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# Do selenium and glutathione (GSH) detoxify mercury in marine invertebrates? II. Effects on gill adenosine triphosphatase and related blood factors in an arcid clam *Anadara granosa*

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ABSTRACT: Selenium, because of its affinity for SH/SS, thiol group, offers protection against or detoxifies mercury in marine vertebrates. This study explores the possibility of a similar effect for a marine invertebrate. Arcid blood clams *Anadara granosa* (L.) with erythrocytic haemoglobin were exposed to sublethal concentrations of $Hg^{2+}$ (0.1 mg $l^{-1}$, as $HgCl_2$), $Se^{4+}$ (1.0 mg $l^{-1}$, $SeO_2$) and reduced glutathione (GSH, 1.0 mg $l^{-1}$), either individually or in combination, for 96 to 144 h *in vivo*. Parameters studied included gill ATPase, haemoglobin (Hb), haemolymph, haematocrit, protein, free amino acids (FAA) and plasma $Na^+$, $K^+$, $Ca^{++}$, $Mg^{++}$ and osmolality. Hg reduced gill ATPase activity by at least 50 %. Se alone and Hg + Se also inhibited enzyme activity. No similar changes occurred on exposure to GSH alone. GSH in the presence of Hg, on the other hand, completely nullified the inhibitory effect of Hg. In the clams transferred to GSH (for 72 h) after initial exposure to Hg (72 h) and vice versa, enzyme activity was inhibited by 45 % compared to controls. Haematocrit increased significantly in clams exposed to Hg alone and to Se alone, but exposure to GSH alone and GSH + Hg had no significant effect. Erythrocyte sedimentation rate (ESR) increased significantly in clams exposed to Se, but was lowered to varying degrees in all other experimental clams. Hb content and haemolymph protein levels in experimental groups did not change significantly compared to controls. Methaemoglobin increased significantly in all experimental groups, the maximum increase (ca 137 %) being in those exposed to Hg. Free amino acid (FAA) levels were appreciably raised in clams treated with Hg alone and Hg + Se, but exposure to Se alone and GSH alone had no significant effect. Plasma cations ($Na^+$, $K^+$ and $Ca^{++}$) showed significant changes following exposure to Hg. However, in clams exposed to Hg + Se only $K^+$ and $Ca^{++}$ levels increased. Concentration of $Mg^{++}$ in haemolymph of groups treated with Hg, Se and GSH remained practically unaltered, but in groups exposed to Hg + Se and Hg + GSH it increased appreciably. Plasma osmolality, however, was reduced in all experimental clams; the lowest being in clams exposed to Hg. Unlike its effects on marine vertebrates, Se failed to provide protection against deleterious effects of Hg on the marine clams. GSH, on the other hand, nullified the impact of mercury and/or exerted its own effect.

## INTRODUCTION

As recently as 2 decades ago there were comparatively few studies evaluating biological effects in the marine environment of strongly divalent metals, especially Cu, Zn, Cd and Hg. Most early efforts were restricted to the understanding of the effects of metals on survival and growth. Since then substantial advances have been made in studying their toxic effects. However, parameters currently used to measure cation-induced stress are rather limited. There have been many studies on metal uptake and biomagnification, but those evaluating impact at cellular and subcellular levels are scanty. Lately it has been realised that greater emphasis should be given to histopathological, biochemical and behavioural parameters as early indicators of growth impairment, reproductive success, survival, and metabolic imbalance (Brown 1976, Eisler 1979, Sabbioni 1981).

One such approach to the study of the effect of

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pollutants is through observations on changes in the activity of various enzymes. Enzyme assays performed in the presence and absence of a given pollutant can provide information on the nature and intensity of its effect and loci of action. The measurement of enzyme systems may be useful in detecting effects of pollutants at a subclinical level. In rats, urinary γ-glutamyl transferase activity, and in rainbow trout *Salmo gairdneri*, erythrocyte σ-amino levulinic acid dehydratase activity, have been identified as specific short-term indicators of adverse effects of mercury and lead respectively (Hodson et al. 1977, Dierickx 1980).

