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Embryotoxicity of Lead on *Bufo arenarum*

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Lead, one of the oldest and more widely distributed pollutants, produces serious toxicological effects (for a review, see Goyer, 1981). In man, these alterations, known as saturnism, are mainly characterized by neurological disorders and anemia. In experimental animals, including mammals, this heavy metal also produces reproductive and developmental alterations, the most significant being the reduced litter size, retarded growth, delayed development and reduced body size (Goyer, 1981). From an ecotoxicological point of view, amphibians are useful as indicators of environmental contamination because they are sensitive to a great variety of toxic agents (Cook, 1981). Considering that *Bufo arenarum* is one of the most widely distributed toads in South America, in the present work we study the LC50 and teratogenical effects of lead on *Bufo arenarum* embryos obtained from different couples of parents exposing them from the 2-cell stage onwards. A differential susceptibility to this heavy metal in embryos obtained from five different couples of parents is described.

MATERIALS AND METHODS

Ovulation of *Bufo arenarum* females (about 250g each) was induced by injection of a suspension of homologous hypophysis or 1000 IU of pregnant mare serum gonadotrophin (PMSG) plus 2000 IU of human chorionic gonadotrophin (HCG). Oocytes were fertilized *in vitro* with a sperm suspension in 10 °/o Holtfreter's solution (HS). Developing embryos were staged according to Del Conte and Sirlin (1951). Jelly coats were removed with a 2 °/o thioglycolic acid solution, neutralized with NaCl at pH 7.2 and the eggs were thoroughly washed with HS.

Toxic effects caused by continuous treatment of lead were studied by placing two batches of 30 embryos (a total of 60 individuals for each condition) in Petri dishes containing 40 mL of lead solutions at different concentrations from the 2-cell stage onwards. Solutions were prepared by diluting a Pb (NO_3) stock solution (1.20 g/L Pb^{++}) in HS to give concentrations of 0.12, 0.25, 0.50, 1.0, 2.0, 4.0, 8.0, 16.0 and 32.0 mg/L Pb^{++} ; control embryos were simultaneously maintained in HS without additions.

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Experiments were conducted at 20-21°C. Solutions were changed daily and dead individuals were recorded and inmediately removed. The 48 h LC50 values were analitically calculated (based on linear regression procedure) for embryos obtained from five different couples of parents and recorded separately in order to evaluate a probable differential susceptibility. Teratological, behavioral and lethal effects were evaluated under a Wild stereoscopical microscope. Individuals of each group were prepared for scanning electron microscopy (SEM) (Herkovits, 1977) and observed in a Jeol JSM-35 CF scanning electron microscope operated at 10 Kw.

RESULTS AND DISCUSSION

The 48 h LC 50 values for embryos obtained from five different *Bufo arenarum* couples of parents ranged between 0.47 and 0.90 mg/L Pb^{++} . In the 8.0-32.0 mg/L Pb^{++} range, none of the embryos survived beyond 48 h (mid-late blastulae stages). These embryos showed pigment displacement from animal to vegetative pole. Progressive dissociation occurred, beginning with loss of intercellular contacts followed by the appearance of spherical cells mainly in the marginal area, and finally complete embryonic dissociation. Effects were concentration-dependent.

Concentrations ranging between 2.0 to 4.0 mg/L Pb^{++} arrested the development of embryos between late gastrulae (S.12) and early neurulae (S.13) stages (Table 1). Embryos treated with 4.0 mg/L Pb^{++} exhibited loss of intercellular contacts in ectodermal cells from the dorsal and lateral lips of the blastopore, while individuals exposed to 2.0 mg/L Pb^{++} showed persistent yolk plugs with pigmented and spherical cells.

