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THE EFFECT OF CHRONIC CADMIUM EXPOSURE ON PHOSPHOADENYLATE CONCENTRATIONS AND ADENYLATE ENERGY CHARGE OF GILLS AND DORSAL MUSCLE TISSUE OF CRAYFISH

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Abstract — The sensitivity of two measures of tissue energy metabolism to environmental stress was examined in the present study through chronic exposure of crayfish to low concentrations of cadmium. Freshwater crayfish, *Procambarus acutus acutus*, were exposed to 5 and 10 μ Cd · l^{-1} (as $CdCl_2$) under flow-through conditions for a period of 21 days. After 1, 7, 14, and 21 days of exposure, gill and dorsal muscle tissue samples were collected to determine Cd and Zn content, phosphoadenylate (ATP, ADP and AMP) concentrations and gill tissue respiration rates. Two potential sublethal stress indicators, the adenylate energy charge [AEC = (ATP + ½ ADP)/(ATP + ADP + AMP)] and ATP turnover rate were calculated. Cd was observed to concentrate over time in gills, but not in muscle tissue. No evidence was found to indicate replacment of Zn by Cd in the tissues analyzed. In addition, Cd tissue concentrations were not correlated ($p > 0.05$) with phosphoadenylate concentrations or respiration rate. After seven days exposure, ATP and total phosphoadenylate concentrations in gill tissue were significantly ($p < 0.05$) lower in crayfish exposed to Cd. This decrease was associated with lower gill tissue respiration rates, probably due to enzyme dysfunction caused by Cd. The return of ATP concentrations and gill tissue respiration rates to control values observed after day 7 may be associated with the induction of replacement enzymes and metallothioneins stimulated by Cd. ATP turnover rate (based on 1 mole O_2 → 6 moles ATP) in gill tissue was not correlated ($p > 0.05$) with the two measures of aerobic respiration recorded, and was not considered to represent an accurate indication of cellular energy turnover. In contrast to an earlier study utilizing a different crayfish species, no significant ($p > 0.05$) decreases in tissue AEC were observed in the present study in crayfish exposed to Cd. The absence of AEC

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changes under the exposure conditions imposed in this investigation could be related to life history differences or in species specific sensitivity to the toxic effects of Cd. Under the experimental conditions utilized, the adenylate energy charge and ATP turnover rate did not provide indications of Cd toxicity at lower concentrations than those previously determined through conventional chronic lethality testing. However, these physiological indicators did permit a more complete understanding of the toxic action of cadmium on the tissues examined.

Keywords — Cadmium Phosphoadenylates Adenylate energy charge Crayfish Physiological toxicity testing Bioaccumulation

INTRODUCTION

Increasing amounts of cadmium (Cd), a highly toxic metal, have been released into the environment through man's activities [1]. Cadmium is bioaccumulated [2-4] and has been found to be hazardous to both plants and animals, including humans [5-8]. Cadmium has been implicated as both a carcinogen and mutagen, and an agent causing cardiovascular disruption, renal dysfunction and hypertension [6,9].

Biochemically, the mechanism of Cd toxicity is through binding to particular bonding sites (sulfhydryl and hydroxyl groups) of various proteins, causing enzyme dysfunction [10-11]. Some of the cellular consequences of this enzyme disruption in aquatic invertebrates and fish include decreased intracellar amino acid concentrations [12], loss of feedback control in sequences of carbohydrate metabolism [11,13], changes in tissue respiration rates [14-16] and disturbance of divalent ion concentrations [11].

Though Cd induced enzyme dysfunction could occur in numerous metabolic systems, it would be expected that the amount of biochemical energy available in cells and tissues would be decreased. In addition to possible disruptions of catabolic (energy yielding) sequences, Cd exposure has been found to induce anabolic (energy requiring) sequences, by inducing production of replacement enzymes [13] and protein complexes (metallothioneins) which may aid in detoxification [17].

The adenylate energy charge (AEC = (ATP + ½ADP)/(ATP+ADP+AMP); (ATP — adenosine triphosphate, ADP — adenosine diphosphate, AMP — adenosine monophosphate), which represents the amount of available energy that is stored in the adenylate system, has been proposed as a measure of the overall metabolic energy status of cells and tissues [18]. This ratio may provide a valuable biochemical measure of environmental stress.

Another measure of metabolic energy status can be determined through calculation of ATP turnover rates of individual tissues. The actual biochemical energy transfer occurring within cells or tissues can theoretically be measured by determining the turnover rate of ATP (adenosine triphosphate — the major energy transfer molecule of all living organisms). The rate of ATP turnover could provide insight into the energy "costs" of exposure to pollutants.

