CHAPTER 3
PHOSPHATASE ACTIVITY IN THE LIVER AND KIDNEY
AFTER EXPOSURE TO COPPER AND MERCURY

The biotransformation and eventual excretion of the many biotransformed derivatives of toxicants, can be realized at different levels of functional complexity such as cellular, subcellular and molecular levels. In many cases the earliest detectable changes of primary events are associated with a particular type of subcellular organelles such as lysosomes, endoplasmic reticulum (ER) and mitochondria.

The lysosomal membrane is often a target of injury by xenobiotics or by their metabolites in addition to its role in sequesteration. Lysosomes are noted for their compartmentalization and accumulation of a wide variety of organic chemicals or metals (Allison, 1969; Sternlieb and Goldfischer, 1976; Moore, 1980). The role of lysosomes may be important as a detoxification system, particularly for metals (Viarengo et al., 1984; George, 1983). As with other detoxification systems, this process is effective until the storage capacity of the lysosome is overloaded or the lysosomes are damaged directly by the contaminant (Moore et al., 1985). Assessment of this type of injury has been confirmed as an index of cellular conditions (Moore, 1980, 1982; Moore et al., 1982, 1985). Cell membrane and the associated endoplasmic reticulum (ER) are also susceptible to the effect of pollutants as they bind the lipoprotein layer of the membrane and induce variation in the permeability which upset the whole cellular systems.

At the molecular level, the accumulation of inclusion bodies like lipofuscin granules, phosphate and carbonate granules and enzymes reflect the impact of xenobiotics. It appears that structural and other properties as well as activities of enzymes can be affected by exposure to pollutants, possibly leading to loss of cellular metabolic flexibility (Gould et al., 1976; Gould, 1977). Many metals have been reported to alter the activity of various enzymes in marine organisms (Webb, 1966; Moore and Stebbing, 1976).

Apart from this, the impact of xenobiotics on cellular and subcellular levels can also be perceived by a study at the molecular level ie., by the study of particular enzymes. Injury of lysosomes by the xenobiotics will result
in the destabilization of lysosomal membrane, resulting in the release of hydrolytic enzymes from the lysosomal compartment into the cytosol (Moore, 1976; Baccino, 1978) and such destabilization may also increase lysosomal fusion with other intracellular vacuoles, leading to the formation of pathologically enlarged lysosomes. The consequence of these lysosomal changes would be increased autolytic activity leading to the atrophy of the cells. This type of injury, resulting in destabilization of the lysosomal membrane bears a quantitative relationship to the magnitude of stress response (Bayne et al., 1979, 1982) and this presumably contributes to the intensity of catabolic or degradative effects as well as to the level of pathological change that results. Hence study of acid phosphatase (ACP) activity in the liver and kidney of fish exposed to heavy metals provides a measurement of the hydrolase latency and lysosomal membrane stability and furnish information on mechanisms involving molecular alterations in the lysosomal membranes which undoubtedly contribute to disturbances of the integration of cellular function (Slater, 1978).

In the present study the enzymes, acid phosphatase (EC 3.1.3.2) and alkaline phosphatase (EC 3.1.3.1) are selected to study the sublethal effects of copper and mercury on Oreochromis mossambicus. Acid phosphatase (ACP) is a lysosomal marker enzyme (De Duve and Wattiaux, 1966; Kendall and Hawkins, 1975). Gupta et al. (1975) reported that ACP is a good indicator of stress condition in biological systems. Racicot et al. (1975) established the diagnostic use of the enzyme by inducting carbon tetrachloride (CCl₄) toxicity and aeromonas infection in rainbow trout.

Intracellular alkaline phosphatase is associated with the plasma membrane and ER (Davison and Gregson, 1965; Bogitsh, 1974). It has been suggested that ALP is involved in membrane transport (Posen, 1967; Cornelius, 1971; Neville, 1974), conversion of NADP to NAD (Morton, 1955) and in several processes including food absorption and calcium deposition (McComb et al., 1979).

