3.1 Introduction

Numerous cytochemical and cytological responses have been developed and recommended as potential tests for quantifying and monitoring the environmental impact of xenobiotics in bivalve molluscs.

The hepatopancreas of molluscs is a large digestive gland formed by a vast number of blind ending tubules, the digestive diverticula. This organ is involved in several functions including the extracellular and intracellular digestion of food, storage of lipids, glycogen and minerals; it is also the main site of nutrient absorption and plays a major role in detoxification (Nelson and Morton, 1979; Morton, 1983; Beeby and Richmond, 1988; Henry et al., 1991).

The digestive diverticula consists of an epithelium with a single layer of cells, separated from the surrounding connective tissue and muscle cells by a basal lamina. In several molluscs this epithelium is formed by the digestive and basophilic cells (Pal, 1971; 1972; Owen, 1973; Lobo-da-Cunha, 1997).

Mollusc digestive cells are mainly characterized by the presence of a large number of heterolysosomes, in which the digestion of the food is completed. These
columnar shaped cells are the most abundant in the hepatopancreas of molluscs and their apical surface is covered by microvilli. Lipid droplets and glycogen granules are usually present in the cytoplasm of digestive cells. The basophilic cells, also called secretory or crypt cells, are typical protein secreting cells. These pyramidal shaped cells contain large amounts of rough endoplasmic reticulum, a well-developed Golgi complex and accumulate granules secreted in the cells. They seem to be responsible for the secretion of digestive enzymes, that undertake the extracellular digestion of the food (Pal, 1971; 1972; Owen, 1973; Henry et al., 1991; Franchini and Ottaviani, 1993).

The branched ducts that link the digestive diverticula to the stomach are also important component of the digestive glands. In mollusc hepatopancreas, duct cells can be involved in lipid storage and nutrient absorption (Mathers, 1972). Moreover, some results suggest that these cells also play a role in digestion (Henry et al., 1991). Studies of the hepatopancreas of molluscs are especially interesting because this organ can be useful in ecotoxicology research. The peroxisomes and lysosomes of mollusc hepatopancreas can suffer morphological and functional changes caused by pollutants, and may be valuable as bioindicators of pollution (Moore, 1985; Cajaraville et al., 1989; Fahimi and Cajaraville, 1995). Ultrastructural and cytochemical aspects of lysosomes and peroxisomes have been reported in some molluscs (Owen, 1972a; Pipe, 1986; Cajaraville et al., 1992; Lobo-da-Cunha et al., 1994; Loboda-Cunha, 1997), but it would be interesting to extend those studies to a larger number of species, in order to obtain a more general picture.

The digestive gland of bivalves is a target organ for the bioaccumulation of PAHs; furthermore, the lysosomes of the digestive cells are generally considered as target organelles (Owen 1972; Viarengo 1989; Marigomez et al. 1990a; Chassard-Bouchaud 1996), while the gills have also been shown to accumulate various xenobiotics either in the field (Balogh and Salanki 1987) or in the laboratory (George and Coombs 1977; Marshal and Talbot 1979; Nolan et al. 1984).
The histology and ultrastructure of the digestive gland of bivalves has been studied extensively (Owen 1970, 1972, 1973, Pal 1972, Morton 1983, Pillai and Menon, 1996, Menon and Menon, 1998). However, light and electron microscopical data concerning the functional morphology of the digestive gland of the common mussel *P. viridis* and *P. indica* after PAHs exposure are scarce, in spite of the importance of this organism in environmental biomonitoring studies.

To enlarge the knowledge about these marine molluscs, the hepatopancreas, gill, mantle, adductor muscle, foot and pedal disc of *P. Viridis* and *P. indica* were studied by electron microscopy.

### 3.2 Materials and Methods

Small pieces of hepatopancreas, gill, adductor muscle, mantle and pedal disc of toxicant exposed and control animals were fixed for 2 h at 4°C in 2.5% glutaraldehyde, diluted in 0.4 M cacodylate buffer pH 7.4 with 5 mM CaCl₂. After washing in buffer, the fragments were post fixed with 2% osmium tetroxide buffered with cacodylate, dehydrated in ethanol and embedded in Epon. Ultrathin sections were stained with uranyl acetate and lead citrate, before being observed in a Morgagni 268 D Transmission Electron Microscope Fei Netherland and relevant areas photographed (Department of Anatomy, All India Institute of Medical science, New Delhi).