In aquatic species Hg induces changes in blood and enzyme chemistry through inhibition of sulphydryl (SH) and non-SH enzymes (Webb 1966, Waku & Nakazawa 1979). Enzymes involved in homeostasis, particularly $Na^{+}/K^{+}$ and $Mg^{++}$ activated adenosine triphosphatase (ATPase), are comparatively sensitive to lipophilic xenobiotics and heavy metals, and it has been strongly suggested that toxicosis from any of these chemicals may develop primarily from ATPase inhibition (Riedel & Christensen 1979). The most important $Na^{+}$ exchange between the internal and external media occurs across the gill filaments and ATPase plays a central role in this process (Towle 1981). Mercurials, by virtue of their strong lipophilic action and affinity towards the thiol group, penetrate plasma membranes and inhibit ATPase activity, the inhibition being dependent upon membrane thiol reactivity with mercurials. The inhibition of ATPase in turn, disrupts ionic balance, and is therefore of special significance to estuarine animals, which are subjected to abrupt changes in ambient salinity.

Similarly, blood parameters also have been recognised as valuable tools in assesing the condition of the organism and its responses to physico-chemical changes in the environment (Carr & Neff 1984). However, the usefulness of these parameters is often impaired by a lack of information about the normal range of values (Dawson 1979). This is particularly true in the case of molluscs about which little is known. The present study explores the relation between gill adenosine triphosphatase activity and haematological factors in an arcid blood clam *Anadara granosa* (L.), exposed to mercury, selenium and reduced glutathione (GSH).

## MATERIALS AND METHODS

Details of collection and maintenance of *Anadara granosa* and the experimental set-up are discussed in Chandy & Patel (1985). A group of 10 clams of similar size (3 to 5 cm shell length) and age (2 to 3 yr) were exposed to $Hg^{2+}$ ($HgCl_2$), $Se^{4+}$ ($SeO_2$) and reduced glutathione (GSH) at a salinity of about 33 ppt at 25 ± 2 °C and dissolved oxygen level around 6.0 mg $l^{-1}$. A control group was maintained without any addition of chemicals. Bleeding and moribund specimens, if any, were removed, and the water replaced twice daily with appropriate additions of fresh chemicals to maintain concentrations. No attempt was made to feed the clams during the exposure period. Separate series of experiments were performed to study the combined effects of Se + Hg and GSH + Hg. In this set a group of clams each was exposed to combination of 1.0 mg $l^{-1}$ Se + 0.1 mg $l^{-1}$ Hg and 1.0 mg $l^{-1}$ GSH + 0.1 mg $l^{-1}$ Hg for 96 h. In another series a group of clams was first exposed to Hg (0.1 mg $l^{-1}$) for 72 h and then transferred to GSH (1 mg $l^{-1}$) for the next 72 h and vice versa. Another batch was exposed for 144 h to Hg or Se individually at the same ambient levels. On completion of the desired exposure (96 or 144 h), gills from both experimental and control groups were dissected under chilled conditions. Gills from 4 or 5 clams were pooled and homogenized in about 8 vols (w/v) Tris buffer (0.05 M, pH 7.4) using a Potter-Elvehjem type homogenizer with teflon pestle. Total ATPase activity was determined for 10 replicates by adding 0.2 ml of appropriately diluted homogenate to an assay tube containing 2 ml incubation medium (5 mM $MgCl_2$, 15 mM KCl and 0.1 M NaCl in Tris buffer). Blank values for non-enzymatic phosphate production were determined by using heat-denatured homogenate (1 min on a boiling water bath). All tubes were pre-incubated for 10 min at 25 °C, and then 0.2 ml (20 mM) vanadium-free equine muscle adenosine triphosphate (ATP; Sigma) made in incubation medium was added. After 60 min incubation at 25 °C the reaction was terminated by adding 2 ml 10 % trichloroacetic acid. The mixture was centrifuged at 3000 × g for 10 min and precipitated protein removed. The supernatant was assayed for inorganic phosphate released (Fiske & Subbarow 1925).

Blood samples from 4 or 5 clams were pooled, and haematocrit and erythrocyte sedimentation rate (ESR) measured. Haemolymph was separated by centrifugation at 1500 × g for 5 min. Osmolality was determined by the freezing point depression technique (Ganotec, Osmomat-030). Concentrations of cations ($Na^{+}$, $K^{+}$, $Ca^{+}$, $Mg^{++}$) in the haemolymph were measured by atomic absorption spectrometry. The ninhydrin method (Rosen 1957) was adopted for determination of free amino acid content in the haemolymph. Haemoglobin was estimated following the cynamethaemoglobin method and the percent methaemoglobin calculated from the difference in absorbance at 630 nm after the addition of KCN to the assay cuvette (Fairbanks 1976). Protein content in gills and haemolymph was determined following the Biuret method (Gornall et al. 1949).

