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Functional micro-anatomy of the digestive gland of the scallop *Pecten maximus* (L.)

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Abstract

The digestive gland of *Pecten maximus* consists, as in other lamellibranchs, of numerous blind-ending tubules which communicate with the stomach by partially ciliated main ducts and non ciliated secondary ducts. The non-ciliated cells of the main ducts are characterized by a well developed brush border constituted by high and dense microvilli and a strong pinocytotic activity. Ciliated and non ciliated cells have a very similar fine structure. The digestive tubules have a large lumen and contain digestive cells at different stages of absorption , digestion and excretion, one part of the tubules being functional while the other is disintegrating. The dark crypts contain the flagellated secretory cells, characterized by a well developed granular endoplasmic reticulum, and the young immature cells which may replace both the secretory and digestive cells. The numerous lipid droplets occurring in the digestive duct cells and in the digestive cells reveal the lipid storage function of the digestive gland.

Several enzyme activities involved in digestion have been localized in the digestive gland. High amylase activity and cellulase and lysozyme activities have been found in the ducts and in the tubules, whereas no proteolytic activity could be detected histochemically. Some intracellular peptidases and glycosidases have been localized in the cells of the digestive gland, especially in the brush-border cells of the ducts and in the functional part of the tubules. High alkaline and acid phosphatase activities are displayed by the duct brush-border cells and the digestive and secretory cells. These results show the main role of the digestive gland, both in extracellular digestion (secretion of the digestive enzymes) and in absorption and intracellular digestion and provide information on the respective functions of the different cells within these processes.

Keywords : Scallop, *Pecten maximus*, digestive gland, enzymes, ultrastructure.

Micro-anatomie fonctionnelle de la glande digestive de la coquille Saint-Jacques Pecten maximus *(L.).*

Résumé

La glande digestive de *Pecten maximus* consiste, comme chez les autres lamellibranches, en nombreux tubules aveugles qui communiquent avec l'estomac par des conduits principaux partiellement ciliés et des conduits secondaires non ciliés. Les cellules non ciliées des conduits principaux sont caractérisées par une bordure en brosse bien développée constituée de microvillosités nombreuses et hautes ainsi que par une forte activité pinocytaire. L'ultrastructure générale des cellules ciliées et non ciliées est similaire. Les tubules digestifs présentent une grande lumière et contiennent des cellules digestives à différents stades d'absorption, digestion et excrétion, une partie du tubule étant fonctionnelle tandis que l'autre se désintègre. Les cryptes sombres contiennent les cellules sécrétrices flagellées caractérisées par un reticulum endoplasmique granulaire bien développé et les jeunes cellules immatures qui doivent remplacer à la fois les cellules sécrétrices et digestives. Les nombreuses gouttelettes lipidiques observées

dans les cellules des conduits digestifs et dans les cellules digestives révèlent la fonction de stockage des lipides de la glande digestive.
Plusieurs activités enzymatiques impliquées dans la digestion ont été localisées dans la glande digestive. Une forte activité amylasique ainsi que des activités cellulasiques et lysozymiques ont été révélées dans les conduits et dans les tubules mais aucune activité protéolytique n'a pu être décelée histochimiquement. Des peptidases et des glycosidases intracellulaires ont été localisées dans les cellules de la glande digestive, plus particulièrement dans les cellules à bordure en brosse des conduits et dans la partie fonctionnelle des tubules. De fortes activités de phosphatases alcalines et acides ont été trouvées dans les cellules sécrétrices et digestives. Ces résultats montrent le rôle essentiel de la glande digestive, à la fois dans la digestion extracellulaire (sécrétion d'enzymes digestives) et dans l'absorption et digestion intracellulaire et fournit des informations sur les fonctions respectives des différentes catégories cellulaires au cours de ces processus.

Mots-clés : *Pecten maximus*, enzymes, fonction, glande digestive, ultrastructure.

INTRODUCTION

It is well established that the digestive cells of Bivalves engulf and digest food particles intracellularly (*see* the review of Morton, 1983); but it is still controversial whether the digestive process is continuous (Owen, 1955, 1970; Robinson and Langton, 1980; Robinson *et al.*, 1981; Robinson, 1983; Henry, 1987) or discontinuous (Morton, 1956; McQuinston, 1969; Mathers, 1972, 1976), the controversy being often related to the various environmental and experimental conditions. Additionally, the functions of the other cell types of the digestive gland are still under discussion. Basiphilic cells are sometimes considered to be a possible source of digestive cell replacement, alternatively they may function as mature secretory cells (Morton, 1983). In the latter case, this cell type could be the source of extracellular enzymes acting in the lumen of the tubules or the stomach, another disputed hypothesis. Although many studies have been carried out on the digestive tubules, the digestive ducts have not been well investigated, although they constitute an important part of the digestive gland. They have been recognized to have both absorptive (Mathers, 1972; Boucaud-Camou *et al.*, 1985) and storage (Henry, 1987) functions in some species, however they may also play a digestive role. Moreover, to our knowledge, the digestive gland of *Pecten maximus* has rarely been studied at the ultrastructural level (Beninger and Le Pennec, 1991).

In an attempt to answer some of these questions and to further investigate the controlling mechanisms for the synthesis and secretion of digestive enzymes, we undertook both ultrastructural and histoenzymological studies of the digestive gland of the juvenile and adult scallop, *Pecten maximus* (L.).

MATERIAL AND METHODS

Materials

Adult scallops *Pecten maximus* (L.) were dredged in the Bay of Seine, from a population studied for growth, metabolism and sexual maturation over the last 5 years (Lubet *et al.*, 1987). Juveniles (1-2 cm diameter) were obtained from the IFREMER nursery of Argenton. The animals were maintained for 48 hours at 15°C in filtered (0.2 µm) sea water before experimentation. Fasted (48 hours) and fed (for 4 to 6 hours with *Pavlova* or *Tetraselmis*) animals were used for histology, cytology and histoenzymology. These two experimental conditions being performed to look for eventual changes in histoenzymology and ultrastructural features.

Methods

Histology

Pieces of digestive gland (adults) or the whole stomach-digestive gland complex (juveniles) were fixed in Bouin-Hollande fixative or saline formalin, dehydrated and embedded in paraffin. Sections were stained with the Prenant-Gabe trichrome (Gabe, 1968).

Electron microscopy

Very small pieces of digestive gland were fixed in 2% glutaraldehyde in 0.1 M (pH 7.4) Sörensen buffer with 8% NaCl, then post-fixed in 2% osmium tetroxide in the same buffer dehydrated and embedded in Epon. Semi-thin sections were stained by Azure blue. Ultrathin sections were cut with a diamond (Diatome). The sections were cut at SCEM ([1]) and observed on a TEM (Philips 400 T) at CMEMA ([2]), Faculty of Sciences, Saint-Jérôme, Marseille, France.

