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Ultrastructural aberrations in the hepatopancreas of *Metapenaeus dobsoni* (Miers) exposed to mercury

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Abstract

Toxic effect of mercury on, the flower-tail shrimp, *Metapenaeus dobsoni* (Miers) was investigated by exposing the animal to sub-lethal concentrations of 0.005 and 0.015 ppm of the metal. As pollutant-induced stress leads to cellular responses and often gets manifested as ultrastructural alterations, an attempt was made to delineate the alterations and damage caused to fine structure level with the help of electron microscopy. The hepatopancreas, which has been identified as a target organ, depicted considerable ultrastructural alterations. Mitochondrial membrane was damaged and the cristae were swollen, assuming circular shape at many instances. Endoplasmic reticulum was totally damaged. Excessive accumulation of lipid was noticed in the R-cells. Electron-dense inclusions were found "nipped off" from the basal lamina. Merging of vesicles resulted in collection of electron-dense materials in larger residual vacuoles. The damage seemed to be dose-dependent. Ultrastructural damage of the cellular components was found to render the cardinal organelles less or non-functional, thus affecting the functional integrity of the organism. Pollutant induced injuries of this kind often prove to be useful biological tools to assess the stress profile.

Keywords: *Metapenaeus dobsoni*, hepatopancreas, ultrastructure, mercury toxicity

Introduction

It is a matter of concern that heavy metals get increasingly concentrated along successively higher trophic levels of the marine food chains. Crustaceans, which form important links in the food chains, would play an active role in this process. Trace metals, including those that are essential, are known to turn harmful above the threshold level. Although information is available on the lethal toxicity and load of heavy metals in the aquatic system, their biological effects on marine organisms have been a subject of considerable debate.

The present work delineates the deleterious effects of mercury at sublethal level on *Metapenaeus dobsoni* (Miers), a commercially important penaeid shrimp contributing substantially to the shrimp fishery in India. Mercury, which has no established biological function, is one of the highly toxic heavy metals, affecting biological systems even at very low concentrations. Among the harmful effects reported in the aquatic organisms are inhibition of acid and alkaline phosphatases (Renfro *et al.*, 1974), disruption of ionic balance and cell membrane permeability (Bouquegnau and Gilles, 1979), blocking of phosphorylation sites of enzymes (Skou and Norby, 1979) and inhibition of sodium transport and Na-K-ATPase (Murti *et al.*, 1985). According to Trump *et al.* (1975) mercury toxicity results in structural alterations which are generally correlated with functional changes at the cellular level. Andersen and Baatrup (1988) made an in-depth study of absorption, transport and deposition of mercury in *Crangon crangon.*

Both biochemical and physiological responses to pollutant-induced stress ultimately lead to cellular responses which often get manifested in ultrastructural alterations in the cellular organelles. Biological effects of this kind are generally dose-dependent and quantifiable and hence are considered as valuable biological indicators of stress. These are often employed as useful tools to predict possible effects of xenobiotics on the vital activities of the organisms and to identify the specific organelles, cells or organs involved as target response sites of the pollutants. Crustacean hepatopancreas has been identified as a target organ which gets extensively damaged when exposed to heavy metal pollution. Since the hepatopancreas in Crustacea plays a key role in several important physiological functions, the potential harm that could be done to it by xenobiotics is considerably high. It is keeping this in view

that hepatopancreas was chosen for the present study on mercury toxicity. According to Heath (1987) liver is probably the primary organ for the excretion of harmful trace metals and hence of importance when considering the action of polluting chemicals.

Materials and methods

Test animal *M. dobsoni* (25-35mm from the tip of rostrum to the tip of telson) were collected from the aquaculture farm at Vypeen (76°10' E, 10°0' N) and transported to the laboratory in oxygen-filled polyethylene bags. They were fed *ad libitum* on fresh clam meat and acclimatized to a salinity of 20±2 ppt. Sub-lethal levels of 0.005 and 0.015 ppm mercury were determined with reference to LC_{50} values for the species (Sivadasan *et al.*, 1986). Experimental medium was prepared by dissolving mercuric chloride ($HgCl_2$) in filtered sea water to give the required concentration in terms of ppm metal ion, the amount of this in the salt being calculated from the atomic weight. Three replicates were run simultaneously for each treatment and the control (n=10 animals/replicate). The animals were fed *ad libitum* on fresh clam meat and the left over feed and faecal matter were siphoned out every 24 h. Test solutions were made from a stock solution and 2/3rd of the test solution in each tub was replenished every 24 h. The medium was well aerated. The experiment was run for 15 days, at a salinity of 20±2 ppt, at room temperature (28±2 ° C).

