Proc. Indian Acad. Sci., Vol. 87 B, (Animal Sciences-4) No. 12, December 1978, pp. 377-380,

Effect of malathion on free amino acids, total proteins, glycogen and some enzymes of pelecypod *Lamellidens marginalis* (Lamarck)

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MS received 6 February 1978; revised 7July 1978

Abstract. Changes in glycogen, total proteins, total free amino acids, succinate dehydrogenase, aspartate and alanine aminotransferases were estimated in foot, mantle and hepatopancreas of normal and malathion exposed mussels. All the organic-constituents along with succinate dehydrogenase showed a decrease while the activities of aspartate and alanine aminotransferases were enhanced. The probable significance of the observed changes were correlated with the phenomena of metabolic compensation and co-operativity among enzymes studied, to meet the toxic stress.

Keywords. Malathion; succinate dehydrogenase; aspartate and alanine aminotransferase; *Lamellidens marginalis*.

1. Introduction

Though the use of pesticides served to ameliorate the crops from the ravages of pests and insects, nevertheless it causes untold hazards to non-target species (Matsumura *et al* 1972). Several attempts have been made to evaluate their hazardous effects on many species by several workers (Cooke and Pollard 1973; Boyer 1975; Robert *et al* 1975; Rand 1977; Kabeer *et al* 1978). However, the effect of the pesticides on pelecypods remains to be studied. Since mussel also forms an integral part of the fresh water ecosystem, it is felt worthwhile to have an insight into the effect of pesticides on the metabolism of this animal. Due to the wide applicability of malathion (the organophosphatic pesticide) in recent times (Patel and Rai 1964) we have chosen this pesticide for our present study.

2. Materials and methods

The mussels *Lamellidens marginalis* (Lamarck) were collected from ponds and streams around Tirupati. They were fed with fresh water plankton and were adapted to laboratory conditions. The commercial grade malathion (50%) was obtained from Auditya mineral traders, Kondapuram, Cuddapah, AP and 5 ppm concentration (sublethal) was prepared by taking one mg per one ml equivalent to 1000 ppm as standard.

A total of 120 animals weighing 24·4±2·6 grams were separated into 12 batches of 10 each. Of these 6 batches were exposed to tap water which served as controls, while the remaining 6 batches were exposed to 5 ppm malathion (sub-lethal) for 48 h. In each batch the proposed studies were conducted as follows.

After sacrificing the animal, three tissues viz., foot, mantle and hepatopancreas were isolated and kept in cold in an ice jacketed pyrex petridish. For enzyme assays, the tissues were homogenised in cold 0·25M sucrose solution using yarco homogeniser (Yarco Scientific industries, New Delhi) and centrifuged at 600 g for 15 min in a Remi T8A centrifuge (Remi Udyog, Bombay, India). The supernatants were employed for enzyme assays.

The aspartate (AAT) (EC 2.6.1.1) and alanine (AIAT) (EC 2.6.1.2) aminotransferases were estimated by the method of Reitman and Frankel (1957), while succinate dehydrogenase (SDH) (EC 1.3.99.1) activity was estimated by the method of Nachlas *et al* (1960). The glycogen content was estimated by using anthrone reagent (Carrol *et al* 1956). The proteins were estimated by the method of Lowry *et al* (1951). The free amino acids were determined by the method of Moore and Stein (1957). Statistical evaluation of the data was made by using students *t* test as described by Snedegor (1956).

3. Results and discussion

The activity of succinate dehydrogenase was found to decrease, while the catalytic output of aminotransferases enhanced, with an overall decrease in all the organic constituents, like glycogen, proteins free amino acids, in all the three tissues of malathion exposed fresh water mussels (table 1).

The increase in aspartate and alanine aminotransferases suggests the existence of heavy drain on the metabolites during malathion exposure. Since stress is known to induce elevation of aminotransferases (Knox and Greengord 1965; Kulkarni and Mehrotra 1973), the toxic impact caused by malathion should be the reason for their elevation (table 1).

