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Uptake and release of $^{63}Ni^{2+}$ by *Xenopus* embryos during early cleavage stages

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Summary. Uptake and release of ^{63}Ni was studied in dejellied *Xenopus laevis* embryos exposed to $^{63}Ni^{2+}$ (0.3–30 μmol/l) for 0.5-h intervals during the period 1–4.5 h post-fertilization (i.e. from first cleavage to early blastula stage). At first cleavage, the mean uptake of ^{63}Ni by embryos was 12–17 times that by non-fertilized eggs, suggesting that conversion of the vitelline envelope to the fertilization envelope enhanced integumental permeability to $^{63}Ni^{2+}$. ^{63}Ni uptake by embryos at the 1–2-cell stage averaged 1.8–2.5 times that at the early blastula stage. An average of 5% of total ^{63}Ni in washed embryos was recovered in isolated fertilization envelopes, indicating that $^{63}Ni^{2+}$ passed through the envelope into internal compartments. Progressive increases of ^{63}Ni uptake were seen with increasing exposure levels; after exposure during 1–1.5 h post-fertilization to the highest concentration of $^{63}Ni^{2+}$ (30 μmol/l), ^{63}Ni uptake averaged 11.4 (SD ± 5.1) pmol/embryo. Rapid efflux of ^{63}Ni was noted after $^{63}Ni^{2+}$-exposed embryos were transferred to nickel-free medium; mean ^{63}Ni contents at 0.25 h and 2 h post-exposure diminished to 50% and 15% of the initial values, regardless of the exposure level. The finding that *Xenopus* embryos are permeable to $^{63}Ni^{2+}$ during early cleavage stages provides a convenient experimental system to investigate the embryotoxicity and teratogenicity of nickel.

Key words: Nickel metabolism – Nickel toxicology – *Xenopus laevis* – Embryogenesis

Introduction

The literature on embryotoxicity and teratogenicity of nickel compounds has been summarized in several reviews (Coogan et al. 1989; Leonard and Jacquet 1984; Mas et al. 1985; Sunderman et al. 1983). Signs of embryotoxicity (e.g. reduced litter size, runts, enhanced neonatal mortality) and sundry fetal malformations (e.g. exencephaly, anophthalmia, palatine and skeletal anomalies, cystic lungs) have been observed following administration of nickel compounds to female rodents during early gestation (see cited reviews). In a study of preimplantation mouse embryos, Storeg and Jonsen (1980) found that addition of Ni^{2+} (10 μmol/l) to the culture medium inhibited in vitro development of two-cell embryos, whereas 300 μmol/l was needed to impair the development of eight-cell embryos, suggesting that embryos are especially susceptible to Ni^{2+} toxicity during the earliest phase of embryogenesis.

Information is scanty about possible mechanisms for the embryotoxic and teratogenic effects of nickel compounds. The present study of embryonic uptake and release of $^{63}Ni^{2+}$ is a preliminary step in our program to elucidate the molecular mechanisms whereby Ni^{2+} affects embryonic development, using the South African clawed toad, *Xenopus laevis*, as the test species. Another preliminary step is our use of a Western blotting technique to demonstrate three major $^{63}Ni^{2+}$-binding proteins in *Xenopus* eggs before and after fertilization and in embryos studied during the two-cell to eight-cell stages (Lin et al. 1989).

Xenopus embryos provide a convenient system for analyzing many aspects of early development, since they are available in large numbers, are fertilized externally, are amenable to numerous experimental interventions, and develop rapidly (i.e. organogenesis is underway within one day post-fertilization). They have been a major subject of embryological investigation for over a century (see a review by Gerhart 1980) and have recently come under intense scrutiny at the molecular level, as a result of the discovery that certain molecules related to mammalian growth factors are involved in cell-signalling processes that specify cell fate during *Xenopus* embryogenesis (see reviews by Brennan 1987; Gurdon 1987; Smith 1989).