The present investigation of freshwater crayfish was designed to: (1) determine the sensitivity of the adenylate energy charge and ATP turnover rate to chronic exposure to low Cd concentrations, (2) examine the association between tissue Cd concentration and energy charge, and (3) correlate changes in the adenylate energy charge and ATP turnover rate with another metabolic indicator, gill tissue respiration rate.

METHODS AND MATERIALS

Crayfish were exposed to Cd in a flow-through system. Dilutions of $CdCl_2$ were mixed and stored in polyethylene-lined 208 L (55 gal) drums. The chemical characteristics of the artesian well water used for dilution [21] were similar to those of the water

Table 1. The concentration of cadmium ($\mu g \cdot l^{-1}$) in unfiltered water used in the crayfish exposure experiment. Water samples were collected from crayfish tray inflow tubes.

	Mean (SD)		
	Control	Nominal Treatment	
		5 $\mu g \cdot l^{-1}$ Cd	10 $\mu g \cdot l^{-1}$ Cd
Day 6	<0.002	3.30	9.42
$n = 2$	(<0.0002)	(1.23)	(4.02)
Day 12	<0.002	3.46	8.23
$n = 2$	(<0.0002)	(1.40)	(3.82)
Day 19	<0.002	3.97	[illegible]
$n = 2$	(<0.0002)	(1.31)	(2.78)

from which the crayfish were originally collected (L. Briese, unpublished data). Water from the drum was gravity-fed into a 26 L polyethylene distribution container which was connected by Nalgene® couplings and Tygon tubing to twenty covered, 7 L, opaque, polyethylene trays (25.5 × 30.0 × 8.5 cm) containing individual crayfish. The flow into the carboy was adjusted to provide constant head pressure, allowing an equal flow by gravity to all trays. Hose clamps for each crayfish tray were adjusted daily to maintain a flow of 6-12 ml · min^{-1}; giving 16-32 exchanges of water per day in each tray. An exit hole on the side opposite the point of water entry maintained a water depth in the trays of 2 cm. This depth allowed crayfish to adjust to any possible decreases in dissolved oxygen content of the water by aerial oxygen uptake. The trays were housed in large, wooden trays outside to allow exposure to natural temperature and light fluctuations. Each of the wooden trays was covered by an awning to protect the crayfish from extreme temperature increases associated with direct exposure to sunlight. Three separate flow-through systems of the preceding design were employed: a control and two Cd concentrations (5 and 10 μgCd · l^{-1}).

Adult, intermolt specimens of the crayfish, *Procambarus acutus acutus* (Girard), were collected by baited traps from an artesian spring on the Savannah River Plant, South Carolina, USA. Sixty crayfish of approximately the same size were selected for use in this exposure experiment, 20 per treatment. Crayfish were acclimated in the flow-through systems for three days using untreated well water. After the acclimation period, appropriate concentrations of $CdCl_2$ were mixed every 24 h, in the 208 L drums of the two experimental groups to give nominal concentrations of 5 and 10 μgCd · l^{-1} (Table 1). The control group continued to receive untreated water. Cadmium concentration in the pelleted shrimp meal utilized as food was 3.1 μgCd · g^{-1} dry weight.

After exposure to Cd for 1, 7, 14, and 21 days, crayfish from each group (sample size dependent upon mortality, see Table 2) were transferred from the trays into appropriately labelled, individual polyethylene buckets. The buckets contained water from each appropriate control or experimental group. The crayfish were transported to the laboratory for analyses of metal concentratins, adenylate concentrations and gill respiration.

In the laboratory, the crayfish were left undisturbed for 30 minutes. After this period, individual crayfish were captured with a dip net in a manner precluding escape responses [22] and killed by rapid destruction of the supraesophageal ganglia. A sample of dorsal tail muscle was immediately removed for phosphoadenylate (ATP, ADP and AMP) determination and freeze-clamped by methods previously described

Table 2. Cadmium and zinc concentrations in sampled crayfish tissues during the 21 day experimental period. Means within each sample day for both metals in gill and muscle tissues which do not have the same letters in their superscripts are significantly different ($\alpha = 0.05$). (Comparison among treatments within tissue and metal.)

