*, 1998). This organ is also involved in the synthesis of digestive enzymes and the detoxification of xenobiotics (Gibson and Barker, 1979; Icely and Nott, 1992; Vogt, 1994). It consists of one or

more lobules formed by tubules with blind ends at the distal zone and open ends at the proximal zone. Tubules converge into a primary duct, which connects the organ with the pyloric stomach (Icely and Nott, 1992; Sousa and Petriella, 2000).

Hepatopancreas epithelium is composed of four main cell types: E (embryonic) cells, confined to the blind distal ends, and F (fibrillar), R (resorptive) and B (blister-like) cells, distributed throughout the whole tubule with some variations according to the species (Icely and Nott, 1992; Petriella and Fonalleras, 1997; Sousa and Petriella, 2000). The organ undergoes histological and histochemical modifications in response to different physiological demands (moult, reproduction) (Al-Mohanna and Nott, 1989; Sousa and Petriella, 2001)

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and environmental changes (physicochemical factors, pollution). As a result, there is a correlation between the physiological condition of the organism and the hepatopancreas structure (Popescu-Marinescu *et al.*, 1997). In hepatopancreas of *Penaeus monodon* exposed to pesticides, Vogt (1987) found disorganization of tubule morphology, epithelium autolysis, reduction of endoplasmic reticulum, and swollen mitochondria.

The prawn *P. argentinus* is widely distributed in the littoral region of Argentina, Paraguay, Uruguay and southern Brazil (Boschi, 1981; Morrone and Lopreto, 1995). This species lives in freshwater and brackish water streams and lagoons and plays an important trophic role in the environments in which it inhabits (Spivak, 1997; Collins, 1999). Some of these lagoons receive direct discharges of chemicals from terrestrial ecosystems, from which *P. argentinus* accumulates important amounts of organochlorine pesticides in its tissues (Gonzalez Sagrario *et al.*, 1998). The present study is part of a project which aims to use the histological changes in this organ as indicators of environmental pollution. This work describes the fine structure and function of the different cell types (E, F, R and B) of *P. argentinus* hepatopancreas.

Materials and Methods

Intermoult adults of both sexes at sexual rest (Boschi, 1981), 0.100-0.200g in weight and 22.2-29.6 mm in total length, were collected from Sotelo stream, tributary of Mar Chiquita lagoon (Argentina, 38°S 55°W). This stream is a shallow creek (<1m in the channel) where pesticide concentrations are below the toxic levels and are not related to adverse biological effects (Miglioranza *et al.*, 2003). The moult stage was determined by microscopic examination of the setae of the uropod exopodite, following the criteria established by Díaz *et al.* (1998).

The animals were placed on ice and the cephalothorax integument was removed. The hepatopancreas were dissected and placed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH= 7.2-7.4) overnight at 4°C. They were postfixed for 1h in 1% OsO₄. Later the material was dehydrated through an ethanol series and embedded in Spurr resin. Semithin sections (1µm) were stained with toluidine blue. Ultrathin sections were mounted on copper grids (400mesh) and stained with lead citrate and uranyl acetate. TEM images were obtained with a Hitachi HU 11C-1 transmission electron microscope.

Results

Each hepatopancreatic tubule is lined by a simple epithelium which consists of four cell types: E (embryonic), F (fibrillar), R (absorptive) and B cells (blister-like) (Fig. 1).

E-cells are located at the distal blind end of the tubules. They are cubical, lack of brush border and have a high nucleus to cytoplasm volume ratio, typical of embryonic cells (Fig. 2). The cytoplasm is homogeneous with few organelles and a poorly developed ER. Slight concentrations of mitochondria are observed at the apex and the base. E-cells lack of lipid or glycogen reserves. They can be observed at different mitotic stages.

F-cells are located at medial and proximal zones of the tubules among R and B-cells. They are cylindrical or dome-like bodies with a centrally or basally located nucleus and apical brush border. Their mitochondria are uniformly distributed around the nucleus, and the Golgi bodies have heavily dilated cisternae. The most conspicuous features of these cells are the dilated Golgi cisternae, and the great development of the RER that surrounds the nucleus and occupies most of the cytoplasm (Fig. 3).

