

## FUNCTIONS OF THE ENDOSTYLE IN THE TUNICATES

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## ABSTRACT

For more than two decades, electron microscope observations on the endostyle of different species of tunicates have revealed the presence of a densely packed rough endoplasmic reticulum in the glandular cells of the organ. The presence of such a developed ergastoplasm fully supports the hypothesis that these cells are capable of synthesizing digestive enzymes. Comparative analyses of extracts of the endostyle and the stomach provide evidence that several kinds of enzymes are secreted and mixed with the food cords. The glycosidases are well represented. Other cells of the endostyle, able to bind iodine, are involved in its incorporation into tyrosine and proteins, a biochemical proof that the endostyle can be considered as the forerunner of the thyroid gland of vertebrates. The endostyle is also an important constituent of the stolon of *Pyrosomas* and salps (Thaliacea), as it gives these to the whole digestive apparatus of the blastozooids. The unique role played by the endostyle strictly opposes the Thaliacea to the other Tunicata. Another distinctive trait of taxonomic importance is their single protostigma while the tadpoles of ascidians bear at least two. Probably *Pyrosomas* and salps branched out very early from the common stock.

The endostyle is a ciliated and glandular groove extending along the floor of the branchial cavity of the Tunicata (Urochordata) and of the Cephalochordata (Amphioxus). A similar organ, the subpharyngeal gland, is present in the ammocoete larvae of the Cyclostomata (lampreys), but transforms into the thyroid gland at metamorphosis (Mueller, 1873).

The presence of the endostyle in the lower chordates was early related to their microphagous diet (filter-feeders). As long ago as 1876, Fol considered the endostyle a mucus secreting organ (Schleimdrüse).

The endostyle of tunicates displays a complex histological structure. Its U-shaped cross-section reveals (Fig. 1) that the organ consists of several longitudinal, one cell thick, cellular tracts located symmetrically on both sides of a mid-ventral zone bearing long flagella. These flagella often extend the whole depth of the groove. The lateral cellular tracts are either glandular or ciliated: two ciliated bands separate three glandular zones. The epithelium is lined by a basal membrane and the mesenchym lacunae.

The structure of the endostyle is remarkably constant as variations only concern either the number of cells of the tracts or the number of tracts constituting the organ (e.g., Doliolids, Godeaux, 1971; 1981).

For a long time the ideas about the role of the endostyle were quite simple: the mucus secreted by the glandular cells is propelled by the cilia and flagella onto the internal pharyngeal walls and allows the trapping of the food particles (microalgae and particulate organic matter) entering the buccal siphon as water is drawn in.

The investigations during the last several decades showed that the endostyle is far from a simple mucus forming system. Histochemical investigations revealed that the cytoplasm of the large glandular cells is basophilic attesting the presence of ribonucleic acid (RNA) as the reaction becomes negative after a pretreatment with ribonuclease (Olsson, 1963; Barrington, 1957). These observations strongly suggest that proteins are synthesized in the glandular cells. Secretion products are visible at the top of most of the cells, even those bearing cilia, as claimed by

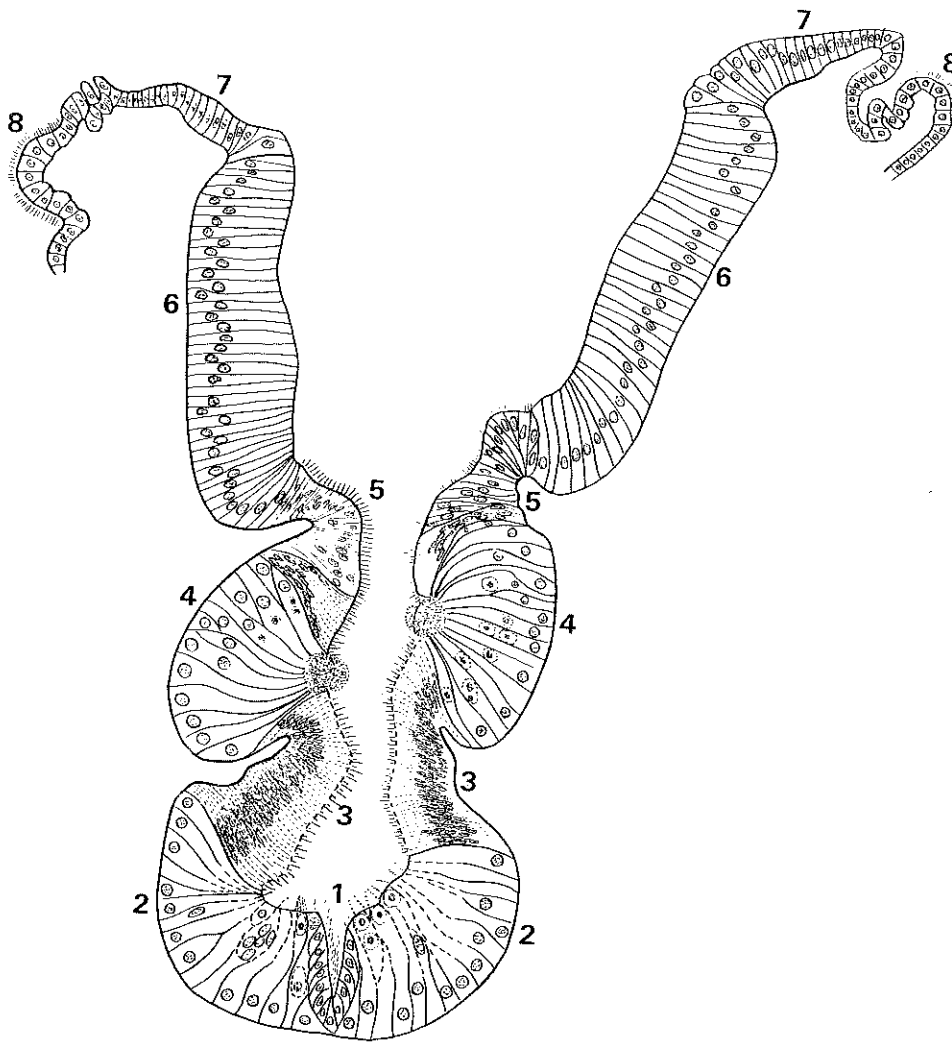


Figure 1. Cross-section through the endostyle of *Molgula manhattensis*. The different epithelial zones are numbered from 1 to 8 (modified after Godeaux and Firket, 1968).

