MORPHOLOGY AND HISTOLOGY OF THE DIGESTIVE GLAND OF *Oxychilus* (Drouetia) *atlanticus* (MORELET & DROUËT) (GASTROPODA: PULMONATA)

M. LOPES, A. RODRIGUES & I. MARIGOMEZ

LOPES, M, A. RODRIGUES & I. MARIGOMEZ. 2001. Morphology and histology of the digestive gland of *Oxychilus* (Dt-ouetia) *atlanticus* (Morelet & Drouet) (Gastropoda: Pulmonata). Arquipélago, Life and Marine Sciences. Supplement 2(Part B): 71-76. Ponta Delgada. ISSN 0873-4704.

Little information exists on the histology of Zonitidae digestive system. This study deals with a detailed characterisation of the differeni types of cells comprising epithelium lining the digestive gland of *Oxychilus atlanticus*. Frorn light and scanning electron microscope (SEM) studies, three types of differentiated cells can be identified in the digestive gland: digestive cells, cxcretory cells and calcium cells. Digestive cells are the most numerous, and are present in two forms, one believed to be absorbing food material and the other secreting material. Excretory cells are distinguished by having a large central vacuole, containing excretory granules. Calcium cells contain spherules of calcium salts, which have a characteristic birefringence.

M. Lopes, A. Rodrigues,(e-mail:rodrigues@notes.uac.pt), Universidade dos Açores, Departamento de Biologia, Rua da Mãe de Deus 58, PT – 9501-801 Ponta Delgada, Portugal; I. Marigomez, Biologia Zelularra Arloa, Zoologia eta Animali Zelulen Dinamika Saila, Euskal Herriko Unibertsitateu. 644 P. K. ES – 48080 Bilbao, Spain.

INTRODUCTION

Oxychilus (Drouetia) *atlanticus*, a terrestrial zonitid gastropod endemic to São Miguel island (Azores), has been successfully subjected to predatory experiments on *Lymnaea truncatula*, thus showing the carnivorous behaviour of the species under laboratory conditions (CUNHA 1991). Further works, concerning the analyses of stomach contents from individuals collected in the field, have shown the omnivorous tendency of this group in natural conditions (LOPES et al. 1997).

Some studies have been undertaken concerning the alimentary preferences within the family Zonitidae (BOYCOTT 1934; FRÖMMING 1954; RONDELAUD 1975; MORDAN 1977; RONDELAUD 1977; DIDIER & RONDELAUD 1989) but only a few on the anatomy and histology of the alimentary tract (RIGBY 1963; LOPES et al. 1998).

The digestive system of gastropods includes, besides the alimentary tract, the digestive gland, a voluminous organ, which develops from the endoderm and constitutes a large part of the visceral mass (KRESS et al. 1994). This gland, also called mid-gut gland, is a much-branched organ ramifying through most of the visceral mass and ending in numerous blind, small tubules. The tubules open into ductules which join to form ducts, and latter combine to form the hepatic ducts which empty into the stomach (GRIEBEL 1993).

According to OWEN (1966) the digestive gland is thought to perform the following functions: absorption of ingested food material, extracellular and intracellular digestion, secretion, excretion and osmoregulation.

Histological studies on the gastropod digestive gland have shown that cells structure and function vary even within the same group (KRESS et al. 1994). Accordingly, several different types of cells have been identified and described in the digestive gland. The most common types of cells are: digestive cell, microtubule-containing cell, mineral-containing granule cell, excretory cell and calcium cell (RIGBY 1963; LUFTY et al. 1967;

WALKER 1970; KRESS et al. 1994; GRIEBEL 1993).

The present study provides a detailed characterisation of the different types of cells lining the digestive gland epithelium of *O. atlanticus* and, in the process, attempts to describe the morphological organisation of the gland.

MATERIAL AND METHODS

Specimens of *Oxychilus atlanticus* were collected at Abelheira near Ponta Delgada (São Miguel Island).

For light microscopy, specimens were dissected and the digestive gland removed and fixed in Bouin. Following fixation, the material was dehydrated and then, embedded in paraplast and stained with Haematoxylin/Eosin (MARTOJA & MARTOJA-PEARSON 1970). For the detection of calcium, material was fixed in Carnoy and subject to the Stoeltzener process (MARTOJA & MARTOJA-PEARSON 1970). The blocks of paraplast were cut to the thickness of 7 μm.

