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Alterations in the Activity of Some Digestive Enzymes of *Channa punctatus*, Exposed to Lead Nitrate

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INTRODUCTION

Trace elements present in the body may be divided into essential and non-essential metals. The levels of essential metals are maintained by some biological processes whereas those of non-essential metals is through external sources. Non-essential metals like lead, cadmium and mercury produce cumulative toxic effects if taken in small doses and acute toxicity in higher doses (HARRISSON et al. 1971).

The input of heavy metals into the environment whether terrestrial or aquatic presents an important chapter in environmental pollution. The fact that harmful effects are produced in man and other organisms makes it essential that biological investigations must be taken into consideration,both for man and his environment. In view of this necessity, experimental studies with lead assume a two fold purpose, first to learn about its accumulation and distribution in edible fishes, and second to observe whether any physiological changes and alterations to enzyme activity are produced due to lead accumulation. Work on the first aspect is well known (CASTILLINO and ALOJ 1969, GREEN et al. 1976, MAKARAV et al. 1976 MERLINI and GIANNI 1977, MORGAN et al. 1973). Pathological effects of lead are mainly confined to kidney, brain, erythrocytes and haeme synthesis (GOYER and RHYNE 1973). Some histochemical studies have also been made on liver and kidney (ZEGARSKA and ZEGARSKI 1968). At least a few of the effects of lead are known to be due to enzyme inhibition and lead appears to act at a large number of biochemical sites (ULMER and VALLEE 1969). In view of the paucity of literature on the effect of lead on the digestive tract, the present work has been undertaken to examine the alterations in the activity of few digestive enzymes after chronic exposure to lead nitrate in a teleost fish Channa punctatus.

MATERIALS AND METHODS

Living fishes were collected from local fresh water sources and maintained in laboratory aquaria. Specimens weighing 70 + 8 g each were selected and prior to experimentation they were acclimatized to the laboratory conditions for 4 days . Preliminary bioassays

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conducted in the laboratory under static conditions have shown that 3.80 mg/l of lead nitrate is a sublethal dose for the experimental animals. First group of 30 fishes were treated with this concentration for 30 days while second group of 30 fishes were maintained in lead free distilled water served as control. 15 fishes from both groups were sacrificed after 15 and 30 days respectively. 10% (W/V) homogenates of stomach, intestine. pyloric caeca and liver were prepared in 0.25M sucrose solution and 0.02M phosphatase buffer using a chilled Potter Elvehjem homogenizer. The homogenates were centrifuged for 20 minutes at 1000 g and the clear supernatant fluids were used as the source of enzymes. 0.016M 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 BODANSKY (1933). Amylase was estimated according to BERNFIELD (1955). The substrate used was 0.5M starch solution. Activities of maltase and lactase were estimated by quantitatively determining the reducing sugars according to the method of GRAY and ROTCHILD (1941) using 0.2M maltose and 0.25M lactose solution as substrates respectively. ANSON'S (1939) method was adopted for the estimation of activities of trypsin and pepsin. Protein in the homogenates was estimated by the method of LOWRY et al. (1951) using bovine serum albumin as standard. All the incubations were carried out at 37°C. 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.

The present work aims to observe whether chronic treatment with lead nitrate produces any alterations in the activity of the digestive enzymes. Lead compounds are known to produce severe damage in liver and kidney (ZEGARSKA and ZEGARSKI 1968), kidney and brain (GOYER and RHYNO 1973). Though some amount of lead enters the digestive system through food and inorganic forms of lead are also absorbed from the intestine (NEATHERY and MILLER 1975), very little information is available on its effect in the digestive system. In the present chronic treatment it has been observed that there is no significant change in alkaline phosphatase activity in liver and stomach. Intestine and pyloric caeca showed marked inhibition after 15 days of exposure of fishes. The inhibition in the enzyme activity may be either due to direct binding of lead

