INTRODUCTION

Metal ions represent a typical example of what is referred to as a 'double edged sword'. On the one hand, metal ions are integral and functional components of many enzymes and transcriptional regulatory proteins (Brouwer and Brouwer, 1991). However, the binding of these same metals to biological macromolecules may lead to a disruption of some essential activity (Vallee and Ulmer, 1972). For example, metal ions act as catalysts in the formation of free radicals derived from oxygen; these free radicals have been implicated in inflammatory disorders as well as in mutagenicity and carcinogenicity (Floyd, 1990). To cope with these potentially hazardous elements, organisms possess various finely-tuned mechanisms to control their concentration and availability in the body. Toxic elements are more easily eliminated from the body if their water solubility is relatively high. Thus the bodies metabolic action on these substances is primarily directed towards increasing the water solubility of lipophilic material that would otherwise tend to accumulate in the body. A few enzymes are reported to serve the detoxification function since it facilitates the elimination of electrophiles (Meister, 1981). One other mode of detoxification of metals by the organism is the sequestration of metals by the formation of metal-binding proteins (Hamer, 1986) (discussed in Chap. 5).

\[ \text{Glutathione S-transferases (GSTs, EC 2.5.1.18) are a family of intracellular dimeric proteins that participate in the metabolism of electrophiles and in ligand transport (Jakoby, 1978). GSTs catalyse the nucleophilic addition of the thiol group of glutathione to potentially toxic compounds, including trace metals (Trakshell and Maines, 1988; Reddy et al., 1995), to form a glutathione-conjugate (Meister, 1981); these conjugates are then transformed to more water soluble, and thus excretable, metabolites (Boyland and Chasseaud, 1969). In this way, GST protects cells against toxic effects. A simple schematic representation of the GST/GSH system is given below.} \]
These enzymes are widely distributed and have wide substrate specificities. GSTs are multifunctional proteins and play a major role in the intracellular transport of endogenous compounds. Substantial GST activity has been detected in the gills, kidney, intestinal caeca, and in particular, the liver of rainbow trout (Bauermeister et al., 1983); all four tissues have been shown to have glutathione as well. Keeran and Lee (1987) demonstrated GST activity in the Hp of the blue crab, *Callinectus sapidus*, thereby confirming the function of this enzyme as an intracellular carrier protein for a variety of hydrophobic compounds.

There have been a number of studies on insect GSTs. One reason for this may be that these GSTs conjugate with a wide range of pesticides possibly resulting in the development of resistance to insecticides (Oppenoorth et al., 1979, Motoyama et al., 1980). GSTs have been reported to metabolise various electrophilic xenobiotics including halogenated compounds, nitro compounds, alpha, beta-unsaturated compounds, iso thiocyanates, organo phosphates and oxides (Yu, 1996).
GSTs are reported to be an important component of the detoxification system of aquatic organisms (James et al., 1979; Bauermeister et al., 1983; Ramage and Nimmo, 1984). According to George (1994), GST is present in fishes, including cod (*Gadus morhua*), winter flounder, brook trout, turbot and bluegill sunfish, with ethacrynic acid as substrate. Hepatic GST activity, following exposure to styrene oxide and benzopyrene, had been reported in black drum (*Pogonias cromis*), mangrove snapper (*Lutjanus griscus*) (James et al., 1979) and rainbow trout (Lauren et al., 1989).

Glutathione (GSH) is a substrate in the GSH S-transferase system and the availability of GSH can be a major factor in the metabolism of xenobiotics by this enzymic system. As early as 1975, Richardson and Murphy had proposed that GSH is capable of chelating and detoxifying metals as soon as they enter the cell. Glutathione (GSH L-γ-glutamyl-cysteinyl-glycine) is a tripeptide of intracellular origin and is the most prevalent low-molecular weight thiol (Meister and Anderson, 1983; Shelton et al., 1986). It participates in a number of cellular functions including translocation of amino acids across cell membranes, the maintenance of protein sulphhydryl groups and detoxification of electrophilic compounds through the action of GST. Glutathione has been reported to protect cells against radiation (Bump et al., 1982), different forms of oxidative stress, such as lipid peroxidation (Burk, 1983), the redox cycling of adriamycin (Russo and Mitchell, 1985), free radicals (Shan et al., 1990), and hyperoxia (Weber et al., 1990). Glutathione also forms a substrate for GSH peroxidase, an enzyme capable of both removing H₂O₂ from the cells and repairing peroxidatively damaged membranes (Meister and Anderson, 1983). GSH has also been reported to act as a first line of defence against cadmium and copper toxicity in mammals before the induction of metallothionein occurs (Singhal et al., 1987; Freedman et al., 1989). In crustaceans, an enormously active copper metabolism is displayed that is correlated with the turnover of haemocyanin. Recent studies suggest that GSH plays a role in delivery of copper to apohaemocyanin and apometallothionein (Freedman et al., 1989) and other copper dependent enzymes (Steinkuhler et al., 1991).
Keeping in view the importance of the GST/GSH system in the enzymatic detoxification of metals, and recognising the paucity of studies relating to the exposure of decapod crustaceans to trace metals, this study was initiated to examine changes in the activity of GST and the concentration of GSH in the tissues of fresh water prawn, Macrobrachium malcolmsonii following exposure to Hg and Cu.