For scanning electron microscope (SEM) examination, a particular procedure was followed. The material was fixed in Bouin and embedded in paraplast, a method usually followed for routine optical microscopy. Following this procedure, 60 μm sections were cut, producing blocks with regular surfaces. The blocks were then submitted to a dehydration procedure and finally immersion in hexamethyldisilazane. The liquid phase of the hexamethyldisilazane evaporates in a few minutes, leaving cells whose ultrastructure is comparable to that of the cells dried by critical-point drying (GOLDSTEIN et al. 1992). For stereology studies, in order to obtain the relative frequency of the different cells types, was used the reticule Weibel Multipurpose Test System M168 (WEIBEL 1979). The reticule was superimposed on the image of the digestive gland tubules using a drawing-tube adapted to a microscope Leitz Laborlux S (Leica). The sections of each portion of the digestive gland were randomly selected and viewed at 100x.

RESULTS

The hepatic ducts of *O. atlanticus* are lined by a ciliated columnar epithelium laying on a thin basement membrane.

The fresh digestive gland is generally yellowish-brown and consists of a single layered epithelium, supported by a thin basement membrane bound by connective tissue and muscle fibres.

According to structural features, three types of cells are identified within the digestive gland epithelium: Cell type I, Cell type II and Cell type III (Figure 1).

Cell type I (Figure 2, A)

The Cell type I is the most abundant in the digestive gland, corresponding to 82% of the lining epithelium. They are columnar in shape with apical walls either level or round in contour. The cells with level apical surfaces possess a well developed brush border, in contrast with the ones that have rounded apical surfaces, where the same structure is less conspicuous. These cells average 50 μm in height and 17 μm in basal width. Nuclei are basal and oval, and nuclear chromatin can be observed as small granules. Much of the cell volume is filled with granules that give a range of colours, according to their stain reaction.

Cell type II (Figure 2, B)

These cells are globular, averaging 30 μm in height and 13 μm in basal width. Much of the cell volume is taken up by a spherical central vacuole. Within this, there are excretory granules, which either occur singly or are joined in small groups. The free surface of the cell presents a well defined brush border. The nucleus is basal when the cell reaches the mature stage, corresponding to the vacuole maximum volume. The cytoplasm presents granules that give a similar stain reaction as the ones found in the cell type I. Cell type II corresponds to 10% of the lining epithelium.

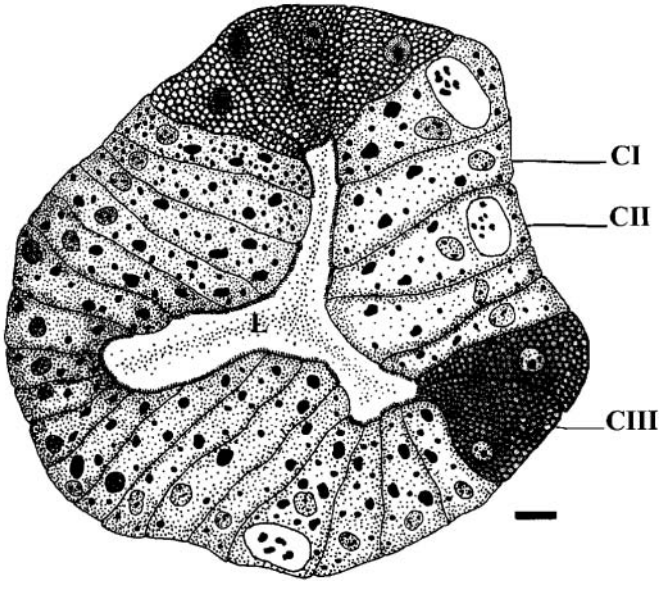

Figure 1. Digestive gland epithelium. C I, Cell type I; C II, Cell type II; C III, Cell type III. Scale bar 10 μm.