As far as the present authors are aware, the metabolism or toxicity of nickel compounds have not been

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previously studied in *Xenopus* embryos. Birge and Black (1980) tested Ni^{2+} embryotoxicity in two other species of toads, estimating the LC_{10} and LC_{50} concentrations of nickel in ambient water to be 0.07 and 0.85 μmol/l, respectively, for the narrow-mouthed toad (*Gastrophyrne carolinensis*), versus 6.9 and 188 μmol/l, respectively, for Fowler's toad (*Bufo fowleri*). These results were consistent with an earlier report that the narrow-mouthed toad was far more susceptible than Fowler's toad to toxicity from several other metals (Birge et al. 1979).

Timourian and Watchmaker (1972) studied $^{63}Ni^{2+}$ uptake by sea urchin embryos (*Lytechinus pictus*), observing that, whereas ^{63}Ni uptake was scarcely detectable in non-fertilized eggs, active uptake of $^{63}Ni^{2+}$ occurred throughout early embryonic development. In embryos exposed to a $^{63}Ni^{2+}$ concentration of 24 μmol/l in sea water, the rate of ^{63}Ni uptake was highest immediately after fertilization and gradually diminished during development to the gastrula stage. Sea urchin embryos grown in Ni^{2+} concentrations of 1–100 μmol/l were able to gastrulate, but failed to develop dorsoventral symmetry and formed radialized larvae. Based upon the Ni^{2+} concentrations found to be toxic for embryos of other species (Birge and Black 1980; Storeng and Jonsen 1980; Timourian and Watchmaker 1972) the present authors selected $^{63}Ni^{2+}$ concentrations of 0.3–30 μmol/l for testing in this study of *Xenopus* embryos.

Materials and methods

Animals and materials. Adult South African clawed toads (*Xenopus laevis*, purchased from Xenopus I, Inc., Ann Arbor, MI) were housed at 24° ± 1° C in plastic aquaria that contained NaCl solution (10 mmol/l) to a depth of 10 cm, and were fed Purina trout chow (Ralston Purina Co., St. Louis, MO). Modified Barth's saline solution (concentrated 10-fold, designated '10 × MBS') was prepared from distilled water and reagent-grade chemicals according to the following recipe: Hepes, 0.1 mol/l; NaCl, 0.88 mol/l; KCl, 10 mmol/l; $NaHCO_3$, 24 mmol/l; $MgSO_4$, 8.2 mmol/l; $Ca(NO_3)_2$, 3.3 mmol/l; $CaCl_2$, 4.1 mmol/l; pH 7.4 (Gurdon 1977). Prior to use, 1 × -MBS and 0.1 × -MBS solutions were prepared by diluting 10 × MBS solution 10-fold or 100-fold with distilled water. The 0.1 × -MBS solution was analyzed by electrochemical atomic absorption spectrophotometry, as previously described (Sunderman et al. 1988), to verify that its nickel concentration was < 0.05 μmol/l. The test materials were nickel chloride ($NiCl_2$, ultrapure reagent, Ventron Corp., Beverly, MA) and $^{63}NiCl_2$ (specific activity = 680 Ci/mol, New England Nuclear Corp., Billerica, MA). By adding stock solutions of $^{63}NiCl_2$ and $NiCl_2$ to 0.1 × -MBS solution, five test solutions were prepared with total nickel concentrations of 0.3, 1.0, 3.0, 10, or 30 μmol/l and ^{63}Ni specific activities that ranged over 680–13.6 Ci/mol. The test solutions were adjusted to pH 6.8 to avoid the possibility that Ni^{2+} might precipitate as $Ni(OH)_2$.