MATERIAL AND METHODS

Two batches, each containing four groups (each group containing one hundred and twenty intermoult juvenile prawns each of M. malcolmsonii), were used in this study. In the first batch, the first group served as control; groups 2, 3 and 4) were exposed to the 1/15th, 1/10th and 1/6th of LC50 value of Hg respectively. Likewise, in the second batch, the first group served as control, and the 2nd, 3rd and 4th groups were exposed to the 3 sublethal concentrations of Cu (1/15th, 1/10th and 1/6th of LC50 value). The experiment was carried out for a period of 22 days and sampling was performed on days 4, 8, 15 and 22. On each sampling day, mortality was observed and it was found to be negligible. 18 prawns were sampled from each concentration, with 3 animals being pooled to constitute a single observation; hence, 6 such observations were made for each concentration tested. Control prawns were similarly sampled.

The activity of GST and the content of GSH were estimated in the gills and Hp of control prawns and test prawns. GST activity was assayed by using 1-chloro-2,4-dinitrobenzene (CDNB) and reduced glutathione (GSH), the methodology being essentially as described by Habig et al. (1974); calculations were performed taking the millimolar extinction coefficient value to be 9.6. The values were expressed as nmol mg⁻¹ protein min⁻¹. The concentration of reduced glutathione content was estimated in these 2 tissues by the method of Anderson (1985). The values were expressed in μmol g⁻¹ wet tissue.

RESULTS

Exposure of prawns to sublethal concentrations of Hg appeared to result in an increase in GST activity of both the gill and Hp (Table 20). The activity of GST was
found to be significantly higher in the gill and Hp of test prawns irrespective of the sublethal concentration of Hg to which exposed than that in the same tissues of the control prawns and this was noted on all sampling days (P<0.001; Table 20; Fig. 36).

In prawns that had been exposed to Cu (Table 20), the activity of GST was significantly higher in the gills and Hp of test prawns than that in the same tissues of controls. In prawns that had been exposed to the $1/15^{th}$ sublethal concentration of Cu, the highest activity of GST in the gills was noted on the $22^{nd}$ day of exposure ($P < 0.001$; Table 20), whereas in prawns exposed to the $1/10^{th}$ and $1/6^{th}$ sublethal concentrations of Cu, the activity of GST in the gills was maximal on the $15^{th}$ day of exposure. In the case of the Hp, the highest activity was observed on the $22^{nd}$ day of exposure in all test prawns ($P < 0.001$; Table 20), irrespective of the sublethal concentrations of Cu to which exposed (Fig. 37).

Regression analysis revealed a significant ‘r’ value for the variations in the activity of GST of gills and Hp between days of exposure (Appendix 5, 6, 7 & 8) following exposure to both Hg and Cu.

On the $4^{th}$ and $8^{th}$ days of sampling, the concentrations of GSH in the gills of test prawns that had been exposed to the $1/10^{th}$ and $1/6^{th}$ sublethal concentrations of Hg were found to exceed those in the same tissue of control prawns ($P<0.005$, $P<0.05$ respectively; Table 21). However, on extended exposure i.e. on the $15^{th}$ and $22^{nd}$ day of sampling, the reverse was noted, that is, the concentration of GSH in the gills of test prawns was lower than that in the gills of control prawns (Table 21; Fig. 38). The quantum of GSH in the gills of test prawns that had been exposed to the $1/15^{th}$ sublethal concentration of Hg did not significantly differ from that in controls on the $4^{th}$ sampling day; significantly exceeded that in controls on the $8^{th}$ day of exposure; was significantly lower than that in controls on the $15^{th}$ and $22^{nd}$ days of sampling (Table 21; Fig. 38). The concentration of GSH in the Hp of test prawns that had been exposed to Hg (irrespective of the sublethal concentration to which exposed) significantly exceeded that in the Hp of controls, on the $4^{th}$ and $8^{th}$ days of sampling.
(Table 21; P < 0.05), but did not significantly differ from control values on the 15th and 22nd day of sampling (Table 21).