Cell type II (Figure 2, B)

These cells are globular, averaging 30 μm in height and 13 μm in basal width. Much of the cell volume is taken up by a spherical central vacuole. Within this, there are excretory granules, which either occur singly or are joined in small groups. The free surface of the cell presents a well defined brush border. The nucleus is basal when the cell reaches the mature stage, corresponding to the vacuole maximum volume. The cytoplasm presents granules that give a similar stain reaction as the ones found in the cell type I. Cell type II corresponds to 10% of the lining epithelium.

DISCUSSION

The hepatic ducts are responsible for the transport of materials to and from the digestive gland. This function is assisted by features of its lining epithelium, where the movement of the cilia creates a current that aids the transport of particles into the digestive gland, whereas a continuously produced secretion flows out of it (VAN WEEL 1950).

The digestive gland epithelium of O. *atlanticus* is composed of three distinct types of cells: Cell type I, Cell type II and Cell type III.

Following the structural description of the digestive gland of *Agriolimax reticulatus* (WALKER 1970), the cell types found in O. *atlanticus* correspond, respectively, to digestive, excretory and calcium cells. Therefore, our results are in contrast with the two types of cells described by RIGBY (1963) for O. *cellarius*, digestive and excretory cells. Other authors have also described the same three types of cells for various pulmonates (VAN WEEL 1950; GUPTA 1977; DIMITRIADIS & HONDROS 1992).

As suggested by DIMITRIADIS & HONDROS (1992) for *Helix lucorum*, in *O. atlanticus* the digestive cells constitute the major part of the lining epithelium.

The range of colours observed in the digestive cells cytoplasm of O. *atlanticus* could be explained, according to KRESS et al. (1994), by food vacuoles at different stages of digestion.

