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Reduction of Heavy Metal Toxicity to *Xenopus* Embryos by Magnesium Ions

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Use of vertebrate embryos as test organisms for determining heavy metal toxicity provides an excellent opportunity to evaluate what concentrations will cause damage at the most sensitive stage of the life cycle (BIRGE et al., 1973). Recent studies in this laboratory (MILLER and LANDESMAN, 1977) have expanded on an earlier report of an absolute magnesium requirement by developing amphibian embryos (BROWN, 1961). This report shows that there is also a direct relationship between environmental levels of magnesium ions and the toxicity of some metal ions.

METHODS

Early stage Xenopus laevis embryos were obtained from hormone-induced spawnings. The embryos were chemically dejellied, divided into groups of 50 and then placed in plastic culture dishes containing 100 ml of rearing medium. The culture dishes were arranged in a grid so that magnesium ion concentrations (0, 0.2, 2, 20, 200 ppm) added to the 10% Holtfreter's Solution rearing medium increase along one axis and the concentrations (generally 10, 1, 0.1, .01, .001 ppm) of the heavy metal ions to be tested (Pb, Hg, Cd, Mn) decrease along the other axis. Test grids were run twice using different spawnings. Each run took 6 days at 18 C.

Xenopus embryos were reared under these conditions from blastula to early feeding stages. Daily changes of the rearing medium were made for the first three days to insure magnesium deficiencies in those embryos reared without magnesium in their medium. When the embryos reached the feeding stages the number surviving and their general condition were recorded.

RESULTS AND DISCUSSION

Morphological deformities occurring in Xenopus embryos as the result of chronic exposure to heavy metal ions are fairly uniform regardless of the ion used. For the sake of simplicity these effects can be divided

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TABLE 1

Effects of metal vs magnesium ions on *Xenopus* development

Mg ppm	Hg 1	Hg .1	Hg .05	Hg .01	Hg .001	Pb 10	Pb 1	Pb .5	Pb .1	Pb .01	Pb .001	Cd 10	Cd 1	Cd .5	Cd .1	Cd .01	Cd .001	Mn 10	Mn 5	Mn 1	Mn .1	Mn .01	Mn .001
200	D	M-S	M-S	N	N	D	M	SL-M	N	N		D	N-SL	N	N	N	N	N	N	N	N	N	N
	0	79	86	93	90	0	99	88	93	88		0	94	88	95	93	94	94	98	92	94	84	86
20	D	M-S	M-S	N	N	D	M-S	M	N	N	N	D	M	N	N	N	N	SL	N	N	N	N	N
	0	61	96	94	97	0	81	100	78	96	96	0	59	94	91	91	91	94	90	91	100	90	94
2	D	M-S	M-S	N	N	D	M-S	M	N	N	N	D	S	M-S	N	N	N	M-S	SL-M	N	N	N	N
	0	75	88	93	89	0	59	92	95	95	97	0	30	100	93	98	90	96	96	88	93	96	87
.2	D	M-S	M-S	SL	SL	D	S	M-S	M-S	SL	SL	D	D	S	M	SL	SL	S	S	M-S	SL	SL	SL
	0	51	96	93	96	0	73	94	94	95	93	0	0	60	96	91	91	72	50	92	74	96	93
0	D	S	S	S	S	D	S	S	S	S	S	D	S	D	S	S	S	S	D	S	S	S	S
	0	70	98	94	94	0	59	100	74	96	82	0	2	0	87	91	91	54	0	79	87	87	92

NOTES: D = death prior to NIEUWKOOP and FABER (1956) stage 46; S = severely deformed; M = moderately deformed; SL = slight effects; N = no effects or normal embryos; numbers = percent survival from blastula to feeding stage.

Controls: 200 = N 20 = N 2 = N .2 = SL 0 = S Survival = 85-99%

arbitrarily into the categories: slight, moderate and severe deformity. Low concentrations of metal ions first cause a slight decrease in swimming activity and reduction in the amounts of eye and body pigment formed. At somewhat higher exposure levels there is a decrease in the growth rate and a loosening of the coiling in the developing intestine in addition to further reductions in pigmentation and general physical activity. Severely affected embryos exhibit arrested growth, edema, very poor pigmentation, paralysis and almost no coiling of the intestine. The most interesting feature of these deformities is that they may be elicited by increasing levels of heavy metal ions or by decreasing levels of magnesium ions below 2 ppm. Furthermore, at some levels of metal ion exposure increased levels of magnesium ions may mitigate or prevent deformity (see Table I).

The data demonstrates the ability of magnesium ions to moderate the toxicity of lead, cadmium and manganese ions. Since the magnesium content of Xenopus embryos is constant regardless of environmental levels, this suggests that there may be competition occurring for some common carrier mechanism which transports divalent cations into the embryos. Similar mechanisms have been shown to exist in microorganisms (see NELSON and KENNEDY, 1972 or ROTHSTEIN et al., 1958). Therefore, we feel that the relationship of magnesium to heavy metal toxicity in vertebrates must be clarified.

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