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Effects of Mercury on Muscle Cells of Atlantic Herring (*Clupea harengus* L.) Larvae: An Ultrastructural Study

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Abstract.- Atlantic herring *Clupea harengus* L. eggs were exposed to different concentrations of mercury (0.2, 0.5, 1.0 mgl^{-1}). Larvae hatched in the mercury solutions were examined under electron microscopy and compared with the controls. The ultrastructure of the muscle cell showed several cellular changes in the larvae in 0.5 and 1.0 mgl^{-1} mercury solutions. These changes were swollen mitochondria and their cristae, dilation of sarcoplasmic reticulum and reduction in the myofibrils. In high concentrations (1.0 mgl^{-1} mercury), mitochondria and the sarcoplasm become vacuolated, cristae were degenerated and the matrix pushed on one side. Morphometric analysis indicates the swelling of sarcoplasmic reticulum and a significant reduction in the muscle fibres. The surface to volume ratio of the mitochondria and their cristae is reduced.

Key Words: Herring, larvae, mercury, muscle cells, ultrastructure.

INTRODUCTION

Mercury is a biological non-essential heavy metal, which normally occurs in small amounts in oceans but the highest deposits of mercury result from industrial discharges (Konrad, 1972; Diltri, 1972). Pollutants including heavy metals produce malformation in fish larvae and reduce the survival potential of the fish population (Somasundaram *et al*., 1984; Abbasi and Shackley, 1995). Effects of zinc and copper on the eggs and larvae of *C. harengus* have been reported. Such effects were abnormal development of fertilized eggs, deformities in the larvae and ultrastructural changes in brain cells, muscle and epidermal cells (Somasundaram, 1985; Abbasi and Shackley, 1995; Abbasi *et al*., 2000a). Ackfors *et al*. (1970) and Friberg and Vostal (1970) reviewed dealing with mercury pollution. Huckabee and Griffth (1974) found that the eggs of *Cyprinus carpio* failed to hatch when incubated in >4 µgl mercury. Jastania and Abbasi (2003) reported the ultrastructural changes in brain cells of *C. harengus* exposed to mercury. Mercury also altered the development of eggs and caused abnormalities in the larvae (Abassi *et al*., 2000b).

Atlantic herring, *Clupea harengus* L., spawn in estuarine waters and may thus be subjected to metal pollution, though the effects of mercury on early development stages in this species is of interest. The present study was conducted to examine the possible effects of mercury on the muscle cells of herring *Clupea harengus* L. larvae hatching from the eggs previously exposed to mercury.

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MATERIALS AND METHODS

Egg incubation

Clupea harengus, adults, which were about to spawn, were gill netted from the Castle Reach, Milford Haven, Wales, U.K. Eggs were stripped from these fish and artificially fertilized (Alderdice *et al*., 1979). Samples approximately equal numbers of fertilized eggs were placed in 2-1 glass jars containing either clean artificial sea water (Tropical Marine Salts) diluted to 21% (ambient salinity) or tested solutions containing 0.02, 0.05 and 0.1 $\mu g/l^{-1}$ mercury, prepared from a stock solution of mercuric chloride. The controls and test solutions were kept at pH 7.5. The jars were continuously aerated at 8±1°C (ambient temperature). Controls and test solutions were renewed after every 2 days in order to maintain the level of mercury and to counter the 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 the each concentration were fixed for 1 hour at 0-4°C in 5% cacodylate-buffered

Table I.- Morphometric analysis of the muscle cells of *C. harengus* L. yolk sac larvae hatched from eggs previously exposed to mercury for 13 days. For each group 20 larvae were examined.

Mercury concentration mgl^{-1}	Myofibrils	Sarcoplasmic reticulum	Mitochondria	Surface to volume ratio of cristae
Control	0.07	0.038	0.26	0.60
0.20	0.06 (>0.01)	0.036 (>0.01)	0.27 (>0.01)	0.55 (<0.05)
0.50	0.39 (<0.001)	0.050 (<0.05)	0.32 (<0.001)	0.50 (<0.001)
1.00	0.19 (<0.05)	0.128 (<0.001)	0.12 (<0.001)	0.31 (<0.001

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 0-4°C. After further washing in the buffer solution for 1 hour 0-4°C, 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 Ultra microtome and were mounted on copper grids. They were then double stained in 30% uranyle acetate (20 min) followed by lead citrate (8-10 min) and viewed in a Corinth AE 1 electron microscope.

Morphometric analysis

Electron micrograde were enlarged to a final magnification of 39x10 and analyzed by differential point counting in order to test the relative volumes of the muscle fibres, sarcoplasm reticulum, mitochondria and surface to volume ratio of the mitochondria cristae. In order to test the surface-to-volume ratio of the mitochondrial cristae, individual mitochondria were enlarged to final magnification of 167.45x10 and analyzed using multipurpose test system consisting of 100 points enclosing 50 short test lines (Weible *et al*., 1966).

RESULTS

Two muscle layers were observed, the red muscle layer which lies just under the epidermis followed by a white muscle layer. The muscle cell consist a large nucleus, mitochondria, myofibrils, a large number of ribosomes and sarcoplasmic reticulum (Figs. 1-3). The sarcoplasm reticulum forming a net work of interconnected cisternae around myofibrils. The mitochondria are with electron dense matrix with closely packed cristae (Fig. 2). The mean relative volumes of the myofibrils, sarcoplasmic reticulum, mitochondria and surface to volume ratio of cristae in control and mercury contaminated specimens are presented in Table I.