Hence a study was conducted to find out the effects of two sublethal concentrations of copper and mercury on the acid phosphatase and alkaline phosphatase activity in the liver and kidney of Oreochromis mossambicus.
MATERIAL AND METHODS

Collection of specimens, acclimatization and experimental set up were the same as described in Chapter 2.

Fishes were exposed to two concentrations of copper (100 μg/l and 200 μg/l) and mercury (100 μg/l and 150 μg/l) for 168 h. For the estimation of phosphatase activity, tissue samples from the liver and kidney were dissected out by immobilising the fish with a hard blow on the head at 24, 72, 120 and 168 h of exposure and then were homogenized in ice cold water (1:100 w/v). The homogenate was centrifuged in a refrigerated centrifuge at 12000 rpm for 15 minutes. The supernatant was collected and used for the analysis of phosphatases. Care was taken to maintain the temperature below 4°C during homogenization and collection of the supernatant.

Assay of acid phosphatase activity

Acid phosphatase activity was determined following the method given in the Sigma Technical Bulletin (No. 104) with slight modification. To 1 ml of 0.1 M citrate buffer of pH 4.5 containing 100 mM NaCl, 0.1 ml of the enzyme extract was added. This buffer enzyme mixture was kept in a water bath at 37°C, 0.1 ml of the substrate (2 mg of P-nitrophenyl phosphate (Merck) in 0.1 ml of distilled water) was added to start the reaction. After incubating for one hour at 37°C, the reaction was stopped by adding 2 ml of 0.25 N NaOH. The yellow colour of p-nitrophenol in the alkaline medium was read at 410 nm. The concentration of p-nitrophenol liberated by the action of the enzyme was found out from the calibration curve prepared. Simultaneously the protein content of 0.1 ml of the enzyme extract, was estimated following the method of Lowry et al. (1951). The specific activity of acid phosphatase as uM of p-nitrophenol liberated per mg of protein per hour was calculated. The results were analysed statistically. The significant difference between controls and experimental fishes was determined using student's 't' test (Zar, 1974).

Assay of alkaline phosphatase activity

To 1 ml of frozen 0.05 M glycine-NaOH buffer (pH 8.6), containing 100 mM NaCl and 0.1 mg of MgCl₂, 0.1 ml of the enzyme extract was added. The buffer-enzyme mixture was kept in a waterbath at 37°C and 0.1 ml of substrate
(2 mg of p-nitrophenyl phosphate in 0.1 ml of distilled water) was added to initiate the reaction. After incubating the mixture for one hour, the reaction was stopped by adding 2 ml of 0.25 N NaOH. The yellow colour of p-nitrophenol liberated by the action of alkaline phosphatase was read at 410 nm. After estimating the protein content of 0.1 ml of the enzyme extract following the method of Lowry et al. (1951), the specific activity of the alkaline phosphatase, as μM/mg protein/h was calculated and the results were analysed statistically (Zar, 1974).

RESULTS

Acid phosphatase activity in the liver

Acid phosphatase activity in the liver is represented in Table 5 and Fig. 5. When values of the ACP activity of the controls of different days were compared, no significant difference was observed. Similarly there was no significant difference in ACP activity between controls and 100 μg/l copper-dosed fishes throughout the experimental period. But fishes exposed to 200 μg/l copper showed a significant increase (P < 0.01) at 168 h when compared to controls.

In fishes exposed to 100 μg/l mercury a significant increase (P < 0.05) was observed at 168 h whereas significantly high values (P < 0.01) were observed at 120 and 168 h in fishes exposed to 150 μg/l mercury.

