Copper-induced damage to the hepatopancreas of the penaeid shrimp *Metapenaeus dobsoni* — an ultrastructural study

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**ABSTRACT** The hepatopancreas of decapod crustaceans has been recognised as an important target organ for studying the effects of heavy metal pollution. This dynamic organ, with its capability of detoxifying heavy metals through lysosomal activities, often indicates distinct pathological disturbances. This led to the present investigation in which we examined the ultrastructure of hepatopancreas of the penaeid shrimp *Metapenaeus dobsoni* (Miers) exposed to culture medium containing 50 and 150 ppb of copper. After an exposure period of 15 d, the hepatopancreas was dissected from individuals belonging to the intermoult stage and the structure studied employing transmission electron microscopy. Structural deformities of the cells were not confined to a specific inclusion. Instead, subcellular organelles including the endoplasmic reticulum, mitochondria, basal lamina, and nucleus showed damage of varying degrees. Damage to the nucleus and its inclusions would have a profound effect on its functioning. Endoplasmic reticulum and mitochondria showed partial to total disintegration which probably would have made them non-functional. Smooth endoplasmic reticulum, the prime organelle for detoxification and lipid synthesis, also showed damage, indicating the possibility of a disruption of the cellular detoxification mechanism. Although at lower levels (50 ppb) copper was found to induce a biphasic effect on the histology and histochemistry of the hepatopancreas, this was not reflected in the nature of structural damage of the subcellular organelles performing vital functions. Presence of electron-dense granules in the haemolymph and epithelial cells is indicative of the role played by these granules in detoxification or demobilisation of copper.

**KEY WORDS:** Copper-induced damage - Hepatopancreas - *Metapenaeus dobsoni*

**INTRODUCTION**

Cellular assay techniques are employed to study pollutant-induced injuries on the internal organ systems of organisms. Such injuries serve as reliable biological indicators of pollution and are effectively used in assessing stress effects. The crustacean hepatopancreas displays considerable cytological, cytochemical and ultrastructural alterations at chronic exposure to low levels of heavy metals. Hence, it has been identified as a target organ of interest in toxicity investigations. Ultrastructural studies aid in locating the specific cell types in different organs which constitute targets. Such studies thus answer questions concerning specific alterations at the cellular level.

Crustaceans play a significant role in marine food chains. They also form a group of animals which are frequently sensitive to heavy metals. With the exception of mercury and silver, copper is the most toxic metal to marine organisms (Bryan 1976). The pathways of entry of copper into the body of crustaceans, the organs or organelles where accumulation takes place and the processes by which the metal gets sequestered and when possible eliminated from the body have been the subject of much investigation over the past 3 decades (Kerkut et al. 1961, Miyawaki et al. 1961, Gibson & Barker 1979, White & Rainbow 1986, Arumugam & Ravindranath 1987). Structural compartmentalisation of copper in membrane-limited vesicles has been reported in *Carcinus maenas* (Chassard-
Bouchard 1982), *Oniscus asellus* (Marcaillou et al. 1986) and *Penaeus semisulatus* (Al-Mohanna & Nott 1987). In a study on accumulation and toxicity of copper in *Penaeus orientalis*, Liu Fayi et al. (1988) reported maximum accumulation of copper in hepatopancreas.

Xenobiotic-induced sublethal cellular pathology reflects perturbations of function and structure at the molecular level. Earlier detectable changes are associated with subcellular organelles such as the mitochondria, lysosomes or endoplasmic reticulum (Moore 1985). The interaction of heavy metals with nuclear proteins and ribosomes, and their possible mutagenic effects, have been documented by Viarengo et al. (1982) and Viarengo (1985). Conney & Burns (1972), Duss & Gibson (1979) and Livingshine (1985) studied the deleterious effects of xenobiotics on the membranes of endoplasmic reticulum.

In the present study, the ultrastructural alterations brought about by copper toxicity in the hepatopancreas of a commercially important penaeid prawn *Metapenaeus dobsoni* (Miers) have been elucidated. The rationale arose out of the concept that the cells and cell organelles of the hepatopancreas might depict alterations or damage which could be associated with stress at organismic or organic levels.

**MATERIALS AND METHODS**

Juvenile *Metapenaeus dobsoni* (measuring 25 to 35 mm from the tip of the rostrum to the tip of the telson) collected from prawn culture fields at Vypeen (76° 10' E, 10° 00' N) were acclimatized to a salinity of 20 ± 2 ppt. The medium was well-aerated and the shrimp were fed ad libitum on fresh clam meat. Copper sulphate was used as the source of heavy metal. Ten shrimp each were exposed to 50 and 150 ppb of copper in a semi-flow-through system for 15 d at room temperature (28 ± 2°C). Test solutions were prepared from a stock solution and 1/5 of the test solution in each tub was replenished every 24 h. A control was also run along with the treatments, in which the shrimp were kept in filtered seawater, which also was changed at 30 min each in 50, 25, 50:50, 25:75 and 75:25 concentrations of ethanol and Spurr’s resin without DMEA (75:25, 50:50, 25:75) 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; sections were mounted on copper grids and stained with uranyl acetate (Watson 1958) 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.

