EFFECT OF SUBLETHAL CONCENTRATION OF METHYL PARATHION ON SELECTED OXIDATIVE ENZYMES AND ORGANIC CONSTITUENTS IN THE TISSUES OF THE FRESHWATER FISH, *TILAPIA MOSSAMBICA* (PETERS)

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ABSTRACT

Levels of succinate dehydrogenase and lactate dehydrogenase enzyme activities, carbohydrate and glycogen contents decreased, while protein and free amino acid contents increased in muscle, gill and liver tissues of methyl parathion exposed fishes. The results are discussed in relation to the compensation of proteins and amino acids to meet the increasing energy demand.

INTRODUCTION

THE extensive use of pesticides by the agriculturists[1,2] results in the pollution of the freshwater ecosystems[3] causing toxic effects on several nontarget species[4]. Methyl parathion is also a widely used pesticide[5]. *Tilapia mossambica*, available in this region, is chosen to evaluate the functional modulation on selected oxidative enzymes, choosing succinate dehydrogenase (SDH) and lactate dehydrogenase (LDH) as models along with certain organic constituents in the metabolically active tissues of the fish, during methyl parathion exposure (MPE). Sublethal concentrations of methyl parathion are preferred in the present study, since for this fish, it is a safe dose with high tolerance and nil mortality rate (LC_0), thus facilitating the study of enzymatic changes and associated metabolic responses.

MATERIALS AND METHODS

Tilapia mossambica (Peters) was collected from streams around Tirupati. They were fed with groundnut cake and the water was changed daily and acclimatised to the laboratory conditions for one week. The technical grade methyl parathion of 95% purity was used for experimentation. The methyl parathion was dissolved in methoxyethanol to form stock solution by taking 1 mg/ml equivalent to 1,000 ppm, which was added to the aquaria at 0·1 ml/l of water to yield 0·1 ppm (Methoxyethanol alone was non-toxic in the quantity used)[6].

Fishes (120 each weighing 13·3 ± 1·7 g) were sorted into 20 batches of 6 each and were exposed to 0·1 ppm (Sublethal concentration) for 48 hours after due standardisation by probit analysis[7]. The troughs containing the fishes were aerated to prevent hypoxic or anoxic conditions in the medium. Controls also received similar treatment.

After exposure, the control and MPE fishes were sacrificed and the tissues, muscle, gill and liver were isolated and kept in cold. For enzyme assays, the tissues were homogenised in 0·25 M cold sucrose solution using Yarco homogeniser and centrifuged at 1000 g for 15 min to remove cell debris. The supernatants were employed for enzyme assays.

The lactate dehydrogenase (E.C. 1.1.1.27) was estimated by the method of Srikantan *et al.*[8], and succinate dehydrogenase (E.C. 1.3.99.1) by the method of Nachlas *et al.*[9], as modified by Reddanna and Govindappa[10]. The total carbohydrate and glycogen contents were determined by using anthrone reagent[11]. The protein content was determined using Folin's reagent[12] and the free amino acid content was determined by using ninhydrin reagent[13]. The mean values of control and MPE fishes were subjected to statistical analyses using student '*t*' test as described by Bailey[14].

RESULTS AND DISCUSSION

Data presented in Table I show a general decrease in both SDH and LDH enzyme activities and also a decrease in the total carbohydrate and glycogen contents in the three tissues of MPE fishes. However, the total free amino acids and the total protein contents were enhanced. Within the tissues, the decrease in SDH was as follows : liver > muscle > gill, while LDH decrease showed the following trend : gill > muscle > liver. The decrease in the total carbohydrates and glycogen indicated the following trend : muscle > liver > gill, while the increase in the total proteins and free amino acid contents in the tissues were as follows : liver > gill > muscle.

Though the oxidative enzymes and its associated substances decreased in MPE fishes, the tissue specific trend showed the gill to be relatively more aerobic than the muscle and the liver (Table I). However, the lower decrease of dehydrogenases in the muscle as compared with the liver, is due to the choice of the red muscle which is aerobic and which contains the myoglobin pigment. Glycogen shows a rapid decrease, suggestive of its immediate utilisation in the

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TABLE I

Levels of carbohydrates, glycogen, total free amino acids, total proteins (mg/gm wet wt.) and succinate and lactate dehydrogenase activities (μM formazan/mg protein/h) in tissues of control and methyl parathion exposed (MPE) fishes

Sl. No.	Enzyme/organic constituents	Muscle		Gill		Liver	
		Control	MPE	Control	MPE	Control	MPE
1.	Carbohydrates	4·34 ±0·43	2·64 ±0·06 −39% P < 0·01	0·81 ±0·02	0·68 ±0·02 −16% P < 0·002	43·98 ± 1·26	29·81 ± 1·41 −32% P < 0·001
2.	Glycogen	1·36 ±0·02	0·30 ±0·01 −78% P < 0·001	0·23 ±0·02	0·17 ±0·02 −26% P < 0·05	15·71 ± 0·38	8·73 ±0·06 −44% P < 0·001
3.	Total free amino acids	17·92 ± 1·39	21·60 ± 1·30 +25% P < 0·05	8·40 ± 0·87	14·44 ± 0·98 +72% P < 0·01	13·96 ±1·35	25·28 ± 3·50 +81% P < 0·02
4.	Total proteins	116·49 ± 2·66	127·11 ± 2·09 +9% P < 0·02	83·36 ± 6·71	98·54 ± 2·66 +18% P < 0·05	91·76 ± 6·19	126·39 ± 3·76 +38% P < 0·01
5.	Succinate dehydrogenase	0·251 ±0·005	0·157 ±0·007 −37% P < 0·001	0·022 ±0·001	0·015 ±0·002 −32% P < 0·01	0·142 ±0·015	0·067 ±0·004 −53% P < 0·01
6.	Lactate dehydrogenase	0·025 ±0·001	0·015 ±0·001 −40% P < 0·001	0·009 ±0·002	0·003 ±0·001 −66% P < 0·05	0·053 ±0·001	0·035 ±0·001 −34% P < 0·001

Each value is the mean of six individual observations. ± indicates SD.
The signs + or − indicate per cent increase or decrease over control.
P = '*t*' test (significant).

tissues; this suggests that such of those carbohydrates that are converted to glycogen or glucose are utilised to meet the excess demands of the energy metabolism.

The total free amino acids and the protein contents increased in the three tissues of the MPE fishes and the increase was found to be more in liver, because the liver was a major site for the sythesis of proteins[14]. *In vitro* studies using labelled amino acids in the same tissues of malathion exposed *Tilapia*, sp. confirmed that protein synthesis was more in the liver than in the other tissues[15].

The high increase in the total free amino acids suggests that it is partly utilised for the protein synthesis as observed by Kabeer[15] and partly for glyconeogenesis through the transamination and the transdeamination reactions to supply the necessary keto acids to act as precursors for the maintenance of carbohydrate metabolism to meet the energy requirements during the methyl parathion exposed stress condition. Documented evidence shows that transamination and transdeamination reactions are prominent under stress conditions[15-17].

Hence it can be inferred that in the MPE fishes, there is compensation by proteins and free amino acids to meet the energy demands.

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