The Effects of Mercury on Fertilization and Cleavage of *Tripneustes gratilla*

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ABSTRACT

Tripneustes gratilla ova were fertilized in 500 ppb, 200 ppb, 100 ppb, 70 ppb, 50 ppb, and 25 ppb $HgCl_2$. Fertilized ova were observed for the presence of fertilization membranes 15 minutes after insemination. Cleavage was observed 2.5 hours after fertilization and again after 18 hours as a check on the development of the embryos.

With increasing concentrations of Hg^{2+}, the number of fertilization membranes decreased. Cleavage, 2.5 hours after insemination also followed this trend, markedly decreasing at 500 ppb Hg^{2+} with 4.5% cleavage.

Abnormalities in the developing embryos were also observed. Cytolysis was predominant at concentrations of 100 ppb, 200 ppb and 500 ppb Hg^{2+}. Development of the embryos was arrested at first cleavage in 500 ppb and at second cleavage in 200 ppb Hg^{2+}. Abnormalities consisted of wrinkled cleavage planes, lack of cleavage planes despite multiple nuclei in the embryos, assymetric cleavage and lysing of the fertilization membrane with extrusion of cleaving cells. The number of abnormalities declined with decreasing concentrations of Hg^{2+}.

INTRODUCTION

Mercury toxicity has concerned investigators since the early 1900's. It is known that cyclic changes in the distribution of marine invertebrates are often related to changes in the chemical composition of sea water (Waterman, 1937). Extensive industrial and agricultural usage can significantly affect the redistribution of heavy metals such as mercury in the ocean, posing a threat to marine flora and fauna.

Kobayashi (2) proposed the use of sea urchin eggs as a suitable material for a bioassay for marine pollution. Different anomalies induced by polluted waters containing various heavy metals at certain developmental stages of sea urchin eggs and embryos may be available as reliable indicators in bioassay. Kobayashi's preliminary experiments were inspirational in generating some ideas for the present study.

A previous student in Zoology 420 (Crudele, 1973) studied the effects of various concentrations of $HgCl_2$ vs. fertilization, based on cleavage rates. I have attempted to expand his work by including qualitative observations to illustrate the detrimental effects of Hg^{2+} on the development of the sea urchin embryo.

MATERIALS AND METHODS

Sea water was filtered with a 2 micron Millipore filter to screen out bacteria that might be capable of converting inorganic mercury to methyl mercury. Dilutions of 500 ppb, 200 ppb, 100 ppb, 70 ppb, 50 ppb and 25 ppb $HgCl_2$ were prepared in sea water from a stock solution of 10 ppm $HgCl_2$. (See Appendix.) It was essential to prepare these solutions twice during the experiment because Hg^{2+} ions are readily adsorbed by glassware.

Tripneustes gratilla were collected from Kaneohe Bay and spawned by injecting 0.55 M KCl into the coelomic cavities of the male and female sea urchins. The optimum sperm concentration used was 1 drop of sperm in 50 ml of filtered sea water. 5 drops of this solution was added to 5 drops of ova (approximately 1000 eggs) in 5 ml of sea water containing the specified concentration of Hg^{2+}.

Eggs were fertilized and observed after 15 minutes, 2.5 hours, and at 18 hours. The first observation was made to count fertilization membranes; the 2.5 hour observation measured success of cleavage; the 18 hour observation was used as a check to observe how far development had proceeded. The experiment was repeated five times over a four week period.

The control for this experiment was fertilized eggs in sea water containing no mercury. An additional control consisting of unfertilized ova in the various Hg^{2+} dilutions was suggested during the Preliminary Report to assure that Hg^{2+} did not induce parthenogenesis. I incorporated this suggestion into the experiment and found that Hg^{2+} did not induce parthenogensis in unfertilized eggs.

The pH of the sea water was also checked. The pH of the sea water used was 8.1. No pH change was observed in any of the dilutions made with $HgCl_2$.

