Ultrastructural Changes in the Posterior Gut of Atlantic Herring, *Clupea harengus* L. Larvae Exposed to Mercury

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Abstract

Cellular changes attributed to mercury solutions (0.02, 0.05, 0.1 µg·l^{-1}) were observed in columnar cells in the posterior gut of Atlantic herring, *Clupea harengus* L. larvae. Significant changes occurred in the specimens exposed to 0.05 and 0.1 µg·l^{-1} mercury. These changes were (a) cytoplasm contained vacuoles and few ribosomes (b) increase in the relative volume of the mitochondria (c) reduction in the surface-to-volume ratio of the mitochondria cristae (d) swelling of smooth and rough endoplasmic reticulum (e) decrease in the number of microvilli at the lumen surface. The morphology of the columnar cells from control specimens is discussed.

Introduction

Metal ions are serious pollutants in the aquatic environment (Eisler et al. 1972, Sorentino 1979). Effects of some metals on the fine structure of aquatic animals have been studied (Gardner and LaRoche 1973, Somasundaram et al. 1984, 1985). Pathological conditions of copper such as hepatic and renal disorders (Baker 1969), nephrotoxic and neurotoxic,

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lateral line damage (Eisler and Gardner 1973), lesions in olfactory organs and renal tissues, hemorrhage in brain periorbital connective tissues (Gardner and LaRoche 1970) have been recorded in fish. Eggs of Atlantic herring (*C. harengus*) exposed to copper show altered rate of development, larval deformities and cellular disruption in the brain, muscle and epidermis (Abbasi et al. 1995a,b,c 2000). Similar effects were also recorded when the fish were exposed to zinc or cadmium (Alderdice et al. 1979, Moore and Ramamoorthy 1984, Somasundaram et al. 1984 1985). Mercury also induces physiological and morphological abnormalities in adult and early life stages of fish (Wobeser 1975, Daost 1981, Lock et al. 1981). Pathological effects of mercury were reported in fish such as sloughing of gill epithelia, necrosis of epidermal cells, damage to epidermal mucous cells, lesions on skin and degeneration of olfactory organs (Gardner 1975, Miller 1981, Olson et al. 1973). Trump et al. (1975) reported that liver, kidney, brain cells and epidermal cells were affected when fish were exposed to mercury.

In the larvae of Atlantic herring, the gut and pronephros are not functional (Blaxter and Holliday 1963) and dependent on the yolk for nutrition. When feeding commences, a few days after hatching, the food is passed almost immediately to the posterior end of the gut where digestion is thought to take place (Blaxter 1962). Any disruption in the gut during the larval life would affect their function when feeding begins. Many estuaries and coastal waters are polluted with high concentration of metals. Jastania and Abbasi (2003a,b) reported that the brain cells and epidermal cells were severely affected when Atlantic herring larvae were exposed in 0.05 and 0.1 $\mu g \cdot l^{-1}$ mercury. Atlantic herring stocks spawn in estuarine waters and may thus be exposed to pollutants. It was considered of interest to examine the posterior gut of the larvae of *C. harengus* L. hatching from eggs previously exposed to mercury.

Materials and Methods

Egg incubation

C. harengus adults, which were about to spawn, were gill netted from the Castle Reach, Milford Haven, Wales, U.K. during March 1988. Eggs were stripped from these fish and artificially fertilized (Alderdice et al. 1979). Approximately equal numbers of fertilized eggs were placed in 2 l glass jars containing either artificial sea water (Tropical Marine Salts) diluted to 21‰ (ambient salinity) or test solutions containing 0.02, 0.05 and 0.1 $\mu g \cdot l^{-1}$ mercury, prepared from a stock solution of mercuric chloride. The control and test solutions were kept at pH 7.5. The jars were

