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Selective Protection of Temperature against Cadmium Acute Toxicity to *Bufo arenarum* Tadpoles

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Cadmium is widely distributed and quantities of this potential pollutant found in natural systems are increasing (US EPA 1985; Nriagu and Pacyna 1988). Cadmium toxicity to aquatic ectothermal animals depends on complex biochemical interactions and a balance between temperature-dependent rates of absorption, detoxification and excretion. The main sites of cadmium deposit are the kidney and liver of vertebrate animals (Foulkes 1986; Ravera 1984).

Toxicity of cadmium in aqueous medium also may be related to its availability, which in turn could be a function of chemical speciation. The free divalent cation is reportedly the most toxic cadmium form (Jacobson and Turner 1980).

The aim of this study was to examine the effect of temperature on the acute toxicity of Cd (II) to larvae of the toad Bufo arenarum.

MATERIALS AND METHODS

Larvae used in our bioassays were obtained using techniques described by Muiño et al. (1990). Prior to cadmium exposure animals were held in artificial pond water (APW) at room temperature (20-22°C) and fed pulverized fish food every other day. The composition of the APW was: 1.3 mM NaCl, 0.1 mM KCl, 0.8 mM $CaCl_2$ and 0.2 mM $NaCO_3H$ (Alvarado and Johnson 1966).

Tadpoles were staged according to Echeverría and Fiorito de López (1981). Young (stage 26) and prometamorphic (stage 28 III-29) larvae were used. Young tadpoles (YT) are characterized by an incipient development of hind limbs. Prometamorphic tadpoles (PT) are distinguished by the presence of five digits on

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hind limbs (stage 28 III) as well as an articulation between the stylopodium and the zeugopodium. Also, the posterior legs of PT larvae are completely developed (stage 29).

Static, renewal bioassays were conducted following procedures of the American Public Health Association (APHA 1975). A test began when tadpoles were distributed to glass containers and acclimated 48 hr prior to cadmium additions at 20°C and 25°C and a photoperiod of 12:12 hr. Test solutions with nominal test concentrations of 1, 2, 4, 8 and 16 mg Cd/l were prepared daily from a stock solution of $CdCl_2$ in APW. A solution without cadmium was considered a control. Each cadmium concentration was prepared in duplicate. Two tests were completed with each larval stage at each temperature. Tadpoles were examined at 24 hr intervals following cadmium additions for 96 hr. Those exhibiting no heartbeat or which did not respond to gentle prodding were considered dead and were removed from assay containers.

Median lethal concentrations (LC-50) were calculated by probit analysis (Finney 1971) after adjusting for mortality among tadpoles in the control treatment with the Abbott´s correction. The 95 % confidence limits for each estimate was calculated by the Fieller´s theorem. In instances where confidence limits for replicate test results overlapped, data of both assays were combined and the LC-50s recalculated. All regressions were significatively lineal ($p<0.05$).

RESULTS AND DISCUSSION

Since the tadpoles used in our tests were hatched in the laboratory and grown in APW, their responses cannot be attributed to a spurious previous contact with the metal.

Survival among YT larvae in control treatments at 20°C and 25°C was always greater than 95 % (Fig. 1A). Also, survival at 1 and 2 mg Cd/l was comparable and at least 75 % at both temperatures. In comparison, survival at 4 mg Cd/l decreased exponentially over the course of the exposure period. It was about 20 % higher at 25°C than at 20°C, however. At cadmium concentrations in excess of 4 mg/l survival within 24 hr was negligible and these data were not used in calculation of LC-50s.

Estimated medial lethal concentrations decreased with exposure time (Table 1). They were slightly, but consistently, higher at 25°C than 20°C and thus support the graphical interpretation of results presented in Fig. 1A that YT tadpoles were more resistant to cadmium