RESULTS

Results of the experiment reveal some general trends. With increasing concentrations of Hg^{2+}, the appearance of fertilization membranes decreased. See Fig. 1. It should also be noted that despite the decreasing trend in the number of fertilization membranes, there was still a relatively high percentage of membranes that appeared even at concentrations of 500 ppb and 200 ppb Hg^{2+}.

At 2.5 hours following fertilization, the number of eggs which successfully underwent cleavage decreased. There was a sharp decline at 500 ppb $HgCl_2$. See Fig. 2.

Certain abnormalities of the developing embryos were more frequent at a particular concentration of Hg^{2+} than others. See Table I. 2.5 hours after fertilization control eggs were in first and second cleavage. Cleavage spindles were observed in eggs treated with 500 ppb, 200 ppb, and 100 ppb Hg^{2+}. No cleavage furrow was seen in eggs treated with 500 ppb Hg^{2+}. Incomplete cleavage planes or no cleavage furrows were seen in eggs treated with 200 ppb Hg^{2+}, despite the presence of multiple nuclei. Ova in 100 ppb $HgCl_2$ showed wrinkled cleavage planes or no planes formed with the presence of multiple nuclei. Cleavage occurred without delay in 70 ppb, 50 ppb and 25 ppb.

Abnormalities were present in all concentrations, declining in frequency

with decreasing amounts of mercury. Membrane damage followed by cytolysis was prominent in concentrations of 500 ppb, 200 ppb, and 100 ppb. Assymetric cleavage was commonly observed at 70 ppb Hg^{2+}. Membrane damage occurred at 70 ppb and 50 ppb with cells cleaving outside of the boundaries of the fertilization membrane. See Fig. 3. A small percentage of abnormalities occurred in eggs treated with 25 ppb Hg^{2+}. Of the concentrations used, 25 ppb Hg^{2+} affected development of the embryos minimally.

Observations made 18 hours after fertilization showed 500 ppb Hg^{2+} to be lethal to the development of the embryos. Development proceeded through first cleavage and was arrested at this stage. 200 ppb Hg^{2+} was also lethal; the embryos died after second cleavage. In 100 ppb, development was arrested in over 50% of the embryos after second cleavage. 12% were in the early gastrula stage but moved sluggishly. In 70 ppb Hg^{2+}, about 50% of the embryos were viable in the blastula and early gastrula stages. 70 ppb was approximately the LD_{50} for this experiment. A majority of the embryos (81%) were viable in 50 ppb Hg^{2+}. Development proceeded through the blastula and gastrula stages. Normal development of embryos through the blastula and gastrula stages was observed in 25 ppb Hg^{2+}.