continuously aerated at 8±1ºC (ambient temperature). Control and test solutions were renewed every 2 days in order to maintain the level of mercury and to counter possible adsorption by glassware and uptake by the eggs. At hatching (14 days after fertilization), larvae were removed for electron microscope fixation.
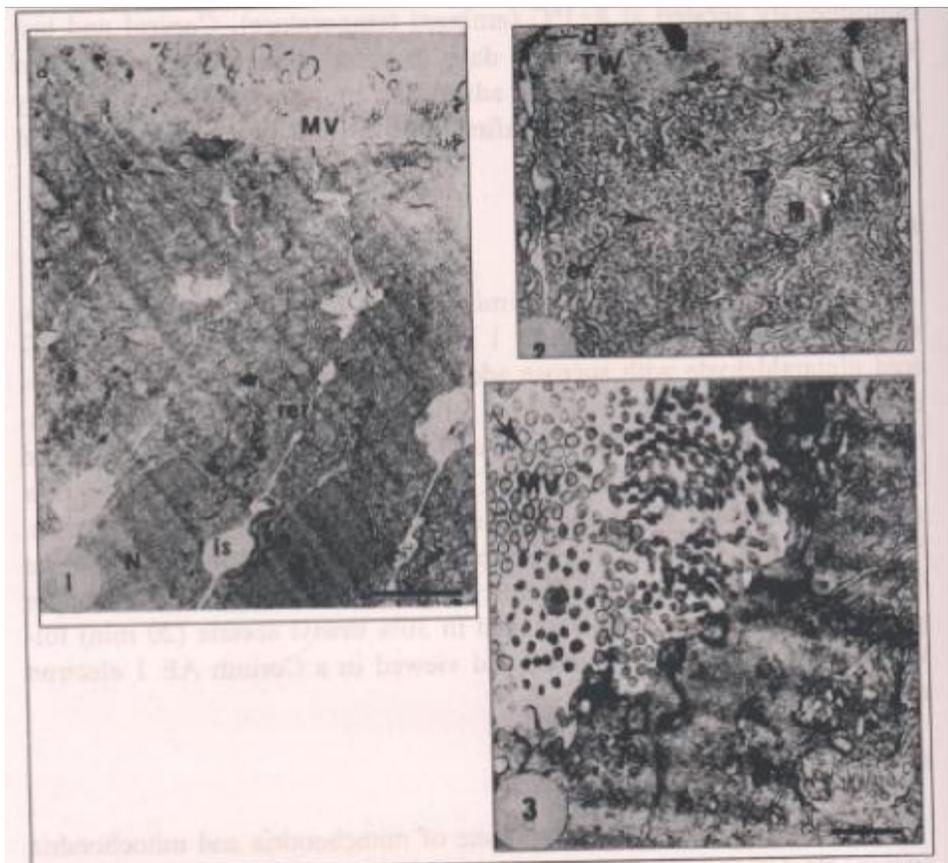
Electron microscopy

At hatching, five larvae of similar length from the control and from each concentration were fixed for 1 hour at 0-4°C in 5% cacodylate-buffered glutaraldehyde with sucrose added. These were then washed in several changes of the buffer solution followed by post fixation in 1% osmium tetroxide solution for 1 hour at 0-4ºC. After further washing in the buffer solution for 1 hour at 0-4ºC, the material was dehydrated in a graded cold acetone series and then embedded in TAAB embedding resin. Sections with gold or silver interference colors were obtained using a Cambridge Huxley Mark 1 Ultramicrotome and were mounted on copper grids. They were then double stained in 30% uranyl acetate (20 min) followed by lead citrate (8-10 min) and viewed in a Corinth AE 1 electron microscope.

Morphometric analysis

To measure of the relative volume of mitochondria and mitochondria cristae, electron micrographs were enlarged to a magnification of $39x10^3$ and analysed by counting the number of squares covered by the organells of the cell divided by total number of squares covered by the whole cell. To measure the surface-to-volume ratio of mitochondria cristae, individual mitochondria were enlarged to a final magnification of 167.45×10^3 and analyzed using multipurpose test system consisting of 100 points inclosing 50 short test lines (Weible et al. *1*966).