R-cells are most abundant, and are located at medial and proximal zones of the tubules. They present a polar organization of organelles in three areas: the apical, with a brush border and numerous mitochondria, sER tubules and little vacuoles (Fig. 4); the medial, with the nucleus surrounded by the RER, and the basal, dominated by big mitochondria and a sER-like tubular system (Fig. 5). Glycogen was not observed; however several cells presented lipid droplets of heterogenous sizes and homogenous contents (Fig. 6). R-cells exhibit small primary lysosomes, autophagosomes of different sizes and electron dense residual bodies (Fig. 7).

B-cells are more frequent at the proximal zone of the tubules than at the other zones. They were observed at different stages of their life cycle. At the beginning of the differentiation, they evidence an apical complex which comprises numerous endocytotic channels and vesicles. Endocytotic channels invaginate from the brush border deep into the cell and give rise to endocytotic vesicles. The endocytotic vesicles fuse with Golgi vesicles originating subapical vacuoles (Fig. 8). The nucleus, surrounded by the RER, elongated Golgi cisternae, small lysosomes and autophagosomes can be observed at the medial area of the cell (Fig. 9). At an early maturation stage the apical complex become more important: the apical vacuoles are enlarged by the incorporation of endocytotic vesicles and Golgi vesicles.

The coalescence of vacuoles originates a large supranuclear vacuole (Fig. 9), which increases in size by the incorporation of new vacuoles (Fig. 10). In the mature cell the nucleus and the rest of the organelles are displaced towards the cell base by the expansion of the supranuclear vacuole. The completely mature cell is like a blister, the large vacuole occupies nearly the whole cytoplasm (Fig. 11) and the brush border is reduced in length. At the end of the maturation period, the large vacuole is eliminated by holocrine secretion. The complete cell protrudes from the epithelium and loses contact with the basal lamina.

Discussion

The general epithelial cytology of the hepatopancreas in *P. argentinus* is consistent with that observed in other decapods (Al-Mohanna *et al.*, 1985; Icely and Nott, 1992; Johnston *et al.*, 1998).

E-cells are undifferentiated, with embryonic ultrastructural features, and are the only cells showing mitotic activity. These findings confirm the previous observations by Sousa and Petriella (2000) in the same species and are coincident with those observed in other decapod species (Travis, 1955; Al-Mohanna and Nott, 1989; Petriella and Fonalleras, 1997).

F-cells are located among R and B cells at the

medial zone of the tubules and have basophilic cytoplasm because of the high RER content (Vogt *et al.*, 1989; Al-Mohanna and Nott, 1989; Sousa and Petriella, 2000). Ultrastructurally, they are characterized by a considerable amount of RER and prominent Golgi bodies. Both characteristics evidence an active protein synthesis. These cells are considered as the only site of digestive enzyme synthesis (Icely and Nott, 1992). Different enzymes were localized by immunohistochemistry and immunofluorescence, such as trypsin, α -amylase, astacin, and chymotrypsin, in the F-cells of various decapods (Malcoste *et al.*, 1983; Vogt *et al.*, 1989; Johnston *et al.*, 1998). In the present study, no reserves were found in this cell type; apparently the main function of these cells is the synthesis and secretion of proteins, presumably digestive enzymes.

The absorptive function of *P. argentinus* R cells is supported by the presence of lipid droplets in the cytoplasm, the conspicuous brush border and the apical tubular system. The apical sER system in association with the apical mitochondria is also observed in other decapods and is involved in nutrient absorption (Vogt, 1994; Johnston *et al.*, 1998). The nutrient reserves, stored during intermoult, are mobilized to provide energy during starvation periods (late premoult) (Vogt, 1996; Sousa and Petriella, 2001). Glycogen was not found in R cells, coincidentally a little amount of this nutrient was found in a previous study during intermoult in the

