

HISTOCHEMISTRY OF THE BUCCO-OESOPHAGEAL GLANDS OF *MYTILUS EDULIS*: THE IMPORTANCE OF MUCUS IN INGESTION

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Histochemical techniques were used to investigate the possible role of the bucco-oesophageal glands in the blue mussel, *Mytilus edulis* L. (Mollusca: Bivalvia). No activity was observed for any of the eight major digestive enzymes tested; however, the glands contained large amounts of both neutral and acid mucopolysaccharides. These results confirm the importance of mucus in the ingestive process in *M. edulis*, and do not support the hypothesis of ingestion of particles suspended in water alone.

Until recently it was thought that the Bivalvia were the only class of molluscs in which some type of secretory gland of extracellular digestive function in the bucco-oesophageal region was totally absent (Table 1). However, in a study of the mode of particle ingestion in five species of suspension-feeding bivalves, an extensive glandular complex was reported in the bucco-oesophageal region of *Mytilus edulis* L. only (Beninger *et al.*, 1991). Although these glands were observed to liberate secretions into the oesophageal lumen, it was not known whether they performed any digestive function. The present study reports the results of histochemical tests to determine the possible presence of digestive enzymes and the role of this glandular complex.

Table 1. Occurrence of secretory glands with extra-cellular digestive function in the bucco-oesophageal region of the extant molluscan classes

Class	Glands present	References
Caudofoveatea	+	Scheltema (1981)
Solenogastres	+	Baba (1940), Salvini-Plawen (1978)
Placophora	+	Fretter (1937)
Monoplacophora	+	Salvini-Plawen (1981)
Scaphopoda	+	Salvini-Plawen (1988)
Gastropoda	+	Fretter & Graham (1962)
Cephalopoda	+	Fuchs (1973)
Bivalvia	-	Morton (1979), Salvini-Plawen (1988)

Adult *Mytilus edulis* were maintained in running sea-water aquaria at 0-10°C (ambient temperature) and fed daily with a mixture of *Chaetoceros muelleri*, Tahitian *Isochrysis galbana*, and the 3H strain of *Thalassiosira pseudonana*. Specimens for histochemical study were quickly dissected using microsurgical instruments to remove the oesophagus and some of the surrounding digestive gland in the posterior region. The digestive gland served as a positive control for most of the enzymatic tests. Details of histochemical protocols are presented in Table 2. For lipase, whole tissue was used rather than sections; the digestive gland was therefore carefully removed to avoid cross-contamination. Enzymes necessary for digestion of the major food components were tested (amylase, polysaccharidases; arylamidase, proteases; lipase and non-specific esterase, lipids), as well as enzymes involved in extracellular digestion and transmembrane transport (acid phosphatase, alkaline phosphatase) and carbohydrate preparation for intermediary metabolism (glucose-6-phosphatase, α -D-glucosidase). All these enzymes have previously been reported in

Table 2. Histochemical protocol for studies of *Mytilus edulis* bucco-oesophageal glands

Enzyme/Stain	Fixation	Sections	Technique
Alkaline phosphatase	Fresh frozen	Frozen 8 µm	Vacca (1985)
Acid phosphatase			
Glucose-6-phosphatase			
Non-specific esterase	10% neutral formalin	Paraffin 8 µm	Vacca (1985) modification of Pearse (1972)
	Fresh frozen	Frozen 6 µm	Perse (1972)
Amylase	Fresh liquid	Freeze-dried 6 µm	Burstone & Folk (1956)
Arylamidase (Aminopeptidase)	N ₂ frozen		
α-D-Glucosidase	10% neutral formalin	Frozen 10 µm	Rutenburg et al. (1960)
Lipase	Fresh	Macerated	George (1952)
Periodic acid-Schiff (PAS)-diastase	Bouin's	Paraffin 7 µm	Vacca (1985)
Alcian blue pH 2.5-diastase			
Best's carmine			

the digestive system of various bivalve species (Zacks, 1955; Reid, 1966; Wojtowicz, 1972; Mathers, 1973; Brock *et al.*, 1986; Le Pennec *et al.*, 1991). In addition, alcian blue and periodic acid-Schiff (PAS) were used to detect acid and neutral mucopolysaccharides, respectively. The specificity of the PAS and alcian blue stains was confirmed using diastase-digested controls (Vacca, 1985). Best's carmine technique was used as a supplementary control for glycogen (Vacca, 1985).

