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LYSOSOMAL MEMBRANE STABILITY IN BLOOD CELLS OF THE SOFT SHELLED CLAM (*Mya arenaria* L.)

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ABSTRACT

Lysosomal membrane stability was investigated in soft shelled clams (*Mya arenaria* L.) from natural populations of the Romanian Black Sea coast. The lysosomal ability to retain the neutral red was higher in the blood cells of clams collected from Mamaia Bay than those from Navodari (polluted area). The correlation between the lysosomal membrane stability in the blood cells of *Mya arenaria* L. and the sexual maturation of the individuals was also demonstrated.

KEY WORDS : Black Sea, *Mya arenaria*, lysosomal membrane stability

INTRODUCTION

Lysosomal membrane damage induced by environmental pollutants, or under experimental conditions, has been demonstrated in vertebrates (LOWE *et al*., 1992) and invertebrates (LOWE & PIPE, 1994; LOWE *et al*., 1995). At the cellular level, the lysosomal system has been identified as a particular target for the toxic effects of many contaminants (MOORE, 1990). Some environmental xenobiotics are metabolised by lysosomes to inoffensive forms, while some of them are sequestrated ans concentrated in lysosomes, or transformed in high toxic substances.

The functional and structural lysosomal membrane injuries are induced by the contaminant concentration and their enhanced toxicity, followed by the acid hydrolyses release in cytosol and cellular damage (LOWE & FOSSATO, 1994).

The speed at which contaminant exposure will results in lysosomal damage depends on nature and concentration of xenobiotics, but also the physiological state of the animals (LOWE, 1995).

The correlation between the lysosomal membrane stability and the gonadal maturation, in the mussels (*Mytilus edulis* L., *Mytilus galloprovincialis* Lmk.) was also demonstrated. When mussels are spawning, the lysosomal membrane stability is failed, the gametes release being a stress factor.

The lysosomal membrane stability technique, using neutral red dye was successfully applied on digestive cells of mussels, *Mytilus edulis* L. (LOWE & PIPE, 1994), on mussel blood cells (LOWE & PIPE, 1994; LOWE & FOSSATO, 1994), on blood cells from *Perna viridis* (LOWE, 1995).

The objective of this investigation consists in carrying out the neutral red technique (LOWE, 1992, 1994) on soft shelled clam blood cells (*Mya arenaria* L.), in a field study, to demonstrate the cellular damages induced by environmental contaminants, from a high polluted area, Navodari.

MATERIAL AND METHODS

Clams (*Mya arenaria* L.) were collected from Navodari (300 m offshore, 6 m depth, 3^0C water temperature) and Mamaia Bay (4 km north from Constantza, 3 m depth, 3^0C water temperature), on February 1997. At the end of April 1997, 14^0C water temperature, another 10 individuals were collected from Mamaia Bay. Ten clams from each station (40-50 cm length) were selected for neutral red test.

Neutral Red Retention Assay (LOWE *et al.*,1992; LOWE & PIPE, 1994)

The valves were carefully prised apart and a scalpel inserted between the valves in order to hold them apart, whilst 0.5 ml of haemolymph withdrawn from the anterior adductor muscle into a 2.5 ml hypodermic syringe, fitted with a 25 gauge needle, containing 0.5 ml of physiological saline (PEEK & GABBOT, 1989). The needle was then removed from the syringe and discarded, in order to reduce shearing forces, and the contents of the syringe ejected into a 2 ml siliconised Eppendorf tube that was held in water ice until required.

For the viability test of the cells, 20 µl cellular suspension was dispensed onto microscope slide, 20 µl 0.2% eosin solution was added. The preparations were inspected after 5 minutes to determine the percentage of the coloured cells.

A stock solution of neutral red was prepared by dissolving 20 mgs of dye in 1 ml dimethylsulphoxide (DMSO); the working solution was then prepared by diluting 10 µl of the dye stock solution with 5 ml of physiological saline.

A 40 µl aliquot of the cell suspension was dispensed onto microscope slide and suspended on a rack in a humidity chamber, for 15 mins, to allow the cells to attach. The excess solution was then carefully removed and 40 µl of the neutral red working solution was added to the area containing the attached cells and a coverslip applied. After 15 mins incubation, the preparations were inspected under a microscope (x 400 mags). Following a further 15 mins incubation the preparations were examined again and systematically thereafter at 15 mins intervals, to determine when the dye, which had been readily taken up into the lysosomal compartment of the cells, was lost to the cytosol. The test for an individual was terminated when dye loss was evident in 50% (assessed qualitatively) of the small granular haemocytes and the time recorded.