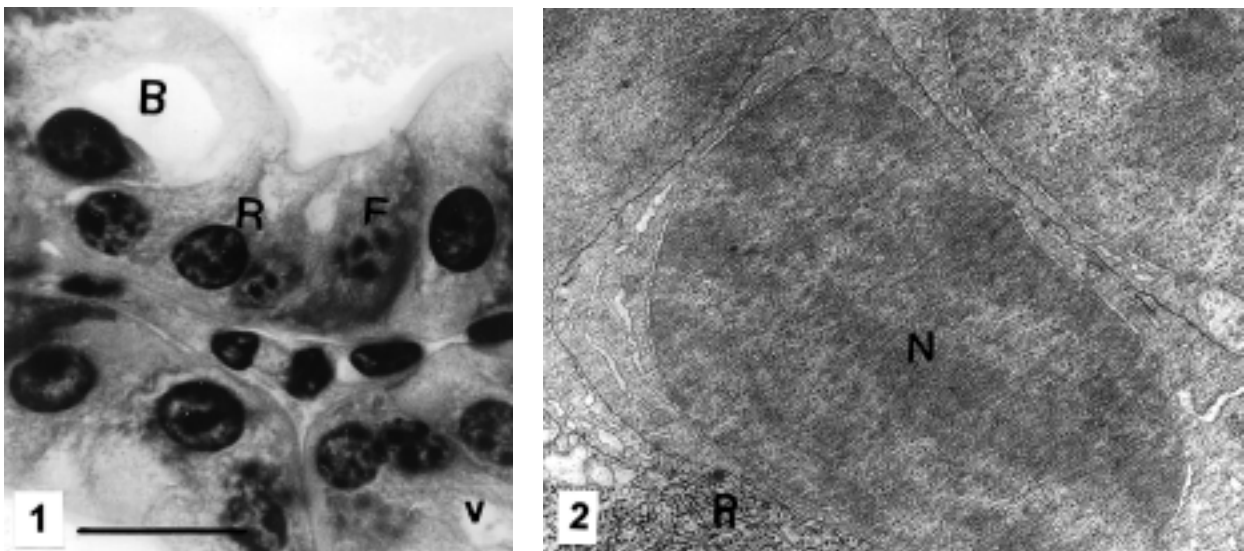


FIGURE 1. Cross section of a tubule. Detail of the specialized cellular types. B: B cells, F: F cells, R: R cells, v: vacuole (From Sousa and Petriella, 2000). H&E. X 1,000. Scale bar: 50 μ m.

FIGURE 2. E cells: prominent nucleus and scarce organelles. N: nucleus, R: R cell. MET. X 8,000