### 3.3 Results

In semithin sections of both *P. viridis* and *P.indica* hepatopancreas, many digestive cells could be observed in the epithelium of the digestive diverticula. They were columnar cells, but many presented club shape with a round apex projected into the lumen of the digestive diverticula. (Plates 3.1 a-d, and 3.2a-d)

Numerous heterolysosomes were found in these cells, but in some of them the heterolysosomes seemed to be fusing with one another. Other cells were almost
entirely occupied by a single large vacuole, and their cytoplasm was reduced to a very thin peripheral layer. In some cases, a few thin strands of cytoplasm could still be observed in the interior of these cells, suggesting the previous existence of smaller heterolysosomes which must have fused to form the large vacuole.

The apical surface of digestive cells was covered with microvilli, (Plate. 3.2a-d and 3..8a-d). In some digestive cells the apical zone contained many mitochondria and only a few endocytic vesicles but in others the number of endocytic vesicles was very high, and only a few mitochondria were found in the area .(3.12a-d)

These vesicles contained electron dense materials and many presented a cup-shape .These vesicles were more abundant in the apical region of digestive cells, but some could also be found in deeper regions of the cytoplasm .Vacuoles with a clear background, usually containing low amounts of dense material, were also observed near the cell apex (Plates:3.13 a-d).

In the digestive cells of *P.indica*, a large part of the cytoplasm was occupied by heterolysosomes, but the dimensions and the content of these organelles were variable, reflecting different stages of intracellular digestion. Heterolysosomes were more abundant in the middle region of the cell but some could also be found in the apical zone (Plate. 4). In general, heterolysosomes were filled with material of medium density, their shape could be almost spherical or oval. Vacuoles containing dense material were identified as lysosomal residual bodies (Plate. 3.1a), many were spherical but some had an oval shape. The residual bodies were frequent in the middle and basal regions. In some digestive cells the nucleus was oval but in others it had an irregular form adjusted to fit in the free space among the heterolysosomes and their residual bodies.

Digestive cells store glycogen granules (and lipid droplets (Plate:3.1d), but large variations in the amounts of these substances were detected between cells.
Golgi stacks were frequently found, each one formed by 4 or 5 cisternae. Most cisternae showed dilated regions filled with an electron dense material in which a pattern of oblique dark lines could be recognized at high magnification (3.1d). Cells with a single large vacuole were also observed with the electron microscope. In these cells a spherical dense mass was usually found within the vacuole.

Several mitochondria, Golgi stacks, endoplasmic reticulum cisternae, a few peroxisomes and lipid droplets were present in the extremely thin layer of cytoplasm surrounding the vacuole. A flattened nucleus was also localized in the cell periphery. Rare images showed cells with a single large vacuole filled with a homogeneous material which seemed about to be extruded into the lumen of the digestive diverticula. A thin layer of cytoplasm with a very high electron density surrounded the vacuole and some mitochondria were the only organelles that could be recognized in these cells that appear to be degenerating (Plate:3.13 a-d).

Digestive Diverticula

The digestive gland of bivalve molluscs is a paired gland consisting of numerous blind ending tubules, the epithelium of which is composed of two cell types, the basophilic cells and the digestive cells, the fine structure of which has already been described (Yonge 1926; Sumner 1966; Owen 1970, 1972; Pal 1972). The basophilic cells possess a well-developed granular endoplasmic reticulum and a cup-shaped Golgi zone above the nucleus that produces membrane-bound vesicles, probably secretory (Sumner 1966; McQuiston 1969; Owen 1973), located mainly in the apical cell part (Owen 1972). Various functions have been attributed to the basophilic cells, although their nature and exact role are uncertain (Owen 1970, 1972; Bush 1986). On the other hand, the digestive cells contain a well-developed lysosomal vacuolar system (De Duve and Wattiaux 1966) that consists of small coated and uncoated tubulovesicular endosomes, irregularly shaped endolysosomes, large heterolysosomes, and
morphologically heterogeneous residual bodies (Cajaraville et al. 1995), where intracellular digestion takes place (Owen 1972; Morton 1983). The heterolysosomes of the lysosomal vacuolar system are usually very large vesicles, up to 10 nm, located in the apical or in the mid region of the digestive cells, containing either evenly distributed, finely granular, moderately electron-dense material or clumps of electron-dense material. On the other hand, the residual bodies are smaller lysosomes, up to 3 nm, show an electron-lucid halo and a granular matrix clumped in circles or rings in the center of them. The residual bodies together with the heterolysosomes are involved in the endocytotic uptake and digestion of nutrients in the digestive cells and may appear in different portions of the cell depending on the stage of digestion (Owen 1972; Morton 1983; Cajaraville et al. 1995).

