Bull. Environm. Contam. Toxicol. 22,009-016 (1979)

The *In Vivo* Effect of Mercuric Chloride on some Digestive Enzymes of a Fresh Water Teleost Fish, *Channa punctatus*

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Mercury is one of the environmental pollutants highly toxic to animals. Toxic effects of metals and chemicals may result from their binding with biologically active constituents of the body such as lipid, aminoacids, enzymes and other proteins (PASSOW et al. 1961). Mercurials are known to produce structural damage in the nervous system (CHANG & HARTMANN 1972a,b) and kidney (SAHAPHONG & TRUMP 1971, WARE et al. 1973) of mammals and digestive system of fishes (SASTRY & GUPTA 1977c). Alterations in enzymic and metabolic processes have been observed in animals treated with mercury and other heavy metals (WEBB 1966). Though, mercury interferes with the physiological functions of liver, brain and kidney (BLACKWOOD et al. 1965), and digestive tract (SASTRY & GUPTA 1977a) the exact mode of action is not known. After its administration, mercury accumulates in the liver (TAKAHASHI et al. 1971) and it has been reported that most of the mercurials are eliminated through the bile, and the diffusible complex is rapidly absorbed in the intestine into the blood stream giving rise to a large enterohepatic circulation of mercury (NORSETH & CLARKSON 1971). As the liver is the main detoxificating organ, it is important to study the physiological alterations in the enzyme activities due to exposure to this metal. Some mercury enters the body of fish through food chain and most likely produces toxic effects in the digestive system. Emphasis is mostly attached to liver and very little information is available on the pathological and physiological alterations produced by mercury in the digestive system. The present communication deals with the alterations in the enzyme activities that accompany chronic mercury intoxication in liver and different parts of the digestive tract of a teleost fish, Channa punctatus.

MATERIALS AND METHODS

Living fishes were collected from local fresh water sources and maintained in laboratory aquaria. 60 specimens weighing 70 $\pm$ 8 g each were selected and divided into two equal groups. Prior to experimentation

0007-4861/79/0022-0009 $01.60

they were acclimatized to the laboratory conditions for 5 days. Preliminary bioassays conducted in the laboratory under static conditions have shown that 0.30 mg/L of mercuric chloride is a sublethal concentration. The first group of fishes was treated with this concentration of mercury while the second group maintained in mercury-free tap water served as control. All the fishes were sacrificed after 20 days.

10% (W/V) homogenates of the different regions of the alimentary canal and liver were prepared in 0.25M sucrose solution in a Potter-Elvehjem homogenizer. The homogenates were centrifuged for 20 min at 1000 G in a cold room and the clear supernatant fluids were used as the source of enzymes. The enzyme extracts were kept frozen until required.

The activity of alkaline and acid phosphatases was determined adopting the method of BODANSKY (1933). The substrate used was 0.16M sodium β-glycerophosphate at pH 9.3 and 5 for alkaline and acid phosphatases, respectively. The mixture was incubated at 37°C for exactly 1 hr. For the estimation of glucose-6-phosphatase activity, 0.01M glucose-6-phosphate solution, having pH 6.5, was used as the substrate. The activity was determined following the method of SWANSON (1965). The incubation period was 15 min. Amylase activity was determined according to the method of BERNFIELD (1955). The substrate used was 0.5M starch solution in 0.02M phosphatase buffer, pH 6.9. Incubation was carried upto 1 hr at 37°C. The reducing sugars liberated were quantitatively estimated by the method of Folin and Wu as given by OSER (1965). The activity of the two proteases was determined by the method of RICK (1965) with haemoglobin as the substrate. Peptidases were determined by the method of SMITH et al. (1955). 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. Total protein content in the homogenates was determined in TCA precipitate 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 AND DISCUSSION

