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Effects of Reversible Incubations of Zebrafish Eggs in Copper and Lead Ions with or without Shell Membranes

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Despite the progress made in the treatment of environmental wastes,heavy metals still pose great health hazards to humans and biota. Unlike other classes of pollutants which can be biodegraded and destroyed completely,metals can neither be created nor destroyed. And knowledge about the chemistry of their formation and concentration is very incomplete. Some metals can be methylated to more toxic forms while others can be complexed to stable and less toxic compounds. With time, some bounded metals are released as metals by natural chemical processes into aquatic environment where they interfer with biota. Some biota can accumulate and magnify these metals. Continuos monitoring of aquatic environment is, therefore, a necessity and zebrafish has been recommended as a standard species for acute toxicity testings in environmental intoxication(INTERNATIONAL ORGANISATION FOR STANDARDISATION 1976). Besides, its eggs are very sensitive to low copper concentrations (OZOH 1979).

The purpose of this communication is to demonstrate that reversible incubations of Brachydanio rerio eggs with or without their shell membranes did not affect the malformation due to copper ions. Inhibitory tendencies of both copper and lead to hatchings of zebrafish (OZOH 1979) were not affected. Rather new defects on the yolks and the interguments which sustend the hearts were detected. Dechorionated embryos reversed from copper to lead ions survived the 24-hours'exposure but those from lead to copper did not. Copper appears to confer some tolerance to lead,while the shell membranes appear to provide some protective action against the toxicity of copper.

MATERIALS AND METHODS

Brachydanio rerio eggs which spawn naturally and between high and flat blastula stages(HISAOKA and BATTLE 1958),were used in the bioassay. Test containers capable of housing 10 L of toxicant concentrations and 250 mL conical flasks were provided for incubating the

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eggs.Lead and copper nitrate salts,reagent grade, supplied the metallic ions. Separate concentrations of 58 ppb of copper and lead were prepared. Another test container contained a mixture of 58 ppb of lead and 8 ppb of copper. All the solutions were aerated and the alkalinity of distilled water used in preparing the media was 1.6 ppm by weight of calcium carbonate. All the solutions were thermostatically maintained at 26 ± 0.5°C. All the solutions were prepared a day before use.

A total of 500 eggs were incubated for 24 hours in five batches of 100 eggs each in 58 ppb of copper concentrations. Similarly, 500 eggs were incubated for 24 hours in 58 ppb of lead ions concentrations. Another set of 500 eggs were incubated in five batches of 100 eggs each in distilled water.

After 24 hours' exposure in both the toxicants and the control,the dead embryos were removed. Four hundred eggs were pipetted out randomly from each toxicant concentration and the control, and dechorionated under stereo-dissecting microscope with sharp scalpels. Three hundred dechorionated eggs from each toxicant concentration were reversed. That is, embryos without shell membranes from copper exposure were transferred to lead ions and those from lead to copper. Another 25 embryos exposed to copper and dechorionated were transferred to a mixture of 58 ppb of lead and 8 ppb of copper.

All the embryos dechorionated and with shell membranes were removed from the toxicants and distilled water after 24 hours and incubated in a mixture of (1:1) distilled and tap water. Because of the inhibitory tendencies to hatching induced by copper and lead ions (OZOH 1979),some intact embryos were dechorionated with scalpels after two days in incubating media.

RESULTS

Effect of removal of shell membranes

The eggs incubated for 24 hours in distilled water and then dechorionated developed normally. The few embryos that failed to survive suffered mechanical injuries during the process of dechorionation. Figure 1 shows the dechorionated larva 4 days old.

Developmental abnormalties

All the embryos dechorionated after 24 hours' copper exposure and then transferred to lead ions for 24 hours were abnormal. Besides the spiral formation of the notochord(Fig.2) inverted boat-like cavities were made in the yolk near the heart (Figs.

