Morphofunctional Study of the Haemocytes of the Bivalve Mollusc
Mytilus galloprovincialis with Emphasis on the Endolysosomal Compartment

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ABSTRACT. In the present work the haemocytes of mussels Mytilus galloprovincialis (Mollusca, Bivalvia) have been studied by light and electron microscopy in order to describe their main morphological features and to relate these to their roles in immune defence. The haemocytes belong to two definitive differentiated types, hyalinocytes and granulocytes. The former shows the presence of several fine pseudopodial protrusions, large nucleus with clumps of dense chromatin, scant cytoplasm, a well developed Golgi apparatus, lysosomes, several mitochondria (some with characteristic inclusions), coated pits and peripherally placed membrane-bound endocytic vesicles, considerable amounts of endoplasmic reticulum and ribosomes. The granulocytes generally possess an organelle-free ectoplasmic zone, numerous membrane-delimited dense granules of various types, coated pits and vesicles, endocytic and phagocytic vesicles, multivesicular bodies, several peroxisome-like organelles, mitochondria with inclusions, scant endoplasmic reticulum and small Golgi apparatus. These cells show the presence of few lipid droplets and variable amounts of glycogen particles. Some of the substructural features of the granules are documented here to indicate their probable biogenesis, growth and relationship with the endolysosomal compartment. In addition, in vitro phagocytosis experiments demonstrate that both hyalinocytes and granulocytes uptake latex and zymosan particles, granulocytes being much more active in phagocytosis than hyalinocytes.

The haemocytes of bivalve molluscs are involved in various physiological functions including wound repair, shell formation and repair, nutrient digestion and transport, excretion, and internal defence (Cheng 1981, 1984). Most recently attention has been focused on the roles of haemocytes in internal defence. Phagocytosis by haemocytes is the major line of defence against invading foreign materials (Bayne 1990). Granule-containing haemocytes or granulocytes appear to be the main phagocytic cells in bivalves (Foley and Cheng 1975; Rodrick and Ulrich 1984). Accordingly, granulocytes are also the main cell type involved in generation of toxic oxygen free radicals upon stimulation with both phagocytizable particles and soluble agents (Pipe 1992; Takahashi et al. 1993; Cajaraville et al. 1994). Cells containing few or no granules, commonly named hyalinocytes, have been considered either as the immature precursor cells giving rise to granulocytes (Mix 1976) or as a distinct mature cell type belonging to a separate cell lineage (Cheng 1981; 1984; Auffret 1989). No definite function has been associated with the hyalinocytes although very recently the involvement of agranular haemocytes in hemostasis and extracellular matrix production during wound healing has been highlighted (Suzuki et al. 1991; Suzuki and Funakoshi 1992).

Conventional transmission electron microscopy forms one of the basic approaches to probe the immunological roles of the diverse kinds of invertebrate blood cells or haemocytes (Millar and Ratcliffe 1989; Sima and Vetvicka 1993). In bivalve molluscs, several important and detailed electron microscopical studies have been restricted to a few commercially valuable species (Cheng and Foley 1975; Cheng 1981; 1984; Hawkins and Howse 1982; Rodrick and Ulrich 1984; Rasmussen et al. 1985; Fisher 1986; Auffret 1988; 1989; Hinsch and Hunte 1990; Pal and Cajaraville 1994). These studies have provided invaluable information on the morphology of the diverse haemocyte types but details of the fine structure of the endo- and phagocytic vacuolar compartments have rarely been reported. Thus the present work was undertaken to study the ultrastructural fea-
tures of the haemocytes of *Mytilus galloprovincialis*, special emphasis being placed on the organelles involved in two key processes of cellular defence mechanisms, *i.e.*, phagocytosis and intracellular degradation via lysosomal digestion. Phagocytosis has also been studied with isolated haemocytes *in vitro* using latex and zymosan particles.

**MATERIALS AND METHODS**

**Animals.** Mussels, *Mytilus galloprovincialis* Lmk, of 3.5 to 4.5 cm shell length were collected from Plentzia (Bay of Biscay, 43°24'N, 2°56'W) at low tide level at different periods throughout the year. The animals were sacrificed immediately upon collection. Due to the open circulatory system of molluscs, blood cells or haemocytes are found in the circulating haemolymph and also in the connective tissues throughout the animal. The following organs were then dissected out to search for haemocytic cells: digestive gland, mantle, gonad and heart.

