The Pathology of Cadmium Poisoning

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ABSTRACT

Exposed to a concentration of 0.1 mg/L cadmium chloride from hatching to 4 weeks, Tilapia nilotica Linnaeus fry exhibit erratic behavior, retarded growth and impaired organogenesis. Cell damage is clearly shown in several developing organs such as the gills, liver, gastrointestinal tract, kidneys, and brain. Developing muscles and fins exhibit no distinct damage at the concentrations used.

INTRODUCTION

Cadmium (Cd) is an occupational hazard and environmental pollutant affecting the biological systems of many organisms. The National Environmental Protection Council determined the Cd concentration in Philippine rivers to range from 0.002 to 0.11 mg/L. Kapauan et al. reported the concentrations of Cd, in all species of aquatic fauna in microgram per gram on a wet basis, to be nil up to 0.13, with an average of 0.009. Studies by Andaya and Gotopeng (1982) revealed that the 24 hr median lethal concentration (LC50) in *Tilapia nilotica* was 1.6 mg/L. The same study also showed uptake level to be significant at 0.1 mg Cd/L. Although Cd in our rivers is of a sublethal concentration, it is interesting to find out if it causes significant effects on a hardy species of commercial importance, *T. nilotica*.

Cadmium affects the cellular systems of not only the higher organisms such as man (Chung et al., 1986) and other mammals (Mukherjee et al., 1984), but also fishes and birds (White and Finley, 1978) as well as invertebrates (Carmichael and Fowler, 1981; Ward, 1982) and fungi (Morselt et al., 1986).

Physiological studies show that Cd accumulation occurs highest in the kidney (Kariya et al., 1978; Kumada et al., 1980; Tokumaru et al., 1980b), followed by the liver (White and Finley,

1978; Morselt et al., 1987), the gills (Noel-Lambot et al., 1978), and the gatrointestinal tract (Banerjee et al., 1978; Jennings and and Rainbow, 1979). According to Rowe and Massaro (1974), the brain does not accumulate Cd, but this was later contradicted by the findings of Benoit et al. (1976). Both authors, however, agree that muscle is the least affected of all organs.

This study aims to determine the effects of sublethal Cd on the organogenesis of the kidney, liver, gills, intestine, brain, fin and muscle using light and electron microscopy.

MATERIALS AND METHODS

Four-day old *Tilapia nilotica* fry were obtained from the College of Fisheries. They were distributed to 15-liter aquaria at 60 fry per aquarium. A stock solution of cadmium chloride of concentration 10,000 mg/L was mixed and from it 0.1 and 0.5 mg/L were prepared. Total water replacement was done every 24 hrs in the duration of 4 weeks. Fry were fed daily to satiation with a commercial fish food and aeration was continuous. Processing was done weekly.

For light microscopy, fry were halved and fixed in Bouin's solution, then processed using the paraffin technique.

For electron microscopy, organs were dissected free from other tissues. They were cut into small cubes (1-2 mm^3), fixed in 2.5% glutaraldehyde, washed in buffer and postfixed in osmium tetroxide. Infiltration was done in propylene oxide and Araldit resin and tissues were embedded in pure resin. Ultrathin sections were mounted on uncoated grids and stained with uranyl acetate and lead citrate. Observation was done on a JEOL U-100 electron microscope at the Natural Science Research Institute of the University of the Philippines (UP-NSRI).

RESULTS AND DISCUSSION

Gross morphological examination of Cd-treated fish shows the following results: Lateral curvature of the vertebral column, changes in the color and markings, retarded growth and muscular spasms or convulsions, as had been observed by Rehwoldt and Karimian-Teherani (1976), Spehar (1976), Koyama and Itazawa (1977 ab), Tokumaru et al. (1980 c) and Mukherjee et al. (1984).

After exposure to 0.1 ppm $CdCl_2$ for one week, discernible effects of Cd poisoning on the kidney, as observed under the light microscope, are the following: presence of cellular debris at the lumen of tubules and at the Bowman's space, and the decrease in the number of glomeruli.

Under the electron microscope, the kidney epithelium shows degeneration. Irregularly shaped and smaller nuclei, abundant and bigger lysosomes and extensive vacuolation are evident. Mitochondria are contracted and dense with indistinct cristae (Fig. 1).