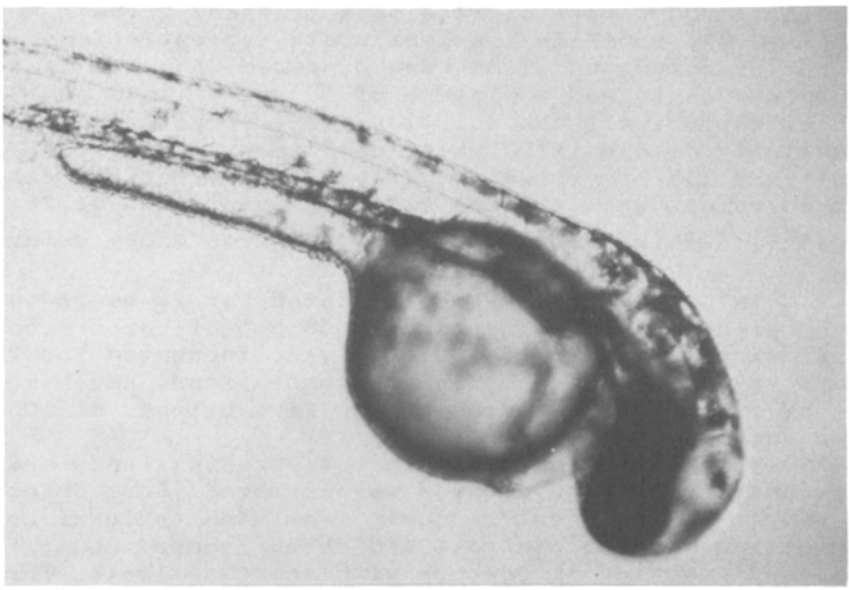

Fig.1. Dechorionated Larva 4 days old.Mag.x 100.
2 and 3). Ballon-like interguments sustended the hearts (Figs.2,3 and 4).

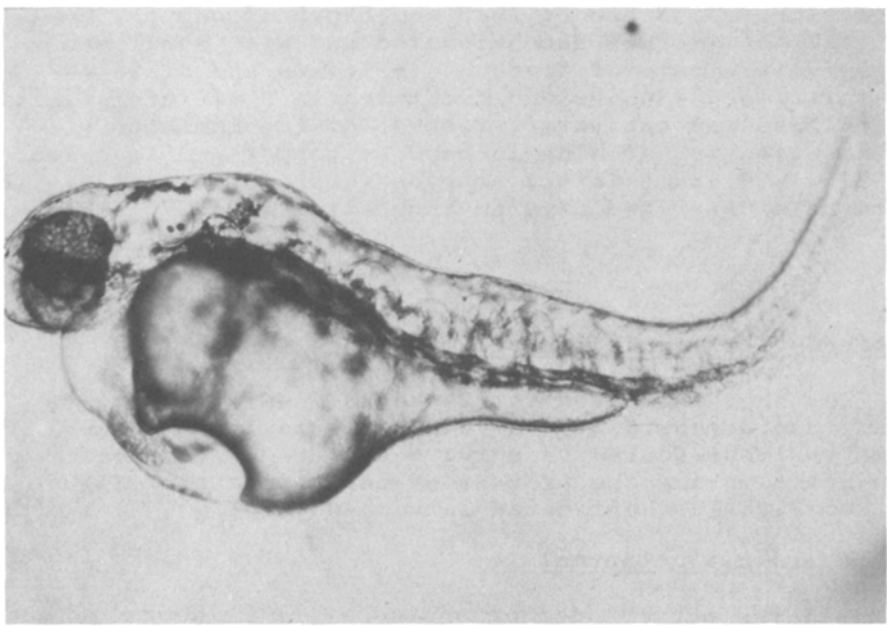

Fig.2. Larva,4 days old.Spirality,ballon-like intergument near the heart.Mag.x 100.