## RESULTS

### Adenosine triphosphatase (ATPase)

Tables 1 & 2 summarize the results of effects of Hg, Se and GSH on ATPase activity expressed as µmol Pi $min^{-1}$ $g^{-1}$ (IU). In *Anadara granosa* gills ATPase activity ranged from 1.5 to 2.0 IU (mean 1.70 ± 0.2 IU, n = 10). After 96 h exposure to Hg (0.1 mg $l^{-1}$) the enzyme activity (0.82 ± 0.3 IU) in the clams was inhibited by more than 50 % ($p < 0.05$) compared to that observed in the control groups. On similar exposure to Se (1.0 mg $l^{-1}$) and GSH (1.0 mg $l^{-1}$) individually, mean ATPase activity decreased to 1.29 ± 0.3 and 1.50 ± 0.2 IU respectively. However, the effect due to GSH was not statistically significant. On exposure up to 144 h either to GSH or to Hg, no further significant decrease in ATPase activity was observed (Table 2). The inhibitory effect of Hg on ATPase activity in the gills of the clams when exposed in the presence of either Se or GSH was reduced by 33 and 94 % respectively compared to that observed in those exposed to Hg alone (Table 1). ATPase activity was inhibited by 46 % on initial exposure to GSH (72 h) followed by Hg (72 h). This effect shows amelioration of enzyme activity by at least 30 %, if the individual effects of GSH (19 %) and Hg (56 %) are considered on a purely additive basis (~ 75 %, Table 2). Similar effects were observed in the enzyme activity in the group first exposed to Hg and then to GSH (0.95 ± 0.3 IU, Table 2).

Table 1. Adenosine triphosphatase (ATPase) activity (IU = µmol Pi $min^{-1}$ $g^{-1}$) and protein (mg $g^{-1}$) in the gills on 96 h exposure to mercury (0.1 mg $l^{-1}$), selenium (1.0 mg $l^{-1}$) and GSH (1.0 mg $l^{-1}$). (Mean ± SD, n = 10, n refers to 10 separate experiments, each experiment with a group of 10 clams

| Treatment | ATPase activity (IU) | Protein (mg $g^{-1}$) |
|---|---|---|
| Control | 1.70 ± 0.2 | 68.2 ± 9.6 |
| Hg | 0.82 ± 0.3 | 73.6 ± 21.2 |
| Se | 1.29 ± 0.3 | 65.0 ± 19.8 |
| GSH | 1.50 ± 0.2 | 69.3 ± 18.2 |
| Hg + Se | 1.11 ± 0.1 | 72.5 ± 7.5 |
| Hg + GSH | 1.65 ± 0.2 | 65.5 ± 16.3 |

Table 2. *Anadara granosa*. Effect of mercury (0.1 mg $l^{-1}$) and glutathione (GSH) (1.0 mg $l^{-1}$) on ATPase activity and protein in the gills. Mean ± SD of 3 experiments, each with 10 clams

| Treatment/duration | ATPase acitvity (IU) | Gill protein (mg $g^{-1}$) |
|---|---|---|
| Control (144 h) | 1.74 ± 0.1 | 69.4 ± 12.2 |
| Hg (144 h) | 0.77 ± 0.5* | 67.8 ± 11.3 |
| GSH (144 h) | 1.41 ± 0.5 | 66.1 ± 9.0 |
| GSH for 72 h followed by Hg for 72 h | 0.94 ± 0.2** | 63.2 ± 8.2 |
| Hg for 72 h followed by GSH for 72 h | 0.95 ± 0.3** | 64.4 ± 4.6 |

* $p<0.05$, ** $p<0.01$

### Protein

Protein levels in gill of control clams varied between 56 and 79 mg $g^{-1}$, the mean being 68.2 ± 9.6 mg $g^{-1}$ (Table 1). In experimental clams exposed to various chemicals for different time intervals, no significant changes in protein content were evident. The variations observed were well within experimental errors as presented in Tables 1 & 2.

### Haematological parameters

**Haematocrit.** Effect of Hg, Se and GSH on the haematocrit (% packed cells) is summarized in Tables 3 & 4. Control clams had a mean haematocrit of 8.5 ± 0.8 %. This increased to 11.8 ± 1.0 % ($p < 0.05$) following exposure for 96 h to Hg. Exposure to Se alone reduced haematocrit by 21 % ($p < 0.01$). Treatment with GSH, on the other hand, did not show any change in haematocrit irrespective of time of exposure (96 to 144 h) compared to controls (Tables 3 & 4). On exposure to a combination of Se + Hg the packed cell volume increased by 32 % (11.2 ± 1.2 %, $p < 0.05$). Exposure to GSH for 96 h restored haematocrit to control levels when administered together with Hg (Table 3). On exposure to Hg alone for the same period, it increased significantly by 24 % ($p < 0.05$, Table 4). In clams transferred to Hg after 72 h initial exposure to GSH and vice versa haematocrit values were found to be appreciably higher than observed in control clams ($p < 0.02$; Table 4).