Fig. 1: % Fertilization Membranes vs. $[Hg^{2+}]$

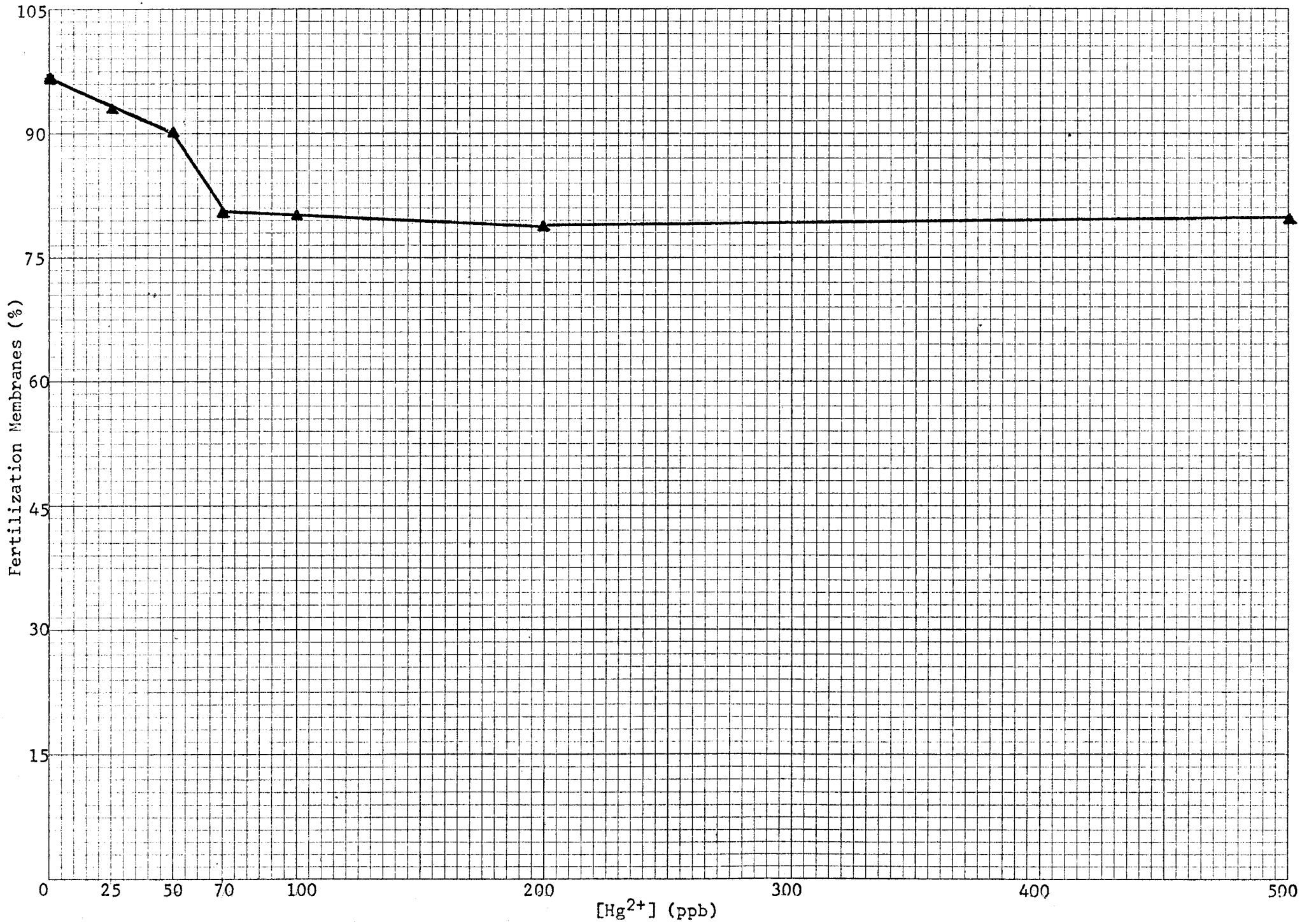


