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Effects of Lead Nitrate on the Activities of a Few Enzymes in the Kidney and Ovary of *Heteropneustes fossilis*

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Heavy metals produce toxic effects on the tissues of various terrestrial and aquatic animals. Among the aquatic fauna, fishes are the most sensitive group (MATHIS & KEVERN 1975). The heavy metals of pricipal toxicological concern are lead, mercury and cadmium (HAMMOND 1973) which produce cumulative toxic effects if taken in small doses and acute toxicity in higher doses (HARRISSON et al. 1971). Lead is more toxic due to its long lasting effects on the tissues of animals. The chronic effects of lead on reproduction of sexually mature male and female rats have been reported by HILDERBRAND et al. (1973) and in the ovary of rhesus monkey by VERMANDE-VANECK (1960). Lead treated female rats showed a significant loss of ovary weight (DER et al. 1974). Some histochemical studies have also been performed on the liver and kidney of mammals (ZEGARSKA & ZEGARSKI 1968 and WHITE 1977). But in fishes very little information is available on the physiological changes produced by chronic and acute lead intoxication. Kidney being one of the most sensitive organs easily affected by heavy metals, it is important to study the physiological alterations produced by lead poisoning. The present communication deals with the alterations in enzyme activities that accompany lead intoxication in the kidneys and ovaries of a teleost fish, Channa punctatus after treatment with LC(50) for 96 hr and a sublethal concentration for 30 days.

MATERIALS AND METHODS

Living fishes were collected from local fresh water sources in the month of February 1978 and maintained in laboratory aquaria in dechlorinated tap water under constant conditions of temperature and nutrition. The fishes were allowed to acclimatize for four days prior to experimentation. Preliminary bioassays conducted in the laboratory under static conditions have shown that LC(50) for 96 hr and the sublethal concentration for 30 days are 13.2 mg/L and 3.8 mg/L of lead nitrate respectively. 90 fishes weighing 70 $\pm$ 6 g each were selected and divided in to three equal groups. The first two groups of fishes were treated with LC(50) and sublethal concentration respectively while the third group served as controls.

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10% (W/V) homogenates of kidneys and ovaries were prepared in 0.25 M sucrose solution in cold. The homogenates were centrifuged for 20 min at 1500 G and the clear supernatant fluids were used as the source of enzymes. 0.016 M sodium-B-glycerophosphate was used as the substrate at pH 5.0 and 9.3 for acid and alkaline phosphatases respectively. The enzyme activity was estimated according to the method of MORTON (1955). For the estimation of glucose-6-phosphatase activity, 0.01 M glucose-6-phosphate solution was incubated for 15 min at pH 6.5. The method of SWANSON (1955) was followed. The inorganic phosphate liberated was quantitatively estimated by the method of FISKE & SUBBAROW (1925). The activity of urease was determined by the method of HOFMANN & SCHMIDT (1953) and lipase activity was estimated following the method of BIER (1955) with Tween 20 as substrate. The protein content in the homogenates was determined by the method of LOWRY et al. (1951) with bovine serum albumin as standard. The test described by FISHER (1950) was employed to calculate the statistical significance between control and experimental values.

RESULTS AND DISCUSSION

Previous reports from this laboratory have shown that lead in the surrounding water is highly toxic to Channa punctatus and inhibits the activities of various enzymes in the digestive system (SASTRY & GUPTA 1978a & b). The present work has been undertaken to examine whether this heavy metal is equally toxic to the kidney and ovary. The results of the experiments are presented in Table 1. It is evident from the results that the activities of all the five enzymes are significantly inhibited in kidney and ovary at both the experimental stages. However, the inhibition is more marked in fishes treated for 30 days with a sublethal concentration than those treated for 96 hr with LC(50). This indicates that chronic exposure is more toxic and lead produces cumulative effects. According to PICKERING & HENDERSON (1964) lead toxicity is different at higher concentration and short term treatment than at low concentration and long term treatment. The inhibition in enzyme activities by heavy metals may be due to the direct binding of the metal with enzyme protein (PASSOW et al. 1961) or the toxic effects produced by them on the tissues (BLACKWOOD et al. 1961) leading to decreased synthesis of enzymes. HIRTH (1964) in his in vitro studies has shown that the mechanism of enzyme inhibition by heavy metals revolves mainly around the affinity of mercury and lead to the sulphydryl groups.

Alkaline phosphatase is a brush border enzyme, localized in the intestinal mucosa and kidney tubules. According to HICKMAN & TRUMP (1969) it plays an active role in the reabsorption of glucose from the renal

TABLE 1

Enzyme activities in experimental and control fishes[a]

Enzyme	Tissue	Control	Experimental	
			96 hr	30 days
Acid phosphatase[c]	Kidney	0.189 ± 0.0020	0.120 ± 0.0022(+)b	0.071 ± 0.0035(+)
	Ovary	0.160 ± 0.0030	0.126 ± 0.0017(+)	0.085 ± 0.0018(+)
Alkaline phosphatase[c]	Kidney	0.226 ± 0.0017	0.187 ± 0.0038(+)	0.091 ± 0.0011(+)
	Ovary	0.225 ± 0.0119	0.194 ± 0.0033(+)	0.107 ± 0.0024(+)
Glucose-6-phosphatase[c]	Kidney	0.217 ± 0.0014	0.139 ± 0.0044(+)	0.056 ± 0.0040(+)
	Ovary	0.223 ± 0.0071	0.168 ± 0.0027(+)	0.147 ± 0.0040(+)
Lipase[d]	Kidney	51 ± 0.574	38 ± 0.818(+)	31 ± 1.08(+)
	Ovary	87 ± 1.224	78 ± 1.153(+)	49 ± 0.707(+)
Urease[e]	Kidney	0.0071 ± 0.00077	0.0043 ± 0.000039(+)	0.0026 ± 0.000048(+)

a. Values are Mean ± S.E.
b. (+) indicates statistically significant difference from control values at 95 percent confidence interval.
c. Activity is expressed in mg of inorganic phosphate liberated per mg of enzyme protein per hour at 37°C.
d. Activity is expressed in lipase units.
e. Activity is expressed in mg of nitrogen per mg of enzyme protein per hour at 37°C.

tubules. Inhibition of this enzyme suggests that glucose reabsorption and transphosphorylation reactions catalyzed by this enzyme are adversely affected by treatment with lead. Further, the inhibition in the activity of glucose-6-phosphatase indicates disturbances in the metabolism of the kidney. The present experiment also points out that lead can affect the growth and maturation of oocytes as all the enzymes examined here in the ovary have shown inhibition. A clear picture can emerge only when other key enzymes in the metabolism of oocytes are studied. This aspect will be taken up at a later occassion as investigations are still under progress.

SUMMARY

The effect of LC(50) and a sublethal concentration of lead nitrate on the activities of alkaline phosphatase, acid phosphatase, glucose-6-phosphatase, lipase and urease in the kidneys and ovaries of a teleost fish, Channa punctatus has been examined after 96 hr and 30 days respectively. The results show that all the five enzymes in the two tissues are inhibited significantly at both the experimental stages. However, the inhibition produced after 30 days by the sublethal concentration is higher indicating the cumulative action of lead. Further, the inhibition of enzymes is more marked in kidney than in the ovary.

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