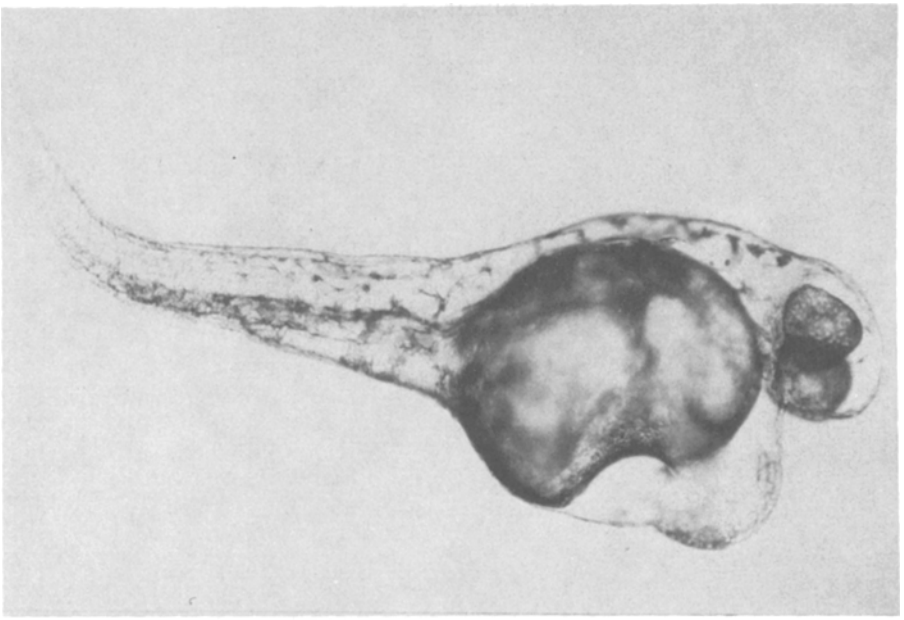

Fig. 3. Larva 4 days old, inverted boat-like cavity near the heart and ballon-like intergument. Mag. x100.

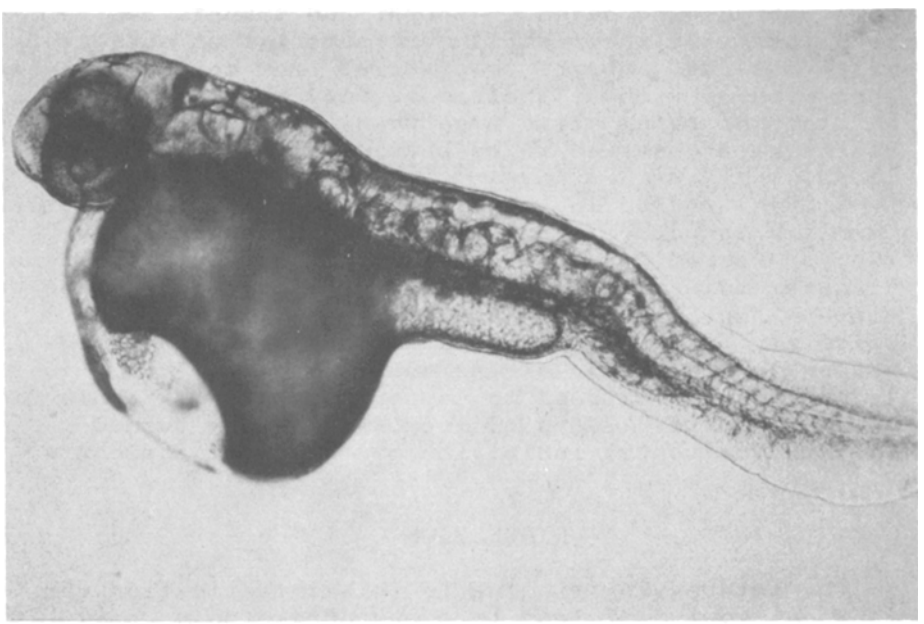

Fig. 4. Larva from copper to Lead. Malformed yolk and notochord. Mag. x 100.

