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# *In Vivo* Tissue Enzyme Activities in the Rosy Barb (*Barbus conchonius* Hamilton) Experimentally Exposed to Lead

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Lead (Pb) is biologically nonessential and if present in excessive levels in the body, it can cause clinical disorders both in humans and animals (Goyer 1971). Pathologies associated with experimental Pb poisoning have been described in fishes, and several markers have been utilized to monitor effects of short- and long-term exposures. Blood seems to be an important target of the deleterious effects of Pb. Dawson (1935) noted an anemic response in the Pb-exposed catfish, *Ameiurus nebulosus*. As in the higher vertebrates, basophilic stippling in the erythrocytes was observed following Pb poisoning in the rainbow trout, *Salmo gairdneri* (Haider 1964). Inhibition of δ-aminolevulinic acid dehydratase (δ-ALAD), which catalyzes the formation of porphobilinogen from δ-aminolevulinic acid in the hemoglobin biosynthesis, is perhaps the most conspicuous and specific effect of Pb observed both in fish as well as in mammals. Partial inhibition of δ-ALAD, however, may not result in serious health effects *per se* as the enzyme has substantial reverse capacity. Jackim (1973) found depressed δ-ALAD activity in the liver and kidney of the mummichog, *Fundulus heteroclitus*, and the winter flounder, *Pleuronectes americanus*. In the rainbow trout, brook trout, *Salvelinus fontinalis*, goldfish, *Carassius auratus*, and pumpkinseed, *Lepomis gibbosus*, erythrocyte δ-ALAD was inhibited by Pb but not by Cd, Cu, Zn, and Hg (Hodson et al. 1977). Besides causing a strong inhibition of δ-ALAD in the erythrocytes, spleen, and kidney of the rainbow trout, chronic Pb poisoning induced anemia and basophilic stippling of erythrocytes but the leucocytes remained unaffected (Johansson-Sjobeck and Larsson 1979).

Information on Pb effects on variables other than the δ-ALAD is rather limited (Helmy et al. 1978, Haux and Larsson 1982, Gill et al. 1991). Davies and Everhart (1973) showed that low concentrations of Pb in water caused black tails and spinal curvature in the rainbow trout. Degeneration of the caudal fin, muscular atrophy, paralysis, and lordoscoliosis are also stated to be related to Pb poisoning (Haider 1964, Davies et al. 1976, Holcombe et al. 1976). In the rainbow trout, Haux and Larsson (1982) found a significant reduction in blood glucose, a dose-dependent increase in plasma potassium both after exposure (30 days at 10, 75, and 300 μg Pb/L) and recovery (49 days), and a dose-depenedent decrease in plasma chloride after recovery.

The specific aim of this study was to examine the effects of acute Pb poisoning on the enzymes concerned with membrane transport, neurotransmission, and energy metabolism in selected tissues of the rosy barb, *Barbus conchonius*, a freshwater fish.

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## MATERIALS AND METHODS

Live rosy barbs (*Barbus conchonius* Hamilton; Cypriniformes), mean total body length 50 mm, hand netted from the Nainital Lake, India, were acclimatized to laboratory conditions for 2 weeks at 18±1°C (room temperature), natural light/dark cycle (13L/11D), constant aeration, and fed commercial fish food (flake ingredients: dried shrimp, rice bran, fish meal, potato protein, gelatin, and animal fat) *ad libitum* once a day. *In vivo* tests were carried out in 50-L glass aquaria filled with reconstituted soft water; EDTA hardness $\leq$6 mg/L, pH 7.1, dissolved oxygen content 8.2 mg/L, and temperature 17±1°C. Fish were divided into two groups (n=25 individuals each); group I was exposed to 378 µg/L Pb (96-h median tolerance limit, TLm) as nitrate salt, while group II served as a control.

