STUDIES ON THE METAL-CONTAINING GRANULES IN THE MUSSELS, *MYTILUS GALLOPROVINCIALIS* AND *VELESUNIO ANGASI*

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Submitted by

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I declare that this thesis is my own account of my own research and contains, as its main content, work that has not previously been submitted for a degree at any tertiary education institution.

Frances Jean Stanley

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SIR WALTER MURDOCH

ON SITTING STILL

"Thinking is the hardest work in the world; most of us are too lazy to attempt it. We prefer what we call the Strenuous Life, which means being busy and fussy, and joining a dozen committees, imagining that we are doing a great deal of good in the world, and blinding ourselves to the fact that we are all suffering from St Vitus' Dance – a disease which we can cure only by shaking off our laziness and acquiring the difficult art of sitting still."

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ABSTRACT

There is a continuing need to develop a simple and reliable biomonitoring technique for pollutants in aquatic systems. Mytilid mussels have been used extensively around the world for monitoring a range of pollutants. However the analysis of soft tissues in such programmes has some drawbacks, one of which is the large amount of variation between individuals in levels of specific pollutant. In order to assess the suitability of lysosomal kidney granules for such a role, it must be established that they are involved in the accumulation and detoxification of pollutants, and the range of change within the granules must also be assessed.

The structure and composition of intracellular metal-containing granules extracted from the kidney of the marine mussel *Mytilus galloprovincialis* from a non-polluted site have been examined using a variety of techniques. Comparisons were made between granules extracted from these mussels and those loaded with zinc in the laboratory, and those exposed to excess levels of zinc in the field. In addition, intracellular and extracellular metal-containing granules have been extracted from the freshwater mussel, *Velesunio angasi* and examined using similar techniques.

Granules isolated from the kidney tissue of fifty *M. galloprovincialis* (using a centrifugation process) were pooled to produce a single sample. Inductively-coupled plasma spectroscopy (ICP) revealed that the concentration of zinc in the kidney granules increased with the level of zinc-loading from a mean value of 5 260 $\mu g.g^{-1}$ in freshly harvested animals to 12 487 $\mu g.g^{-1}$ in animals loaded with 2.5 $\mu g.g^{-1}$ zinc for 28 days. The other main elements present in the granules were phosphorus, sulfur, sodium, calcium, iron and copper. Granules from mussels exposed to excess zinc in the field had a greater concentration of zinc (13 570 $\mu g.g^{-1}$) than both field control animals (1 424 $\mu g.g^{-1}$) and the mussels loaded at the highest level of zinc in the laboratory. Silicon was also present in the granules extracted from the field-contaminated mussels.

Both light and transmission electron microscopy (TEM) of kidney tissue from *M. galloprovincialis* loaded with zinc in the laboratory showed signs of increasing degeneration as the level of zinc-loading increased. All cells examined contained membrane-bound granules. The kidney tissue of the field-contaminated mussels, despite containing high levels of zinc and many granules, showed no signs of degeneration.

Scanning electron microscopy (SEM) of the granules revealed that they were spherical in shape, while energy dispersive spectroscopy (EDS) of the isolated granules confirmed the results of the ICP analysis with regard to elemental composition. TEM also revealed that the granules themselves underwent structural changes as the level of zinc-loading increased. Thus, granules extracted from freshly harvested animals were electron-dense and regular in shape while zinc-loading produced granules with a concentric ring formation. In kidney tissue from mussels that were maintained in clean seawater for 14 days following 28 days of zinc-loading, the majority of the granules visible had the concentric ring formation. Granules with concentric rings were not found in kidney cells of the field-contaminated mussels. The number of multivesicular bodies in individual kidney cells also increased during zinc-loading and a positive relationship exists between the number of these bodies and the mean diameter of the granules in the cells.

The lack of degeneration in the cells of the kidney tissue in the field-contaminated mussels is almost certainly due to the fact that these animals were subjected to a chronic excess of zinc, rather than an acute dose as occurred in the laboratory-loaded animals. Presumably, this longer time period allows the animal to adapt and establish defence mechanisms, including the production of granules. The acute exposure to zinc, combined with the stress of being kept in the laboratory, may also have resulted in the appearance of granules with concentric rings in the laboratory zinc-loaded mussels.

In the freshwater mussel *Velesunio angasi*, extracellular granules were found mainly on the labial palps, mantle edge, gills and the junction between the foot and the visceral mass. ICP of pooled granules and EDS analysis of isolated granules showed that calcium, phosphorus, iron, manganese and barium were the major elements present.

Two types of intracellular granules were isolated from the kidney cells of *V. angasi*, the first time this has been recorded. The first was similar in composition to the extracellular granules and it was hypothesised that these two groups of granules are actually insoluble deposits of metal phosphates, despite being membrane-bound in the kidney cells. The second type of intracellular granule was mainly composed of phosphorus and sulphur and was possibly lysosomal.

It appears that difficulties with extraction of the granules and the analysis of the samples preclude the use of the metal-containing kidney granules of either *M. galloprovincialis* or *V. angasi* as biomarkers. The use of other biomarkers for pollutant contamination in mussels is discussed.