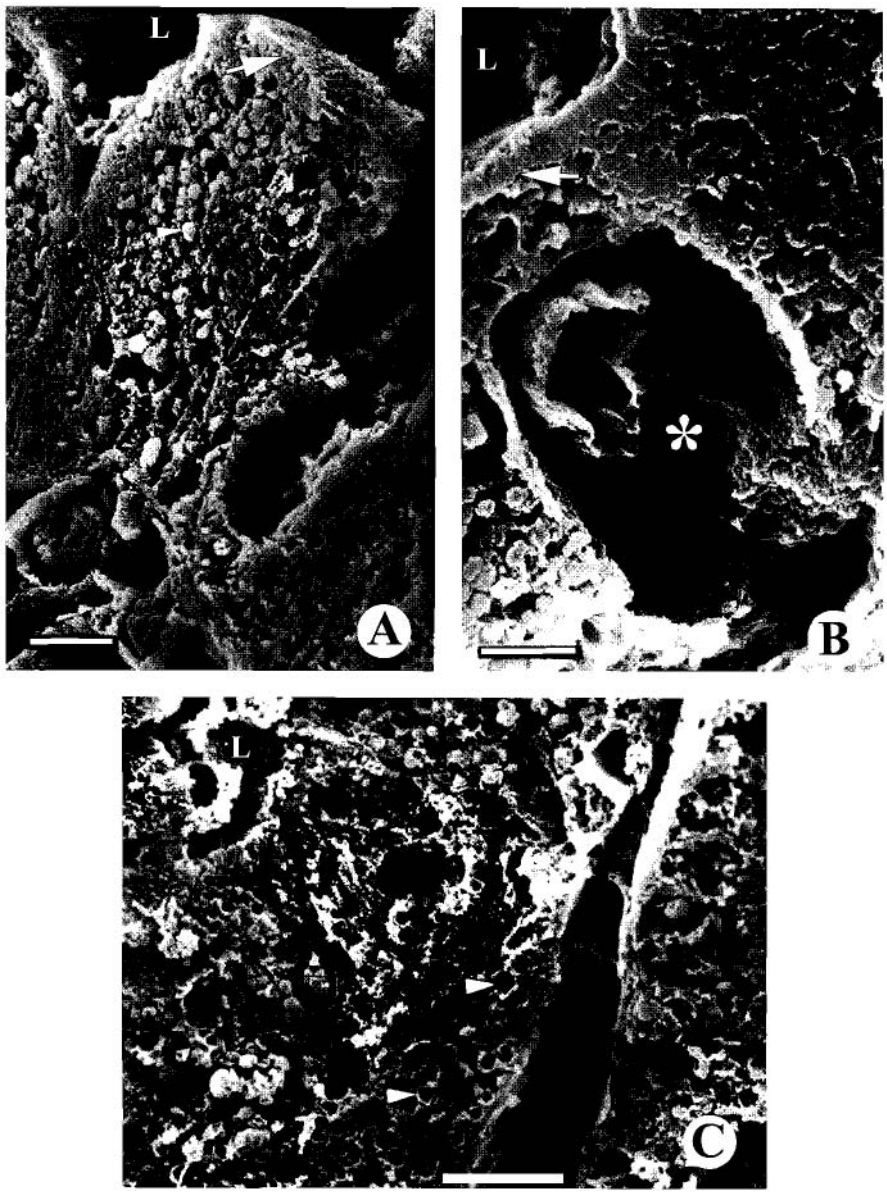

Fig. 2. Digestive gland epithelium (S.E.M.). A (scale bar 10 ym), Digestive cell; B (scale bar 5 ym), Excretory cell; C (scale bar 16 μm), Calcium cell. L, Lumen; * Vacuole; ➨ Brush border; ‣ calcium spherules.

On account. of their structural features, the digestive cells can be divided in two different forms: one, believed to be absorbing food material, has a level apical surface that possess a well developed brush border, while the other, mainly involved in secretion, presents a rounded apical surface with a less conspicuous brush border. The presence of two digestive cell forms

has been referred to in other pulmonates (DAGUZAN 1985; WALKER 1970).

The similar reaction of the granules found in digestive and excretory cells of O. *atlanticus* suggests a close affinity between both types of cells. This reaction was also noted for other species by WALKER (1970) and DAGUZAN (1985), who considered that excretory cells are derived from digestive cells.

Calcium cells are characterised by the presence of calcium salts, giving a strong positive reaction to the Stoeltzener test. The function of the calcium reserves is not yet certain but, according to the literature, it is believed to participate in many important calcium dependent metabolic reactions, such of shell construction and repair, pH regulation at the digestive tract, and in the reproductive process (WALKER 1970; GUPTA 1977; SIMKISS & MASON 1983; IRELAND & MARIGOMEZ 1992).

The use of special procedures to prepare SEM material provided clear cuts (see figure 2, A, B and C) for 3-D observation and interpretation. Besides, they could be compared with histological sections for light microscopy, which were used as templates.

ACKNOWLEDGEMENTS

Thanks are due to Patrícia Garcia, Dr. Regina Cunha and Dr. Manu Soto Lopez for helpful discussions, and Dr. Frias Martins for his help and comments upon the first drafts of the manuscript of this paper. This work was supported by the project Praxis /2/2.1/BIA/169/94 - Biodiversidade.

REFERENCES

BOYCOTT, A.E. 1934. The habitats of land mollusca in Britain. *Journal of Ecology* 22: 1-38.

CHÉTAIL, M. & G. KRAMPITZ. 1982. Calcium and skeletal structures in mollusks: concluding remarks. *Malacologia* 22 (1-2): 337-339.

CUNHA, R. T. 1991. Predação em *Lyinnaea truncatula* (Muller) por *Oxychilus atlanticus* (Morelet & Drouet), 105 pp. Trabalho de síntese no âmbito das Provas de Aptidão Pedagógica e Capacidade Científica. Universidade dos Açores, Ponta Delgada.

DAGUZAN, J. 1985. Principales caractéristiques de l'appareil digestif, de la nutrition et de la digestion chez les mollusques. *Année Biologique* 4: 367-406.

DAS, S., K.K. MISRA & K.C. GHOSE. 1992. The digestive gland of gastropods eith different feeding habits. *Proceedings of the Ninth Eurupean Malacological Congress:* 117-124.

DIDIER, B. & D. RONDELAUD. 1989. Les caractéristiques des proies consommées par le mollusque Zonitoides nitidus Müller et leur dynamiqye en Juin, Juillet et Août. *Bulletin de la Société d'Histoire Naturelle* 125: 111-117.

DIMITRIADIS, V.K. & D. HONDROS. 1992. Effect of starvation and hibernation on the fine structural morphology of the digestive gland cells of the snail *Helix lucorum*. *Malacologia* 34 (1-2): 63-73.

FRÖMMING, E. 1954. *Biologie der mitteleuropaischen landgastropoden*. Duncker & Humblot. Berlin. 404 pp.