Ghiani et al. (1965), Ghiani and Orsi (1966), and Orsi and Relini (1966) in their exhaustive investigations on several species of ascidians.

These investigations have been completed by electron microscopic observations carried out by different workers during more than two decades (Levy and Porte, 1964; Godeaux and Firket, 1966; 1968; Ghiani et al., 1965; Godeaux, 1971; 1981; and unpublished results; Bogoraze et al., 1979).

#### MATERIAL AND METHODS

Investigations were carried out on different species of tunicates: *Molgula manhattensis* (De Kay) collected in an oysterpark at the Belgian coast, *Ciona intestinalis* (L.), *Phallusia mammillata* (Cuvier) and *Halocynthia papillosa* Gun. obtained from the Biological Station Stareso in Calvi (France, Corsica), *Thalia democratica* (Forskål), *Doliolina muelleri* (Krohn), *Doliolum nationalis* Borgert and *Pyrosoma atlanticum* Peron caught in the area of the Biological Station of Villefranche-sur-Mer (France).

Electron microscopic observations were performed with Siemens Elmiskop 1 and 101. After dissection, the endostyles were fixed into chilled 6% glutaraldehyde solution in phosphate buffer (pH 7.4) and, after rinsing, postfixed in 1% osmium tetroxide. After dehydration, organs were embedded in Epon 812, sectioned with LKB ultratome and stained with uranyl acetate and lead citrate.

The enzymatic activities of extracts of endostyle and stomach were tested by two methods: a) the API-ZYM<sup>1</sup> semi-quantitative micromethod (Plantevin and Nardon, 1972; Monget, 1978). It allows a rapid study of 19 different enzymatic reactions and needs small sample quantities. This method uses a set of 20 cells (API-ZYM Kit), one with no substrate (control), and 19 containing different naphthyl derivatives as synthetic substrates, at a suitable buffer. Every cell receives two micro-drops (about 75  $\mu$ l) of the extract. After a 4 h incubation at 40°C, the activities of the extract are tested colorimetrically (using a reading scale) after addition of Fast Blue BB, a dye which combines with the naphthyl radicals set free by the enzymes. b) The second method is micro-spectrophotometric. Proteases were determined according to Rick (1974: trypsin), Del Mar et al. (1974: chymotrypsin) and Appel (1974: carboxypeptidase A), the lipase (triacylglycerol-acylhydrolase) using the Boehringer monotest (triolein as a substrate: Verduin et al., 1973) and the glycosidases following Dahlqvist (1968: invertase, lactase and maltase) and Samain et al. (1977:  $\alpha$  amylase). Concentrations of soluble proteins in the extracts were measured according to Schacterle and Pollack (1973) and to Bradford (1976).

*Halocynthia papillosa* was preferably used for the determinations owing to its large size, robustness and availability. The organs were ground with white sand and the homogenates extracted with 1.5 volumes of cold water. Centrifugations at 12,000 rotations  $\cdot$  min<sup>-1</sup> for 30 min at 4°C. This allowed the separation of a clear supernatant fluid. Sometimes, the extracts of the complex stomach + liver of *H. papillosa* had to be filtered through glass wool to eliminate fat deposits.

Observations on the budding of *Pyrosoma atlanticum* were done on whole mounts of stolons stained with carmine and on 5 to 10  $\mu$ m thick serial sections stained mainly with haematoxylin-eosin according to Heidenhain.

## RESULTS AND DISCUSSION

*Endostyle as a Secretory Organ.*—The sketch of a cross section of an endostyle (Fig. 1) shows the glandular tracts (2, 4 and 6) separated by the ciliated zones (1, 3 and 5).

The cells of the fan-shaped zones 2 and 4 are giant, several times bigger than the neighboring cells and may be polyploid. They are not very numerous. Their base is extended on the basal membrane, contrasting with the narrow apex; the shape of the cell is somewhat triangular. The nucleus, large and spherical, lies near the base of the cell; its nucleolus is deeply stained. The cytoplasm displays a densely packed granular reticulum with some beautiful mitochondria and a Golgi apparatus. The membranes of the ergastoplasm, in the zones 2 and 4 cells, are running parallel to the cell walls, evoking that of the exocrine pancreas cells (Figs. 2 and 3).

Epithelium 6 is formed of elongated columnar cells and is relatively extended. The nucleus of the cell bearing a conspicuous nucleolus is near the base; the main part of the cytoplasm is filled up with a dense but very irregular ergastoplasm.

The top of the glandular cells is occupied by free ribosomes and well colored droplets of dense secretion products inside of microvesicles (Figs. 2 and 3). Some cells may bear a more or less long flagellum. The same general picture of the glandular bands was found in all the species examined.

Ciliated cells of the tracts 1, 3 and 5 are slender; their cytoplasm is poor in ribosomes, contains few mitochondria often close to the rootlets of the cilia, and microvesicles. Microtubules and granules of glycogen are conspicuous in the zone 1 cells (*Molgula manhattensis*, Fig. 2C). The cells are choanocyte-like with their flagellum surrounded by rows of microvilli (Fig. 4A). Different authors do not agree about possible secretory activity of the ciliated cells. Barrington (1957), Levy and Porte (1964), and Ghiani et al. (1965) credit these cells (especially the zone

<sup>1</sup> Appareils et Procédés d'Identification S.A. La Balme-les-Grottes, F-38390 Montalieu-Vercieu (France).

