

**DNA SYNTHESIS AFTER EXPOSURE TO HEAVY METALS IN THE
TESTIS OF THE SPINY DOGFISH (*SQUALUS ACANTHIAS*)**

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EXTENDED ABSTRACT ONLY – DO NOT CITE

Contamination of aquatic environments with metal compounds poses a serious risk to the health of aquatic species and terrestrial species that rely on food from aquatic environments. Widespread metal contamination of both marine and freshwater systems has been reported, and a large body of literature documents deleterious effects of such pollution on various species. Of particular concern is the tendency of animals, especially carnivores, to accumulate metals from dietary sources, thereby increasing their risk for dose-dependent toxic effects. The spiny dogfish, *Squalus acanthias*, and other elasmobranchs are especially susceptible to such cumulative effects because they are long-lived, carnivorous animals whose home range includes coastal marine habitats which tend to have the highest concentrations of metals. Several studies have documented high metal concentrations in tissues of the spiny dogfish and other members of the genus *Squalus* (e.g., Taguchi, 1979). Metal intoxication may be directly detrimental to the health of the dogfish populations by decreasing survival or reproductive fitness.

The known toxic effects of metals are diverse. Effects are thought to be exerted via the formation of stable complexes with many different biological molecules including proteins, DNA, RNA, and phosphorylated compounds. Specific mechanisms of metal toxicity have been characterized extensively for mammalian systems, but much less is known about non-mammalian vertebrates. Information on the specific cellular effects of metals on male reproductive systems in vertebrates is sparse but suggestive of profound disturbances (e.g., Clarkson, et al., 1983).

The dogfish testis has proven to be an excellent model for studying the regulation of vertebrate spermatogenesis (DuBois and Callard, 1991). Distinct developmental stages of spermatocysts (germ cell:Sertoli cell units) can be

isolated and cultured in vitro for at least two weeks. Moreover, mitotic activity, as indicated by DNA synthesis, is maintained quantitatively during this period and is responsive to stimulatory and inhibitory factors (Piferrer, et al., 1993). Thus, this model system would seem suitable for toxicological studies of vertebrate spermatogenesis. The purpose of this study was to determine the effects of various metals on DNA synthesis in the testis of the spiny dogfish.

For each experiment, spermatocysts were isolated from zone I tissue from testes of 2-4 sharks and maintained in culture with Leibovitz L-15 medium, modified for use with elasmobranch tissue (DuBois and Callard, 1991). After various periods of treatment with metals, 5.0 uCi/ml of ^3H -thymidine was added to the cyst cultures for 6-24 hr before harvesting the cysts. Harvested cysts were washed twice with saline solution augmented with excess unlabelled thymidine. Then, cysts were treated with ice-cold 10% trichloroacetic acid for 1-24 hr. Cysts were then washed again before solubilizing overnight in 0.2 M NaOH. Aliquots of the solubilized cysts were analyzed for radioactivity (cpm). Data were not standardized by sample protein concentration as previously reported; in these experiments such standardization would not significantly change the results. Results from cysts treated with metals were standardized as a percentage of the mean of untreated controls. The standardized means of treatment groups were compared to that of untreated control groups by an unpaired t-test with a pooled variance estimate.

Preliminary experiments showed that mercuric chloride (HgCl_2) at concentrations greater than 100 uM inhibited synthesis of DNA. Subsequently, the effects of equivalent concentrations (100, 500, 1000 uM) of HgCl_2 , the organic mercurial parachloro-mercuric-phenol-sulfonic acid (PCMBs), sodium vanadate (NaVO_3), zinc chloride (ZnCl_2), and cadmium chloride (CdCl_2) were evaluated. Effects of these metals were compared to untreated controls, positive controls treated with bovine insulin (10 ug/ml), and negative controls treated with isobutyl-methylxanthine (1 mM, IBMX). Results of this experiment are shown in Table 1.

Mercurial compounds showed dose-dependent inhibition of DNA synthesis. Of these HgCl_2 was most potent, virtually negating DNA synthesis between 100 and 500 uM. Cadmium stimulated DNA synthesis at 100 uM but markedly inhibited it at 500 and 1000 uM. Vanadate was relatively ineffective, reducing DNA synthesis only to 71% of controls at 1000 uM. In contrast, zinc slightly stimulated DNA synthesis at all concentrations. Beyond simply identifying their effective concentrations, these results demonstrate the specificity of

various metals with respect to their effects on DNA synthesis in shark testis. This specificity may reflect differences in the capacity and affinity of metal binding proteins, such as metallothionein, that effectively sequester metals and prevent them from affecting critical cellular processes. It is evident from these results that DNA synthesis in the *Squalus* testis is sensitive to metal intoxication, generally supporting previous results from mammalian models (Clarkson, et al., 1983). These results support the use of *Squalus* testis as a model system for toxicological studies of vertebrate spermatogenesis.

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Table 1. DNA synthesis rates of *Squalus* zone I spermatocysts cultured *in vitro* with metals, insulin (10 ug/ml, positive control), or IBMX (1 mM, negative control) for 24 hr and exposed for the last 12 hr to radiolabelled thymidine. Results are shown as a mean (SE) percentage of control cultures. Sample size was four for each treatment and eight for the untreated control. All means except those noted by "ns" were significantly ($P < 0.01$) different from the control.

Treatment	Metal Concentration (uM)			
	0	100	500	1000
Control	100 (2)	---	---	---
Insulin	192 (7)	---	---	---
IBMX	34 (3)	---	---	---
HgCl ₂	---	84 (2)	3 (1)	0 (0)
PCMBs	---	95 (3) ns	62 (3)	8 (3)
CdCl ₂	---	128 (6)	69 (2)	21 (3)
NaVO ₃	---	98 (3) ns	---	71 (4)
ZnCl ₂	---	117 (6)	117 (6)	127 (12)

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