Histo-enzymology

Pieces of digestive gland (adults) or the whole stomach-digestive gland complex (juveniles) were frozen

([1]) SCEM : Service Commun de préparation des Echantillons pour la Microscopie.

([2]) CMEMA : Centre Commun de Microscopie Electronique et de Microanalyse X.

in liquid nitrogen cooled freon, then cut in a cryostat (10-14 µm sections) at −30°C. Substrate film methods (Daoust, 1965) were used whenever possible (for amylase, cellulase, laminarinase and lysozyme, table 1). In the methods the unfixed fresh frozen sections are laid on a coloured film of the natural substrate; after incubation in a moistered chamber at 37°C, the sites of enzymes are digested and appear in clear (*fig.* 13, 14, 15). Gelatin, cellulose and starch are filmogen substrates whereas laminarine and *Micrococcus lysodeikticus* have to be incorporated into gelatin film (gelatin was used instead of agar preconised in Speece's method). Gelatin films were always fixed by formalin (modification of Chrétien's method). Precipitation methods were used for the other enzymes. In these methods, the artificial substrate, a naphthol or a naphthylamide compound, is cleaved by the enzyme action and the free naphthol or naphthylamide reacts with a diazonium salt to give an insoluble azo-dye. Simultaneous coupling was used (*table* 1). These methods are mostly qualitative. However, the intensity of the enzyme activity is roughly estimated by the rate of digestion of the substrate for substrate-film methods, by the intensity of the azo coloration for precipitations methods.

Table 1. – Localization of enzymatic activities in the digestive gland of *Pecten maximus*.

Enzymes	Methods	Localization	
		Ducts	Tubules
Amylase	Shear and Pearse (1963)	+ + +	+ + +
Cellulase	Arnould and Bouchez-Decloux (1978)	+	+ + +
Laminarinase	Arnould and Bouchez-Decloux (1978)	−	−
Lysozyme	Speece (1963) (modified)	+	+
N-acetyl-glucosaminidase	Lojda *et al.* (1979)	+ +	+
β-glucuronidase	Lojda *et al.* (1979)	−	+ + (*)
Acid phosphatase	Grogg and Pearse (1953) mod. by Gabe (1968)	+ + +	+ +
Alkaline phosphatase	Pearse (1953) mod. by Lojda *et al.* (1979)	+ + +	+ +
Non specific esterase	Gomori (1952) mod. by Lojda *et al.* (1979)	+ + +	+ +
Proteases (modified)	Chrétien (1965)	−	−
Chymotrypsine	Lagunoff (1967)	−	−
Dipeptidylamino-peptidase I (D.A.P. I)	Lojda *et al.* (1979)	+	−
Dipeptidylamino-peptidase II (D.A.P. II)	Lojda *et al.* (1979)	+ + +	−
Dipeptidylamino-peptidase IV (D.A.P. IV)	Lojda *et al.* (1979)	+ + +	−
Aminopeptidase I	Moore *et al.* (1980)	+ + +	−

− : no activity; + : low activity; + + : medium activity; + + + : strong activity.

(*) Between the tubules.

RESULTS

Structure

The digestive gland of the scallop *Pecten maximus* consists, as in other Bivalvia, of primary ducts arising from the stomach, followed by thinner, branched secondary ducts opening into the digestive diverticula. At the light microscopy level, the main ducts classically show an epithelium composed of tall cells characterized by a well developed brush border and a deep ciliated groove. The cells of the secondary ducts possess a lower non ciliated brush border and contain abundant lipid droplets.

On semi-thin sections, the digestive tubule are ovoid or tubular, and for the most part, consist of two distinct regions (*fig.* 1). On one side they present

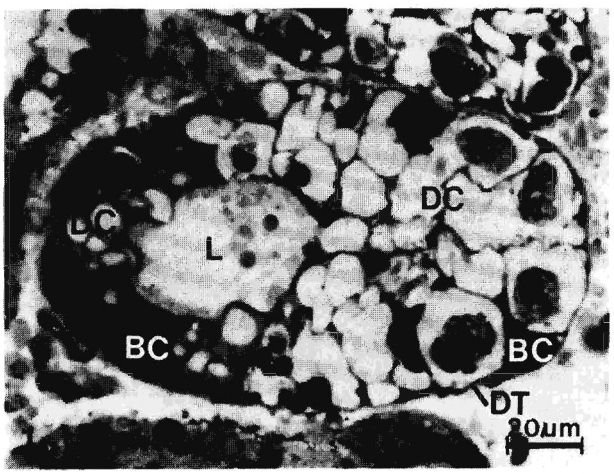


Figure 1. – Photomicrograph of a digestive tubule (DT) with two distinct regions: in one region (left) functional digestive (DC) and basiphilic (BC) cells surround a large lumen (L); the other region (right) is disintegrating.

a wide lumen surrounded by digestive and basiphilic cells. In this region, the digestive cells contain numerous vacuoles and lipid droplets; some fragmentation spheres also occur in the lumen. The basiphilic cells extend from the basal lamina to the lumen. In other parts of the tubule, the central lumen is occluded. Here, the digestive cells are disintegrating and comprise only large clear vacuoles with residual bodies. The basiphilic cells lie in clusters within the confines of the basal lamina of the tubule. Between the digestive tubules amoebocytes are observed.

Ultrastructure

The ducts

At the ultrastructural level, the brush border has long and numerous microvilli and demonstrates numerous pinocytotic vesicles (*fig.* 2). The subapical zone shows many mitochondria, multivesicular bodies

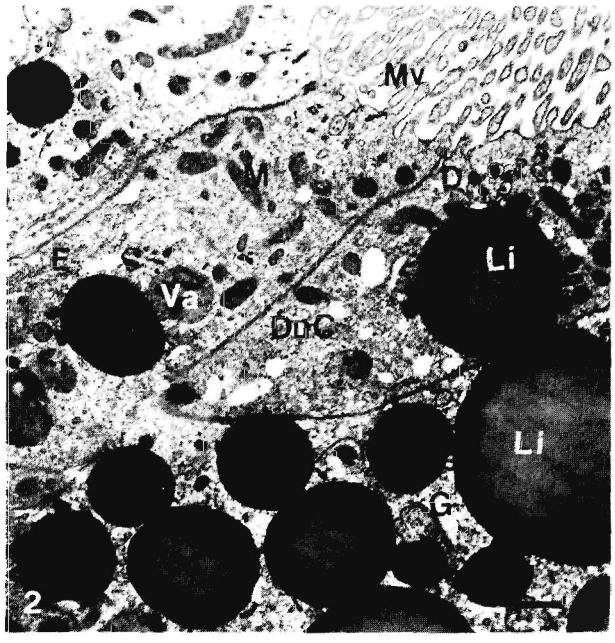


Figure 2. – Electromicrograph. Epithelial duct cells (DuC) united by desmosomes (D). They present microvilli (Mv), numerous mitochondria (M), few ergastoplasmic lamellae (E), autophagic vacuoles (Va), two Golgi bodies (G) and abundant lipid droplets (Li). The scale bar represents 1 µm.

