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# Light and electron microscopy studies of the oesophagus and crop epithelium in *Aplysia depilans* (Mollusca, Opisthobranchia)

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**Abstract**

The oesophagus and crop epithelium of *Aplysia depilans* consist in a single layer of columnar cells with apical microvilli, and some of them also possess cilia. Cell membrane invaginations, small vesicles, multivesicular bodies and many dense lysosomes were observed in the apical region of the cytoplasm. In most cells, a very large lipid droplet was observed above the nucleus and a smaller one was frequently found below the nucleus; glycogen granules are also present. Considering these ultrastructural features, it seems that these cells collect nutritive substances from the lumen by endocytosis, digest them in the apical lysosomes and store the resulting products. The cell bodies of mucus secreting flask-shaped cells are subepithelial in the oesophagus and intraepithelial in the crop. Histochemistry methods showed that the secretion stored in these cells contains acidic polysaccharides. Secretory vesicles with thin electron-dense filaments scattered in an electron-lucent background fill most of these cells, and the basal nucleus is surrounded by dilated rough endoplasmic reticulum cisternae containing small tubular structures. Considering the relatively low number of secretory cells, mucus production cannot be high. Moreover, since protein secreting cells were not observed in either oesophagus or crop, extracellular digestion in the lumen of these anterior segments of the digestive tract most probably depend on the enzymes secreted by the salivary and digestive glands.


*Keywords:* Cytochemistry; Digestive tract; Gastropoda; Mucus cells; Ultrastructure

## 1. Introduction

The genus *Aplysia* has a worldwide distribution, comprising 37 species of sea slugs (Eales, 1960; Carefoot, 1987). They have short life spans, ranging from 3 to 16 months (Carefoot, 1967; Gev et al., 1984), but a high rate of food consumption allows them a rapid growth (Carefoot, 1987). The largest specimens of *A. depilans* collected on the North Portuguese coast had a length of about 25 cm and weigh almost 500 g (Lobo-da-Cunha, 2001), but specimens of *A. gigantea* from Australia can reach a length of 60 cm (Eales, 1960).

Species of *Aplysia* eat mainly green and red algae that are cut into pieces by the combined action of the radula and jaws (Howells, 1942). Algae pieces pass through the oesophagus and reach the crop where they can be stored in such amounts that in satiated specimens of *A. depilans* the total weight of the anterior gut contents may represent about 12% of the total body weight (Susswein and Markovich, 1983). From the crop, alga fragments pass into the gizzard where they are macerated by the chitinous teeth. A filter chamber with long acicular teeth retains the larger food fragments that return to the gizzard during the forward movements of the anterior gut content. A fluid with fine food particles passes through the filter chamber and enters the stomach, a sac-like compartment ending in a comma-shaped caecum. The wall folds and complex ciliary currents of the stomach provide a sorting mechanism necessary to conduct useful substances into the digestive gland ducts (Howells, 1942; Fretter and Hian, 1979). The smallest food particles coming from the stomach

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reach the digestive gland diverticula, where a major part of the digestion and absorption of nutrients takes place. However, a fluid containing digestive enzymes coming from both the salivary glands and the digestive gland is present in the crop and gizzard and can start acting on the alga fragments in the anterior part of the digestive tract. Waste coming from the digestive gland returns to the stomach and is conducted into the caecum where the faecal rods are formed. Stomach wall folds form a groove separated from the major cavity of this organ, through which the faecal rods are transported to the intestine that ends in the anus situated on the posterior face of the siphonal fold of the mantle (Howells, 1942; Fretter and Hian, 1979).

In *A. depilans*, several cell types found in the digestive gland, salivary glands and stomach were previously characterised on the basis of light and electron microscopy observations (Lobo-da-Cunha, 1999, 2000, 2001, 2002; Lobo-da-Cunha and Batista-Pinto, 2003). With the present article the study of the digestive system in *A. depilans* is extended to the oesophagus and crop. These segments of the alimentary canal were investigated with light and electron microscopy techniques in some species of prosobranch and pulmonate gastropods (Voltzow, 1994; Luchtel et al., 1997), but ultrastructural studies on the oesophagus and crop of opisthobranch gastropods still have to be performed. Although the digestive gland is generally regarded as the major site for nutrient absorption and storage in gastropods, some studies showed that in pulmonates nutrient uptake could also occur in the crop (Walker, 1972; Bourne et al., 1991). Thus, one aim of this work was the detection of intracellular digestion and nutrient storage in the anterior part of the alimentary canal of the opisthobranch gastropod *A. depilans*. Additionally, histochemical methods were used to characterise the secretory cells in order to compare them with the secretory cells previously described in this and in other gastropods.