Fig. 2: % Cleavage vs. $[Hg^{2+}]$

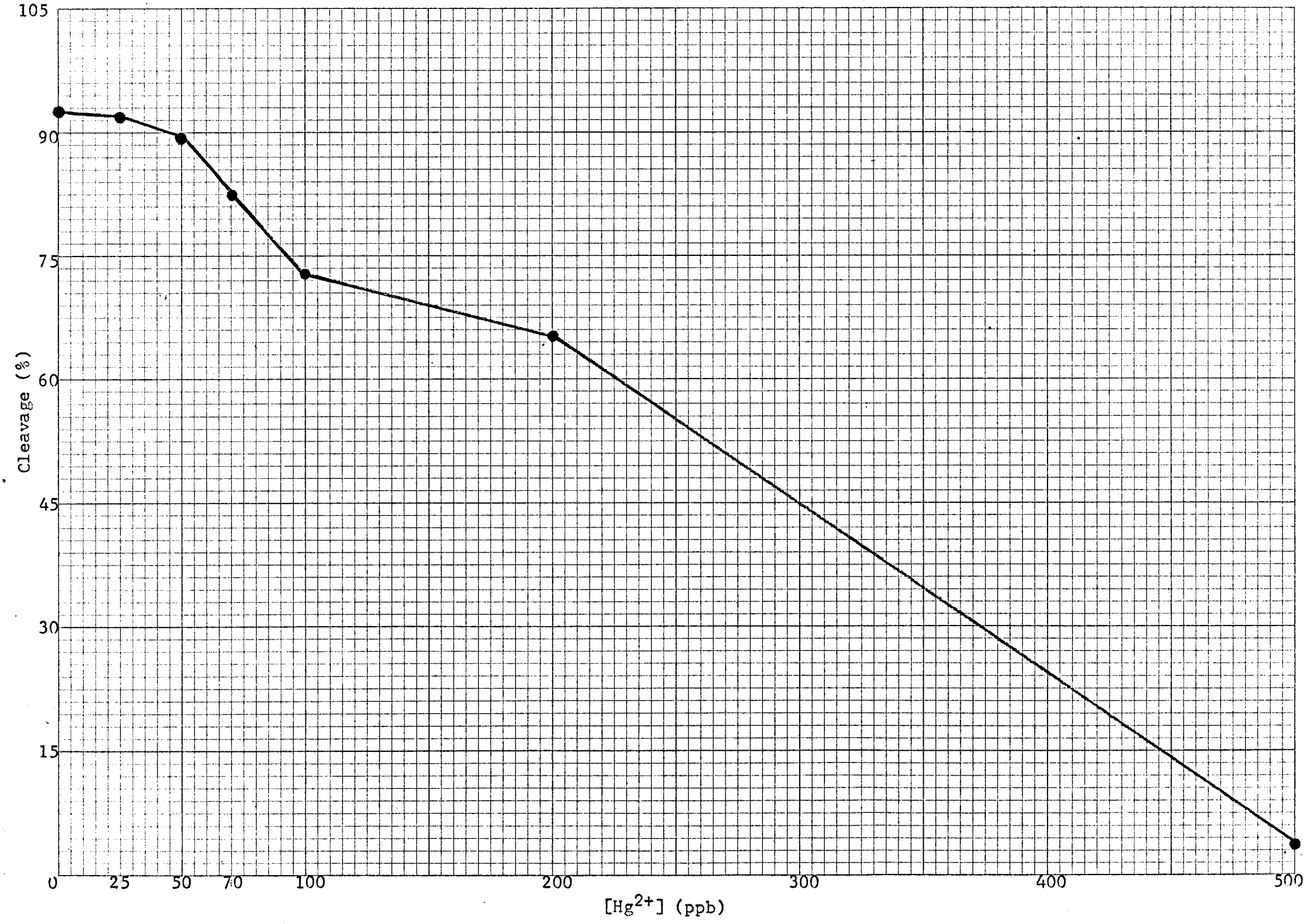
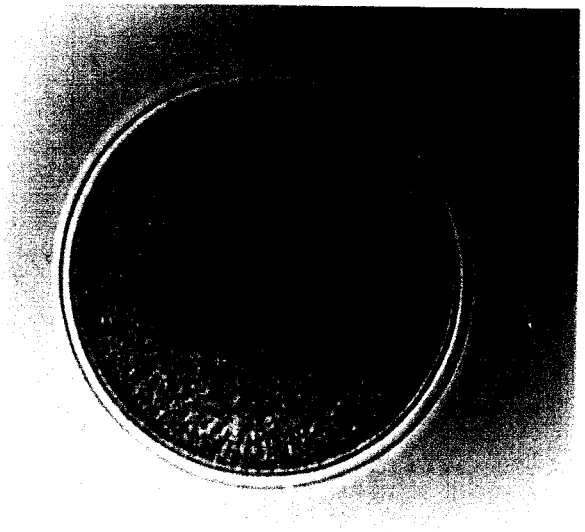