**Electron Microscopy.** Small pieces of the different organs were fixed for 2 h at 4°C by immersion in 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, containing 7.5% sucrose and 2.5% NaCl. After fixation, the tissues were rinsed in the same buffer at 4°C and postfixed for 1 h at 4°C in 1% osmium tetroxide or in reduced osmium (Karnovsky 1971). Thereafter the tissues were washed in buffer, dehydrated in a graded series of ethanols, cleared in propylene oxide and embedded in Durcupan or Epon. Semithin sections were mounted on glass slides, stained with toluidine blue and examined by light microscopy. Ultrathin sections were cut with a diamond knife on a LKB ultramicrotome, collected on copper grids and stained with uranyl acetate and lead citrate. Sections were examined in a Philips (EM 300) electron microscope operated at 80 kV or in a Jeol (JEM 100 SX) electron microscope at 60 kV.

**Haemolymph collection and phagocytosis experiments.** For each experiment, the haemolymph of 3 mussels was withdrawn immediately before use from the posterior adductor muscle with disposable plastic syringes and pooled. An aliquot was put on a haemocytometer to determine total cell counts and cell viability by the Tripan blue exclusion assay. Cell viability was found to be 80–90%. For the preparation of haemocyte monolayers, 1–2 × 10^6 live cells per ml were put on round glass coverslips (10 mm in diameter) located in 24-well microtiter plates and cells were allowed to adhere and spread for 45 min at room temperature. After haemocyte spreading, the haemolymph was discarded and a solution of dyed latex particles (0.8 μm diameter, Sigma) in saline (2.5% NaCl, 7.5% sucrose) was added to each well (0.476% solid contents). Cells were incubated for 45–60 min at room temperature. For zymosan uptake experiments, cells were incubated for 90 min with zymosan A (Sigma) at a cell : zymosan ratio of 1 : 10. After incubation, cells were rinsed in saline, fixed in methanol (5 min in 20%, 5 min in 100%) or in Baker's formol calcium for 15 min, washed again in saline and mounted in glycerine. In some experiments cells were stained with Giemsa or with a 0.1% Fast Green solution.

**RESULTS**

There are morphologically two distinct kinds of haemocytes, agranulocytes or hyalinocytes and granulocytes, in the tissue spaces of the various organs of mussels. Granulocytes show a conspicuous number of cytoplasmic electron-dense specific granules (Fig. 1) which are not present in the hyalinocytes (Fig. 2).

**Hyalinocytes.** The hyalinocytes are small cells showing several fine and long pseudopodial projections. They contain a large nucleus, scant cytoplasm, free ribosomes, considerable amounts of rough- and smooth-surfaced endoplasmic reticulum (RER and SER), a highly developed and curved Golgi apparatus, a few small membrane-bound dense bodies and cortically placed groups of clear-looking endocytic vesicles. The latter originate as small bulbs having narrow connections to the extracellular space (Fig. 3). Internally the limiting membrane of such vesicles is covered uniformly by a 200 Å-thick coat and on the cytoplasmic side there occur, on occasion, several fine fibrillar entities.

The nucleus in the hyalinocytes bears irregular dense chromatin clumps and a prominent but eccentric nucleolus (Figs. 2, 3). The Golgi apparatus is well developed, consisting of 2–3 stacks per cell, and maintains usually a bipolar location in the cytoplasm with typical concave and convex appearance (Fig. 3). Often the termini of

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**Fig. 1.** Low power electron micrograph of a mature granulocyte showing high numbers of cytoplasmic electron-dense specific granules (G) of various sizes and shapes. The nucleus (N) is spherical and eccentrically placed. The organelle-free peripheral endoplasm (EC) contains several electron-lucency glycogen fields (GL). Electron-lucency endosomal fields (E) are also placed cortically. × 7,000. Bar: 1 μm

**Fig. 2.** Low power electron micrograph of a hyalinocyte illustrating various ultrastructural features including the large nucleus (N) with a prominent eccentric nucleolus (NL), few small lysosomes (L), cortically placed endocytic vesicles (E) and numerous mitochondria (M). Of particular interest are the crystalline inclusions found in the matrix of some mitochondria (arrowheads). × 14,500. Bar: 0.5 μm