## 2. Materials and methods

### 2.1. Animals

Four adult specimens of *A. depilans* Gmelin, 1791, measuring 20–25 cm, were collected during low tides in beaches near Oporto. The animals were transported to the laboratory in a container with seawater and dissected shortly after collection.

### 2.2. Morphology

For light microscopy, pieces of oesophagus and crop were fixed for 24 h with 4% formaldehyde in 0.4 M cacodylate buffer (pH 7.4), dehydrated in ethanol and embedded in paraffin. Tissue sections were stained with haematoxylin and eosin, dehydrated in ethanol and mounted with DPX. For electron microscopy, small pieces of the oesophagus and crop were fixed for 2 h at 4 °C in 2.5% glutaraldehyde, diluted in 0.4 M cacodylate buffer pH 7.4 with 5 mM $CaCl_2$. After washing in buffer, the fragments were postfixed with 2% $OsO_4$ buffered with cacodylate, dehydrated in ethanol and embedded in Epon. Ultrathin sections were stained with uranyl acetate and lead citrate, before being observed in a JEOL 100CXII transmission electron microscope operated at 60 kV. Semithin sections of Epon embedded tissues (2 μm) stained with methylene blue and azure II were also used for light microscopy observations.

### 2.3. Histochemistry

The tetrazonium reaction for protein detection, PAS technique for polysaccharides and Alcian blue staining (pH 2.5 and 1.0) for acidic mucopolysaccharides, based on the procedures described by Ganter and Jollès (1970), were applied to 15 μm sections of tissues embedded in paraffin. These sections were additionally stained with haematoxylin, dehydrated in ethanol and mounted with DPX. These histochemical techniques were also applied to 2 μm semithin sections of Epon embedded tissues (Lobo-da-Cunha, 2001).

Moreover, the Sudan black staining for lipids (Ganter and Jollès, 1970) was also applied on 2 μm semithin sections of Epon embedded tissues. Before staining, floating sections were treated for 10 min with a 0.02% $H_2O_2$ solution to remove osmium tetroxide. After washing in water, sections were stained for 3 min with a saturated solution of Sudan black in 70% ethanol, washed in 70% ethanol and finally in water. Sections just treated with $H_2O_2$ were used as control, and Epon was used to mount all the sections.

### 2.4. Cytochemistry

#### 2.4.1. Catalase detection

Very small fragments of the oesophagus and crop were fixed for 1 h at 4 °C in 1.25% glutaraldehyde, diluted in 0.4 M cacodylate buffer pH 7.4 with 5 mM $CaCl_2$. After several rinses in cacodylate buffer, the fragments were preincubated for 30 min at 4 °C, in 0.2 M 2-amino-2-methyl-1,3-propanediol buffer pH 9.5 with 5% sucrose. Incubation was performed for 2 h at 35 °C in a medium containing 0.06% $H_2O_2$ and 2 mg/ml of 3,3′-diaminobenzidine (DAB) in 0.2 M 2-amino-2-methyl-1,3-propanediol buffer pH 9.5 with 5% sucrose (Novikoff and Goldfischer, 1969). For control, 50 mM 3-amino-1,2,4-triazol was added to the incubation medium to inhibit catalase activity. Postfixation was carried out for 2 h at room temperature in 1% $OsO_4$ in cacodylate buffer with 1.5% potassium ferrocyanide. Ultrathin sections were observed without further staining.

#### 2.4.2. Arylsulphatase detection

Very small pieces of the oesophagus and crop were fixed for 1 h at 4 °C in 2.5% glutaraldehyde, diluted in 0.4 M cacodylate buffer pH 7.4 with 5 mM $CaCl_2$. After washing in