**Erythrocyte sedimentation rate (ESR).** ESR was modified in clams exposed to Hg, Se or GSH after 96 h. Thus on exposure to Hg, ESR was reduced by 41 % (10.6 ± 0.6, $p < 0.001$) compared to controls (18.1 ± 0.6, Table 3). Se increased ESR by 17 % ($p < 0.01$). In the group exposed to combinations of Se + Hg, ESR remained practically the same as observed in clams exposed to Hg alone (Table 3). On the other hand, ESR decreased both in the clams exposed to GSH alone (16.6 %) and in the presence of Hg (22.1 %) compared to control values (Table 3). On further exposure to GSH and Hg separately for a period up to 144 h no further decrease in ESR was evident (Table 4). Similarly, no

significant change in ESR was observed in clams first exposed to GSH and then followed by to Hg and vice versa (Table 4).

**Haemoglobin and methaemoglobin.** Haemoglobin content remained practically the same in both control and experimental groups of *Anadara granosa* exposed to various chemicals (Tables 3 & 4). However, methaemoglobin showed an appreciable increase in the experimental (exposed) groups (Tables 3 & 4). The mean haemoglobin level in control clams was about 5.8 ± 0.2 g per 100 ml whole blood; of this 15.0 ± 2.7 % was methaemoglobin (Table 3). On exposure to Hg for 96 h, methaemoglobin increased dramatically by 137 % (to 35.5 ± 9.2 %). Methaemoglobin content in the clams exposed to Se or GSH individually and to a combination of Se + Hg, also increased significantly by 40, 38 and 68 % respectively. Although methaemoglobin level increased in clams exposed to combinations of Hg + GSH (23.4 ± 8.0 %), compared to those exposed to GSH alone (20.8 ± 4.2 %) and control (15.0 ± 2.7 %), it was well below that observed in those exposed to Hg (35.5 ± 9.2 %) alone ($p < 0.05$, Table 3).

On exposure over a period of 144 h haemoglobin level showed no significant change in clams treated either with Hg or with GSH (Table 4). However, methaemoglobin content showed an appreciable increase in clams exposed to these chemicals. Thus in the clams exposed to Hg, methaemoglobin increased by 118 % (to 33.6 ± 5.2 % of the total pigment). On exposure to GSH for the same period a 23 % increase in methaemoglobin was observed. After 72 h exposure to GSH followed by exposure to Hg for the same period and vice versa, methaemoglobin increased significantly ($p < 0.01$), whereas no appreciable change was noted in haemoglobin content (Table 4).

**Free amino acids (FAA).** Changes observed in free amino acid levels in haemolymph of *Anadara granosa* exposed to Hg, Se and GSH for 96 h are shown in Table 5. In the control clams FAA level was 0.19 ± 0.02 mM. Exposure to Se and Hg individually and to a mixture of Se + Hg resulted in an appreciable increase (10 to 58 %) in FAA compared to controls. On exposure to GSH, FAA level decreased marginally (ca 10 %), whereas it increased by 21 % after exposure to a combination of GSH + Hg, compared to controls (Table 5). Marginal changes in FAA levels were observed following 144 h exposure to GSH and Hg (Table 6). In the

Table 3. *Anadara granosa*. Effect of mercury, selenium and GSH on haematological parameters after 96 h exposure (ambient concentrations same as in Table 1). Values are mean ± SD of 5 experiments, each with 10 clams