After 48 h, the control and Pb-treated fish were sacrificed by decapitation and tissue samples collected and processed immediately for enzyme assays. Preweighed tissues were homogenized in a Potter-Elvehjem Teflon glass homogenizer in cold (4°C) 0.85% saline at the following dilutions (w/v): liver, gill lamellae, kidneys, ovary, testis, gut including intestinal bulb, and skeletal muscles from the epiaxial musculature, 6%, ventricular portion of heart, 10%, and brain excluding the olfactory bulbs, 1%. Homogenates were centrifuged (1200 rev/min) at room temperature for 10 min and the supernatant kept at 4°C until used for enzyme assays (usually within 1-3 h) and determination of the protein content (Gornall et al. 1949). The tissue enzymes examined were; alkaline and acid phosphatases (AlP, EC 3.1.3.1; AcP, EC 3.1.3.2), glutamate-oxaloacetate and -pyruvate transaminases (GOT, EC 2.6.1.1; GPT, EC 2.6.1.2), lactic dehydrogenase (LDH, EC 1.1.1.27), and acetylcholinesterase (AchE, EC 3.1.1.7). The diagnostic kits used for enzyme assays were based on the following procedures; AlP (Bessey et al. 1946), AcP (Fishman and Lerner1953), GOT (Bergmeyer et al. 1976, 1978), GPT (Bergmeyer et al. 1978, Bergmeyer and Horder 1980), AchE (Ellman et al. 1961), and LDH (Amador et al. 1963). Samples were read on MODULAB SYSTEM 4010 (Boehringer-Knoll, Mannheim), and the catalytic activity calculated and expressed in terms of units/mg protein.

Each enzyme was asayed in 6-8 samples, each consisting of pooled tissues of two (four in case of the heart) individual animals and averaged values for the Pb-treated group were compared with their respective controls by Dunnett's t-test. Differences were considered significant at $p<0.05$ or less.

## RESULTS AND DISCUSSION

Acute exposure to a sublethal concentration of dissolved Pb resulted in noticeable impact on the enzyme activities in certain tissues of the rosy barb. There was an increase in the AcP activity in the liver (39%), kidney (54%), gut (30%), ovary (94%), and testis (53%); the difference from control being statistically significant ($p<0.05$) only in the kidney and ovary (Fig. 1). The AcP activity was not raised in the gills. The AlP activity conspicuously increased in the kidney (149%, $p<0.001$) and ovary (141%, $p<0.01$); the rise in the testis (52%) was, however, not significant (NS) (Fig. 2). On the other hand, Pb exposure inhibited the AlP in the gut (-63%, $p<0.05$) and the gills (-19%, NS).

Both AcP and AlP are stimulated in the kidney, ovary, and testis but not in the gills, and whereas AcP activity is moderately increased in the liver and gut, it is unchanged in the liver and significantly depressed in the gut in the case of AlP. Elevated AcP activity is usually indicative of structural damage to tissues due to increased lysosomal activity resulting in cellular degeneration and necrosis. Sastry and Gupta (1978) also found increased AcP activity in the tissues of the snakehead, *Channa punctatus* exposed to Pb. Necrotic changes in the internal organs of the rosy barb have been previously described following Pb