TABLE 1

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

Enzyme	Tissue	Number of experiments conducted	Control	Experimental	
				15 days treated	30 days treated
Alkaline* Phosphatase	Liver	3	0.049 ± 0.0020	0.050 ± 0.0007 (-)	0.047 ± 0.0007 (-)
	Stomach	3	0.057 ± 0.0100	0.062 ± 0.0017 (-)	0.068 ± 0.0014 (-)
	Intestine	3	0.047 ± 0.0010	0.035 ±0.0007 (+)	0.046 ± 0.0007 (-)
	Pyloric caeca	3	0.050 ± 0.0007	0.046 ± 0.0010 (+)	0.072 ± 0.0074 (+)b
Acid* Phosphatase	Liver	3	0.050 ± 0.0015	0.052 ± 0.0014 (-)	0.056 ± 0.0012 (+)
	Stomach	3	0.052 ± 0.0014	0.056 ± 0.0013 (-)	0.063 ± 0.0011 (+)
	Intestine	3	0.043 ± 0.0010	0.035 ± 0.0004 (+)	0.048 ± 0.0013 (+)
	Pyloric caeca	3	0.045 ± 0.0025	0.054 ± 0.0015 (+)	0.064 ± 0.0009 (+)
Trypsin**	Intestine	3	0.065 ± 0.0034	0.093 ±0.0102 (+)	0.112 ± 0.0168 (+)
	Pyloric caeca	3	0.052 ∓ 0.0080	0.074 ∓ 0.0036 (+)	0.100 ∓ 0.0030 (+)
Pepsin**	Stomach	3	0.380 ±0.0094	0.461 ± 0.0120 (+)	0.529 ± 0.0360 (+)

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

TABLE 2

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

Enzyme	Tissue	Number of experiments conducted	Control	Experimental	
				15 days treated	30 days treated
Amylase*	Liver	3	0.281 ± 0.0016	0.294 ± 0.0016 (+)	0.304 ± 0.0096 (+)b
	Stomach	3	0.195 ± 0.0016	0.216 ± 0.0076 (+)	0.154 ± 0.0076 (+)
	Intestine	3	0.170 ± 0.0080	0.232 ± 0.0102 (+)	0.138 ± 0.0066 (+)
	Pyloric caeca	3	0.173 ± 0.0065	0.263 ± 0.0044 (+)	0.150 ± 0.0044 (+)
Maltase*	Liver	3	0.065 ± 0.0050	0.080 ± 0.0056 (-)	0.055 ± 0.0044 (-)
	Stomach	3	0.064 ± 0.0014	0.087 ± 0.0035 (+)	0.058 ± 0.0061 (-)
	Intestine	3	0.082 ± 0.0084	0.124 ± 0.0097 (+)	0.064 ± 0.0072 (-)
	Pyloric caeca	3	0.087 ± 0.0050	0.121 ± 0.0035 (+)	0.064 ± 0.0044 (+)
Lactase*	Liver	3	0.121 ± 0.0200	0.197 ± 0.0270 (+)	0.140 ± 0.0168 (-)
	Stomach	3	0.154 ± 0.0150	0.156 ± 0.0043 (-)	0.252 ± 0.0100 (+)
	Intestine	3	0.139 ± 0.0060	0.100 ± 0.0037 (+)	0.150 ± 0.0120 (-)
	Pyloric caeca	3	0.138 ± 0.0090	0.112 ± 0.0050 (+)	0.154 ± 0.0100 (-)

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

with the enzyme proteins or due to the toxic effects produced by it on the tissues. The decrease in the alkaline phosphatase activity shows that the absorption process in the intestine and pyloric caeca is inhibited. After 30 days of exposure also there is no significant change in the activity of alkaline phosphatase except in pyloric caeca where marked elevation is noted. Cellular damage is usually accompanied by an increase in acid phosphatase activity. In the present experiment also an increase has been noted in acid phosphatase activity after 15 and 30 days of treatment. The increase in acid phosphatase activity may be due to increased histiocytic reaction resulting from an excess of lead. WHITE (1977)WAGSTAFF (1973) and CHANDRA and IMAM (1973) have also reported an increase in acid phosphatase activity of different tissues treated with lead, mercury and manganese respectively, which according to ZONEK et al. (1966) is due to increased pinocytosis. All the three carbohydrases examined herr have shown an initial increase in activity followed by a subsequent decline after 30 days of treatment. In contrast to carbohydrases, the activity of the two proteases remained elevated above the normal level upto the end of the experimental period. This may be possibly related to the difference in enzyme structure and behaviour. No possible explanation can be given at this stage as further experiments are in progress.