Acid phosphatase activity in the kidney

ACP activity in the kidney of controls did not vary significantly between days. ACP activity in fishes exposed to 100 μg/l copper showed a significant increase (P < 0.05) at 168 h when compared to controls. But fishes exposed to higher concentration of copper (200 μg/l) showed a significant increase at 72 h, 120 h (P < 0.05) and 168 h (P < 0.01). Fishes dosed with 100 μg/l mercury showed a significant (P < 0.05) increase in ACP activity at 168 h, from that of controls. But 150 μg/l mercury-dosed fishes showed an increase in ACP activity at 120 h (P < 0.05) and 168 h (P < 0.01). The values are given in Table 6 and Fig. 6.
Table 5. Acid phosphatase activity in the liver of *O. mossambicus* exposed to copper and mercury

<table>
<thead>
<tr>
<th>Concentration µg/l</th>
<th>24 h</th>
<th>72 h</th>
<th>120 h</th>
<th>168 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu 100</td>
<td>1.25 ± 0.39</td>
<td>1.09 ± 0.31</td>
<td>1.29 ± 0.41</td>
<td>1.30 ± 0.29</td>
</tr>
<tr>
<td>200</td>
<td>1.18 ± 0.34</td>
<td>1.21 ± 0.27</td>
<td>1.49 ± 0.39</td>
<td>1.79** ± 0.37</td>
</tr>
<tr>
<td>Hg 100</td>
<td>1.29 ± 0.42</td>
<td>1.36 ± 0.30</td>
<td>1.38 ± 0.45</td>
<td>1.76* ± 0.35</td>
</tr>
<tr>
<td>150</td>
<td>1.17 ± 0.26</td>
<td>1.09 ± 0.25</td>
<td>2.06** ± 0.45</td>
<td>2.10** ± 0.42</td>
</tr>
<tr>
<td>Control</td>
<td>1.35 ± 0.31</td>
<td>1.27 ± 0.22</td>
<td>1.38 ± 0.36</td>
<td>1.29 ± 0.36</td>
</tr>
</tbody>
</table>

Table 6. Acid phosphatase activity in the kidney of *O. mossambicus* exposed to copper and mercury

<table>
<thead>
<tr>
<th>Concentration µg/l</th>
<th>24 h</th>
<th>72 h</th>
<th>120 h</th>
<th>168 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu 100</td>
<td>1.10 ± 0.34</td>
<td>1.30 ± 0.28</td>
<td>1.42 ± 0.35</td>
<td>1.71* ± 0.37</td>
</tr>
<tr>
<td>200</td>
<td>1.20 ± 0.28</td>
<td>1.52* ± 0.36</td>
<td>1.58* ± 0.27</td>
<td>1.86** ± 0.33</td>
</tr>
<tr>
<td>Hg 100</td>
<td>1.13 ± 0.31</td>
<td>1.36 ± 0.34</td>
<td>1.38 ± 0.30</td>
<td>1.62* ± 0.33</td>
</tr>
<tr>
<td>150</td>
<td>1.35 ± 0.29</td>
<td>1.35 ± 0.31</td>
<td>1.57* ± 0.32</td>
<td>2.15** ± 0.39</td>
</tr>
<tr>
<td>Control</td>
<td>1.24 ± 0.25</td>
<td>1.18 ± 0.31</td>
<td>1.21 ± 0.28</td>
<td>1.28 ± 0.32</td>
</tr>
</tbody>
</table>

* P < 0.05         ** P < 0.01
FIGURE 5. ACID PHOSPHATASE ACTIVITY IN THE LIVER OF O. MOSSAMBICUS EXPOSED TO COPPER AND MERCURY

ACP activity µ M/mg protein/h

hours

Cu 100  Cu 200  Hg 100  Hg 150  Control
FIGURE 6. ACID PHOSPHATASE ACTIVITY IN THE KIDNEY OF O. MOSSAMBICUS EXPOSED TO COPPER AND MERCURY

ACP activity $\mu$M/mg protein/h

hours

Cu 100  Cu 200  Hg 100  Hg 150  Control
Alkaline phosphatase activity in the liver

Alkaline phosphatase (ALP) activity in liver is represented in Table 7 and Fig. 7. No significant difference in ALP activity were observed among control fishes between days. ALP activity in the liver of 100 µg/l copper-dosed fishes showed no significant difference from that of controls. In 200 µg/l copper-dosed fishes ALP activity was significantly lower (P < 0.01) than that of the controls at 72 h and 120 h. ALP activity of 100 µg/l mercury-dosed fishes showed lower values at 72 h (P < 0.01) when compared to the ALP values of the controls. In the 150 µg/l mercury-dosed fishes, a decrease in ALP activity was found at 24 h (P < 0.05), 72 h (P < 0.01) and 120 h (P < 0.01).