Results

At hatching, the posterior gut is a simple straight tube containing columnar epithelial cells (Figs. 1-4). The surface of the cells from control specimens possess numerous regularly spaced 2.0-2.5µm microvilli with a diameter of 0.06-0.14µm. Microvilli are interspersed with a number of cilia extending into the gut lumen (Fig. 1). Cytoplasm immediately adjacent to the microvilli contains a fine filamentous web (Fig. 2). The filamentous terminal webs are lying closely to each other. Nucleus is oval and located in the basal half of the cell. Cytoplasm contains smooth and

Figs. 1, 2 and 3. Electron micrograph of the epical region of epithelial cell of posterior gut of *C. harengus* larvae at hatching showing lumen consists microvilli (MV), nucleus (N), rough endoplasm reticulum (rer), interacellular space (IS), terminal web (TW), Desmosomes (d) ribosomes (arrow) and mitochondria (M), and cilia (arrow). Scale bar 2 µm.

Fig. 4. Electron micrograph of the basal region of the epithelial cell showing a basement membrane (BM), nucleus (N), and elongated mitochondria (M), rough endoplasmic reticulum (arrow), intercellular space (is). Scale bar 1.0 µm

rough endoplasm reticulum; elongated mitochondria lie near the invaginations of the basal plasma membrane (Fig. 4). In the basal part of the cell, double membranes arise from the plasma membrane and are closely associated with mitochondria. Desmosomes hold cells together and occur near the apical terminal web region.

Significant changes occurred in the posterior gut of the specimens hatched from the eggs previously exposed to 0.05 or 0.1 $\mu g \cdot l^{-1}$ mercury (Figs. 5-10); however, in the specimens exposed to 0.02 $\mu g \cdot l^{-1}$ mercury, there was no significant cellular change. The relative volume of the mitochondria and the surface-to-volume ratios of their cristae in the posterior gut cells are given in Table 1. The increase in the relative volume of the mitochondria suggests swelling of the mitochondria (Fig. 8). The mitochondria cristae show significant reduction in surface-to-volume ratio and in some cases breakdown of the cristae (Figs. 7-8). Swelling of the smooth and rough endoplasm reticulum also occurred. Multivesicular bodies appeared at the base of microvilli and in the cytoplasm (Fig. 5). Slouging of the microvilli from the cell membrane (Figs. 6-7) occurred in conjunction with the formation of vacuoles at their base. Lysosomes and other vesicles appeared in the apical half of the cell (Fig. 9). A reduction in the number of microvilli present at the terminal surface of the cell was observed and finally some remnants of the microvilli lie in the lumen region (Figs. 9-10).

Discussion

The columnar cells of the posterior gut of *C. harengus* larvae are similar to those in the posterior gut of feeding larvae of rainbow trout, *Salmo gairdneri*, bass, *Lateolabrax japonicus* and goldfish, *Carassius auratus* (Iwai 1968). At hatching, herring larvae with yolk sacs, take food by darting movements following an S-shaped flexing of the body (Blaxter and Holliday 1963). This suggests that at hatching the posterior gut cells

Table 1. Morphometric analyses of relative volume of mitochondria and surface - to - volume ratio of their cristae in the posterior gut of *C. harengus* larvae at hatching

