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In vivo Effects of Cadmium Chloride on Certain Aspects of Carbohydrate Metabolism in the Tissues of a Freshwater Field Crab *Barytelphusa guerini*

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Heavy metal input into the media, either terrestrial or aquatic, is an important aspect of environmental pollution. Cadmium is a toxic, non-essential heavy metal inhibiting numerous enzymes with functional sulfhydryl groups (Vallee and Ulmer 1972). It belongs to group II B of the periodic table. Its use in electroplating industry, smelting and refining industry, the application of phosphate fertilizers and in mineleaching, releases cadmium into the environment. Among the animals, aquatic organisms are most sensitive to heavy metals. Various aspects of toxic effects of cadmium pollution on fishes have been extensively reviewed (Cornelius et al. 1983). Survey of literature reveals that relatively few attempts have been made on the various aspects of cadmium toxicity in crustaceans (Collier et al. 1973; Thurberg et al. 1973; Calabrese et al. 1977; Frank and Robertson 1979) and these studies were mainly devoted to marine forms. The freshwater crustaceans, particularly the freshwater field crab, Barytelphusa guerini, received less attention. This crab forms one of the major components of the paddy field ecosystem and has an edible importance among local populations. Apart from this, these crabs are easily available, maintainable in the laboratory and data obtained in this study can be extrapolated to other crustaceans.

The present study reports the influence of cadmium on certain aspects of carbohydrate metabolism in the tissues of the freshwater field crab, Barytelphusa guerini, exposed to sublethal concentration of cadmium chloride.

MATERIALS AND METHODS

Healthy, uniform sized male crabs, Barytelphusa guerini, were collected from and around Hyderabad and were acclimatized to the laboratory conditions for a period of 15 days. The animals were fed fishmeat ad libitum. To determine the LC50 value, the crabs were exposed to six serial concentrations of cadmium chloride. A density of 10 crabs per 8 litres of water was used with 10 individuals in each tub. The physico-chemical characteristics of water were as follows: pH 7.4; dissolved oxygen 4.6 ppm; total alkalinity expressed as HCO_3^- 16 ppm; and carbonates (CO_3^-) 4 ppm. Free carbon dioxide was absent. The

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bioassay experiment of each concentration was repeated six times with parallel controls, and mortality was noted in each concentration at the end of 96 hours. No mortality was observed in controls. All the experiments were conducted at 26.5 ± 0.5° C. The average mortality in each concentration was taken to determine LC50 by plotting a graph, taking log concentration on X-axis and % mortality on Y-axis (Finney 1964). According to graphical plots the 50% mortality corresponds to log concentration 1.26 which is equivalent to 1.82 mg/L of $CdCl_2$. The crabs were exposed to sublethal concentration (1/3 of LC50, i.e., 0.6 mg/L) as suggested by Konar (1969) for a period of 15 days. The toxicant water and normal water were renewed every 24 hours after feeding. The animals were starved a day prior to experimentation to avoid metabolic differences, if any due to differential feeding and food reserves. Six crabs each from experimental and control tubs were sacrificed on the 4th and the 15th day of exposure. The tissues, chelate legmuscle, hepatopancreas, heart, gills and thoracic ganglion, were isolated from both control and toxicant-treated animals and were immediately transferred to a deep-freezer for analysis of various biochemical parameters glycogen and total free sugars (Nicholas et al. 1956) lactic acid (Barker and Summerson 1941), glycogen Phosphorylase "a and ab" (Cori et al. 1955), Succinate dehydrogenase and Lactate dehydrogenase (modified method of Nachlas et al. 1960). The protein content in the enzyme source was estimated according to Lowry et al. (1951), using bovine serum albumin (Sigma) as a standard.

RESULTS AND DISCUSSION

The effect of cadmium chloride on glycogen, total free sugars, glycogen phosphorylase, succinate dehydrogenase, lactate dehydrogenase and lactate levels in the tissues gill, chelate legmuscle, hepatopancreas, heart and thoracic ganglion has been investigated. The results presented in the Tables 1-3 reveal that the carbohydrate metabolism was significantly altered during sublethal toxicosis of cadmium chloride. The alterations were found to be tissue-specific and time-dependent. Reports in the higher animals reveal that cadmium exposure affects overall carbohydrate metabolism (Sporn et al. 1970). A fall in glycogen levels in the tissues indicates the possibility of active glycogenolysis. As evidence of this, the activities of enzymes phosphorylase 'a & ab', involved in the glycogenolysis, were found to be enhanced in the present study (Table 2). Such an observation, a drop in glycogen, has been reported in higher animals (Singhal et al. 1974). The decrease in the ratio of active to total phosphorylase activities in this study (Table 2) suggests the possible involvement of endocrine system in the stimulation of glycogen breakdown (Ramamurthi et al. 1968). 5-Hydroxy tryptamine (Seratonin) is considered as a neuroregulatory biogenic amine. The catabolism of 5-Hydroxy tryptamine proceeds via oxidative deamination by monoamine oxidase. The animal subjected to inhalational mercury showed a progressive fall in MAO activity and a consequential cerebral accumulation of 5-Hydroxy tryptamine. This kind of neurological phenomenon is a characteristic of heavy metal poisoning (Corsi et al. 1963). Studies on the influence of 5-Hydroxy tryptamine to freshwater field crab, Oziotelphusa senex senex, resulted in an increase of haemolymph total carbohydrates and a fall in free sugars, glycogen and elevated phosphorylase activity in the tissues