| Treatment | Haemolymph protein (mg ml$^{-1}$) | Haematocrit (%) | ESR (mm h$^{-1}$) | Haemoglobin (g 100ml$^{-1}$) | Methaemoglobin (% of haemoglobin) |
|---|---|---|---|---|---|
| Control | 10.1 ± 1.7 | 8.5 ± 0.8 | 18.1 ± 0.6 | 5.8 ± 0.2 | 15.0 ± 2.7** |
| Hg | 11.6 ± 1.6 | 11.8 ± 1.0* | 10.6 ± 0.6** | 6.2 ± 0.3 | 35.5 ± 9.2** |
| Se | 8.9 ± 0.8 | 6.7 ± 0.7** | 21.1 ± 0.4** | 5.3 ± 0.6 | 21.0 ± 6.1 |
| GSH | 10.3 ± 0.8 | 8.9 ± 0.9 | 15.1 ± 0.4** | 5.3 ± 0.2 | 20.8 ± 4.2 |
| Hg + Se | 12.7 ± 2.4 | 11.2 ± 1.2* | 11.0 ± 0.5** | 6.2 ± 0.5 | 25.2 ± 7.1* |
| Hg + GSH | 11.6 ± 1.6 | 8.5 ± 0.8 | 14.1 ± 0.6** | 5.4 ± 0.7 | 23.4 ± 8.0 |

* $p < 0.05$, ** $p < 0.01$

Table 4. *Anadara granosa*. Changes observed in haematological parameters on 144 h exposure to mercury and GSH (ambient concentrations same as in Table 2). Values are mean ± SD of 3 experiments, each with 10 clams

| Treatment | Haemolymph protein (mg ml$^{-1}$) | Haematocrit (%) | ESR (mm h$^{-1}$) | Haemoglobin (g 100ml$^{-1}$) | Methaemoglobin (% of haemoglobin) |
|---|---|---|---|---|---|
| Control | 10.6 ± 0.9 | 7.9 ± 0.9 | 17.8 ± 0.9 | 5.8 ± 0.3 | 15.4 ± 1.8 |
| Hg | 10.8 ± 0.9 | 10.5 ± 0.8* | 12.6 ± 0.8** | 6.4 ± 0.6 | 33.6 ± 5.2** |
| GSH | 9.8 ± 1.2 | 7.8 ± 0.5 | 14.9 ± 1.3 | 5.7 ± 0.5 | 19.0 ± 3.1 |
| GSH for 72 h followed by Hg for 72 h | 12.0 ± 1.9 | 10.1 ± 1.2* | 16.5 ± 1.1 | 5.6 ± 0.7 | 25.4 ± 1.3** |
| Hg for 72 h followed by GSH for 72 h | 13.2 ± 1.6 | 9.6 ± 0.9 | 18.1 ± 2.0 | 6.2 ± 0.6 | 23.4 ± 3.2 |

* $p < 0.05$, ** $p < 0.01$

Table 5. *Anadara granosa*. Change in haemolymph cations, free amino acids and osmolality after 96 h exposure to mercury, selenium and GSH (ambient concentrations same as in Table 1). Values are mean ± SD of 5 experiment, each with 10 clams

| Treatment | Cation concentration (mM) | | | | Free amino acids | Osmolality |
|---|---|---|---|---|---|---|
| | $Na^+$ | $K^+$ | $Ca^{++}$ | $Mg^{++}$ | (mM) | (mOsm $kg^{-1}$) |
| Control | 622 ± 25 | 10.7 ± 1.8 | 15.0 ± 2.4 | 58.9 ± 11.7 | 0.19 ± 0.02 | 1068 ± 16 |
| Hg | 544 ± 27•• | 13.3 ± 1.3• | 17.9 ± 3.6 | 53.6 ± 7.3 | 0.26 ± 0.06 | 940 ± 37•• |
| Se | 623 ± 98 | 11.1 ± 2.2 | 16.1 ± 3.8 | 57.7 ± 12.1 | 0.21 ± 0.04 | 1000 ± 25•• |
| GSH | 622 ± 79 | 9.7 ± 1.5 | 14.1 ± 1.4 | 50.8 ± 8.2 | 0.18 ± 0.01 | 993 ± 25•• |
| Hg + Se | 623 ± 20 | 15.8 ± 1.6 | 26.0 ± 4.0 | 63.9 ± 13.0 | 0.30 ± 0.02 | 1000 ± 22•• |
| Hg + GSH | 616 ± 51 | 10.1 ± 1.2 | 16.0 ± 1.2 | 68.5 ± 11.8 | 0.23 ± 0.01 | 993 ± 13•• |

