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Functional Alterations with Reference to Lipofuscin Production in the Hepatopancreas of Metal Stressed Prawn

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Abstract

In the present investigation, we examined the effects of heavy metals on formation of lipofuscin, in the hepatopancreas of the penaeid shrimp *Metapenaeus dobsoni* (Miers), exposed to culture medium containing 0.05 and 0.15 ppm copper and 0.005 and 0.015 ppm mercury. Impact of mercury on lipofuscin accumulation is found to be rather limited. Intensity of lipofuscin is much more in the case of copper exposed animals. It is more so in animals exposed to 0.05 ppm of copper as the epithelial cells in the middle and proximal region of the tubules seem to be heavily laden with lipofuscin granules. The increase is noticed not only in the number of granules but also in the size. The distal tubular region with practically no secondary or tertiary lysosomes is devoid of lipofuscin granules.

Introduction

Excessive accumulation lipofuscin granules can be induced in organisms by normal aging as well as dietary, stressful or toxic environmental factors. The process of autophagy of lipoprotein membranous components in the cytoplasm by lysosomes and lipid peroxidation of these components resulting in the formation of lipofuscin have been explained by earlier workers. Formation of lipid peroxides in the biological membranes often results in extensive disturbance of their basic structure and function. Lipofuscin granules also complex metals, making them unavailable to the cells (George *et al.*, 1982). Increase in the lipofuscin content is often associated with increase in the number of tertiary lysosomes, facilitating the process of elimination of toxic metals by exocytosis. Studies on the toxic effects of heavy metals on crustaceans are limited. Among the studies on the effects of copper on prawn are those of Chen (1985) on *Penaeus monodon* and Liao and Hsieh (1988, 1990) on *P. japonicus* and *Macrobrachium rosenbergii*. Mercury, which has no established biological function, is also one of the highly toxic heavy metals affecting biological system at very low concentrations. Toxic effects of mercury have been studied in *Crangon crangon* (Snderen and Baatrup, 1988), *Palaemonetes pugio* (Kraus *et al.*, 1992), *Penaeus japonicus* (Chuabng *et al.*, 1991), *Penaeus* shrimp and crab (Palmer *et al.*, 1992), *P. aztecus* (Palmer and Presley, 1993), *Callinassa tyrrenna* (Thaker and Haritos, 1993) and *Pleoticus muelleri* and *Artemesia longinaris* (Marcovecchio, 1994). It has been a matter of concern that heavy metals get accumulated along successively higher trophic levels in the marine food chains. Evidently, crustaceans which form important links in the marine food chains would play an active role in this process. In the light of this, the present study on the effect of copper and mercury on accumulation of lipofuscin in the hepatopancreas of *Metapenaeus dobsoni*, a commercially important penaeid

shrimp abundant in tropical waters, assumes topical importance and relevance.

Material and Methods

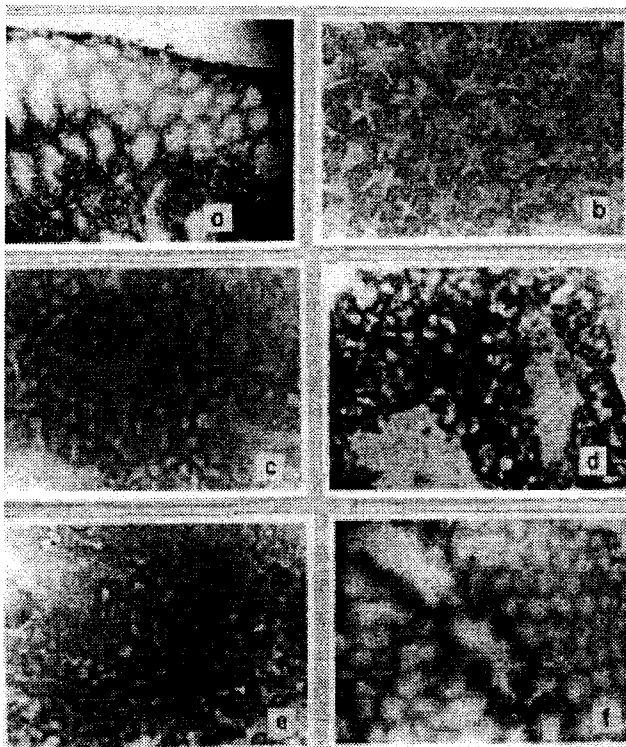
Juvenile *Metapenaeus dobsoni* measuring 25-35 mm from the tip of rostrum to the tip of telson were collected from an aquaculture farm at Vypeen (76° 10' Long E, 10° 0' Lat N). The animals were transported to the laboratory in oxygen filled polythene bags and acclimatised to a salinity of 20 ± 2 ppt. They were fed *ad libitum* on fresh clam meat. The test animals were exposed in batches of ten to sublethal doses of 0.05 and 0.15 ppm copper and 0.005 and 0.015 ppm mercury in a semi flow-through system for 15 days at room temperature ($28 \pm 2^\circ\text{C}$). Experiments were run using 3 replicates for each treatment and the control. Copper sulphate and mercuride were used as sources of heavy metals. The test medium was well-aerated. Left over feed and faecal matter were siphoned out and 2/3rd of the test solution in each treatment was replenished every 24h. Three animals (belonging to the intermoult stage) were selected from each replicate of the different treatments on closure of the experiment for histochemical studies. Hepatopancreatic studies. Hepatopancreatic tissue was dissected out immediately after sacrificing the animals and fixed in hexane using liquid nitrogen. Sections, 10 microns in thickness, were cut using a Bright Cryostat, maintaining a chamber temperature of $-20 \pm 1^\circ\text{C}$. Lipofuscin contents in the hepatopancreas of animals belonging to the various treatments were determined using the Schmorl reaction (Moore, 1988). Sections were post-fixed in calcium formal for 15 min at 4°C ; rinsed in distilled water and stained in a reaction medium (1% ferric chloride and 1% potassium ferricyanide in a ratio of 3:1) for 5 min. After staining, the sections were immersed in 1% acetic acid for 1 min; rinsed in distilled water and mounted using an aqueous mounting medium (glycerin jelly). Lipofuscin granules were

indicated as blue reaction products and their sizes and abundance were determined by microscopical assessment of the sections.