Alkaline phosphatase activity in the kidney

There was no significant difference in ALP activity of the controls between days. ALP activity of 100 µg/l copper-dosed fishes showed higher values at 168 h (P < 0.05) when compared to controls, whereas 200 µg/l copper-dosed fishes showed higher values at 72 h, 120 h, (P < 0.05) and 168 h (P < 0.01). Similarly ALP activity of 100 µg/l mercury-dosed fishes showed higher values at 168 h (P < 0.05) when compared to controls. The 150 µg/l mercury-dosed fishes showed significantly higher values at 72 h, 120 h (P < 0.05) and 168 h (P < 0.01) (Table 8, Fig. 8).

DISCUSSION

The result of acid phosphatase (ACP) activity in the liver reveals that it is dose dependent, the higher concentration of heavy metals used eliciting a more significant increase in activity. It also appears that mercury-dosed fishes showed a more significant increase than copper-dosed fishes.

Increase in ACP activity in response to heavy metals and other toxicants have been reported earlier. Elevation of ACP activity in the liver, among other tissues has been reported in Heteropneustes fossilis in response to different organic pesticides (Thomas and Murthy, 1976); in Ophiocephalus punctatus in
Table 7. Alkaline phosphatase activity in the liver of O. mossambicus exposed to copper and mercury

<table>
<thead>
<tr>
<th>Concentration µg/l</th>
<th>24 h</th>
<th>72 h</th>
<th>120 h</th>
<th>168 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu 100</td>
<td>0.46 ± 0.04</td>
<td>0.39 ± 0.09</td>
<td>0.46 ± 0.07</td>
<td>0.44 ± 0.05</td>
</tr>
<tr>
<td>200</td>
<td>0.40 ± 0.04</td>
<td>0.33** ± 0.05</td>
<td>0.39** ± 0.03</td>
<td>0.47 ± 0.05</td>
</tr>
<tr>
<td>Hg 100</td>
<td>0.40 ± 0.07</td>
<td>0.29** ± 0.05</td>
<td>0.47 ± 0.07</td>
<td>0.39 ± 0.08</td>
</tr>
<tr>
<td>150</td>
<td>0.37* ± 0.07</td>
<td>0.27** ± 0.04</td>
<td>0.30** ± 0.06</td>
<td>0.46 ± 0.07</td>
</tr>
<tr>
<td>Control</td>
<td>0.45 ± 0.06</td>
<td>0.47 ± 0.09</td>
<td>0.44 ± 0.04</td>
<td>0.46 ± 0.07</td>
</tr>
</tbody>
</table>

Table 8. Alkaline phosphatase activity in the kidney of O. mossambicus exposed to copper and mercury

<table>
<thead>
<tr>
<th>Concentration µg/l</th>
<th>24 h</th>
<th>72 h</th>
<th>120 h</th>
<th>168 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu 100</td>
<td>1.12 ± 0.32</td>
<td>1.18 ± 0.26</td>
<td>1.22 ± 0.37</td>
<td>1.50* ± 0.26</td>
</tr>
<tr>
<td>200</td>
<td>1.01 ± 0.28</td>
<td>1.47* ± 0.31</td>
<td>1.51* ± 0.29</td>
<td>1.67** ± 0.33</td>
</tr>
<tr>
<td>Hg 100</td>
<td>0.99 ± 0.34</td>
<td>1.06 ± 0.29</td>
<td>1.28 ± 0.32</td>
<td>1.58* ± 0.35</td>
</tr>
<tr>
<td>150</td>
<td>1.25 ± 0.36</td>
<td>1.38* ± 0.29</td>
<td>1.52* ± 0.28</td>
<td>1.71** ± 0.32</td>
</tr>
<tr>
<td>Control</td>
<td>1.08 ± 0.17</td>
<td>1.12 ± 0.21</td>
<td>1.18 ± 0.25</td>
<td>1.24 ± 0.23</td>
</tr>
</tbody>
</table>