• $p < 0.05$, •• $p < 0.01$

Table 6. *Anadara granosa*. Effect of mercury and GSH (ambient concentrations same as in Table 2) on haemolymph cations, free amino acids and osmolality after 144 h exposure. Values are mean ± SD of 3 experiments, each with 10 clams

| Treatment | Cation concentration (mM) | | | | Free amino acids | Osmolality |
|---|---|---|---|---|---|---|
| | $Na^+$ | $K^+$ | $Ca^{++}$ | $Mg^{++}$ | (mM) | (mOsm $kg^{-1}$) |
| Control | 619 ± 19 | 10.3 ± 2.6 | 14.9 ± 1.2 | 51.2 ± 2.6 | 0.19 ± 0.02 | 1055 ± 14 |
| Hg | 522 ± 16•• | 10.9 ± 2.4 | 16.5 ± 0.9 | 50.1 ± 5.7 | 0.23 ± 0.2 | 964 ± 22• |
| GSH | 565 ± 22 | 9.3 ± 3.2 | 13.0 ± 1.6 | 51.2 ± 4.3 | 0.17 ± 0.01 | 1011 ± 27 |
| GSH for 72 h followed by Hg for 72 h | 510 ± 24•• | 17.0 ± 1.5• | 15.8 ± 2.0 | 51.1 ± 3.6 | 0.22 ± 0.02 | 800 ± 19•• |
| Hg for 72 h followed by GSH for 72 h | 500 ± 13•• | 12.3 ± 0.7 | 15.2 ± 1.7 | 49.9 ± 1.9 | 0.20 ± 0.03 | 900 ± 16•• |

• $p < 0.05$, •• $p < 0.01$

clams transfered to Hg after initial acclimation to GSH and vice versa, FAA remained practically within the same range as that observed in the controls (Table 6).

### Haemolymph cations

**Sodium ($Na^+$).** $Na^+$ concentration in the haemolymph of controls clams was 622 ± 25 mM (Table 5). On exposure to Hg for 96 h it dropped to 544 ± 27 mM ($p < 0.01$). In the clams exposed to Se, GSH, and to mixtures of Se + Hg and GSH + Hg, $Na^+$ levels, however, did not show any significant changes (Table 6). In clams transferred to Hg (72 h) after initial acclimation to GSH (72 h) and vice versa, $Na^+$ levels dropped by ca 18 % below controls (Table 6).

**Potassium ($K^+$).** Significant changes amounting to 24 and 48 % increase in $K^+$ level over controls (10.7 ± 1.8 mM) was observed in clams exposed to Hg (13.3 ± 1.3 mM) and to a mixture of Hg + Se (15.8 ± 1.6 mM) (Table 5). On the other hand, in clams exposed to Se and GSH individually and to a combination of GSH + Hg, $K^+$ level remained within the control values (Table 5). Following 144 h exposure to GSH and Hg individually, $K^+$ levels were more or less the same as observed in controls (Table 6). Clams first exposed to GSH for 72 h and then followed by to Hg for the same period showed 65 % increase in $K^+$ level (17.0 ± 1.5 mM) in the haemolymph. In the clams initially exposed to Hg and then followed by to GSH, $K^+$ concentration increased only by 21 % (Table 6).

**Calcium ($Ca^{++}$).** Changes observed in haemolymph $Ca^{++}$ concentration after 96 h exposure to Hg, Se and GSH are summarized in Table 5. In control groups, $Ca^{++}$ level was 15.0 ± 2.4 mM. No appreciable change in $Ca^{++}$ level was noticed (Table 5) on exposure to Se, GSH and to a mixture of GSH + Hg. $Ca^{++}$ increased marginally by 19 % following 96 h exposure to Hg (17.9 ± 3.6 mM) alone and significantly by 73 % on treatment with a mixture of Hg + Se (26.0 ± 4.00 mM). A similar increase in $Ca^{++}$ was also evident in clams exposed to Hg for 144 h (Table 6). However, in clams exposed to Hg after 72 h initial acclimation to GSH and vice versa, $Ca^{++}$ concentration in haemolymph did not change appreciably (Table 6).

**Magnesium ($Mg^{++}$).** Magnesium content in the haemolymph also showed no significant change in clams exposed to Se, Hg and GSH for various time

intervals compared to the controls (Table 5). Clams exposed to GSH for 72 h after an initial exposure to Hg over the same period, however, showed about 20 % reduction in $Mg^{++}$ level (Table 6).

**Osmolality.** Average haemolymph osmolality in control and experimental clams is given in Tables 5 & 6. Significant reduction in osmolality was evident in the clams exposed to Hg for 96 h (940 ± 37 mOsm $kg^{-1}$, $p < 0.001$) and 144 h (964 ± 22 mOsm, $p < 0.05$) compared to controls (1068 ± 16 and 1055 ± 14 mOsm $kg^{-1}$ respectively). Similarly, osmolality decreased significantly in clams exposed either to GSH or a mixture of GSH + Hg ($p < 0.01$, Table 5). Haemolymph osmolality was practically the same in clams exposed to Se both alone and in the presence of Hg (1000 mOsm $kg^{-1}$, Table 5). Initial exposure to GSH followed by exposure to Hg and vice versa also reduced osmolality significantly ($p < 0.001$, Table 6).