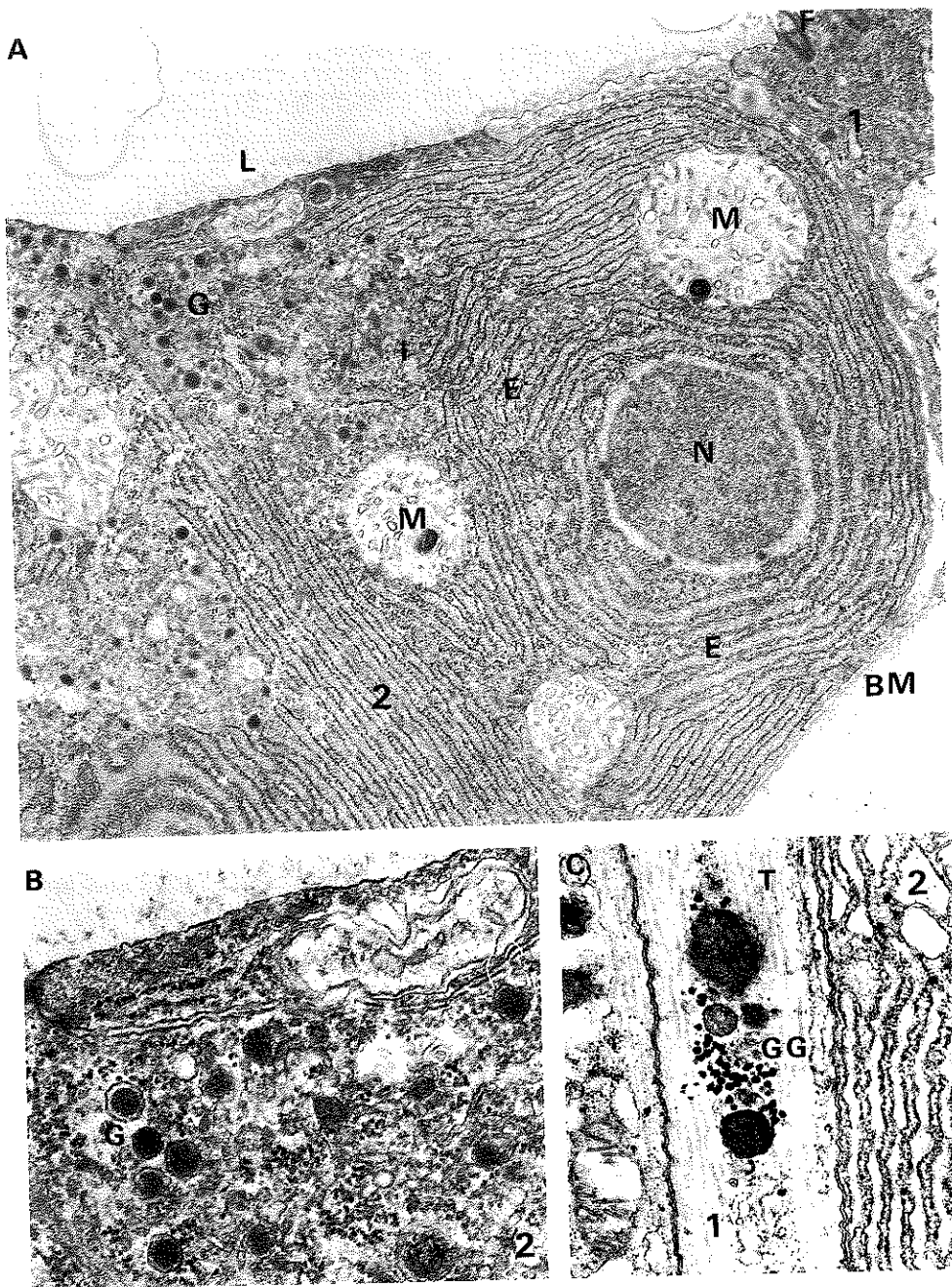


Figure 2. A) Electron microscope section through a part of the flagellate tract 1 and two cells of the glandular band 2 of the endostyle of *Thalía democratica*. 1) flagellate cell with rootlets of flagella F, 2) glandular cells, BM, basal membrane; E, rough endoplasmic reticulum; F, flagella; G, granules of secretion; L, lumen of the endostyle; M, mitochondria; N, nucleus ( $\times 3,500$ ). B) Apical zone of a glandular cell with intravacuolar dense granules G ( $\times 10,000$ ). C) View of two cells of the flagellate tract 1 of the endostyle of *Molgula manhattensis* with glycogen granules GG and bundles of microtubules T. On the right, membranes of the reticulum of the next cell 2 (after Godeaux and Firket, 1968) ( $\times 18,500$ ).

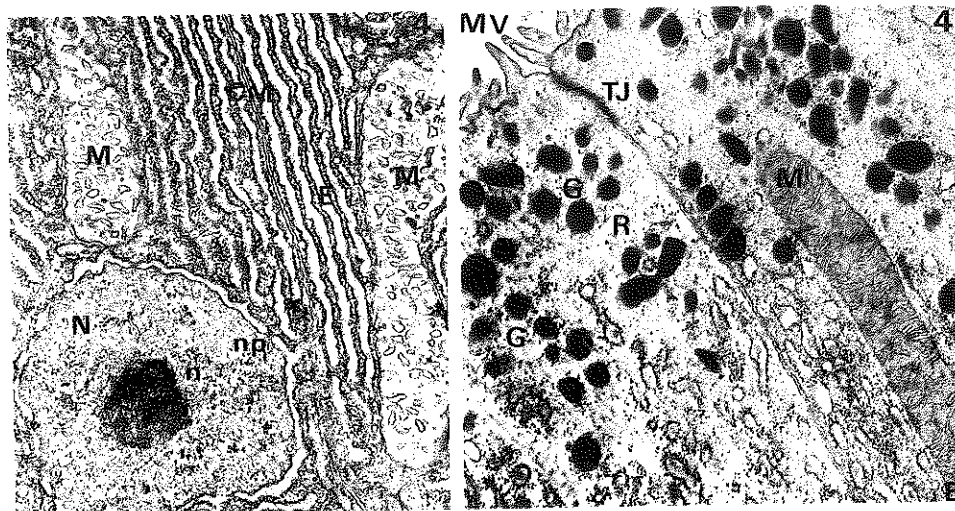


Figure 3. A) Longitudinal section through 2 glandular band 4 cells of *Doliolum nationalis* (phorozooid). CM, cellular membranes; E, membranes of the rough endoplasmic reticulum; M, mitochondrion; N, nucleus with conspicuous nucleolus n and nuclear pores np (after Godeaux, 1981) ( $\times 11,000$ ). B) Apical zone of two bands four cells of *Molgula manhattensis* G, intravacuolar dense secretion granules; M, mitochondrion; MV, microvilli; R, free ribosomes; TJ, thigh junction ( $\times 14,400$ ).

