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Correlation between Subacute Toxicity of Malathion and Acetylcholinesterase Inhibition in the Tissues of the Teleost *Tilapia mossambica*

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The organophosphorus insecticides are known to disrupt the impulse transmissions of the central and peripheral nervous systems in the vertebrates by inhibiting the acetylcholinesterase (METCALF 1957, FUKUTO 1971, RAINSFORD 1978). Considering this action of organophosphorus pesticides, several attempts were made to correlate the toxicity of the pesticides with the acetylcholinesterase inhibition and thereby estimate the anticholinesterase activity of the insecticide (WILSON, 1969, METCALF, 1971). Hence, in the present study an attempt is made to assess the toxic impact of the sublethal malathion exposure (2 mg/l)to the fish (Tilapia mossambica) by correlating the degree inhibition of acetylcholinesterase to the toxicity potential. Besides, the toxic potency of sublethal concentration of malathion, in terms of acetylcholinesterase inhibition, is compared with the inhibitory potency of eserine (the specific inhibitor of AChE), to evaluate malathion impact in terms of eserine equivalence.

MATERIALS AND METHODS

The LC_{50} value of malathion was determined and 2 ppm concentration was chosen to represent sublethal concentration (KABEER, 1979). All exposures were restricted to 48 hours. The acetylcholinesterase (AChE) (EC 3.1.1.7) was assayed as per METCALF (1957), with slight modifications, by determining the amount of unhydrolysed acetylcholine. 3% homogemates of brain, muscle (red), gill and liver tissues were prepared in cold 0.25 M sucrose solution and used for enzyme assays. The reaction mixture of 3 ml contained : 12 µ moles of acetylcholine chloride, 100 µ moles of sodium phosphate buffer (pH 7.4) and 1.0 ml of the homogenate.

The protein content in the tissue homogenates was estimated as per LOWRY *et al.* (1951).

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Kinetic studies

The mean values of the enzyme activity levels of triplicates were emplcyed for all the experiments. The reciprocal plots (1/v versus 1/s, where 'v' is the reaction velocity and 's' is the substrate concentration) are plotted as per the method of LINEWEAVER and BURK (1934). The slope, intercept, Vmax and Michaelis-Menten constants (Km) were calculated by the method of least squares and were found to coincide with the observed values.

RESULTS AND DISCUSSION

Exposure of fishes to sublethal (2 mg/l) concentrations of malathion produced a significant decrease in the AChE activity (Table 1). COPPEGE et al. (1975) observed similar inhibition of AChE in the fish brain exposed to sublethal concentrations of malathion for 72 hours. Since the inhibition of AChE by organophosphorus pesticides is well known (O' BRIEN 1967 and CORBETT, 1974), the reduction in AChE activity in the four tissues of malathion exposed (ME) fishes observed in the present study, should represent the action of malathion on the enzyme. Brain AChE showed highest inhibition (47.8%) followed by muscle (45.9%), gill (39.1%) and liver (35.4%) tissues. (Table 1). This trend may be true since, tissues with high innervation exhibit greater sensitivity, the differential inhibition of AChE by malathion should represent differences in sensitivity of four tissues. Thus brain exhibited high sensitivity followed by muscle, gill and liver tissues.

When different concentrations of eserine were tested on AChE of normal tissues (Table 1), 0.03 mM in brain, 0.0266 mM in muscle, 0.02 mM in gill and 0.016 mM in liver is found to induce the same percentage of inhibition of AChE as in the respective tissues of ME fishes. (Fig. 1, A to D). Thus the brain tissue having relatively higher degree of innervation (more AChE) needs higher concentration of eserine than muscle gill and liver tissues. While the same brain tissue being relatively more sensitive than muscle, gill and liver, showed greater inhibition in (in vivo) ME fishes (Fig.1, Table 1). Thus both in vivo (malathion) and in vitro (eserine) effects were antagonistic in the sense that, it is the sensitivity of AChE in in vivo, while quantity of AChE in in vitro that contributes differences in inhibition of AChE.

TABLE 1

Acetylcholinesterase (AChE) activity of brain, muscle, gill and liver tissues of normal with different concentration of eserine and malathion-exposed (ME) fishes.

