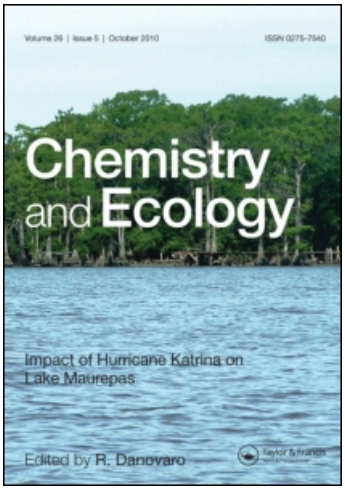

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ALTERATIONS IN CARBOHYDRATE METABOLISM IN SELECTED TISSUES OF CRAB, *OZIOTELPHUSA SENEX SENEX* (FABRICIUS) UNDER FENVALERATE-INDUCED STRESS

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Changes in carbohydrate metabolism in hepatopancreas and muscle of the crab, *Oziotelphusa senex senex* exposed to a sublethal concentration (0.2 ppm) of fenvalerate, a pyrethroid insecticide, were studied. The glycogen and total carbohydrate levels decreased significantly in the tissues of crab exposed to fenvalerate. An increase in phosphorylase 'a' and decrease in aldolase activity levels suggested increased glycogenolysis, and decreased glycolysis during fenvalerate toxicity. Krebs cycle enzymes such as NAD-isocitrate dehydrogenase, succinate dehydrogenase and malate dehydrogenase were decreased, suggesting reduced mitochondrial oxidative metabolism. Glucose-6-phosphate dehydrogenase activity was increased significantly, indicating enhanced oxidation of glucose through the hexose monophosphate shunt pathway. Lactate dehydrogenase activity was elevated indicating the development of anaerobic conditions at tissue level in the stressed crab. Cytochrome C oxidase and Mg^{2+} ATPase activity levels were also decreased, indicating the impaired energy synthesis and prevalence of energy crisis. These results suggest that fenvalerate has a profound effect on the glucose metabolism of crab.

KEY WORDS: Pyrethroid insecticide, crab tissues, fenvalerate, enzyme function

INTRODUCTION

Water pollution has been increasing in recent years due to indiscriminate and widespread use of insecticides. Recently, synthetic pyrethroid compounds with a broad spectrum of insecticidal action are being used extensively for crop protection in India as well as in other countries, because of their non-persistent nature in the environment and high insecticidal toxicity with low mammalian toxicity (Elliot and James, 1978; Casida *et al.*, 1983). The continued use of these chemicals for agriculture in both small and large amounts leads to various physiological changes in aquatic non-target species (Clark and Matsumura, 1982; Ghosh, 1989). Among these physiological changes, energy metabolism has a key role as the animal is forced to spend more energy to mitigate the imposed toxic stress.

The crab, *Oziotelphusa senex senex*, is part of the food chain and is fished commercially for human consumption. There is interest in *O. senex*, first as part of a biocoenose accumulating pollutants, and secondly as a source of potential toxicants in human diet. Fenvalerate is a broad spectrum pyrethroid insecticide and is toxic to several aquatic biota in low concentrations, which include a variety of crustaceans and fish (Radhaiah, 1989). In the present investigation, the changes in glucose

metabolism in selected tissues of the crab, *O. senex*, were studied during induced toxicity of a sublethal concentration of fenvalerate. The alterations in the metabolites and in enzyme activities during fenvalerate sublethal toxicity may therefore have diagnostic significance in evaluation of adverse health effects of toxic substances.

MATERIALS AND METHODS

Animals: Adult healthy, male specimens of *Oziotelphusa senex senex* (body weight, 30–32 g; carapace length, 30–35 mm) were collected from local rice fields and fed *ad libitum* with frog muscle. Prior to use they were acclimatized to laboratory conditions for one week. The media in which they were placed were changed every 24h and analyzed for various physico-chemical characteristics. The average values are as follows: temperature, 25 ± 1°C; pH, 7.3; dissolved oxygen content, 6.2 ppm; acidity as $CaCO_3$, 3.3 mg/l; alkalinity as $CaCO_3$, 9.7 mg/l; total hardness, 35 mg/l.

Pesticide: Technical grade fenvalerate of 96% purity was obtained from Gujarat Insecticides Ltd., Ahemadabad, India and a stock solution was prepared in acetone as described earlier (Reddy *et al.*, 1991). The LC_{50} value was determined by the probit method (Finney, 1964) and was found to be 0.6 ppm for 48h exposure.

