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Alteration in the Activities of Three Peptidases and Lipase in the Digestive System of the Fish *Channa punctatus* Exposed to Lead Nitrate

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Heavy metals produce toxic effects on the tissues and alter the physiological functioning of various systems of animals. Toxic effects of metals and chemicals may result from their binding with biologically active enzymes and other proteins (PASSOW et al. 1961). Lead compounds are known to produce toxic effects in the kidney and brain (GOYER and RHYNE 1973). Though several workers have reported that lead interferes with the physiological functions of the liver, brain and kidney (BLACKWOOD et al. 1965) and digestive tract (SASTRY and GUPTA 1978b) the exact mode of action is not known. Alteration in enzymic and metabolic processes has been observed in animals treated with heavy metals (WEBB 1966, SASTRY and GUPTA 1978a,b). Histochemical studies on liver and kidney have been made by WHITE (1977) and ZEGARSKA and ZEGARSKI (1968). ULMER and VALLEE (1969) observed some effects of lead on enzyme activities. Lead produces cumulative toxicity in low doses (HARRISON et al. 1971). As liver is the main detoxificating organ, it is important to study the physiological alteration in the enzyme activities due to exposure to this metal. Some lead enters the body of fish through food chain, and most likely produces toxic effects in the digestive system. Much attention is paid to the toxic effects of lead on the liver, kidney and brain and very little information is available on the pathological and physiological changes produced in the digestive system. The present investigation, therefore, deals with the alteration in the activities of some digestive enzymes that accompany acute and chronic lead intoxication in the liver and different parts of the digestive tract of a teleost fish, Channa punctatus.

MATERIALS AND METHODS

Living fishes collected from local fresh water sources were maintained in the laboratory aquaria. Prior to experimentation, fishes were allowed to acclimatize to the laboratory conditions for 4-5 days. Specimens weighing 60-70 g each were selected and divi-

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ded into 4 groups of 30 fishes each. The first group of fishes were treated with 6.8 mg/L of $PbNO_3$ (LC_{50}) for 96 hr. while the second group was maintained in a sublethal medium containing 2.8 mg/L of $PbNO_3$ for 30 days. The third and fourth groups served as controls respectively. The surviving fishes from first group were sacrificed after 96 hr. From the second group 15 fishes each were dissected after 15 and 30 days of treatment. 10% (W/V) homogenates of liver, stomach, intestine and pyloric caeca were prepared in 0.25 M sucrose solution using a chilled Potter-Elvehjen homogenizer. The homogenates were centrifuged for 20 min at 1000 G and the clear supernatant fluids were used as the source of enzymes. The activities of the peptidases were determined by the method of SMITH (1955). Lipase activity was estimated following the method of BIER (1955) with Tween 20 as substrate. For each enzyme, triplicate samples were analysed and the incubations were repeated three times. Enzyme protein in the homogenate was estimated by the method of LOWRY et al. (1951) using bovine serum albumin as standard. The incubation period was 1 hr at 37°C. The test described by FISHER (1950) was employed to calculate the statistical significance between the control and experimental values.

RESULTS AND DISCUSSION

The results of the experiments conducted are presented in Tables 1 and 2.

The present short and long term experiments have been undertaken to observe the effect of lead on the activities of some peptidases and lipase in the digestive system of Channa punctatus. The results reveal that lead inhibits the activity of all the enzymes examined here. However, the inhibition in lipase activity is statistically insignificant. Lead compounds are known to produce severe damage in liver, kidney and brain (ZEGARSKA and ZEGARSKI 1968, GOYER and RHYNE 1973). In our earlier studies (SASTRY and GUPTA 1978b) also an inhibitory effect was observed by lead on the activities of some phosphatases and carbohydrases. The inhibitory effect of heavy metals on enzymes may be due to the direct binding of lead with the enzyme protein (PASSOW et al. 1961). This aspect will be dealt with in a separate communication, as investigations are under progress. From our earlier studies (SASTRY and GUPTA 1978a) and the present one, it may be pointed out that the inhibition in enzyme activities produced by lead is more than that of mercury. However, mercury produces more tissue damage than lead.