and polysomes. A few ergastoplasmic lamellae, autophagic vacuoles, peroxisomes and lipid droplets are observed in the mid cytoplasm. One or two Golgi bodies are observed in the juxtanuclear zone; at their bases are some dense bodies. The elongated nucleus comprises marginated heterochromatin and abundant

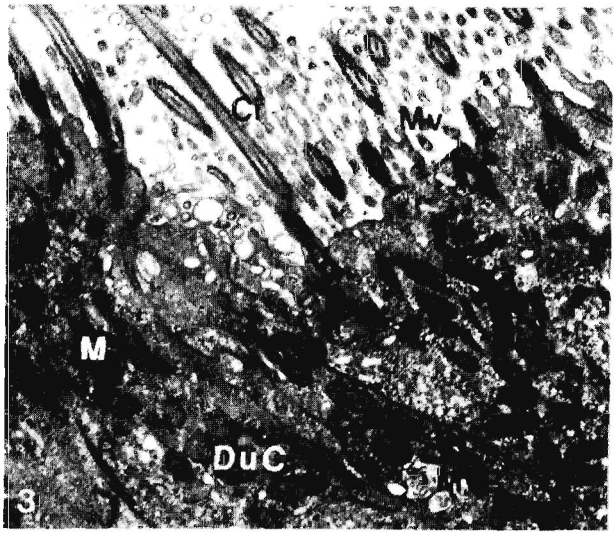


Figure 3. – Electromicrograph. Ciliated duct cells (DuC) characterized by long cilia (Ci) scattered between the microvilli (Mv). The ciliary roots (R) are long and in close contact with mitochrondria (M). Abundant multivesicular bodies (MVB) are observed. The scale bar represents 1 µm.

euchromatin; the nucleolus is round and voluminous; it shows a fibrillar core surrounded by a granular cortex.

In the groove of the main ducts, the cells (*fig.* 3) are characterized by several long cilia scattered through the microvilli. The roots of the cilia are classically in contact with long mitochondria. We noticed numerous multivesicular bodies in the apical region. We sometimes observed mucus cells between ciliated cells: their apical zone is filled with amorphous, voluminous granules, some of which are seen empty in the lumen.

The digestive tubules

The epithelium of the digestive tubules consists of digestive cells, with crypts of electron dense cells scattered among them (*fig.* 4 to 12).

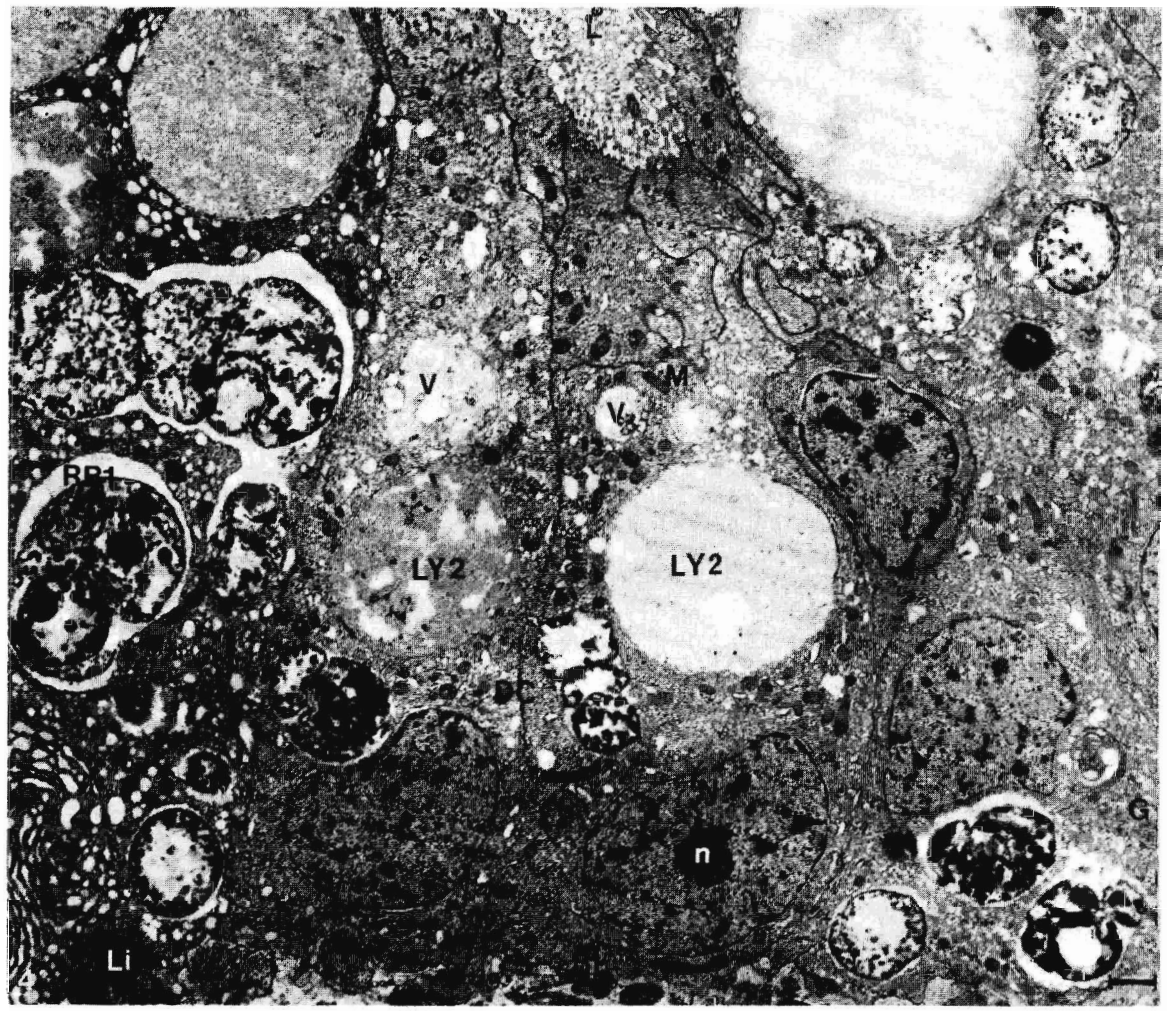
The digestive cells

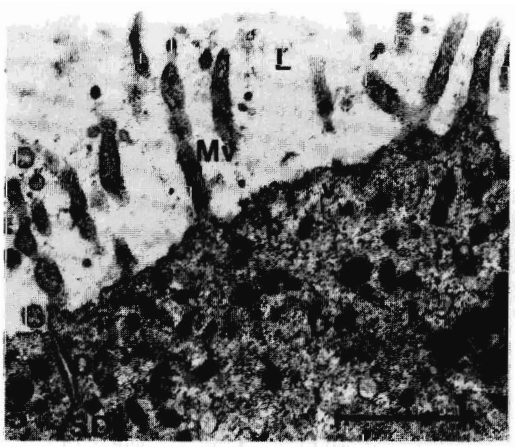
Different types of digestive cells are observed in a single digestive tubule, reflecting different phases of activity:

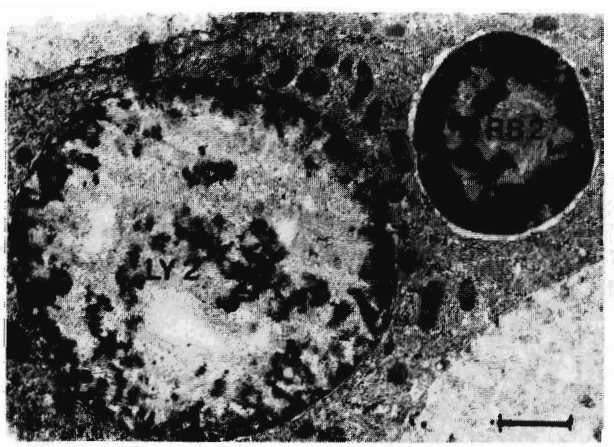
The first type consists of rectangular, elongate absorptive cells extending from the basal lamina to the lumen of the tubule (*fig.* 4). They usually show an oval nucleus containing a voluminous nucleolus located at the basal pole. The Golgi apparatus, always situated in the juxtanuclear zone, is small and generally horseshoe shaped. The apical surface (*fig.* 5) always bears microvilli with pinocytotic vesicles at their bases. They are easily recognizable by their fuzzy coat. These cells are united by a belt desmosome followed by a septate junction. The subapical cytoplasm is filled with dense short tubules and clear vesicles (*fig.* 5). In the cytoplasm (*fig.* 4 and 6) we found different types of vacuoles, constituting the digestive vacuolar apparatus: large vacuoles are clear and have a more or less regular shape. They exhibit considerable variations in size and are always located in the apical region. In contrast, the secondary lysosomes are found in the mid cytoplasm. They are characteristically round, large, and have a heterogeneous content of fibrillar and/or granular material (*fig.* 4 and 6). All residual bodies concentrate an electron dense material separated by a clear halo from the limiting membrane. The residual bodies of type 1 (*fig.* 4) contain several clumps enclosed within a large vacuole and are bigger than the residual body of type 2 (*fig.* 6). These RB2 are electron dense and always show a single mass of residues.

The second type of digestive cell is recognizable by the fragmented apical zone protruding into the lumen (*fig.* 7, arrows). The plasma membrane of the spherule has lost most of the microvilli. In the cytoplasm, numerous secondary lysosomes and residual bodies of both types accumulate.

The third type of digestive cell present in the same tubule are disintegrating digestive cells (*fig.* 8): they





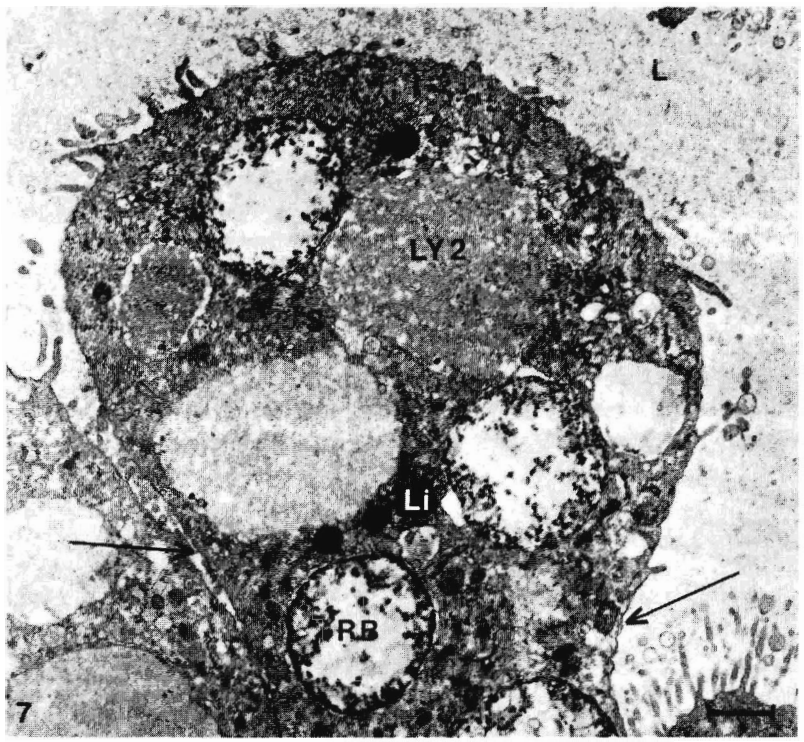


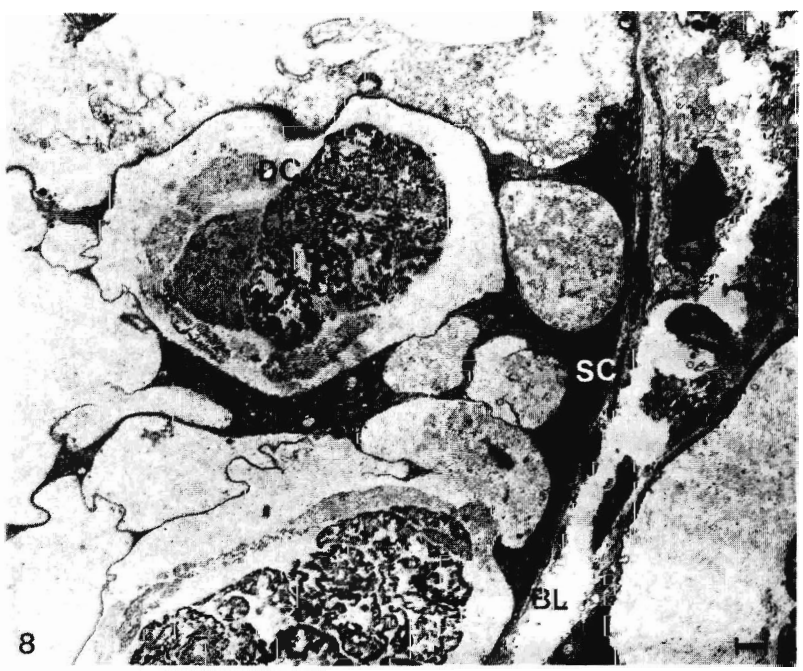
Figures 4 to 6. – Electromicrographs. Digestive cells (DC) in the absorptive-digestive phase. The scale bar is 1 µm.

