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Histopathological changes induced in the gill of an estuarine mullet, *Liza parsia*, by sublethal exposure to mercuric chloride

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ABSTRACT

Sublethal exposure of *Liza parsia* to $HgCl_2$ (0.5 ppm) elicited erratic swimming pattern, loss of equilibrium and respiratory distress during the initial 24 hrs. On day 2, copious secretion of mucus, swelling (hypertrophy) and vacuolization of the epithelial lining cells of secondary gill lamellae were noticed. Oedema and oedematous separation of epidermis from the basement membrane as well as necrosis and desquamation of epithelial cells were observed by day 4. Oedematous changes, particularly at the base of primary lamellae, tissue hyperplasia and fusion of secondary gill lamellae were seen on day 8 of the treatment. Extensive cellular hyperplasia, desquamation of epithelial cells, congestion of blood vessels and lamellar telangectases (aneurysms) were frequently encountered on day 10 and the entire interlamellar spaces became filled with the hyperplastic epithelium by day 15 of $HgCl_2$ intoxication.

Introduction

Mercury (Hg) pollution in the aquatic (both freshwater and sea) environment has been reported from many parts of the world (Ruvio, 1972; Scott and Armstrong, 1972; FAO, 1986; Zingde, 1989). High concentrations of Hg have been recorded in various tissues of freshwater as well as marine fishes (Scott and Armstrong, 1972; Drummond *et al.*, 1974; Richard and Krzynowek, 1979; Buggiani and Yannucchi; 1980; FAO, 1986). Recent studies have demonstrated the distribution of Hg along the Indian coasts too (Tejam and Halder, 1975; Singbal *et al.*, 1978; Sanzgiry *et al.*, 1979, 1988; Zingde and Desai, 1981; Krishnakumar and Pillai, 1990). Though Kureishy *et al.* (1979, 1983) have found mercurial accumulation in the marine teleosts collected from Indian Ocean and Andaman waters the concentration is far below the level recommended by WHO.

The toxic effects of Hg have been well documented among freshwater fishes (Pickering and Henderson, 1966; Wobeser, 1975; Deshmukh and Marathe, 1980; Khangarot and Somani, 1980; Menezes and Qasim, 1983; Gill *et al.*, 1988). Though Helmy *et al.* (1979) and Krishnakumari *et al.* (1983) have recorded toxicity to heavy metals on *Liza macrolepis* and *Therapon jarbua*, re-

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spectively, we lack information on the organal damage caused by Hg in the fish inhabiting estuarine/marine environment (Zingde, 1989). Since the gill covers more than 60% surface area of the fish and its external location as well as intimate contact with water makes it the most vulnerable target for the pollutants (Roberts, 1989), an attempt was made to record the branchial histopathology in response to the sublethal mercuric chloride ($HgCl_2$) treatment.

Materials and methods

Immature specimens (average length 6.5 cm, weight 5.8±0.5 g) of *Liza parsia* (Hamilton - Buchanan), collected from the Chinese dip net of Fort Cochin (09°57'06"N, 76°14'02"E), were transported to the laboratory. They were kept in aquaria of 120 l capacity containing well-aerated sea water (salinity 28 ppt, average water temperature 26°C) for a period of one week prior to use. Thereafter, they were randomly divided into two equal groups of 40 specimens.

Group A : Fishes were maintained in sea water (control).

Group B : Fishes were exposed to the sublethal concentration of 0.5 ppm of $HgCl_2$ (one-third of LC50 value for 96 hrs) in sea water.

Since fish actively extract Hg from the water (Drummond *et al.*, 1974; Cuvin-Aralar and Furness, 1990), the medium was renewed every alternate day. Fishes were not fed during the entire course of investigation and dead fishes were removed from the water. Five specimens from each group were killed on days 2, 4, 7, 10 and 15 of the treatment. The gills were removed and fixed immediately in freshly prepared aqueous Bouin's solution. After 24 hrs, the tissues were washed thoroughly and routinely dehydrated in ascending series of alcohol, cleared in xylene and embedded in paraffin wax at 60°C. Serial sections were cut at 8 μ and stained in hematoxylin-eosin (H&E).