The results of the experiments conducted are presented in Tables 1 and 2. Mercury, both in organic and

inorganic forms is highly toxic to fishes. As some amount of mercury enters through the food chain and surrounding aquatic environment into the digestive system of fishes, and may produce toxic effects, it is desirable to examine the alterations in the activity of the digestive enzymes to assess the extent of damage caused by this metal on the process of digestion. Our earlier studies have revealed that considerable structural damage is produced by mercury in the alimentary tract and liver (SASTRY & GUPTA 1977c). The present results point out that the activity of a number of digestive enzymes like amylase, aminotripeptidase, glycylglycine dipeptidase and carnosinase is inhibited by mercury treatment and this can adversely effect the digestive process in the alimentary canal. However, it is interesting to note that the two proteolytic enzymes, trypsin and pepsin, and alkaline phosphatase in the intestine and pyloric caeca have shown an increase in activity.

The inhibition in the activity of enzymes may be either due to the direct binding of mercury with the enzyme protein (PASSOW et al. 1961) or due to the toxic effects produced in the tissues (BLACKWOOD et al. 1961) leading to decreased synthesis of enzyme protein. According to BATTIGELLI (1960) the ultimate effect of mercury and its compounds is due to enzyme inhibition. Alkaline phosphatase is a brush border enzyme involved in the transphosphorylation reactions. This has been correlated with the absorption of nutrients across the intestinal wall (VERZAR & MC DOUGALL 1936). In liver and stomach, the decrease in the activity of this enzyme indicates that transphosphorylation reaction is inhibited. HINTON et al. (1973), KENDALL (1975) and (SASTRY & GUPTA 1977a,b) reported similar inhibition in hepatic alkaline phosphatase activity. However, HINDRICKS et al. (1973) have shown that zinc and magnesium are necessary for alkaline phosphatase activity in mammalian intestine and placenta, and mercury can replace them. If similar condition exists in fishes also, the elevation in alkaline phosphatase activity observed in intestine and pyloric caeca may be due to the greater availability of mercury. Acid phosphatase is a lysosomal enzyme and cellular damage is usually accompained by an increase in the activity of this enzyme. However, in the present study inhibition is observed in all tissues except liver. The fall in the activity of glucose-6-phosphatase noted here lends support to similar findings by CHANDRA & IMAM (1973) in the gastro-intestinal mucosa of guinea pigs treated with manganese. This indicates disturbances in the general metabolism of the cell under the toxic influence of mercury.

TABLE 1

The activities of phosphatases in experimental and control fishes[a]

Enzyme	Tissue	Control	Experimental	Significant difference
Alkaline phosphatase	Liver	0.0494 ± 0.00073	0.0349 ± 0.00086	7.2(+)[b]
	Stomach	0.0621 ± 0.00106	0.0387 ± 0.00093	21.3(+)
	Intestine	0.0398 ± 0.00020	0.0643 ± 0.00083	34.5(+)
	Pyloric caeca	0.0360 ± 0.00076	0.0624 ± 0.00110	26.4(+)
Acid phosphatase	Liver	0.0458 ± 0.00073	0.0513 ± 0.00077	6.5(+)
	Stomach	0.0545 ± 0.00093	0.0506 ± 0.00080	4.0(+)
	Intestine	0.0588 ± 0.00150	0.0535 ± 0.00093	4.0(+)
	Pyloric caeca	0.0577 ± 0.00063	0.0472 ± 0.00120	9.5(+)
Glucose-6-phosphatase	Liver	0.0566 ± 0.00141	0.0504 ± 0.00098	4.5(+)
	Stomach	0.0729 ± 0.00130	0.0549 ± 0.00196	9.4(+)
	Intestine	0.0626 ± 0.00085	0.0456 ± 0.00066	19.8(+)
	Pyloric caeca	0.0580 ± 0.00210	0.0379 ± 0.00054	11.9(+)

a. Activity is expressed in mg of inorganic phosphate liberated per mg of tissue protein per hour at 37°C. (Mean ± SE).

b. (+) indicates statistically significant difference from control values at 95 percent confidence interval.