The data reveal that the pattern of alterations in enzyme activity is not similar in all organs for liver shows an entirely different pattern in comparison to digestive tract which may be due to as pointed out by ZIMMERMAN (1976) that liver injury is not a single entity but rather a variety of abnormal tissue responses.

SUMMARY

The effect of exposure of Channa punctatus to a sub-lethal concentration of lead nitrate on the activities of alkaline phosphatase, acid phosphatase amylase, maltase, lactase, trypsin and pepsin has been investigated. A decrease in the activity of alkaline phosphatase has been recorded after 15 days of exposure but there was no significant change after 30 days. Acid phosphatase showed an elevation in activity of both stages. All the three carbohydrases shows elevation after 15 days, followed by an inhibition after 30 days of treatment. The activity of pepsin and trypsin remained above the normal level throughout the tenure of the experiment reveal that the pattern of alteration in enzyme activities is different in liver and digestive system.

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LITERATURE CITED

ANSON, M.L. : J. gen. Physiol. 22 : 79 (1939).

BERNFIELD, P. : Methods in Enzymology Vol. I., (ed. S.P. COLOWICK and N.O. KAPLAN) New York. Academic Press p. 149 (1955).

BODANSKY, A.: J. Biol. Chem. 101 :93-104 (1933).

CASTELLINO, N. and S.ALOJ : Br. J. Ind. Med. 26 : 139 (1969).

CHANDRA, S.V. and Z. IMAM : Acta Pharmacol. et Toxicol. 33 : 449-458 (1973).

FISHER, R.A. : Statistical Methods for Research Workers, 11th ed. (OLIVER and BOYD) London (1950).

GOYER, R.A. and B.C.RHYNE : Int. Rev. of Experimental pathology Vol. 12 (ed G.W. Richter and M.A. EPSTEIN) New York. Academic Press (1973).

GRAY, P.P. and H.ROTHCHILD : Ind. Eng. Chem. 13 : 902 (1941).

GREEN, V.A., G.W. WISE and J. COLLENBACH : Clin. Toxicol. 9 : 33-51 (1976).

HARRISON, P.R., W.R. MATSON and J.W. WINCHESTER : Atmos. Environ. 5 : 613 (1971).

LOWRY, O.H., N.J. ROSEBROUGH, A.L. FARR and R.J. RANDALL : J. Biol. Chem. 193 : 265-75 (1951)

MAKAROV, V.K., T.G. ZHDANOVA, I.I. ISAKHANOVA and G.S. GOLIKOVA : Gig. Tr. Prof. Zabol. 4 : 40-43 (1976).

MANEVSKA, B : Gig. Tr. Prof. Zabol. 12: 29-32 (1976).

MERLINI, M. and G. POZZI : Environ. Pollution. 12 : 167-172 (1977).

MORGAN, A., A. HOLMES and J.C. EVANS : Brit. J. of Ind. Med. Vol. 34 : 37-43 (1977).

NEATHERY, M.W. and W.J. MILLER : J. Dairy Sci. 58 : 1767-1781 (1976).

ULMER, D.D. and B.L. VALLE : Environ. Heth. Proc. Uni. Mo. Ann. Conf. 2nd. : 7 (1969).

WAGSTAFF,D.J. : Bull. Environ. Contam. & Toxicol. 9 : 10-14 (1973).

WHITE, D.J. : Brit. J. of Exp. Pathol. 58 : 101 (1977).

ZEGARSKA, Z. and W. ZEGARSKI : Acta Med. Pol. 9 : 119 (1968).

ZIMMERMAN, H.J.: Environ. Hlth. Pres. 15 : 3-12 (1976).

ZONEK, J., Z.OLKOWSKI and G. ZONDERKO : Int. Arch. Gewerbepath. Gewerbehyg. 22 : 1-9 (1966)