		Mean (SD)			
		Gill		Muscle	
	Treatment	Cd	Zn	Cd	Zn
		$\mu g \cdot g^{-1}$ dry wt			
Day 1	Control	6.91[a]	177.45	4.76	48.73
	$n = 4$	(6.20)	(56.85)	(9.10)	(28.72)
	5 $\mu g \cdot l^{-1}$ Cd	19.70[b]	129.10	1.59	74.62
	$n = 5$	(14.84)	(98.08)	(1.87)	(11.48)
	10 $\mu g \cdot l^{-1}$ Cd	2.60[c]	143.33	0.35	72.78
	$n = 4$	(1.69)	(106.87)	(0.36)	(10.71)
Day 7	Control	2.40[a]	89.03	0.19	66.38
	$n = 4$	(0.61)	(44.86)	(0.08)	(8.17)
	5 $\mu g \cdot l^{-1}$ Cd	41.46[b]	632.86	4.11	69.60
	$n = 5$	(34.04)	(840.58)	(4.65)	(19.92)
	10 $\mu g \cdot l^{-1}$ Cd	44.55[c]	160.63	0.32	67.23
	$n = 4$	(46.12)	(114.00)	(0.07)	(17.90)
Day 14	Control	3.30[a]	177.43	5.90	53.58
	$n = 4$	(1.55)	(199.82)	(6.73)	(37.83)
	5 $\mu g \cdot l^{-1}$ Cd	125.64[b]	121.55	3.35	54.90
	$n = 4$	(97.31)	(46.59)	(3.76)	(19.85)
	10 $\mu g \cdot l^{-1}$ Cd	155.38[c]	156.30	4.65	45.52
	$n = 5$	(133.12)	(76.98)	(7.98)	(25.26)
Day 21	Control	2.53[a]	55.28	3.98	104.73
	$n = 4$	(0.58)	(25.41)	(3.88)	(53.60)
	5 $\mu g \cdot l^{-1}$ Cd	204.04[b]	65.90	7.10	90.90
	$n = 4$	(261.65)	(44.06)	(5.06)	(37.10)
	10 $\mu g \cdot l^{-1}$ Cd	179.36[c]	142.64	1.05	85.74
	$n = 4$	(81.19)	(80.59)	(1.49)	(22.08)

[19,20] and stored in liquid nitrogen prior to adenylate extraction. A second muscle sample was taken and stored on ice for subsequent metal analyses. The right gill chamber was then opened and all of the gill filaments placed in a labeled plastic bag. These excised gills were freeze-clamped and stored in liquid nitrogen prior to adenylate extraction. Phosphoadenylates were extracted and assayed from both muscle and gill tissues by methods previously discussed [20,22].

Gill tissue respiration rates were determined for each crayfish in a Gilson® (Gilson Medical Electronics, Inc.) respirometer. Immediately after samples for adenylate analyses had been collected, the set of gills from the left gill chamber were carefully removed and placed into a single respirometer flask (15 ml), which had been filled with 5 ml of the appropriate treatment water (control, 5 or 10 μg Cd $\cdot l^{-1}$) to which the crayfish had been experimentally exposed. Five hundred microliters of 20% KOH (w/v) were added to flask center wells for CO_2 absorption. Nutrients were not added to the tissue media based on the observation that addition or deletion of 10^{-3} M pyruvate did not significantly alter gill tissue respiration rates in blue crabs [23]. After a 10 minute equilibration period, O_2 consumption was recorded every 15 minutes for a period of 60 minutes. Respiration analyses were conducted at 29°C to simulate after-

noon water temperatures in the flow-through system. After completion, tissues were removed from the flasks, rinsed with deionized water and blotted dry and dried at 60°C for 24 hours and weighed.

Turnover time of ATP in gill tissues was calculated from respiration rates and ATP concentrations as reported by Engel et al. [24]. The assumption is made that 1 mole O_2 yields 6 moles of ATP under aerobic conditions at 100% conversion efficiency of ADP to ATP.

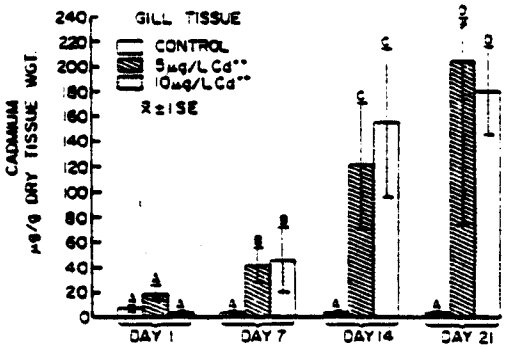

Fig. 1. Mean concentration of cadmium in excised gill tissue of *P. a. acutus* under control conditions and exposure to cadmium. Within control and experimental groups, bars which do not have the same letter above them are significantly different ($\alpha = 0.05$).

Gills used previously in respiration analyses and dorsal muscle samples were lyophilized prior to wet oxidation with HNO_3. Cadmium and Zn concentrations in these two tissues were measured by the methods of Dickson et al. [25]. The experimental design was a randomized block design, blocked by sex. Differences among means were examined by one-way ANOVA and Scheffe's multiple range test.