Crabs were exposed to a sublethal concentration of 0.2 ppm (1/3 of LC_{50}/48h) of fenvalerate for 1, 3 and 7 days. After stipulated time intervals, hepatopancreas and muscle were quickly dissected out, weighed and used for the estimation of metabolites and for assay of enzymes.

Estimate of total carbohydrate content: Total carbohydrate levels in tissue homogenates were estimated following the method of Carroll *et al.* (1956). Tissue homogenates of 1% (W/V) were made in 10% (W/V) trichloroacetic acid (TCA) solution. To 0.5 ml of the centrifuged (3000 rpm for 15 min) clear supernatant, 5.0 ml of anthrone reagent was added and the combination boiled for 10 minutes in a water bath. The tubes, with their contents, were then immediately cooled. A standard sample containing a known quantity of Analar glucose solution was always run along with the experimental samples. The colour was measured at 620 nm in a Bausch and Lomb Spectronic 20 against a reagent blank.

Estimate of glycogen content: Tissue glycogen level was estimated in the ethanolic precipitate of TCA supernatants as described above following the method of Carroll *et al.* (1956).

Enzyme assays: The isolated tissues were minced with scissors and homogenized (5% W/V) at 4°C in an ice-jacketed glass homogenizer with a motor-driven teflon-coated pestle. The homogenate was centrifuged at 750 g for 10 minutes. The pellet obtained was discarded. The supernatant was again centrifuged at 17,000 g for 20 minutes and then the pellet suspended in the homogenizing medium and again centrifuged at 17,000 g for 20 minutes. The pellet thus obtained was resuspended in the homogenizing medium and used for the assay of succinate dehydrogenase, malate dehydrogenase, NAD-isocitrate dehydrogenase, cytochrome C oxidase and Mg^{2+} ATPase. The activity levels of phosphorylase, aldolase, lactate dehydrogenase and glucose-6-phosphate dehydrogenase were measured in 105,000 g supernatant (cytosol) fractions.

Two extraction media were employed: Medium A, for all enzymes except phosphorylase, and medium B for phosphorylase. (Medium A: 0.25M sucrose solution adjusted to pH 7.4; medium B: 0.1M sodium fluoride, 0.037M EDTA, 0.03M cysteine, 0.015M glycerophosphate adjusted to pH 6.5.)

Enzyme activities were measured at 37°C with appropriate enzyme and reagent blank using a spectrophotometer. The optimal conditions for each enzyme with respect to pH, and substrate and co-factor concentration in the tissues of crab were established earlier (Reddy, 1981, 1990; Bhagyalakshmi, 1981; Bhagyalakshmi *et al.*, 1984a). Optimal assay conditions for individual enzymes (nomenclature, IUB, 1978) were as follows:

Phosphorylase (1-4-glucon: orthophosphate glucosyl transferase; EC 2.4.1.1.) was assayed in the direction of glycogen synthesis (Cori *et al.*, 1955). The cytosol fraction (0.3 ml) was added to 0.2 ml of 2% glycogen and incubated for 20 minutes at 37°C. The reaction was started by the addition of 0.2 ml of 0.016 M glucose-l-phosphate (G-l-P) to one tube (phosphorylase 'a'), 0.2 ml of G-l-P and 0.004 M AMP to the other (phosphorylase 'ab'). After incubation for 15 minutes for phosphorylase 'ab' and 30 minutes for phosphorylase 'a', the reaction was stopped by the addition of 1 ml of 10% sulphuric acid. The inorganic phosphate liberated was estimated (Fiske and Subbarao, 1925).

Aldolase (fructose-1,6-diphosphate, D-glyceraldehyde-3-phosphate lyase; EC 4.1.2.13) was assayed (Burns and Bergmeyer, 1965) in a medium of 3.0 ml containing 1.75 ml of collidine hydrazine buffer (pH 7.4), 0.2 ml of fructose-1,6-diphosphate (0.1M, pH 7.4) and 1.0 ml of enzyme source.

Glucose-6-phosphate dehydrogenase (G-6-PDH) (D-glucose-6-phosphate: NADP oxidoreductase, EC 1.1.1.49) was assayed (Georg and Waller, 1965) in a medium of 2 ml containing: 20 μmoles of glucose-6-phosphate, 0.5 μmoles of NADP, 10 μmoles of triethylamine buffer (pH 6.8), 4 μmoles of INT (2-4 Iodophenyl-3(4-nitrophenyl)-5-phenyl tetrazolium chloride), and 0.3 ml of enzyme source.

