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Effects of Mercury and Lead on Tissue Glutathione of the Green Mussel, *Perna viridis* L.

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Glutathione (GSH) is the major non-protein thiol in animals, and involved in a number of important physiological and detoxification processes. It has been suggested that this tripeptide protects thiol groups in proteins from oxidation, functions as an intracellular redox buffer and serves as a reservoir of cysteine (Moron et al. 1979). Tissue GSH is also known to be involved in the metabolism and detoxification of endogenous and exogenous substances (Ketterer et al. 1983), including the binding of inorganic mercury ions (Ballatori and Clarkson 1984). Therefore, a change in the amount of GSH in tissues maybe considered to reflect the effects of deposited mercury on tissue function (Sin et al. 1990; Sin and Teh 1992). The functions of the tripeptide have been studied less thoroughly in invertebrates than in vertebrates.

The purpose of this study was to determine whether there were changes in the amounts of tissue GSH in the green mussel, *Perna viridis,* at various time intervals after metal exposure. Mussel tissues contain GSH (Viarengo et al. 1990) and possess glutathione S-transferase (Fitzpatrick et al. 1995) and glutathione peroxidase (Gamble et al. 1995). Heavy metals, especially mercury and lead, are accumulated by the mussels to a high concentration (Kureishy and D'Silva 1993). Mercury sequestered in the tissues is mostly bound to metallothionein, whereas lead is not (Marshall and Talbot 1979). Moreover, metals may cause lipid peroxidation in the mussel cells (Viarengo et al. 1988). Ail these studies suggest that an interaction may exist between metal intoxication and GSH metabolism in the mussel. In this experiment, GSH levels in gill, digestive gland and kidney of green mussel were measured and effects of mercury and lead on GSH content in the tissues were determined, to reveal the role of GSH in metal detoxification in marine mussels.

MATERIALS AND METHODS

Mussels, length 8-10 cm, were collected on floating rafts along the coast of Changi Village in the northeastern part of Singapore. The mussels were acclimatized for 1 week in a running seawater system (salinity: 30% and temperature 29°C). The mussels were fed with cultured algae, *Pavlova salina.*

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Twenty-five mussels were transferred to a 100-L control or test tank. The animals in the test tank were exposed to a sublethal concentration of mercuric chloride (0.25 µg/L) and lead nitrate (l.0 mg/L) at a sublethal concentration for up to 3 wk. The tank water was replaced every 3 d. Algal culture was added to the tanks to maintain the algal concentration at 1.5 x 10^4 cells/mL.

Five mussels were sampled every 7 d for 3 wk from each group for GSH determination. Glutathione content in kidney, gill and digestive gland was determined calorimetrically using DTNB method (Moron et al. 1979). Two-hundred mg tissue was homogenized in 2 mL of ice-cold 5% TCA. The homogenate was then centrifuged at 4°C (Julan MRl812) at 17,000 x g for 30 min. A volume of 75 µL of the clear supernatant was added to a cuvette containing 300 µL of phosphate buffer (0.2 *M*, pH 8.0) and 750 µL of 0.6 m*M* DTNB (5,5'-dithiobis-2-nitrobenzoic acid) in phosphate buffer. The absorbance at 412 nm was read and gluthathione content was derived against a standard curve prepared with known amounts of glutathione in 5% TCA. Glutathione content in tissue was expressed as µg GSH per g wet weight tissue.

Three mussels were sampled every 7 d for 3 wk from each group and the tissues pooled for measurement of tissue burdens of the metals. Mercury was extracted according to the method of Thorpe (1971). Mercury concentration was analyzed by a Perkin-Elmer MAS 50B Mercury Analyzer System against a Hg^{+2} standard prepared with known amounts of mercury chloride, Mercury concentration in tissues was expressed as µg Hg per g dry weight tissue. Lead in tissues was extracted with a dry-ashing method, and analyzed by a Hitachi Z-7000 AAS against a lead nitrate standard (Sigma). Lead concentration in tissues was expressed as mg Pb per g dry weight tissue.

The significance of the results was determined using the Student-t test. A value of $p<0.05$ was considered to be significant.

RESULTS AND DISCUSSION

Glutathione content varied with the tissues examined (Fig, 1). In control animals, the highest concentrations of GSH were observed in the kidney (258 µg/g wet wt). These concentrations were approximately half of that obtained from mullet (480 µg/g wet wt, Thomas et al. 1982). On the other hand, GSH content in the digestive gland was only 175 µg/g wet wt, which is much lower than the reported value for mullet (657 µg/g wet wt, Thomas et al. 1982; Wofford and Thomas 1984). Viarengo et al.(1990), using the fluorometric method, reported a much higher GSH concentration (640-730 µg/g wet wt) in the digestive gland of blue mussel, *Mytilus edulis*, and this is much higher than the value obtained in the present experiment. The GSH content in the gill tissue of control mussels was low (139 µg/g wet wt) compared with those in the kidney and digestive gland. Glutathione represents the major antioxidant compound present in the soluble and membrane fraction of the cells (Viarengo et al. 1990). Therefore, the levels of