Concentration $\mu g \cdot l^{-1}$ (mercury)	n	mean relative volume of mitochondria	p	mean surface-to-volume ratio of cristae	p
control	15	0.116	0.23		
0.02	15	0.117	>0.001	0.21	>0.001
0.05	15	0.123	<0.05	0.25	>0.01
0.1	15	0.143	<0.05	0.18	<0.001

n - number of observations; p - significant difference from control where $p < 0.05$
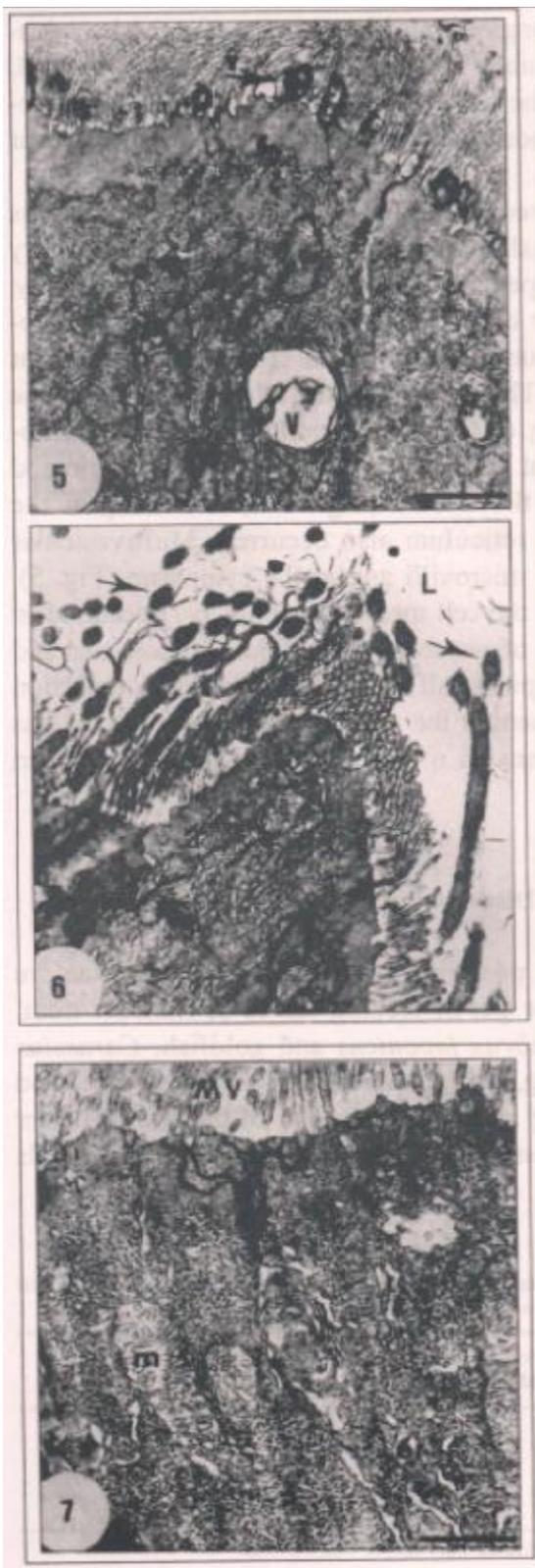
Figs. 5 and 6. Electron micrograph of epithelial cells of posterior gut of *C. harengus* larvae exposed to 0.05µg·l⁻¹ mercury, showing the vesicle formed at the bottom of microvilli and in the cytoplasm. Note the ploughing off microvilli (MV) and cilia in the lumen gut (L). Scale bar 1.0 µm

Fig.7. Electron micrograph of epithelial cells of posterior gut of the *C. harengus* larvae exposed to 0.05µg·l⁻¹ mercury, showing the reduced number of microvilli (MV), swollen mitochondria (m) and endoplasmic reticulum (er). Scale bar 1.0 µm

Figs. 8 and 9. Electron micrograph of posterior gut of *C. harengus* larvae exposed to 0.1μg·l^{-1}mercury, showing few microvilli (MV), inter-spaced Desmosomes (d), swollen mitochondria (m), degenerate cristae, swollen endoplasmic reticulum (er) and Vacuolated cytoplasm . Scale bar 1.0 μm