For light microscopy, the tissues were fixed in Bouin’s fixative, dehydrated using ethyl alcohol, cleared in xylene and embedded in paraffin wax (58 to 60°C). Sections of 7 µm thickness were obtained, stained in Delafield’s haematoxylin and eosin and mounted in DPX (Dextrene Plasticizon Xylene), following standard methodology.

**RESULTS**

We analysed the fine structure of *Metapenaeus dobsoni* hepatopancreas both with and without exposure to copper. Fig. 1a, b (light micrographs) presents the different cell types of the hepatopancreas of shrimp maintained under control conditions. Electron micrographs (Fig. 2a, b) depict the fine structure of the R- and F-cells of normal hepatopancreas. Cytoplasm of the cell is traversed with endoplasmic reticulum with varying sizes are found in the cytoplasm. A distinct basal lamina separates the haemolymph space from the cell. Clumps of chromatin are found dispersed at the center and along the periphery of the nucleus. The B-cells contain vacuoles with inclusions which are generally found to be uniformly distributed, forming homogeneous bodies.

The hepatopancreas of prawns exposed to 50 ppb of copper showed structural damage to the cells. The variations found in the ultrastructure of cells included damaged nuclei, disfigured and disintegrated endoplasmic reticulum, and excessive accumulation of glycogen in the endoplasmic reticulum. The mitochondrial membrane was distended in some locations and partially degenerated in others (Fig. 3). Numerous electron-dense
bodies, distributed near the inner side of the basal lamina, appeared to be inclusions into which copper has been incorporated (Fig. 4). In one instance, a large electron-dense area was identified inside the haemolymph space. We believe that this was a copper-rich inclusion being transported into the cell through the basal lamina. Totally disarrayed lipid droplets were distributed inside the cells. The microvilli of the cells did not display any structural deformity.

In the case of the hepatopancreas of *Metapenaeus dobsoni* exposed to 150 ppb of copper, almost all the cardinal inclusions of the cell were destroyed to various degrees. Many nuclei had lost their typical elliptical shape due to extensive shrinkage of the nuclear membrane (Fig. 5). This resulted in denser distribution of chromatin. An enlarged view of the nucleus showed characteristic ruffling of the nuclear membrane. The shrinkage could be due either to disruption of the tensile strength of the nuclear membrane or to dehydration of the nuclear content. The rough endoplasmic reticula lacked their characteristic arrangement. They were broken down into very minute pieces (ca 8 pm) and were dis-

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**Fig. 1.** *Metapenaeus dobsoni*. Cross section of hepatopancreas of shrimp maintained under controlled conditions showing tubules (t) and different cell types (E-, R-, F- and B-). Light microscopy; (a) x20, (b) x200.

**Fig. 2.** *Metapenaeus dobsoni*. Ultrastructure of normal R- and F-cells of the hepatopancreas of shrimp maintained under control conditions: (a) x16200; n: nucleus; er: endoplasmic reticulum; (b) x25700.
Fig. 3. *Metapenaeus dobsoni*. Ultrastructure of the R-cell of the hepatopancreas of shrimp showing swollen mitochondria when exposed to 50 ppb copper. ×25700

Mitochondria were also found to lose their characteristic shape. In a few instances, mitochondria were found totally disintegrated in the R-cells (Fig. 6). The basal lamina had developed characteristic foldings and the folds were found to contain electron-dense material which could have been copper-rich inclusions (Fig. 7); these may possibly be 'nipped off' and taken onto the cell, thereby resulting in transfer of copper from the haemolymph to the cell.

DISCUSSION

Heavy metals at sublethal levels are known to affect the structure and functioning of cellular components, leading to impairment of vital functions of many marine organisms. It is in this context that histopathological and ultrastructural alterations are employed as effective indices of physiological and biochemical changes caused by copper-induced stress. These biological indices provide insight into cellular injuries...
before any irreversible alteration occurs. The hepatopancreas is a vital organ in crustaceans, with secretory, absorptive, digestive and excretory functions. This organ has been identified as a target organ for heavy metal pollution because it shows critical histopathological and ultrastructural alterations at very early stages of exposure to toxicants. The present study on copper-exposed *Metapenaeus dobsoni* elucidates the major ultrastructural alterations brought about in the vital organelles of hepatopancreas cells.