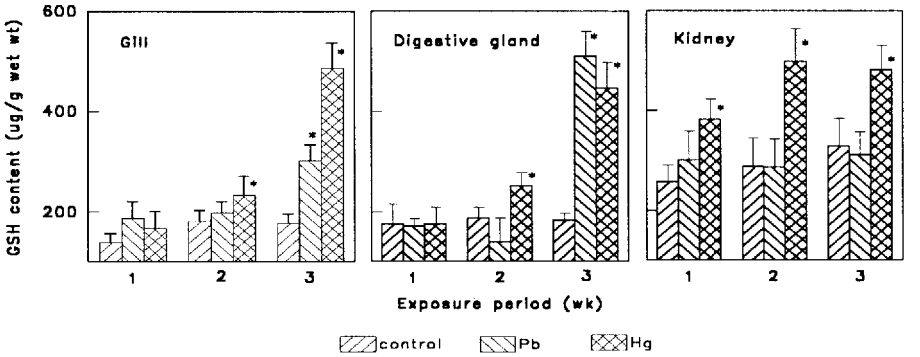

Figure 1. Glutathione concentrations in the tissues of green mussels *(Perna viridis)* exposed to 0.25 µg /L Hg^{+2} or 1.0 mg/L Pb^{+2} at different time intervals. The bars represent a mean plus standard error (n=5), and asterisks denote means significantly different from controls ($p<0.05$)

glutathione in control animals suggest that the gills and digestive gland could be more prone to oxidative stress than the kidney.

A time-dependent elevation of glutathione concentrations was observed in the present experiment in response to both mercury and lead (Fig. 1). The increase of kidney GSH in Hg-treated mussels was observed after 1 wk exposure, whereas the GSH levels in digestive gland and gill did not show any changes within 1 wk. After exposure to mercury for 2 wk, the gill and digestive gland showed a marked increase in GSH level.

Mercury ions are known to bind tissue GSH (Ballatori and Clarkson 1984) and cause a significant increase of kidney GSH in $HgCl_2$-treated rats (Sin et al. 1989, 1990). Theoretically, tissue GSH is mainly synthesized in the liver and transported to other organs via systemic circulation. However, considering the higher levels of mercury in the kidney than in the other tissues measured (Table 1), it appears more likely that the increase of kidney GSH in Hg-treated mussels for 1 wk was due to the greater amount of mercury accumulated in that tissue. Exposure to mercury can induce the synthesis of metallothioneins in mussel tissue, and binding of mercury by metallothioneins protects other fictional targets from interaction with toxic metals (Wong and Klaassen 1981), and may prevent the elevation of GSH at mercury concentrations at which the accumulated metal is found mainly conjugated with metallothioneins.

In contrast, GSH level in the tissues of green mussels exposed to lead showed a different profile (Fig, 1). No alteration of GSH level in the kidney was observed even though a high concentration of lead accumulated in the tissue of animals exposed for up to 3 wk (Fig. 1 and Table 1). The GSH levels showed a significant increase in the gill and digestive gland of animals exposed to lead for 3 wk, reaching up to 302 and 510 µg/g wet wt respectively. It is interesting to note that

Table 1. Concentrations of metals in the tissues of green mussels exposed to Hg^{+2} (0.25 µg /L) or Pb^{+2} (1 mg/L) for up to 3 weeks. Each value was obtained from the pooled tissues of 3 animals (Unit: µg metal per g dry tissue weight)

Week		Gill		Digestive gland		Kidney	
	Group	Hg	Pb	Hg	Pb	Hg	Pb
1	cont.	0.10	3.27	0.16	3.59	0.16	2.08
	expt.	15.20	124.31	14.29	103.21	43.08	232.19
2	cont.	0.12	3.00	0.14	2.98	0.12	3.01
	expt.	15.51	186.87	17.10	148.08	45.50	293.82
3	cont.	0.14	3.18	0.18	3.29	0.17	3.88
	expt.	24.69	371.16	36.43	773.60	48.11	1002.35

lead can cause a significant increase in GSH levels in some mussel tissues but not in others. This same phenomenon was observed by Thomas et al. (1982) with the effects of Cd on GSH contents of mullet tissues. According to Marshall and Talbot (1979), lead was found in mussel gills as extracellular crystalline deposits of mixed or complex carbonate associated with Ca in equiatomic ratio. Amiard et al. (1989) observed that storage of Pb correlated with an increase of phosphorous amounts, and suggested the storage of Pb as phosphate, Schultz-B aides (1978) observed electron-dense granules in the excretory cells of the kidney of *Mytilus edulis*, and suggested that lead was stored as phosphorous- and sulfur-rich complex in membrane-bound vesicles. It is possible that binding of accumulated lead in the kidney in these ways prevented its conjugation with -SH, and hence, prevented any change in GSH level in this tissue.

Among the GSH-dependent enzymes, three (glutathione-S-transferase, glutathione reductase and glutathione peroxidase) have been identified in mussel tissues (Livingstone et al. 1992; Fitzpatrick et al. 1995). Under normal conditions glutathione regulates its own synthesis from cysteine by -glutamylcysteine synthetase in mammals. Lead could increase GSH levels in hepatic tissues by stimulating the activity of this enzyme, but lead-poisoning does not change the activities of glutathione reductase and glutathione peroxidase (Hsu 1981). Cadmium could increase GSH concentrations by reducing glutathione peroxidase activity (Thomas et al. 1982). GSH levels in the tissues are also dependent on the availability of hepatic sulfhydryl groups such as cysteine (Gallagher and Di Giulio 1992; Sin and Teh 1992). These studies suggest that several different mechanisms elevate GSH content in organs exposed to pollutants. The same variety of mechanisms may be reflected in the GSH increases noted in the present experiment

In conclusion, results of the present experiment indicated that elevations of the GSH levels in the tissues are associated with the mercury concentrations in the

tissues. Influence of lead on the GSH levels in the tissues showed a different profile. This result suggested that there might exist different mechanisms between toxicity of lead and mercury in terms of GSH metabolism in the mussel. Although mechanisms of metal-induced perturbations of GSH metabolism in mussel are still poorly understood, the fact that mercury and lead cause alterations in the GSH levels in the tissues to some extent suggests that exposure to the metals may influence many of the functions performed by glutathione.

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