**Fig. 3.** Detail of the cytoplasm of a hyalinocyte showing a well developed Golgi apparatus (GA). The lumina of the flattened cisternae appear electron-dense. Membrane-bound small vesicles and larger bodies with moderately electron-dense materials (L) are common near the GA. Note also the presence of a coated pit (arrowheads) and an endocytic vesicle (E) pinching off the plasma membrane. The latter shows a narrow connection to the extracellular space. N nucleus. × 46,000. Bar: 0.25 μm

**Fig. 4.** Electron micrograph of an immature granulocyte illustrating the large elongate nucleus (N) with a prominent nucleolus (NL), the small-sized specific granules (G) and cisternae of the RER containing electron-dense materials similar to those found in the specific granules (arrowheads). × 16,000. Bar: 0.5 μm
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Fig. 1-4.
Fig. 5–10.
the cisternae appear dense and swollen. Equally, the lumina of the flattened sacs of the Golgi apparatus are also electron-dense. Near the Golgi zone there occur, on occasion, few membrane-bound small bodies with moderately electron-dense materials. Such bodies show an electron-lucent space between the contents and the membrane and are interpreted as lysosomes (Figs. 2, 3). The mitochondria show dense matrical substances and some contain crystalline bodies (Fig. 2). These are of rectangular shape, measuring up to 0.4 μm in length and showing distinct alternate dark and light parallel lines. Pigment bodies are common in the cytoplasm of these cells. Besides, there are glycogen particles in the cytoplasm; the lipid globules are few in number. Occasionally cytoskeletal elements are observed towards the peripheral cytoplasm in the hyalinocytes, mainly in the spatula-like projections.

**Granulocytes.** The granulocytes are large cells which may have both broad and several fine elongated pseudopodial processes. In mature cells filled with numerous granules, the nucleus is spherical (Fig. 1) but in the smaller immature cells the nucleus may also be elongate (Fig. 4). It is generally eccentrically placed and presents dense chromatin clumps. The cytoplasm is very characteristic and thick; this surrounds most of the endoplasmic granules and organelles (Fig. 1). The ectoplasm or periphery of these cells shows the presence of several electron-lucent zones having large glycogen deposits or fields (Figs. 1, 5).

One of the important features associated with the plasma membranes of the cells is the presence of several small depressions or pits (Figs. 5, 7, 9), some of these showing clear internal coat materials and fine dense particles (approximately 60 Å in diameter) lying on the cytoplasmic aspect of the limiting membrane. Endocytic vesicles (with internal hairy processes), channels and multivesicular bodies (MVB) measuring 0.7 to 1.4 μm in diameter are frequent in the cortical cytoplasm (Figs. 5–8). The cortically placed endocytic vesicles show a uniform inner coat and lie attached with the periphery of other similar vesicles or with the specific dense granules (Figs. 5, 6). The MVBs show different morphologies, the degree of structural complexity being possibly related to the maturation of the organelle. Early MVBs possess one or two small membranous vesicles and fine granular substances in their interior (Figs. 5, 7). More complex late MVBs may be pleomorphic and contain several vesicles and other granular materials (Fig. 8). The internal vesicles seem to originate from the budding of the limiting membrane towards the lumen of the organelle (Fig. 8). Large-sized phagosomal vesicles containing bacterial cells or algal vegetative cells are also common in the granulocytes (Fig. 9). Similar large vesicles are also frequently seen containing residual bodies from digestive cells (Fig. 10).

The Golgi apparatus is not extensive and possesses 3–4 flattened cisternae. Small coated vesicles may be observed budding from the last trans cisterna (Fig. 11). In addition, the Golgi area is surrounded by several different kinds of small vesicles and dense granules. Both SER and RER are scanty in the mature granulocytes. The granules are the most prominent organelles of mature granulocytes in terms of occupied volume (Fig. 1). They are usually dense and compact and their sizes and forms are extremely variable. These so-called "specific" granules have contents separated from the kinky limiting membrane by a uniformly thick and electron-lucent space (Figs. 12–18). The smallest granules measure 0.1 to 0.3 μm in diameter, while the larger ones measure from 0.3 to 1.5 μm in diameter. The electron-lucent halo is decreased or even absent in the larger granules. These larger granules are usually spherical in shape but smaller ones, which are dominant in immature cells (Fig. 4), show worm-like elongated shapes. Few of the granules are observed to be interconnected (Figs. 12, 13), frequently adopting a reticular appearance. Microtubular profiles and small vesicles (Fig. 13) may be found inside the tubular structures connecting or bridging the granules. In addition, such configurations may show few ribosomes adhering on the surface of the