**Basophilic cells**

Basophilic cells (Plate.3.1a) contain a well developed granular endoplasmic reticulum and a cup-shaped Golgi zone above the nucleus that produces membrane bound secretory granules usually located in the apical cytoplasm (Plate 3.1d). Most of the latter granules, depending on the section, present more or less extended electron-dense intra-granular areas or cores.

In some instances, these secretory granules are seen in a close proximity to the microvillous border just prior to their membranes being fused with the plasma membrane and their content secreted into the tubule lumen. The endocytic canal system is not very well-developed as in the digestive cells. Nevertheless, a prominent endocytic activity is evidenced by the formation of pits, coated on their cytoplasmic face at the base of the microvillus border of basophilic cells.

**Digestive cells**

In the digestive cells (Figure 2a), the elements of a well developed lysosomal vacuolar system, such as heterolysosomes and residual bodies are apparent. Also
evident is the Golgi complex, located in the supranuclear portion. It is cup-shaped and displays characteristic distended regions located in the periphery or the center of the Golgi stacks (not shown). A condensed material, less regularly arranged, is often localized in vesicles located in the apical cell part. Sometimes in a close proximity to a Golgi complex. In some cases, elongated membranous structures are found near the apical cell part and arranged parallel to the lateral plasma membranes. Inside the elongated membranous structures, clumps of electron-dense material, probably similar to that found inside the peripheral regions of the Golgi complex and the apical vesicles described above, are localized.

The endocytic canal system, in the apical cytoplasm of the digestive cells, branches through the cytoplasm and in section has an appearance of short, tubular profiles and vesicles.

**Gill**

The gills consist of various columnar epithelial, squamous endothelial, and mucous cells (Sunila 1986; Cajaville et al. 1990–1991) and deal with gas exchange, excretion, osmoregulation, transport of food material, formation of a protective coat and lubrication of the surface of the epithelium (Cajaraville et al. 1990–1991). (Plates: 3.2c-d). Plate (3.14a-d) describe in detail about gill epithelium exposed PAHs. WAF treated mussel tissues. Ciliary axonemes appearance occurred in gills of *Perna indica* (Plate 3.3a-d).

Election micrograph of the foot tissue of *Perna viridis* exposed to 2ppm WAF of BHC changes was described in (Plate: 3.5a-d).

Adductor muscle structural changes were given in (Plates: 3.4a, 3.6a-d and 3.10). Degenerative changes in the mantle tissue was in (Plates: 3.7a-d and 3.9a-d). Autophagic vacuole are clearly visible in exposed mantle tissues (Plates: 3.4c-d).

