Chitosan for the Removal of Mercury, Hg

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水中에서 카이토산에 의한 수은 제거

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ABSTRACT

Experiments were conducted with goldfish exposed to various levels of mercuric chloride (control group) and mercuric chloride with chitosan (experimental group). Dilutions of these two solutions were made in the concentration ranges 0.6 to 1.0 mg/ℓ and 1.2 to 2.0 mg/ℓ, respectively. Fifty percent lethal concentration of mercuric chloride(LC_{50}) for 48 hours with the species was between 0.6 and 0.7 mg/ℓ. Exposure of goldfish to mercury produced a marked, dose-dependent mortality with elevation of concentration ($P<0.05$). However, at each concentration of mercuric chloride treated with chitosan, mortality decreased significantly compared to control group ($P<0.05$).

요 약

카이토산이 수중의 수은 제거에 미치는 영향을 알아보기 위하여, 금붕어를 사용하여 염화수은을 사용한 군 (대조군, n=75, 농도=0.6 mg/ℓ1.0 mg/ℓ)과 염화수은에 카이토산을 첨가한 군(실험군, n=75, 농도=1.2 mg/ℓ~2.0 mg/ℓ)의 폐사율을 비교 분석하여 다음과 같은 결과를 얻었다. 염화수은에 대한 금붕어의 48 시간 동안의 반수치사 농도 (LC_{50})는 0.6 mg/ℓ 와 0.7 mg/ℓ 사이였다. 대조군에 있어서 수은의 농도가 증가함에 따라 폐사율이 현저하게 증가되었다 ($P<0.05$). 그러나, 카이토산이 첨가된 실험군에 있어서는 폐사율이 감소되었으며 수은 농도가 높은 경우 100 % 폐사에 이르는 시간이 연장되었다($P<0.05$). 상기 결과로서 카이토산이 수중의 수은 제거와 금붕어에 수은의 독성을 완화시키는 효과가 있는 것으로 사료된다.

INTRODUCTION

Mercury is one of the most toxic heavy metals and nearly ubiquitious in the aquatic environment. Many reports have been published on acute and chronic toxicity of mercury to various aquatic organisms (Jensen ahd Jernelov 1969 ; Peakall and Lovett 1972 ; Gillespie 1973). A rapid, simple, and easy to score toxicity is the mortality test. In most studies, Hg (usually as $HgCl_2$) has been dissolved directly into water.

Chitosan, which is a deacethylated form of chitin produced by treating the polymer with sodium hydroxide, is believed to mediate the stress response by serving as a chelating agent. Muzzarelli (1977) indicated how effective chitosan is in removing heavy metal ions from dilute aqueous solutions. The potential utility of chitosan for the removal of undesirable metals such as mercury, lead, chromium, and uranium has been demonstrated (Masri et al. 1974 ; Eiden et al. 1980 ; Galun et al. 1983). So applying chitosan to goldfish may protect them from heavy metals. Nevertheless, studies on the combination effects of mercuric chloride and chitosan are very limited.

This report summarizes the use of goldfish to study the effects of mercury and chitosan. The objectives of this study were to determine (a) the acute toxicity of mercuric chloride, (b) mortality levels of fish held at 10 different concentrations, and (c) the relationship between mercuric chloride and chitosan in order to provide a measure of utility of chitosan. The goal was to determine the feasibility of using chitosan as a chelating agent of heavy metal.

MATERIALS AND METHODS

In this study, goldfish (*Carassius auratus*) were used as the test species. This choice was dictated by (1) ease of conducting to accurate tests with the experimental apparatus, (2) relevance to toxicologic questions, and (3) survival in the laboratory (Levy and Gucinski 1964). Most lists of relative sensitivity to toxicants (measured by survival) show goldfish to be intermediate (McKee and Wolf 1963).

All goldfish were obtained from a local pet store. Overall length of the fish ranged 4. 0 to 5. 0 cm, and weight of the fish ranged 2. 0 to 2. 5 gm. They were held in 10-gallon tanks at 20 ℃, and while in the tanks, they were fed daily once between 0800 and 0900 hours. The pH of the water was 7. 0 ($\pm$ 0. 3) during the period. The fish required about 2 days to adjust to the tanks, and tests were started after the adjustment. Fish were randomly divided into test groups.