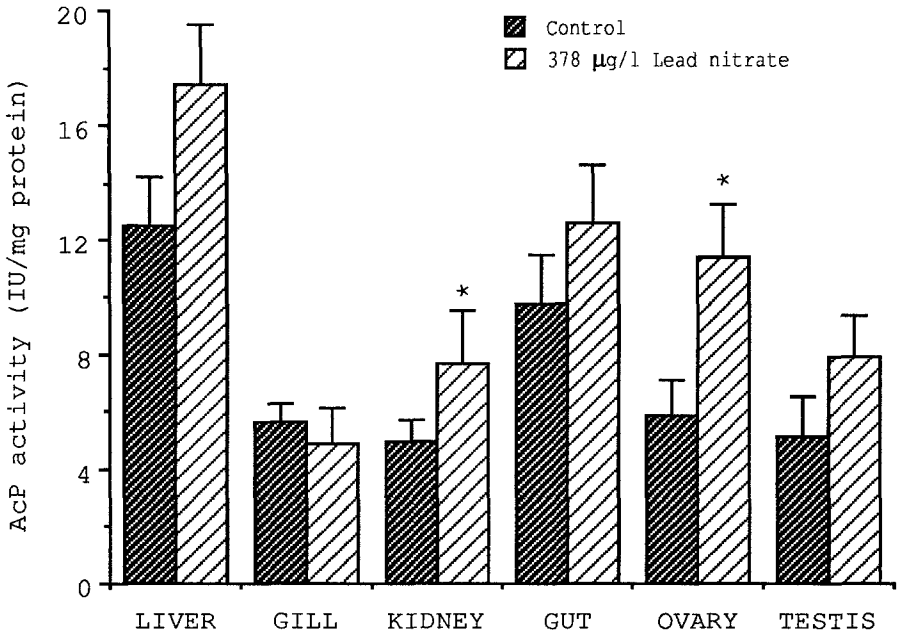


Figure 1. Effect of acute Pb exposure on the AcP activity in different tissues of the rosy barb. Mean±SE (n=6-8). *p<0.05, **p<0.01, ***p<0.001.

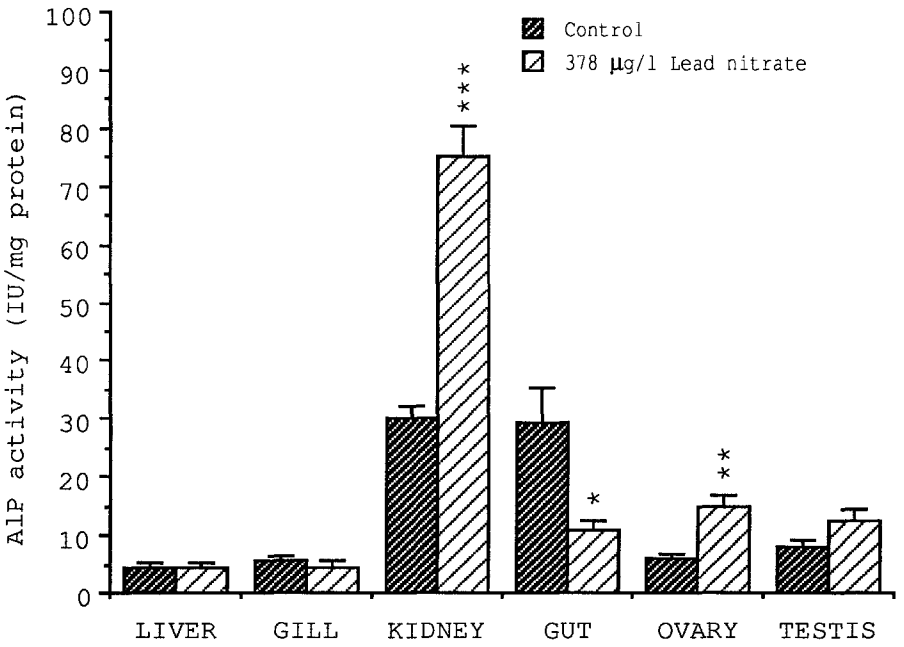


Figure 2. Effect of acute Pb exposure on the AlP activity in different tissues of the rosy barb. Mean±SE (n=6-8). Symbols as in fig. 1.