Collection, fertilization, and preparation of Xenopus eggs. Three or four days before each experiment, an adult *Xenopus* female was primed to ovulate by an injection into the dorsal lymph sac of reconstituted pregnant mare serum (0.1 ml, containing 50 IU, gonadotropin). The injection solution was prepared by dissolving lyophilized pregnant mare serum (Calbiochem Corp., La Jolla, CA; 3370 IU, gonadotropin/mg) in NaCl solution (140 mmol/l). On the evening before the experiment, the female was given an injection into the dorsal lymph sac of human chorionic gonadotropin (600 IU, Sigma Chemical Co., St. Louis, MO) dissolved in 0.3 ml sodium phosphate buffer (10 mmol/l, pH 7.2). The female was kept overnight at 16° C. Next morning, by pressing gently on the female's lower back, batches of approximately 250 eggs were elicited into a plastic Petri dish (6 cm diameter). The eggs were fertilized immediately by adding a fresh suspension of *Xenopus* sperm. To prepare the suspension, a testicle was excised from an adult *Xenopus* male, following anesthesia by immersion for 15 min in an aqueous solution of ethyl *m*-aminobenzoate methanesulfonate (Tricaine, 20 mmol/l, Sigma Chemical Co.). One-third of the testicle was gently minced with a polypropylene micropestle in a polypropylene microfuge tube (1.5 ml volume, Kontes Scientific Co., Vineland, NJ) that contained 0.3 ml 0.1 × -MBS solution. After the sperm suspension was poured onto the eggs, an interval of 45 s was allowed for sperm attachment, before sufficient 0.1 × -MBS solution was added to cover the eggs in the Petri dish. After 20 min, the fertilized eggs were dejellied by swirling gently for 7 min in 1 × -MBS solution that contained L-cysteine · HCl (0.13 mol/l) adjusted to pH 8.0. The L-cysteine was removed by washing the fertilized eggs four times with 1 × -MBS solution, transferring them to a clean Petri dish, and washing them four more times with 0.1 × -MBS solution. The developing *Xenopus* embryos were kept in the 0.1 × -MBS solution at 24° ± 1° C for periods up to 4.5 h post-fertilization, during which time first cleavage (two-cell stage) occurred at about 1.5 h, second cleavage (four-cell stage) at about 2 h, third cleavage (eight-cell stage) at about 2.25 h, fourth cleavage (16-cell stage) at about 2.75 h, fifth cleavage (32-cell stage) at about 3 h, sixth cleavage (morula stage) at about 3.5 h, and seventh cleavage (large-cell blastula stage) at about 4 h, as previously noted (Nieuwkoop and Faber 1967).

Exposures of Xenopus embryos to $^{63}Ni^{2+}$. *Xenopus* embryos, during early cleavage stages, were exposed to specified concentrations of $^{63}Ni^{2+}$ in 0.1 × -MBS solution for 0.5 h, beginning at 1 h, 2.5 h or 4 h post-fertilization. After exposure to $^{63}Ni^{2+}$, the embryos were immediately rinsed three times with 0.1 × -MBS solution and samples of 3, 5, or 10 embryos were removed in duplicate or triplicate for ^{63}Ni counting. In experiments to monitor the subsequent release of ^{63}Ni, groups of embryos that had been exposed to $^{63}Ni^{2+}$ 1–1.5 h post-fertilization were kept in 0.1 × -MBS solution (without ^{63}Ni) for further intervals of 0.25, 0.5, 1, and 2 h, prior to ^{63}Ni counting. In experiments to measure ^{63}Ni binding to fertilization envelopes, groups of washed embryos were dissected with microforceps under a stereomicroscope; the envelopes were removed and placed directly into scintillation vials. In experiments to test the effect of fertilization on ^{63}Ni uptake, eggs deposited by a *Xenopus* female were divided between two Petri dishes: eggs in one dish were fertilized with sperm, as described above, while the other eggs were left unfertilized. Beginning 1 h later, the eggs or embryos were exposed for 0.5 h to $^{63}Ni^{2+}$ (0.3 or 3 μmol/l), and groups of fertilized and non-fertilized eggs were then assayed for ^{63}Ni uptake.