TABLE 1

Enzyme activities in acutely treated experimental and control fishes[a]

Enzyme	Tissue	Control	Experimental	Level of Significance
Aminotripeptidase[c]	Liver	0.182±0.0033	0.146±0.0093	2.92 (+)[b]
	Intestine	0.205±0.0063	0.149±0.0033	4.02 (+)
	Pyloric caeca	0.200±0.0107	0.151±0.0026	5.50 (+)
Glycylglycine dipeptidase[c]	Intestine	0.152±0.0080	0.120±0.0063	3.90 (+)
	Pyloric caeca	0.140±0.0054	0.112±0.0030	5.60 (+)
Leucyl-l-glycine dipeptidase[c]	Intestine	0.156±0.0033	0.120±0.0030	10.00 (+)
Lipase[d]	Liver	62±4.12	55±7.07	1.04 (-)
	Stomach	32±4.12	30±7.07	0.23 (-)
	Intestine	42±4.12	33±4.12	1.89 (-)
	Pyloric caeca	43±4.12	37±4.12	1.26 (-)

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 glycine liberated per mg of tissue protein per hour at 37°C.
d. Activity is expressed in lipase units per hour at 37°C.

TABLE 2

Enzyme activities in chronically treated experimental and control fishes[a]

Enzyme	Tissue	15 days			30 days		
		Control	Experimental	Sig. Diff.	Control	Experimental	Sig. Diff.
Amino-tripeptidase[c]	Liver	.172±0.0043	.168±0.0064	0.64(-)	.141±0.0052	.087±0.0066	7.94 (+)[b]
	Intestine	.187±0.0120	.145±0.0046	4.20(+)	.129±0.0090	.071±0.0093	5.80 (+)
	P. caeca	.203±0.0030	.170±0.0047	7.11(+)	.129±0.0026	.090±0.0066	6.84 (+)
Glycyl-glycine dipeptidase[c]	Intestine	.121±0.0038	.092±0.0013	9.06(+)	.116±0.0013	.085±0.0066	5.74 (+)
	P. caeca	.131±0.0033	.116±0.0026	4.41(+)	.111±0.0070	.084±0.0050	4.40 (+)
Leucyl-L-glycine dipeptidase[c]	Intestine	.129±0.0023	.097±0.0041	8.42(+)	.122±0.0096	.084±0.0053	4.32 (+)
	P. caeca	.125±0.0023	.099±0.0030	8.66(+)	.118±0.0073	.084±0.0063	5.00 (+)
Lipase[d]	Liver	53±4.12	48±4.12	1.05(-)	45±7.07	32±4.12	1.95 (-)
	Stomach	28±4.12	27±4.12	0.21(-)	27±4.12	22±4.12	1.05 (-)
	Intestine	40±7.07	35±7.07	0.61(-)	33±4.12	23±4.12	2.10 (-)
	P. caeca	39±3.31	32±4.12	1.70(-)	32±4.12	22±4.12	2.10 (-)

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

Another important observation is that chronic treatment produces more inhibition in comparison to short term acute treatment. According to PICKERING and HENDERSON (1964) lead toxicity appears to be different at higher concentration and short term treatment than at long term exposure to low concentration. Further, the inhibitory activity of lead on enzymes increases with time. More inhibition results after 30 days of treatment than after 15 days. This confirms the findings of CRANDALL and GOODNIGHT (1962) that lead produces chronic intoxication and occurrence of this chronic toxicity makes the determination of "safe pollution level for fish difficult".

SUMMARY

The effect of exposure of Channa punctatus to LC(50) (6.8 mg/L) and sublethal concentration (2.8 mg/L) of lead nitrate on the activities of amino tripeptidase, glycylglycine, leucyl-l-glycine dipeptidases and lipase has been investigated. An inhibition in the activities of all the enzymes is noted after 96 hr, 15 and 30 days of treatment period. The inhibition in lipase activity is statistically insignificant in both the acute and chronic treatment cases. The experiments indicate that lead induced toxicity is more severe in chronic treatment as compared to acute treatment.

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