There were two experiments for this study. The first one was LC_{50} test of mercuric chloride for 48 hours of the species. Fifteen groups, with five fish each were, used for the LC_{50} test. Dilutions for this test were made in the concentration ranges 0. 4 to 0. 8 ㎎/ℓ with 0. 1 ㎎/ℓ increament. Each dilution was replicated three times. Five-gallon glass aquaria were used for the experiment. According to the results of LC_{50} test, the second experiment was carried out. For the second experiment, thirty groups, five fish in each group, were transferred from holding tank to experiment tanks. Five gallon glass aquaria also were served as experiment tanks for the second experiment.

Chitosan was obtained as 0. 1 % solution from Dr. D. D. Culley, Deparment of Fisheries and Wildlife, Louisiana State University at Baton Rouge, U.S.A.

Stock solutions of mercuric chloride and chitosan were prepared with distilled water on the basis of $1 / 10^{-2}$ M. Then the stock solutions were dilluted with tap water, and they were dispensed into the 20 liter glass aquaria. For the control group, 5 different concentrations, 0.6, 0.7, 0.8, 0.9, and 1.0 ㎎/ℓ of mercuric chloride were made. For the chitosan test group, 1.2, 1.4, 1.6, 1.8, and 2.0 ㎎/ℓ of chitosan were added to each of the above concentrations of mercuric chloride, respectively.

Fish were introduced into each aquarium 30 minutes after dispersal of the compounds. Five goldfish were exposed to each concentration of mercuric chloride (LC_{50} and control group) and each concentration of mercuric chloride with chitosan (experimental group). Each treatment was triplicated. The aquaria were aerated but not filtered, and food was withheld during the study period. The solutions were stirred manually once every six hours when mortality was assessed. However, each aquarium was checked every three hours for the first two days.

RESULTS

During the toxicity tests, all fish treated with mercury showed tremors, erratic swimming, darting, and rapid opercular beats. Whereas fish treated with chitosan became more quiet and appeared not to "fight" the toxicant. Mercury exposed goldfish appeared to be restless until death.

Figure 1 shows the relative change in mortalities for control and experimental groups. Each treatment group showed different responces to any given concentration of mercuric chloride with or without chitosan. High mortalities were observed in both control and experimental group through this experiment ($P<0.05$).

After the fish were exposured to the mercury, mortality increased steadily until 60 hrs in the control group. One hundred percent mortality occurred between 36 hrs and 60 hrs in all mercury treated groups except 0.6 ppm-Hg group. For 0.6 ppm-Hg the mortality occurred significantly after 42 hours (Fig. 1). For the experimental group, mortality decreased significantly compared to the control group ($P<0.05$). Fifty percent lethal concentration occurred between 60 to 160 hours after the fish were exposured except 0.6 ppm-Hg with chitosan. At 0.6 ppm-Hg with chitosan treated group, LC_{50} levels were extended over 190 hours after the fish were exposured. It was striking that 0.6 ppm-Hg with 1.2 ppm-chitosan had lower mortality than any other dilutions.

DISCUSSION

Because this experiment was a static toxicity test under laboratory conditions, one would might think that hypersensitivity would result in violent swimming movement at high concentrations of Hg. The toxic concentrations of these compounds were somewhat artificial ; however, the versatility and usefulness of the techniques for such measurements is obvious. Mortality had a completely different mode at each concentration of Hg with or without chitosan.

These findings must be related to those of chronic toxicity studies. The long-term effects of chitosan on the goldfish have not been determined to our knowledge. However, the results of this experiment clearly show that (1) chitosan had higher binding or inhibition capacity with mercuric chloride, and (2) chitosan was not equally effective. The greatest effectiveness of chitosan for mercuric chloride binding may due in part to the maximum strength of chitosan. Although the highest effectiveness of the chitosan came from the lowest concentration, 1.2 ppm, the higher concentration groups showed appreciable mortality decrease.