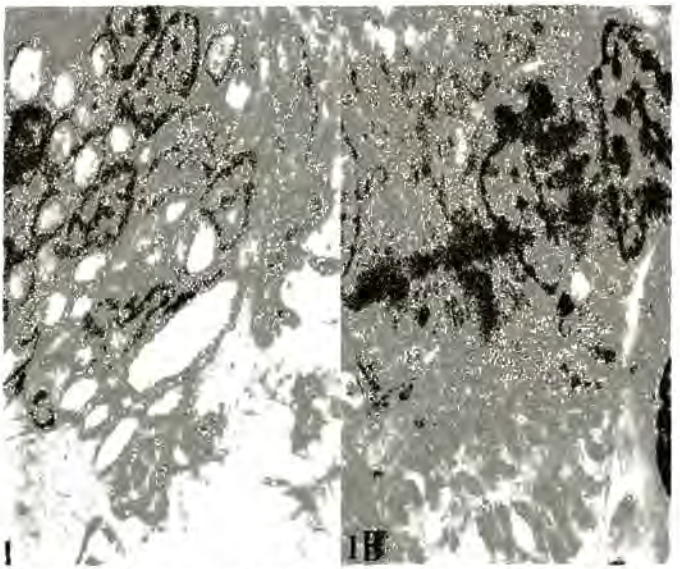

Fig. 1 Extensive vacuolations and necrotic mitochondria are signs of damage in the kidney after four weeks exposure. x5,000 B. untreated. x 5,000.

Various authors made similar observations such as the loss of cytoplasm of the kidney epithelium due to increase in number of vacuoles (Koyama and Itazawa, 1977 b; Koyama et al., 1979; Hawkins et al., 1980), degeneration of cell organelles (Tokumaru et al., 1980 b; Hawkins et al., 1980) and the increase in number and size of lysosomes (Koyama et al., 1979) signifying increase in lysosomal activity.

The mitochondrial defects may signify changes in cell permeability that may have led to an influx of Ca^{++} into the cell and a subsequent loss of mitochondrial function (Hawkins et al., 1980). The presence of cellular debris found in the lumen of kidney tubules is due to the detachment of some cell components, even whole cells such as rodlet cells (Hawkins, 1984). Friberg et al. (in Diliman, 1980) have proposed a model for renal injury in vertebrates. According to them, protective Cd-binding proteins (Cd-MT), which are small enough to be filtered by the renal glomerulus (Templeton

& Cherian, 1985), are reabsorbed in the tubules just like other proteins but when the Cd-binding potential of such protein is exceeded, free Cd in the cells adversely affects numerous enzyme systems. As a plateau is reached, cellular contents may be excreted together with Cd thus leaving behind a tubule which is in the process of degeneration.

Electron micrographs of the liver show degeneration of the cytoplasm, increase in size and number of lysosomes, evolution of discrete vacuoles, cloudy swelling or condensation of the mitochondria, hypertrophy of the rough endoplasmic reticulum (ER) and Golgi complex (Fig. 2), and peripheral chromatin condensation in the nuclei of the hepatocytes.

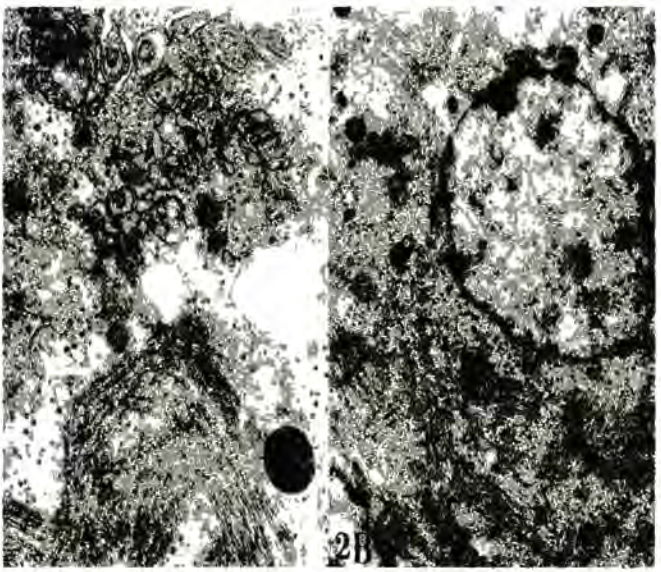

Fig. 2. Hepatocytes have enlarged vacuoles, swollen mitochondria and atrophic cristae and dilated endoplasmic reticulum. x5,000. B. untreated, x5,0000.