Morphometric analysis of muscle cells indicates similar results in control and the specimens exposed to 0.2 mgl^{-1} mercury. The ultrastructural changes occurred in specimens exposed to 0.5 and 1.0 mg^{-1} mercury. In specimens exposed to 0.5 mgl^{-1} mercury, the sarcoplasm reticulum was significantly swollen ($P<0.001$). The mean relative volumes of the mitochondria showed significant increase in size and contained degenerated cristae (Fig. 4). The surface to volume ratio of the mitochondrial cristae was reduced ($P<0.05$). The sarcoplasm faded and the ribosomes reduced in number (Fig. 4). Specimens exposed to 1.0 mgl^{-1} mercury showed significant reduction in the surface to volume ratio of myofibrils ($P<0.001$) compared to control. The relative volume of mitochondria and surface to volume ratio of cristae were significantly reduced ($P<0.001$) and the sarcoplasmic reticulum was swollen ($P<0.001$). In affected cells vacuoles appeared in mitochondria and in the sarcoplasm. Due to large vacuoles in mitochondria the cristae and the matrix were lying on one side of the mitochondria.

DISCUSSION

In the present study, mercury caused ultrastructural changes in the muscle cells of *C. harengus* larvae. Similar changes in the muscle cells were also observed when *C. harengus* larvae were exposed to zinc, aluminium and copper (Somasundaram *et al*., 1984; Abbsi *et al*., 1995). Cameran and Smith (1980) described the cellular damage in the larvae of Pacific herring, *C. harengus pallasi* exposed to crude oil. In winter flounder,

Figs. 1-5. Electron micrograph of muscles of transverse section of *Clupea harengus* larvae at hatching; 1, the muscle fibres consist of myofibrils (fi) which contain myosin filaments (arrow) surrounded by actin filaments; 2, muscle cell larvae containing ribosomes (R), mitochondria (M). The cristae (small arrow) of the mitochondria are well arranged and developed and closely packed with dense matrix; 3, showing a large nucleus (N). Sarcoplasm contains mitochondria (M), myofibrils (fi) and sarcoplasmic reticulum (Sr); 4, muscle cell of *Clupea harengus* exposed to 0.5 mgl^{-1} mercury showing faded sarcoplasm with few ribosomes. Mitochondrial cristae and myofibrils (fi) become shrinked and sarcplasmic reticulum is dilated; 5, muscle cell of *Clupea harengus* exposed to 1.0 mgl^{-1} mercury showing reduction in muscle fibre and swollen sarcoplasm reticulum, vacuoles in sarcoplasm and mitochondria and degenerating cristae lying on the sides of mitochondria. Scale bar 1 µm.

exposed to mercury resulted severe epithelial necrosis (Wobeser, 1975). Degeneration and necrosis of the nerve cells of the brain of human were also noted during acute dose of mercury (Mattet, 1971). Roulle (1960) suggested that in the cells swelling of the mitochondria is due to osmotic disorder of the cell. Segner (1987) described that the change in mitochondrial morphology is the result of heavy metal interference with mitochondrial functions. Trump *et al*. (1975) suggested that the swelling of membrane bound organelles such as mitochondria, endoplasmic reticulum and nuclear envelop is the result of an accumulation of cation, anions and water in the cell. Vernberg *et al*. (1974) suggested that mercury produces swelling of the mitochondria which could affect oxygen uptake. Jastania and Abbasi (2003) suggested that mercury treated larvae of *C. harengus* have reduced oxygen utilization due to affect on mitochondria which ultimately result in energy loss of the muscle cells. Trump *et al*. (1975) concluded that mercury reduced the activity of enzymes affecting liver, kidney, brain cells and epidermal cells of fish. Bubel (1976) suggested that mercury changed the membrane ATPase system, which led to an increase in the permeability of the cellular membrane causing swelling of endoplasmic reticulum and disturbance of mitochondrial morphology. Jastania and Abbasi (2003) suggested that mercury inhibitied the activities of enzymes in brain cells necessary for the development and function of brain cells. In the present study the sarcoplasmic reticulum was swollen and the number of ribosomes was reduced, which resulted in reduction of protein production. The reduction in the protein synthesis might be the cause of shrinkage of myofibrils and inhibition of protein synthesis would affect the normal function and development of the muscle cells. Heath (1984) reported that copper elicited changes in oxygen dependent ATP production by acting on biochemical reactions in the muscle tissue. Exposure of fish to heavy metals generally resulted in reduction of skeletal muscle metabolism and disruption of cellular organization by depressing the oxidation process and enzymatic activity (Hubschman, 1967; Bilinski and Jones, 1973; Smith, 1982).

In the present study, mercury affected the components of muscle cells, which would impair movement and hence affect the viability of the larvae. Ellgaard and Guillot (1988) reported that copper induced locomotor hypoactivity in bluegill, *L. maxochirus* Rafinesque, as low as 0.01 ppm. Abbasi *et al*. (2000) reported that larvae of *C. harengus* L. exposed to mercury resulted in deformities of mouth, eyes and vertebral column.

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