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**Fig. 5.** Portion of a granulocyte illustrating the presence of several small invaginations of the plasma membrane (asterisks). A cortically placed endocytic vesicle is lying attached to the periphery of a specific dense granule (arrowheads). Note also the presence of an early multivesicular body (MVB) and glycogen (GL). × 58,500. Bar: 0.2 μm

**Fig. 6.** Portion of a granulocyte showing various events of apparent fusions between (1) cortical endosomes and larger vesicles possibly belonging also to the endosomes (arrow) and (2) cortical endosomes and the specific granules (arrowheads). E endosome, G granule, N nucleus. × 21,000. Bar: 0.5 μm

**Fig. 7.** Early multivesicular bodies (MVB) contain few small membranous vesicles, fine granular substances and filamentous structures (arrowheads). The presence of a coated pit (CP) in the nearby plasma membrane is also evidenced. G granule. × 45,000. Bar: 0.25 μm

**Fig. 8.** Late multivesicular bodies (MVB) are complex pleomorphic organelles containing several vesicles and coarsely granular materials. In the electron micrograph many electron-lucent endosomes (E) are shown to be located in the proximities or even attached to the MVB (arrowheads). Small specific granules (G) are also seen close to the MVB. Note the budding of the MVB limiting membrane towards the lumen of the organelle (small arrows). × 29,500. Bar: 0.25 μm

**Fig. 9.** Granulocyte showing large-sized phagosomal vesicles containing algal cells (arrows). Asterisks indicate the location of coated pits. L lipid, G granule, MU muscle, N nucleus. × 11,200. Bar: 1 μm

**Fig. 10.** Granulocyte showing large-sized phagosomal vesicles (arrows) containing residual bodies from digestive cells (RB). G granules, N nucleus. × 8,300. Bar: 1 μm
Fig. 11-18.
membrane of the connecting structures, suggesting they may be elements of the RER. In agreement with this idea, in the immature granulocytes the cisternae of the RER are observed to contain electron-dense materials similar to those found in the specific granules (Fig. 4), thus suggesting a continuity between the developing elongated specific granules and the RER.

Inside the dense contents of the specific granules lie, on occasion, fingerprint-shaped membranous whorls (Fig. 14) or dense inclusions, either amorphous or having microtubular outlines (Fig. 15). Additionally, several granules show localized peripheral sites where indistinct stacks of membranous lamellae lie (Fig. 16). Some of the granules may contain eccentric clear zone(s) (Fig. 17).

The specific granules are usually observed to lie in groups near the cortical cytoplasm of the granulocytes and some frequently show contacts with the plasma membrane (Fig. 18). At these sites of apparent "fusion" of membranes, the definition of the two membranes is not clear, while the identity of the granular membrane at the sub-plasmalemmal cytoplasmic side is sharp. Close to the sites of fusion lie the exocytosed electron-dense materials.

Membrane-bound organelles resembling peroxisomes are characterized by the possession of a finely granular dense matrix and an eccentric osmiophilic inclusion (Fig. 19). These peroxisome-like organelles are rectilinear in outline, with a diameter of 0.4-0.5 \( \mu \text{m} \), and do not present any space separating the limiting membrane from the contents. Mitochondria are also present among the granules and some contain an extensive dense crystalline body in the matrix (Fig. 20). These crystalline bodies are similar or equal to those described before in the hyalinocytes. It is rarely observed that clusters of mitochondria encircle a dense fine granular mass, near the nucleus (Fig. 20). This resembled the so-called "juxtanuclear bodies" of the cardiac haemocytes of oysters (Hawkins and Howse 1982). There is more than one such organelle in the cell. The lipid droplets appear as non membrane-bound electron-lucent spherical entities in the cytoplasm. On occasion, one or two irregular dense bodies of pigment-like materials are observed in the granulocytes. Few microtubules are present towards the cell cortex or in the cellular projections of the granulocytes.