1 cells) with the capacity of secretion of mucous substances, on account of the existence of glycogen in the cytoplasm. This secretory capacity is denied by Olsson (1963) and Godeaux and Firket (1968).

Glandular band 6 is connected with the flattened pharyngeal epithelium by two rows of cells, the naked band 7 and the ciliated band 8. Zone 7 cubic cells have a large nucleus, few mitochondria, numerous electron-lucent vesicles and multivesicular bodies in the apical cytoplasm, a Golgi apparatus and a poorly developed ergastoplasm. They look like ordinary epithelial cells. Numerous investigations done by different authors on many ascidians gave the same general picture of these cells (Fig. 4B).

Attention was focused on the zone 7 cells after the discovery by Barrington (1957) of their ability to accumulate radioactive iodine. Electron microscopic autoradiography revealed the presence of dense clusters of small black dots (Ag) along the apical membrane of the cell and even of large inclusions in the multivesicular bodies. Dense extracellular accumulations of iodinated products are also apparent at the level of the ciliated band 8 (Barrington and Thorpe, 1965b; Thorpe et al., 1972; Thiebold, 1972; Dunn, 1974). After work by Barrington and Franchi (1956), different authors later separated by chromatography the extracts: monoiodotyrosine (MIT), diiodotyrosine (DIT), triiodotyrosine ( $T_3$ ) and tetraiodotyrosine ( $T_4$  or thyroxine) were identified, mainly in free form (Roche et al., 1959; 1962; Barrington and Thorpe, 1965a; Thorpe et al., 1972; Dunn, 1980a).

The presence of "mammalian" thyroglobulin was proved by Thorndyke (1978). Furthermore histochemical reagents proved that the zone 7 cells are stained for peroxidase (Tsuneki et al., 1985), an I-oxidizing enzyme bound to the cell membrane and known as facilitating the iodination of free tyrosine and proteins in vitro (Dunn, 1975; 1980b). The cells of band 7 are obviously unable to synthesize proteins as they are lacking the necessary apparatus, but they can receive the raw

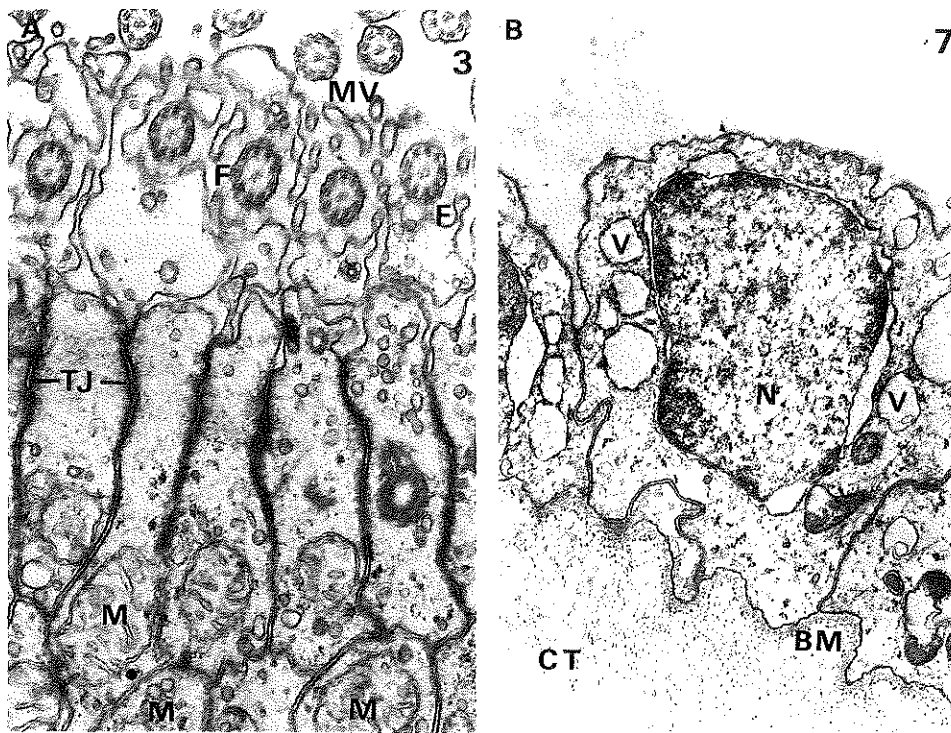


Figure 4. A) Section through the apical zone of the flagellate band 3 of *Molgula manhattensis*. The cells are very slender, show numerous mitochondria M but display no secretory activity; a few empty vesicles are visible. The flagella F are surrounded by a ring of microvilli MV. The tight junctions TJ with dense tonofibrils are conspicuous ( $\times 17,500$ ) B) Section through zone 7 cells of *Molgula manhattensis*. BM; basal membrane; CT, conjunctive tissue; N, nucleus; V, vesicles ( $\times 13,000$ ).

proteins from the other parts of the organ; so their action would be limited to the iodination of secretions.

Cilia of zone 8 carry out the secretions towards the branchial basket where they mixed with the food; iodinated substances have been traced in the gut. Their role remains unknown although thyroxine seems able to induce the onset of metamorphosis of ascidian tadpoles (Patricolo et al., 1981). More recently, a calcitonin-like substance has been detected in a group of cells (human anti-calcitonin rabbit serum positive) lying above the zone incorporating iodine. The granules ( $\theta$ : 100–250 nm) are located in the lower part of the cells below the nucleus and facing the hemocoel. The presence of calcitonin-like molecules was visualized by the immunofluorescence method. The association of I-binding cells and of calcitonin producing cells could be the forerunner of that previously observed in the higher vertebrates (Thorndyke and Probert, 1979).