The concentrations of GSH in the gills and Hp of test prawns exposed to Cu (irrespective of the sublethal concentration to which exposed) significantly exceeded those in the same tissues of control prawns on the 4th sampling day (Table 21), but were significantly lower than control values on the 15th and 22nd sampling days (Fig. 39).

Two way Anova showed a significant F ratio for the variations in the GSH content in the gill and Hp of test prawns exposed to the different sublethal concentrations of Hg and Cu and the period of exposure (Hg- gill F (9,81) = 4.000, P< 0.001; Hg- Hp F (9,81) = 5.535 , P< 0.001; Cu - gill F (9,81) = 5.125, P< 0.001; Cu - Hp F (9,81) = 4.000, P< 0.001).

**DISCUSSION**

This study confirms that substantial activity of glutathione S-transferase activity is present in the gills and hepatopancreas of the prawn, *M. malcolmsonii* on exposure to both Hg and Cu (Fig. 36 and 37). GSTs, in general, are found to be present in tissues which interact with the external environment and which are highly metabolic in nature (Bauermeister *et al.*, 1983; Clark, 1989).

Hg and Cu appear to cause an increase in the GST activity of both gills and Hp of the test prawns. An increase in the GST activity has been reported in the housefly due to phenobarbital toxicity (Ottea and Plaap, 1981; Hayaoka and Dauterman, 1982), in cray fish on exposure to cadmium (Almar *et al.*, 1987), in Hp of the field crab, *Paratelphusa hydrodromus* on exposure to endosulfan (Yadwad, 1989), in *M. edulis* when exposed to sediments contaminated with pollutants (Sheehan *et al.*, 1995), in the liver of rats on treatment with ferric nitrilo triacetate (Fukuda *et al.*, 1996) and in the liver of the channel catfish on exposure to benzopyrene (Gallagharp *et al.*, 1996). In mammals, it is generally believed that the conjugation of toxic substances with GSH occurs primarily in the liver and that conversion of this conjugate to corresponding
mercapturic acid occurs in the kidney (Hughey et al., 1978; Meister, 1981). In aquatic invertebrates Hp seems to a major organ where the cysteine conjugate is formed (Keeran and Lee, 1987). There are few reports to substantiate the involvement of other tissues as well, in this enzymatic detoxification of electrophiles. It has been proven that tissues, such as gills, that interact with the external environment can also form glutathione conjugates (Parker et al., 1980; Bauermeister et al., 1983). The present study appears to demonstrate the involvement of gills in the enzymatic detoxification of Hg and Cu.

Since GSH forms the substrate in the GSH S-transferase system, the availability of GSH seems to be a major factor in the detoxification of the metals by the detoxification enzyme system. Aside from its involvement in the detoxification of electrophilic compounds via the GST S-transferase system, GSH is also involved in the intracellular transport of Cu and many amino acids, and in the maintenance of the sulfhydryl content of the tissue.

In the present study, there was an increase in the GSH content in the gills till the 8th day and in the Hp till the 15th day on exposure to Hg (Fig. 38). Since exposure to Hg resulted in an increase in GST activity, there would have been utilisation of GSH for formation of glutathione conjugate. As the basal GSH level has a feedback control over the GSH-synthesizing enzymes, the low GSH level would have resulted in an increase in the activity of the GSH-synthesizing enzyme. Thus, the elevated GSH levels could be attributed to the increase in GSH synthesis. An increase in GSH level was reported by Thomas and Wofford (1984), in the tissues of Mugil cephalus on exposure to cadmium. Fukino et al. (1986) reported an increase in the GSH levels in kidneys of rats exposed to Hg and Zn, and this was attributed to the increased GSH synthesis. An increase in the GSH level was reported by Yadwad (1989) in the liver tissue of the crab, Paratelphusa hydrodromus following exposure to endosulfan. The decrease in the GSH content on the 22nd day, observed in the present study, suggests that the prawn had lost its compensatory ability and could not maintain its GSH level on prolonged exposure to Hg.
In prawns that had been exposed to Cu, there was a decrease in the GSH content of both Hp and gills of the prawn on all sampling days except day 4 (Fig. 39). A possible explanation for this could be the utilisation of GSH for the formation of GSH conjugates, which is catalysed by GST. Secondly, it is a well known fact that Cu, like Fe, interacts with physiologically-produced reduced oxygen species, like H$_2$O$_2$, catalysing the formation of highly toxic hydroxyl radicals, and GSH peroxidase activity could have increased (although this study does not deal with GSH peroxidase) for the elimination of H$_2$O$_2$. Here again GSH forms the substrate for GSH peroxidase activity. Additionally, GSH is required for the conversion of intracellularly available Cu II to Cu I oxidation state, which is incorporated into apohaem and MT (Freedman et al., 1989; Brouwer and Brouwer, 1991; 1992). A decrease in GSH levels following exposure to metal was reported to be the result of GSH utilisation by GSH peroxidase during the enzymatic repair of lipid peroxides (Freedman et al., 1989). Depletion of GSH level was reported by Bauman et al., (1992) in mice following exposure to diethyl maleate and also in mice exposed to paraquat (Nakagawa et al., 1995).

