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Chronic Malathion Toxicity: Effect on Carbohydrate Metabolism of *Oziotelphusa senex senex*, the Indian Rice Field Crab

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The use of organophosphate pesticides has increased extensively in recent years to control a wide variety of agricultural pests due to the ban on chlorinated pesticides. However, because of misuse these chemicals in agricultural and public health operations, many 'non-target' species, some of them very important members of food chain, are adversely affected. The tragic incidence of 'Handigodu syndrome' of Karnataka (India) has been attributed to be due to the 'long-term' consumption of pesticide-poisoned crabs and fish by the local population (National Institute of Nutrition 1977). In view of this, an elaborate program to evaluate the impact of pesticides on the physiology and biochemistry of some non-target species has been undertaken. The present report, the first one of the series, deals with the effects of Malathion on carbohydrate metabolism in the tissues of crab. Malathion has a wide range of applications in agriculture as an effective systematic pesticide. The crabs are abundantly available locally and are usually considered as 'poor-man's protein'.

MATERIALS AND METHODS

Specimens of Oziotelphusa senex senex having an average weight of 30 g were collected from local rice fields where no evidence of any contamination by anticholinesterases were reported (Nandakumar et al. 1983) and were acclimatized singly in 1 L glass aquaria (temperature 30°C; relative humidity, 75% photoperiod, 12:12 L:D) for 10 days before experimentation. Only intermoult (Stage C_4), uninjured, male crabs were selected for the present study. Technical grade (95% W/V) Malathion (O, O-dimethyl S-)1,2-dicarboxyethyl) phosphorothioate $C_{10}H_{19}O_6PS_2$) obtained gratis from Cyanamid India Ltd., Bombay was used. $^{L}C_{50/24h}$-value computed by the probit method (Finney 1964) was found to be 15mg Malation L^{-1} of water and 2 mg malation L^{-1} was a sublethal concentration during 60 days exposure.

Two hundred and forty crabs were selected for the present study and divided into six equal groups. The first three groups were exposed to 2 mg Malathion, L^{-1} for 10, 20, and 30 days respectively, whiel the remaining three groups were maintained as controls. The experimental and control crabs were fed daily ad libitum with frog

muscle and the medium in which they were placed was renewed every 24 h. Hemolymph (about 2 mL from each crab) was collected with a hypodermic syringe inserted through the arthrodial membrane into chilled glass tubes. Hepatopancreas and muscle were isolated on ice, placed in crab ringer (Van Harreveled 1936) and used for the estimation of following parameters.

Glycogen and total carbohydrates were estimated using anthrone reagent (Carroll et al. 1956). Glycogen phosphorylase (EC.2.4.1.1) was assayed in the direction of glycogen synthesis (Cori et al. 1955) and the liberated inorganic phosphate was determined using molybdate reagent (Fiske and Subba Rao 1925). Aldolase was assayed by the method of Burns and Bergmeyer (1965). Succinate dehydrogenase (EC. 1.2.99.1) was assayed according to the method of Nachlas et al. (1960). Lactate dehydrogenase (EC,1.1.1.27) and pyruvate dehydrogenase EC.1.2.3.3) was estimated by the method of Srikanthan and Krishnamurthi (1955). Protein content was determined by the method described by Lowry et al. (1951) using bovine serum albumin as standard.

RESULTS AND DISCUSSION

No crabs died during exposure to 2 mg Malathion.L^{-1} for 30 days. The first reaction following exposure to Malathion appeared after 15 days. At that time, it was noted that treatment crabs failed to consume the normal ration of food. After 25 days, treated crabs completely rejected food, while the control crabs continued to feed normally.