Results

Fishes exposed to $HgCl_2$ initially exhibited symptoms of distress like erratic swimming pattern, increased opercular movements, loss of equilibrium and lethargy. Excessive mucus secretion was also noticed which was reduced by day 7. Skin of the treated fish turned pale yellow, coarse and scaleless at some places by day 10 onwards. About 40% mortality was observed after day 7 of $HgCl_2$ exposure.

Primary gill lamella (filament) of control *Liza parsia* comprised of a central core of cartilaginous rod, lining epithelial cells and blood vessels whereas the secondary gill lamella consisted of a layer of flattened epithelial cells attached to the basement membrane, contractile pillar cell system and blood spaces (Fig. 1). Sublethal exposure of the fish to $HgCl_2$ for 2 days resulted in swelling (hypertrophy), cytoplasmic vacuolization and an increase in the weakly staining eosinophilic cytoplasm of the epithelial cell (Fig. 2). Oedema and oedematous separation of epidermis from the basement membrane were noticed on day 4 of the intoxication. Also, epithelial lining cells of the secondary lamellae depicted necrosis (Fig. 3). Severe oedema, particularly near the base of primary lamellae, fusion of secondary gill lamellae and general hyperplasia were observed by day 7 of $HgCl_2$ treatment (Fig. 4). By day 10, extensive cellular hyperplasia, desquamation of epithelial cells, congestion of blood vessels and lamellar

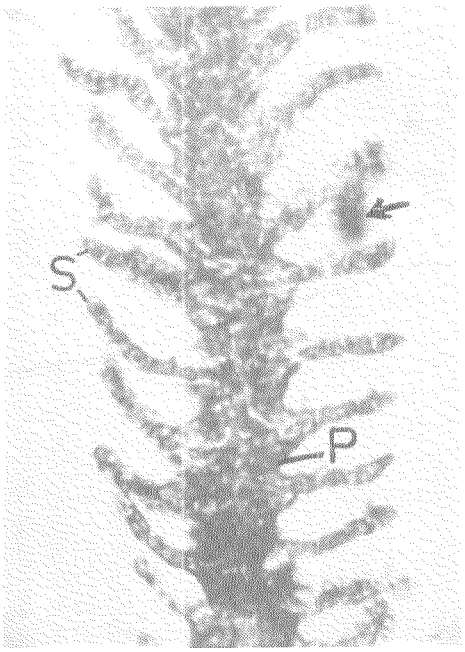

Fig. 1. Gill of control *Liza parsia* showing primary (P) and secondary (S) gill lamellae. (arrow-an artifact). H & E. x 240.

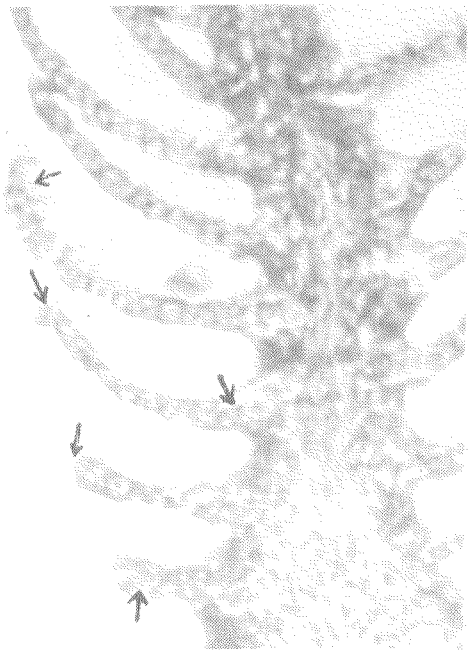

Fig. 2. Gill of *Liza parsia* on day 2 of $HgCl_2$ treatment exhibiting hypertrophied (swollen) epithelial cells of secondary lamellae. Mark the cytoplasmic vacuolization (arrow) of the epithelial cells. H & E. x 400.

telengectases (or aneurysms) were frequently seen (Fig. 5). However, such changes were drastic in $HgCl_2$-treated fish for 15 days to the extent that the entire interlamellar spaces became filled with the hyperplastic epithelium (Fig. 6).

