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In Vitro Inhibition of Three Phosphatases by Mercuric Chloride and Their Reversal by Chelating Agent EDTA

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Industrial wastes cause contamination of world water ways and endanger the life of aquatic fauna, of which fishes are the most sensitive group (MATHIS & KEVERN 1975). Non-essential metals like lead, cadmium and mercury produce cumulative toxic effects in small doses and acute toxicity in higher doses (HARRISON et al. 1971). According to PASSOW et al. (1961) toxic effects of non-essential metals and chemicals may result from their binding with biologically active constituents of the body such as lipids, amino acids, enzymes and proteins. The exact mode by which heavy metals interfere with the physiological functions of body is not known. Alteration in the activity of certain enzymes is observed by SASTRY & GUPTA (1978a,b) in Channa punctatus exposed to mercury. The essential factor in the treatment of metal poisoning is to prevent the accumulation of excess of metals which may cause irreversible damage to the biomolecules. In vitro inhibition of some enzymes in the digestive system of Channa punctatus by mercury and lead was observed in our earlier experiments (SASTRY & GUPTA 1978c,d). SINGH & TANDON (1975) studied the in vitro removal of manganese by chelating agents from brain and liver of manganese treated rats. The present experiments have been undertaken to observe whether mercury has any direct action on brain enzymes and the effectiveness of the chelating agent EDTA in reversing the early stages of intoxication.

MATERIALS AND METHODS

Living specimens of Channa punctatus were collected from the local fresh water sources. After acclimatization for 4-5 days to the laboratory conditions, the fishes were dissected and the brain was immediately removed. The tissue was weighed to the nearest milligram and homogenized in cold 0.25 M sucrose solution using a chilled Potter-Elvehjem homogenizer. The homogenates were centrifuged for 20 min at 2000 G and the clear supernatant fluids adjusted to 10% (W/V) strength were used as the source of enzymes. 0.016 M sodium B-glycerophosphate was used as the substrate at pH 9.3 and 5.0 for alkaline phosphatase and acid phosphatase respectively. The

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enzyme activities were determined by the method of MORTON (1955). Glucose-6-phosphatase activity was determined by the method of SWANSON (1955). Enzyme protein in the homogenates was estimated 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.

Mercuric chloride and EDTA were dissolved in distilled water so as to form a 10 µM stock solution and further dilutions were made as desired.

RESULTS

The present in vitro studies reveal that mercury is quite effective in inhibiting the activities of all the three enzymes examined here. Maximum inhibition is observed in the activity of glucose-6-phosphatase. The degree of inhibition is directly proportional to the increase in the concentration of mercuric chloride in the incubation medium. To observe the effect of EDTA in restoring the inhibitory action of mercury on enzymes, normal tissue homogenates were preincubated with mercury and these samples were then incubated with different concentrations of EDTA. The results are presented in Tables 1 & 2. EDTA is capable of restoring the activity of all the enzymes studied here. There is a gradual restoration and the degree of restoration increased with the increase in the concentration of EDTA and time.

DISCUSSION

A number of workers (CHANG & HARTMANN 1972a,b,c; CHANG & YAMAGUCHI 1974; SASTRY & GUPTA 1978a,b) have shown that mercury adversely affects the metabolic functions of the body. Toxic effects of heavy metals and chemicals may result from binding with biologically active constituents of the body such as lipids, amino acids, enzymes and other proteins (PASSOW et al. 1961). The exact mechanism by which this metal interferes with the physiological functions of the body of organisms is not clearly understood. Heavy metals inhibit the activity of enzymes not only in vivo but in vitro also. In vitro inhibition of succinic dehydrogenase by manganese has been reported by SETH & HUSAIN (1974). In our earlier in vitro experiments inhibition of certain digestive enzymes by mercury and lead was observed (SASTRY & GUPTA 1978c,d). According to VALLEE & ULMER (1972) heavy metals inhibit the reactions of the energy generating processes. Our data indicate that the degree of inhibition increases with an increase in the concentration of the toxicant.

