Bull. Environ. Contam. Toxicol. (1986) 36:199–203

Bulletin of Environmental Contamination and Toxicology

Chromatin Condensation in the Erythrocytes of Fish Following Exposure to Cadmium

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Experimental cadmium poisoning in the fishes has been investigated and a variety of hematological changes have been observed (Smith et al. 1976; Johansson-Sjobeck and Larsson 1978; Garofano and Hirschfield 1982; Gill and Pant 1983, 1985; Nomiyama and Nomiyama 1984). In rabbits Cd poisoning lead to marked neutrophilia and lymphopenia although the total leucocyte count remained more or less stable (Stowe et al.1972). It has been suggested that Cd affected the leucocyte subpopulations by disturbing their formation and/or destruction, and possibly by influencing their transformation (Oshawa and Kawai 1981; Oshawa et al. 1983). Studies on mammals have also revealed that Cd interferes with basic cellular processes and the metal has been shown to cause chromatin condensation and emptying of interchromatin spaces in cultured hepatocytes (Puvion and Lange 1980; Puvion-Dutilleul and Puvion 1981). The present work illustrates the effect of chronic Cd poisoning on the erythrocytes of a freshwater fish, Puntius conchonius with reference to chromatin condensation.

MATERIALS AND METHODS

Adults of Puntius conchonius (Teleostei), weighing 4.5-5 g, were collected from the local lake and maintained in laboratory aquaria for two weeks prior to Cd exposure. Fish were maintained during both acclimatization and Cd exposure in test water of the following quality; pH 7.3, hardness 46 mg/l as $CaCO_3$, dissolved oxygen content 8.5 mg/l, and an average temperature 11.8°C. Fish were allowed food ad libitum and natural photoperiod throughout the experimental period. Ten fish each were distributed into six 25 L aquaria and treated as follows; group I, II and III were exposed to 630 µg/l $CdCl_2$ (1/20th of the 96-hr LC50) for 4, 8, and 12 weeks, respectively. The remaining three groups served as controls. The test water was changed every week and

kept sufficiently aerated. At the end of stipulated exposure period, the fish from control and Cd-treated groups (n=6-8 each) were sacrificed and blood taken into tuberculin syringes. Blood smears were prepared, two slides per individual, and stained with Leishman's or Giemsa's stain (Wintrobe et al. 1981). Erythrocytes were examined light microscopically under immersion oil and photographed with an Olympus PM6 camera.

RESULTS AND DISCUSSION

Exposure to Cd elicited hyperexcitability and increased opercular movements in the first few days but subsequently the fish appeared quiet. The fish autopsied after 4 weeks were generally sluggish while those sampled after 12 weeks were almost moribund. Further, despite a sufficient D.O. level of about 8 mg/l, most Cd-exposed fish tended to swim at the surface. Control fish, on the other hand, appeared active and healthy during the experimental period. No mortality occurred in any group.

Erythrocytes in control fish revealed an elliptical shape and an oval nucleus with an evenly dispersed chromatin material (Figure 1). After 4 weeks of Cd exposure, signs of nuclear aberrations were visible. The chromatin condensation occurred at the periphery of erythrocyte nuclei and their central part appeared unstained (Figure 2). At the end of 12 weeks, nuclei in most erythrocytes revealed nuclear 'puffs' which appeared to have been formed due to leakage of chromatin material from the nuclear membrane. The accumulation of chromatin material occurred at certain places along the nuclear periphery (Figure 3). The nuclei themselves contained very little chromatin. The affected erythrocytes also appeared hypochromic.