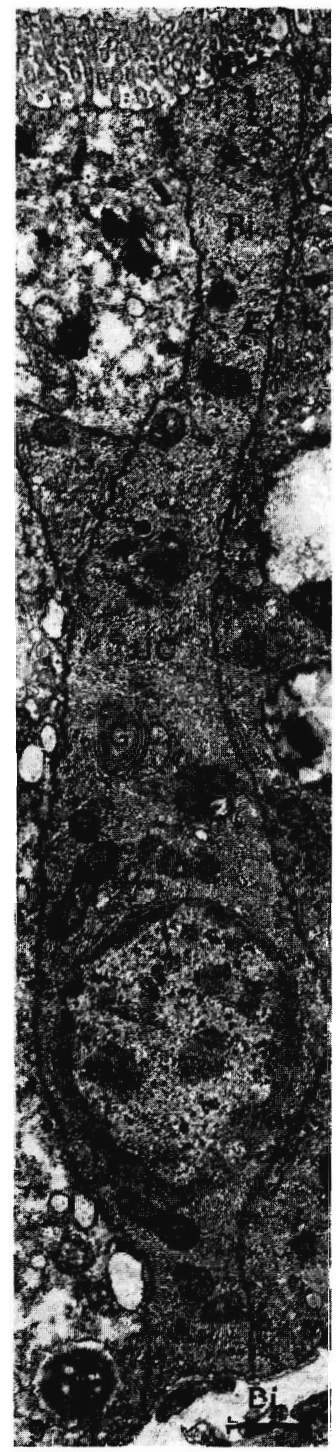
Figure 4. – The cells extend from the basal lamina (BL) to the lumen (L) and show a basal nucleus (N) containing a voluminous nucleolus (*n*). Numerous mitochondria (M), a Golgi body (G), a few ergastoplasmic lamellae (E), vacuoles (V), secondary lysosomes (Ly2) and residual body of type 1 (RB1) are present.

Figure 5. – The digestive cells (DC) are united by a belt desmosome (D) followed by a septate junction (SD). Their apical surfaces exhibit microvilli (Mv) with numerous pinocytotic vesicles (*p*) at the base. In the subapical cytoplasm are dense tubules (*t*) and clear vesicles (*v*).

Figure 6. – Detail of secondary lysosomes (LY2) and residual body of type 2 (RB2).







Figures 7 to 12. – Electromicrographs. The scale bar on figures 7-12 represents 1 µm.

Figure 7. – Spheres (S) of fragmentation protruding (arrows) in the lumen (L) and containing lipids (Li) secondary lysosomes (LY2) and residual bodies (RB).

Figure 8. – Part of the digestive tubule limited by the basal lamina (BL). Note the disintegrating digestive cells (DC) and small secretory cells (SC).

Figure 9. – The undifferentiated cell (UC) is united to the digestive cells by belt desmosomes (D) followed by septate junctions. It bears numerous microvilli (Mv). Note the abundance of ribosomes (Ri), the poorly developed ergastoplasm (E), lysosomes (LY), mitochondria (M) and Golgi apparatus (G).

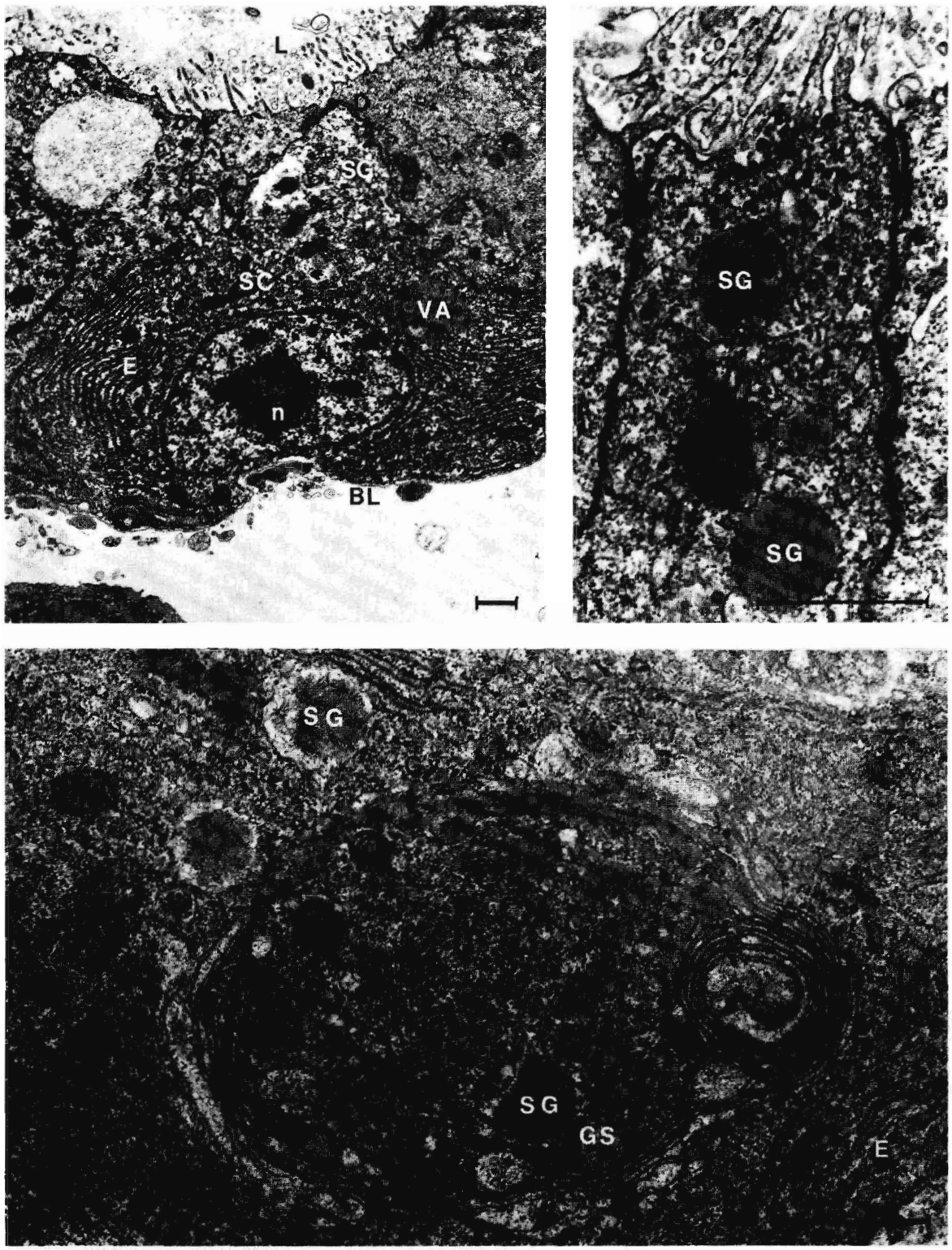


Figure 10. – The pyramidal secretory cell (SC) extends from the basal lamina (BL) to the lumen (L); the basal nucleus (N) shows a voluminous nucleolus (*n*). The cytoplasm exhibits numerous long parallel ergastoplasmic lamellae (E) autophagic vacuoles (VA) and secretory granules (SG).