RESULTS AND DISCUSSION

The neutral red retention test demonstrated that the blood cells lysosomes from the Mamaia Bay clams (individuals collected in February) have the capacity to retain the dye for 60-90 mins, whilst those from the Navodari site exhibited dye loss within 15-30 mins (Fig.1). For the Mamaia Bay collected clams in April 1997, the neutral red retention time was very short: 0-15 mins (Fig.2).

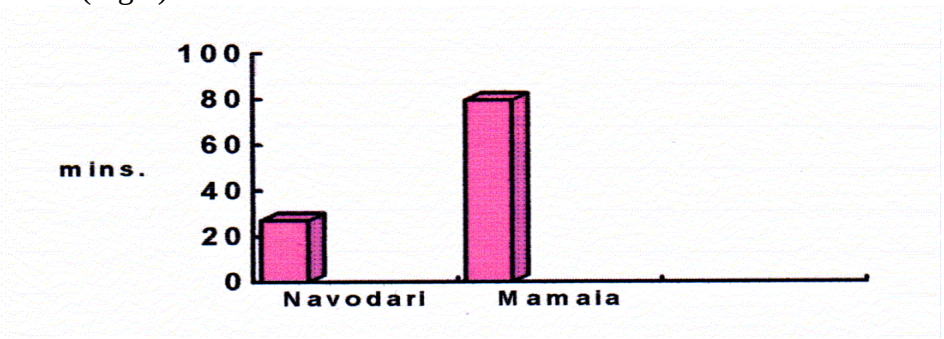

Fig.1 - Neutral red retention time in blood cells lysosomes
from clams collected in February 1997

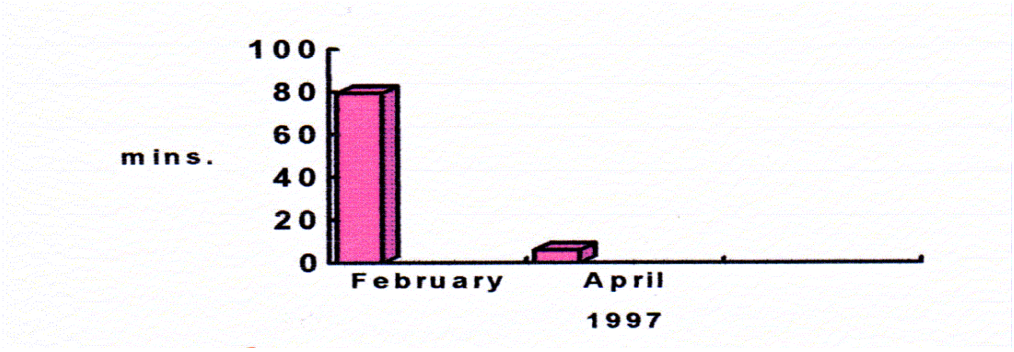

Fig.2 - Neutral red retention time in blood cells lysosomes
from Mamaia Bay clams

The objective of this study was to demonstrate the regress of the blood cells lysosomal capacity to retain the neutral red dye, in soft-shelled clam (*Mya arenaria* L.) collected from a polluted site (Navodari).

The result are very similar with those obtained for mussels (*Mytilus galloprovincialis* Lmk.) collected from the same site (CIOCAN, 1996/1997).

Lysosomal membrane damages are induced, probably, by the failure of the Mg^{2+} ATPase dependent proton pump (HOLZAM, 1989) resulting from the presence of environmental xenobiotics in lysosomal compartment, in this case, industrial waste, especially heavy metals as a result of producing chemical fertilisers in Navodari Chemical Fertiliser Plant.

The dramatically regress of the neutral red retention time in soft shelled clams collected in April 1997 from Mamaia Bay is the consequence of spawning. MULLER *et al.* (1986) have shown that the gonadal maturation of the clams (*Mya arenaria* L.) under the climatic variations of the Romanian shore, is strictly correlated with the environmental temperature. The temperature interval of 13-14^{0}C is characteristic and restricted for the clams, as concerns the lowest temperature required for gametes emission. The l4^{0}C water temperature is the start point for spawning.

The neutral red retention results demonstrated a lower lysosomal membrane stability for the animals collected from polluted sites, showing, once again, that the lysosomes are the target for the environmental xenobiotics.

Structural and functional lysosomal damages in the blood cells of clam (*Mya arenaria* L.) are induced by environmental stressors, but are also a consequence of the gameted release process.

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