Pedal disc tissue also showed certain degenerative changes like appearance of electron dense materials (Plate: 3.11a-d). Pedal disc groove is visible in (Plate 3.4b).
Plate 3.1.a) General ultra structural aspect of a digestive tubule of *P. viridis* showing digestive cells with heterolysosomes (HL), residual bodies (RB), and basophilic cells (BC) possessing a well developed granular endoplasmic reticulum; l, digestive tubule lumen. b) Normal structure of digestive diverticula of *Perna viridis* c) Secretory granules in the apical region of a basophilic cell. Two of them are just prior to their membranes being fused with the plasma membrane (small arrows) and their content secreted into the tubule lumen. In the apical membrane, note the formation of a coated endocytic vesicle (arrow) d) Electron-dense material is found in small vesicles (arrows) located in the apical part of a digestive cell. HL: heterolysosome, Li: Lipid inclusion, Mv: microvillous border, RB: residual body 21,000×.
Plate 3.2: a) *Perna indica* Hepatopancreas control b) Electron microscopic view of ciliated epithelial cells of gill filament showing the typical structure; dense bodies (DB) are interspersed throughout the cytoplasm (Ci cilia, Mi mitochondria; Nu nucleus; Go golgi apparatus). c) *Perna indica* gill 1 ppm LDO endocytic vesicle with electron dense content were abundant. d) In the apical cytoplasm of a digestive cell, near the tubule lumen (L), the endocytic canal system, comprising of short tubules and vesicles (arrows), is prominent.
Plate 3.3.a) *Perna viridis* 5 ppm BHC WAF: The irregularly shaped nucleus contains a prominent nucleolus. b) Damaged microvillae and cilia of *Perna indica*. c) *Perna indica* gill treated with 0.4 ppm LDO. These ciliary axonemes from gill of *Perna indica*. d) Digestive cell of *Perna indica* treated with 5 ppm BHC WAF showing fusion of lysosomes (arrow). H, heterolysosomes; and filled with electron dense Materials.
Plate 3.4: a) *Perna viridis* Adductor Muscle 2 ppm LDO
b) *Perna viridis* Pedal disc
c) *Perna indica* 3.5 ppm LDO exposed mantle
d) *Perna indica* mantle: treated with 2 ppm BHC WAF showing autophagic vacuole containing partially degraded mitochondria
Plate 3.5: Election micrograph of the foot tissue of *Perna viridis* exposed to 2ppm WAF of BHC showing a) Patchy distribution of blebs b) fusion of lysosome c) Residual bodies of mussel showing a dense appearance and granular appearance respectively (scale bar 1µm)
Plate 3.6: Adductor muscle tissue of *Perna viridis* exposed to 5 ppm BHC showing a – b) moderately dense, flocculent substances and dense irregular inclusions in lysosomes c) distorted endoplasmic reticulum d) localization of lipid droplets. (scale bar 2μm)
Plate 3.7: Mantle tissue of *Perna viridis* 2ppm WAF BHC exposed animals a) poor development of epithelium b) abundant lipid droplets c) digestive tubule showing sloughed off materials from the cell d) vacuole formation and fusion of cells.
Plate 3.8 Electron micrograph of digestive cell of *P.indica* showing a) fusion of lysosomes b) Endoplasmic reticulum and mitochondria containing electron dense material c) phagolysosomes oval in shape d) electron dense material in mitochondria.
Plate 3.9  Electron micrographs of mantle of *Perna viridis* exposed to 5ppm WAF of BHC a) Group of extracellular dense granules b) marked degenerative changes are apparent c) Numerous vacuolated and degenerating mitochondria are visible in the cells d) appearance of electron dense material in the cell. (scale bar 2 µm)
Plate 3.10: Adductor muscle of *Perna viridis* exposed to 8ppm of BHC WAF a) more electron dense material of a heterolysosomes condensed towards residual body b) aggregation of flattened vesicles with the residual vacuole of an F/B cell c) Plasma membrane of the microvilli specialized to form hemidesmosomes at the apex of an F/B cell d) dilated cell enter into the plasma membrane (scale bar 2µm)
Plate 3.11  Electron micrograph of the apical region of a pedal disc cell showing a) apocrine surface activity with granular endoplasmic reticulum; golgi vesicles b) cells in degenerative condition c) basal region of pedal disc cell Note the much folded basal region with cytoplasmic processes and endocytotic vesicles d) structure of pedal groove filled with electron dense materials. (scale bar 2µm)
Plate 3.12: Electron micrograph of digestive diverticula of *P. indica* exposed to 5ppm BHC WAF showing a) lipid droplet accumulation b) The close association between golgi redie, endocytotic vesicle containing fibrous material c) Food particles are apparently being directed between the microvilli d) appearance of irregular resides within the residual vacuole. (scale bar 2 µm)
Plate 3.13: Digestive cell of *Perna viridis* exposed to 5ppm of BHC WAF showing a) degenerative mitochondria, loss of material from lysosomes b) digestive tubule showing localization of lipid droplets c) apical region of an F cell in the hepatopancreas. The nucleus is not visible in this section. d) Residual vacuole of a B cell in the lumen of the hepatopancreas filled with electron dense material. (scale bar 2 μm)
Plate 3.14 a): Transmission electron micrograph of the distal regions of several cells of the gill epithelium of *P. indica* exposed to 3.5ppm LDO WAF showing a) sloughed cell fragment b) a membrane bound cytoplasmic protrusion c) Numerous cytoplasmic vacuoles d) Nuclear vacuole (scale bar 1 µm)
3.4 Discussion

Earlier transmission electron microscopic analyses (TEM) of bivalve gill filaments have shown that cilia projecting from various cell types have a unique morphology (Good et al., 1990; Stephens and Good, 1990; Gregory et al., 1996). In the case of *P. perna*, frontal cilia have a well-defined neck while cilia projecting from lateral squamous cells do not (Gregory et al., 1996). It was considered possible that a careful examination of abnormal lateral cilia may enable the cell type to be determined. Unfortunately, the “undergrowth” of lateral cilia made it impossible to either conclusively prove or disprove the presence of a ciliary neck.