Liquid scintillation counting and statistical analyses. The duplicate or triplicate samples of embryos or non-fertilized eggs were homogenized in 0.2 ml distilled water using a polypropylene micropestle in a polypropylene microfuge tube (1.5 ml volume, Kontes Scientific Co., Vineland, NJ), and the homogenates were transferred, along with two 0.5-ml washings, into glass scintillation vials. Scintillation counting fluid (15 ml of 'Opti-Fluor', Packard Instrument Co., Downers Grove, IL) was added and the β-emission of ^{63}Ni was counted (± 1% precision or 10 min) with a liquid scintillation spectrometer (Tri-Carb model 4530, Packard Instrument Co.) using automatic quench correction. For quality assurance, random vials were recounted following addition of ^{63}Ni; the recovery of added ^{63}Ni averaged 96% (SD ± 2%). Data for ^{63}Ni radioactivity (cpm/sample) were converted to dpm/sample by reference to ^{63}Ni calibrators (New England Nuclear Corp., Billerica, MA); nickel concentrations (pmol/embryo) were computed

from the specific activities of the ^{63}Ni test solutions (Ci/mol) and the numbers of embryos/sample. Results are reported as means ±SD, with N equal to the number of maternal *Xenopus* females. Statistical significance ($P<0.05$) was tested by one-way or repeated-measures ANOVA, followed, when appropriate, by the Student-Neuman-Keuls (SNK) multiple-range test (Zar 1974). Statistical certainty ($P<0.05$) of dose-effect and time-course relationships was tested by regression analysis, using the trend test of Tukey et al. (1985).

Results

^{63}Ni uptake by embryos versus non-fertilized eggs

When batches of non-fertilized eggs from four females were exposed to $^{63}Ni^{2+}$ (0.3 or 3.0 µmol/l) for 0.5 h, beginning 1 h post-deposition, ^{63}Ni uptake averaged 0.06±0.02 and 0.19±0.11 pmol/egg, respectively (Fig. 1, open circles). In contrast, when batches of embryos from nine or ten females were exposed to the same concentrations of ^{63}Ni for 0.5 h, beginning 1 h post-fertilization, ^{63}Ni uptake averaged 0.74±0.35 and 3.25±1.44 pmol/embryo, respectively (Fig. 1, closed circles). Thus, the mean uptake of $^{63}Ni^{2+}$ by fertilized embryos was 12–17 times that of non-fertilized eggs ($P<0.05$).

^{63}Ni binding to fertilization envelopes

Batches of embryos from five females were exposed to $^{63}Ni^{2+}$ (0.3 or 3.0 µmol/l) for 0.5 h, beginning 1 h post-fertilization; the embryos were washed, and the fertilization envelopes were isolated by dissection and assayed for ^{63}Ni. The ^{63}Ni contents averaged 0.04±0.03 and 0.17±0.12 pmol/envelope, respectively, which comprised 5.5%±3.8% and 4.8%±2.2% of the corresponding ^{63}Ni contents of the intact embryos ($P<0.05$).

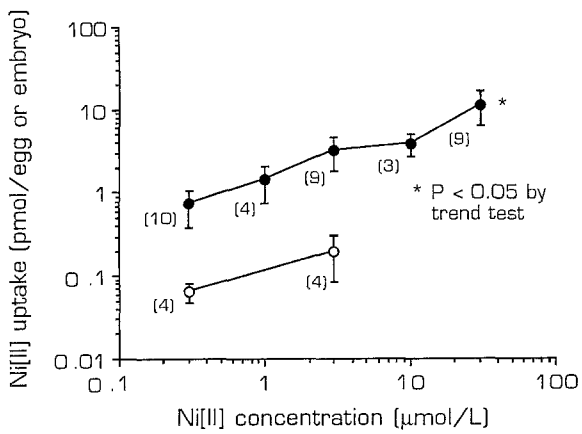

Fig. 1. ^{63}Ni uptake in non-fertilized eggs (○) or embryos (●) exposed to specified concentrations of $^{63}Ni^{2+}$ for 0.5 h, beginning 1 h after collection or fertilization. The number of *Xenopus* females whose eggs or embryos were tested at each exposure level are given in parentheses; the error bars denote ±1 SD