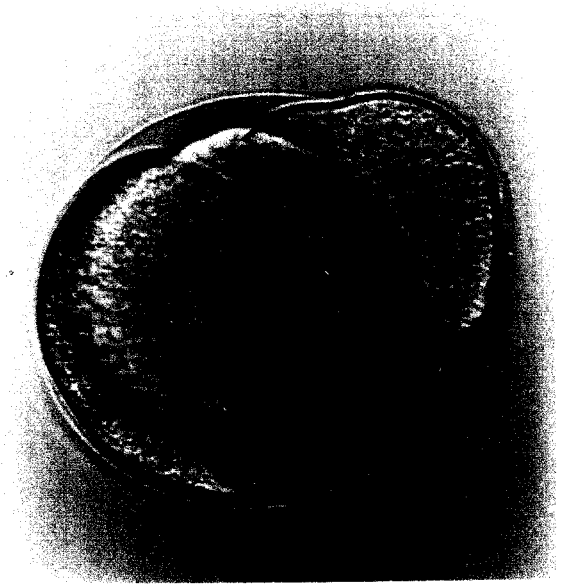
TABLE I. SUMMARY OF OBSERVATIONS

	Fertilization Membranes (%)	% Cleavage 2.5 Hours After Fertilization	OBSERVATIONS 2.5 Hours After Fertilization	OBSERVATIONS 18 Hours After Fertilization
CONTROL	97.2	92.7	Normal first and second cleavage	Late blastula/early gastrula
500 ppb	79	3.95	Cleavage spindle formed but no furrow present	Lethal; development up to first cleavage and arrested
200 ppb	79.2	65.2	Incomplete cleavage planes; nuclei present but without cleavage plane formation; 32.8% abnormal	After second cleavage embryos die; the rest do not cleave
100 ppb	80.5	73.4	Nuclei present but without cleavage plane formation; 27.3% abnormal	Development arrested after second cleavage in over 50%; 12% in early gastrula stage; sluggish movements
70 ppb	81.7	82.5	16.2% Abnormal Assymetric cleavage	About 50% viable Blastula/early gastrula stage; sluggish movements
50 ppb	90	89.2	11.1% Abnormal	81% viable Blastula/gastrula stages Sluggish movements
25 ppb	93	92.5	.63% Abnormal	Appear to be developing normally Blastula/gastrula stages
			*Extensive cytolysis observed in 500 ppb, 200 ppb, and 100 ppb Hg^{++}	

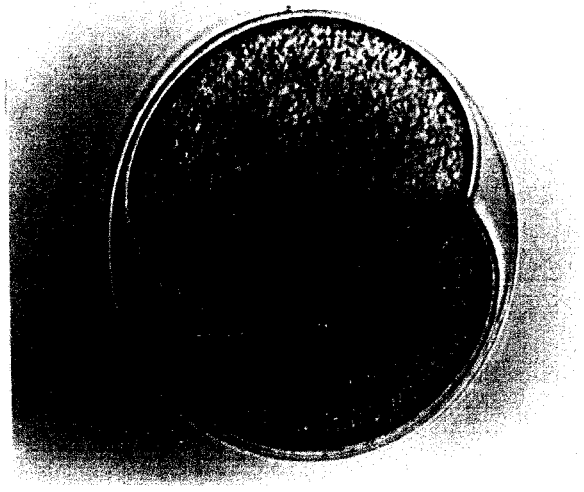


Control: Normal cleavage

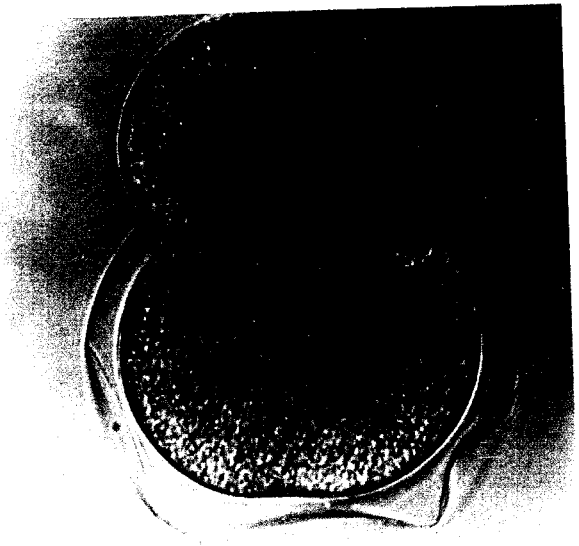
500 ppb Hg^{2+}: Cleavage spindle formed but no furrow present