While microvillous swelling, cell necrosis and other pathomorphological alterations might be expected as a consequence of toxic insult, it is more difficult to explain the reason for ciliary hyperplasia in lateral cells. Under normal conditions, the few cilia projecting from lateral squamous cells probably help with the circulation of oxygenated water to the epithelium. We postulate that extended exposure to PAHs may deleteriously affect respiration. If this is the case, then the observed increase in lateral cilia may be a response by the animal to increase flow of oxygenated water over the epithelium thereby enhancing the rate of respiration.

The digestive gland of bivalve molluscs is comprised mainly of blind-ending digestive tubules, the epithelium of which is composed of two major cell types: the columnar digestive cell and the pyramidal basophilic cell (Owen 1972, Morton 1983). The digestive cells are subject to cyclical changes and contain a well-developed lysosomal vacuolar system dealing with the uptake and the intra-cellular digestion of endocytosed substances deriving from the extra-cellular digestion of food particles in the stomach (Owen 1972, Morton 1983, Cajaraville *et al.* 1995, Lobo-da-Cunha 2000). The basophilic cells of molluscs are considered mainly as enzyme-secreting cells,
since the presence of enzymes in their secretory granules has recently been reported (Lobo-da-Cunha 1999).

**Basophilic cells**

The above-mentioned results, in accordance to other reports show that the basophilic cells are responsible for the secretion of digestive enzymes that undertake the extracellular digestion of food particles (Owen 1970, Morton 1983, Henry et al. 1991, Lobo-da-Cunha 1999). The secreting nature of basophilic cells of molluscs is partly based on their morphological similarity with the enzyme-secreting cell of the mammalian exocrine pancreas (Owen 1970, 1972).

The electron microscope results in the present study showing granules of the basophilic cells very close to the apical cell membrane, just before their membranes fuse with the plasma membrane and their content is secreted into the tubule lumen, constitutes a further indication of the secretory nature of these granules.

**Digestive cells**

The Golgi complex of the digestive cells of bivalves is less orderly arranged compared to that in other cell types and commonly displays swollen regions located either in the peripheral or in the central portion of the stacks (Robledo and Cajaraville 1996). These regions contain electron-dense material with characteristic tubular or filamentous substructure, with different degree of condensation (Pal 1972, Owen 1973, Robledo and Cajaraville 1996). Concerning the chemical nature of the electron-dense material inside the tubulofilamentous structures of the Golgi complex observed in the present study. The elongated structures containing similar tubulo-filamentous material could belong to (1) the cisternae of the rough endoplasmic reticulum that has lost most of the ribosomes artefactually or (2) could belong to smooth endoplasmic reticulum or even to the elements of the endosomal system.
The canal system observed in the apical part of the digestive cells of *P. viridis* and *P. indica* is also reported in *M. edulis* (Owen 1972), *Nucella* (Dimitriadis and Andrews 2000), *Risoea* (Wigham 1976), *Lasaea* (McQuiston 1969) and *Cardium* (Owen 1970). The canal system has been regarded as the compartment where the endocytosed material enters the lysosomal pathway (Lloyd 1996). In *Perna*, as well as in the above-mentioned molluscs, no connection of the canal system with the apical membrane is reported. In *Patella vulgata*, an association of the tubules of the canal system with heterolysosomes was reported (Bush 1986). Hypotheses about how pinocytotic vesicles can empty their contents indirectly (Owen 1972) or directly (McQuiston 1969, Wigham 1976) into the secondary lysosomes have been stated. In addition, Bush (1986) suggests a temporary connection of the canal system to the lumen, to establish a concentration gradient along the canal/heterophagosome complex.