Table 1. Glycogen (A) and total free sugar (B) content in the tissues of a fresh water field crab, Barytelphusa guerini exposed to sublethal concentration of cadmium chloride.

Tissues		4 days			15 days		
		Control	Experimental	%Difference	Control	Experimental	%Difference
Gills	A	0.268±0.027	0.132 ± 0.018**	-50.65	0.298 ± 0.032	0.243 ± 0.024	-18.38
	B	2.774 ± 0.211	1.086 ± 0.078*	-60.88	2.833 ± 0.167	2.077 ± 0.189@	-26.67
Muscle	A	2.100 ± 0.125	0.490 ± 0.110*	-77.14	2.329 ± 0.112	2.050 ± 0.114	-11.97
	B	3.192 ± 0.298	1.393 ± 0.110*	-56.35	3.443 ± 0.325	2.043 ± 0.084**	-40.67
Hepato-pancreas	A	3.891 ± 0.800	1.941 ± 0.445	-50.11	4.240 ± 0.734	3.306 ± 0.406	-22.02
	B	6.805 ± 0.407	3.711 ± 0.206*	-45.47	5.551 ± 0.369	4.347 ± 0.221@	-21.59
Heart	A	6.028 ± 0.837	2.920 ± 0.485**	-51.56	6.540 ± 0.927	4.370 ± 0.769	-33.13
	B	5.639 ± 0.290	2.965 ± 0.162*	-47.43	5.794 ± 0.278	4.098 ± 0.182*	-29.27
Thoracic ganglion	A	3.162 ± 0.323	1.481 ± 0.351**	-53.15	3.268 ± 0.347	2.401 ± 0.347	-26.51
	B	4.238 ± 0.251	2.383 ± 0.214*	-43.77	4.338 ± 0.218	3.895 ± 0.192@	-10.20

Values expressed as mg glucose per gm wet weight of the tissue; *P < 0.001; **P< 0.01; @P < 0.05

Table 2. Glycogen phosphorylase 'a' (A), phosphorylase 'ab' (B) activities and ratio of a/ab (C) in the tissues of a fresh water field crab Barytelphusa guerini exposed to sublethal concentration of cadmium chloride.

Tissues		4 days			15 days		
		Control	Experimental	%Difference	Control	Experimental	%Difference
Gills	A	0.083 ± 0.004	0.139* ± 0.002	+67.45	0.083 ± 0.003	0.133* ± 0.009	+56.47
	B	0.137 ±0.012	0.247* ± 0.020	+80.29	0.142 ± 0.007	0.193* ± 0.006	+35.92
	C	60.58	56.28		59.86	68.91	
Muscle	A	0.560 ± 0.012	0.749* ± 0.020	+33.75	0.606 ± 0.021	0.781* ± 0.026	+28.88
	B	1.030 ± 0.021	2.382* ± 0.090	+131.86	1.070 ± 0.020	2.102* ± 0.092	+96.45
	C	54.37	31.44		56.64	37.16	
Hepato-pancreas	A	0.021 ± 0.001	0.040 ± 0.006	+90.48	0.028 ± 0.002	0.045* ± 0.003	+60.71
	B	0.046 ± 0.004	0.102* ± 0.003	+121.74	0.052 ± 0.001	0.098* ± 0.007	+88.46
	C	45.65	39.22		53.85	45.92	
Heart	A	0.310 ± 0.120	0.760** ± 0.042	+145.16	0.350 ± 0.020	0.703** ± 0.076	+100.85
	B	0.638 ± 0.020	1.638* ± 0.032	+156.74	0.595 ± 0.021	1.172* ± 0.062	+96.97
	C	48.59	46.90		58.82	59.98	
Thoracic ganglion	A	0.034 ± 0.002	0.057* ± 0.004	+67.64	0.042 ± 0.004	0.067@ ± 0.009	+59.52
	B	0.058 ± 0.002	0.142* ± 0.009	+143.44	0.066 ± 0.001	0.102** ± 0.008	+54.54
	C	58.62	40.14		63.64	65.69	

Values expressed as μ moles of Pi/mg Protein/h *P < 0.001; **P < 0.01 @P < 0.05

Table 3. Succinate dehydrogenase (A), Lactate dehydrogenase (B) activities and Lactate (C) levels in the tissues of a fresh water field crab Barytelphusa guerini exposed to sublethal concentration of cadmium chloride.