GOLDSTEIN, J.I., D.E. NEWBURY. P. ECHLIN, D.C. JOY, A.D. ROMING Jr, C.E. LYMAN, C. FIORI & E. LIFSHIN. 1992. *Scanning electron microcopy and X-ray microanalysis* (2nd ed.).Plenum Press, New-York-London. 820 pp.

GRIEBEL, R. 1993. Fine structure of the three cell types found in the digestive gland of *Elysia viridis* (Opisthobranchia: Sacoglossa). *The Veliger* 36 (2): 107-114.

GUPTA, A.S. 1977. Calcium storage and distribution in the digestive gland of *Bensonia moticola* (Gastropoda: Pulmonata): A histophysiological study. *Biological Bulletin* 153: 369-76.

IRELAND. M.P. & 1. MARIGOMEZ. 1992. The influence of dietary calcium on the tissue distribution of Cu, Zn, Mg & P and histological changes in the digestive gland cells of the snail *Achutina fulica* Bowdich. *Journal of Molluscan Studies* 58: 157-168.

KRESS, A., L. SCHMEKEL & J.A. NOTT. 1994. Ultrastructure of the digestive gland in the opisthobranch mollusk, *Runcina*. *The Veliger* 37 (4): 358-373.

LOPES. M., I. MARIGOMEZ & A. RODRIGUES. 1998. Anatomy and histology of the alimentary tract of *Oxychilus* (*Drouetia*) *atlanticus* (Morelet & Drouet, 1857) (Pulmonata: Zonitidae) *Abstracts of the World Congress of Malacology*, Washington, D. C.: 197.

LUTFY, R.G. & E.S. DEMIAN. 1967. The histology of the alimentary system of *Marisa cornuarietis* (Mesogastropoda: Ampullariidae). *Malacologia* 5 (3): 375-422.

MARTOJA, R. & M. MARTOJA-PEARSON. 1970. *Técnicas de Histologia Animal.* Toray-Masson. Barcelona, 350 pp.

MORDAN, P.B. 1977. Facts affecting the distribution and abundance of *Aegopinella* and *Nesovitrea* (Pulmonata: Zonitidae) at Monks Wood National Nature Reserve, Huntingdonshire. Biological *Journal of the Linnean Society* 9:59-72.

OWEN, G. 1966. Digestion. Pp. 53-96 in: WILBUR, K.M. & C.M. YONGE (Eds). *Physiology of Mollusca.* Volume 2. Academic Press, New-York-London. 645 pp.

RIGBY, J.E. 1963. Alimentary and reproductive systems of *Oxychilus cellarius* (Müller) (Stylommatophora). *Proceedings of rhe Zoological Society of London* 141: 311-359.

RONDELAUD, D. 197. La predation de *Lymnaea (Galba) truncatula* Muller par *Zonitoides nitidus* Muller, moyen de lutte biologique. *Annales de Parasitologie Humine Comparée* 50: 55-61.

RONDELAUD, D. 1977. Les aptitdes malacophages de quelques mollusques Zonitidae et leur intérêt dans le contrôle biologique de *Lymnaea (Galba) truncatula* Muller. *Annales de Parasitologie Humaine Comparée* 52 (4): 411-420.

RUNHAM, N.W. & P.J. HUNTER. 1970. *Terrestrial slugs*. Hutchinson & Co LTD. London, 175 pp.

SIMKISS, K & A.Z. MASON. 1983. Metal ions: Metabolic and toxic effects. in: SLEUDDIN, A.S.M. & K.M. WILBUR (Eds). *The Mollusca.* Volume 2. Academic Press, New York.

WALKER, G. 1970. The citology, histochemistry and ultrastructure of the cell types found in the digestive gland of the slug, *Agriolimax reticulatus* (Muller). *Protoplasma* 71: 91-109.

WEEL, P.B. 1949. Contribution to the physiology of the glandula media intestini of the african giant snail, *Achatina fulica* Fér., during the first hours of digestion. *Physiology and Comparative Oecology* 2: 1-19.

WEIBEL, E.R. 1979. *Stereological methods.* Vol 1. Academic Press Inc., London, 415 pp.

Accepted 17 November 2000.