TABLE 2

The activities of digestive enzymes in experimental and control fishes[a]

Enzyme	Tissue	Control	Experimental	Significant difference
Amylase (mg maltose/mg protein/hr)	Liver	0.187 ± 0.0083	0.170 ± 0.0014	2.5(-)
	Stomach	0.066 ± 0.0007	0.072 ± 0.0033	2.2(-)
	Intestine	0.081 ± 0.0010	0.109 ± 0.0055	6.2(+)b
	Pyloric caeca	0.132 ± 0.0069	0.142 ± 0.0046	1.5(-)
Trypsin[c]	Intestine	0.260 ± 0.0700	0.619 ± 0.0408	5.4(+)
	Pyloric caeca	0.255 ± 0.0164	0.404 ± 0.0130	8.8(+)
Pepsin[c]	Stomach	0.417 ± 0.0633	0.775 ± 0.0298	14.8(+)
Aminotri-peptidase[d]	Liver	0.152 ± 0.0290	0.133 ± 0.0061	0.8(-)
	Intestine	0.156 ± 0.0190	0.133 ± 0.0017	1.8(-)
Glycylglycine dipeptidase[d]	Intestine	0.172 ± 0.0057	0.147 ± 0.0042	3.9(+)
	Pyloric caeca	0.187 ± 0.0070	0.148 ± 0.0046	5.8(+)
Carnosinase[d]	Intestine	0.143 ± 0.0070	0.121 ± 0.0044	4.1(+)
	Pyloric caeca	0.164 ± 0.0070	0.130 ± 0.0033	5.4(+)

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 tyrosine liberated per mg of tissue protein per hr at 37°C.
d. Activity is expressed in mg glycine liberated per mg of tissue protein per hr at 37°C.

Amylase shows inhibition in the liver and elevation in intestine and pyloric caeca. In our earlier studies with 1.8 mg/L of mercury also similar elevation in the activities of amylase, trypsin and pepsin was observed (SASTRY & GUPTA 1977a). Inhibition in the activities of the dipeptidases is statistically significant but that of aminotripeptidase is insignificant. Similar inhibition in dipeptidase activity was observed by SASTRY & GUPTA (1977a,d) in the same fish acutely treated with mercuric chloride and lead nitrate. The elevation in the proteolytic activity, as pointed out by CHRISTENSEN (1975), may be due to enzyme induction.

The results reveal that the pattern of alteration in enzyme activity by mercury is not similar in all organs. Most of the enzymes examined are inhibited in liver but in the digestive tract some enzymes have shown elevation in activity. The reason may be due to the fact that mercury is rapidly eliminated from the liver through bile (TAKAHASHI et al. 1971) and it undergoes an extensive enterohepatic recirculation (NORSETH & CLARKSON 1971). Some of the mercury reenters the liver even after discontinuation of its administration. The persistence of pathological degeneration of some hepatocytes throughout the recovery period may represent the effect of residual and recirculating mercury in the liver. Further, ZIMMERMAN (1976) pointed out that liver injury is not a single entity but rather a variety of abnormal tissue responses.

SUMMARY

Alterations in the activities of alkaline phosphatase, acid phosphatase, glucose-6-phosphatase amylase, trypsin, pepsin, aminotripeptidase, glycylglycine dipeptidase and carnosinase due to exposure of Channa punctatus to a sublethal concentration (0.30 mg/L) of mercuric chloride by bath for 20 days have been studied in the different parts of the digestive system. A fall in the activities of the three phosphatases was recorded except for alkaline phosphatase which showed a slight elevation in activity in intestine and pyloric caeca. An increase in the activity of amylase and the two proteases was observed in all the portions of the digestive system. The three peptidases revealed a decrease in activity.

ACKNOWLEDGEMENT

The authors are grateful to Dr. V.P. Agrawal, Principal of this institution for his keen interest and guidance in the work. Authors are also thankful to University Grants Commission for financial assistance.

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