Figure 11. – Dense and homogenous secretory granules at the apical pole of the secretory cell.

Figure 12. – The prominent Golgi apparatus (G) of the secretory cell: remark the small dense Golgi vesicles (Gv) and huge secretory granules (SG) showing a more or less granular content.

have an empty cytoplasm and lack cytoplasmic organelles except residues enclosed in large clear vacuoles. They cluster on one side of the tubule; a basal lamina always surround the digestive diverticula. Small secretory and undifferentiated cells occur between degenerating digestive cells.

The electron dense crypts

The electron dense crypts of the digestive tubule, show different flagellated cell types principally composed of undifferentiated cells and secretory cells.

The undifferentiated cells (*fig.* 9) extend from the basal lamina to the lumen and are united by apical belt desmosomes to adjoining digestive or secretory cells. They are columnar; between their microvilli occur one or two flagella. They possess numerous ribosomes, free ergastoplasmic lamellae, mitochondria, rare lysosomes and autophagic vacuoles. The dictyosomes are composed of four or five concentric thin saccules and small vesicles. The basal nucleus is round and contains usually one central nucleolus.

The secretory cells (fig. 10 to 12)

In the active part of the digestive tubule, secretory cells are pyramidal in shape, flagellated and extend from the basal lamina to the lumen. They are characterized by an oval basal nucleus containing a voluminous nucleolus with a clear fibrillar core and a dense granular periphery. The cytoplasm is filled with long numerous parallel ergastoplasmic lamellae, mitochrondria, peroxisomes, voluminous autophagic vacuoles and abundant secretory granules. The secretory granules often accumulate in the apical region and contain a homogenous concentrated material (*fig.* 10 and 11). The Golgi apparatus (*fig.* 12) is prominent and consists of stacks of saccules concentrically arranged and clumps of Golgi vesicles. On the *cis* face, the first dilated saccule is electron lucent and shows a fibrillar material; the other saccules are thinner and contain a dense granular material; their extremities are swollen. Secretory granules are seen on the *trans* face, but are also present scattered throughout the cytoplasm, their content becoming more and more dense as they approach the apical pole of the cell.

Secretory cells observed in the degenerating part of the tubule are small and clustered close to the basal lamina.

Localization of enzymatic activities

A high amylasic activity was found in the lumen of the digestive gland (*table* 1 and *fig.* 13). Lower cellulolytic and lysozyme activities were also found (*table* 1, and *fig.* 14-15). A high N-acetylglycosaminase activity was localized in the ducts, especially in the brush-border cells (*fig.* 16-17) and a lower one in the active part of the tubules (*fig.* 16). A β-glucuronasic activity has been detected between the digestive tubules, probably located in amebocytes (*fig.* 18).

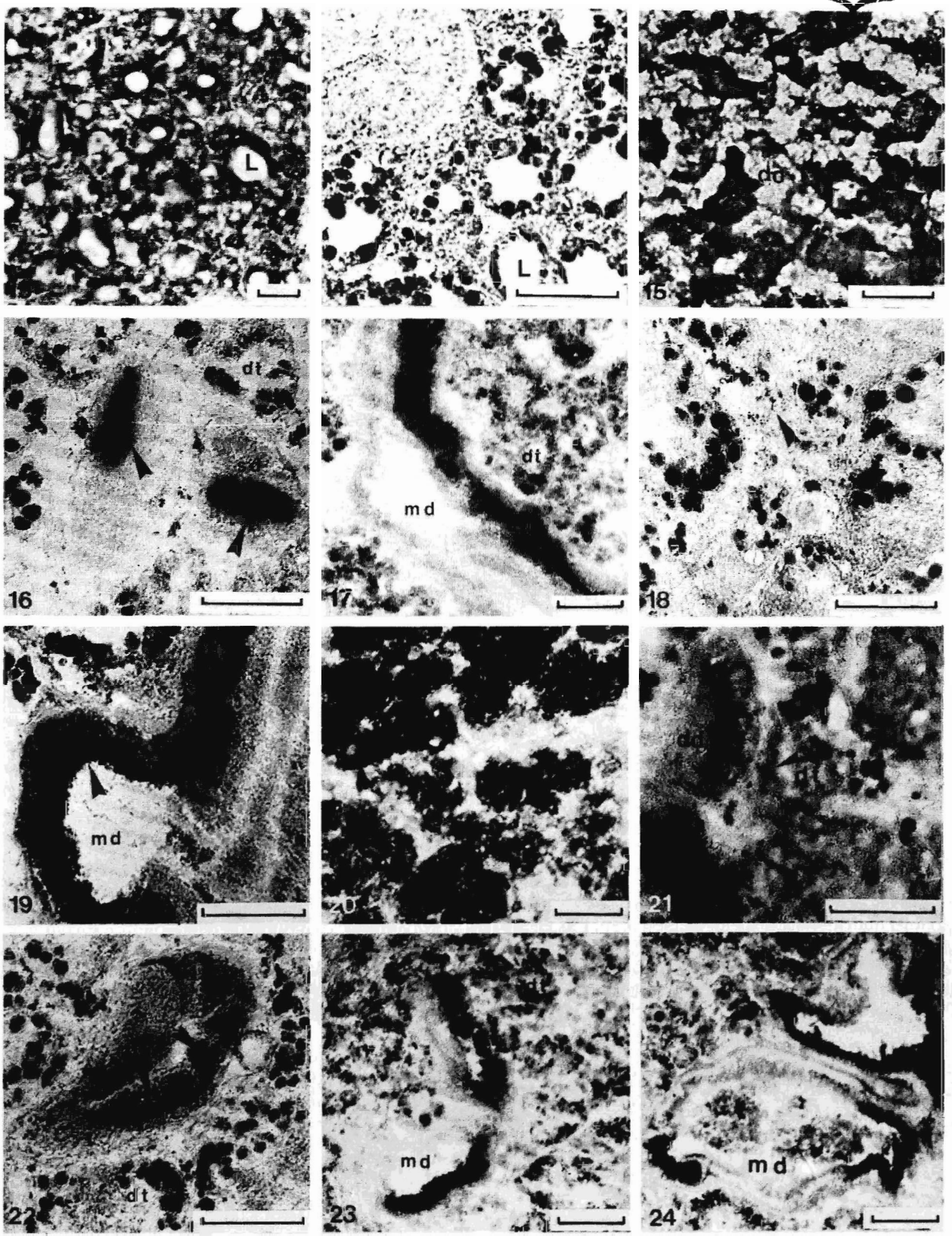
Non-specific esterase activity was localized in the whole tissues of the digestive gland and was very strong in the brush-border cells of the ducts (*fig.* 19). In the tubules, the activity appeared to be localized mostly in the secretory cells (*fig.* 19). Alkaline phosphatase (*fig.* 20) and strong acid phosphatase activities (*fig.* 21) were found in the ducts and in the tubules.

