

MECHANISMS OF SILVER ION (Ag^+) TOXICITY IN FERTILIZED EGGS
OF ILYANASSA OBSOLETA

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We have demonstrated previously that microtubule distribution in fertilized eggs of the common marine mudsnail, Ilyanassa obsoleta Stimpson (=Nassarius obsoletus Say) is very sensitive to the presence of silver ions (Ag^+) in the sea water (A. Conrad, et al. Cell Motil. & Cytoskel. 27: 117-132 (1994)): a narrow range of Ag^+ concentrations ($5-7 \times 10^{-11}\text{M}$) causes a marked increase in the numbers of microtubules in a normally transient cytoplasmic neck (polar lobe constriction), followed by great elongation of the neck and its eventual severing, an abnormal developmental event. This response largely mimics the cellular response to the reference standard microtubule stabilizing agents, taxol and hexylene glycol (A. Conrad et al. J. Exp. Zool. 262: 154-165 (1992) and J. Exp. Zool. 269: 188-204 (1994)). Heavy metal ions, such as Ag^+ , are thought to interact with proteins via a cage formed from three sulfhydryl groups. We therefore asked if any other metal ion could duplicate the Ag^+ response and whether it could be mimicked by agents that could form cross-links between sulfhydryl groups, in the absence of Ag^+ .

Effects of other metal ions: In a narrow range of concentrations ($0.75-1.5 \mu\text{M}$), we observed that Cu^{2+} causes 13- 37% of Ilyanassa fertilized eggs to form very long polar lobe necks resembling those formed in response to Ag^+ . This indicates that Ag^+ is not the only heavy metal ion to cause this effect.

Effects of cross-linking reagents: Four homobifunctional sulfhydryl cross-linking reagents were assessed for their ability to elicit Ag^+ -like cellular deformation of fertilized Ilyanassa eggs (very long, thin polar lobe necks; a shape not seen during normal development): N,N'-p-phenylene dimaleimide (p-PDM), N,N'-bis(3-maleimidopropionyl)- 2-hydroxyl-1,3-propanediamine (N,N'-bis), Bismaleimidohexane (BMH), and 1,4-Di-[3'-(2'pyridyldithio)propionamido]butane (DPDPB). When applied to cells in sea water, p-PDM, N,N'-bis, and BMH either caused no cells to form abnormally elongated polar lobe necks, or caused very few to form (N,N'-bis; <5% of cells). In contrast, exposure of cells to DPDPB caused as many as 47% of cells to assume this unusual shape. The range, $75-250 \mu\text{M}$, causes an average of 6 % or more cells to form long necks, with the optimum concentration of $125 \mu\text{M}$ causing an average of 11% with long necks. If eggs are pretreated with a reducing agent, dithiothreitol, followed by DPDPB, the percentage of responding cells increases to 24%, whereas if the pretreatment is with an oxidizing agent, H_2O_2 , the percentage of responding cells is 19 % (continuous treatment with dithiothreitol gives only 3% responding cells, whereas continuous exposure to H_2O_2 gives 4% responding). We conclude that Ag^+ -like morphological effects can be produced by treatment with a homobifunctional

sulfhydryl reactive cross-linking reagent. (Support: NASA NAGW-4491, NASA-NSCORT NAGW-2328, & NSF REU 9322221)