NAD-isocitrate dehydrogenase (NAD-ICDH) (Isocitrate: oxidoreductase, EC 1.1.1.41) was assayed by the method of Kornberg and Pricer (1951). The reaction mixture in a final volume of 2 ml contained: 20 μmoles of DL-isocitrate, 100 μmoles of phosphate buffer (pH 7.4), 4 μmoles of INT, 10 μmoles of magnesium chloride, 0.2 μmoles of ADP, 0.2 μmoles of NAD and 0.4 ml of enzyme source.

Succinate dehydrogenase (SDH) (Succinate: oxidoreductase; EC 1.3.99.1) was assayed by the method of Nachlas *et al.* (1960) using 100 μmoles of phosphate buffer (pH 7.4), 40 μmoles of sodium succinate, 2 μmoles of INT and 0.5 ml of enzyme source in a 2.0 ml volume.

Malate dehydrogenase (MDH) (L-malate: NAD^+ oxidoreductase; EC 1.1.1.37) was assayed (Nachlas *et al.*, 1960) in a medium of 2 ml containing 100 μmoles of phosphate buffer (pH 7.4), 40 μmoles of sodium malate, 2 μmoles of INT, 0.1 μmole of NAD and 0.5 ml of enzyme source.

Lactate dehydrogenase (LDH) (L-lactate: NAD^+ oxidoreductase; EC 1.1.1.27) was assayed (Prameelamma *et al.*, 1975) in a medium of 2 ml containing 100 μmoles of

phosphate buffer (pH 7.4), 40 μmoles of sodium lactate, 2 μmoles of INT, 0.1 μmole of NAD and 0.4 ml of enzyme source.

Cytochrome C oxidase was assayed by the method of Oda *et al.* (1958). The reaction mixture consisted of 0.2 ml of 0.2 M 1-4-phenylene diamine, 0.2 ml of 0.2 % neo-tetrazolium chloride, 0.2 ml of 10^{-4} M cytochrome C and 0.2 ml of enzyme source.

Mg^{2+} ATPase (ATP phosphohydrolase EC 3.6.1.3) was assayed according to the method of Tirri *et al.* (1973). The reaction mixture in a final volume of 2.0 ml contained: 40 μmoles of ATP (pH 7.6), 40 μmoles of magnesium chloride, 0.5 mM of EGTA, 0.04 M sucrose and 0.2 ml of enzyme source.

The protein content in the enzyme source was estimated by a Folin Phenol reagent (Lowry *et al.*, 1951) using bovine serum albumin as standard.

Statistical treatment: The data obtained from the study was tested with Student's 't'-test (Pillai and Sinha, 1968) and the significance is represented at $P < 0.001$ and $P < 0.01$ levels.

RESULTS AND DISCUSSION

The alterations in carbohydrate metabolism in hepatopancreas and muscle tissues of crab, *O. senex*, exposed to sublethal concentration of fenvalerate are given in Tables 1 and 2.

A significant decrease in the glycogen and total carbohydrate levels was observed in the tissues of crab on exposure to fenvalerate. The decrease was more pronounced in the hepatopancreas after exposure to 7 days. This decrease in carbohydrate levels signifies a possible demand to meet the higher energy demands warranted by the toxic environment. Radhaiah (1989) has reported that the synthesis and utilization of glycogen are altered during pyrethroid stress. The decrease in glycogen and total carbohydrate levels observed in the present study might be attributed to a hypoxic condition, which normally increases glycogen utilization (Dezwaan and Zandee, 1972). A hypoxic condition in the crab, *O. senex*, exposed to fenvalerate has been reported (Bhagyalakshmi, 1981).

Phosphorylase occupies a strategic position in the glycolytic sequence, since it is the initial catalytic force in the chain of chemical events that leads to the degradation and utilization of glycogen. In the present study, the activity of phosphorylase 'a' was increased with a concomitant decrease in phosphorylase 'b'. The ratio between phosphorylase 'a' and phosphorylase 'b' suggests possible conversion of inactive phosphorylase 'b' to active phosphorylase 'a' in the tissues of exposed crabs. The increase in phosphorylase 'a' confirms glycogen breakdown during fenvalerate intoxication.

Aldolase activity was significantly decreased, indicating lowered operation of glycolysis. Decreased aldolase activity and increased glycogenolysis suggest the possible release of glucose molecules into haemolymph. Radhaiah (1989) earlier observed hyperglycemia in the crab after fenvalerate intoxication, and the source of haemolymph glucose was hepatopancreatic glycogen.