To visualise the comparative catalytic output of the aminotransferases and to ascertain the possible shift in feeding of metabolites into krebs cycle, the velocity ratios of AAT/AIAT were calculated. The ratios showed the same trend in the malathion exposed animals as found in the controls. However there is a shift in emphasis more towards AIAT in the tissues of malathion exposed mussels, which envisages, enhanced incorporation of amino acids, through alanine and pyruvate (table 2).

The depletion in amino acid pool, brought about by enhanced aminotransferase activities corroborates with the decrease in the protein content (table 1), suggestive of proteolysis, possibly to compensate the decrease in the amino acid pool and to overcome the impeding energy demands under toxic stress.

Since anoxia and hypoxia were known to increase the carbohydrate consumption (Dezwaan and Zandee 1972), the depletion in glycogen content and consequent decrease in succinate dehydrogenase acitvity in malathion exposed mussels suggests the possibility of anoxia or hypoxia.

Since carbohydrates form the key substrates for energy metabolism (Peter 1973), the decrease in glycogen is in consonance with the decreased succinate dehydrogenase

Table 1. Changes in organic constituents and certain enzymes in the selected tissues of controls and malathion exposed (ME) mussels *Lamellidens marginalis* (Lamarck)

S. No.	Enzyme/organic constituents	Foot		Mantle		Hepatopancreas	
		Control	ME	Control	ME	Control	ME
1.	*Aspartate amino transferase**	3·01±0·15	4·17±0·35 +38·40 P<0·001	6·45 ±0·45	8·66±0·47 +34·20 P<0·001	5·08 ±0·18	5·86±0·21 +14·40 P<0·001
2.	*Alanine amino-transferase** μ moles of sodium pyruvate formed/ mg protein/hour	3·62±0·14	5·63±0 38 +55·50 P<0·02	6·13 ±0·23	9·12±0·52 +48·20 P<0·01	5·04 ±0·17	7·78±0·48 +35·23 P<0·02
3.	*Succinate de-hydrogenase* μ moles of formazan formed/ mg protein/hour	0·61±0·02	0·55±0·02 −10·42 P<0·001	0·23 ±0·02	0·21±0·02 −9·22 P<0·02	0·53 ±0·02	0·46±0·02 −12·48 P<0·01
4.	*Glycogen* mg glycogen/gm wet weight of tissue	18·65±2·30	14·83±0·81 −20·48 P<0·01	33·30 ± 3·50	25·09±1·85 −24·65 P<0·01	22·45 ±2·01	17·70±1·67 −21·15 P<0·01
5.	*Total proteins* mg protein/gm wet weight of the tissue	48·88±3·08	41·83±4·47 −15·30 P<0·02	24·13 ±1·87	19·80±1·95 −17·85 P<0·01	27·58 ±2·33	24·80±2·52 −9·69 P<0·02
6.	*Total free amino acids* μ moles of TFAA/ gm wet weight of the tissue	1007·83±25·16	874·10±30·87 −13·26 P<0·001	390·30 ±26·50	285·00±21·90 −26·90 P<0·001	866·60 ±30·76	754·10±40·86 −12·98 P<0·01

Each value is the mean of six individual observations, ± indicates SD. The signs + or − indicates per cent increase or decrease over normal. P = 't' test. All values are significant.

Table 2. AAT/AIAT ratios in selected tissues of controls and malathion exposed (ME) mussels.

Treatment	Foot	Mantle	Hepatopancreas
Controls	0·8316	1·052	1·008
ME (5 ppm)	0·7578	0·9495	0·7530

activity in tissues, which in turn necessitates the enhancement of other alternative mechanisms like amino transferase reactions to feed the ketoacids into the TCA cycle, but fails to retain its original activity, thus showing a decline in its activity suggests of availability of insufficient substrate during toxic stress. However with an overall decrease in all the organic constituents, there appears to be the occurrence of the phenomena of compensation and co-operativity between aminotransferases and succinate dehydrogenase under the malathion exposed stress condition.

Acknowledgement

The authors thank Prof. K S Swami, for his encouragement.

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