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Effect of Water Hardness on the Toxicity of Cadmium to the Egg of the Teleost *Oryzias latipes*

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The tolerance of aquatic organisms to cadmium varies considerably. One of the factors having the most influence on the toxicity of cadmium seems to be the hardness of fresh water or the salinity of sea water. Fresh-water fishes are more resistant to cadmium in hard water than in soft water (BROWN 1968; MCCARTY et al. 1977). Similarily, the toxic effect of cadmium is inversely related to the increase in salinity for eury-haline crabs and fishes (O'HARA 1973a, 1973b; WRIGHT 1977a, 1977b, 1977c; FRANK and ROBERTSON 1979; EISLER 1971). A few investigations have been performed on this matter using fish eggs. WESTERNHAGEN et al. (1974) observed the influence of cadmium on embryonic development of marine herring eggs under different salinities and found that the effect of cadmium in diluted sea water was more pronounced than in natural sea water.

On the other hand, the influence of cadmium on the development of eggs of the fresh-water fish, Oryzias latipes, was observed in a previous investigation (MICHIBATA, unpublished data), and it was found that almost all the cadmium absorbed by the egg was located in the egg chorion. According to YAMAMOTO (1936), the electric charge of the chorion readily reverses upon a change in the concentration of electrolytes in the surrounding medium. It is probable that water hardness is intimately involved in the combination of cadmium with the chorion.

The main purpose of the present study is, therefore, to examine to what extent cadmium accumulation by the egg chorion depends on the hardness of the medium and to learn whether or not the cadmium absorbed by the chorion is closely correlated with egg survival.

MATERIALS AND METHODS

Blastulae of the medaka, Oryzias latipes, spawned every morning were used as materials. They were rinsed thoroughly in distilled water prior to the experiment. The experimental media having four different water-hardnesses were prepared on the basis of artificial sea water (1 M ASW), which contained 820 mM NaCl, 18 mM KCl, 19 mM $CaCl_2$ and 87 mM $MgCl_2$ (YAMAMOTO 1936). The hardnesses of the media were as follows; distilled water (0 mg/L as $CaCO_3$), M/1,000 ASW (12.2 mg/L as $CaCO_3$), M/100 ASW

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(122.0 mg/L as $CaCO_3$), and M/10 ASW (1,220.0 mg/L as $CaCO_3$).

In order to determine the survival rate of the embryos in each hardness, 100 blastulae were incubated at 28°C in 50 ml of each solution in which cadmium chloride was dissolved at the concentration of 10.0 mg Cd/L. The pH of each solution was adjusted to 7.3 with M/10 $NaHCO_3$. Although it is possible that the concentration of cadmium in the solution might change during the incubation period, this could be kept to less than 10 % by changing the medium every day.

In the second experiment, median tolerance limits (TL50) were determined for embryos exposed to cadmium under the different hardnesses. The embryos were exposed for 96 hrs to the solutions with concentrations ranging logarithmically from 0.1 to 1,000.0 mg Cd/L, respectively. Other experimental conditions were the same as those described above.

To determine the cadmium content of the embryos exposed to the medium containing 10.0 mg Cd/L in the different hardnesses, the surviving embryos were removed 3 hrs after they were immersed and their cadmium content was determined. Before the determination of the cadmium content, it is necessary to remove the cadmium that may be loosely combined on the surface of the chorion. The surviving embryos were washed either by rinsing them in distilled water or by rinsing them in glycine buffer solution the pH of which was adjusted to 2.0. The latter procedure may reverse the electric charge of the egg chorion. The eggs were then dried at 110°C and mineralized in $HClO_4$ and H_2O_2 at 220°C. For the determination of the cadmium content, flameless atomic absorption spectrometry was applied to the digested solution.

RESULTS

It was previously confirmed that the mortality rate of Oryzias embryos did not vary with water hardness if cadmium was absent. The death rate of the control embryos was 3.7 %. Figure 1 shows the effect of hardness on the mortality of the embryos exposed to cadmium at the concentration of 10.0 mg/L. Clearly the embryos exposed to cadmium in soft water were more sensitive than those exposed in hard water. The mortality was complete within 48 hrs when the embryos were immersed in either distilled water or M/1,000 ASW. The death rate in M/100 ASW was less than 5 % during the first 10 hrs after immersion; thereafter, it rose gradually and reached more than 30 %. The mortality of the embryos in M/10 ASW was quite low and was comparable to that in the control lacking cadmium.