The differential effect of lead on embryos obtained from each couple of parents was expressed with concentrations below 1.0 mg/L Pb^{++} . Two patterns of effects were observed:

1) high incidence of malformations (persistent yolk plug, partial neurulation, microcephaly, pear shaped embryos) followed by lethality at neurulae with 0.5 and 1.0 mg/L Pb^{++} (couples 2 and 4 respectively, Table 1). The few surviving embryos beyond 72 h exhibited underdeveloped gills, microcephaly, delayed development, stunted tail, axial incurvations (Fig 1 D, F);

2) gradual increase in teratogenesis and lethality (couples 1, 3 and 5) as follows: With 1.0 mg/L Pb^{++} , about 80-100°/o embryos were unable to survive beyond 48 h exhibiting malformations such as those described for 2.0 mg/L Pb^{++} . 35-50 °/o embryos treated with 0.50 mg/L Pb^{++} died within 48 h of treatment (Table 1) and the remainder showed abnormal development (failure in the closure of the blastopore and disorders during the neurulation process. Embryo survival was constant from the gill circulation stage (S.20) onwards although embryos continued developing 1-2 stages behind controls. These embryos showed stunted tail, hidropsy (Fig. 1 A, B, C, E and F) and rugged cellular surfaces which finally produced epithelial desquamation. The underdeveloped gills of these embryos were functional. Sigmoidal body shapes due to marked axial incurvations (Fig 2 A, B) and bifid tails also occurred. Neurological disturbances such as circular swimming and spasmodic movements were observed.

25 °/o embryos treated with 0.25 mg/L Pb^{++} stopped their development at neurulae (S.16) (Table 1). The remaider developed either normally or with some of the alterations describes for treatments with 0.50 mg/L Pb^{++} .

No significant effects were obtained with 0.12 mg/L Pb^{++} .

Table 1. Mortality and malformations produced by lead (nitrate) treatment from the 2-cell stage onwards of *Bufo arenarum* embryos obtained from different couples of parents.

	Couple 1						Couple 3						Couple 5					
	Hours of treatment and stage						Hours of treatment and stage						Hours of treatment and stage					
	24 (S.12)[1]		48 (S.18)		72 (S.21)		24 (S.12)		48 (S.18)		72 (S.21)		24 (S.12)		48 (S.18)		72 (S.21)	
mg/L Pb^{++} [2]	Mo[3]	Mf[4]	Mo	Mf	Mo	Mf	Mo	Mf	Mo	Mf	Mo	Mf	Mo	Mf	Mo	Mf	Mo	Mf
0	0	0	0	1.7	0	5	0	0	6.7	11	16.7	8	0	0	3.3	6.9	8.3	5.5
0.12	0	0	0	3	0	5	0	0	6.7	3.7	10	3.7	0	0	5	3.5	6.7	5.4
0.25	0	19.7	24.6	10.9	24.6	11	0	13.3	26.7	4.5	26.7	4.5	0	15	25	6.7	25	6.7
0.50	12.7	21.8	34.9	75.6	54	100	3.3	62	50	76	53	100	8.3	40	44.3	61.8	48.3	100
1.00	18.8	19.2	81.3	100	100	-	10	100	100	-	100	-	13.3	23.1	93.3	100	100	-
2.00[5]	29.5	100	100	-	100	-	20	100	100	-	100	-	23.3	100	100	-	100	-

	Couple 2						Couple 4					
	Hours of treatment and stage						Hours of treatment and stage					
	24 (S.12)		48 (S.18)		72 (S.21)		24 (S.12)		48 (S.18)		72 (S.21)	
mg/L Pb^{++}	Mo	Mf	Mo	Mf	Mo	Mf	Mo	Mf	Mo	Mf	Mo	Mf
0	3.3	7	3.3	0	6.7	3.6	0	0	1.6	0	1.6	1.6
0.12	3.3	13.8	3.3	13.8	3.3	17.2	0	0	1.7	0	1.7	0
0.25	0	73.3	6.6	78.6	13.3	100	0	0	0	0	0	5
0.50	6.7	100	83.3	100	100	-	0	0	1.7	0	1.7	100
1.00	3.3	100	100	-	100	-	0	100	95.1	100	100	-
2.00	30	100	100	-	100	-	0	100	100	-	100	-

1. Stage of control embryos. S.12: late gastrulae, S.18: neuromuscular activity, S.21: open mouth
2. Number of embryos treated at each concentration (in duplicate): 30
3. Mo: $^{o}/_{o}$ mortality
4. Mf: $\frac{\text{Number of abnormal embryos}}{\text{Number of surviving embryos}}$
5. Concentrations higher than 2.0 mg/L Pb^{++} produced 100 $^{o}/_{o}$ lethality at 48 h (see Results)

The dashed line indicates the threshold concentration which exerted the effects.