From this study, it could be inferred that both Hg and Cu, like any other electrophilic organic toxic substance, cause an increase in the GST activity to form glutathione conjugate for further stepwise formation of water-soluble mercapturic derivative for the easy elimination of the same. The GSH content was higher in test prawns that had been exposed to Hg, and this was probably due to an enhanced synthesis of GSH. GSH content of the gill and Hp of test prawns that had been exposed to Cu was lower than that of control prawns and could be due to the utilisation of the same for GST activity and for the transport of Cu to apoproteins. Further studies are required to specify the substrate specificities, if any, and types of isozymes present in the GSTs of *M. malcolmsonii*. 
TABLE 20. Glutathione S-Transferase activity (GST) in various tissues of *Macrobrachium malcolmsonii* following exposure to Hg and Cu.

<table>
<thead>
<tr>
<th>Metal</th>
<th>Tissues</th>
<th>Days of exposure</th>
<th>GST activity (nmol ng(^{-1}) protein min(^{-1})) in tissues of Prawn*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Control prawns</td>
</tr>
<tr>
<td>Hg</td>
<td>Gill</td>
<td>4</td>
<td>4.110±0.159</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
<td>4.165±0.049</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15</td>
<td>4.140±0.047</td>
</tr>
<tr>
<td></td>
<td></td>
<td>22</td>
<td>4.235±0.109</td>
</tr>
<tr>
<td>Hg**</td>
<td>Gill</td>
<td>4</td>
<td>3.531±0.020</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
<td>3.452±0.051</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15</td>
<td>3.587±0.122</td>
</tr>
<tr>
<td></td>
<td></td>
<td>22</td>
<td>3.596±0.112</td>
</tr>
<tr>
<td>Cu</td>
<td>Gill</td>
<td>4</td>
<td>4.110±0.159</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
<td>4.165±0.049</td>
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<td></td>
<td>22</td>
<td>3.596±0.122</td>
</tr>
</tbody>
</table>

* P< 0.001
* Each value is the mean ± SD of 6 pooled observations
** Hepatopancreas
TABLE 21. Glutathione content in various tissues of *Macrobrachium malcolmsonii* following exposure to Hg and Cu.

<table>
<thead>
<tr>
<th>Metal</th>
<th>Tissues</th>
<th>Days of exposure</th>
<th>Concentration of GSH (μmol g(^{-1}) wet tissue) in tissues*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control Prawn</td>
<td>Test Prawns exposed to Metals</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1/15 of LC(_{50})</td>
</tr>
<tr>
<td>Hg</td>
<td>Gill</td>
<td>4</td>
<td>0.572±0.004</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
<td>0.561±0.027</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15</td>
<td>0.604±0.015</td>
</tr>
<tr>
<td></td>
<td></td>
<td>22</td>
<td>0.590±0.019</td>
</tr>
<tr>
<td></td>
<td>Hp**</td>
<td>4</td>
<td>1.104±0.012</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
<td>1.072±0.043</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15</td>
<td>1.121±0.014</td>
</tr>
<tr>
<td></td>
<td></td>
<td>22</td>
<td>1.130±0.026</td>
</tr>
<tr>
<td>Cu</td>
<td>Gill</td>
<td>4</td>
<td>0.572±0.004</td>
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<td></td>
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<td>22</td>
<td>1.130±0.026</td>
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</tbody>
</table>

* P< 0.001;  † P< 0.005;  ‡ P< 0.05
* Each value is the mean ± SD of 6 pooled observations
** Hepatopancreas
Figure 36. CHANGES IN ACTIVITY OF GST IN THE TISSUES OF *Macrobrachium malcolmsonii* FOLLOWING EXPOSURE TO Hg
Figure 37. CHANGES IN ACTIVITY OF GST IN THE TISSUES OF *Macrobrachium malcolmsonii* FOLLOWING EXPOSURE TO Cu

4, 8, 15 & 22 - DAYS OF EXPOSURE
Figure 38. CHANGES IN GSH CONTENT IN THE TISSUES OF *Macrobrachium malcolmsonii* FOLLOWING EXPOSURE TO Hg.
Figure 39. CHANGES IN THE GSH CONTENT IN THE TISSUES OF *Macrobrachium malcolmsonii* FOLLOWING EXPOSURE TO Cu

4, 8, 15 & 22 - DAYS OF EXPOSURE