Krebs cycle enzymes, NAD-ICDH, SDH and MDH, decreased significantly, indicating possible impairment of mitochondrial oxidation during fenvalerate toxicity. A similar kind of inhibition of mitochondrial enzymes by pyrethroids has been

Table 1 Changes in biochemical parameters in hepatopancreas of control and fenvalerate-exposed crab, *Oziotelphusa senex senex*.

Parameter	Control	Fenvalerate exposed (days)		
		1	3	7
Glycogen (mg/g. tissue)	1.51 ± 0.21	1.32** ± 0.17 (–12.58)	1.21* ± 0.19 (–19.87)	1.02* ± 0.19 (–32.45)
Total carbohydrates (mg/g. tissue)	13.88 ± 1.74	11.09** ± 1.82 (–20.10)	9.89* ± 1.26 (–28.75)	9.03* ± 1.76 (–34.94)
Phosphorylase 'a' (μmol Pi formed/ mg protein/h)	2.52 ± 0.28	2.96* ± 0.31 (+17.46)	3.33* ± 0.29 (+32.14)	3.47* ± 0.32 (+37.70)
Phosphorylase 'b' (μmol Pi formed/ mg protein/h)	2.47 ± 0.29	2.01** ± 0.26 (–18.62)	1.92* ± 0.22 (22.26)	1.73* ± 0.21 (–29.96)
Phosphorylase a/b	1.02	1.47	1.73	2.01
Aldolase (μmol FDP cleaved/ mg protein/h)	24.78 ± 4.51	20.21* ± 3.41 (–18.44)	17.66* ± 3.02 (–28.73)	14.31* ± 2.42 (–42.25)
G-6-PDH (nmol formazan formed/mg protein/h)	64.71 ± 4.62	82.61* ± 8.09 (+27.66)	96.73* ± 7.66 (+49.48)	100.94* ± 9.04 (+55.99)
NAD-ICDH (nmol formazan formed/mg protein/h)	68.81 ± 6.16	59.73** ± 6.39 (–13.20)	54.09* ± 5.71 (–21.39)	50.66* ± 5.43 (–26.38)
SDH (nmol formazan formed/mg protein/h)	132.69 ± 7.91	114.77** ± 11.09 (–13.51)	107.93* ± 9.47 (–18.66)	96.41* ± 7.31 (–27.34)
MDH (nmol formazan formed/mg protein/h)	80.74 ± 5.33	70.69** ± 7.18 (–12.45)	65.43* ± 5.47 (–18.96)	63.19* ± 6.31 (–21.74)
LDH (nmol formazan formed/mg protein/h)	8.35 ± 1.09	12.47* ± 1.73 (+49.34)	14.38* ± 2.44 (+72.22)	15.09* ± 2.31 (+80.72)
Cytochrome C oxidase (μmol diformazan formed/mg protein/h)	84.73 ± 7.31	75.44** ± 5.67 (–10.96)	70.69* ± 4.39 (–16.57)	59.07* ± 6.71 (–30.28)
Mg^{2+} ATPase (μmol Pi formed/ mg protein/h)	35.45 ± 4.78	29.47* ± 3.71 (–16.87)	26.05* ± 5.01 (–26.52)	21.44* ± 4.0 (–39.52)

Values are mean ± S.D of 8 individual crabs. Values in parentheses are % change over control. (+) indicates increase; (–) indicates decrease. Values are significantly different at *$P < 0.001$; **$P < 0.01$.

reported (Reddy *et al.*, 1987; Ghosh, 1989). LDH, which catalyses the conversion of pyruvic acid into lactic acid under anaerobic conditions, was significantly increased. The decrease of SDH, MDH and NAD-ICDH, and the increase of LDH, indicate anaerobic metabolism in fenvalerate-stressed crabs. In support to this, several workers have reported the inhibition of SDH and elevation of LDH, and consequent impairment of oxidative metabolism, during malathion, sumithion and parathion treatment (Kohli *et al.*, 1977; Dikshit *et al.*, 1978; Bhagyalakshmi *et al.*, 1984b). In the fenvalerate-stressed crabs, various deleterious structural alterations occurred in the gills (author's unpublished data), which affect respiratory exchange and deplete oxygen uptake. This view has been supported by a number of other workers, who

Table 2 Changes in biochemical parameters in muscle of control and fenvalerate-exposed crab, *Oziotelphusa senex senex*.