The 96-hr TL50 values for cadmium in different hardnesses show a linear relationship to the hardness of the medium (Fig. 2). The lowest value was obtained for the embryos in distilled water, that is, the concentration of 0.3 mg Cd/L caused 50 % mortality

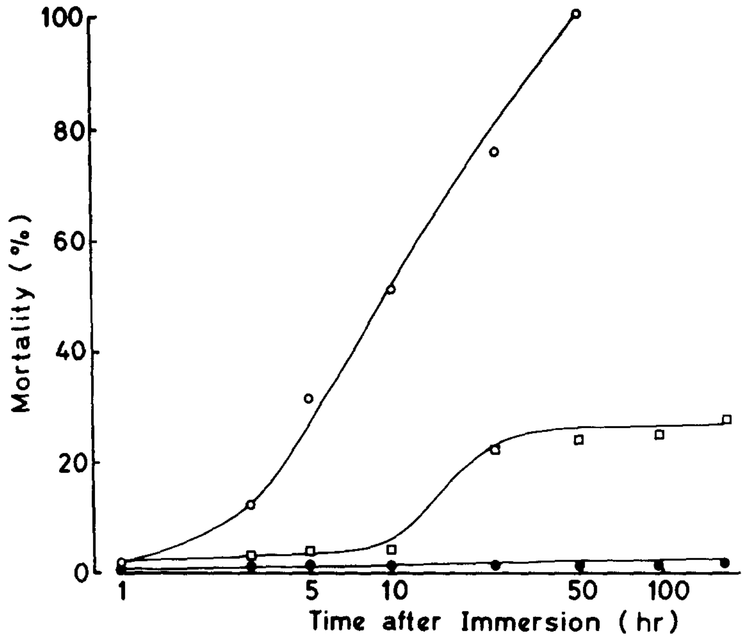

Figure 1. Mortality of Oryzias embryos exposed to 10.0 mg/L of cadmium with different water hardnesses.
○ : distilled water and M/1,000 ASW, □ : M/100 ASW,
● : M/10 ASW and control.

at 96 hrs. On the other hand, in M/10 ASW (hardness; 1,220.0 mg/L as $CaCO_3$), half of the embryos survived at the concentration of 560.0 mg Cd/L. These results seem to reflect the dependence of cadmium t-xicity on the hardness of the medium.

Figure 3 shows the highly inverse relationship between the cadmium content of the embryos and the hardness of the medium. The amount of cadmium in the embryos decreased markedly with the rise in the hardness. A small amount of cadmium remained in the embryos after they were rinsed in the acidic solution. The amount of cadmium detected was 0.6 ng/egg in each case and was independent of the hardness.

DISCUSSION

It has been shown that an increase in the hardness of the medium reduces the mortality rate of fish exposed to cadmium (BROWN 1968; EISLER 1971; MCCARTY et al. 1977). At the same time, some investigations have established an inverse relationship between the susceptibility of crabs to cadmium and the salinity of sea water (O'HARA 1973a, 1973b; WRIGHT 1977a, 1977b,

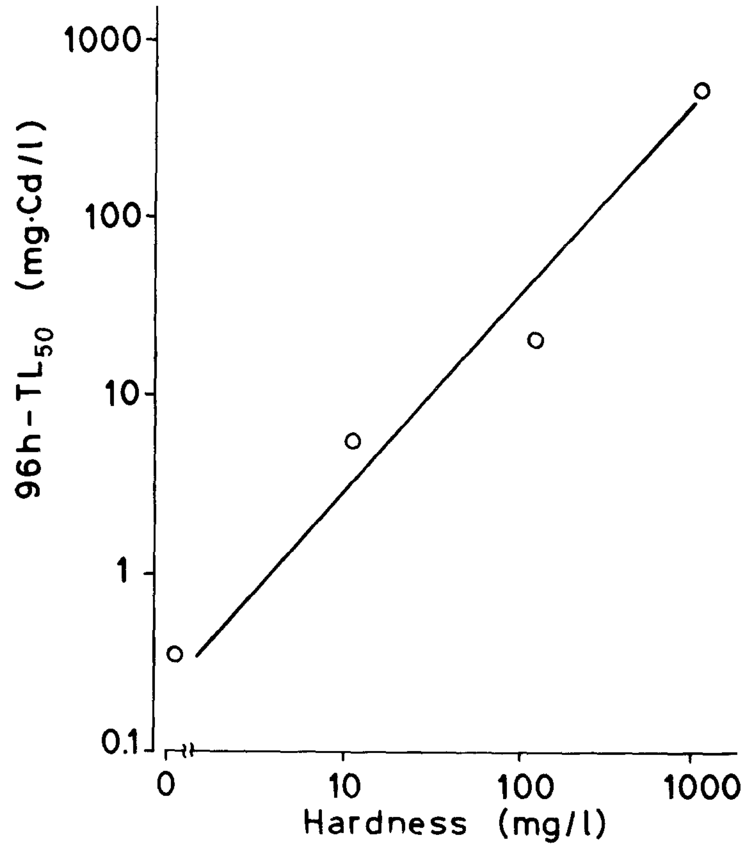

Figure 2. Relationship between 96-hr TL50 value for cadmium and hardness of the medium.