In molluscs, the products of extracellular digestion found in the lumen of digestive diverticula are collected by digestive cells for further breakdown in the heterolysosomes. In some species digestive cells can collect relatively large food particles by phagocytosis (Morton, 1979), but in others extracellular digestion is more complete and only dissolved substances are captured by small endocytic vesicles (McLean, 1971; Walker, 1972). Many endocytic vesicles filled with electron dense material were observed in the apical region of *P. viridis* digestive cells. Probably, these vesicles contained the products of extracellular digestion that would be transferred to the heterolysosomes of digestive cells, to complete the digestive process. In some digestive cells the number of endocytic vesicles was very high, indicating a very intense endocytic activity, but in others only a few vesicles could be found, suggesting the existence of stages with low endocytic activity. That would be transferred to the heterolysosomes of digestive cells, to complete the digestive process. In some digestive cells the number of endocytic vesicles was very high, indicating a very
intense endocytic activity, but in others only a few vesicles could be found, suggesting the existence of stages with low endocytic activity.

Several studies have proved that shortly after endocytosis the substances captured by endocytic vesicles will appear in early endosomes; sometime later those substances will be present in late endosomes (or prelysosomal compartment) and finally they will be found in the matured heterolysosomes that contain the largest amounts of acid hydrolases (Thilo et al., 1995; Tjelle et al., 1996). Electronlucent structures limited by a single membrane, containing only small amounts of internal material and practically devoid of acid hydrolases were identified as early endosomes (Tjelle et al., 1996). Structures with identical characteristics were observed in the apical region of A. depilans digestive cells and probably correspond to early endosomes. The heterolysosomes and their residual bodies observed in the digestive cells of P. viridis were very similar to those described in the hepatopancreas of other mollusc species (Owen, 1972a; Henry et al., 1991; Rebecchi et al., 1996). In the basophilic cells of P. viridis, bundles of tubular structures were observed inside some cisternae of endoplasmic reticulum (Lobo-da-Cunha, 1999), but these tubular structures were never found in the digestive cells.

Golgi stacks with dilated cisternae containing dense substances were reported in digestive cells of bivalves (Owen, 1973; Robledo and Cajaraville, 1996). In the bivalve Nucula sulcata, the dilated regions of golgi cisternae contained electron-dense material forming parallel thin lines (Owen, 1973), and a very similar pattern was observed in the golgi cisternae of P. viridis digestive cells.

The excretory cells described in pulmonate gastropods (Sumner, 1965; Walker, 1970) are morphologically very similar to the cells with a large vacuole as that of P. viridis. Some authors considered that the excretory cells were degenerated calcium cells (Thiele, 1953; Sumner, 1965), but others view them as a final stage in the cycle of
digestive cells (Walker, 1970; Morton, 1979; Almendros and Porcel, 1992). Other authors did not recognize intermediate stages and concluded that excretory cells were an independent cell type not derived from digestive or calcium cells (Dimitriadis and Hondros, 1992). In P. viridis these cells seem to be an advanced stage of digestive cell maturation. This hypothesis is supported by the observation of cells in an intermediate stage, showing a partial fusion of the lysosomes.

According to the observations made in semithin and ultrathin sections of P. viridis hepatopancreas, the single large vacuole seems to result from the fusion of all heterolysosomes and residual bodies of a digestive cell. In the end, the undigested substances accumulated in the large vacuole will be excreted to the lumen of digestive diverticula, and after that the cell will probably die. Some basophilic cells of A. depilans possess a large number of vacuoles filling a great part of the cell volume, but they maintain a characteristic pyramidal shape, some apical secretion granules and a large oval nucleus (Lobo-da- Cunha, 1999). Because of these morphological aspects, vacuolated basophilic cells seem not to be precursors of the excretory type cells. Ultrastructural examination of the digestive cells revealed that secondary lysosomes of mussels were wider due to the enhancement of lysosomal fusion.

This role of the lysosomal system may reflect a general metabolic pattern for the detoxification. However, other molluscs with a lysosomal system less developed than mussels could be limited to detoxify cadmium via binding to insoluble compounds (Marigomez et al., 2002).

After progressed PAHs-exposures the digestive cells become vacuolated and reduced in number, while basophilic cells become hypertrophied and are relatively more numerous (Cajaraville et al. 1990a, Marigomez et al. 1990b, 1998). The functional equivalence of basophilic cells with calcium cells of terrestrial gastropods (Marigomez et al. 1995), which have been reported to accumulate many xenobiotics.
Ultrastructure Studies in *Perna* spp.