The nucleus appears to be the primary target for the toxic action of Cd. Ultrastructural studies on isolated liver cells have demonstrated chromatin condensation as an initial response to Cd poisoning (Puvion and Lange 1980). Morselt et al. (1983 a,b) reported chromatin condensation in the nuclei of cultured hepatocytes and endothelial cells of small uterine vessels after $CdCl_2$ poisoning in the Wistar rats. The irreversible phenomenon of chromatin condensation was found to precede membrane leakage. The administration of $ZnCl_2$, Pb-acetate, $HgCl_2$ and $CuCl_2$ in equimolar concentration, however, failed to evoke chromatin condensation 'in vitro' (Morselt et al. 1983 a). Emptying of interchromatin space due to chromatin condensation has also been observed in the endothelial cells of medium renal arteries of the rat (Fowler et al. 1975),

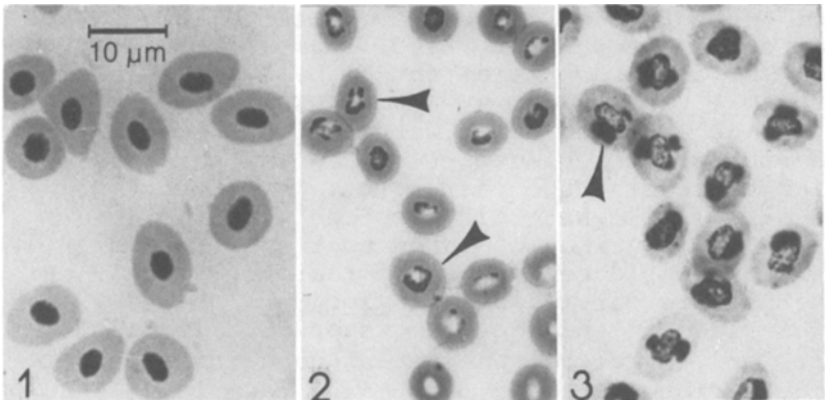

Figure 1. Control. Scale of magnification is common to all figures.
Figure 2. Erythrocytes of fish exposed to $CdCl_2$ (630 µg/l) for 4 weeks. Note chromatin condensation.
Figure 3. Erythrocytes of fish exposed to $CdCl_2$ (630 µg/l) for 12 weeks. Note nuclear 'puffs'.

and proximal tubule cells of rat kidney (Nishizumi 1972) after chronic Cd administration.

Results of present investigation show that Cd causes nuclear anomalies including clumping of chromatin material and an increase in interchromatin spaces in the erythrocyte nuclei. In the circulating erythrocytes of this fish, other morphological aberrations such as cytoplasmic stippling, deterioration of cellular membrane, hypochromia and schistocytosis have been described, in addition to a persistent anemia following long term exposure to sublethal levels of Cd (Gill and Pant 1985). Qualitatively, these nuclear and cytoplasmic changes after Cd poisoning are suggestive of disturbed RNA synthesis. Ribosomal abnormality and the resultant decrease in Hb synthesis (Wintrobe et al. 1981) are likely to affect oxyphoretic capacity of the erythrocytes. The general debility observed in Cd-exposed P. conchonius might have been due to hypoxemia related to an anemic state. Evidences are available which suggest that the chromatin condensation and an increase in interchromatin space reflect deranged mRNA and rRNA synthesis (Puvion and Moyne 1981). In rat, Cd induced nuclear and cytoplasmic changes in hepatocytes and uterine vessels were interpreted as a disturbance in rRNA synthesis which ultimately lead to cytolysis (Morselt et al. 1983 b).

Chromatin condensation may be reversible without damaging the cell (Trump et al. 1980). However, Morselt et al. (1983 a) found the effect to be irreversible. Exposure to 1 µg/ml $CdCl_2$ evoked chromatin condensation in cultured liver cells within 15 min to 1 hr, and the subsequent replacement in Cd-free water for 24 hr failed to reverse the phenomenon. This investigation reveals a somewhat delayed effect of Cd on the nuclear morphology when compared to the situation in mammals. This could be due to two reasons, firstly the experiments were carried out *in vivo*, and secondly, the $CdCl_2$ was added to the aquarium water at a level of 630 µg/l, and therefore, the actual concentration of Cd in the peripheral blood might have been low although no data are available to support this presumption. Besides, resistance to Cd-cytotoxicity, described both *in vitro* and *in vivo*, has been associated with increased content of metallothionein (Gick et al. 1981).

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Received December 26, 1984; accepted March 15, 1985