TABLE 1

In vitro inhibition of phosphatases by mercuric chloride

Enzyme	Concentration of $HgCl_2$	μ moles of inorganic phosphate/mg protein/hr					
		15 min	% inhibition	30 min	% inhibition	60 min	% inhibition
Alkaline phosphatase	Control (Dist. wat.)	0.49±0.018		0.50±0.005		0.53±0.005	
	0.4 μM	0.39±0.005	20(+)[a]	0.45±0.002	10(+)	0.40±0.018	25(+)
	1 μM	0.33±0.007	33(+)	0.43±0.003	14(+)	0.36±0.005	32(+)
	2 μM	0.30±0.014	39(+)	0.37±0.000	26(+)	0.31±0.019	42(+)
Acid phosphatase	Control (Dist. wat.)	0.20±0.011		0.23±0.009		0.29±0.051	
	0.4 μM	0.17±0.056	15(+)	0.16±0.003	30(+)	0.23±0.024	21(+)
	1 μM	0.12±0.011	40(+)	0.13±0.007	43(+)	0.23±0.002	21(+)
	2 μM	0.12±0.008	40(+)	0.12±0.012	48(+)	0.22±0.033	24(+)
Glucose-6-phosphatase	Control (Dist. wat.)	0.35±0.054		0.32±0.006		0.26±0.000	
	0.8 μM	0.23±0.006	34(+)	0.20±0.000	38(+)	0	100(+)
	2 μM	0.17±0.003	51(+)	0	100(+)	0	100(+)
	4 μM	0.09±0.021	74(+)	0	100(+)	0.04±0.005	85(+)

Values are mean ± S.E.
a (+) indicates statistically significant difference from control value at 95% confidence interval.

TABLE 2

Restoration of *in vitro* mercuric chloride inhibited phosphatase activity by EDTA

Enzyme	Concentration of EDTA	μ moles of inorganic phosphate/mg protein/hr					
		15 min	% restoration	30 min	% restoration	60 min	% restoration
	Control (Dist.wat.)	0.30±0.001		0.32±0.006		0.31±0.018	
Alkaline phosphatase	0.7 μM	0.35±0.013	17(+)[a]	0.36±0.013	13(+)	0.36±0.031	16(+)
	1.75 μM	0.40±0.029	33(+)	0.38±0.019	19(+)	0.38±0.013	23(+)
	3.5 μM	0.42±0.016	40(+)	0.49±0.008	53(+)	0.38±0.024	23(+)
	Control (Dist.wat.)	0.11±0.014		0.14±0.009		0.20±0.041	
Acid phosphatase	0.7 μM	0.12±0.002	9(+)	0.15±0.017	7(+)	0.23±0.028	15(+)
	1.75 μM	0.14±0.007	27(+)	0.16±0.023	14(+)	0.26±0.020	30(+)
	3.5 μM	0.14±0.005	27(+)	0.18±0.011	29(+)	0.29±0.009	45(+)
	Control (Dist.wat)	0.14±0.017		0.14±0.017		0.14±0.017	
Glucose-6-phosphatase	1 μM	0.24±0.016	71(+)	0.17±0.021	21(+)	0.31±0.016	121(+)
	2.5 μM	0.26±0.000	86(+)	0.24±0.000	71(+)	0.28±0.012	100(+)
	5 μM	0.28±0.000	100(+)	0.28±0.026	100(+)	0.31±0.012	121(+)

Values are mean ± S.E.
a (+) indicates statistically significant difference from control value at 95% confidence interval.

HIRTH (1964) in his in vitro study has stated that the mechanism of enzyme inhibiting effect revolves mainly around the affinity of mercury and lead to the -SH groups. JONDERKO (1964) observed similar inhibition of alkaline phosphatase and dehydrogenases by lead and sodium salts. MUSTAFA et al. (1971) also reported that inhibition of ATPase activity in alveolar macrophages and lung tissue is related to the cadmium in the incubation medium. Restoration of activity by the chelating agent EDTA confirms the view that this metal is bound to enzyme protein. Restoration of enzyme activity by a chelating agent depends on its electron donating ability, its accessibility to the cellular system or the nature of the linkage between the metal and the biomolecules of tissues. The degree of restoration observed in the activity of the three phosphatases in the present study is related to the concentration of EDTA. This is in agreement with our earlier observations on the digestive system (SASTRY & GUPTA 1978c,d) and suggests that the functional activity of metal affected biomolecules is restored due to the formation of a metal chelator complex and degree of restoration depends upon concentration of chelating agent used and the incubation period of chelating agent with the metal. According to PENALVER (1957) and WYNTER (1962) chelating agents are more effective in restoring the physiological changes in early stages of poisoning by metal than in chronic stages where permanent alterations take place.

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