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Stage-Dependent Susceptibility of *Bufo arenarum* Embryos to Cadmium

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Cadmium (Cd) is one of the most toxic heavy metals and affects a wide spectrum of organisms (Ravera 1984). Its natural occurrence in the environment is rather low, but it is significantly increased by anthropogenic inputs, such as agricultural and industrial applications (Davis 1984). From an ecotoxicological point of view, amphibians are useful indicators of freshwater contamination (Fernández et al. 1989), while on the other hand amphibian embryos and larvae are higher in the food chains in which Cd is accumulated (Dmowsky and Karolewsky 1979). According to previous studies, the effects of Cd on amphibian embryos treated from the two blastomere stage onwards include delayed development, reduced body size, congenital malformations, behavioral disorders and eventually death (Pérez-Coll et al. 1985). The degree of heavy metal toxicity could be related to stage susceptibility. For instance, lead exposure to different stages of Bufo arenarum embryos show that the neuromuscular activity stage (stage 16) is twice as sensitive to lead than neurulae or gill circulation stages (Pérez-Coll and Herkovits 1990). The purpose of this study is to report the minimal Cd concentration causing Bufo arenarum embryos at six different stages to arrest their development and die within 24 hours.

MATERIALS AND METHODS

Ovulation was induced by subcutaneously injecting Bufo arenarum adult females with 2000 IU of human chorionic gonadotrophin (HCG) plus 1000 IU of pregnant male serum gonadotrophin (PMSG). Oocytes were fertilized in vitro and the eggs were maintained in 10% Holtfreter solution (HS) until the 2-blastomere stage (Del Conte and Sirlin 1951) was reached.

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The gelatinous coats were removed with 2% thioglycolic acid solution neutralized with NaOH at pH 7.4. Three mating pairs of adult Bufo arenarum contributed in the same proportion to the experimental groups. Batches of 30 embryos (in duplicate, a total of 60 individuals for each condition) were placed in 20 different Cd concentrations in 10% Haltfreter solution ranging between 0.1 and 4 mg Cd^{2+}/L (Cd atomic absorption standard solution, Sigma) from the following stages: A) 2 blastomeres (S.3); B) late gastrulae (S.12); C) neural tube (S.16); D) gill circulation (S.20); E) opercular folds (S.23) and F) complete operculum (S.25). Control groups were maintained in 10% HS. Survival of control embryos was over 90%. Embryos were kept at temperatures ranging between 18-21°C. The results were statistically analyzed by using X^2 test.

RESULTS AND DISCUSSION

This study confirms the severe toxicity of Cd to amphibian embryos. A concentration of 0.25 mg Cd^{2+}/L arrests the development of 100% of Bufo arenarum embryos at neurulae (stage 16) within 24 hours (Fig.1).

With respect to this stage-dependent susceptibility study, we emphasize embryo lethality instead of malformations or other disorders because: 1) although the same teratogenic effect can be obtained by different toxic agents and even at different embryonic stages (Pérez-Coll et al. 1985 and 1986; Pérez-Coll and Herkovits 1990), some other teratogenic effects are stage-dependent (Herkovits and Fernández 1978), and 2) this study also includes embryonic stages in which the morphogenic processes are advanced or even completed.

The survival of Cd-treated embryos is markedly stage-dependent ($p<0,01$), with the neural tube stage 16 times more sensitive than the blastulae. During postneural stages, the embryos become gradually more resistant. At the end of embryonic development, however, the tolerance to Cd is still one fourth that of the blastula stage. The interference that Cd exerts on embryonic development appears to be related to its multiple effects upon nucleic acids and structural and enzymatic proteins (Jacobson and Turner 1980), as well as on the availability of essential elements, energy-rich molecules (Reddy et al. 1988) and oxygen consumption (Herkovits and Jatimtinsky 1986) which result in a critical reduction of embryonic performance and finally death.

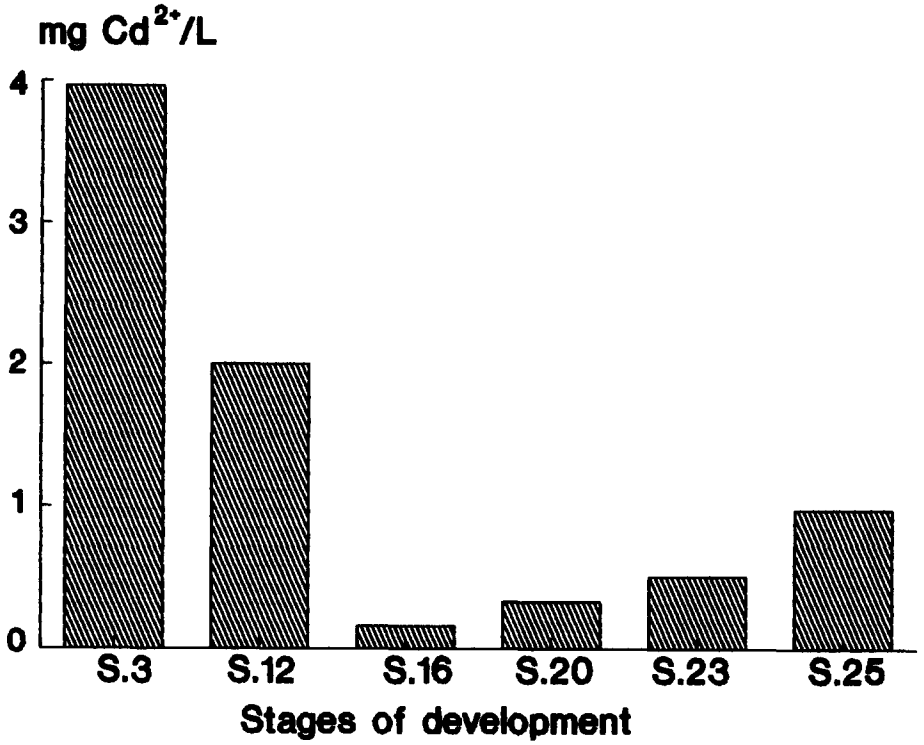

Figure 1. Concentrations of cadmium that cause 100% mortality of Bufo arenarum embryos within 24 hours of treatment.

The stage-dependent susceptibility to Cd of Bufo arenarum embryos could be related to stage-dependent features in the uptake of this heavy metal (Michibata 1981). Preliminary results on uptake of Cd by Bufo arenarum show that Cd incorporation is not stage-dependent, at least between the last developmental stages (Zeni et al. 1988). Although embryos at the gill circulation (S.20) and complete operculum (S.25) stages present similar levels of Cd uptake, S.25 is about three times more resistant to Cd than S.21. Therefore, in the stage-dependent susceptibility other mechanisms such as changes in the detoxification capacity, e.g., synthesis of metallothioneins (Suzuki et al. 1986) could also be involved. It is noteworthy that the most susceptible period of embryonic development of Bufo arenarum to Cd,as well as other toxic agents, is during the beginning of organ and tissue differentiation (Herkovits and Fernández 1978).

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