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Carbohydrate Metabolism in Tissue of Fresh Water Crab (*Oziotelphusa senex senex*) Exposed to Methyl Parathion

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Organophosphate insecticides are now increasingly used on account of their biodegradable nature and less persistence in the environment. However, indescriminate use of these pesticides for crop protection sometimes causes much havoc to other aquatic fauna. Continued presence of these chemicals in irrigation canals both in small and large amounts produces various physiological changes in aquatic animals, of which we have unfortunately very little knowledge. Among these physiological changes, energy metabolism has a key role as the animal is forced to expend more energy to mitigate toxic stress. Based on this, an attempt was made to study the sublethal effect of Methylparathion (an organophosphate pesticide) on the carbohydrate metabolism taking the key enzymes, phosphorylase, aldolase, succinate dehydrogenase and lactate dehydrogenase in the tissues of the crab Oziotelphusa senex senex. The crabs are abundantly available locally, and are usually considered as poor man's food.

MATERIALS AND METHODS

Oziotelphusa senex senex were collected from the fresh water resources in and around Tirupati, India. Prior to use they were acclimatized to laboratory conditions for one week and starved before the day of experimentation. During acclimatization, water was changed daily and they were fed earthworms ad libitum.

The technical grade Methylparathion (0.0 dimethyl-0-(4-nitrophenyl) mono-thiophosphate) of 95% purity was used for experimentation. The preparation of stock solution and other test conditions were essentially similar to the procedure described earlier (Nagarathnamma 1982). The lethal limits were determined by probit analysis (Finney 1964) and the LC50 value was found to be 1 ppm for 48 h (Nagarathnamma 1982). Sixty crabs exposed to a sublethal concentration (0.2 ppm) of Methylparathion for 48 h were used in the present study.

Methylparathion was first dissolved in acetone and diluted with tap water so that the final concentration was 0.2 ppm containing 0.01% acetone. The sixty control crabs were kept in tap water containing the same acetone concentration (0.01%) as in tap water containing Methylparathion. The water was analysed for various physico-chemical characteristics and the average values are as follows: pH 7.3; dissolved oxygen content, 6.2 ppm; hardness 38 ppm of $CaCO_3$; temperature, 30°C. Hepatopancreas and muscle tissues isolated from control and experimental crabs were used for the present study.

The tissue total carbohydrates, glycogen (Carroll et al. 1956) and lactate (Barker and Summerson 1941) levels were estimated in Trichloroacetic acid (10% W/V) supernatants of control and experimental animals. The activities of phosphorylase (Cori et al. 1955), aldolase (Fructose-1-6-bisphosphate, D-glyceraldehyde-3-phosphate lyase) (Burns and Bergmeyer 1965), succinate dehydrogenase (succinate oxidoreductate, SDH) and Lactate dehydrogenase (Lactate NAD oxidoreductase, LDH) (Srikanthan and Krishnamurthi 1955) were assayed in tissue homogenates. Two extraction media were employed for enzyme assays; medium A for all enzymes except phosphorylase; and medium B for phosphorylase. Medium A: 100 M phosphate buffer, 0.25 M sucrose, adjusted to pH 7.5. Medium B: 0.03 M cysteine hydrochloride, 0.015M β-glycerophosphate buffer, 0.1 M NaF, 0.037 M EDTA, adjusted to pH 6.5. The protein content in the enzyme source was estimated using folin phenol reagent (Lowry et al. 1951). The liberated inorganic phosphate was estimated according to Fiske and Subbarao's (1925) method. Statistical significance of the difference between control and experimental values was calculated using student's t-test.

RESULTS AND DISCUSSION

The glycogen and total carbohydrate levels were decreased in hepatopancreas and muscle on exposure to Methylparathion. The decrease was more in hepatopancreas compared with muscle, whereas the lactate levels were increased and the increase was more in the muscle compared with hepatopancreas (Table 1). An increase in aldolase activity and a decrease in SDH and LDH activity levels were observed in both the tissues of experimental crabs (Table 2). In general these changes were more pronounced in hepatopancreas compared with muscles. The active phosphorylase ('a') increased significantly in both the tissues, while phosphorylase 'b' was decreased (Table 2).

Table 1. Levels of total carbohydrates, glycogen and lactate (mg/g wet wt tissue) in the tissues of control and Methylparathion exposed crabs

Component	Hepatopancreas		Muscle	
	Control	Experimental	Control	Experimental
Total carbohydrates	12.83 ± 1.14	5.22 ± 1.02* (-59.31)	6.35 ± 0.71	4.61 ± 0.81* (-27.40)
Glycogen	1.86 ± 0.17	0.98 ± 0.07* (-47.31)	0.85 ± 0.21	0.66 ± 0.21** (-22.35)
Lactate	0.79 ± 0.09	1.42 ± 0.21* (+79.75)	0.88 ± 0.11	1.97 ± 0.26* (+123.86)

