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Effects of Cadmium on the Biochemical Composition of the Freshwater Crayfish *Procambarus clarkii* (Girard, 1852)

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Lake Albufera of Valencia (Spain) and the surrounding rice field waters are subjected to very heavy loads of sewage and toxic residues. Among these residues include heavy metals which have been deposited from the many urban and waste waters in this area.

The American red crayfish, Procambarus clarkii (Girard, 1852) from Albufera Lake has high resistance to heavy metals (Del Ramo et al. 1987). We have also found that P. clarkii shows a high capacity for cadmium accumulation in experimental conditions and natural conditions since crayfish collected in this area contained considerable amounts of cadmium in several tissues even after 15 days of depuration in clean water (Díaz Mayans et al. 1986).

In previous work we utilized physiological and biochemical approaches to assess the sublethal impact of cadmium in P. clarkii, however the results were not conclusive. In these studies neither gill oxygen consumption nor gill NaK ATPasa activity of P. clarkii showed alterations after sublethal cadmium exposure that can be easily differentiated from the background variability (Díaz Mayans et al. 1986, Torreblanca et al. 1989).

Since the physiological changes that take place when organisms are exposed to sublethal levels of stress could include rate of feeding as well as respiration and excretion, the net result could be a change in energy available for growth and reproduction. Although there are several studies which describe the effects of sublethal exposure to contaminants on the biochemical composition of freshwater and marine crustaceans (Dhavale and Masurekar 1985, Radhakrishnaiah and Busappa 1986, Wang and Stickle 1988), carbohydrate levels were mainly studied and, when the size of the organism allowed tissue measurements, hepatopancreas and muscle tissues were usually chosen. Since for most pollutants uptake from water is the most important route, gill is the primary target organ and may be one of the first organs to exhibit symptoms of sublethal toxicity. Furthermore, there are biochemical components other than carbohydrates such as proteins and lipids that can also act as energy sources. In this work, we have studied the biochemical energy composition of hepatopancreas, muscle and gills of P. clarkii after short term sublethal exposure to cadmium.

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MATERIALS AND METHODS

Adult intermolt specimens (males and females) of Procambarus clarkii were collected from Lake Albufera (Valencia, Spain) and carried immediately to the laboratory where they were transferred into 300-L aquaria. They were maintained and starved for 15 days at 20°C. Afterwards, they were fed pork liver *ad libitum*. Two days after feeding forty crayfish ranging in weight from 19.5 to 30.2 g selected from the stock at random were divided in two groups. One group was kept in clean water (control) and the second group was exposed to 10 mg Cd/L. Cadmium exposure was carried out in a semistatic way. Cadmium solution was removed every day to reduce the buildup of metabolic wastes and to keep the concentration of cadmium near the nominal level. After 12, 48 and 96 hr of cadmium exposure animals from each experimental group were transferred to clean water and kept there for an additional 2 hr. Gills, hepatopancreas and muscle were removed by dissection. During the test, mean water temperature was 20°C (±0.2), pH 7.9 (±0.2), hardness 250 (±30) mg/L as $CaCO_3$.

One gram of accurately weighed tissue was placed in a 6 mL homogenizing tube. Three milliliters of ice-cold 6% of aqueous perchloric acid solution were added and the mixture was immediately homogenized with a motor-driven teflon pestle. The sample was kept ice-cold throughout all procedures. Following homogenization, a 50 μL aliquot was removed and added to 950 μL of 1N NaOH. It was stored at -20°C and was analyzed for total protein as described later. To isolate other biomolecules the method described by McKee and Knowles (1986) was used with several modifications. The homogenate was centrifuged for 10 min. at 10000 x *g* at 4°C. The supernatant which contained glycogen, was neutralized by $KHCO_3$ and stored at -20°C.

Lipid material in the pellet was extracted twice with 2 mL of a mixture of 2:1 chloroform:methanol. The combined supernatants were brought to a constant volume, washed for 24 hr with a 0.9 % aqueous sodium chloride solution, and the resulting organic phase was analyzed for lipids by the phosphovanillin method (Boheringer kit n° 124 303). Lactate concentrations were measured spectrophotometrically by means of NADH produced in a reaction catalyzed by lactate dehydrogenase (Boheringer kit n° 256773). To determine glucose and glycogen the method described by Keppler and Decker (1974) was used. Glycogen was hydrolyzed to glucose by amyloglucosidase from Aspergillus niger and the liberated glucose was measured by the hexoquinase/glucose-6-phosphate dehydrogenase method. Glycogen concentrations were calculated as the difference in glucose concentrations before and after enzymatic hydrolysis. Mollusc glycogen was used as reference standard. The protein samples in 1 N NaOH were resolubilized by incubating at 100°C for 60 min prior to analysis. Protein was analyzed by the method of Lowry et al. (1951) using bovine serum albumin as standard.