## DISCUSSION

Cellular ATP and associated enzymes play an important role in the normal physiological activities of an organism. Besides ionic regulation, where Na/K-activated ATPase has been identified as an enzymatic equivalent to an 'Na-pump', ATPase mediates vital functions like propagation of nerve impulses and oxidative phosphorylation (Desaiah et al. 1980, Towle 1981). Most of the available evidence suggests that epithelial Na/K-ATPase is important in blood ion regulation. However, some uncertainty exists regarding the function of this enzyme in ion/volume regulation in aquatic species (Kirschner 1977, 1980, Schulz 1978, Evans 1979). The role of branchial Na/K-ATPase in electrolyte movement between the body fluids and the ambient medium in osmoconforming species has not yet been established. Both in osmoregulating as well as in osmoconforming invertebrates, little or no Na/K-ATPase has been so far detected, when the body fluids osmotically conform to the external salinity (Mangum et al. 1980, Towle 1981). During the present studies on *Anadara granosa*, an osmoconforming bivalve, likewise little Na/K-dependent (ouabain sensitive) ATPase was detected in the gill tissue. In the clams exposed to mercury alone and in the presence of selenium, although there was appreciable reduction in gill ATPase, expected changes in haemolymph characteristics did not occur. We had assumed that in the blood clam, inhibition of ATPase activity would increase plasma osmolality, but instead it decreased. These observations clearly suggest that branchial ATPase, at least in *A. granosa*, does not play any significant role in the regulation of electrolytes. This is not surprising, since in 3 species of osmoconforming mussels (*Modiolus* spp.) regulation of ions was also not detected (Pierce 1970, 1971, Virkar & Webb 1970). Moreover, ions did not function as a solute source in the maintenance of cellular volume.

Inhibition of ATPase due to mercury observed in *Anadara granosa* could be explained in terms of the metal's attachment to lipid membrane and subsequent inactivation, considering its strong lipophilic nature, although $Hg^{2+}$ is less lipophilic than methyl mercury. A wide variety of lipophilic xenobiotics have been demonstrated to reduce ATPase activity following *in vivo* treatment in many aquatic species (Kuhnert et al. 1976, Bouquegneau 1977, Miller & Kintner 1977, Verma et al. 1978, Riedel & Christensen 1979, Boese et al. 1982, Rao & Rao 1984). Further, the degree of ATPase inhibition has been found to be either selective, so that it cannot be reversed by substrate (Waku & Nakazawa 1979), or dependent upon the extent to which mercurials have reacted with the membrane thiols (Knight et al. 1968). In the present study also, significant reduction in branchial ATPase coincided with similar reduction in reactive thiol groups. Alternatively, a decline in ATP availability could in turn result in decreased enzyme activity. This possibility assumes significance since Mg-activated ATPase is involved in the terminal ATP-producing step of oxidative phosphorylation (Desaiah et al. 1980).

In marine bivalves, modification in concentration of internal free aminoacids has been recognized as a means of regulation of cell osmotic pressure. Thus intertidal mussels have more expendable intracellular solute than the subtidal forms and can therefore maintain their intracellular volumes over a much wider range of salinities (Pierce 1970). In *Anadara granosa*, following exposure to selenium, mercury and GSH individually, free amino acids in the haemolymph showed appreciable changes. The most profound change was noticed in clams exposed to mercury and to a mixture of selenium and mercury, when a substantial reduction in ATPase activity was observed. However, the increase in free amino acid levels cannot be solely considered as an effort to maintain osmotic pressure. This may well be the consequence of cellular injury, leading to leakage of free amino acids into the haemolymph. The elevated concentration of $K^{+}$ in haemolymph could also be the result of cellular damage. Mercury is reported to induce structural changes in the gills of teleosts (Olson et al. 1973, Gardner 1975), leading to impairment of normal gas exchange across the gill surface, so inducing anaerobiosis. Accumulation of anaerobic end products has been found to decrease the pH of haemolymph so leading to dissolution of Ca (Crenshaw & Neff 1969, Shaffi 1981). The increase in Ca levels in haemolymph may therefore be a consequence of this buffering response.