Ultrastructural studies were carried out on the hepatopancreas of *M. dobsoni* belonging to the intermoult stage. Animals from different treatments and control were sacrificed on closure of the experiment. Hepatopancreas was dissected out from 10 animals from each treatment and fixed immediately in cold (4°C) cacodylate-buffered glutaraldehyde solution (5%) at *p*H 7.2 for 12 h. The tissue was then washed 3 times in cacodylate buffer solution, trimmed and washed again in fresh buffer solution. The tissue was further fixed in 1% osmium tetroxide solution at 4°C for 2 h. After draining, the tissue was washed in several changes of fresh buffer solution followed by washing in double-distilled water. Dehydration was done in ascending series of ethanol concentrations (15 min each in 30%, 50%, 70%, 90% and absolute) at 4°C, giving two washes at each step. Ethanol and Spurr's resin were used for infiltration. Finally the tissue was embedded in Spurr's embedding resin, keeping the moulds at 70°C for 24 h. Silver-coloured ultra thin sections were taken with an 'Ultracut' E (LKB) ultramicrotome. The sections were mounted on copper grids and stained with uranyl acetate (Watson, 1968) and lead citrate (Reynolds, 1963). The sections were examined and electron micrographs were taken in a Philips EM 300 transmission electron microscope, operating at 60 kV.

Results

Exposure of *M. dobsoni* to mercury led to severe ultrastructural alterations in the hepatopancreas. The fine structure of R- and F-cells in the hepatopancreas of control animal is depicted in Figures 1 & 2. The nucleus maintains the normal shape and the nuclear membrane is intact. Chromatin is found dispersed at the centre and along the periphery of the nucleus adjacent to the nuclear membrane as lumps. Cytoplasm of the cell is traversed with endoplasmic reticulum with uniformly arranged ribosomes. Mitochondria and lysosomes of different sizes are found in the cytoplasm. The basal lamina, which separates the cells from the haemolymph space, is provided with numerous invaginations. The B-cells contain vacuoles with electron-dense inclusions, which are generally distributed uniformly forming homogenous bodies.

The nuclei of the epithelial cells did not register much evidence of damage in animals exposed to 0.005-ppm

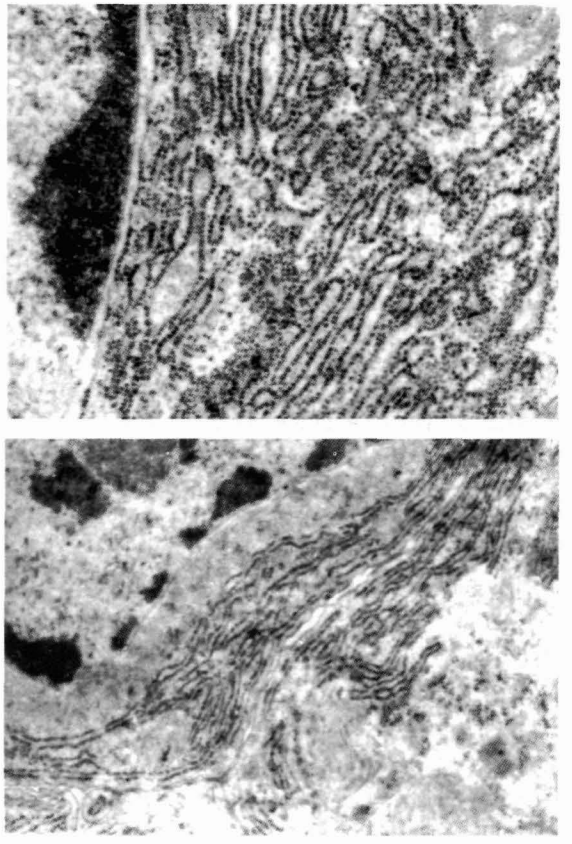

Fig. 1 & 2 Ultrastructure of normal R- and F- cells of the hepatopancreas of *M. dobsoni* maintained under controlled conditions. x16200 (Fig.1), x25700 (Fig. 2).

mercury. However, the endoplasmic reticulum showed considerable alteration, it being disfigured and disintegrated in some cells. The reticulum in some regions enclosed electron-dense material by bending of cisternae (Fig. 3). Mitochondrial membrane also showed damage. At a few instances the membranes were diffused so as to give an impression of total damage. It was also noticed that mitochondira could lose their characteristic shape. Excessive quantities of lipid were found in the R-cells. Merging of vacuoles resulting in collection of electron-dense materials was noticed (Fig. 4). These inclusions might subsequently be released into the lumen. The cristae of the mitochondria were swollen, giving the mitochondrial structure a hazy nature. The plasma membrane was also found swollen at a few places suggesting mercury-induced damage of biological membranes (Fig. 5). The microvilli of the cells did not display any structural deformity.