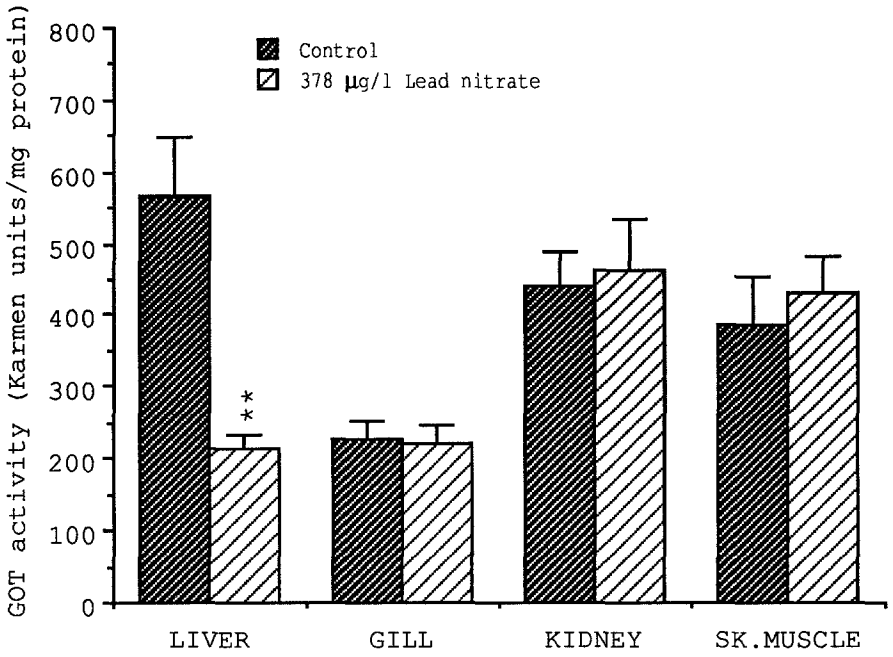


Figure 3. Effect of acute Pb exposure on the GOT activity in different tissues of the rosy barb. Mean±SE (n=6-8). Symbols as in fig. 1.

intoxication (Pant 1982), and, therefore, the observed effects in the AcP activity are more likely to occur as a consequence of tissue damage rather than enzyme induction.

Effects of Pb exposure on the AlP activity were most pronounced in the kidney, gut, and ovary. Vallee and Ulmer (1972) proposed that whereas most enzymes bearing a single functional -SH group are inhibited by Pb, others are stimulated, among them the transaminases (GOT and GPT), LDH, and AlP. In the rosy barb, enzyme induction in the intact cells which escape structural damage by toxic cations may explain the enhanced AlP activity in the kidney and ovary. On the other hand, disruption of intestinal mucosa could be the cause for reduced AlP activity in the gut.

The GOT and GPT activities in the blood and tissues are altered under certain clinical conditions and, therefore, these enzymes have considerable diagnostic value in humans. However, their diagnostic significance in fish is not clearly understood. Nevertheless, changes in their activites in the specific organ may reflect Pb toxicity at the cellular level. Impact of Pb on the tissue transaminases was most obvious in the liver and kidneys. The GOT activity fell significantly in the liver (-62%, $p<0.01$) but remained close to control values in gill, kidney, and skeletal muscle (Fig. 3). The GPT activity was, however, seriously impaired in the liver (-52%, $p<0.05$), kidneys (-39%, $p<0.05$), and the skeletal muscle (-29%, NS). In gills, enzyme activity was increased (27%) but not significantly (Fig. 4).

Hepatocellular disorders cause plasma GPT levels to go up sooner, faster, and higher than GOT. In the present study, hepatic GOT and GPT fell significantly below the control levels, probably related to cytolysis and enzyme leakage into