The alarm phase of stress (Selye 1950) was characterized by an immediate increase in Hg-levels. Resistance is the phase of the stress response following the initial reaction (alarm) to the stressing

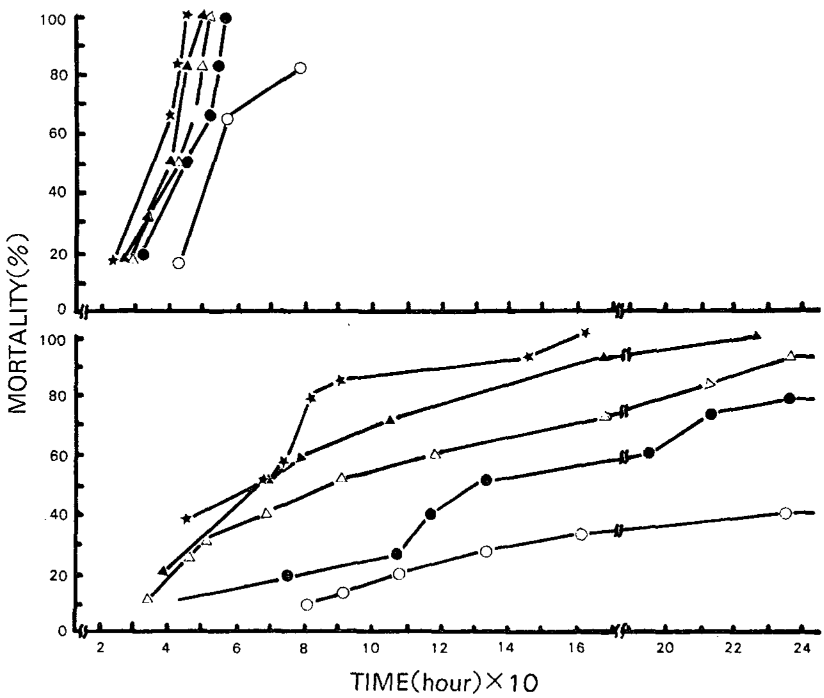

Figure 1. Cumulative mortalities among 10 groups of goldfish *Carassius auratus* exposed to $HgCl_2$(upper) and $HgCl_2$ with chitosan (lower)

Upper($HgCl_2$ only) ; ○——○ 0.6 ppm
●——● 0.7 ppm ; △——△ 0.8 ppm
▲——▲ 0.9 ppm ; ★——★ 0.1 ppm

Lower($HgCl_2$+chitosan)
○——○ 0.6 ppm+1.2 ppm ; ●——● 0.7 ppm+1.4 ppm
△——△ 0.8 ppm+1.6 ppm ; ▲——▲ 0.9 ppm+1.8 ppm
★——★ 1.0 ppm+2.0 ppm

agent and during the organism tries to maintain or regain homeostasis (Schreck and Lorz 1978). One would presume that the early mortality resulted from shock stress. This response lasted a few hours (both groups) and was followed by the resistance phase (experimental group). The alarm, resistance, and death phase could not be readily distinguished at Hg concentration 0. 7 to 1. 0 ppm without chitosan. This pattern indicated that although the stress (Hg) was still present at the same severity, fish might return to prestress conditions owing to chitosan.

From the results of the mortality test, one can postulate that when fish were exposed to mercuric chloride with chitosan, the mortality rates should not same as that of any given mercuric chloride concentration without chitosan. Added chitosan level and reduced mortality were probably related either to inhibiting Hg toxicity or to the process of trying to restore the values to normal activity of goldfish.

Other studies conducted in different laboratories were not comparable because of water quality or species used. Objection to uncontrolled variables were acknowledged, but it was felt that uniform water quality was more important for comparision to other results than definition of exact toxicity figures. The importance of these experiments lies in the demonstration that the mortality test is feasible and imformative.

In conclusion, this study showed the potential utility of chitosan, one of the natural waste by-products and readily available material for removing toxic metals(Muzzarelli 1977). It was striking that chitosan showed relatively high uptake or inhibition of mercuric chloride from the aqueous environment. Thus this material, chitosan, appears potential usefulness for the removal of heavy metals from the aquesous environment. Finally, it may be feasible to develop a series of chitosan concentrations in a fixed concentration of mercuric chloride to find out the more precise values because of the variation in response among individual fish exposed to mercuric chloride and chitosan. Such a series would help in slecting a maximum value with minimum concentration. One would suggest that physiological studies directed toward assessment of fish health or water quality shall include detrimentations or response curves for various concentrations of the factors of interest over a substantial period of time (Schreck and Lorz 1978).

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