The activity expressed in μ moles of acetylcholine hydrolysed/mg protein/hour
Each value is the mean ± S.D. of 6 individual observations.
P = 't' test, NS = Not significant. Plottings are shown in Figure 1, A to D

Tissue	NORMAL ESERINE CONCENTRATION IN mM									ME
	Control	0.0066	0.0133	0.0200	0.0260	0.0333	0.0400	0.0466	0.0533	
Brain	4.62 ±0.324	3.88 ±0.137	3.35 ±0.228	2.98 ±0.304	2.59 ±0.462	2.23 ±0.371	2.02 ±0.234	1.69 ±0.631	1.40 ±0.882	2.41 ±0.224
		P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
% inhibition	--	16.0	27.5	35.5	45.9	51.7	56.3	63.4	69.7	47.8
Muscle	3.51 ±0.421	3.01 ±0.621	2.69 ±0.562	2.27 ±0.388	1.88 ±0.224	1.60 ±0.928	1.32 ±0.864	1.05 ±0.523	0.83 ±0.684	1.90 ±0.366
		NS	P<0.005	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
% inhibition	--	14.2	23.4	35.3	46.4	54.4	62.4	70.1	76.4	45.9
Gill	2.43 ±0.334	2.01 ±0.433	1.74 ±0.452	1.48 ±0.342	1.15 ±0.366	0.93 +0.471	0.68 ±0.382	0.49 ±0.244	0.28 ±0.088	1.48 ±0.411
		P<0.05	P<0.005	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
% inhibition	--	17.3	28.4	39.1	52.7	61.7	72.0	79.8	88.5	39.1
Liver	1.47 ±0.342	1.18 ±0.333	1.00 ±0.420	0.81 ±0.066	0.59 ±0.087	0.38 ±0.182	0.24 ±0.094	0.11 ±0.073	0.04 ±0.022	0.95 ±0.201
		NS	P<0.025	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
% inhibition	--	19.7	32.0	44.9	59.9	74.2	83.7	92.5	97.3	35.4

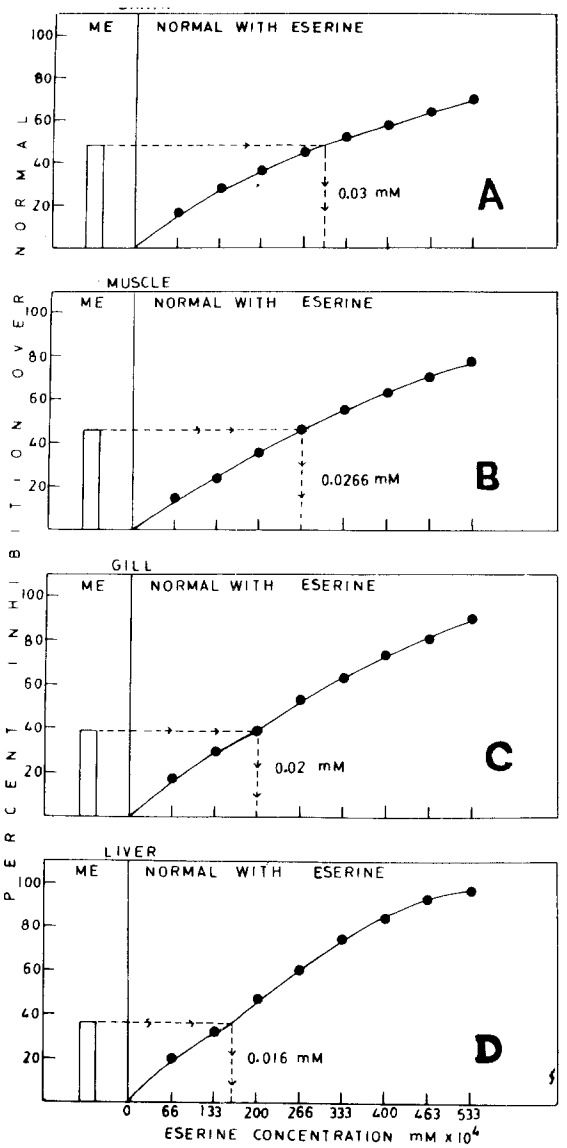

Figure 1. Acetylcholinesterase activity in brain (A), muscle (B), gill (C) and liver (D) tissues of normal with different concentrations of eserine (in vitro) and malathion exposed (ME) fishes.

The four concentrations of eserine determined as equipotent in inhibiting AChE of normal tissues as in the respective ME fish tissues were further tested for similarity in enzyme-substrate affinity (Km) with the AChE in ME fish tissues (Fig. 2 A to D). The Vmax and intercept values of the AChE of brain, muscle, gill and liver tissues of normal, normal with eserine assayed and ME fishes were almost similar, while the Km values showed variations (Table 2). Thus both malathion (in vivo) and eserine (in vitro) exerted a competitive type of inhibition on AChE. Inspite of the structural resemblence (CORBETT 1974, HARPER 1977) competitive inhibitors are known to compete with the substrates for the same active site resulting in the competitive type of inhibition as in the present study. Thus the competitive inhibition obtained by eserine and malathion might be due to their resemblence with acetylcholine. Though both malathion and eserine exerted a competitive type of inhibition, the Km values differed suggesting that, the extent of inhibition is same but the inhibitory potency is different.