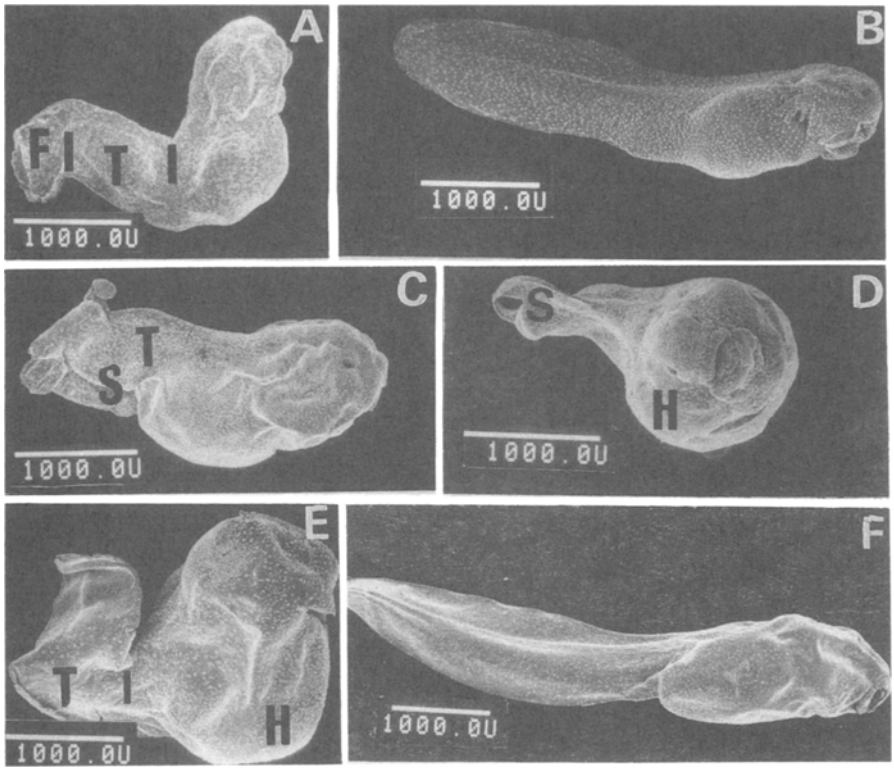

Fig. 1. A Panoramic view of a *Bufo arenarum* embryo treated with 0.50 mg/L Pb^{++} (couple 1) at the open mouth stage (S.21) X 24. Notice the sigmoidal shape due to the incurvations in the body axis (I), stunted tail (T), partially developed fin (F) and generally reduced body size. B. Control embryo fixed at S.21 X 24. C. Embryo treated with 0.50 mg/L Pb^{++} (couple 1) fixed at the end of embryonic development (S.25) X 24. Observe the generally reduced body size, stunted tail (T) with irregular borders and bifid spine (S). D. Embryo treated with 0.25 mg/L Pb^{++} (couple 2) at S.25 X 27. Observe the reduced body size, bifid spine (S) and hydropsy (H). E. Embryo treated with 0.50 mg/L Pb^{++} (couple 1) at S.25 X 24. Notice the incurvated body axis (I) with irregular borders, hydropsy (H) and the stunted tail (T). F. Control embryo fixed at S.25 X 20.

SEM studies of control as well as experimental embryos showed normal glandular and ciliated cells in the epithelium.

The susceptibility of amphibians to heavy metals such as copper, mercury, arsenic and cadmium at early stages of embryonic development has been already reported (Landé and Guttman, 1973; Ghate and Mulherkar, 1980; Vega and Pisanó, 1980; Pérez-Coll et al, 1985).

Our results indicate that lead interferes with the normal development of *Bufo arenarum* from 0.25 mg/L Pb^{++} onwards. These alterations do not seem to be lead specific since the same malformations have been produced using other adverse physicochemical conditions such as Cd, Li, Cn, D_2O or thermal shock (Pérez-Coll, 1985;Herkovits and Fernández, 1979; Rosenthal and Alderdice, 1976) in amphibian, fish and mammalian embryos.