The caloric concentration (cal/mg) was calculated by assuming caloric values of 9.5 cal/mg for lipids, 4.3 cal/mg for glycogen, and 4,1 cal/mg for proteins (White et al. 1978).

Two-way Analysis of Variance was used to determine treatment and time effects on the various parameters studied. Mean separation was accomplished with test Fisher PLSD. The significance level in all instance was probability of ≤ 0.05.

RESULTS AND DISCUSSION

Procambarus clarkii has a LC50-96 hr for cadmium of 58.5 mg at 20°C (Del Ramo et al. 1987). The concentration utilized in this work is less than one fifth of LC50-96 hr. We did not observe mortality during this experiment.

The most remarkable effect of cadmium on gill tissue was a decrease of protein concentration (Table 1). Statistically significant differences between metal treated and control groups were found after 12 hr exposure. This effect can be related to the structural alterations that we observed in gill epithelium of cadmium exposed crayfish (Torreblanca et al. 1989). It was also shown that cadmium causes a decrease in caloric content in gill tissue. This decrease can be explained by the protein depletion. Although gill cannot be considered to play a major role as reserve storage this tissue contributes to the caloric content of the whole organism. No toxicant effects were documented on gill lactate levels.

Table 1. Biochemical composition of gills corresponding to crayfish exposed to cadmium and their corresponding controls.

	12 hr		48 hr		96 hr	
	Control	Exposed	Control	Exposed	Control	Exposed
Lipid (mg/g)	5.6±4.9[a] (n=3)	3.9±1.2 (n=10)	4.7±4.3 (n=4)	9.5±7.4 (n=11)	15.8±10.1 (n=6)	5.3±2.0 (n=7)
Protein (mg/g)	65.4±14.8 (n=3)	25.2±19.6[b] (n=10)	57.1±10.0 (n=4)	46.7±32.5 (n=10)	85.0±15.0 (n=6)	39.8±18.9[b] (n=7)
Glucose (μmol/g)	2.0±0.9 (n=3)	1.2±0.9 (n=8)	0.9±0.5 (n=4)	1.4±0.7 (n=11)	1.8±0.7 (n=6)	2.4±1.1 (n=7)
Glycogen (μmol glycosil/g)	3.9±0.2 (n=2)	4.1±3.2 (n=8)	2.1±2.0 (n=4)	8.0±8.4 (n=11)	10.8±5.8 (n=6)	7.5±7.7 (n=7)
Lactate (μmol/g)	1.9±1.6 (n=3)	2.5±2.0 (n=9)	3.1±1.0 (n=4)	2.7±1.6 (n=10)	5.3±1.6 (n=6)	2.5±1.8 (n=7)
Caloric concentration(Cal./g)	325±13 (n=3)	137±71[b] (n=8)	282±49 (n=4)	276±175 (n=10)	505±133 (n=6)	221±87[b] (n=7)

[a] Mean and standard deviation

[b] Significantly different from control organisms Fisher PLSD test with $\alpha=0.05$

Exposure to cadmium showed changes in the biochemical composition of crayfish hepatopancreas (Table 2). Lipid concentration was less than a half of the control value after 96 hr. Cadmium also produced a decrease in protein concentration in hepatopancreas that was more evident at the end of the experiment. Similarly, glucose levels were decreased by cadmium. As the result of the above effects caloric content was also reduced. The differences in caloric content between cadmium exposed and control group became significant 96 hr after the start of the experiment.

Only glucose concentration was significantly decreased by cadmium exposure in crayfish muscle (Table 3). Our results agree with the decrease in carbohydrate content of crustacean muscle after short term exposure to pollutants that has been reported by several authors (Reddy et al. 1986, Repetto et al. 1988).

Table 2. Biochemical composition of hepatopancreas corresponding to crayfish exposed to cadmium and their corresponding controls.

	12 hr		48 hr		96 hr	
	Control	Exposed	Control	Exposed	Control	Exposed
Lipid (mg/g)	52.3±43.9[a] (n=2)	37.8±49.3 (n=10)	17.2±8.4 (n=4)	45.3±34.5 (n=11)	121.3±31.7 (n=6)	53.3±14.1[b] (n=10)
Protein (mg/g)	124.3±25.4 (n=2)	78.8±35.9 (n=10)	71.8±39.4 (n=4)	89.2±24.9 (n=11)	129.8±58.5 (n=6)	73.7±34.1[b] (n=10)
Glucose (μmol/g)	19.5±3.6 (n=2)	5.1±7.2[b] (n=10)	3.1±4.1 (n=4)	5.4±6.0 (n=11)	13.2±9.1 (n=6)	5.6±4.7[b] (n=10)
Glycogen (μmol glycosil/g)	36.2±32.8 (n=2)	10.2±18.4 (n=9)	2.5±2.3 (n=4)	10.6±12.9 (n=9)	28.8±14.2 (n=6)	15.3±20.4 (n=8)
Lactate (μmol/g)	3.9±1.9 (n=2)	2.6±1.1 (n=10)	2.2±0.5 (n=4)	2.7±1.6 (n=11)	2.8±2.0 (n=6)	2.3±1.0 (n=10)
Caloric concentration(Cal./g)	1049±341 (n=2)	693±484 (n=10)	463±228 (n=4)	808±364 (n=11)	1717±909 (n=6)	823±505[b] (n=10)