Nevertheless, in brain and muscle tissues, high Km values were found to be associated with malathion, while in the gill and liver tissues high Km values were associated with tissues assayed with eserine, indicating that the eserine concentration determined through inhibition studies is lower in brain and muscle tissues, but higher in gill and liver tissues. Thus, a particular concentration of eserine, although it produced similar percentage of AChE inhibition in normal tissues to that of the respective ME tissues, it does not seem to have similarity in their effects on enzyme-substrate affinity of AChE in the respective tissues.

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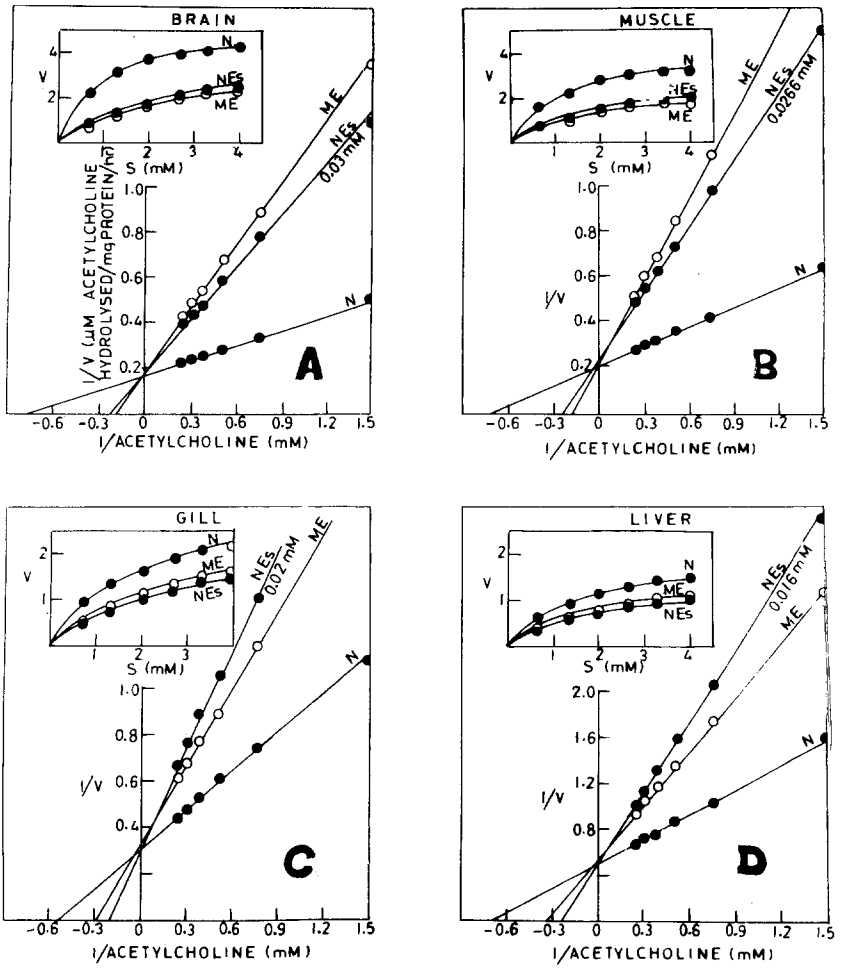

Figure 2. Line weaver-Burk plots of acetylcholinesterase in brain (A), muscle (B), gill (C) and liver (D) tissues of normal (N, ●—●), normal with eserine (NEs, ●—●) and malathion exposed (ME, O—O) fishes. In the inserts are given the substrate versus enzyme velocity curves.

TABLE 2

Kinetic parameters of acetylcholinesterase in brain, muscle, gill and liver tissues of normal, normal with eserine added and malathion-exposed (ME) fishes. Plottings are shown in Figure 2, A to D.

Kinetic Parameter	Normal	Normal with eserine	ME	Normal	Normal with eserine	ME
		Brain			Muscle	
Slope	0.192	0.765	0.919	0.285	0.979	1.25
Intercept	0.188	0.196	0.194	0.214	0.237	0.218
Vmax	5.33	5.09	5.16	4.68	4.22	4.58
Km(mM)	1.02	3.90	4.74	1.34	4.13	5.72
		Gill			Liver	
Slope	0.531	1.45	1.11	0.705	2.03	1.53
Intercept	0.328	0.319	0.339	0.505	0.550	0.573
Vmax	3.05	3.14	2.95	1.98	1.82	1.75
Km(mM)	1.62	4.55	3.27	1.40	3.69	2.67

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