(Marigomez *et al.* 1986; Reico *et al.* 1988), could account for our suggestion. In the present study, all visualized PAHs were localized mainly in the frontal epithelium, *i.e.* frontal, laterofrontal, lateral, and postlateral gill epithelial cells and endothelial cells (see Sunila 1986 and Wright *et al.* 1987. Since the gill epithelium of many molluscs and *Perna* spp are comprised of various cell types (Nuwayhid *et al.* 1978; Wright *et al.* 1987; Cajaraville *et al.* 1990–1991), differences in the PAHs deposition probably reflect differences in the physiology of the cells along the gill filament.

In the digestive cells, a morphological alteration caused by the PAHs treatment was the aggregation of complex structures containing residual bodies, enclosed by a single membrane. Membrane fusion is a possible mechanism for the formation of these structures, since PAHs interact with biomembranes leading to a decrease in membrane fluidity (Webb 1979). Similar structures named “compound residual bodies” were commonly found in the digestive cells of some bivalves (Owen 1972), however, their large number observed in treated mussels could probably be a result of PAHs exposure.

Studies in *M. galloprovincialis* suggested that the basophilic cells also undergo important alterations under stress conditions (Cajaraville *et al.* 1990). In the present study, fragmentation or vacuolization of the rough endoplasmic reticulum of basophilic cells was noted after BHC and LDO treatment. Since this histopathological alteration observed in our experiments has also been reported in basophilic cells after petroleum hydrocarbon exposure (Carles *et al.* 1986; Lowe 1988), it probably represents a general and not a specific stress response. So a suggestion of the present study, is the use of the vacuolization of the rough endoplasmic reticulum of the basophilic cells, as a potential general stress indices in marine pollution monitoring studies (Cajaraville *et al.* 1990; Soto and Marigomez 1997a).
Increased number and size of the dense granules in the basophilic cells compared to that of controls was observed. The increase in the number and size of these granules probably represents an increase in the enzyme activity of the basophilic cells after PAHs treatment. The latter hypothesis is supported by Marigomez et al. (1990b), who reported that the increase in the number of the secretory granules of the basophilic cells after xenobiotic exposure may be necessary to augment enzyme secretion for extracellular digestion. Thus, this response may be a defense mechanism to compensate the disturbance of intracellular digestion after PAHs exposure.

The main gill lesions observed in some of the treated mussels were fusion of the lateral epithelium of the filaments in the BHC and LDO-treated mussels. Similar abnormalities have been described in the gills of *M. edulis* either after an experimental exposure to Cu or after field sampling (Sunila 1986, 1987). Sunila (1986) observed empty intercellular spaces and detachment of the abfrontal cells after Cu and Ag treatment in *M. edulis*. Detachment of the cells from the chitinous rod may be a sign of enhanced gill regeneration. Despite the fact that regeneration of the gill epithelium is rather a normal cyclic activity, the gill lesions are probably related to regeneration errors of the gill (Sunila 1986).

In conclusion, by using light and electron microscope observations, the present study provided useful information on PAHs sequestration by the cells of the digestive gland and the gills. When viewed by TEM, intracellular dense granules were observed distributed in the cytoplasm of the digestive gland cells. In addition, these granules are close to endoplasmic reticulum, forming clusters in association with lipid droplets.

Chapman et al. (1996) related the molluscs capacity in maintain high PAHs concentration in their tissues within discrete ranges. These organisms may accumulate large amounts of PAHs in their tissues mitigating toxic effects by sequestering then in granular form.
The digestive gland of *P. viridis* and *P. indica* and other marine bivalves consists of numerous blind-ending tubules, the epithelium of which is composed of digestive and basophilic cells. In the digestive cells, the existence of a well-developed endocytic lysosomal vacuolar system, mainly consisting of heterophagosomes, heterolysosomes and residual bodies, evidences intra-cellular digestion processes; in addition, the lysosomal content is characterized by the presence of acid hydrolases dealing with the intra-cellular digestion of nutrients. In the abovementioned studies, there are few data concerning the role of basophilic cells, which display a highly developed rough endoplasmic reticulum and numerous secretory granules, in enzyme-production and secretion.

It may be concluded that *P. indica* and *P. viridis* were similar to other bivalves in that it efficiently accumulates PAHs in its soft tissues. In addition, it would appear that chronic exposure to increased PAHs induces significant morphological changes in its gill tissues. While exposure to other pollutants, either singly or in combination may produce other responses, it is hoped that these data will provide an initial comparative baseline for future studies.