The results of the enzymatic tests show that the bucco-oesophageal glands of *Mytilus edulis* are devoid of any detectable enzyme activity. Although it is possible that these glands may liberate enzymes not tested in the present study, the results cogently suggest that the bucco-oesophageal glands have no digestive function. The strong positive alcian blue and periodic acid-Schiff reaction confirm earlier reports (Beninger *et al.*, 1991), and suggest that the primary function of these glands is the secretion of both acid and neutral mucopolysaccharides into the oesophageal lumen. Although it had previously been affirmed that the mechanism of particle ingestion in *Mytilus edulis* did not involve mucus (Jørgensen, 1981, 1990; Kiørboe & Møhlenberg, 1981; Jørgensen *et al.*, 1984), the importance of mucus in the ingestive process was underlined by Beninger *et al.* (1991).

Of the five bivalve species previously examined (Beninger *et al.*, 1991), bucco-oesophageal glands were found only in *Mytilus edulis*. Since mixed-secretion mucocytes were found to be abundant in the mussel oesophageal epithelium, the presence of such an extensive glandular system apparently devoted to the addition of more of the same secretion to the oesophageal

Table 3. Histochemical tests on *Mytilus edulis* oesophagus and digestive gland

Enzyme/histochemical stain	Result			
	Oesophagus Epithelium	BOG	Digestive gland Ducts	Tubules
Acid phosphatase	-	-	++(L)+(E)	-
Alkaline phosphatase	-	-	++(L)+(E)	++(L)+(E)
Non-specific esterase	-	-	-	-
Lipase	-	-	-	+(1)
Arylamidase	-	-	++(1)	++(1)
Amylase	-	-	-	-
Glucose-6-phosphatase	-	-	-	-
α-D-Glucosidase	-	-	+(L)+(E)	+(L)++(E)
Periodic acid-Schiff (PAS)-diastase	++(2)	++	-	+(L)+(E)
Alcian blue-diastase	++(2)	++	-(L)+(E)	-
Best's carmine	-	-	-	-

BOG, bucco-oesophageal gland; E, digestive gland epithelium; L, digestive gland lumen; -, no reaction; +, positive reaction; ++, strongly positive reaction. (1) Lipase and amylase tests unable to localize enzyme activity. (2) In mucocytes of oesophageal epithelium.

lumen suggests that this type of mucus is of primary importance in the mussel ingestive process. Endoscopic studies and observations of mucocyte distribution (Beninger *et al.*, 1992; in preparation) indicate that mixed secretions present low to moderate viscosity; it is thus not yet certain whether the 'capestan' model of winding mucus strings around the crystalline style is accurate (Purchon, 1977). In any event, it is clear that large quantities of endogenously-produced mucus are added either continuously or in response to certain particles; this mucus must also be broken down in order to minimize accumulation in the digestive tract. The strong amylase activity observed in the digestive gland could be related to such recycling, and could also explain the lack of activity for α -D-glucosidase and glucose-6-phosphatase in the digestive gland (Table 3). A survey of the literature, however, shows that the presence of different enzymes in the digestive gland varies between species and according to physiological status and environmental conditions (Reid, 1966; Wojtowicz; 1972; Mathers; 1973; Brock *et al.*, 1986); non-positive reactions in the digestive gland positive controls are thus not to be construed as definitive negative reactions.

Recent studies using video-endoscopy have revealed the importance of mucus in feeding processes at the gill in several bivalve species (Ward *et al.*, 1991; Ward *et al.*, in press; Beninger *et al.*, 1992). Current work on the distribution of mucocyte types on the gill frontal surface details the roles of different mucus secretions on the bivalve gill, and also shows that the *Mytilus edulis* bucco-oesophageal glands are part of an extensive sub-epithelial system present in the lips and labial palps (Beninger *et al.*, in preparation). Taken together, these studies establish the fundamental importance of mucus in the feeding processes of bivalves.

The apparent lack of digestive function for the bucco-oesophageal glands of *Mytilus edulis* is a departure from the general situation in the other molluscan classes; however, it is consistent with its anatomical relationship to the sub-epithelial gland system of the peribuccal organs. Further study will be required to ascertain the eventual prevalence and significance of such glands in other members of the Bivalvia.

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