* P < 0.05    ** P < 0.01
FIGURE 7. ALKALINE PHOSPHATASE ACTIVITY IN THE LIVER OF O. MOSSAMICUS EXPOSED TO COPPER AND MERCURY

ALP activity µM/mg protein/h

0.50
0.45
0.40
0.35
0.30
0.25

0 24 48 72 96 120 144 168 192

hours

- Cu 100
- Hg 150
- Cu 200
- Hg 100
- Control
FIGURE 8. ALKALINE PHOSPHATASE ACTIVITY IN THE KIDNEY OF O. MOSSAMBICUS EXPOSED TO COPPER AND MERCURY

ALP activity (µM/mg protein/h)

0 24 48 72 96 120 144 168 192

hours

Cu 100  Cu 200  Cu 100  Hg 150  Hg 100  Control

0.9

1.1  1.3  1.5  1.7
response to endrin (Sastry and Sharma, 1979); in carp in response to PCB (Ito et al., 1980); in *Tilapia mossambica* in response to monocrotophos (Joshi and Desai, 1981); in *H. fossilis* in response to mercuric chloride (Gupta and Sastry, 1981); in *Clarias batrachus* in response to lithium nitrate intoxication (Goel et al., 1985) and in *Cyprinus carpio* in response to cadmium chloride (Koyama et al., 1985). There are also many reports that the ACP activity increased in different other tissues on exposure to different metals (Gupta and Sastry, 1981; Hilmy et al., 1985; Sastry and Subhadra, 1985).

However, decreased ACP activity in the liver of fishes exposed to toxicants are also reported. These include a decrease in ACP activity in the liver of *Fundulus heteroclitus* in response to Cd, Cu, and Hg (Jackim et al., 1970); in *C. batrachus* in response to mercuric chloride (Sastry and Gupta, 1978 b) in *Channa punctatus* in response to mercury (Rana and Sharma, 1982); in *Sarotherodon mossambicus* in response to 1 ppm of mercury (Naidu et al., 1984); in different fishes in response to lead (Shaffi and Jeelani, 1985); in blue gill fish in response to cadmium (Versteeg and Giesy, 1985); in *Mugil cephalus* in response to cadmium (Hilmy et al., 1985); and in *H. fossilis* in response to cadmium (Sastry and Subhadra, 1985). It is also interesting to note that Sastry and Malik (1979) have reported no significant differences in ACP activity in the liver of *C. punctatus* in response to dimecron.

From the above, it can be inferred that there is no consistency in the ACP activity in the liver of fishes exposed to toxicants. This is equally true if we take the case of fishes exposed only to heavy metals as well. In the present study the liver of *O. mossambicus* registered an increase in activity in response to copper and mercury. Mercury induced high ACP activity than copper at both the concentrations employed.

As in the liver, the ACP activity in the kidney of fishes exposed to copper and mercury also showed an increase in activity and here also it is dose-dependent i.e., the kidney of fishes exposed to higher concentration of copper and mercury registered higher ACP activity earlier and also for longer duration.
However unlike in liver it is to be noted that significantly higher ACP activity sets in earlier in the kidney and also its duration is longer in fishes exposed to higher concentration of copper than those exposed to higher concentration of mercury.

Increase in ACP activity in the kidney of fishes exposed to toxicants are also reported earlier. This include an increase in activity in the kidney of C. punctatus in response to mercury chloride (Sastry and Agrawal, 1979 a); in O. punctatus in response to endrin (Sastry and Sharma, 1979); in T. mossambica in response to monocrotophos (Joshi and Desai, 1981); in H. fossilis in response to cadmium (Sastry and Subhadra, 1985); in C. batrachus in response to lithium intoxication (Goel et al., 1985) and in the kidney of C. carpio dosed with cadmium chloride (Koyama et al., 1985). However, decrease in ACP activity in the kidney of H. fossilis exposed to lead nitrate (Sastry and Agrawal, 1979 b) and in the kidney of different fishes in response to lead (Shaffi and Jeelani, 1985) have also been reported.