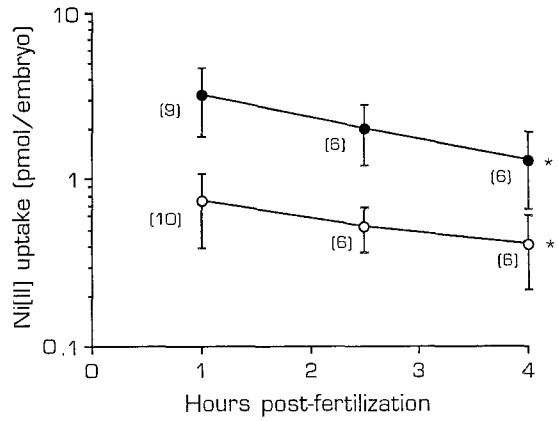

Fig. 2. ^{63}Ni uptake in embryos exposed to $^{63}Ni^{2+}$ (3.0 µmol/l, ● 0.3 µmol/l, ○) for 0.5 h, beginning at specified times post-fertilization. The number of *Xenopus* females whose embryos were tested at each time are given in parentheses; the error bars denote ±1 SD. *$P<0.05$ by trend test

Exposure-effect relationship for ^{63}Ni uptake

The uptake of ^{63}Ni by embryos exposed to five concentrations of $^{63}Ni^{2+}$ (0.3–30 µmol/l) for 0.5 h, beginning 1 h post-fertilization (i.e. during first cleavage) is shown in Fig. 1. The progressive increase of ^{63}Ni uptake at increasing exposure levels was statistically significant, based upon ANOVA and the trend test. At the highest exposure level (30 µmol/l), ^{63}Ni content averaged 11.4±5.1 pmol/embryo.

Time-effect relationship for ^{63}Ni uptake

As illustrated in Fig. 2, ^{63}Ni uptake by *Xenopus* embryos diminished progressively during 4 h post-fertilization ($P<0.05$ by trend test). Thus, when batches of embryos were exposed to $^{63}Ni^{2+}$ (0.3 or 3.0 µmol/l) from 4–4.5 h post-fertilization (i.e. during large cell blastula stage), ^{63}Ni uptake averaged 0.41±0.19 and 1.29±0.62 pmol/embryo, which comprised 55% and 40%, respectively, of the corresponding ^{63}Ni uptake of embryos exposed to $^{63}Ni^{2+}$ 1–1.5 h post-fertilization (i.e. during first cleavage, $P<0.05$).

Release of ^{63}Ni from Xenopus embryos

Batches of embryos from six *Xenopus* females were exposed to $^{63}Ni^{2+}$ (0.3, 3.0, or 30 µmol/l) for 0.5 h, beginning 1 h post-fertilization, washed, an then kept in 0.1×-MBS solution at 24°±1° C for intervals of 0.25, 0.5, 1, or 2 h prior to ^{63}Ni counting. Fig. 3 shows that ^{63}Ni was progressively released from the embryos during the period of observation, decreasing to 50%±18% of initial values at 15 min and 15%±9% at 2 h post-exposure ($P<0.05$ by ANOVA and SNK tests). The groups of embryos exposed to the three concentrations of $^{63}Ni^{2+}$ did not differ significantly in their rates of ^{63}Ni release.

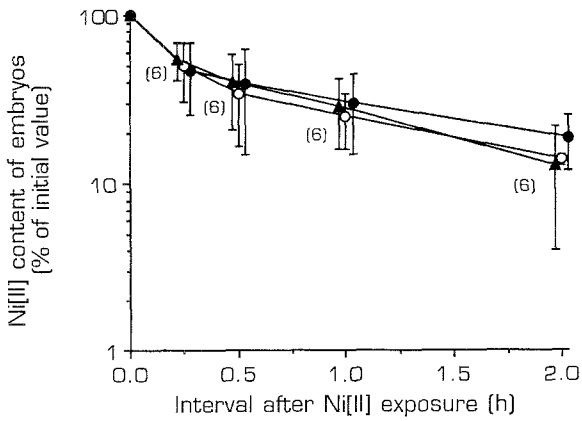

Fig. 3. ^{63}Ni contents of embryos exposed to $^{63}Ni^{2+}$ (30 μmol/l, ●; 3.0 μmol/l, ○; 0.3 μmol/l, ▲) 1–1.5 h post-fertilization, and then kept in nickel-free medium for the specified intervals post-exposure. The results are expressed as percentages of the initial values measured immediately post-exposure. The number of *Xenopus* females whose embryos were tested at each interval is given in parentheses; the error bars denote ±1 SD