1977c). Similar results with the embryos of a marine fish have been reported by WESTERNHAGEN et al. (1974). They found that the deleterious effects of cadmium on herring embryos were more pronounced in diluted sea water than in natural sea water. The results of their experiment seem to agree with those in the present study. As shown in figure 1, the mortality rate was significantly higher in the soft water than in the hard water when Oryzias embryos were exposed to cadmium at the concentration of 10.0 mg/L. It is noteworthy that no distinct difference in the death rate of the embryos was found between those in M/10 ASW containing cadmium and that lacking cadmium. In M/10 ASW, the highest hardness tested in the present experiment, the toxic effect of cadmium seemed to be eliminated. The relationship between the 96-hr TL50 value for cadmium and the hardness (Fig. 2) confirms that the toxicity of cadmium is inversely proportional

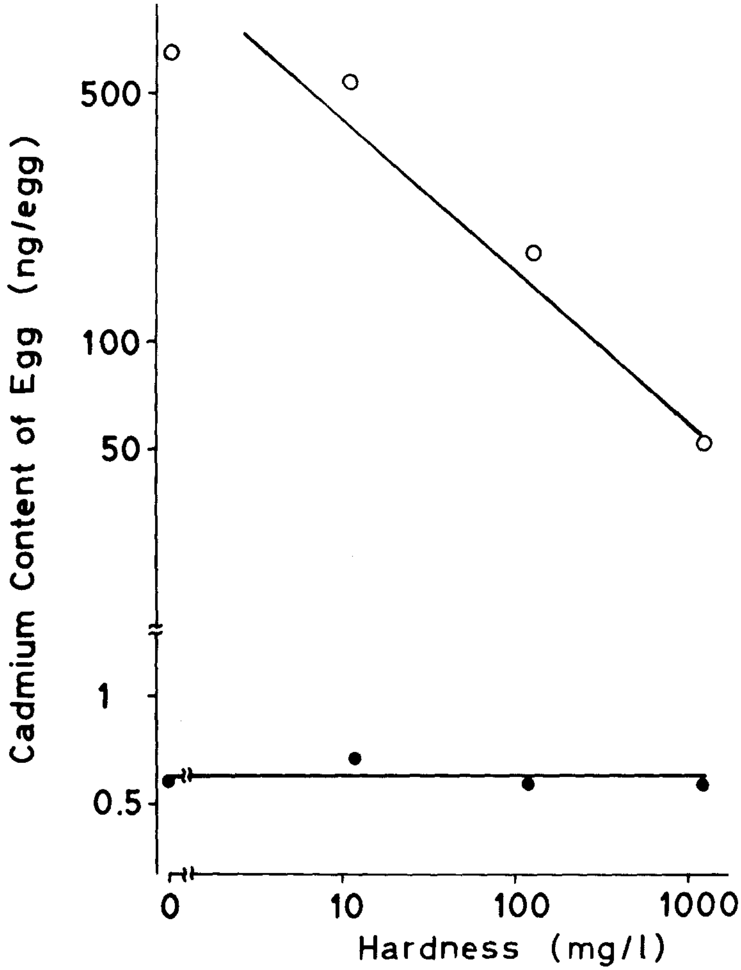

Figure 3. Relationship between cadmium content of embryos and water hardness. The cadmium content of embryos exposed to cadmium in water with different hardnesses and rinsed in distilled water (○) is compared with that of embryos rinsed in an acidic solution (●).

to the hardness.

In the previous investigation (MICHIBATA, unpublished data), it was shown that almost all the cadmium absorbed by Oryzias embryos was combined loosely with the egg chorion and could be rinsed out in acidic solution. ROSENTHAL and SPERLING (1974) also insisted that most of the cadmium was localized in the chorion of herring eggs exposed to cadmium. In the present study, therefore, the cadmium detected in the embryos after they were rinsed in the acidic solution was considered to be the real amount of cadmium absorbed by the embryo proper. It is, therefore, concluded that the amount of cadmium absorbed by the embryo proper is not influenced by water hardness, but the amount of cadmium combined with the chorion does depend on the hardness of the medium, provided the incubation time is limited to 3 hrs.

The higher the hardness of the incubating medium, the less cadmium is combined with the chorion and the more resistant the embryo becomes to cadmium toxicity. Because of these results, the author is inclined to believe that the toxic effect does not depend on cadmium absorbed by the embryo proper but that the combination of cadmium with the egg chorion may induce the death of the embryos. By what mechanism the cadmium accumulated in the chorion kills the embryo remains problematic.

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