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Immunolocalization of the Induced Metallothionein and Tissue Damages in Gills of the Clam *Ruditapes philippinarum* following Exposure to Heavy Metals

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Metallothioneins (MTs) are a group of low molecular weight proteins about 6 to 7 kDa comprising 61-62 amino acids residues with a high cysteine content and generally assumed that play a central role in metal homeostasis. They can function as storage proteins for essential metals such as Zn and Cu, or as chelating agents to bind toxic metals such as Cd and Hg. Therefore, for detection of differently induction on MTs, we have studied Hg-, Cd-, Pb-induced proteins by exposing the clam, *Ruditapes philippinarum* to selected heavy metals. The immunohistochemical stain detected the presence of MTs from gill tissue exposed to different dose of each heavy metal. Especially in this clam by exposing to Hg this protein increase the immunoreactivity compared with control, Cd and Pb treated samples. The predominant structural effects by exposing heavy metal were tubulovesicular cristae of mitochondria, destruction of the microvilli, cilia and ER in the epithelial cell of the gill filament. In addition to these, the gill tissue showed hypertrophy of the epithelial cells and atrophy gill filament structure.

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Induction of Apoptosis in the Clam, *Ruditapes philippinarum* by Heavy Metal Accumulation

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Apoptosis and cytopathological effect by the heavy metals as mercury, lead and cadmium in the gill tissue of manila clam, *Ruditapes philippinarum* was investigated by the TUNEL method and electron microscopy. The TUNEL method was used to detect cells displaying DNA fragmentation within the gill structure. The results showed that a considerable amount of apoptosis occurred in the apical portion of the epithelial tissue in the gill filament structure of the all selected heavy metal exposed group. In the all heavy metal exposed groups, the TUNEL positive reactions were visible heavy metal treated after 3 days and mainly in the pavement epithelial cell of the gill tissue.

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Ultrastructure and Immunoreactivity of Glutamic Acid Decarboxylase (GAD) Isoforms in the Central Nervous System of Spiders, *Achaearanea tepidariorum*

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The CNS of the spider, *Achaearanea tepidariorum*, consists of a dorsal brain or supraesophageal ganglion and circumesophageal connectives joining it to the subesophageal mass. The subesophageal nerve mass underneath the brain is the foremost part of the ventral nerve cord. All of this nerve mass is totally fused together, and forming subesophageal ganglia in this spider. The γ -aminobutyric acid (GABA) has long been considered as an inhibitory neurotransmitter in the central nervous system (CNS) of both vertebrates and arthropods. The glutamic acid decarboxylase (GAD) catalyzes the conversion of L-glutamate to GABA. GAD has a restricted tissue distribution and it is highly expressed in the cytoplasm of GABAergic neurons in the CNS. It is, however, also present in other non-neuronal tissues such as testis, oviduct and ovary. Recently, it has been reported that a GABA-like immunocytochemical reactivity and a ninhydrin-positive GABA derivative, GABamide, exist in the visual ganglia and in the water-soluble fraction of the spider web, respectively. Therefore, this experiment will contribute to identify the exact distribution of the GAD isoform with immunoreactivity in the CNS of the spider to reveal the ecophysiological significance of GABA for spider's behavior.

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Fine Structural Modification of the Developing Cardiac Tissues in the Mealworm Beetle, *Tenebrio molitor*

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The development of the cardiac tissues in the mealworm beetle, *Tenebrio molitor* were studied with both of scanning and transmission electron microscopes. The heart was consisted of three types of layers: epicardium, cardiac muscle, and endocardium. Both of the epicardium and the endocardium encircling the free surface of the heart, and they commonly composed of a single layer of flattened cells. The alary muscles, which suspend the heart, are more delicate in the adults compared to the early pupae. The pericardial cells in both early pupae and adults are connected to the heart by connective tissue radiating from the alary muscles or dorsal diaphragm. The pupal heart and the adult beetle has a chambered appearance and bounded to the dorsal body. The intercalated disk, one of the peculiar structure of the cardiac muscles in higher animals also appeared in this beetle. Moreover, the substructure of the sarcomere, the regular arrangement of sarcoplasmic reticula, and special banding patterns of cardiac muscles were also observed.