[a] Mean and standard deviation

[b] Significantly different from control organisms using Fisher PLSD test with α=0.05

Hepatopancreas and gills are more modified in their biochemical composition than muscle. In a previous work on cadmium accumulation we found that gills and hepatopancreas accumulated higher levels of cadmium than muscle (Díaz Mayans et al. 1986).

On the other hand, lipids, protein and glycogen levels of gills were higher at the end of the experiment than at the start. Increase of lipids and caloric concentration with time have been also found in hepatopancreas. The increase in the concentration of several compounds and caloric concentration in gills and hepatopancreas of control animals with time may be due to the assimilation of food ingested 2 days before the start of the experiment.

All the effects that we have found due to the action of cadmium exposure on the the hepatopancreas are extremely important from the energetic point of view. Hepatopancreas of crustaceans is a storage organ and there is an indication that at least part of the lipids of the hepatopancreas are translocated to the ovary (Sastry 1983). Because of this, a decrease in lipid content in female hepatopancreas could affect reproduction. Hepatopancreas has also been reported as storage of proteins that are accumulated during the intermolt period and can be utilized for the growth of tissues during the molt period (Durliat and Vranckx 1982). Although it is generally accepted that juvenile organisms are more sensitive to pollutants than adult organisms and that any detrimental effect of pollutants on juveniles would affect their growth, adults also depend on their nutritional reserves for surviving natural periods of starvation and reproduction requires extra energy.

Table 3. Biochemical composition of muscle corresponding to crayfish exposed to cadmium and their corresponding controls.

	12 hr		48 hr		96 hr	
	Control	Exposed	Control	Exposed	Control	Exposed
Lipid (mg/g)	4.0±1.1[a] (n=5)	5.3±4.4 (n=9)	2.7±0.6 (n=4)	4.3±1.6 (n=11)	4.1±2.1 (n=6)	4.5±1.9 (n=7)
Protein (mg/g)	66.0±15.1 (n=5)	67.6±21.3 (n=10)	59.4±18.1 (n=4)	82.1±20.2 (n=11)	84.6±9.7 (n=6)	80.4±19.7 (n=7)
Glucose (μmol/g)	14.0±7.0 (n=5)	5.1±7.1[b] (n=10)	3.1±4.1 (n=4)	5.4±6.0 (n=11)	13.2±9.1 (n=6)	3.6±1.9[b] (n=7)
Glycogen (μmol glycosil/g)	2.5±2.9 (n=5)	2.3±2.5 (n=8)	0.6±0.4 (n=4)	1.6±1.8 (n=11)	1.3±0.9 (n=6)	1.2±1.1 (n=6)
Lactate (μmol/g)	6.8±2.6 (n=5)	5.4±2.6 (n=10)	7.4±6.1 (n=4)	6.1±4.3 (n=11)	11.6±7.5 (n=6)	8.3±3.7 (n=7)
Caloric concentration(Cal./g)	322±68 (n=5)	334±126 (n=8)	272±76 (n=4)	384±87 (n=11)	398±47 (n=6)	352±65 (n=6)

[a] Mean and standard deviation

[b] Significantly different from control organisms Fisher PLSD test with α=0.05

It has been proposed that most symptoms of heavy metal sublethal toxicity are a direct effect of anoxia in tissues and lactate has been widely used as a measure of anaerobic metabolism. An increase of lactate levels in different tissues of aquatic invertebrates and fishes after exposure to toxicants has been reported (Tort et al. 1984, Reddy et al. 1986). However, we did not find a clear effect of cadmium exposure on lactate levels in those tissues studied.

Caloric concentration is an index that offers an integrated parameter of the energy reserves. A reduction in the caloric content of whole organisms exposed to several toxicants has been observed in other studies (Capuzzo et al. 1984). The reduction in caloric concentration by pollutant exposure may be due to a toxicant effect on feeding behavior. Since in our experiments animals were not fed during cadmium exposure period, the decrease in caloric content in hepatopancreas and gills must be produced by an increased utilization of reserves to resist toxicant-induced stress or by a toxicant effect on assimilative efficiency.

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