Values are mean of ± S.D of 8 individuals, Values in parentheses are % change over control. Value are significantly different at *$P < 0.001$; **$P < 0.01$

Table 2. Activity levels of Phosphorylase 'a', 'b', Aldolase, LDH & SDH in the tissues of control and Methylparathion exposed crabs

Enzyme	Hepatopancreas		Muscle	
	Control	Experimental	Control	Experimental
Phosphorylase[a] 'a'	3.41 ± 0.23	4.93 ±0.18* (+44.58)	1.29 ± 0.24	2.09 ± 0.22* (+62.02)
'b'	2.69 ± 0.19	1.44 ± 0.20* (-46.47)	1.07 ± 0.17	0.92 ± 0.14[NS] (-14.01)
Aldolase[b]	6.25 ± 1.23	11.81 ± 2.09* (+88.96)	5.44 ± 1.09	9.01 ± 2.01* (+65.63)
LDH[c]	7.66 ± 0.93	3.22 ± 0.71* (-57.96)	23.45 ± 3.08	18.33 ± 4.09** (-21.83)
SDH[c]	136.72 ± 8.66	86.93 ± 6.73* (-36.42)	124.66 ± 9.01	98.09 ± 9.14* (-21.31)

Values are mean of ± S.D of 8 individuals
Values in parentheses are % change over control
Values are significantly different at *$P < 0.001$; **$P < 0.01$; NS = Not Significant

Values expressed as a) n moles of Pi liberated/mg protein/h
b) μ moles of FDP cleaved/mg protein/h
c) n moles formazan formed/mg protein/h

The decrease in total carbohydrate levels in the tissue of crabs exposed to Methylparathion suggests its mobilization to meet the higher energy demands warranted by the toxic environment. Of the carbohydrates, glycogen decreased rapidly, indicative of its immediate utilization by these tissues. Sivaprasada Rao and Ramana Rao (1983) reported that the synthesis and utilization of glycogen are altered during Methylparathion toxic stress. This view has also been supported by a number of workers who found depletion of glycogen in pesticide intoxicated animals (Srivasthava and Singh 1981, Sastry and Siddiqui 1982). Similarly a reduction in glycogen content in the tissues of the crab Oziotelphusa after the sublethal intoxication by sumithion has been observed (Sreenivasula Reddy et al. 1982). The decrease in tissue glycogen levels observed in the present study might be due to the prevalence of hypoxic/anoxic conditions, which normally increase glycogen use (Dezwan and Zandee 1978). Dissolved oxygen concentration (6.2 ppm) did not differ in the experimental solution when compared to normal tap water. An anoxic condition in the crab Oziotelphusa exposed to sumithion was reported earlier (Bhagyalakshmi et al. 1983). Increased lactate levels in the tissues of the experimental crabs also supports this speculation.

The increased phosphorylase 'a' activity in hepatopancreas and muscle tissues of Methylparathion exposed crabs confirms the active breakdown of tissue glycogen for metabolic processes to meet the augmented stress condition. The increase in phosphorylase 'a' form suggests rapid conversion of the inactive 'b' form into phosphorylase 'a' form thereby quantitatively elevating phosphorylase 'a' to manifest its activity under toxic stress. Correspondingly, there is a decrease in the phosphorylase 'b' activity in the tissues of experimental crabs.

The aldolase, that cleaves the hexoses into trioses in the glycolysis increased significantly in both the tissues of Methylparathion exposed crabs, further suggesting that the animal attempts to gear up the mobilization of the reserves as an attempt to maintain high energy potentials. An increase in aldolase activity and a decrease in LDH activity resulted in the elevation of lactate levels in the tissues indicated that the rate of glycolysis in the tissues was higher in crab exposed to Methylparathion. It appeared that aerobic oxidation through Krebs cycle was adversely affected by the inhibition in the SDH activity. The unequivocal depression of SDH and accumulation of lactate indicate favoring anaerobic metabolism in Methylparathion stressed crab. Such an assumption is supported by the findings of Bhagyalakshmi et al (1983), who observed inhibition of SDH

in the tissues of Oziotelphusa exposed to sumithion. Bhagyalakshmi (1981) also reported that oxygen consumption in the tissues was depressed in Oziotelphusa by sumithion. This is due to the 'coagulation film anoxia', in which mucus was lost from gills, adversely affecting absorption of oxygen from the surrounding medium. Histological evidence in fishes shows that Methylparathion damages the surface cells and blood capillaries of the gill filaments by a deposition of a thick mucous layer (Nagarathnamma 1982). A similar situation seems to operate in the crab Oziotelphusa senex senex under the above mentioned stress condition of methylparathion which results in failure to absorb the required amount of oxygen and depend on anaerobic metabolism for energy to meet various metabolic demands.

The present study concludes that the Methylparathion decreased oxidative metabolism in the tissues of crabs. Consequently these crabs switch over to anaerobiosis as an adaptive measure to combat the induced pesticide toxicity.

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