Discussion

Sublethal exposure to $HgCl_2$ elicited behavioural as well as respiratory distress and excessive mucus secretion in the mullet, *Liza parsia*. Similar responses have also been noticed among freshwater fishes (Pickering and Henderson, 1966; Wobeser, 1975; Deshmukh and Marathe, 1980; Khangarot and Somani, 1980; Menezes and Qasim 1983) and estuarine/coastal species (Helmy *et al.*, 1979; Krishnakumari *et al.*, 1983). The loss of equillibrium and erratic swimming activities may probably be due to the effect of Hg on the central nervous system (CNS) of the fish (Menezes and Qasim, 1983; George *et al.*, 1989).

Gills are the major route for the entry of Hg into the fish (Olsen and Fromm, 1973; Drummond *et al.*, 1974) and they also accumulate significant amount of this metal as compared to other tissues (Walczak *et al.*, 1986; Cuvin-Aralar and Furness, 1990). Lethal effects of heavy metals (Hg, Pb, Cu, Zn) have been ascribed to the coagulation of mucus on gill surface and

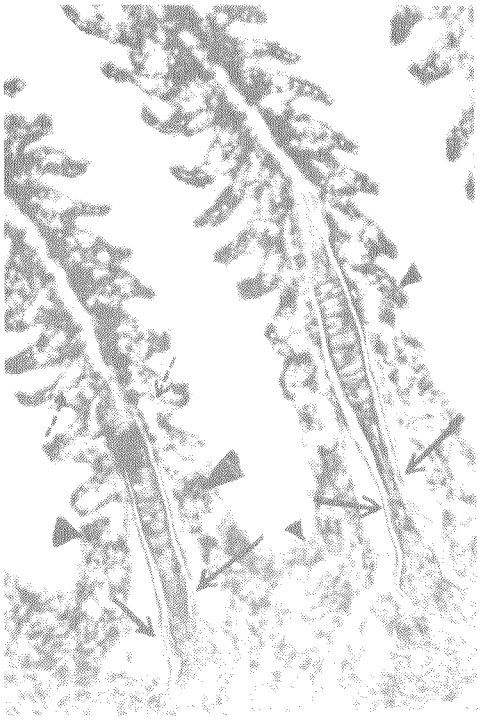

Fig. 3. Gill of *Liza parsia* on day 4 of $HgCl_2$ intoxication depicting oedema (arrow) and separation of epidermis from the basement membrane of the secondary lamellae. Mark the necrosis and desquamation of the epithelial cells (arrow head). Broken arrow = Pillar (Pilaster) cell system. H & E. x 240.

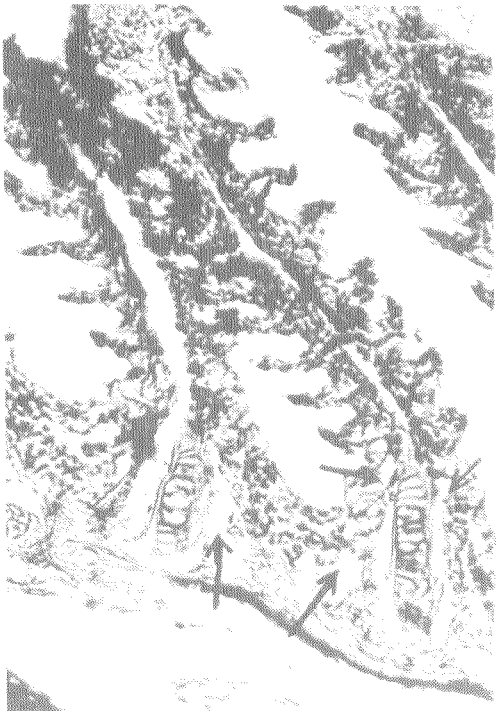

Fig. 4. Gill of *Liza parsia* showing oedematous changes at the base of primary filament (arrow) on day 7 of $HgCl_2$ exposure. Fusion of secondary gill lamellae are also seen. H & E. x 240.

damage to the branchial tissue which culminate in the respiratory failure (Wobeser, 1975; Kumar and Pant, 1981; Menezes and Qasim, 1983; Sultan and Khan, 1983; Gill *et al.,* 1988).