In hepatopancreas of *M. dobsoni* exposed to 0.015 ppm mercury, almost all the cardinal inclusions of the cell displayed damage to various degrees. The rough endoplasmic reticula lost their characteristic arrangement. They were broken down into very small bits which were distributed haphazardly throughout the cell. Total disintegration of the reticula was also noticed in some cells (Fig. 6). The basal lamina developed characteristic foldings and these folds were found to contain electron-dense materials which could be mercury-rich inclusions. The entrapped materials could possibly be "nipped off" from the basal lamina and taken into the cell (Fig.7). Conspicuous thickening of the basal lamina, especially in areas associated

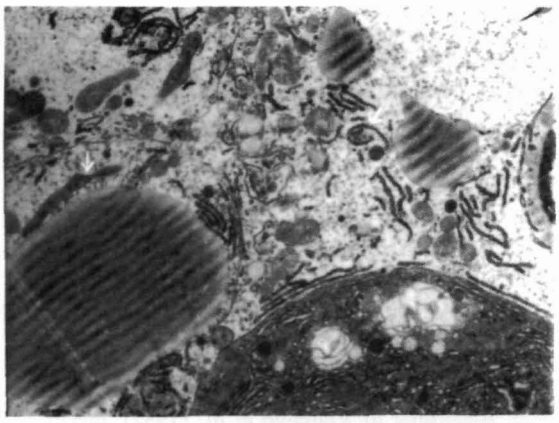

Fig. 3. Distorted mitochondria and enfolded electron-dense inclusions in the R-cells of the hepatopancreas of *M. dobsoni* exposed to 0.005 ppm mercury. x6000.

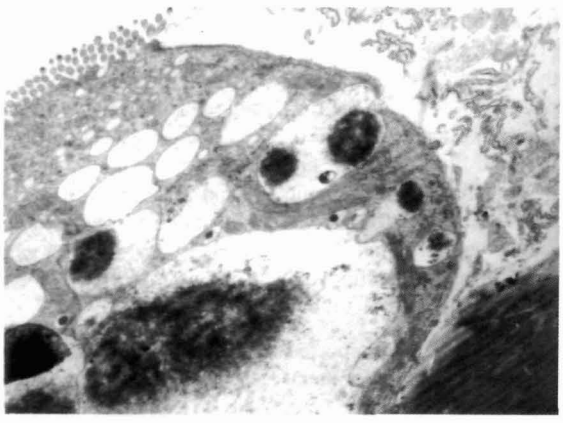

Fig. 4. Merged vacuoles with electron-dense inclusions in the B-cell of *M. dobsoni* exposed to 0.005 ppm mercury. x7300.

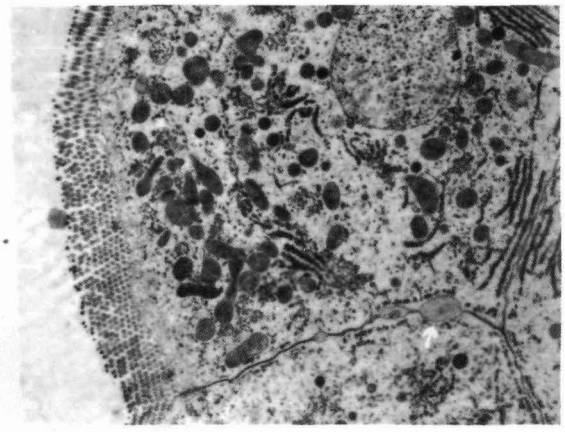

Fig. 5. Swollen plasma membrane in the hepatocytes of *M. dobsoni* exposed to 0.005 ppm mercury. x6000.