Biochemical profiles of tissues before and during Malathion treatment are summarised in Tables 1 and 2. Crabs were hyperglycemic after 10,20 and 30 days of treatment. There was a gradual depletion in both glycogen and total carbohydrate levels in hepatopancreas and muscle at the three time periods. Glycogen phosphorylase activity increased in both the tissues during Malathion exposure. Decrease in glycogen content and increase in phosphorylase activity are indicative of increased rate of glycogenolysis. Aldolase activity was considerably inhibited in both the tissues. In light of depleted aldolase activity, decreased glycogen content suggests the onset of glycogenolysis forming free glucose and its possible exit into the hemolymph. Gopal et al. (1980) reported elevated levels of blood sugar in Clarias batrachus exposed to Endosulfan. Koundinya and Ramamurthi (1979) reported hyperglycemia in Sumithion exposed Sarotherodon mossambicus accompanied by decrease of glycogen levels in liver and muscle. Fingerman et al. (1981) found repeated discharges of crustacean neurons and hyperglycemia in DDT-intoxicated crabs. They suggested that the discharged material was a hyperglycemic hormone. It is conceivable in the present case that Malathion might cause discharge of hyperglycemic hormone from the neurosecretory cells that synthesize it in a similar way. Earlier, Hohnke and Scheer (1970) suggested that the primary role of the so called 'hyperglycemic hormone' is not to elevate hemolymph sugar level but to elevate intracellular glucose through the degradation of glycogen by activating the

Table 1. Alterations in the levels of glycogen and total carbohydrates of hemolymph, hepatopancreas, and muscle in control Oziotelphusa senex senex and those exposed to 2.0 mg Malathion. L^{-1}

	Tissue	10 days		20 days		30 days	
		Control	Exposed	Control	Exposed	Control	Exposed
Total carbohydrate	Hemolymph	71.84 ± 2.28	90.99* ± 3.04 (+26.66)	70.53 ± 5.13	105.83* ± 5.33 (+50.05)	70.08 ± 4.39	130.35 ± 6.17
	Hepatopancreas	13.57 ± 0.73	9.66* ± 0.43 (−28.79)	13.47 ± 0.47	6.37* ± 0.19 (−52.71)	14.20 ± 0.79	5.29* ± 0.21
	Muscle	1.40 ± 0.16	1.03* ± 0.06 (−26.43)	1.36 ± 0.08	0.91* ± 0.07 (−33.09)	1.38 ± 0.21	0.80* ± 0.04
Glycogen	Hepatopancreas	4.37 ± 0.48	3.21** ± 0.39 (−26.55)	4.26 ± 0.53	3.09* ± 0.21 (−27.47)	4.44 ± 0.29	2.67* ± 0.31 (−39.87)
	Muscle	0.67 ± 0.06	0.41* ± 0.03 (−38.81)	0.69 ± 0.04	0.39* ± 0.04 (−43.48)	0.66 ± 0.08	0.32* ± 0.06 (−51.52)

Values are expressed in mg/g wet weight of fresh tissue; mg/100 mL of hemolymph
Each value is mean of ± SD of 8 individuals
Values in parentheses are percent change. (+) indicates increase; (−) decrease
Values are statistically significant at *($P < 0.001$); **($P < 0.01$).

Table 2. Alterations in the activity levels of phosphorylase ('a' and 'ab') aldolase and succinate, lactate and pyruvate dehydrogenases of hepatopancreas and muscle in control Oziotelphusa senex senex and those exposed to 2.0 mg Malathion. L^{-I}

Enzyme	Tissue	10 days		20 days		30 days	
		Control	Exposed	Control	Exposed	Control	Exposed
Phosphorylase[a]	Hepatopancreas	2.52 ± 0.28	4.66* ± 1.09 (+ 84.92)	2.54 ± 0.19	5.23* ± 1.23* (+105.91)	2.61 ± 1.14	5.78* ± 1.19 (+121.46)
	Muscle	4.91 ± 0.56	7.88* ± 1.43 (+ 60.49)	4.88 ± 0.67	8.04* ± 0.91 (+ 64.75)	4.94 ± 0.93	8.88* ± 2.86 (+ 79.76)
'ab'	Hepatopancreas	4.89 ± 0.36	6.93* ± 1.93 (+ 41.72)	4.73 ± 0.91	8.06* ± 2.02 (+ 70.40)	4.69 ± 1.06	9.09* ± 1.77 (+ 93.82)
	Muscle	7.29 ± 0.95	10.46* ± 2.44 (+ 43.48)	7.81 ± 1.46	12.43* ± 1.01 (+ 59.16)	7.66 ± 2.09	12.99* ± 2.03 (+ 69.58)
Aldolase[b]	Hepatopancreas	4.83 ± 0.37	2.69* ± 0.31 (- 44.31)	4.69 ± 0.41	2.09* ± 0.44 (- 55.44)	4.88 ± 0.44	2.01* ± 0.61 (- 58.81)
	Muscle	3.66 ± 0.61	2.73* ± 0.43 (- 25.41)	3.54 ± 0.47	2.29 ± 0.33 (- 35.31)	3.59 ± 0.66	2.00* ± 0.19 (- 44.29)