RESULTS

During the 21 day experiment, water temperatures ranged from 21-30°C. These temperatures were similar to those observed in the original habitat of *P. a. acutus* for the same period [22].

Crayfish exposed to Cd exhibited no major difference in mortality or molting frequency when compared with control animals. Mortality ranged from three in the control group to two in each of the experimental groups. One crayfish was observed to molt in the control group and in the crayfish exposed to 5 µg Cd · l⁻¹, while two molted within the higher cadmiun exposure group. On all four sampling dates, significantly ($p < 0.05$) higher concentrations of Cd were measured in gill tissues of crayfish exposed to 5 and 10 µg · l⁻¹ than in control crayfish (Table 2; Fig. 1). No statistical differences were observed among Cd concentrations of dorsal muscle tissue from the three experimental groups (Table 2; Fig. 2). In both gill and muscle tissues, Zn concentrations exhibited no statistical differences among treatments (Table 2).

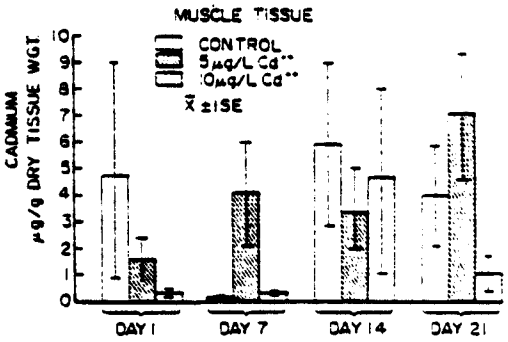

Fig. 2. Mean concentration of cadmium in dorsal tail muscle of *P. a. acutus* under control conditions and exposure to cadmium.

Gill tissue ATP and total adenylate (ATP + ADP + AMP) concentrations were significantly lower on day 7 in crayfish exposed to 5 and 10 µg · l⁻¹ Cd (Table 3). Differences in AEC of gill tissue were not observed at the other samplings.

In muscle tissue, significantly lower ATP and total phosphoadenylate concentrations were observed in only the 10 µG · l⁻¹ exposure group on day 21 (Table 4). No significant differences in AEC were recorded in muscle tissue of any of the crayfish tested (Table 5).

Respiration rates of excised gill tissues were significantly lower in crayfish exposed to both 5 and 10 µg Cd · l⁻¹ on the seventh

Table 3. Adenylate concentrations, adenylate energy charge and respiration rate of gill tissue excised from crayfish utilized in the cadmium exposure experiment. Means within each sample day and parameter which do not have the same letter in their superscript are significantly different ($\alpha = 0.05$). (comparison among treatments within day and adenylate).

	Treatment	Mean (SD) ATP	ADP	AMP	Total[1]	AEC[2]	Respiration Rate
		nM·mg^{-1}					μl O_2·mg^{-1}·hr^{-1}
Day 1	Control	0.15	0.01	0.09[a]	0.25[a]	0.54[a]	1.65
	$n = 4$	(0.13)	(0.01)	(0.03)	(0.15)	(0.24)	(0.42)
	5 μg·l^{-1} Cd	0.28	0.03	0.13[a]	0.44[a]	0.66[a]	1.66
	$n = 5$	(0.07)	(0.02)	(0.03)	(0.06)	(0.09)	(0.44)
	10 μg·l^{-1} Cd	0.28	0.04	0[b]	0.32[ab]	0.94[a]	1.30
	$n = 4$	(0.03)	(0.02)	(—)	(0.05)	(0.03)	(0.46)
Day 7	Control	3.25[a]	0.33[a]	0	3.57[a]	0.96	3.18[a]
	$n = 4$	(0.95)	(0.19)	(—)	(1.13)	(0.01)	(0.61)
	5 μg·l^{-1} Cd	0.74[b]	0.09[b]	0.05	0.87[b]	0.88	1.61[b]
	$n = 5$	(0.34)	(0.05)	(0.07)	(0.36)	(0.08)	(0.94)
	10 μg·l^{-1} Cd	0.47[b]	0.02[b]	0.02	0.51[b]	0.94	0.95[b]
	$n = 3$	(0.10)	(0.02)	(0.02)	(0.14)	(0.05)	(0.17)
Day 14	Control	0.38	0.03	0.05	0.45	0.90	2.87
	$n = 4$	(0.18)	(0.03)	(0.06)	(0.24)	(0.10)	(0.95)
	5 μg·l^{-1} Cd	0.52	0.03	0.05	0.61	0.89	2.89
	$n = 4$	(0.08)	(0.01)	(0.04)	(0.07)	(0.06)	(1.05)
	10 μg·l^{-1} Cd	0.53	0.06	0.04	0.62	0.90	2.08
	$n = 5$	(0.35)	(0.03)	(0.05)	(0.41)	(0.05)	(0.99)
Day 21	Control	0.25	0.02	0.03	0.29	0.87	2.23
	$n = 4$	(0.03)	(0.02)	(0.02)	(0.04)	(0.05)	(1.16)
	5 μg·l^{-1} Cd	0.14	0.01	0.01	0.16	0.94	1.77
	$n = 4$	(0.04)	(0.01)	(0.01)	(0.05)	(0.05)	(0.52)
	10 μg·l^{-1} Cd	0.22	0.02	0.01	0.25	0.94	1.81
	$n = 5$	(0.11)	(0.01)	(0.01)	(0.11)	(0.01)	(0.55)