The results of the present study indicate that the specific activity of ACP in both liver and kidney is influenced by copper and mercury. These metals caused an increase in ACP activity, but the time period of increase in activity varied with the concentration and is also dependent on the tissues.

In the present study the lower concentration of copper employed did not show any effect on the ACP activity in liver of O. mossambicus. But the lower concentration of mercury elicited an increase in the ACP activity at 168 h both in liver and kidney. Even though lower concentration of copper did not change the ACP activity significantly in the liver at 24, 72, 120 and 168 h, the ACP activity increased in kidney at 168 h. This shows a difference in behaviour of the two tissues towards the lower concentrations of these two metals. Hilmy et al. (1985) found that different tissues react differently in vivo exposure to cadmium. Such differences in the tissues behaviour towards toxicants were observed by Sastry and Subhadra (1985), Dalela et al. (1982),
Sharma and Sastry (1979), Sastry and Sharma (1979) and Sastry and Agrawal (1979 a).

ACP activity in response to higher concentration (200 µg/l) of copper showed an increase in liver at 168 h. But in kidney, higher concentrations of copper elicited an increase in ACP activity early at 72 h and after. ACP activity in response to higher concentration of mercury (150 µg/l) showed an increase both in liver and kidney at 120 and 168 h. These observations also indicate a difference in behaviour in response to copper and mercury.

Different concentrations of metals behaved differently in changing the ACP activity. Here the higher concentrations of copper evoked an increase in ACP activity, whereas there was no reaction in the liver of fishes exposed to lower concentration of copper. In mercury-dosed fishes the lower concentration showed an increase in ACP activity only at 168 h in the liver, whereas the higher concentration of mercury caused the effect at 120 h onwards. In the kidney, the effects of the higher concentrations of both metals had a greater effect on the ACP activity at 120 and 168 h. Thus the concentrations of toxicants are also important in causing a change in the activity of ACP in fishes. Changes in the ACP activity in fishes due to different concentrations of toxicants were reported by Hinton and Koenig (1975), Shaffi (1980 a), Dalela et al. (1982), Rana and Sharma (1982) and Arora and Kalshrestha (1985).

There are many ways in which structure and/or function of organelles can be disrupted by toxic contaminant. According to Slater (1978) the changes caused by contaminants can be classified into four major categories: (1) depletion or stimulation of metabolites or coenzymes (2) stimulation or inhibition of enzymes and other proteins, (3) activation of xenobiotic to a more toxic molecular species and (4) membrane disturbance. Many toxic substances or their metabolites result in cell injury by reacting primarily with biological membranes. Examples of membrane damage include changes in cellular compartmentalization, injury to lysosomes or mitochondria, changes in the content or activity of enzymes or other membrane components, lipid peroxidation giving rise to aldehydes and
ketones, changes in membrane fluidity leading to altered rates of fusion of cellular vesicles or alterations of enzyme interaction (Moore, 1985).