Tissues		4 days			15 days		
		Control	Experimental	%Difference	Control	Experimental	%Difference
Gills	A	9.200 ± 0.115	5.530* ± 0.129	-39.89	9.661 ± 0.187	2.360* ± 0.160	-75.87
	B	2.255 ± 0.084	1.215* ± 0.061	-46.12	2.450 ± 0.106	0.467* ± 0.024	-80.94
	C	0.208 ± 0.020	0.956** ± 0.120	+45.67	0.281 ± 0.012	0.553* ± 0.015	+96.88
Muscle	A	3.634 ± 0.138	2.571* ± 0.129	-29.25	3.950 ± 0.110	2.032* ± 0.032	-48.44
	B	4.157 ± 0.072	2.641* ± 0.063	-36.47	3.852 ± 0.095	9.921* ± 0.031	-76.09
	C	1.55 ± 0.093	2.113* ± 0.062	+36.32	1.780 ± 0.051	3.208* ± 0.096	+80.22
Hepato-pancreas	A	7.452 ± 0.182	4.870* ± 0.093	-34.65	7.961 ± 0.200	3.093* ± 0.084	-61.11
	B	1.794 ± 0.115	0.942* ± 0.017	-47.49	1.550 ± 0.077	0.248* ± 0.010	-84.00
	C	0.562 ± 0.030	0.825* ± 0.032	+47.06	0.585 ± 0.035	1.084* ± 0.028	+85.30
Heart	A	6.258 ± 0.092	3.207* ± 0.096	-48.75	6.864 ± 0.046	2.980* ± 0.028	-56.69
	B	4.657 ± 0.124	7.358* ± 0.049	+58.00	4.256 ± 0.081	1.525* ± 0.093	-64.17
	C	1.820 ± 0.115	1.021* ± 0.050	-43.90	1.795 ± 0.057	2.915* ± 0.084	+62.40
Thoracic ganglion	A	10.126 ± 0.101	6.909* ± 0.149	-31.77	10.526 ± 0.201	3.009* ± 0.015	-71.40
	B	2.750 ± 0.079	3.840** ± 0.216	+39.64	2.943 ± 0.145	1.921* ± 0.082	-34.73
	C	1.451 ± 0.093	0.943* ± 0.039	-35.01	1.278* ± 0.078	1.721** ± 0.082	+34.66

Values for A and B expressed as μ moles of Formazan/mg protein/h and for C in mg lactate/gm wet weight of the tissue; *P < 0.001; **P < 0.01

(Venkataramanaiah and Ramamurthi 1981). In the light of these observations, cadmium chloride may alter the normal functioning of the nervous system through altered membrane permeability and induce the increased release of hyperglycemic factor from the eyestalk glands, and hence alter the carbohydrate metabolism. The decrease in % difference between 4th and 15th day in glycogen and total free sugars (Table 1) may be due to enhanced cadmium concentration in the tissues. These differences are clearly reflected in the levels of active and total phosphorylase activities (Table 2).

Inhibition of SDH activity in the crab (Table 3) suggests the dependence of crab on glycolytic metabolism for energy needs. Collier et al. (1973) observed a dose-dependent cadmium-induced decrease in the respiration in the mud crab Eurypanopeus depressus. This suggests that the inhibition of SDH activity in the tissues may be due to the reduced supply of oxygen. Accumulation of cadmium may induce the structural alterations in enzyme molecule of SDH which may lose affinity for substrate and therefore, may result in the inhibition of activity. Inhibition of SDH activity in the kidney of channel cat fish during heavy metal (mercuric chloride) toxicosis (Kendall 1975) also supports the inhibition of SDH activity in the tissues of crab. Lactate profile in various tissues presents a very interesting inference of glycolytic metabolism. The gradual built-up of lactic acid was observed in gill, muscle and hepatopancreas, whereas lactate levels fall in thoracic ganglion and heart after 4 days of exposure with subsequent increase after 15 days of exposure (Table 3). The alterations in lactate levels in the individual tissues could be very well correlated with the changes in LDH activity. The increase in LDH activity in the legmuscle of mud crab observed by Calabrese et al (1977), supports the initial elevatory response in the present animal. The sensitivity of LDH can be taken as a meaningful biochemical index during $CdCl_2$ toxicosis. Isozymal characterization of this enzyme at regular intervals of chronic exposures could present a meaningful picture about the tissue-specific dependence for energy requirements during this toxicity. This aspect will be taken up at the later stages and some of the investigations are still under progress.

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