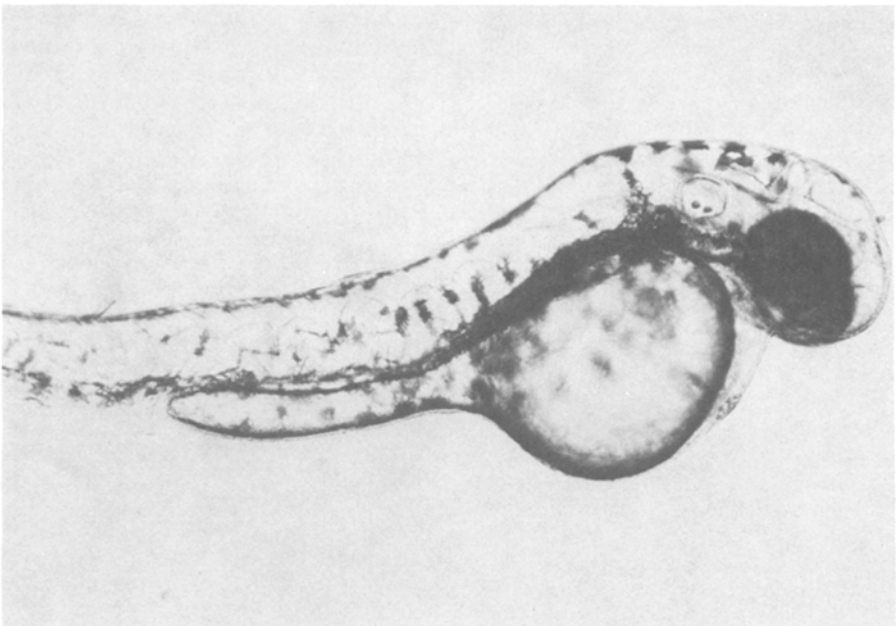

Fig. 5. 4 day old Larva, exposed to copper and then to a mixture of lead and copper ions. Mag. x 100. Some embryos incubated in copper and transferred to lead ions with their shell membranes intact suffered malformations. Embryos transferred from copper to lead ions with or without shell membranes showed some evidence of being alive even when badly malformed. Their hearts were observed to be beating with reduced pulsations.

Embryos reared in lead ions and dechorionated before transfer to copper ions died in copper solutions. Embryos reared in copper ions and dechorionated before transfer to a mixture of 58 ppb of lead and 8 ppb of copper (Fig.5) suffered enhanced maximization in spirality of the notochordal process from rhombencephalon to the region of the anus.

The embryos exposed to copper and lead toxicants did not hatch after 4 days of incubation. They turned whitish due to the inhibition by the metal toxicants.

DISCUSSION

The interesting finding in this investigation was that antagonism of lead to copper broke down when copper occurred in sublethal concentrations. Resistance of zebrafish embryos to copper intoxication was enhanced by shell membranes. This was contrary to what

obtains in zinc ions. SKIDMORE (1966)observed that dechorionated zebrafish embryos survived longer in zinc ions than embryos with unruptured/chorionated membranes. He attributed this to the presence of white materials inside the egg membranes. Copper appeared to confer some tolerance to lead in the present experiments. Embryos incubated first in copper and then dechorionated before reversing to lead survived longer than those from lead to copper. It would appear that the toxic actions of copper and zinc differ in zebrafish embryos.

BROWN(1978) reported that the tolerance for copper by isopod crustaceans, Asellus meridianus,seemed to confer tolerance to lead. Lead was stored in the "cuprosomes" at the expense of copper. In zebrafish embryos,on the other hand,the effect of copper on lead tolerance seemed to work by a different mechanism. In orthogonal interactions of lead and copper in zebrafish eggs and larvae,lead ions were observed to antagonise the inhibitory tendency of copper to hatching (OZOH 1979). Lead might antagonise copper concentration above sublethal levels by mobilising hydrated water around the embryos or adsorb preferentially to the epidermis.

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