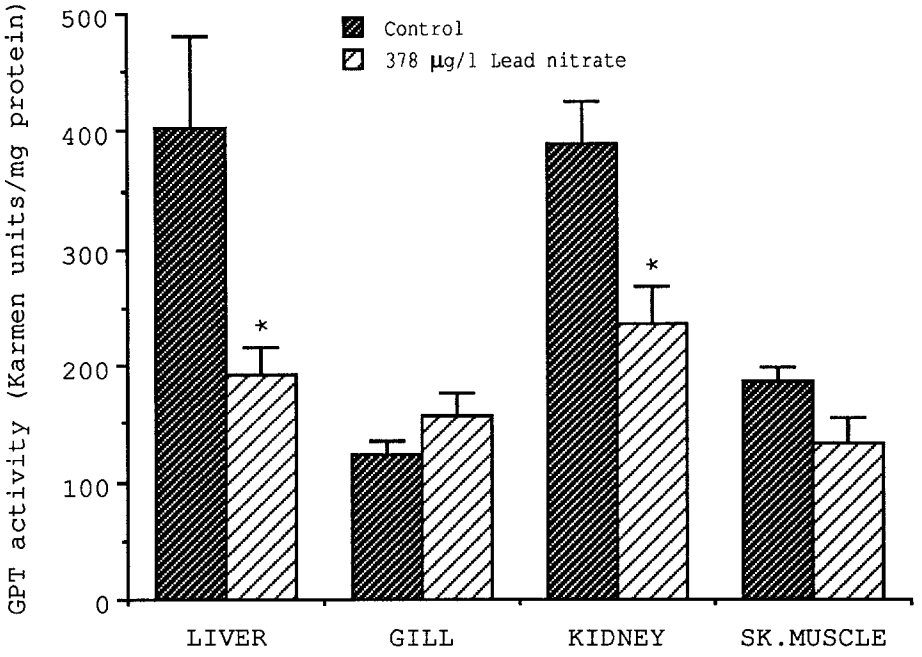


Figure 4. Effect of acute Pb exposure on the GPT activity in different tissues of the rosy barb. Mean±SE (n=6-8). Symbols as in fig. 1.

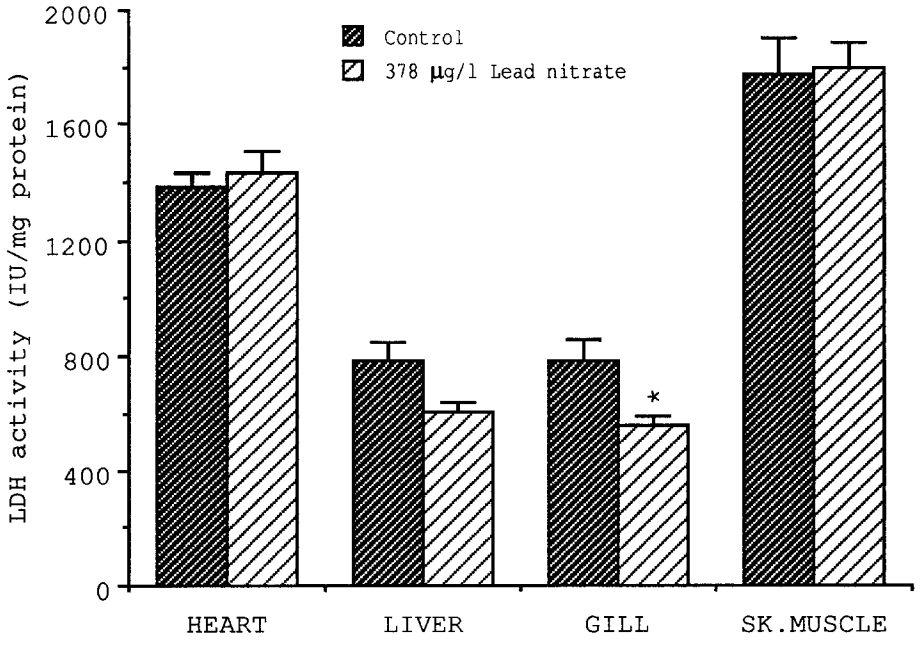


Figure 5. Effect of acute Pb exposure on the LDH activity in different tissues of the rosy barb. Mean±SE (n=6-8). Symbols as in fig. 1.