Increase in haematocrit has been observed in fishes following activities under stress. However, this appears to be due to changes in the volume of erythrocytes rather than the total blood (Houston et al. 1971, Kirk 1974, Casillas & Smith 1977). The haematocrit values have been found to depend upon ambient temperature, age reproductive status, etc. (Barnhart 1969, Wedemeyer & Chatterton 1971). However, no such observations seem to have been made on marine bivalves. In *Anadara granosa* haematocrit showed significant changes in specimens exposed to mercury alone and in combination with selenium, whereas no appreciable change was evident in those exposed to GSH alone and in the presence of mercury. Although haematocrit is also influenced by other biotic and abiotic factors, its usefulness in monitoring the impact of pollutants as an indicator of physiological condition in the blood clam is evident from the present study.

ESR has immense prognostic value in the diagnosis of many diseases. However, this parameter has not been studied in marine invertebrates – not surprisingly since very few such species have erythrocytes. Thus, the arcid clams with erythrocytic haemoglobin provide an unique system for such studies and for assessment of the health of ecosystems in general. It is interesting to note that both haematocrit and ESR in *Anadara granosa* under stress behaved quite differently. Thus when haematocrit was high, ESR was low and vice versa. This could be due to increase either in viscosity of haemolymph or cell volume or both. Unlike the situation in human blood, where pathological conditions elevate ESR, in *A. granosa* there was substantial reduction in ESR when exposed to mercury alone and in the presence of selenium. Furthermore, on exposure to selenium and mercury individually and in combination, the ratio of ESR to haematocrit worked out to 3.2, 0.9 and 1.0 respectively. This clearly indicates that when administered together, the effect of selenium was completely overshadowed by that of mercury. On the other hand, on exposure to GSH alone and in presence of mercury, ESR to haematocrit ratio remained practically the same (1.7), suggesting total amelioration of mercury effect. Consistency in response even at a very low individual variation makes this response an ideal stress indicator.

Heavy metals including mercury are reported to affect haem-biosynthesis and metabolism (Piper et al. 1977, Tephly et al. 1978). Appreciable reduction in haemoglobin content in teleosts following exposure to mercury chloride has been reported (Dawson 1979). In the present study, however, haemoglobin content in *Anadara granosa* remained unchanged even after exposure to various chemicals. On the other hand, methaemoglobin increased significantly in clams exposed to mercury alone and in the presence of selenium. Methaemoglobin formation depends upon the relative rates of oxidation of haemoglobin and its subsequent reduction by NADH-dependent methaemoglobin reductase back to haemoglobin (Miyauchi et al. 1981). Methaemoglobin accumulates in blood cells when it is formed at a rate in excess of the reducing capacity of the normal enzyme systems in erythrocytes. Mercurials, by virtue of their strong lipophilic action, are reported to penetrate the limiting membrane and interact with the mitochondrial inner membrane leading to uncoupling of oxidative phosphorylation and drop in ATP production (Castro et al. 1978). Impairment of normal metabolic processes may decrease the formation of NADH and NADPH and subsequently result in the ineffective reduction of oxidised haemoglobin. Similarly, inhibition of ATPase (Mg-activated) deleteriously affects the terminal step in oxidative phosphorylation and ATP production (Desaiah et al. 1980). Significant reduction in ATPase and appreciable increase in methaemoglobin in clams exposed to mercury and to a mixture of mercury and selenium suggests that methaemoglobinemia could be a consequence of disrupted aerobic metabolism. However, direct effect of mercury on the methaemoglobin-reducing enzyme, NADH-dependant methaemoglobin reductase, could also be a possible reason for accumulation of oxidised haemoglobin. Substantial increase in methaemoglobin in turn may decrease the oxygen carrying capacity of the pigment. Inefficient gas exchange due to branchial epithelial damage together with reduced oxygen carrying capacity of haemoglobin adversely affects normal physiology of the clams and leads to decreased survival rate.

The significant finding of the present study on branchial ATPase and related parameters is the ability of GSH to detoxify the adverse effects of mercury in *Anadara granosa* following *in vivo* exposure. About 50 % reduction in ATPase activity observed on exposure to mercury alone was restored to control levels in clams treated with mercury and GSH together. Similarly, GSH nullified the impact of mercury and/or exerted its own effect on the other biochemical processes. Selenium, on the other hand, failed to offer effective protection against the mercury toxicity. This is in agreement with the earlier observation where, unlike selenium, GSH provided effective protection against the impact of mercury on lysosomal marker enzymes, arylsulfatase and acid phosphatase (Chandy & Patel 1985).

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