Results and Discussions

Colour intensity, an index of lipofuscin content, is minimal towards the peripheral region of the hepatopancreatic tissue. Cell differentiation in the hepatopancreas of decapod crustaceans follows a gradient, the youngest embryonic cells being seen towards the periphery and the relatively older cells towards the middle and proximal regions of the tubules. The less differentiated embryonic cells occupying the distal region are generally devoid of large secondary and tertiary lysosomes containing lipofuscin granules. On the contrary colour intensity indicating the presence of lipofuscin is reasonably high towards the middle and proximal regions of the tubules where the cells contain numerous secondary lysosomes and residual bodies. Concentration of lipofuscin, as evidenced by the colour intensity at the proximal region probably indicates storage of metal sequestered granules for subsequent disposal. (Fig. 1a).

Lipofuscin activity is reasonably good in the hepatopancreas of the control animals (Fig. 1b). Impact of



- Fig. 1a Lipofuscin granules in the hepatopancreas of *M. dobsoni* exposed to 0.05 ppm copper. X20
 Fig. 1b Hepatopancreas of *M. dobsoni* maintained under controlled conditions indicating lipofuscin granules. X50
 Fig. 1c Lipofuscin granules in the hepatopancreas of *M. dobsoni* exposed to 0.015 ppm mercury. X200
 Fig. 1d Intense accumulation of lipofuscin granules in the hepatopancreas of *M. dobsoni* exposed to 0.05 ppm copper. X100
 Fig. 1e Hepatopancreas of *M. dobsoni* exposed to 0.15 ppm copper with less intense accumulation of lipofuscin granules. X200
 Fig. 1f Hepatopancreas of *M. dobsoni* exposed to 0.15 ppm copper, showing lipofuscin granules near the proximal end. X20

mercury on lipofuscin accumulation is rather limited (Fig. 1c). Intensity of lipofuscin is much more in the case of copper exposed animals (Fig. 1d and 1e). It is more so in animals exposed to 0.05 ppm of copper as the epithelial cells in the middle or proximal regions of the tubules seem to be heavily laden with lipofuscin granules. The abundance is reflected not only in the number of these pigment granules but also in their increased sizes. The absence of colouration towards the distal region with practically no secondary or tertiary lysosomes is clearly evident in animals exposed to 0.05 and 0.15 ppm copper whereas just the reverse is noticed at the proximal end where the tubules open to the common lumen (Fig. 1f).

Lipofuscin granules are formed as a result of lysosomal degradation of cellular membranes occurring after fusion of primary vesicles or other cell organelles with lysosomes to form multivesicular bodies, called tertiary lysosomes. Biochemical studies suggest that lipofuscin is the end product of peroxidation of polyunsaturated membrane lipids by free radicals. The fact that these pigment granules are ubiquitous in living organisms (George *et al.*, (1982) explains the presence of lipofuscin, though in small quantities, in the hepatopancreatic cells of the control animals, as observed in the present study. The tertiary lysosomes enable detoxification of excess quantities of heavy metals. Viarengo *et al.*, (1988) reported an increase of lipofuscin in the digestive cells of mussels when exposed to copper and elimination of the metal, bound by lipofuscin in a relatively stable form, by exocytosis of the residual bodies. Totaro *et al.*, (1985) reported involvement of copper in the formation of neuronal lipofuscin in the spinal ganglia of *Torpedo marmorata*. Copper induces lipoperoxidative reaction of free radicals and this in turn results in an increase of both the number and average size of lipofuscin granules. Greater accumulation of lipofuscin pigment granules with an increase in their average size as noticed in the hepatopancreatic cells of *Metapenaeus dobsoni* exposed to copper is in agreement with the observations made by the authors. The greater accumulation of lipofuscin granules observed in *M. dobsoni* exposed to 0.05 ppm copper could probably indicate a biphasic effect of the metal.

Compared to copper, mercury at sublethal levels, causes lesser accumulation of lipofuscin pigment granules in the epithelial cells of the hepatopancreas of *Metapenaeus dobsoni*. A possible explanation is that alteration of the redox balance and stimulation of lipid peroxidation processes in cells, depend on the kind of metal involved. In a study on the effect of different heavy metals on the rate of lipid peroxidation in the tissues of mussels, Viarengo *et al.*, (1988) observed that copper but not cadmium or zinc is able to alter the redox balance stimulating the peroxidation process and leading to the formation of lipofuscin. This clearly shows the differential effects of heavy metals on the formation of lipofuscin granules.

In a study on the effect of heavy metal ions on microorganism aging, Pisanti *et al.*, (1988) stated that lipofuscin

could serve as a marker of the presence and the level of metal pollution in marine environment. According to the authors almost all the transition metals containing unpaired electrons and thus qualifying as radicals, induce lipofuscinogenesis. The quantity of lipofuscin present is generally proportional to the level of pollution and could be used as an index of environmental contamination by these metals. The present study on *Metapenaeus dobsoni* clearly shows that the lipofuscin content of copper or mercury stressed animals is always more than that of the control and serves as an index that could be employed in the assessment of chronic stress from sublethal doses of xenobiotics.

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