The capacity of synthesizing organic molecules of great biological importance is not limited to the endostyle. Recently, using indirect immunofluorescence methods coupled to cytochemical techniques and electron microscopy, several authors have shown that various small polypeptides displaying hormonal properties are present as well in the neurons (and nerves) of the cerebral ganglion as in cells widespread in the digestive tract of the ascidians (see review by Fritsch et al., 1982). Substance P, somatostatin, neurotensin, bombesin, cholecystokinin gas-

Table 1. Comparative enzymatic activities of extracts of endostyle and stomach of three species of ascidians (Method API-ZYM)

	<i>Ciona intestinalis</i>		<i>Phallusia mammillata</i>		<i>Halocynthia papillosa</i>	
	Endostyle	Stomach	Endostyle	Stomach	Endostyle	Stomach + "liver"
Proteins $\mu\text{g ml}^{-1}$	180	250	240	250	210	200
Enzymes						
Alcaline phosphatase	3	5	0/1	2	2	4
C <sub>4</sub> esterase	3	3	1	3	3	3
C <sub>8</sub> esterase	3	2	2	2	1	2
C <sub>14</sub> lipase	0/1	0/1	0/1	1	0/1	1
Leucine arylamidase	0/1	2	0	4	1	5
Valine arylamidase	0/1	0/1	0	1	0/1	2
Cystine arylamidase	0	0/1	0	0/1	0/1	1
Trypsin	0	0	0	0	0/1	0
$\alpha$ -Chymotrypsin	0	0	0	0	0	0
Acid phosphatase	3	5	1	3	3	5
Phosphoamidase	2	4	1	3	3	5
$\alpha$ D-galactosidase	0	$\pm 1$	0	1	0	3
$\beta$ D-galactosidase	3	3	2	4	2	5
$\beta$ D-glucuronidase	3	2	1	1	3	3
$\alpha$ D-glycosidase	0/1	0/1	0/1	1	0	2
$\beta$ D-glycosidase	0	0/1	0	1	0	5
N acetyl $\beta$ D-glucosaminidase	3	4	2	3	3	3
$\alpha$ D-mannosidase	0/1	0/1	0/1	1	0	1
$\alpha$ L-fucosidase	3	4	2	3	5	4

trin-like substances and others can be cited. Some of these polypeptides are also known from various invertebrates (sea anemone, earthworm, freshwater snail) and therefore have a long history. Pharmacological experiments are urged in order to understand the action of these substances in the tunicates where the target organs are not obvious. Bombesin and mammalian cholecystokinin stimulate the protein secretion of the stomach of *Styela clava* (Bevis and Thorndyke, 1981; Thorndyke and Bevis, 1984). It can be suggested that some of the polypeptides are also involved in the regulation of the endostylar secretion.

*Endostyle as a Digestive Gland.* — The possibility that the endostyle is an enzyme-secreting organ was first proposed by Godeaux and Firket (1968) and later on by Timashova (1982), but owing to the lack of suitable micromethods, this hypothesis remained a simple suggestion. The only information available at that time was the presence of a protease in ammocoete endostyle (Clements and Gorbman, 1955).

The hypothesis was tested by two methods: a) API-ZYM micromethod. The extracts of the endostyle and stomach of three ascidians: *Ciona intestinalis*, *Phallusia mammillata* and *Halocynthia papillosa*, were used for that purpose. The extracts were tested either concentrated in order to detect the lesser active enzymes or diluted in order to know the stronger active ones (Tables 1 and 2). For practical reasons, stomach and "liver" of *H. papillosa* were examined together as the digestive complex.

The results obtained with the three species are in good agreement and the presence of different kinds of enzymes was proved in the extracts. Three phosphatases were tested with the method: the alkaline one is rather weak but the acid one (currently associated with lysosomes) and the phosphoamidase are very active



Table 2. Comparative enzymatic activities of extracts of endostyle (at three different concentrations) and stomach + "liver" of *Halocynthia papillosa* (Method API-ZYM)

Proteins $\mu\text{g ml}^{-1}$	<i>Halocynthia papillosa</i> endostyle			Stomach + liver
	2,540	800	480	2,100
Enzymes				
Alcaline phosphatase	5	4	1	5
C <sub>4</sub> esterase	4	3	1	2
C <sub>8</sub> esterase	3	1	0/1	3
C <sub>14</sub> lipase	0	0	0	1
Leucine arylamidase	5	2	1	4
Valine arylamidase	0	0	0	5
Cystine arylamidase	1	0	0	1
Trypsin	0	0	0	4
$\alpha$ -Chymotrypsin	0	0	0	0
Acid phosphatase	>5	5	5	5
Phosphoamidase	>5	5	4	4
$\alpha$ D-galactosidase	0	0	0	1
$\beta$ D-galactosidase	5	4	2	5
$\beta$ D-glucuronidase	5	4	2	4
$\alpha$ D-glycosidase	2	1	0	5
$\beta$ D-glycosidase	5	0	0	5
N acetyl $\beta$ D-glucosaminidase	>5	5	5	3
$\alpha$ D-mannosidase	3	0	0	1
$\alpha$ L-fucosidase	5	5	$\pm 5$	4

both in the endostyle and in the digestive tract. The C<sub>4</sub> and C<sub>8</sub> esterases are not very active in either extract and the lipase (C<sub>14</sub>) is very weak in the stomach. The aminopeptidases with the exception of the leucylaminopeptidase display higher activities in the gut than in the endostyle. Chymotrypsin-like enzyme appears missing to both extracts, but a trypsin-like enzyme is present in the stomach.

Contrasting with the above described observations, the glycosidases are well represented and active in the endostyle, especially the  $\beta$  glycosidases: acid  $\beta$  galactosidase,  $\beta$  glucuronidase (often associated with lysosomes), N acetyl  $\beta$  glycoaminidase together with  $\alpha$  fucosidase (at pH 5.4). The same enzymes are also present in the extracts of the stomach and often with a higher activity; it is the case for the  $\alpha$  and  $\beta$  glycosidases in digestive complex of *Halocynthia papillosa*. Only the  $\alpha$  mannosidase displays very little activity in both extracts of the different species.

It must be stressed that the tunicates are feeding on microalgae and on organic particulate matter of vegetal origin.

Extracts of branchial wall exhibit a similar range of enzymatic activities. The absence of glandular cells in this tissue suggests the origin of the enzymes could be the endostyle.