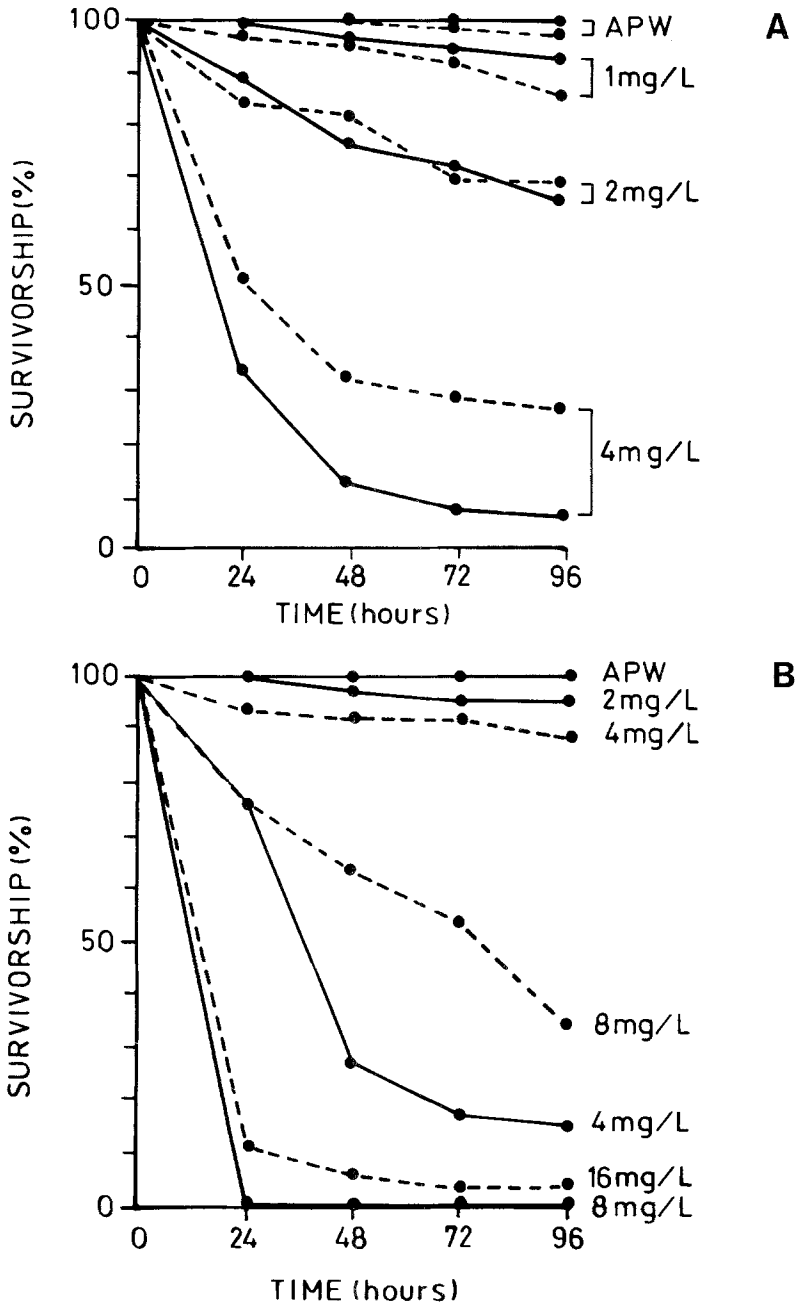

Figure 1. Survivorship of Bufo arenarum tadpoles incubated in Cd-containing solutions at 20 (——) and 25ºC (- - -). Controls in APW. Cadmium concentrations are indicated on the right end of the curves. A: young tadpoles; B: prometamorphic tadpoles.

at the high than at the low experimental temperature.

This effect of temperature was also evident among PT larvae, particularly at 4 and 8 mg Cd/l (Fig. 1B). The magnitude of 96-hr LC-50s at 25°C, for example, was about double that at 20°C. Comparison of LC-50s by larval stage revealed that this thermal effect was more pronounced for PT than for YT (Tables 1 and 2, Fig. 2).

Reductions in activity, arrhythmic contractions, atypical swimming position, axial incurvations and abdominal epithelial peeling (Ferrari and Salibián 1991) were observed among tadpoles exposed to cadmium.

Table 1. LC-50 values of Cd for Bufo arenarum young tadpoles (stage 26) incubated at two temperatures. (n = 80 tadpoles/concentration, degrees of freedom: 3).

	Time (hr)	LC-50 (mg/l)	Confidence limits	Slope	Correl. coeff.
20°C	24	3.41	3.14-3.74	6.71	0.99
	48	2.55	2.33-2.79	5.68	0.98
	72	2.32	2.12-2.55	5.34	0.98
	96	2.19	1.99-2.41	5.16	0.98
25°C	24	4.05	3.47-5.11	3.37	0.97
	48	3.15	2.80-3.63	3.91	0.99
	72	2.87	2.52-3.33	3.53	0.99
	96	2.65	2.31-3.11	3.11	0.95

Table 2. LC-50 values of Cd for Bufo arenarum prometamorphic tadpoles (stages 28 III - 29) incubated at two temperatures. (n= 40 and 50 tadpoles / concentration, degrees of freedom: 3).

	Time (hr)	LC-50 (mg/l)	Confidence limits	Slope	Correl. coeff.
20°C	24	4.76	4.42-5.53	6.97	0.89
	48	3.40	3.04-3.77	8.51	0.99
	72	3.11	2.78-3.45	8.71	0.99
	96	3.06	2.74-3.38	8.87	0.99
25°C	24	9.92	8.76-11.30	4.80	0.97
	48	8.60	7.62- 9.75	4.93	0.98
	72	7.84	6.95- 8.93	5.18	0.95
	96	6.77	5.97- 7.65	4.94	0.99

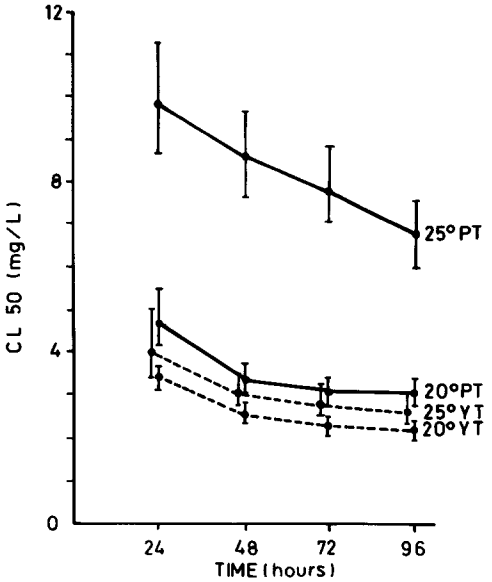

Figure 2. Time-related changes in the LC-50s of Cd (±95% confidence limits) for Bufo arenarum young (YT) and prometamorphic (PT) tadpoles incubated at 20 and 25ºC.