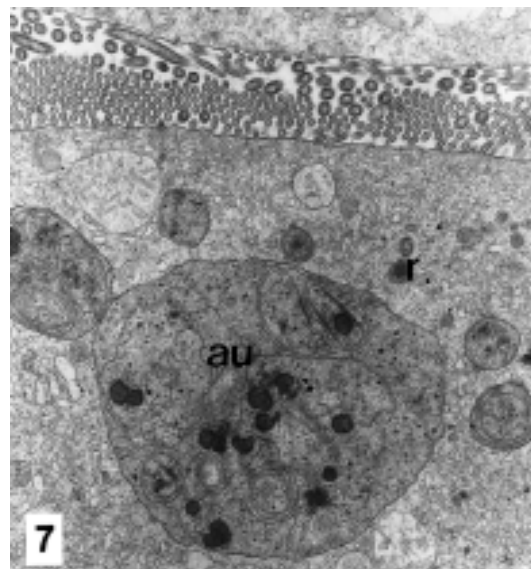
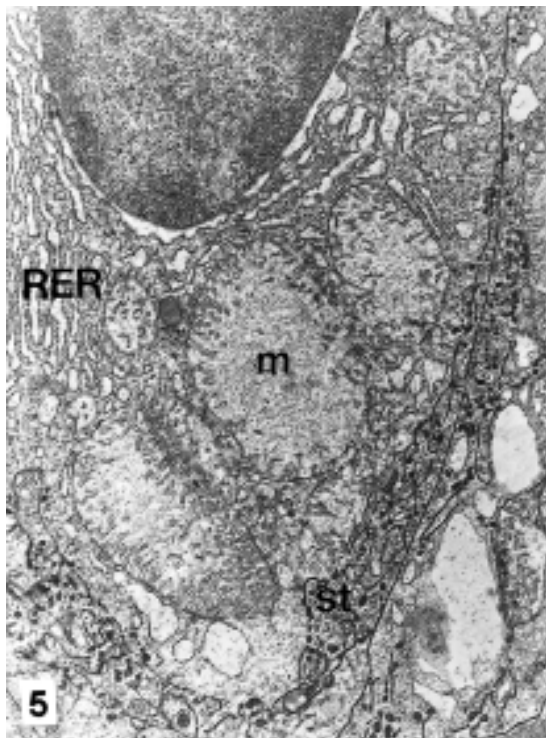
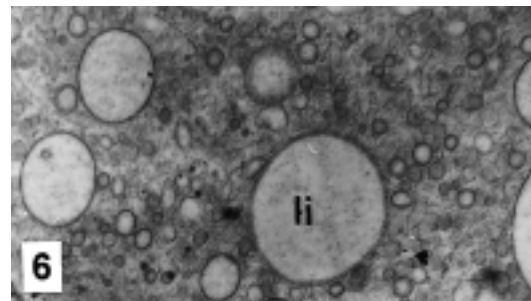
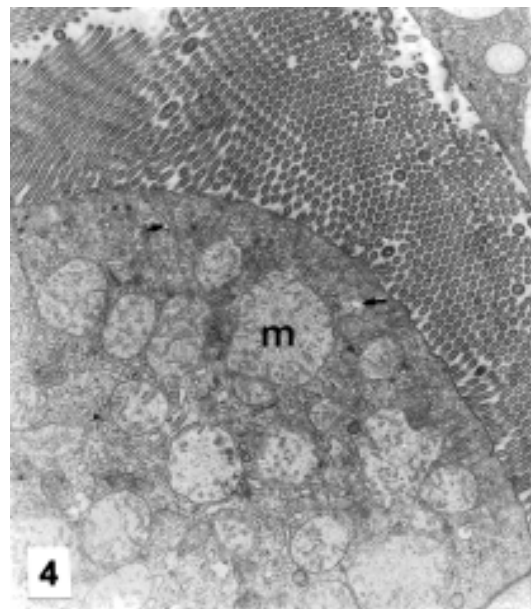
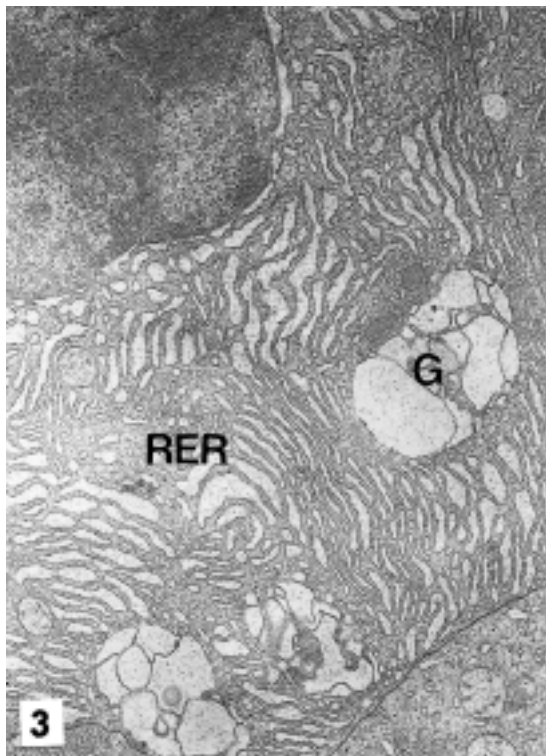


FIGURE 3. F cell. Note the great development of the RER and dilated cisternae of Golgi. G: Golgi, RER: rough endoplasmic reticulum. MET. X 10,000

FIGURE 4. R cell apical area. Observe the conspicuous brush border, endocytotic vesicles (arrows) and the mitochondria concentration. m: mitochondria. MET. X 8,000

FIGURE 5. R cell infranuclear area with RER, large mitochondria and a branched system of sER like tubules. m: mitochondria, RER: rough endoplasmic reticulum, st: sER like tubules. MET. X 12,000

FIGURE 6. Details of lipid droplets in the R cell cytoplasm. li: lipid droplets. MET. X 15,600

FIGURE 7. R cell cytoplasm. Little lysosomes, autophagosomes of different sizes and electron-dense residual bodies. au: autophagosome, r: residual bodies. MET. X 12,000

