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A Comparative Study of the Effect of Acute and Chronic Mercuric Chloride Treatment on the Activities of a Few Digestive Enzymes of a Teleost Fish, *Channa punctatus*

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Heavy metal input into the media, either aquatic or terrestrial is an important aspect of environmental pollution. Heavy metals are reported to produce toxic effects in terrestrial and aquatic animals. Among the aquatic fauna, according to MATHIS & KEVERN (1975) fishes are the most sensitive group. Several reports have appeared in recent years suggesting that heavy metals interfere with the physiological functions of various organs (HIRTH 1964, AHMED & GEORGANAS 1973, WAGSTAFF 1975, WOBESTER 1975, SASTRY & GUPTA 1978a,b,c,d). According to HARRISSON et al. (1971) lead, mercury and cadmium produce cumulative toxicity when consumed in small doses. In our previous investigations (SASTRY & GUPTA 1978a,b,c,d) several alterations in the activity of digestive enzymes of Channa punctatus acutely and chronically treated with mercuric chloride and lead nitrate have been observed. However, much information is required to understand the mechanism of toxicity of heavy metals to the digestive system of fishes. Such studies are important as some amount of mercury enters the digestive system of fishes through the food and may cause considerable damage affecting the growth and nutritive value of fishes. In the present work, acute and chronic experiments have been conducted with mercuric chloride to examine the alterations in the activities of a few carbohydrases, lipase and a peptidase in the digestive system of Channa punctatus.

MATERIALS AND METHODS

Living fishes were collected from local fresh water sources and maintained in laboratory aquaria. Specimens weighing 70 $\pm$ 5 G each were selected and prior to experimentation they were acclimatized to the laboratory conditions for 4 days. Preliminary bioassays conducted in the laboratory under static conditions have shown that 1.8 mg/L of $HgCl_2$ is LC(50) and 0.30 mg/L is a sublethal concentration for the experimental animals. Fishes were divided into 4 groups of 30 fishes each. The first group of 30 fishes were treated with LC(50) for 96 hr and the second group was treated with the sublethal concentration for 20 days. The third and fourth groups served as controls for the first and second

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groups respectively. The surviving fishes from the first group and all the fishes from second group were sacrificed after respective tenures of treatment. 10% (W/V) homogenates of stomach, intestine, pyloric caeca and liver were prepared in 0.02 M phosphate buffer using a chilled Potter-Elvehjem homogenizer. The homogenates were centrifuged for 20 min at 1500 G and the clear supernatant fluids were used as the source of enzymes. Activities of maltase and lactase were estimated by quantitatively determining the reducing sugars according to the method of GRAY & ROTHCHILD (1941) using 0.2 M maltose and 0.25 M lactose solutions as substrates respectively. Lipase activity was estimated following the method of BIER (1955) with Tween 20 as substrate. The activity of leucyl-L-glycine dipeptidase was determined by the method of SMITH (1955). The individual aminoacids liberated by the hydrolysis of the substrate in 10 µl of the incubation mixture were separated by paper chromatography. The intensity of the coloured spots of glycine developed with ninhydrin was scanned in a Systronix densitometer.

For each enzyme, triplicate samples were analysed and the incubations were repeated three times. The protein content in the homogenates was determined by the method of LOWRY et al. (1951) using bovine serum albumin as standard. The test described by FISHER (1950) was employed to calculate the statistical significance between control and experimental values.

RESULTS

The results of the experiments are given in Tables 1 to 3. The elevation in the activity of maltase after 96 hr of treatment is significant in intestine and pyloric caeca. However, after 20 days of exposure to mercuric chloride there is an inhibition in maltase activity in all the tissues examined which is statistically insignificant. In intestine and pyloric caeca a significant inhibition in lactase activity is noted at both the experimental stages. In contrast, liver and stomach revealed an insignificant elevation in lactase activity. Lipase activity is inhibited in all the two stages but it is more marked after 20 days of treatment. Inhibition is also recorded in leucyl-L-glycine dipeptidase activity which is significant only in the chronic treatment stage.