Lysosomes and the hydrolytic enzyme, acid phosphatase, present in the lysosome, play an important role in the detoxification process by compartmentalization and accumulation of metals that enter the cell (Allison, 1969; Viarengo, et al., 1981, 1984; George, 1983). This process is effective in containing the metals in the lysosome until the storage capacity of the lysosomes is overloaded or the lysosomes are damaged directly by the contaminant which is accumulated (Moore et al., 1985). But when the storage capacity of the lysosomes is exceeded, xenobiotics induce alterations in the bounding membrane which leads to destabilization of lysosome (Moore and Clarke, 1982; Moore and Lowe, 1985 and Moore et al., 1985). Destabilization may involve increased lysosomal fusion with other intracellular vacuoles, leading to the formation of pathologically enlarged lysosomes. Destabilization results in release of degradative hydrolytic enzymes from lysosomal compartment into the cytosol (Moore, 1976; Baccino, 1978). This increases the acid phosphatase activity. Several mechanisms have been suggested for the release of ACP from the lysosomes. These are (1) alteration of osteoblasts resulting in more production and liberation of ACP (Cantarow and Schepartz, 1967), (2) proliferation of smooth ER in the parenchymatous cells that lead to more production and release of microsomal enzymes resulting in increased enzyme activity (Hart and Fouts, 1965), (3) peroxidation of lysosomal membrane leading to membrane breakdown and increasing permeability resulting in the release of ACP (Arstilla and Trump, 1968) and (4) degeneration and necrosis induced in tissues and the resultant release of ACP (Trump and Arstilla, 1971). Any change in the activity, number, function of lysosomes or its damage could alter the activity of ACP. Verity and Reith (1967) observed that the lysosomes are structurally altered in response to toxic dosage of methyl mercury. Deung et al. (1978) reported an increase in the number of lysosomes in the liver cells of Carassius carassius exposed to mercuric chloride while Ferri and Macha (1980) observed a change in shape distribution and functional degree of lysosomes in the hepatic cells of Pimelodus maculatus exposed to cadmium. Assessment of this type of injury has been confirmed as an extremely sensitive general index of cellular conditions (Moore, 1980, 1982; Moore et al., 1982, 1985). Injury resulting in destabilization of the lysosomal membrane bears a quantitative
relationship to the magnitude of stress response (Bayne et al., 1979, 1982). Since release of ACP from the lysosome is associated to membrane injury due to xenobiotics, increased activity of ACP in the present study can be related to the lysosomal destabilization.

Failure of the lower concentration of copper to evoke an increase in the ACP activity in liver and also the absence of increase in ACP activity during the initial period of the present experiment may be due to the detoxification and consequent removal of copper and mercury before they could damage the lysosomes. But as the exposure time or the concentration of the metal is increased, the storage capacity of the lysosomes gets overloaded, altering the permeability and damaging the lysosomal membrane resulting in the increased leakage and activity of ACP. Such biphasic responses of ACP activity on exposure to different toxicants were also reported (Sastry and Gupta, 1978 b; Hinton and Koenig, 1973; Joshi and Desai, 1983; Arora and Kalshrestha, 1985). So depending on the concentration and period of exposure and type of tissue, ACP activity can vary from time to time and tissue to tissue and many of the reported stimulation of ACP activity by metals may be due to this phenomena described above.

The results of the present study indicated that the ALP activity of the liver of fishes exposed to lower concentration of copper did not show any significant difference from that of the control fishes, while those exposed to the lower concentration of mercury showed a significant decrease at 72 h. The liver of fishes exposed to higher concentration of copper showed a significant decrease at 72 and 120 h, whereas those exposed to higher concentration of mercury showed significant decrease at 24, 72 and 120 h. It is to be noted that the ALP activity returned to the control values at the later part of the experimental period. Decrease in ALP activity in the liver of _F. heteroclitus_ exposed to beryllium and silver (Jackim, et al., 1970); in _H. fossilis_ exposed to mercuric chloride (Gupta and Sastry, 1981); in the liver of _C. punctatus_ dosed with mercury (Rana and Sharma, 1982); in _S. mossambicus_ exposed to mercuric chloride (Naidu et al., 1984); in _C. punctatus_ exposed to cythion (Narayan Ram and Sathyanesan, 1985) and in _H. fossilis_ exposed to cadmium (Sastry and Subhadra, 1985) have been reported. However increase in ALP activity in the liver of _F. heteroclitus_ exposed to mercury and lead
(Jackim et al., 1970) and no significant change in the ALP activity in F. heteroclitus exposed to copper and cadmium (Jackim et al., 1970) and in M. Cephalus exposed to cadmium (Hilmy et al., 1985) have also been reported.