Probably this absence of specificity is due to the multiple effects that these agents exert on biological systems. Particularly in the case of divalent cations, we have obtained similar effects with cadmium (Pérez-Coll et al, 1985) and lead treatments (present results), which may be related to the existence of similar mechanisms of action for both cations. In fact, lead as well as cadmium interferes with proteins, nucleic acids, energetic molecules and also competes with essential elements (Vallee and Ulmer, 1972). The interaction of lead with calcium might explain the cellular dissociation observed with high concentrations of lead. On the other hand, the neurotoxic action of lead, which is evident when neuromuscular activity begins, may be related to the binding of lead ions at neuromuscular junctions, altering calcium concentrations and hence the spontaneous release of transmitter (Kotton and Yaari, 1982). Lead also inhibits Na-K ATPase activity in diverse types of cells, including cells of the renal tubules, an action that might explain disturbances in the osmoregulatory mechanisms that give rise to the hydropic embryos.
Relative to pigment arragement, lead is known to bind to melanin with a high affinity (Ireland et al, 1979), being thus capable of producing pigment displacement.

The usefulness of adult Anura and Urodele as biological indicators in toxicological bioassays has been informed (Slooff and Baerselman, 1980). Although the data available of lead content in our continental waters (Riachuelo River) range between 0.045 and 0.067 mg/L Pb^{++} (INCYTH - CTUA, 1985) and therefore the embryonic development of *Bufo arenarum* does not seem to be really affected, this specie could be considered as a sensitive indicator of lead contamination since 1.0 mg/L Pb^{++} produced high levels of lethality and malformations in all cases and within a few hours.

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REFERENCES

Cook AS (1981) Tadpoles as indicators of harmful levels of pollution in field. Env Pol 25:123-133

Del Conte E, Sirlin JL (1951) Los primeros estadios embrionarios en *Bufo arenarum*. Acta Zool Lilloana 12:495-499

Ghate HV, Mulherkar L (1980) Effect of mercuric chloride on embryonic development of the frog *Microhyla ornata* Indian J Exp Biol 18:1094-1096

Goyer RA (1981) Lead. In Academic Press Inc (ed) Disorders of mineral metabolism I pp 159-199

Herkovits J (1977) Are shape and morphogenesis independent phenomena? Experientia 33:510-513

Herkovits J, Fernández A (1979) Tolerancia a noxas durante el desarrollo embrionario Medicina (Bs. As.) 39:400-408

INSTITUTO NACIONAL DE CIENCIA Y TECNOLOGIA HIDRICA (Argentina) - CTUA (Ezeiza) (1985) Monitoreo de calidad de aguas en la Cuencia del Río Matanza-Riachuelo en el período Octubre 1982-Septiembre 1984; informe final Comisión municipal de la vivienda, gabinete Riachuelo-Ezeiza I pp 40-50

Ireland MP, Richards KS, Gwynn I The localisation of lead in the skin of light - and dark- adapted *Xenopus laevis* Histochemistry 65:31-34

Kotton L, Yaari Y (1982) Sites of action of lead on spontaneous transmitter release from motor nerve terminals Israel J Med Sci 18:165-170

Landé S, Guttman SI (1973) The effects of copper sulfate on the growth and mortality rate of *Rana pipiens* tadpoles Herpetologica 29:22-27

Pérez-Coll CS, Herkovits J, Salibián A (1985) Effects of cadmium on the development of an amphibian (In Spanish) Arch Biol Med Exp 18:33-40

Rosenthal H, Alderdice DF (1976) Sublethal effects of environmental stressors, natural and pollutional on marine fish eggs and larvae J Fish Res Board Can 33:2047-2065

Sloof WB, Baerselman R (1980) Comparison of the usefulness of the Mexican Axolotl *(Ambystoma mexicanum)* and the Clawed Toad *(Xenopus laevis)* in toxicological bioassays Bull Environm Toxicol 24:439-443

Vallee BL, Ulmer DD (1972) Biochemical effects of mercury, cadmium and lead Ann Rev Biochem 41:91-129

Vega DE, Pisanó A (1980) Teratogénesis experimental por arsénico durante el desarrollo de un anfibio Rev Mus Hist Nat San Rafael, Mendoza 8:29-34

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