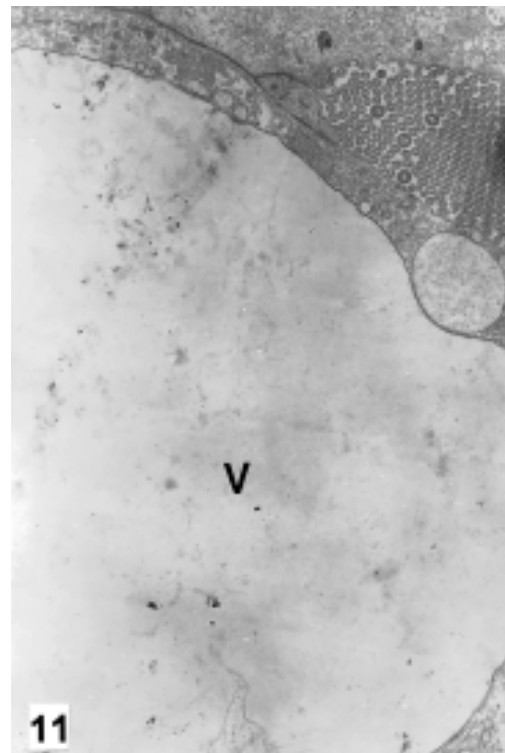
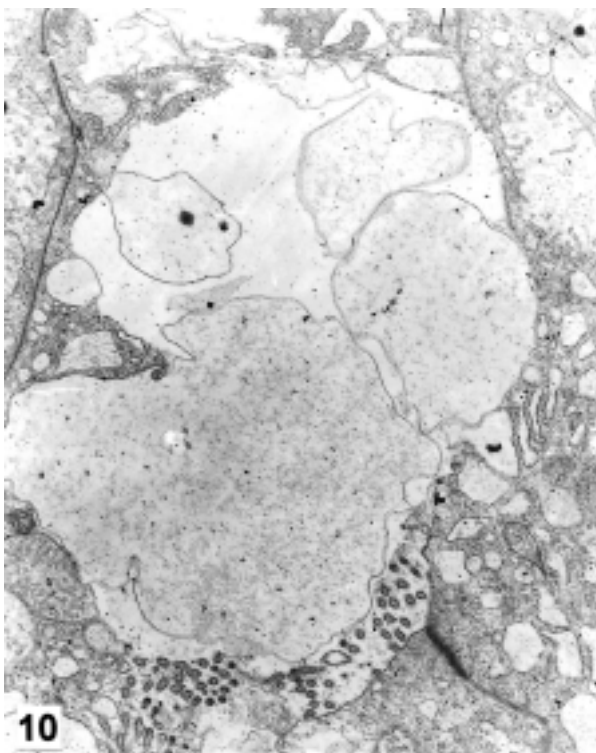
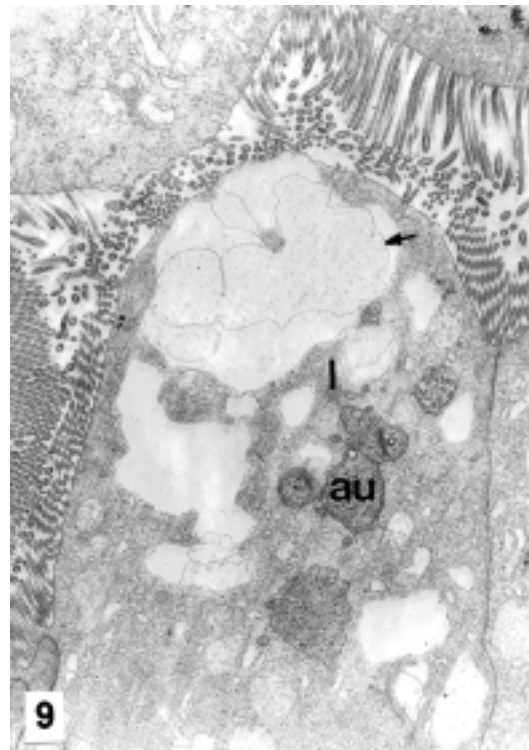
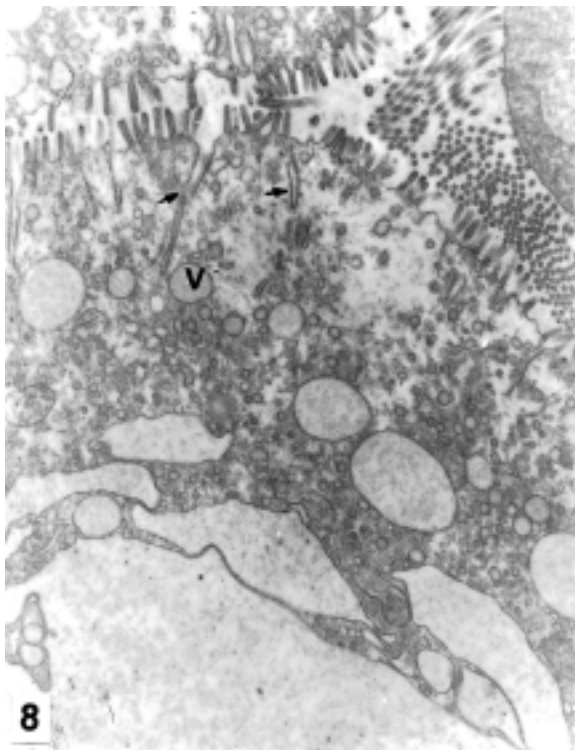