The second test method: b) spectrophotometric micromethods gave more precise results. *Halocynthia papillosa* was the sole species utilized for that purpose (Tables 3 and 4).

After activation by pig enterokinase, the digestive complex trypsin-like enzyme becomes active, but is lacking in the endostyle. In the same manner, after activation with enterokinase, a chymotrypsin-like activity was recorded in both tissues with the same activity. Carboxypeptidase A, after activation with trypsin displays a 2.5 times higher activity in the digestive complex than in the endostyle.



Table 3. Different enzymatic activities of extracts of endostyle and stomach + "liver" of *Halocynthia papillosa* (spectrophotometric methods)

Enzymes	Endostyle	Stomach + liver	
Lipase (C <sub>16</sub> )	0	0.3566	Unit mgr. prot. <sup>-1</sup>
Trypsin	0	12.66	Int. U. mgr. prot. <sup>-1</sup>
Chymotrypsin	111	119	nmol. p-nitraniline mgr. prot. <sup>-1</sup> min <sup>-1</sup>
Carboxypeptidase A	1,130	2,950	nmol. PHE mgr. prot. <sup>-1</sup> min <sup>-1</sup>
Lactase ( $\beta$ galactosidase)	0.205	22.123	nmol hydrol. substrate mgr. prot. <sup>-1</sup> min <sup>-1</sup>
Maltase ( $\alpha$ glycosidase)	5.18	10.04	nmol hydrol. substrate mgr. prot. <sup>-1</sup> min <sup>-1</sup>
Invertase ( $\alpha$ glycosidase)	4.48	2.193	pmol hydrol. substrate mgr. prot. <sup>-1</sup> min <sup>-1</sup>

As far as the lipase (hydrolysing triolein) is concerned, the activity is nil in the endostyle and extremely weak in the nucleus.

The presence of the  $\beta$  galactosidase as lactase (different from the acid galactosidase) and of the  $\alpha$  glycosidases as maltase and saccharase was confirmed in both extracts. The activity of the saccharase is weak especially in the stomachal region; on the contrary, the activities of maltase and especially that of lactase are higher in the digestive tract. Amylase (an  $\alpha$  glycosidase) is also present in the extracts of endostyle and stomach of *Ciona intestinalis*, *Phallusia mammillata* and *Halocynthia papillosa*; except in *Halocynthia papillosa*, the concentrations are identical in both tissues (Table 4). Chitinase is absent in the gut (Jeuniaux, 1963). These results are in rather good agreement with those published by Koch and Marsh (1972) on *Pyura solonifera*.

Catalase can be easily detected: bubbling initiates as soon as the extracts are put into contact with oxygenated water.

The presence of digestive exoenzymes in the extracts of the endostylar tissue leads to the conclusion that this organ functions in the digestion as its secretion products are mixed with the food particles as soon as they are trapped by the mucus, a property evidently bound to the microphagy.

*Endostyle as a Blastogenetic Anlage.*—Many species of Ascidiacea and all the Thaliacea can reproduce both sexually and asexually. The blastogenetic ascidians develop buds in different manners: each bud consists primarily of a double vesicle with blood cells between the walls. The outer vesicle is always derived from the epidermis of the mother and just gives the epidermis of the offspring. On the contrary the inner vesicle is of various origins according to the species considered: it can derive either from the ectodermal, or from the endodermal or from the mesodermal tissues of the mother and will give most of the internal organs and particularly the whole digestive system. But whatever the origin of the inner vesicle, it has nothing to do with the endostyle.

In *Pyrosomas* and also salps, but not in doliolids, the endostyle plays an important role in the constitution of the stolon and thus of the buds. Early in the development of the oozoid of the *Pyrosomas* (cyathozoid) appears a stolon outgrowth. A cross-section shows that the stolon is limited by an epithelium of epidermal origin as usual, and contains extensions of the main organs, say the endostyle, the peribranchial tubes and the pericardium. Blood cells are present between the anlages. At first an important feature must be stressed: the blastogenesis of *Pyrosomas* is more precisely a strobilisation as each tissue retains its original embryonic value. The endostyle caecum will generate the whole digestive system of the blastozoid, as the pericardium and the peribranchial tubes will

Table 4. Amylase activity of extracts of endostyle and stomach (including "liver" in *H. papillosa*) of three species of ascidians (E.U.: enzyme activity hydrolysing 1 mg starch min<sup>-1</sup>)

	<i>Ciona intestinalis</i>		<i>Phallusia mammillata</i>		<i>Halocynthia papillosa</i>	
	Prot. µg/ml	E.U.	Prot. µg/ml	E.U.	Prot. µg/ml	E.U.
Endostyle	135	0.60	75	0.47	210	0.47
	180	0.56	240	0.87	210	0.52
					180	0.50
					270	0.62
Stomach	250	0.46	250	0.50	200	1.0
	250	0.44	250	0.42	200	1.2
					180	0.78
					270	1.07

develop either cardiopericardial or peribranchial cavities. Only the nervous system is neoformed.

Figure 5 shows the cross-sections of a primary stolon and of a secondary stolon of *Pyrosoma atlanticum* which obviously display the same general organization.

In a fullgrown primary blastozoid of *Pyrosoma*, the posterior end of the endostyle remains in an undifferentiated stage, grows and pushes the nearby epidermis. It is accompanied by the peribranchial and pericardial tubes and some blood cells, the future primordia of the offspring. The young stolon progressively protrudes into the common tunic envelope of the colony, increases and divides into successive buds. There are 4 buds in *Pyrosoma atlanticum*, the best known species (Fig. 6) and up to 100 in *Pyrosoma vitjasi* (Ivanova-Kazas, 1958).

The endostylar anlage swells and develops into a large pharyngeal cavity, which is flanked by the peribranchial cavities derived from the peribranchial tubes. Gill slits appear. The intestinal loop comes from a fingerlike caecum arisen from the dorso-posterior wall of the pharynx. The cardiopericardium lies ventrally under the intestine (Fig. 6). Progressively the stolon becomes simplified and reduced to a simple vascular vessel divided into two longitudinal lacunae by a transverse septum, the sole trace of the former endostylar tube; in the lacunae, the blood, moved by the heart of the mother, runs throughout the stolon.