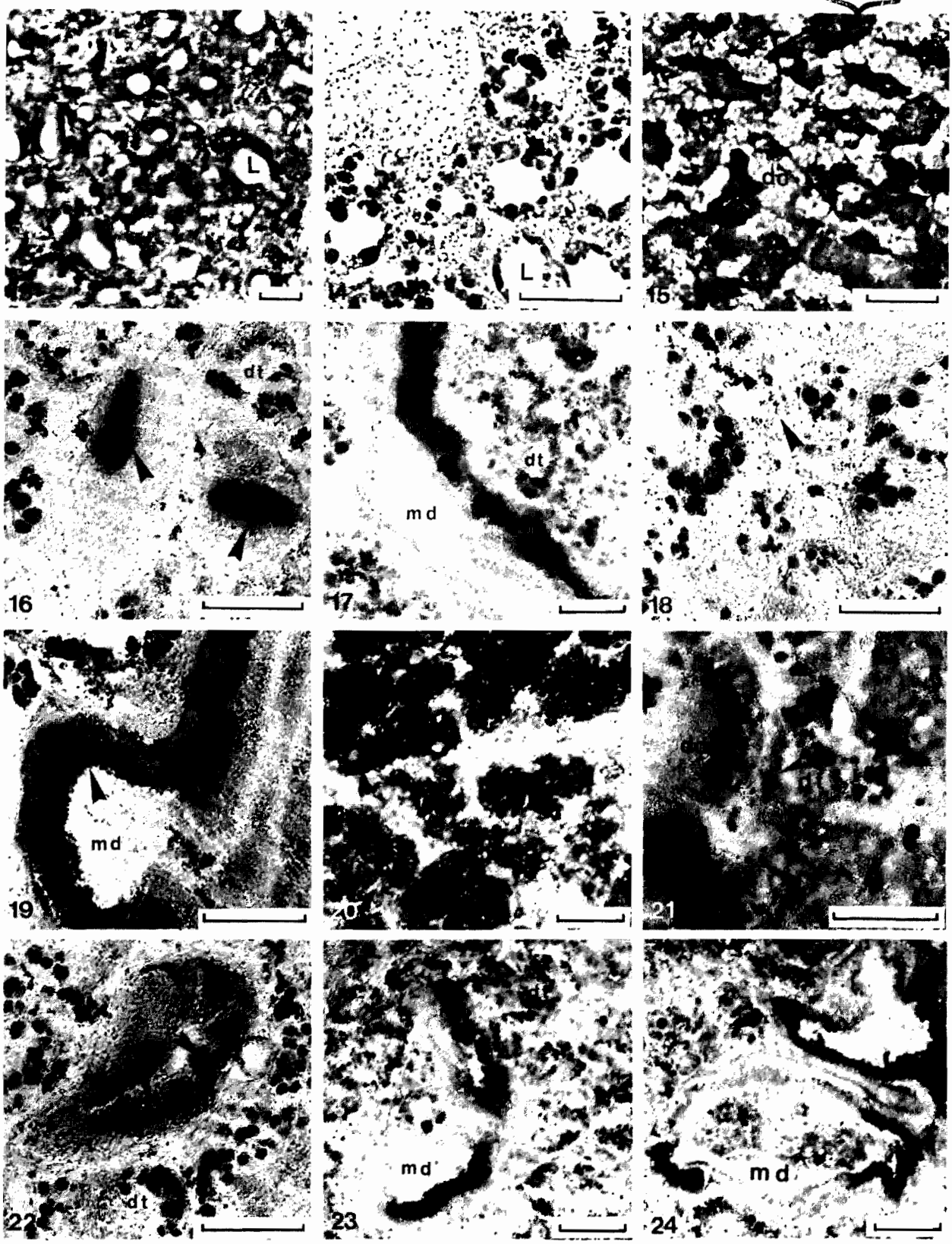
It was not possible to detect any proteolytic or chymotrypsin activity at any stage of digestion or time of incubation.

The digestive gland cells displayed varying levels of intracellular peptidase activities, higher activities being found in the brush-border cells of the ducts (*table* 1 and *fig.* 22 to 24).

Most of the enzymes we detected did not show any changes in their localization with varying experimental conditions except acid and alkaline phosphatase. Alkaline phosphatase was found in the ducts and in the basal part of the tubules in fed scallops, but uniquely in the ducts in unfed animals. Unfed and fed animals showed no differences in their activities. However, the histochemical methods used are more