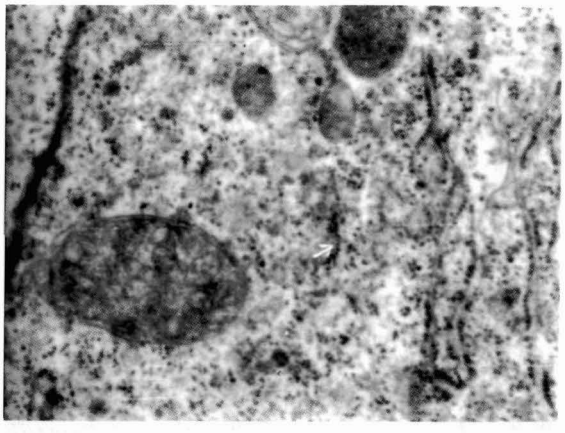

Fig. 6. Damaged endoplasmic reticulum in the R-cell of the hepatopancreas of *M. dobsoni* exposed to 0.015 ppm mercury. x25700.

with invaginations, also was noticed. The lipid droplets showed translucent areas. These electron-translucent areas could indicate breaking down of lipid inclusions. Mitochondria showed widespread damage in structure. The cristae assumed circular shape at many instances. In some cases the mitochondria were found extremely swollen and totally devoid of cristae. Similarly the membrane also showed damage. Large residual body-like structures were seen in the cells which could probably be the result of fusion of degenerating mitochondria with lysosomes (Fig.8).

Discussion

Critical examination of cells and their inclusions forms an important aspect of histopathology accompanying toxicity on target organs. In the decapod midgut gland, there are well defined digestive diverticula. New cells (E-cells) are formed at the apex of each diverticulum and differentiation into the other types of cells viz. R-cells, F-cells and B-cells, proceeds down the tubules. Among the vital functions attributed to this dynamic organ are secretion, digestion, absorption, storage and excretion. Crustacean midgut gland is also known to serve as an organ that inactivates a number of potentially toxic metals (Dall and Moriarty, 1983). While physiological malfunctioning is mainly assessed by rate-functions, histopathological and ultrastructural studies give a true picture of the actual damage caused. Histopathological and ultrastructural alterations resulting from xenobiotic exposure serve as effective indices of physiological and biochemical changes giving an insight to cellular injury. According to Moore (1985), earlier detectable histopathological or ultrastructural changes induced by xenobiotics are associated generally with subcellular organelles such as mitochondria, lysosomes, endoplasmic reticulum and biological membranes.

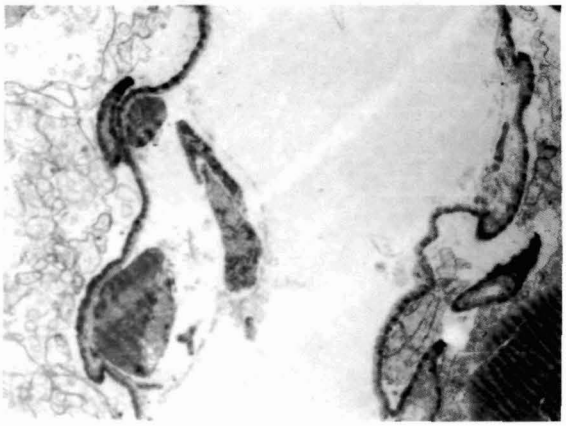

Fig. 7. Electron-dense inclusions adjacent to the basal lamina in the R-cell of *M. dobsoni* exposed to 0.015 ppm mercury. x6000.

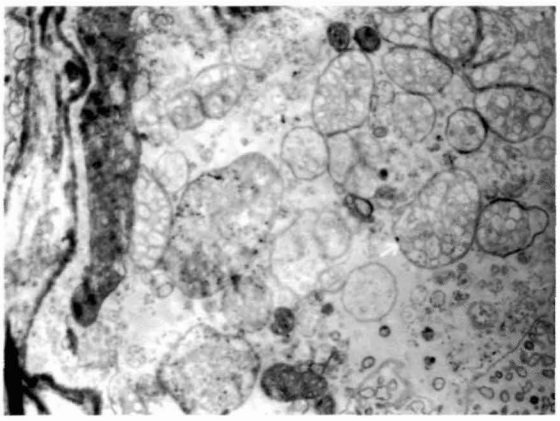

Fig. 8. Degenerating mitochondria in the R-cell of the hepatopancreas of *M. dobsoni* exposed to 0.015 ppm mercury. x12400.