**In vitro phagocytosis.** Phagocytosis experiments were performed with haemocytes collected from the haemolymph and allowed to spread on glass slides. Two cell types are readily distinguished, hyalinocytes and granulocytes, the latter being larger and more numerous than the former. The two cell types spread on glass slides but some remain in rounded condition. Both the non-granular hyalinocytes and the granulocytes are able to uptake zymosan and latex particles (Fig. 21). On a quantitative basis, granulocytes are far more phagocytic than hyalinocytes (Fig. 22).

**DISCUSSION**

The present light and electron microscopical study provides information on the various morphological characteristics of the haemocytes of mussels, *Mytilus galloprovincialis*. In general, in earlier ultrastructural studies a considerable emphasis has been placed on the use of morphological features to classify bivalve haemocytes (Cheng 1984; Rasmussen *et al.* 1985; Auffret 1988; 1989). Nevertheless, the classification of bivalve haemocytes is still a matter of debate (Rasmussen *et al.* 1985; Auffret 1988; Friedl *et al.* 1988). Two general types of haemocytes, agranulocytes or hyalinocytes and granulocytes, have been distinguished in mytilids (Cheng 1981; Rasmussen *et al.* 1985; Pal and Cajaraville 1994) although some studies have further described two subtypes within the granulocytes of mussels *M. edulis*, one type with small granules (0.2-0.3 \( \mu \text{m} \)) and the other type with large granules (0.5-1.5 \( \mu \text{m} \)) (Pipe 1990a). In the present study granules of both sizes were commonly

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Fig. 11. Detail of the Golgi apparatus (GA) of the granulocytes. Note the small coated vesicles (arrowheads) budding from the last trans cisterna. G granule, SER smooth endoplasmic reticulum. \( \times 40,000 \). Bar: 0.25 \( \mu \text{m} \)

Fig. 12. Portion of the cytoplasm of a granulocyte illustrating that groups of specific granules (G) are interconnected (arrows) thus adopting a reticular appearance. Note also the differences in the size of the granules, the smaller ones showing worm-like elongated shapes. \( \times 40,500 \). Bar: 0.25 \( \mu \text{m} \)

Fig. 13. Detail of a granular connexion illustrating the presence of two small vesicles (arrowheads) within the tubular structure connecting the granules. \( \times 85,300 \). Bar: 0.1 \( \mu \text{m} \)

Fig. 14. Detail of the substructure of the specific granules which contain membranous whorls (arrowheads). Note also the figures indicative of intergranular fusion (arrows). \( \times 50,500 \). Bar: 0.2 \( \mu \text{m} \)

Fig. 15. Detail of the substructure of a specific granule containing a dense amorphous inclusion (arrowhead). A second granule showing a membranous whorl is also evidenced (arrow). \( \times 70,500 \). Bar: 0.1 \( \mu \text{m} \)

Fig. 16. Detail of the substructure of the specific granules which show localized peripheral stacks of membranous lamellae (arrowheads). \( \times 50,500 \). Bar: 0.2 \( \mu \text{m} \)

Fig. 17. Detail of the substructure of the specific granules which contain eccentric electron-lucent zones (arrowheads). M mitochondria. \( \times 55,500 \). Bar: 0.2 \( \mu \text{m} \)

Fig. 18. Portion of the cortical cytoplasm of a granulocyte showing a specific granule (G) in close contact with the plasma membrane (arrowheads). GL glycogen. \( \times 64,000 \). Bar: 0.2 \( \mu \text{m} \)
seen side by side within the same cell, although there were also cells containing granules of either one or other size. These results suggest that there might be different stages of granulocyte differentiation or maturation paralleled by growth of their specific granules. Thus, the smaller granules, often showing elongated worm-like profiles, are dominant in immature cells while fully differentiated large granulocytes mostly contain large spherical granules. The latter clearly grow by fusing with similar granules (Fig. 14).