The intensities of these changes were proportional to exposure concentration of cadmium and to exposure time. They were independent of developmental stage.

Pérez-Coll et al. (1986) described an analogous protective effect of temperature on B. arenarum embryos at stages 19 (cardiac beat) and 25 (complete operculum) at cadmium concentrations of 0.07-1.68 mg/l. These results showed that mortality was independent of temperature (20 and 30°C), but that the incidence of malformations was considerably reduced at the higher of these two temperatures. Exposure of 1- and 4-week old Microhyla ornata tadpoles to cadmium at 25.5-26°C yielded results that are comparable to those we present in Table 1 (Rao and Madhyastha 1987). Unfortunately, their figures do not allow one to determine the existence of age-related differences.

Mechanisms by which trace elements enter and are regulated by the organism are poorly understood. The toxicity of cadmium might be related to availability of the free ion instead of its total concentration. The free ion amounts decrease because of its complexation capacity with other ionic species in solution like Cl^- or HCO_3^-. This capacity must be temperature-dependent. Under our experimental conditions the composition of

the external solution remained constant. Only temperature and the age of the animals varied. Thus it is evident that the interpretation of our results cannot be merely reduced to physicochemical considerations; if this was the case the effect must have been basically the same in both YT and PT. However, an increase of 5ºC in the external bath provoked an important decrease in the acute toxicity signs of cadmium in PT. We consider that the increase of ambient temperature must affect the standard metabolic rate of the animals and, consequently, might affect biochemical events associated with the kinetics of detoxification-releasing processes (e.g., induction of metallothionein synthesis) as well as other associated reactions like those involved in the regulation of metal homeostasis (Hochachka and Somero 1984; Simkiss and Watkins 1988). There is recent evidence indicating that induction of MT synthesis and its intracellular accumulation are early events that could occur within the periods of time of our experiments (Gagné et al. 1990).

The negligible or poor differential response of Bufo arenarum late embryonic and young tadpole stages to the same environmental stimuli might be attributed to the fact that the metabolic pathways involved in the endogenous protective mechanisms are either nonfunctional or were not fully developed yet.

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REFERENCES

Alvarado RH, Johnson SR (1966) The effects of neurohypophysial hormones on water and sodium balance in larval and adult bullfrogs (Rana catesbeiana). Comp Biochem Physiol 18: 549-561

American Public Health Association (APHA), American Water Works Association and Water Pollution Control Federation (1975) Standard methods for the examination of water and wastewater. 15 th Ed, Washington DC

Echeverría DD, Fiorito de López LE (1981) Estadios de la metamorfosis en Bufo arenarum (Anura). Physis (Buenos Aires) 40 B: 15-23

Ferrari L, Salibián A (1991) Alteraciones epidérmicas en larvas jóvenes de Bufo arenarum expuestas a cadmio en soluciones no iónicas. Arch Biol Med Exp

24:R141
Finney DJ (1971) Probit analysis. Cambridge University Press
Foulkes EC (1986) Cadmium. Springer-Verlag, Berlin.
Gagné F, Marion M, Denizeau F (1990) Metal homeostasis and metallothionein induction in rainbow trout hepatocytes exposed to cadmium. Fund Appl Toxicol 14: 429-437
Hochachka PW, Somero GN (1984) Biochemical adaptation. Princeton University Press. Princeton, New Jersey.
Jacobson KB, Turner JE (1980) The interaction of cadmium and certain other metal ions with proteins and nucleic acids. Toxicology 16: 1-37
Muiño CV, Ferrari L, Salibián A (1990) Protective action of ions against cadmium toxicity to young Bufo arenarum tadpoles. Bull Environ Contam Toxicol 45: 313-319
Nriagu JO, Pacyna JM (1988) Quantitative assessment of worldwide contamination of air, water and soils by trace metals. Nature 333: 134-139
Pérez-Coll CS, Herkovits J, Salibián A (1986) Teratogenic effects of cadmium on Bufo arenarum during gastrulation. Experientia 42: 1174-1176
Rao J, Madhyastha MN (1987) Toxicities of some heavy metals to the tadpoles of frog, Microhyla ornata (Dumeril & Bibron). Toxicol Let 36:205-208
Ravera O (1984) Cadmium in freshwater ecosystems. Experientia 40: 2-14
Simkiss K, Watkins B (1988) Metal ion activities in oscillating temperatures. Mar Environ Res 24:125-128
US Environmental Protection Agency (1985) Ambient water quality criteria for cadmium-1984. EPA 440/5-84-032. Environmental Research Laboratories, Duluth (MN), Narragansett (RI)

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