Discussion

Shortly after fertilization, the integument that surrounds *Xenopus* eggs undergoes biochemical changes, partly mediated by release of proteases from cortical granules, that convert the vitelline envelope to the fertilization envelope (Gerton 1986; Lindsay and Hedrick 1989). Judging from the 12–17-fold increase of $^{63}Ni^{2+}$ uptake in fertilized embryos compared to non-fertilized eggs, the present study suggests that envelope conversion greatly enhances the integumental permeability to $^{63}Ni^{2+}$. Consistent with previous findings in sea urchin embryos (Timourian and Watchmaker 1972), $^{63}Ni^{2+}$ contents of *Xenopus* embryos were highest at the 1–2-cell stage and gradually diminished thereafter, at least until the early blastula stage. Uptake and subsequent release of $^{63}Ni^{2+}$ could involve the Ca^{2+} channels that have been studied in *Xenopus* embryos by Burgess and Vere (1989), since competition of Ni^{2+} for Ca^{2+} channels has been demonstrated in other experimental systems (Brommundt and Kavalier 1987; Raffa et al. 1987; Saito and Menzel 1986; Wang et al. 1984).

Since the diameter of *Xenopus* embryos averages 1.3 mm during early cleavage stages (Nieuwkoop and Faber 1967), the embryo volume is approximately 1.15 μl, as computed by the equation $V=4\pi r^3/3$. A ^{63}Ni concentration of 1 pmol/embryo would, therefore, approximate 0.87 μmol/l, assuming ^{63}Ni to be uniformly distributed within the embryo. Estimated from the data in Fig. 1, embryos exposed 1–1.5 h post-fertilization to 0.3, 3.0, and 30 μmol/l of $^{63}Ni^{2+}$ in the external medium would have ^{63}Ni concentrations that averaged 0.7, 3.7, and 9.9 μmol/l, respectively, in the internal medium, implying active influx at the low exposure level and partial exclusion at the high exposure level. Most of the ^{63}Ni in *Xenopus* embryos does not appear to be firmly anchored, in view of the rapid efflux of ^{63}Ni that occurred after $^{63}Ni^{2+}$-exposed embryos were transferred to nickel-free medium.

Only 5% of total ^{63}Ni in washed embryos was recovered in isolated fertilization envelopes, indicating that ^{63}Ni retention does not merely reflect adsorption to the integument; most of the $^{63}Ni^{2+}$ evidently traverses the fertilization envelope and enters one or more internal compartments. To date, technical obstacles have hindered attempts to measure the proportion of ^{63}Ni in the perivitelline space versus the embryo proper. In a future study, this point will be addressed by microdissection of $^{63}Ni^{2+}$-exposed embryos; if ^{63}Ni is present in blastomeres, its subcellular localization will be determined by autoradiography. A study in our laboratory showed that *Xenopus* embryos contain at least three ^{63}Ni-binding proteins, based upon *in vitro* affinity for $^{63}Ni^{2+}$ in a Western blotting assay (Lin et al. 1989). Hence, it will be interesting to determine whether $^{63}Ni^{2+}$ that enters embryos *in vivo*, as described herein, binds to the same proteins. In addition, assays of the embryotoxicity and teratogenicity of Ni^{2+} for *Xenopus* are underway in our laboratory.