[1]Total = ATP + ADP + AMP
[2]AEC = (ATP + ½ADP)/(ATP + ADP + AMP)

day of exposure (Table 3). No observable differences in calculated ATP turnover times were present in the tested crayfish (Table 5). However, the validity of the underlying assumption of completely aerobic transformation of ADP to ATP is questioned in this situation, based on the lack of significant correlations of ATP turnover time with either ATP concentrations or respiration rates (Table 5). No significant differences ($p > 0.05$) in the recorded parameters were found between male and female crayfish.

DISCUSSION

Previous cadmium exposure studies conducted with crayfish have not provided adequate data to calculate a chronic toxicity concentration (e.g., MATC). However, based upon the studies of Thorp et al. [27] and Giesy et al. [19], exposure to 5 and 10 μg Cd · l^{-1} is considered a sublethal stress to crayfish. These cadmium concentrations are similar to those reported to cause chronic effects on other aquatic organisms [26].

Bioaccumulation of Cd was recorded in gill tissues of *P. a. acutus* (Table 2; Fig. 1). The increase in gill Cd concentration did not affect metabolic rate since there were no significant correlations (Spearman Rank: $p > 0.05$) between gill tissue Cd concentrations and respiration rates at exposures of 5 or 10 μg · l^{-1} Cd. These results agree with

past work conducted with other crustaceans which indicate Cd uptake from water is a passive process, involving among other mechanisms, complexing with hemolymph proteins [28,29]. No major Cd accumulations were observed in dorsal muscle tissue during the 21 day exposure period (Table 2; Fig. 2). In crustacean studies, muscle tissues were found to accumulate this heavy metal at a lower rate than gills, hemolymph or the hepatopancreas [15,28]. Longer exposure periods may have resulted in greater muscle Cd concentrations.

The configuration of the outer electron shells of Cd and Zn are very similar and it has been speculated that they would react in a similar chemical manner and that Cd could replace Zn in biological molecules [13]. However, no evidence of Zn replacement was observed in the present study (Table 2).

The decreased ATP and total adenylate concentrations of gill tissues recorded in crayfish after 7 days exposure to Cd (Table 3) are thought to reflect the sublethal effects of Cd toxicity and were concomitant with significant decreases in gill tissue respiration rates (Table 3). In other crustaceans, exposures to low Cd concentrations were followed by gill respiration rates either above [15,30] or below [14,16] control values. The exact biochemical mechanisms involved in

Table 4. Adenylate concentrations and adenylate energy charge of dorsal tail muscle of crayfish utilized in the cadmium exposure experiment. Means within each sample day and parameter which do not have the same letters in their superscripts are significantly different ($\alpha = 0.05$). (Comparison among treatments, within day and adenylate.)