DISCUSSION

Mercury compounds are known to produce severe damage in different organs of fishes and alter the activities of enzymes (HIRTH 1964, SASTRY & GUPTA

TABLE 1

Activities of two carbohydrases in experimental and control fishes[a]

Enzyme	Tissues	Control	Experimental			
			96 hr	Sig. Diff.	20 days	Sig. Diff.
Maltase[c]	Liver	0.085±0.0050	0.092±0.0013	1.66(-)	0.080±0.0038	0.98(-)
	Stomach	0.066±0.0090	0.070±0.0066	0.44(-)	0.060±0.0066	0.66(-)
	Intestine	0.092±0.0009	0.099±0.0013	5.83(+)b	0.090±0.0044	0.57(-)
	Pyloric caeca	0.090±0.0042	0.100±0.0023	4.76(+)	0.096±0.0044	1.22(-)
Lactase[c]	Liver	0.129±0.0073	0.107±0.0009	3.73(+)	0.132±0.0023	0.48(-)
	Stomach	0.150±0.0060	0.140±0.0092	1.12(-)	0.156±0.0044	0.99(-)
	Intestine	0.145±0.0082	0.110±0.0043	4.66(+)	0.115±0.0009	4.22(+)
	Pyloric caeca	0.140±0.0027	0.110±0.0043	7131(+)	0.118±0.0083	5.37(+)

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 reducing sugar liberated per mg of tissue protein per hr at 37°C.

TABLE 2

Activity of lipase in experimental and control fishes[a]

Tissues	Control	Experimental			
		96 hr	Sig. Diff.	20 days	Sig. Diff.
Liver	57.00 ± 2.07	51.00 ± 1.67	2.77(+)b	45.00 ± 1.03	6.36(+)
Stomach	30.00 ± 1.00	27.00 ± 2.07	1.50(-)	25.00 ± 1.03	4.33(+)
Intestine	42.00 ± 0.67	36.00 ± 1.65	4.02(+)	31.00 ± 2.07	6.20(+)
Pyloric caeca	41.00 ± 0.88	35.30 ± 1.68	3.16(+)	32.30 ± 1.33	6.66(+)

Activity is expressed in lipase units.

TABLE 3

Activity of leucyl-L-glycine dipeptidase[c] in experimental and control fishes[a]

Tissues	Control	Experimental			
		96 hr	Sig. Diff.	20 days	Sig. Diff.
Intestine	0.160 ±0.0057	0.158 ±0.0013	0.42(-)	0.118 ±0.0066	5.90(+)
Pyloric caeca	0.165 ±0.0063	0.149 ±0.0170	1.14(-)	0.120 ±0.0092	5.00(+)

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 hr at 37°C.

1978a,b,c,d). According to PICKERING & HENDERSON (1964) heavy metal toxicity appears to be different at higher concentration and short term treatment than at long term exposure to low concentration. Therefore, in the present study a comparative study has been made on the effect of short term and long term treatments. Maltase activity showed a significant inhibition after 20 days of treatment whereas in fishes treated with LC(50) for 96 hr there is no significant change. Lactase activity revealed a significant fall at both the stages. In Channa punctatus chronically treated with lead nitrate, similar inhibition was noted in our earlier studies (SASTRY & GUPTA 1978c). The difference in the behavior of the two carbohydrases may be attributed to the differences in enzyme structure and behavior. Further, after 20 days of treatment the mercury content in the body may be greater resulting in increased binding of mercury with the enzyme protein. Further evidence for the production of cumulative toxicity by mercury is provided by the inhibition in lipase activity which is higher in chronically treated fishes than in those treated for 96 hr with LC(50). According to NEATHERY & MILLER (1975) mercury enters the digestive system through food and some amount of this inorganic salt is absorbed into the alimentary canal and accumulates there. However, due to effective homeostasis after absorption, retention time is longer. Accumulation of this metal interferes with the metabolic activities by binding with enzyme proteins, as suggested by PASSOW et al. (1961). The intestinal enzyme leucyl-L-glycine dipeptidase also shows marked inhibition after 20 days of treatment while after 96 hr of treatment, there is no significant alteration in its activity. Our earlier studies with lead nitrate have also yielded similar results (SASTRY & GUPTA 1978d).

The present experimental data reveal that the alteration in the activities of digestive enzymes differs in acute and chronic treatments. The structural damage produced is also more severe in fishes treated chronically with 0.30 mg/L of $HgCl_2$ than in animals treated with LC(50) (1.8 mg/L) for 96 hr (SASTRY & GUPTA 1978e). All these findings point out that heavy metals act as cumulative poisons and alter the physiological functioning of the experimental animals irrespective of the concentration of the metal in the medium. This lends support to the findings of CRANDALL & GOODNIGHT (1962) that heavy metals like lead and mercury produce chronic intoxication and occurrence of this chronic toxicity makes the determination of "safe pollution level for fish difficult".

SUMMARY

In this study a comparison of the effect of exposure of Channa punctatus to LC(50) (1.8 mg/L) and

a sublethal concentration (0.30 mg/L) of mercuric chloride on the activities of maltase, lactase leucyl-L-glycine dipeptidase and lipase has been made. After 96 hr of treatment, a slight elevation in maltase activity was recorded in all the portions of the digestive system. Lipase and leucyl-L-glycine dipeptidase showed inhibition in activity. Chronic treatment for 20 days revealed a significant inhibition in the activities of all the four enzymes.

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