FIGURE 8. B cell. Apical complex at the beginning of the differentiation. Endocytotic channels (arrows), v: endocytotic vesicles. MET. X 8,000

FIGURE 9. B cell apex. Note the endocytotic vesicles fusion originating a big supranuclear vacuole (arrow). au: autophagosome, l: lysosome. MET. X 8,000

FIGURE 10. B cell. Detail of supranuclear vacuole enlargement by coalescence of new vesicles. MET. X 8,000

FIGURE 11. Mature B cell. The central vacuole occupies nearly the whole cytoplasm. V: vacuole. X 13,700

same species (Sousa and Petriella, 2001). These prawns feed actively during intermoult so it is possible that the storage of glycogen is not necessary because of the constant supply (Chang and O'Connor, 1983; Sousa and Petriella, 2001). The basal tubule system of sER found in R cells of *P. argentinus* closely related to mitochondria seems to be involved in the delivery of nutrients to other organs via the hemolymph (Vogt, 1985; 1994; Johnston *et al.*, 1998). Autophagosomes and residual bodies were present in various R cells of *P. argentinus*; supranuclear autophagosomes are interpreted as sites of intracellular waste deposition (Vogt, 1994). Residual bodies observed in *P. argentinus* R cells are consistent with the metal storing vacuoles and residual bodies present in *Thenus orientalis* and *Penaeus semisulcatus*, respectively (Al-Mohana and Nott, 1987; 1989; Johnston *et al.*, 1998). These cells detoxify heavy metals by their accumulation in a soluble form in the cytoplasm before excretion (Vogt, 1987; Johnston *et al.*, 1998).

B-cells of *P. argentinus*, like those in other decapods (Icely and Nott, 1992; Al-Mohana and Nott, 1989; Johnston *et al.*, 1998; Vogt, 1993) are characterized by an endocytotic apical complex and a central vacuole; these ultrastructural features indicate an absorbing and degrading function (Hopkin and Nott, 1980; Vogt, 1993; 1994). The gradual enlargement of the central vacuole in *P. argentinus* indicates the absorption of materials from the tubular lumen, but no nutrients were detected in B-cells cytoplasm. Vogt (1994) proposed that B cells degrade exhausted digestive enzymes and waste products which remain in the tubules after absorption of nutrients.

In summary, the ultrastructure of F-cells in *P. argentinus*, like in most of decapods, is consistent with an active synthesis and secretion of proteins. R-cells are implicated in absorption and storage of nutrients which are exported to other organs through the basal sER-like tubule system. B cells ultrastructural features indicate an absorptive and degrading function; mature B cells, with the central vacuole replete of wastes, are discharged by holocrine secretion to the tubular lumen. The present fine analysis supports a previous conclusion about the origin of F, R and B cells (Sousa and Petriella, 2000), which considers that these specialized cells originate independently from embryonic cells.

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