Table 2. continued

Enzyme	Tissue	10 days		20 days		30 days	
		Control	Exposed	Control	Exposed	Control	Exposed
Succinate dehydrogenase[c]	Hepatopancreas	132.29 ± 4.91	60.39* ± 4.00 (-54.35)	130.44 ± 6.71	49.66* ± 7.31 (-61.92)	134.66 ± 6.67	40.09* ± 8.19 (-70.23)
	Muscle	124.90 ± 8.98	73.81* ± 8.99 (-40.91)	125.09 ± 7.67	70.69* ± 6.44 (-43.39)	122.96 ± 6.63	63.39 ± 3.07 (-48.45)
Pyruvate dehydrogenase[c]	Hepatopancreas	9.66 ± 1.09	5.31* ± 1.14 (-45.03)	9.81 ± 2.01	4.52* ± 1.06 (-53.93)	9.73 ± 0.16	3.98* ± 0.09 (-59.10)
	Muscle	4.31 ± 0.14	2.99* ± 0.16 (-30.63)	4.22 ± 0.26	2.73* ± 0.11 (-35.31)	4.34 ± 0.21	2.55* ± 0.09 (-41.24)
Lactate dehydrogenase[c]	Hepatopancreas	8.35 ± 1.49	11.91* ± 0.93 (+42.61)	8.66 ± 1.33	12.61* ± 1.09 (+45.61)	8.40 ± 1.22	18.09* ± 2.69 (+115.36
	Muscle	25.23 ± 4.45	30.06** ± 6.71 (+19.14)	24.66 ± 4.01	32.33* ± 6.73 (+31.10)	22.09 ± 6.73	39.09* ± 7.33 (+72.43)

Values expressed as a µmol of Pi liberated/mg protein/h
b µmol of PDP cleaved/mg protein/h
c nmol of formazan formed/mg protein/h
Each value is mean of ± S.D of 8 individuals
Values in parentheses are percent changes (+) indicates stimulation; (-) inhibition
Values are statistically significatn at *($P < 0.001$); **($P < 0.01$)

enzyme phosphorylase. These glucose molecules leak into the hemolymph causing hyperglycemia.

Alterations in the activity levels of certain biologically important enzymes - succinate dehydrogenase, pyruvate dehydrogenase and lactate dehydrogenase - were significant during Malathion exposure (Table 2). The maximum stimulation in lactate dehydrogenase was observed in hepatopancreas after 30 days, and the minimum in muscle at 10 days of exposure. However, pyruvate and succinate dehydrogenases were found to be decreased significantly in both the tissue but mainly in hepatopancreas after the Malathion exposure. The inhibition of pyruvate dehydrogenase and succinate dehydrogenase and the stimulation of lactate dehydrogenase indicate that pyruvate is not catabolised via the Kreb's cycle but via lactic acid cycle with the formation of lactic acid in the tissue during Malathion exposure and indicate depression in cellular oxidation and development of anaerobic conditions. Such an inhibition of Kreb's cycle enzymes at the tissue level might in turn be responsible for decreased oxygen consumption at the whole animal level (Bhagyalakshmi 1981). In the stressed crab various deleterious structural alterations occurred in the gill (author's unpublished data) which affect the respiratory exchange, leading to declining oxygen consumption (Bhagyalakshmi 1981). This has also been supported by a number of workers, who found suppressed respiratory metabolism after exposure to Sumithion and Sevin (Koundinya and Ramamurthi 1978), Malathion and Methylparathion (Sivaprasada Rao and Ramana Rao 1979; Nagarathnamma and Ramamurthi 1982).

Since, the activity levels of these enzymes have been an important diagnostic tool to measure the metabolic status of the animal, it is speculated that they may be used as a valuable indicator of the stress condition in the crab. Thus it appears from the present investigation, that the metabolism of the subacute Malathion exposed crab is geared to lowered oxidative and increased glycolytic enzyme activities. The results observed under these experimental conditions would almost certainly change if factors like temperature, salinity, and reproductive state were changed. Further interaction of various pollutants and/or natural variables can also alter the degree of pollutant effect (Hodson and Blunt 1981).

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