Considerable structural deformity is noticed in the cardinal organelles such as endoplasmic reticula, mitochondria and basal lamina of the epithelial cells of the hepatopancreas of *M. dobsoni* exposed to low levels of mercury. According to Hinton and Lauren (1990) stressor-associated alterations of hepatocytes may be found in the nucleus or cytoplasm, or both. Viarengo (1985) has stated that metals can interact with nuclear proteins, altering the complex structure of chromatin or the catalytic activity of enzymes involved in DNA and RNA metabolism. According to Viarengo *et al.* (1982), metals like mercury, copper and cadmium initially decrease the RNA synthesis which subsequently returns to control level or increase further. Extensive degeneration of the endoplasmic reticula, as noticed in the present study would lead to malfunctioning of this organelle. According to Viarengo (1985) mercury, cadmium and copper are able to reduce the rate of protein synthesis by influencing the attachment of polyribosomes to the rough endoplasmic reticulum and probably damaging the ribosomes themselves. Detoxification and lipid synthesis are among the special functions of the smooth endoplasmic reticulum (De Robertis and De Robertis, 1980). A general negative effect exerted by metals on the membranes of both the rough and smooth endoplasmic reticulum is due to lipid peroxidation (Buss and Gibson, 1979).

George (1982) reported active accumulation of mercury and certain other metal cations by mitochondria, the organelles responsible for aerobic ATP production. Mercury is known to block the phosphorylation sites of enzymes by binding to the essential –SH group (Skou and Norby, 1979). It is demonstrated that extremely low

concentrations of heavy metals can inhibit oxidative phosphorylation. As mitochondria are a possible site of lipid peroxidation (Buss and Gibson, 1979), the involvement of heavy metals in this process also could interfere with their structure and physiology. The present study is in agreement with the observations made by De Robertis and De Robertis (1980), who reported that injury to mitochondria may produce degenerative changes consisting of fragmentation, intense swelling, accumulation of material and formation of cytolysosomes or large chondriospheres. Accumulation of large quantities of lipid droplets has been observed in *M.dobsoni* exposed to mercury. Similar observations have been made by Lowe (1988) and Moore (1988) in the case of mussels exposed to pollutants. Papathanassiou and King (1984) reported accumulation of fat in whorls on endoplasmic reticulum when *Palaemon serratus* was subjected to starvations stress.

Lysosomal-vacuolar system has been recognized as the major degradative system within the cell. According to George *et al.* (1982), metals accumulate in lysosomes by a combination of autophagy and generation of acidic groups within aging secondary and tertiary lysosomes. The formation of pathologically enlarged lysosomes is associated with membrane destabilization or increased permeability resulting in the release of degradative hydrolytic enzymes into the cytosol and also in lysosomal fusion with other intracellular vacuoles. This would eventually lead to autolytic activity and atrophy of the digestive cells (Moore, 1985). Andersen and Baatrup (1988), in a study on the effects of mercury on *Crangon crangon*, reported accumulation of the metal within the residual bodies located near the luminal surface of the epithelial cells and suggested exocystosis of the metal into the lumen. According to Manisseri and Menon (1995), vacuolation of the tubular cells may be due to the formation of membrane-bound vesicles containing accumulated heavy metals as electron-dense granules or perhaps be related to vesiculation of endoplasmic reticulum as a result of the failure of ion pump. The incidence of secondary and tertiary lysosomes as a conspicuous feature in the tubular cells suggests xenobiotic induced cellular pathology disturbing both structure and function (Moore 1982; 1985).

Electron-dense inclusions, near the basal lamina are probably metal-rich bodies transported from the haemolymph to the cells for further sequestration and elimination. According to Al-Mohanna and Nott (1987), R-cells in the hepatopancreas of *Penaeus semisulcatus* can take up soluble and particulate material from the haemolymph by pinocytosis at the basal cell membrane. In their study on mercury toxicity in *Crangon crangon*, Andersen and Baatrup (1988) reported accumulation of the metal in the hepatopancreas, major part of the metal being absorbed from the haemolymph. Conspicuous thickening of the basal lamina, especially in areas associated with invaginations as is seen in the present study could be an index of damage as reported by Storch *et al.* (1984) in the hepatopancreas of starvation-stressed *Penaeus monodon.*

According to Palmer *et al.* (1992), shrimps, crabs and oysters have the potential to obtain mercury from both water and food. However, chronic sub-lethal stress, though more common in the environment, often goes unnoticed since adverse effects are generally manifested first at the suborganismal level. Pollutant-induced injuries to the internal organ systems have now been recognized as useful tools to assess the stress profile. Vogt (1987) suggested the use of such studies in monitoring the impact of water pollutants on shrimp. It is amply evident from the present investigation that the functional integrity of hepatopancreas is seriously affected even on chronic exposure of shrimp to very low levels of mercury.

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