Most of the authors who have studied the hepatopancreas of crustaceans have agreed on a classification of the epithelial cells into 4 groups (E-, R-, F- and B-cells) and that they are formed by a sequence of cellular differentiation (e.g. Gibson & Barker 1979, Hopkin & Nott 1980, Dall & Moriarty 1983). In the present study, the structural details of various cellular components of the hepatopancreas in control prawns confirmed with the descriptions given in the literature. The hepatopancreas cells of *Metapenaeus dobsoni* exposed to the low copper level harboured damaged cardinal organelles such as mitochondria, endoplasmic reticulum and nuclear membrane. The nuclear damage observed included distortion of nuclei with 'scalloped' edges, damage of the nuclear membrane, and overall shrinkage resulting in the nuclei losing their characteristic shape. These changes would profoundly influence the normal functioning of the nuclei. Metals can interact with nuclear proteins, altering the complex structure of chromatin or the catalytic activity of the enzymes involved in DNA and RNA metabolism (Viarengo 1985). Metal cations can also induce depolymerisation and favour hydrolysis of RNA, affect the correct replication or transcription of DNA, and alter the fidelity of the translation of RNAs during the process of protein synthesis at the ribosomal level.

Extensive disruption and disintegration of the endoplasmic reticulum observed in the prawns exposed to copper may indicate serious deleterious alterations associated with heavy metal stress. The extent of damage seemed to be directly proportional to the concentration of toxicant in the medium. Detoxification and lipid synthesis are among the special functions of smooth endoplasmic reticulum (De Robertis & De Robertis 1980). Copper reduces the rate of protein synthesis by reducing the rate of RNA synthesis, influencing the attachment of polyribosomes to the rough endoplasmic reticulum, and potentially damaging the ribosomes themselves (Viarengo 1985). A general negative effect exerted by metals on the membranes of both the rough and smooth endoplasmic reticulum is due to lipid peroxidation (Buss & Gibson 1979). Structural damage of the kind noticed in the mitochondria of *Metapenaeus dobsoni* exposed to copper would probably render this organelle less or non-functional. Since various enzymes which participate in the formation and transfer of high energy phosphate bonds are located in the mitochondria, the role of mitochondria appears to be the formation and liberation of compounds of high phosphate transfer potential (e.g. ATP). Injury to mitochondria may produce degenerative changes consisting of fragmentation and intense swelling (De Robertis & De Robertis 1980).

Increased accumulation of residual glycogen enmeshed in the endoplasmic reticulum and the appearance of disarrayed lipid droplets in the prawns exposed to 50 ppb of copper probably indicate a biphasic effect of this metal. In a study of accumulation and toxicity of copper in *Penaeus orientalis*, Liu Fayi et al. (1988) observed an increase in cytochrome oxidase activity in the hepatopancreas on the fourth day of the exposure period. Subsequently, a steady decrease in cytochrome activity was recorded. As cytochrome oxidase is associated with the smooth endoplasmic reticulum, this could indicate a proliferation of the latter towards the beginning of the exposure period. A biphasic effect of copper was reported in the case of *Mytilus edulis* by Calabrese et al. (1984). Moore (1988) also reported a biphasic effect of a mixture of diesel oil and copper in molluscan digestive cells. Accumulation of large quantities of lipid droplets has been observed in *Metapenaeus dobsoni* exposed to copper. Similar observations have been made by Lowe (1988) and Moore (1988) in the case of mussels exposed to toxic substances. *Metapenaeus dobsoni* exposed to copper showed merging of vesicles or vacuoles containing electron-
dense materials. The inclusions were not uniformly spaced as in the control prawns. The lysosomal-vacuolar systems is considered to be the major degradative system within the cell. Tertiary lysosomes in the hepatopancreas and excretory organs can accumulate large quantities of metals. Many invertebrates including crustaceans are to excrete these bodies by a process of exocytosis (George 1982). Numerous electron-dense inclusions seen near the basal lamina of hepatopancreas cells of copper-exposed *M. dobsoni* could be metal-rich bodies in the process of being transported from the haemolymph to the cell for further sequestration and elimination (Fig. 7). According to Al-Mohanna & Nott (1987), the R-cells in the hepatopancreas of *Penaeus semisulcatus* can take up particulate material from the haemolymph by pinocytosis at the basal cell membrane. It is also reported that copper, sulphur and other elements are accumulated in large dense vacuoles. The present findings support this hypothesis.

Icely & Nott (1980) carried out ultrastructural studies on the accumulation of copper within the hepatopancreatic caeca of the amphipod *Corophium volutator*. In the case of amphipods with high tissue burdens of copper, numerous granules of homogeneous electron-dense material were found. Amphipods with lower tissue concentrations of copper had fewer such granules. Weeks (1992) also reported the presence of copper-rich granules containing homogeneous electron-dense material (with no nucleus or core) in the R-F-cells of the ventral caeca of *Orchestia gammarellus* inhabiting copper-polluted localities. The presence of electron-dense granules in the haemolymph and epithelial cells of *Metapenaeus dobsoni* hepatopancreas indicates the role played by these granules in the detoxification and elimination of copper from the tissues of copper-contaminated individuals.

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