		Mean (SD)				
	Treatment	ATP	ADP	AMP	Total[1]	AEC[2]
		$nM \cdot mg^{-1}$				
Day 1	Control	5.23	0.53	0.25	6.01	0.87
	$n = 4$	(2.99)	(0.45)	(0.51)	(2.39)	(0.19)
	5 $\mu g \cdot l^{-1}$ Cd	6.13	1.30	0.62	8.04	0.83
	$n = 5$	(2.22)	(0.44)	(0.41)	(1.72)	(0.11)
	10 $\mu g \cdot l^{-1}$ Cd	7.94	0.70	0.07	8.71	0.95
	$n = 4$	(1.93)	(0.30)	(0.13)	(1.85)	(0.03)
Day 7	Control	6.21	1.22	0[a]	7.43	0.92
	$n = 4$	(1.40)	(0.39)	(—)	(0.49)	(0.02)
	5 $\mu g \cdot l^{-1}$ Cd	6.52	1.20	0.15[ab]	7.87	0.90
	$n = 5$	(1.06)	(0.43)	(0.34)	(0.54)	(0.07)
	10 $\mu g \cdot l^{-1}$ Cd	6.83	0.49	0.67[b]	7.98	0.90
	$n = 4$	(1.61)	(0.56)	(0.46)	(1.29)	(0.06)
Day 14	Control	7.13	0.30[a]	0.43[a]	7.88	0.93
	$n = 4$	(1.22)	(0.47)	(0.32)	(1.43)	(0.02)
	5 $\mu g \cdot l^{-1}$ Cd	8.35	1.47[b]	0[b]	9.82	0.92
	$n = 4$	(2.19)	(0.61)	(—)	(2.83)	(0.03)
	10 $\mu g \cdot l^{-1}$ Cd	8.78	1.01[ab]	0[b]	9.80	0.95
	$n = 5$	(2.29)	(0.56)	(—)	(2.37)	(0.03)
Day 21	Control	9.73[a]	1.18	0	10.92[a]	0.95
	$n = 4$	(1.16)	(0.30)	(—)	(1.03)	(0.02)
	5 $\mu g \cdot l^{-1}$ Cd	9.24[ab]	1.91	0	11.15[a]	0.92
	$n = 4$	(2.03)	(1.56)	(—)	(3.55)	(0.04)
	10 $\mu g \cdot l^{-1}$ Cd	5.81[b]	0.49	0.14	6.44[b]	0.94
	$n = 5$	(2.52)	(0.37)	(0.30)	(2.66)	(0.05)

[1]Total = ATP + ADP + AMP
[2]AEC = (ATP + ½ADP)/(ATP + ADP + AMP)

Table 5. Mean ATP turnover of excised gill tissue and significance levels of correlation with gill ATP concentrations and respiration rates (See Table 3).

		Mean ATP[1] Turnover Time · Sec (SD)	Spearman Rank Correlation	
			Gill ATP	Gill Respiration
Day 1	Control	0.1	NS	NS
	$n = 4$	(0.1)		
	5 $\mu g \cdot l^{-1}$ Cd	0.3	NS	NS
	$n = 5$	(0.2)		
	10 $\mu g \cdot l^{-1}$ Cd	0.4	NS	NS
	$n = 4$	(0.1)		
Day 7	Control	1.5	NS	NS
	$n = 4$	(0.2)		
	5 $\mu g \cdot l^{-1}$ Cd	1.1	NS	*
	$n = 5$	(0.9)		
	10 $\mu g \cdot l^{-1}$ Cd	0.8	NS	NS
	$n = 3$	(0.3)		
Day 14	Control	0.2	NS	NS
	$n = 4$	(0.1)		
	5 $\mu g \cdot l^{-1}$ Cd	0.3	NS	NS
	$n = 4$	(0.1)		
	10 $\mu g \cdot l^{-1}$ Cd	0.6	NS	NS
	$n = 5$	(0.9)		
Day 21	Control	0.2	NS	NS
	$n = 4$	(0.1)		
	5 $\mu g \cdot l^{-1}$ Cd	0.3	NS	NS
	$n = 4$	(0.3)		
	10 $\mu g \cdot l^{-1}$ Cd	0.2	NS	NS
	$n = 5$	(0.1)		

[1] ATP turnover computed with assumption of aerobic respiration (1 mole O_2 → 6 moles ATP)
NS = not significant ($p > 0.05$)
* = $p < 0.05$

these alterations in tissue metabolic rate are not known. Explanations of the effects of sublethal Cd toxicity on respiration include decreases associated with binding and blockage of sulfhydryl groups of respiratory enzymes [10] and enhancements caused by increased production of various enzymes [15]. Following day 7, phosphoadenylate concentrations and respiration rates increased to control levels (Table 3), possibly reflecting compensatory biochemical mechanisms (e.g., induction of replacement enzymes and metallothioneins). Mortality of crayfish exposed to low concentrations of Cd for longer periods [27] is probably associated with the breakdown or saturation of these compensatory mechanisms.

The rate of ATP turnover has been calculated for gill tissues of marine and freshwater decapods with knowledge of aerobic respiration rates and ATP concentrations. These investigations, involving species adaptations to food-poor habitats in crayfish [31] and osmoregulatory adjustment in crabs [24] yielded results which appeared to explain expected tissue energy fluxes. Because ATP turnover rates hypothetically represent the actual energy expenditure of cells and tissues, its use in approximations of biochemical "costs" of pollutant exposure would be of major interest. However, in the present investigation, changes in rates of ATP turnover were not correlated with the two measures of aerobic respiration recorded (Table 5) and could not be considered to represent accurate values. Chronic

exposure to small concentrations of Cd may cause changes in cellular energy production (i.e., increased variability in aerobic respiratory reactions or shifts to anaerobic pathways) making this technique unsuitable under these conditions.