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References

Birge WJ, Black JA (1980) Aquatic toxicology of nickel. In: Nriagu JO (ed) Nickel in the environment. John Wiley, New York, pp 349–366

Birge WJ, Black JA, Westerman AG, Hudson JE (1979) The effects of mercury on reproduction of fish and amphibians. In: Nriagu JO (ed) Biogeochemistry of mercury in the environment. Elsevier/North Holland, Amsterdam, p 629–655

Brennan S (1987) Molecular approaches to the study of mesoderm formation in amphibians. Bio Essays 6:52–57

Brommundt G, Kavaler F (1987) La^{3+}, Mn^{2+}, and Ni^{2+} effects on Ca^{2+} pump and on Na^+-Ca^{2+} exchange in bullfrog ventricle. Am J Physiol 253:C45–C51

Burgess AMC, Vere DW (1989) Teratogenic effects of some calcium channel blocking agents in *Xenopus* embryos. Pharmacol Toxicol 64:78–82

Coogan TP, Latta DM, Snow ET, Costa M (1989) Toxicity and carcinogenicity of nickel compounds. CRC Crit Rev Toxicol 19:341–384

Gerhart JC (1980) Mechanisms regulating pattern formation in the amphibian egg and early embryo. In: Goldberger R (ed) Biological regulation and development, vol 2. Plenum Press, New York, pp 133–316

Gerton GL (1986) Biochemical studies of the envelope transformations in *Xenopus laevis* eggs. Adv Exp Med Biol 207:133–149

Gurdon JB (1977) Methods for nuclear transplantation in amphibia. Methods Cell Biol 16:125–139

Gurdon JB (1987) Embryonic induction: molecular prospects. Development 99:285–306

Leonard A, Jacquet P (1984) Embryotoxicity and genotoxicity of metals. In: Sunderman FW Jr (ed) Nickel in the human environment. Oxford University Press, Oxford, pp 277–291

Lin SM, Hopfer SM, Brennan SM, Sunderman FW Jr (1989) Protein blotting method for nickel-binding proteins. Res Commun Chem Pathol Pharmacol 65:275–288

Lindsay LL, Hedrick JL (1989) Proteases released from *Xenopus laevis* eggs at activation and their role in envelope conversion. Dev Biol 135:202–212

Mas A, Holt D, Webb M (1985) The acute toxicity and teratogenicity of nickel in pregnant rats. Toxicology 35:47–57

Nieuwkoop PD, Faber J (1967) Normal table of *Xenopus laevis* (Daudin), 2nd edn. Elsevier/North-Holland, Amsterdam, pp 1–252

Raffa RB, Bianchi CP, Narayan SR (1987) Reversible inhibition of acetylcholine contracture of molluscan smooth muscle by heavy metals: correlation to Ca^{2+} and metal content. J Pharmacol Exp Therap 243:200–204

Saito K, Menzel DB (1986) Accumulation and efflux of nickel from cultured pneumocytes. Tohoku J Exp Med 148:295–302

Smith JC (1989) Mesoderm induction and mesoderm-inducing factors in early amphibian development. Development 105:665–677

Storeng R, Jonsen J (1980) Effect of nickel chloride and cadmium acetate on the development of preimplantation mouse embryos *in vitro*. Toxicology 17:183–187

Sunderman FW Jr, Reid MC, Shen SK, Kevorkian CB (1983) Embryotoxicity and teratogenicity of nickel compounds. In: Clarkson TW, Nordberg GF, Sager PR (eds) Reproductive and developmental toxicity of metals. Plenum Press, New York, pp 399–416

Sunderman FW Jr, Hopfer SM, Crisostomo MC (1988) Nickel analysis by atomic absorption spectrometry. Methods Enzymol 158:382–391

Timourian H, Watchmaker G (1972) Nickel uptake by sea urchin embryos and their subsequent development. J Exp Zool 182:379–388

Tukey JW, Ciminera JL, Heyse JF (1985) Testing the statistical certainty of a response to increasing doses of a drug. Biostatistics 41:295–300

Wang Z, Bianchi CP, Narayan SR (1984) Nickel inhibition of calcium release from subsarcolemmal calcium stores of molluscan smooth muscle. J Pharmacol Exp Ther 229:696–701

Zar JH (1974) Multiple comparisons. In: Zar JH (ed) Biostatistical analysis. Prentice-Hall, Englewood Cliffs, NJ, pp 151–162