In every secondary blastozoid, the posterior part of the endostyle also remains undifferentiated after rupture of the stolon vessel. As soon as the blastozoid becomes fullgrown, budding initiates. The endostyle embryonic piece of all the blastozoids is the direct descent of the oozoid organ, multiplied by a tremendous budding process.

One can suggest that endostyle anlage induces the growth of the stolon; up to now it remains simply an hypothesis. On both sides of the endostylar pouch stands a transient enigmatic organ called eleoblast, the role of which is still poorly understood: it could be a temporary yolk storing tissue slowly disappearing as the new individual develops. Eleoblast could also play a role in the initiation of budding (Godeaux, 1960).

The structure of the stolon and merely the participation of the endostyle in its building is a valuable argument in favor of a close relationship between salps and *Pyrosomas* among the Thaliacea, despite their quite different habitus. The doliolids are excluded: they appear closer to the stock of Aplousobranchia owing to the presence of the epicardia in the stolon.

Another argument is the existence of a single protostigma in the oozoid embryos of the Thaliacea instead of two or more as in the Ascidiacea. That strongly

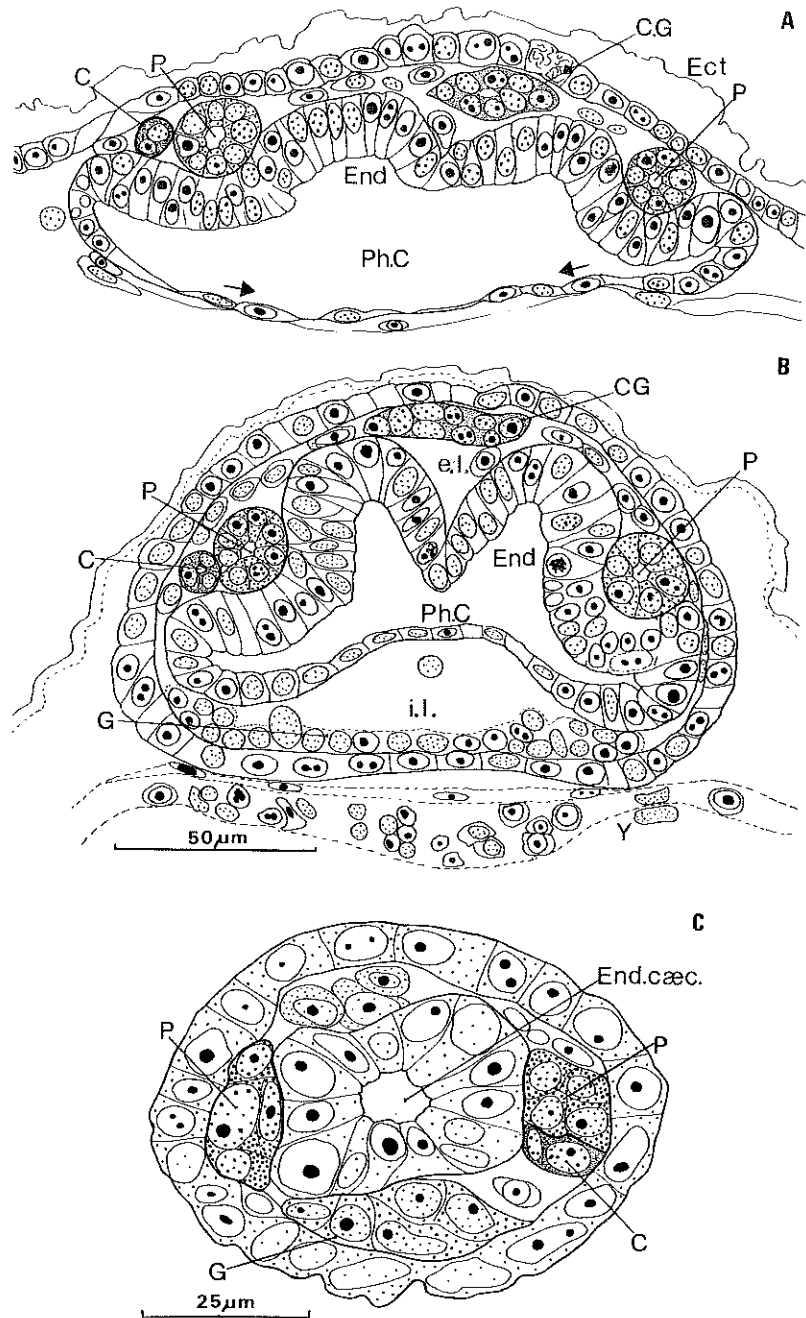


Figure 5. Cross sections through a primary (1–2) and a secondary (3) stolons of *Pyrosoma atlanticum*. A) section through the bud no. 4. The bud is not yet enveloped by the epidermal layer (Ect) and the pharyngeal cavity (Ph.C) has just closed (→) below the endostylar fold (End). B) section through the bud no. 2 of the same stolon. C) section through a young secondary stolon. The blood lacunae are visible above (e.l.) and below (i.l.) the endostylar caecum (End. caec.) C, cardiopericardium; CG, cerebral ganglion; Ect, ectodermal layer; End, endostylar fold; End.caec., endostylar caecum; G, ger-

suggests *Pyrosomas* and salps separated very early from the ancestral stock of the tunicates and surely before the first appearance of any protostigma division. In Aplousobranchia tadpoles, there are always two primary protostigmata (as rows of small stigmata) soon divided into four protostigmata, a condition retained by the colonial Didemnidae (*Didemnids* sp.) and Polycytoridae (*Distaplia* sp.).

#### CONCLUSIONS

The endostyle is an organ with diverse functions. It produces the mucous secretions indispensable to the food trapping by these filter-feeders which are the adult protochordates and the ammocoetes larvae before metamorphosis. All the other chordates (vertebrates) are macrophagous during their whole life. Sorberacea, a class of tunicates recently described by Monniot et al. (1975), which feed on large prey, have the endostyle very reduced or even lacking.