Exposure to 5 and 10 μg Cd · l^{-1} for 21 days did not significantly lower AEC values of either gill or muscle tissue of the crayfish examined (Tables 3,4). During this exposure period, mean Cd concentrations reached 204 and 7 μg Cd · g^{-1} dry weight in gill and muscle tissue, respectively (Table 2). The AEC remained stable even during the significant drops in adenylate concentrations and respiration rates (Table 3) on day 7. These results support the view [18] that the adenylate energy charge is homeostatically maintained within a normal range even during periods of low level stress. Stress conditions in the present experiment apparently did not attain those under which decreases in energy charge could be recorded. The presence of significant decreases in AEC in another crayfish species under similar experimental conditions [19] may indicate differences in toxicity due to species, season or environmental parameters. The logical extension of the present set of experiments is to follow low level Cd effects on respiration, adenylate energy charge and mortality through longer exposure periods, examine other stresses and other species.

CONCLUSIONS

1. Cadmium was accumulated in gills and not in dorsal tail muscle tissue of the crayfish, *P. a. acutus*, during 21 days of exposure to 5 and 10 μg Cd · l^{-1}.
2. After 7 days of Cd exposure, significant decreases were observed in concentrations of ATP and total adenylates, as well as gill tissue respiration rates; but were not significantly different from control values for the duration of the experiment.
3. Exposure to low concentrations of Cd for 21 days did not affect AEC of either gill or dorsal tail muscle tissue of the crayfish species examined.
4. The AEC and ATP turnover rate did not provide indications of Cd toxicity at lower concentrations than those previously determined through conventional chronic lethality testing. These physiological indicators did permit a more complete understanding of the toxic action of cadmium on the tissues examined.

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REFERENCES

1. Friberg L., M. Piscator and G. Nordberg. 1971. Cadmium in the Environment. CRC Press. Cleveland, Ohio. 166 pp.
2. Fassett D.W. 1974. Cadmium. pp. 97-124. H.K. Lee ed. *In* Metallic Contaminants and Human Health. Academic Press. New York, NY. pp. 97-124.
3. Jennings J.R., P.S. Rainbow and A.G. Scott. 1979. Studies on the uptake of cadmium by the crab *Carcinus maenas* in the laboratory. II. Preliminary investigation of cadmium-binding proteins. *Marine Biol.* 50:141-149.
4. Giesy J.P., J.W. Bowling and H.J. Kania. 1980. Cadmium and zinc accumulation and elimination by freshwater crayfish. *Arch. Envir. Contam. Toxicol.* 9:685-699.
5. Lagerwerff J.V. and A.W. Spect. 1970. Contamination of roadside soil and vegetation with cadmium, nickel, lead and zinc. *Environ. Sci. Technol.* 4:583-586.
6. Flick D.F., H.F. Kraybill and J.E. Dimitrott. 1971. Toxic effects of cadmium: A review. *Environ. Res.* 4:71-85.
7. Hiatt V. and J.E. Hugg. 1975. The environmental impact of cadmium: An overview. *Int. J. Environ. Studies* 7:277-285.
8. Chadwick M.H. 1976. Cadmium in the environment. *Biologist* 23:23-29.
9. Perry H.M., G.S. Third and E.F. Perry. 1976. The biology of cadmium. *Med. Clin. N. Amer.* 60:759-769.
10. Lukacsovics F. and J. Salanki. 1964. Effects of substances influencing tissue respiration and the temperature on the O_2 consumption of the gill tissues in *Unio tumidus*. *Annls. Inst. Biol. Tihany.* 31:55-63.
11. Larsson A., B.E. Bengstsson and O. Svanberg. 1976. Some haematological and biochemical effects of cadmium on fish. *In* A.P.M. Lockwood