Parameter	Control	Fenvalerate exposed (days)		
		1	3	7
Glycogen	0.67	0.59**	0.55*	0.47**
	± 0.06	± 0.06	± 0.09	± 0.07
		(–11.94)	(–17.91)	(–29.85)
Total Carbohydrates	4.89	3.97*	3.88*	3.41*
	± 0.51	± 0.67	± 0.72	± 0.62
		(–18.81)	(–20.65)	(–30.26)
Phosphorylase 'a'	4.91	6.03*	6.72*	7.33*
	± 0.62	± 0.91	± 1.02	± 1.03
		(+22.81)	(+36.86)	(+49.29)
Phosphorylase 'b'	3.89	2.94*	2.47*	2.11*
	± 0.81	± 0.71	± 0.64	± 0.66
		(–24.42)	(–29.82)	(–45.75)
Phosphorylase a/b	1.26	2.05	2.46	3.47
Aldolase	20.78	15.43*	13.09*	10.77*
	± 3.91	± 2.66	± 3.32	± 2.71
		(–25.75)	(–37.01)	(–48.17)
G-6-PDH	45.89	54.31*	58.67*	62.09*
	± 4.41	± 5.34	± 5.71	± 6.01
		(+18.35)	(+27.85)	(+35.30)
NAD-ICDH	79.81	70.71**	62.37*	59.41*
	± 6.34	± 5.41	± 6.31	± 5.92
		(–11.40)	(–21.85)	(–25.56)
SDH	124.81	108.09*	98.73*	96.77*
	± 9.88	± 10.12	± 9.73	± 8.70
		(–13.40)	(–20.90)	(–22.47)
MDH	61.31	54.09**	52.67*	49.09*
	± 5.97	± 5.04	± 4.73	± 4.31
		(–11.78)	(–14.09)	(–19.93)
LDH	25.44	31.67*	38.04*	41.77*
	± 5.45	± 5.09	± 4.11	± 4.33
		(+24.49)	(+49.53)	(64.19)
Cytochrome C	63.39	52.11*	50.19*	44.34*
oxidase	± 5.92	± 5.22	± 4.78	± 4.32
		(–17.79)	(–20.82)	(–30.05)
Mg^{2+} ATPase	26.73	20.31*	18.42*	15.01*
	± 5.02	± 2.91	± 2.82	± 3.09
		(–24.02)	(–31.09)	(–43.85)

Values are mean ± S.D of 8 individual crabs. Values in parentheses are % change over control. (+) indicates increase; (–) indicates decrease. Values are significantly different at *$P < 0.001$; **$P < 0.01$.

found that impaired respiratory function induced by pollutants leads to metabolic depression (Koundinya and Ramamurthi, 1978; 1981; Nagarathnamma and Ramamurthi, 1982).

The hexose monophosphate shunt pathway enzyme, G-6-PDH, increased in the tissues during fenvalerate exposure. The increased G-6-PDH indicates oxidation of glucose through the hexose monophosphate shunt pathway thereby facilitating the increased production of dihydronicotinamide adenine dinucleotide phosphate (NADPH2) for the detoxification process (O'Brien, 1970). It seems that the operation of the hexose monophosphate shunt pathway serves as an alternate route for glucose

oxidation. Similar increased catalysis of G-6-PDH in the tissues of *Oziotelphusa* during fenitrothion exposure was reported (Bhagyalakshmi *et al.*, 1983).

Mg^{2+} ATPase, involved in the synthesis and production of ATP molecules and cytochrome C oxidase, which represents the oxygen dependent electron transport system, was inhibited significantly in fenvalerate-exposed crab tissues. The inhibition of cytochrome C oxidase results in respiratory distress which in turn reduces oxidative metabolism (Bhagyalakshmi, 1981). A similar inhibition of cytochrome C oxidase was observed in the tissues of *Penaeus monodon* during phosphamidon-induced stress (Reddy and Ramana Rao, 1988). The decrease of Mg^{2+} ATPase activity could be attributed to damage in the mitochondrial lamellae, the site for oxidation, and may interfere with the conversion of oxidative energy to phosphate bond energy.

The present investigation concludes that fenvalerate reduces the oxidation of glucose through the Krebs cycle and enhances the HMP shunt pathway; this yields a larger number of reduced NADPH2 molecules for detoxification. The stressed crabs also appear to meet their energy requirements through anaerobic oxidation, as indicated by elevated LDH during impaired mitochondrial oxidation. This can be interpreted as a functional/physiological adaptation during fenvalerate intoxication and these enzymes may provide a good indicator for monitoring stress conditions in crabs. These modifications in the tissue metabolic profiles may explain or predict the survival of crabs in pyrethroid polluted habitats.

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