Figures 13-24. – Enzymatic activities revealed by substrate film methods and precipitation methods with simultaneous azo-dye coupling in the digestive gland of *Pecten maximus* (L). The scale on figures 13 to 24 represents 100 µm. Fig. 13: Amylase activity of the digestive tubules of an adult scallop (starch film colored by iodine reagent). Fig. 14: Cellulase activity of the digestive tubules of an adult scallop (carboxymethylcellulose film colored by nuclear fast red and toluidine blue. Fig. 15: Lysozyme activity of the digestive tubules of a juvenile scallop (*Micrococcus lysodeikticus* embedded in a gelatin film and colored by basic fuchsin and alcian blue). Fig. 16 : N-acetyl-glycosaminidase activity in the brush border of the secondary ducts (sd) and in the functional part of the digestive tubules (dt) of an adult scallop (naphthol AS β-N-acetyl-glucosamine coupled with FBB). Fig. 17 : N-acetyl-glycosaminase activity in a main duct (md) at the brush-border level and in the digestive tubules (dt) of a juvenile scallop (same method as for fig. 16). Fig. 18: β-glucuronidase activity revealed in haemocytes (arrows) in the connective tissue of the digestive gland of an adult scallop (naphthol AS-β-glucuronide coupled with fast red violet). Fig. 19: Esterase activity in the brush-border of a main duct (md) of a juvenile scallop (-naphtyl-acetate coupled with FBB). Fig. 20: High alkaline phosphatase activity of the digestive gland of an unfed juvenile scallop-naphthyl phosphate coupled with FBB). Fig. 21: Acid phosphatase activity in the digestive ducts and digestive tubules (functional part) of an adult scallop (-naphthyl-phosphate coupled with fast garnet GBC). Fig. 22: Aminopeptidase activity in a main duct (md), at the brush-border level, and in the functional part of the digestive tubules (dt). (L. leucyl. 4 MNA coupled with FBB). Fig. 23: D.A.P. II activity in the brush border cells of a main duct (md) and in the functional part of the digestive tubules (dt) of a juvenile scallop (lysyl-alanine 4 MNA coupled with FBB). Fig. 24: D.A.P. IV activity in the main ducts of a juvenile scallop (glycyl-prolyl 4 MNA coupled with FBB).
FBB: fast blue B salt; 4 MNA: 4-methoxy- β-naphthylamide.





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qualitative than quantitative, and do not allow to detect small differences in activity.

Little significant difference was observed between the juvenile and adult scallops, the two having the same enzyme complement in their digestive gland. DAPI, however, appears more active in the adult whilst the juveniles display higher phosphatase activities (*fig.* 20).

DISCUSSION

Analysis of the results of the histochemical and ultrastructural studies carried out on the digestive gland allows a better understanding of the processes of the extracellular and intracellular digestion and absorption in *Pecten maximus* (L.).

The digestive activities found in the digestive gland of *Pecten* are in agreement with previous reports on other lamellibranches: glucanases such as cellulase and amylase have been found in the digestive gland of extracts of various Bivalvia (Horieuchi, 1957, 1963; Elyakova, 1972; Wojtowicz, 1972). The high cellulolytic activity found in the digestive tubules of *Pecten* agrees with the results of Payne *et al.* (1972) on *Scrobicularia plana* and Brock (1989) on *Crassostrea gigas* and confirms the endogenous origin of the cellulolytic enzymes in Bivalvia. Lysozyme localized in the digestive gland could be involved in the use of bacteria as food as already suggested for the cockle *Cerastoderma edule* (Conway, 1987). We did not detect any proteolytic histochemical activity; however a low activity has been measured *in vitro* (Lefort, unpublished result). In *Crassostrea gigas* (Boucaud *et al.*, 1985), a low proteolytic activity was found particularly at the ultimate phase of the digestive process.

Our negative results for proteolytic activity can be explained by the fact that we did not systematically follow the enzyme activities throughout the digestive cycle. Our results on the localization of the acid and alkaline phosphatases, esterases, leucine aminopeptidase (arylamidase) are exactly the same as those obtained by Reid (1966) on *Lima hians* which has the same stomach type as *Pecten maximus* (Gastrotetartika) (Purchon, 1986).

Lysosomal enzyme activities were found in the functional digestive cells whereas the degenerative cells did not display any enzymatic activity. These lysosomal enzymes are involved in the intracellular digestion which is known to occur in the digestive cell in Bivalvia (Morton, 1983). The nutriments resulting of the intracellular digestion pass to the hemolymph through the latero basal membranes of the cells as suggested by the alcaline phosphatase activity of the basal part of the digestive tubules.

The observation of the fragmentation of spherules from the digestive cells agrees with the classical theory (Morton, 1983) of the transport of lysosomal enzymes to the stomach for extracellular digestion.

The secretory cell of the digestive tubules has the same ultrastructural features as the exocrine mammalian pancreas cell; some of the enzymes (mostly glucanases) we detected in the digestive tubules would be secreted by these secretory cells and would complete the pool of extracellular gastric enzymes. Immunocytochemical studies are in progress to attempt to identify the enzymes contained in the secretory granules.

Secretory cells and undifferentiated cells are clustered in crypts which are scattered everywhere between the digestive cells, whereas in many Bivalvia the crypts are organized in two, three or four groups with well defined localization (Owen, 1955; Summer, 1966; Owen, 1970; Pal, 1971; Henry, 1987).

In the functional as well as in the degenerative part of the tubule we observe the existence of small dense cells in the scattered dense crypts. As small stem cells located in crypts are usually thougth to be undifferentiated precursors for all of the other cell types seen in the tubule (Owen 1970, Henry 1987, Beninger and Le Pennec 1991) we think that in *Pecten*, new parts of the tubule originating from one of the scattered crypts may become functional as another part of the same tubule degenerates.

The brush-border cells of the duct displayed high enzyme activities likely involved in absorption and achievement of digestion (alkaline phosphatase, dipeptidase, membrane aminopeptidase, acetyl glycosaminidase). Similar results were obtained in previous studies on the mussel, *Mytilus edulis* (Janssen, 1981) and the oyster, *Crassostrea gigas* (Boucaud-Camou *et al.*, 1985).

The enzyme activity of the digestive ducts has already been noticed by Mathers (1973) who wrote that the ducts were "an active region of enzyme secretion". Similar ideas were shared by Palmer (1979), who found that the ducts secreted extracellular exopeptidases. Another explanation, not incompatible with the first hypothesis is that the digestive duct epithelium could also play a role in intracellular digestion of large molecules. The capacity of absorptive epithelia to digest proteins intracellularly is generally recognized (see review in Georgopoulou *et al.*, 1985). The incoming countercurrent presumed to carries fine particles of food from the stomach to the digestive tubules flows along these brush border cells (Owen, 1955). Larger particles fall to the ciliated floor of the ducts and are swept outward to the stomach (Beninger and Le Pennec 1991). A basketwork of smooth muscle fibres surrounding the tubules assist in expulsion of material from the lumen of the tubules into that of the stomach (Purchon, 1971). A large part of the soluble particles is certainly retained and digested by these brush border duct cells while the other part is engulfed by the digestive cells. The ciliated duct

cells which display no enzyme activity appear only involved in the outgoing current.

The different stages of the digestive process which were here described for the digestive tubules: absorption and intracellular digestion, release of fragmentation spherules and lysis were simultaneously observed in the same digestive diverticula in our experimental conditions, the animals being kept in a constant environment in aquaria. This suggests that the digestive process is continuous. The cyclic diphasic rhythms described by Mathers (1973) in *Pecten maximus* reflect tidal fluctuations. These observations suggest that the digestive process is continuous when the food is available (Langton and Gabbott, 1974, Robinson and Langton, 1980) while it becomes cyclic when the nutritional apport is discontinuous.

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