Fig. 10. Electron micrograph of epithelial cells of posterior gut of *C. harengus* larvae exposed to 0.1μg·l^{-1} mercury, showing the lumen region (L). Note the remnants of microvilli (MV). Scale bar 1.0 μm

of *C. harengus* are near complete differentiation. The present study shows that the columnar epithelial cells of the posterior gut are affected by mercury in concentrations 0.05 and 0.1 µg·l^{-1}. However, specimens exposed to 0.02 µg·l^{-1} mercury show no significant change in the ultrastructure of the posterior gut. In higher concentrations of mercury various degrees of degeneration including swelling of the mitochondria and endoplasmic reticulum and reduction of microvilli were recorded. Similar changes were also recorded in the cells of the posterior gut of the *C. harengus* larvae exposed to zinc (Somasundaram et al. 1985). Similar cellular changes were observed in brain and epidermal cells of Atlantic herring larvae tested in 0.05 and 0.1µg·l^{-1} mercury (Jastania and Abbasi 2003a,b). *Fundulus heteroclitus*, exposed to 0.05µg·l^{-1} mercury had severe cytoplasmic and nuclear degeneration and the degeneration of the olfactory cells (Gardner 1975). Bubel (1976) reported that the isopod, *Jaera nordmanni*, exposed to 0.1 µg·l^{-1} mercury, had swelling of mitochondria, endoplasmic reticulum and basal membrane in gill tissue due to increased permeability and stimulated membrane ATPase. Olson et al. (1973) reported that mercuric chloride or methyl mercuric chloride damaged the microvilli and the epithelia of the gills. Abbasi et al. (2000) reported that the microvilli disappeared with degeneration of the outer epidermal cells when *C. harengus* were exposed to copper. In the present study, the gut cells of affected larvae of *C. harengus* showed increased number of lysosomes, multivesicular bodies and vacuoles, suggesting an autolysosomal process, leading to degeneration of the cell may be taking place. Peters (1982) reported a similar breakdown in the stomach cells of adult *Anguilla anguilla* under stress. Bouquegnean (1977) reported that the loss of NaCl balance in *A. anguilla,* exposed to mercury was due to inhibition of gill Na K ATPase activity. In *Pseudo pleuronectes*, mercury reduced ion gradients in the cell membrane by inhibiting Na K ATPase activity as well as increasing membrane permeability to cations (Miller 1981). Lock et al. (1981) concluded that a reduction in plasma Na and Cl concentration in *Salmo gairdneri* exposed to sublethal concentrations of mercury could be attributed to an increase in gill permeability to water rather than to an inhibition of gill Na K ATPase activity. Swollen mitochondria due to stress from mercury concentrations may lead to increased rate of respiration of the larvae. Olson et al. (1973) and Lock et al. (1981) suggested that mercury could affect capability of osmoregulation as well as impairment of respiratory efficiency with respect to both molecular exchanges across the gill surface and transport by the blood. Wobeser (1975) and Daoust (1981) reported that trout, *S. gairdneri* exposed to mercury showed morphological changes in their gills and death was thought to be caused by asphyxiation and acidosis. The tissues with the highest concentration of enzymes such as liver, gills and kidneys tend to accumulate the

greatest amount of mercury. For this reason, many cellular processes, in addition to ion transport, are probably inhibited by mercury (Peterson and Usher 1971). Yu and Philips (1971) reported that in mercury exposed specimens, formation of nodular masses in the smooth endoplasmic reticulum resulted from destruction of the granular endoplasmic reticulum caused by reduction in ATP production within the cell and mercury also caused swelling of the mitochondria which would affect oxygen uptake.

Conclusion

The present study shows that the posterior gut cells of *C. harengus* larvae are affected by mercury and this may ultimately affect digestion. Iwai (1966) reported that the posterior guts of many fish larvae are involved in protein absorption. Thus the effects of mercury on the gut may reduce viability of older larvae by affecting digestion. Abbasi et al. (2000) reported that the larvae of *C. harengus* exposed to mercury had deformities of eyes, jaws and vertebral column.

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