Epithelial cells of the secondary gill lamellae of $HgCl_2$- treated *Liza parsia* depicted an initial hypertrophy and vacuolization (Fig. 2). These appear to be the general response of the gill to heavy metal pollution (Wobeser, 1975; Kumar and Pant, 1981; Gill *et al.,* 1988; Roberts, 1989; Pandey *et al.,* 1995). The oedematous changes observed at the base of primary and secondary gill lamellae of *Liza parsia* (Figs. 3, 4) may probably be due to the increased capillary permeability or lowered efficiency of the epithelial cells in maintaining normal water balance (Roberts, 1989). The progressive hyperplasia, necrosis, desquamation of the epithelial cells and multiple telengectases (aneurysms), as observed in *Liza parsia* to prolonged $HgCl_2$ treatment (Figs. 5, 6), have also been noticed in fry and fingerlings of *Salmo gairdneri* (Wobeser, 1975) and *Puntius sophore* (Khangarot and Somani, 1980) due to the mercurial intoxication. Multiple lamellar telengectases and filling of the entire interlamellar spaces by hyperplastic epithelium in 15 days

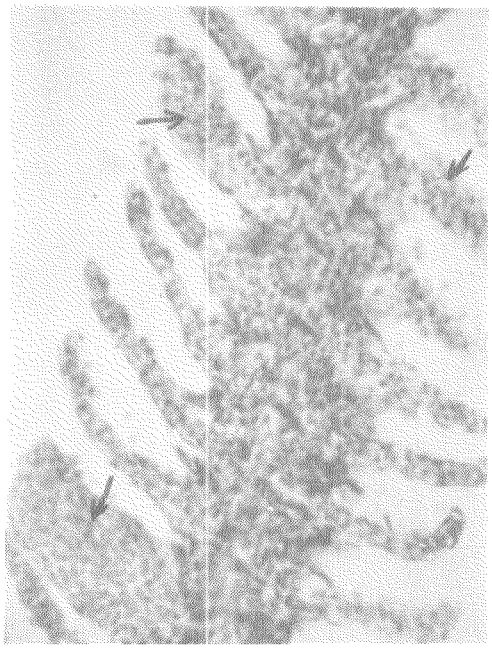

Fig. 5. Gill of *Liza parsia* on day 10 of $HgCl_2$ treatment. Mark the multiple telengectases (aneurysms) on the secondary lamellae (arrow) and cellular hyperplasia. H & E. x 400.

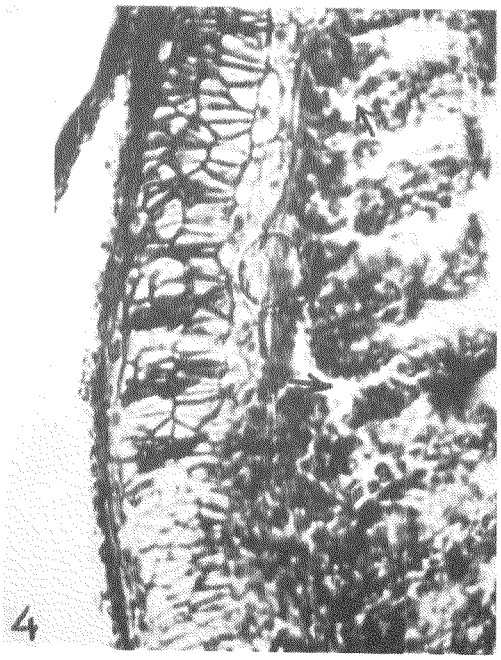

Fig. 6. Gill of *Liza parsia* exhibiting oedema (arrow) and extensive cellular hyperplasia filling the entire inter-lamellar spaces by day 15 of $HgCl_2$ treatment. H & E. x 600.

exposed mullets tend to explain the high mortality of fish due to asphyxia (Roberts, 1989).

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