Some of these granules probably belong to the lysosomes since they are reactive for a number of lysosomal marker enzymes (Huffman and Tripp 1982; Moore and Gelder 1985; Pipe 1990b; Pal and Cajaraville 1994; Cajaraville et al. submitted). As other lysosomes, they present a characteristic uniform thin layer of electron-lucent materials between the dense contents and the limiting membrane (Neiss 1984). As summarized in Fig. 23, the granules frequently fuse with other similar granules and with the electron-lucid endosomal vesicles thus showing the characteristic features of a highly pleomorphic and dynamic lysosomal compartment.

On the other hand, the specific granules seem also able to fuse with the plasma membrane (see Figs. 18 and 23). On the termination of the release of secretory materials, a part of the substances apparently lie attached with the external side of the plasma membrane of the granulocytes. Degranulation has been associated with the release of lysosomal enzymes into serum during active phagocytosis (Cheng 1981; Mohandas et al. 1985; Cheng and Dougherty 1989). According to the latter authors, the released enzymes alter the surface of exogenous cells so that these are recognized as nonself and then attacked by phagocytic cells. This in turn results in the release of additional lysosomal enzymes acting as cytotoxic molecules and enhances phagocytic activity (Cheng and Dougherty 1989). Hinsch and Hunte (1990) have reported the extrusion of dense granules from the haemocytes of oysters *Crassostrea virginica*. Most importantly, the discharge of granules occurred both prior to and during the time of phagocytosis of latex beads, suggesting that degranulation may not be a necessary prerequisite for phagocytosis (Hinsch and Hunte 1990).

In the present study, cells with phagocytosed residual bodies were filled with granules and were not observed to degranulate. Conversely, cells apparently showing exocytosis of granular materials did not appear to be undergoing phagocytosis.
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Fig. 21. Light micrographs of mussel haemocytes adhering on glass and showing in vitro phagocytosis of zymosan (a) and dyed latex particles (b). Arrows point to engulfed zymosan or latex particles. Latex particles appear refringent in the phase-contrast micrograph. ×450. Bar: 35 µm

Fig. 22. Percentage of haemocytes phagocytosing zymosan (a) and dyed latex particles (b). In each case, 100 randomly selected cells were scored in each of 5 preparations from 5 different mussels.
In *M. galloprovincialis* the specific electron-dense granules display a wide range of substructural peculiarities including the presence of membranous whorls, dense inclusions and peripheral stacks of membranes. Small vesicles and microtubular profiles are also observed within the granule-connecting structures. As a consequence of fusion with similar or different intracellular organelles, some of the granules may incorporate and store membrane fragments in their contents, either allowing the contents to be enriched with phospholipids or facilitating the growth of the granular limiting membrane. Alternatively, the various intragranular membranous structures could correspond to digestion products resulting from degradation of exogenous materials. With regard to the question of granulogenesis in these cells, the small growing granules seem to be interconnected so as to form a "reticulum" in some granulocytes. This reticular structure appears to be also connected to the ER or alternatively it may correspond to the ER, as suggested by the presence of a few attached ribosomes.

It is significant to record here that not all the granules in the granulocytes of *Mercenaria mercenaria*, *Mya arenaria*, *Crassostrea gigas*, *Rangia cuneata* and *Mytilus edulis* are reactive for lysosomal enzymes (Yoshino and Cheng 1976; Huffman and Tripp 1982; Auffret 1989; Marsh 1990; Pipe 1990b) as also noted in our cytochemical studies on *M. galloprovincialis* haemocytes (Pal and Cajaraville 1994; Cajaraville *et al.* submitted). From the present report it is apparent that several of the small dense granules and coated vesicles originating from the Golgi apparatus probably belong to the lysosomes (Auffret 1989). In fact, the cases of apparent fusion of dense granules with phagosomes or with the cortical endosomal vesicles are clear stages of the intracellular digestive pathway. Some of the larger granules, however, may well not be lysosomal. Granules of some bivalve granulocytes have been demonstrated to contain calcium-binding phosphoproteins (Marsh 1990), and various hemagglutinin and non-lysosomal hemolysin substances are known to be produced by bivalve haemocytes (Chu 1988). Suzuki *et al.* (1991) and Suzuki and Funakoshi (1992) report that the agranular haemocytes of the bivalve *Pinctada fucata* produce extracellular matrix during wound healing.