ed. Effects of Pollutants on Aquatic Organisms. Cambridge University Press. Cambridge. England. pp. 34-35.
12. Briggs B.R. 1979. Effects of cadmium on the intracellular pool of free amino acids in *Mytilus edulis*. *Bull. Environ. Contam. Toxicol.* 22:838-845.
13. Gould E. 1977. Alteration of enzymes in winter flounder, *Psuedopleuronectes americanus*, exposed to sublethal amounts of cadmium chloride. *In* F.J. Vernberg, A. Calabrese, F.P. Thurberg and W.B. Vernberg eds. Physiological Response of Marine Biota to Pollutants. Academic Press. New York. NY. pp. 209-224.
14. Thurberg F.P., M.A. Dawson and R.S. Collier. 1973. Effects of copper and cadmium on osmoregulation and oxygen consumption in two species of estuarine crabs. *Marine Biology* 23:171-175.
15. Thurberg F.P., D. Calabrese, E. Gould, R.A. Greig, M.A. Dawson and R.K. Tucker. 1977. Response of the lobster, *Homarus americanus*, to sublethal levels of cadmium and mercury. *In* F.J. Vernberg, A. Calabrese, F.P. Thurberg and W.B. Vernberg eds. Physiological Response of Marine Biota to Pollutants. Academic Press. New York. NY. pp. 185-197.
16. Vernberg W.B., P.J. DeCoursey, M. Kelly and D.M. Johns. 1977. Effects of sublethal concentration of cadmium on adult *Palaemonetes pugio* under static and flow-through conditions. *Bull. Environ. Contam. Toxicol.* 17:16-23.
17. Cooke M., A. Jackson, G. Nickless and D.J. Roberts. 1979. Distribution and speciation of cadmium in the terrestrial snail, *Helix aspersa*. *Bull. Environm. Contam. Toxicol.* 23:445-451.
18. Atkinson D.E. 1977. Cellular Energy Metabolism and Its Regulation. Academic Press. New York. NY.
19. Giesy J.P., S. Denzer, C.S. Duke and G.W. Dickson. 1981. Phosphoadenylate concentration and energy charge as measures of response of two freshwater crustaceans to stressors. *Internat. Verein. Limnol.* 21:205-220.
20. Giesy J.P., C.S. Duke, R.D. Bingham, G.W. Dickson and G.J. Leversee. In press. Changes in phosphoadenylate concentrations and adenylate energy charge of the freshwater clam (*Corbicula fluminea*) when exposed to cadmium. *Environ. Toxicol. Chem.*
21. Giesy J.P., H.J. Kania, R.L. Bowling, S. Mashburn and S. Clarkin. 1979. Fate and biological effects of cadmium introduced into channel microcosms. EPA-600/3-79-039. 157 pp.
22. Dickson G.W. 1980. Phosphoadenylate concentrations and adenylate energy charge in freshwater crayfish (Decapoda:Astacidae). Natural and stress related variation. Ph.D. dissertation. University of Georgia. Athens, Ga. 112 pp.
23. Engel D.W. and L.D. Eggert. 1974. The effect of salinity and sex on the respiration rates of excised gills of the bluecrab, *Callinectes sapidus*. *Comp. Biochem. Physiol.* 47A:1005-1011.
24. Engel D.W., R.L. Ferguson and L.D. Eggert. 1975. Respiration rates and ATP concentrations in the excised gills of the blue crab as a function of salinity. *Comp. Biochem. Physiol.* 52A:669-673.
25. Dickson G.W., L.A. Briese and J.P. Giesy. 1979. Tissue metal concentrations in two crayfish species cohabiting a Tennessee cave stream. *Oecologia* 44:8-12.
26. Lake P.S., R. Swain and B. Mills. 1979. Lethal and sublethal effects of cadmium on freshwater crustaceans. Australian Water Resources Council. Technical Paper No. 37. Australia Government Publishing Service. Canberra, Australia.
27. Thorp J.H., J.P. Giesy and S.A. Wineriter. 1979. Effects of chronic cadmium exposure on crayfish survival, growth and tolerance to elevated temperatures. *Arch. Environm. Contamin. Toxicol.* 8:449-456.
28. Nimmo D.R., D.V. Lightner and L.H. Bahner. 1977. Effects of cadmium on the shrimps *Penaeus duorarum*, *Palamonetes pugio* and *Palamonetes vulgaris*. *In* F.J. Vernberg, A. Calabrises, F.P. Thurberg and W.B. Vernberg eds. Physiological Responses of Marine Biota to Pollutants. Academic Press. New York. NY. pp. 131-183.
29. Wright D.A. 1977. The effect of salinity on cadmium uptake by the tissues of the shore crab, *Carcinus maenas* (L.). *J. Exp. Biol.* 63:137-146.
30. Moraitous-Apostolopoulou M., G. Verriopoulous and P. Lentzou. 1979. Effects of sublethal concentrations of cadmium as possible indicators of cadmium pollution for two populations of *Arcatia clausi* (Copepoda) living in two differently polluted areas. *Bull. Environ. Contam. Toxicol.* 23:642-649.
31. Dickson G.W. and R. Franz. 1980. Respiration rates, ATP turnover and adenylate energy charge in excised gills of surface and cave crayfish. *Comp. Biochem. Physiol.* 65A:375-379.