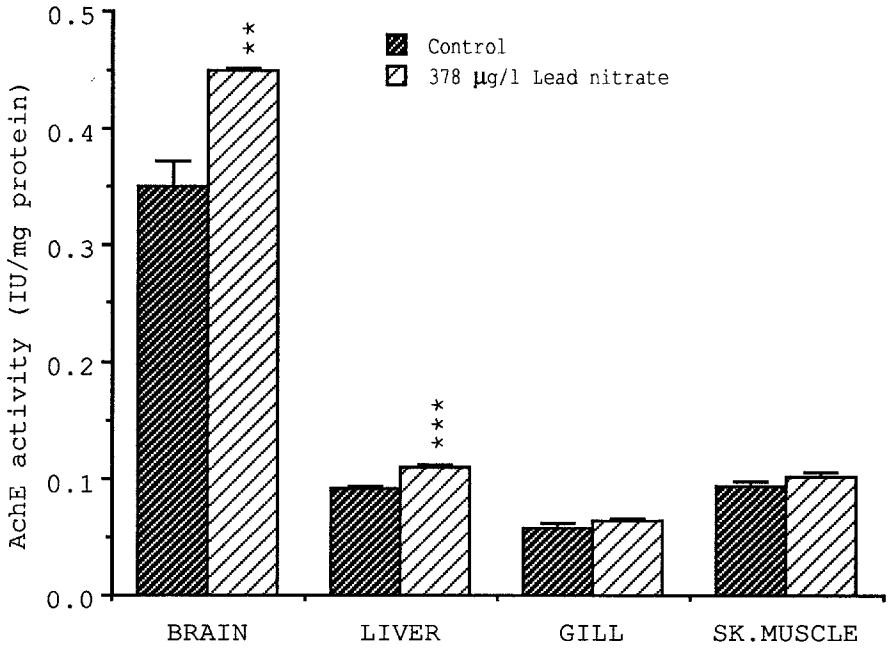


Figure 6. Effect of acute Pb exposure on the AchE activity in different tissues of the rosy barb. Mean±SE (n=6-8). Symbols as in fig. 1.

the blood. Because blood volume was small, we could not get enough plasma for enzyme assays, and, thus, have no data to show that this really occurred. A significant loss of renal GPT activity noted in the present study can be correlated to nephropathological changes in the mesonephros. It is well known that GOT and GPT are located in proximal and distal nephric tubules, and in heavy metal poisoning (e.g., with Cd) there is urinary excretion of these enzymes which are mainly of renal origin rather than of plasma origin (Nomiyama et al. 1975).

A reduction in the LDH activity was noted in the gills (-27%, $p<0.05$) and liver (-22%, NS), whereas cardiac and skeletal muscle enzymes remained more or less unaffected during Pb exposure (Fig. 5). Studies dealing with Pb effects on LDH activity in fishes are few; lead nitrate caused an increase in the LDH activity in the blood of brooktrout, *Salvelinus fontinalis*, during 2- and 8-wk exposure to 474 µg $Pb^{+2}$/L (Christensen et al. 1977). In the rosy barb, changes in tissue LDH activities were not appreciable during Pb exposure although the metal is stated to stimulate this enzyme (Vallee and Ulmer 1972). It is also possible that the toxic impact of Pb under the present set of conditions was not strong enough to force the fish to utilize non-oxidative metabolic pathways.

The AchE activity was not affected in the gills and skeletal muscles, but the brain and liver showed significant increases (29%, $p<0.01$ and 25%, $p<0.001$, respectively) (Fig. 6). The neurotoxicity of Pb is well established in higher vertebrates (Goldstein et al. 1974), and an inhibition of AchE by this metal has been reported (Vallee and Ulmer 1972). Why the AchE activity in the brain and liver of the Pb-exposed rosy barb increased is not quite clear. Perhaps, a large reserve of pseudocholinesterase, which is synthesized in the liver (Bergmeyer 1963), can explain the observed changes in the test fish.

Overall, experimental Pb poisoning seriously impairs the enzyme systems in the rosy barb. Alterations in the activities of enzymes regulating membrane transport seem to be the result of tissue damage caused by toxic cations. On the other hand, changes in the AchE may be related to a direct neurotoxic effect. An appreciable impact of the Pb is, however, not indicated in the case of LDH.

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