Phagocytosis is the most common of the cellular defence reactions in invertebrates (Bayne 1990; Sima and Vetvicka 1993). Haemocytes of bivalve molluscs...
are known to phagocytose exogenous materials such as bacteria, parts of broken cells or own necrotic cells (Bubel et al. 1977; Cheng 1981; Rodrick and Ulrich 1984; Le Gall et al. 1988). In the present work bacterial and algal cells have been observed within phagosomes of granulocytes thus stressing the importance of haemocyte phagocytosis in nutrition and the close linkage between feeding and internal defence (Cheng 1981; 1984; Bayne 1990). The fact that some of the granulocytes show the presence of phagosomes containing digestive cell residual bodies, that could be discharged by digestive and subsequently interiorized by haemocytes during their diapedesis (Fletcher and Cooper-Willis 1982), supports the idea of these cells having an active role in the excretion of non-degradable digestion end products (Cheng 1984; Bayne 1990). Alternatively the phagocytosis of digestive cell residual bodies could also be related to the resorption of degenerating and necrotic digestive cells during the normal phasic activity of the digestive epithelium (Owen 1972; Cajaraville et al. 1991), thus accounting for the recycling of digestive epithelial cells. In addition to in vivo phagocytosis of bacterial and algal cells, and of digestive cell residual bodies, in the present study we have demonstrated in vitro phagocytosis of zymosan and latex particles by mussel haemocytes. In agreement with previous studies with other bivalves (Foley and Cheng 1975; Rodrick and Ulrich 1984), granulocytes are far more phagocytic than hyalinocytes in mussels.

Another remarkable observation in this report concerns the occurrence of numerous surface “pits” or depressions and endocytic vesicles and channels in both the haemocyte types. Some of these are characterised by the possession of an uniform coat lining the internal or luminal aspect of the limiting membrane, which is reminiscent of the glycosylcalyx of animal cells (Neiss 1984). Significantly this substructural peculiarity of the early endosomes probably represents their pre-lysosomal nature. Further, some of these “pits”, prior to their release into the sub-plasmalemmal cytoplasm, show serially arranged fine dense entities or fibrils attached to the cytoplasmic aspect of the membrane. Similarly coated small vesicles are seen budding from the trans-cisterna of the Golgi complex in granulocytic cells (Figs. 11 and 23). It remains to be established whether the coat corresponds to clathrin or to some other coat protein (Schmid 1993). The uncoated endocytic vesicles of granulocytes appear to travel gradually in bivalve haemocytes as well. The underlying mechanisms controlling endocytic processes and vesicular traffic in these haemocytes have not been explored. Importantly, the organisation of the cytoskeletal network is involved in phagocytosis by the bivalve haemocytes (Rodrick and Ulrich 1984; Le Gall et al. 1988; Alvarez et al. 1989; Moore et al. 1992). Application of immunocytochemical tools to detect markers of early and late endosomes, phagosomes and lysosomes should provide information about the origin, growth and fate of the MVBs and other components of the endosomal compartment in the haemocytes and would facilitate a molecular description of the interactions between the endolysosomal apparatus and internal defence.

The presence of considerable amounts of glycogen particles, ribosomes, ER, Golgi bodies, and the numerous dense granules, particularly in the granulocytes supports the idea that these cells are synthetically very active. The occurrence of one or two juxtanuclear bodies in a few granulocytes is special and such features are similar to those described by earlier workers in haemocytes of other bivalves (Hawkins and Howse 1982; Auffret 1988; 1989; Beninger and Le Pennec 1991). Similar structures have been observed also in other molluscan cell types such as digestive duct cells (Cajaraville MP unpubl. obs.) and germ cells (Kessel 1982). The granular mass of the juxtanuclear bodies contains ribonucleoprotein and has been suggested to originate from nucleolar fragmentation and nuclear cytoplasmic exchange (Kessel 1982). The latter author also suggests that the granular mass may be the site of biogenesis of annulate lamellae, but we have not observed such structures within mussel haemocytes. Evidently, it would be instructive to follow the origin of such bodies and to relate their functions with synthesis of ribonucleoprotein particles in the haemocytes. The significance and metabolic role of the intramitochondrial crystals remain also undeciphered. Such crystals have previously been observed in the mitochondria of M. edulis haemocytes (Bubel et al. 1977; Rasmussen 1986). Certainly further studies are needed to determine the nature of these crystalline inclusions.

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