Decrease in ALP activity in some other tissues of fishes dosed with different heavy metals have also been reported (Saleem and Alikhan, 1973; Koyama and Itazawa, 1977; Sastry and Gupta, 1978 a, b; Sastry and Agrawal, 1979 a, b; Koyama et al., 1985; Shaffi and Jeelani, 1985).

Intracellular ALP is associated with plasma membrane and endoplasmic reticulum (Davison and Gregson, 1965). Hence destabilization or damage to these membranes may affect the activity of ALP. It is probable that the membrane systems of the cell encounter the influx of heavy metal ions first and the latency of ALP associated with plasma membrane is lowered, thereby permitting leakage, away from the liver tissues. Moreover the heavy metal ions may also be inhibiting the enzymes directly. Inhibition of ALP activity in different tissues after exposure to metals have been reported (Hiwada and Wachsmuth, 1974; Cathala et al., 1975; Yokota, 1978). Sometimes heavy metals displace or replace metals of metalloenzyme. These changes alter the three dimensional configuration of the enzymes, so that substrate molecules no longer fit binding site (Friedberg, 1974) or splits enzymes into subunits (Gerhart and Schachman, 1965; Jovin et al., 1969) so that regulation of enzyme activity may be lost (White et al., 1968; Brown, 1977; Brown et al., 1977). These processes can give a reduction in the ALP activity in the liver tissue. The absence of any significant difference in the ALP activity of fishes exposed to lower concentration of copper also supports this view. Later, when the detoxifying process gain an upper hand, the ALP activity, returns to comparable values of ALP activity of the controls which may be through the hypersynthesis of this enzyme.

In the kidney of O. mossambicus exposed to copper and mercury, the ALP activity, showed a different trend. A significant increase in the ALP activity in the kidney of experimentals over the control values was obtained. The pattern of increased activity is similar between the concentration of copper and mercury but the duration of increase is greater in the kidney of fishes exposed to higher concentration of copper and mercury. Goel et al. (1985)
have reported an increase in the ALP activity in the kidney of *C. batrachus* in response to lithium. However a decrease in ALP activity in kidney was reported in *C. punctatus* exposed to mercuric chloride (Sastry and Agrawal, 1979 a); in *H. fossilis* exposed to lead nitrate (Sastry and Agrawal, 1979 b), and in *H. fossilis* exposed to cadmium (Sastry and Subhadra, 1985). Many workers have also reported increased ALP activity in different other tissues of fishes treated with metals (Cardeilhac and Hall, 1977; Sastry and Gupta, 1978 b, 1979; Banerjee et al., 1979; Saxena and Tyagi, 1979; Sastry and Sharma, 1980; Hilmy et al., 1985; Sastry and Subhadra, 1985).

In the present study the ALP activity in the liver of experimental fish showed a significant decrease over the controls, whereas in the kidney of experimental fish the ALP activity registered an increase over the controls indicating that the two tissues vary differently with respect to ALP activity in response to copper and mercury. Such differences in the ALP activity in different tissues exposed to same toxicants were reported by Sastry and Sharma (1979), Shaffi (1980 b), Hilmy et al. (1985), Narayan Ram and Sathyanesan (1985) and Sastry and Subhadra (1985). The observed increase in the ALP activity in the kidney could be associated with the functions of kidney.

ALP is reported to play an important role in the active transport of materials through phosphorylated intermediate (Goodman and Rothstein, 1957; Posen, 1967; Neville, 1974). The reports of George and Viarengo (1984) stating that some metals react with phosphate groups of lipid bilayer before being complexed by intracellular ligands supports the need of this enzyme in the transportation of heavy metal ions, by providing the phosphate group. In the kidney, ALP activity is thus important as it is reported that copper can be excreted in the urine, faeces and bile by complexing it with alpha globulin (Peisach et al., 1967). Enhanced urine flow in fishes exposed to heavy metal is also reported (Lock et al., 1981). Thus in the kidney it may be possible that there is hypersynthesis of ALP to facilitate transport and excretion of heavy metal ions resulting in the noted increase in the ALP activity.