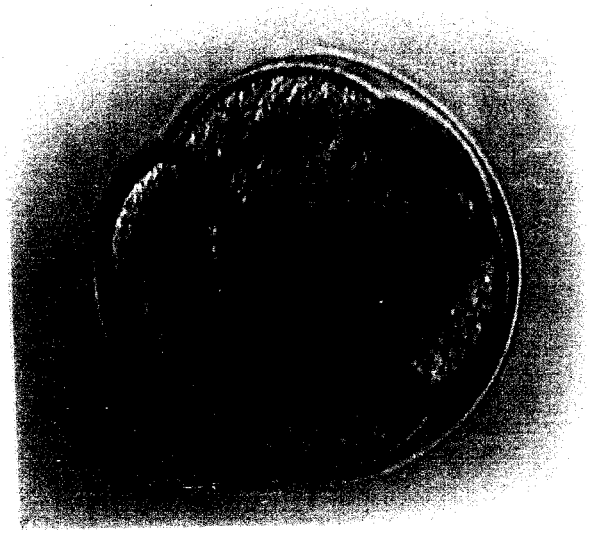


200 ppb Hg^{2+}: Incomplete cleavage planes



100 ppb Hg^{2+}: Membrane damage and cytolysis





70 ppb Hg^{2+}: Assymetric cleavage

OBSERVATIONS 2.5 HOURS AFTER FERTILIZATION

DISCUSSION AND CONCLUSIONS

Several experimental outcomes should be noted. There was a decreasing number of fertilization membranes as the Hg^{2+} concentration increased. The success of cleavage also followed this trend. As Hg^{2+} increased, there was a decline in successful cleavage. In all concentrations of Hg^{2+}, anomalies in development occurred.

The positive correlation between decreasing numbers of fertilization membranes and the decline in successful cleavage as Hg^{2+} concentrations increased might be attributed to the action of mercury as a metabolic poison. Mercury shows a strong affinity for ligands suchs as phosphates, cysteinyl and histidyl side chains of proteins, purines, pteridines and porphyrins. Hence, it can act at a large number of biochemical sites.[4]

The particular site of action of mercury would thus be difficult to pinpoint in an investigation such as this one. A possible initial effect of Hg^{2+} would be a change in membrane permeability. Hg^{2+} ions readily change cell membrane permeability according to Vallee and Ulmer. Even small amounts of heavy metals produce appreciable changes of surface tension and surface of lipid film of cell membranes. This may lead to marked changes of permeability and metabolic activities of enzymes.

Hg^{2+} is also known to interact with sulfhydryl and disulfide groups of proteins. During fertilization, Hg^{2+} ions might have interacted with the sulfated mucopolysaccharides extruded from the cortical granules during the formation of the fertilization membrane. Once within the membrane, cell functions would have been affected. Mercury can bind to and affect the conformation of nucleic acids and disrupt metabolic pathways such as

oxidative phosphorylation.

The effect of Hg^{2+} on the spermatozoa must not be overlooked. Hg^{2+}, if permeable to the membrane, could bind to enzymes in the acrosomal granule responsible for digesting the egg membrane during fertilization. Oxidative phosphorylation in the mitochondria might also be affected.

Many visible abnormalities in the embryos appeared to be related to membrane damage. Fertilization membranes were torn in some eggs with cells cleaving outside of the membrane. This occurred at a single localized area of the egg. Hoadley (1930) observed this phenomenon in Arbacia punctulata and suggested this as a mechanism for the egg to extrude mercury-avid pigments from the embryo. Pigments were not clearly distinguishable in the sections of eggs cleaving outside of the membrane, however, this interpretation may be plausible. Another abnormality observed were embryos with assymetric multi-cells during cleavage. Since membrane damage was evident, these embryos may suggest polyspermy.

Recommendations for future study would include short term exposures of the embryos to mercury solutions followed by transfer to normal sea water to study recovery.

APPENDIX

Mercuric chloride
'Baker Analyzed' Reagent
Lot # 411684

Assay ($HgCl_2$)	99.48%
Residue after ignition	0.012%
Solution in ether	PACST
Iron (Fe)	0.001%

The 10 ppm stock solution of $HgCl_2$ was prepared by dissolving 1 g $HgCl_2$ in 1 liter of filtered sea water. 1 ml of this solution was diluted in 100 ml of sea water resulting in a final dilution of 10 ppm.

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