The glandular cells of the endostyle possess a well developed rough endoplasmic reticulum, similar to that displayed by protein synthesizing organs (e.g., exocrine pancreas). The endostyle produces digestive enzymes, most probably elaborated in its glandular cells, the only ones presenting obvious traces of secretory activities. These enzymes are able to hydrolyse small lipidic molecules (esterases), polypeptides and proteins (amino-peptidases and chymotrypsin) and especially polysaccharides (glycosidases). The same enzymes along with some others are also present in the extracts of the stomach region. It must be stressed that it is not yet possible to decide whether the glandular cells can elaborate all the enzymes or not. As shown above, the band 6 cells are not quite similar to the bands 2 and 4 cells; the differences could reflect different capacities.

The lancet fish endostyle is very similar to the organ of the tunicates. It would be pertinent to investigate its ability to produce digestive organs. If it is so, that capacity could then be related to the protochordates microphagous mode of feeding.

Another interesting feature is the capacity of binding inorganic iodine displayed by cells homologous to those developing the thyroid gland of the lamprey. The binding reaction is rendered easier by a membrane bound peroxidase, also present in vertebrates. The iodinated molecules are derivatives of the amino acid tyrosin and proteins, but the functions of these molecules in the protochordates remains an enigma. Maybe they are stimulating the general metabolism as done in the vertebrates!

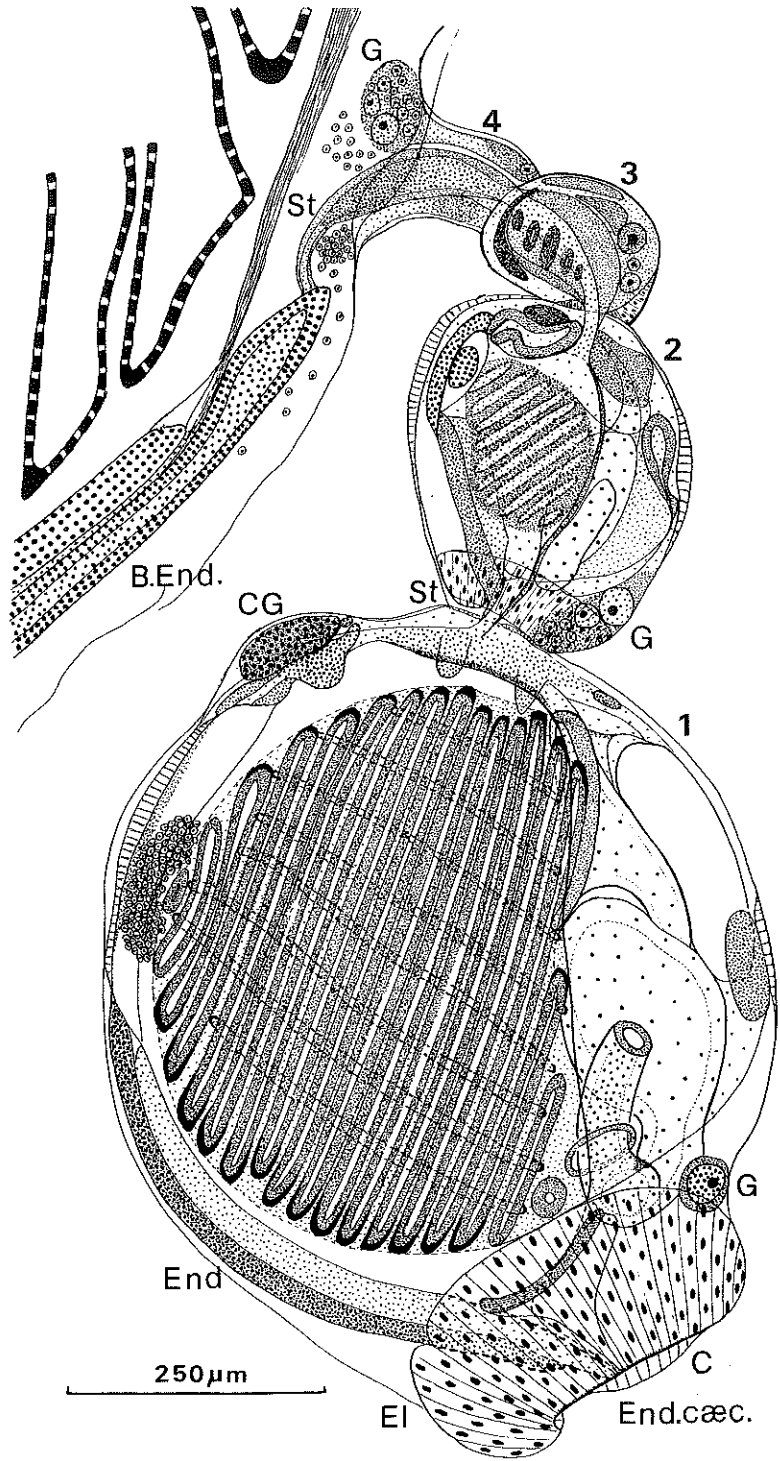
Lastly the endostyle also plays an important role in the blastogenetic processes (strobilisation) of the *Pyrosomas*. Contrary to what is observed in the other budding tunicates, the different components of the *Pyrosomas* stolon keep the potentialities of the primary germ layers which they originate from. There is no depression of the genes, an argument supporting the hypothesis *Pyrosomas* (and salpids) represent an offshoot branched early from the tunicate stock and before budding processes diversify so intensively in the ascidians (see Garstang, 1928). Another argument is the existence of a single protostigma.

Blastogenesis in *Pyrosomas* looks primitive and reminds the regeneration processes known from the lower invertebrates.

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minal anlage; P, peribranchial tubes; Ph.C., pharyngeal cavity of the bud; Y, yolk droplet of the embryo (after Godeaux, 1957).



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Figure 6. Lateral view of a secondary stolon of *Pyrosoma atlanticum* bearing four buds (1 to 4) at different stages of development. B, End, endostyle of the budding zooid; C, cardiopericardium with its stolon outgrowth; CG, cerebral ganglion; El, eleoblast; End, endostyle of bud 1 and End caec; its endostylar caecum, starting zone of its own stolon; G, germinal cells; St, stolon (after Godeaux, 1957).

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