Hepatocytes of fish exposed to Cd show similar structural alterations as reported by other authors: accumulation of black abnormal granules in hepatic blood vessels (Koyama and Itazawa, 1977 b), increase in the number of rough ER in porgy and smooth ER in carp, increased lysosomal activity, decrease of glycogen granules (Koyama et al., 1979), degeneration, focal fibrosis and general congestion of the parenchyma (Tokumaru et al., 1980 b) and hypertrophy of the Golgi complex (Ferri, 1980). Morselt et al.'s

(1983 ab and 1987) study on Wistar rats showed chromatin condensation and emptying of the interchromatin space as well as the increased number of loose cell nuclei and the discharge of cytoplasmic cell constituents in the sinusoidal spaces.

The effects of Cd on liver tissues are caused by the affinity of free metal ions towards hemoglobin which is readily taken up by the hepatocytes where it induces the synthesis of metallothionein, and remains bound to it (Cherian et al., 1983; Templeton & Cherian, 1984 & 1985). Structural alterations occur in hepatocytes even if the induced Cd-MT is passed on to the kidney. The hypertrophy of the rough ER and the Golgi complex are related to the increase in the synthesis of metallothionein, as well as in the enhancement of lysosomal activity (Ferri, 1980). Chromatin condensation in the nuclei of hepatocytes is suggestive of a disturbance in the transfer of heterogenous RNA along the dispersed chromatin thus affecting the production of messenger RNA (mRNA) for the cytoplasm (Morselt et al., 1983 b). Chromatin condensation is said to be the morphological sign in cells destined to die, since upon the disruption of mRNA production, metallothionein synthesis is also hindered, enabling free Cd to adversely affect other cellular processes. Mitochondrial swelling is most probably due to changes in membrane permeability.

In gill tissues, thickening of the mucous layer, fusion of some secondary lamellae, lifting off of the epithelial membrane of the secondary lamellae, and shortening of the length of the secondary lamellae, are observed. Ultrastructurally, such thickening of the mucous layer is caused by the increase in the number of mucous cells (Fig. 3), also causing the interlamellar space to narrow, and in other cases, the secondary lamellae to be completely fused. Other fine features of gill tissue effects of Cd poisoning are: increase in volume of subepithelial spaces leading to the lifting off of the epithelial membrane of the secondary lamellae, and the irregular shape of and presence of vacuoles in the pillar cell leading to the eventual collapse of the pillar cell system or teleangiectasia. Chloride cells which increased in number with Cd concentration, have a vacuolated cytoplasm with well-developed Golgi apparatus (Fig. 4).

The lamellae of gills are in direct contact with the aquatic environment and are considered as potential uptake sites of Cd dissolved in water. Tokumaru et al. (1980 b) observed deterioration in the respiratory epithelium of the secondary lamellae. The increase in the volume of non-tissue or subepithelial spaces (Karlsson-Norgren, et al., 1985), the lack of normal microvilli-like protrusions in the apical membranes and the presence of vacuolated cytoplasmic blebs between neighboring cells (Karlsson-Norrgren

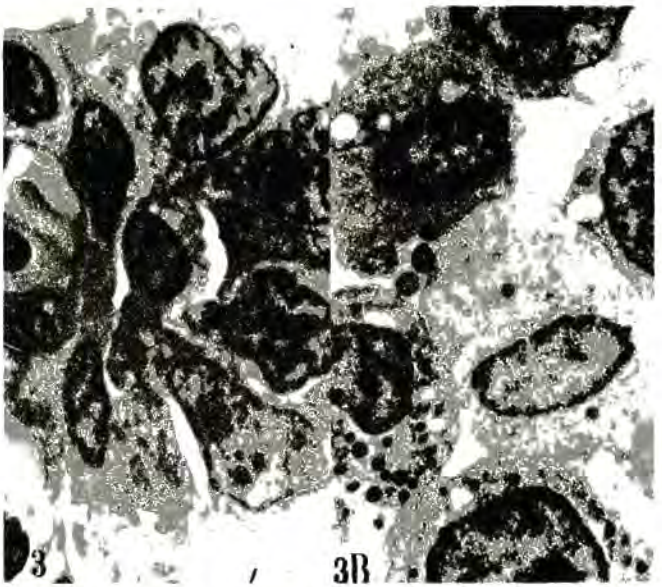

Fig. 3. Gill epithelium cells increase in number. x5,000. B. untreated. x5,000.

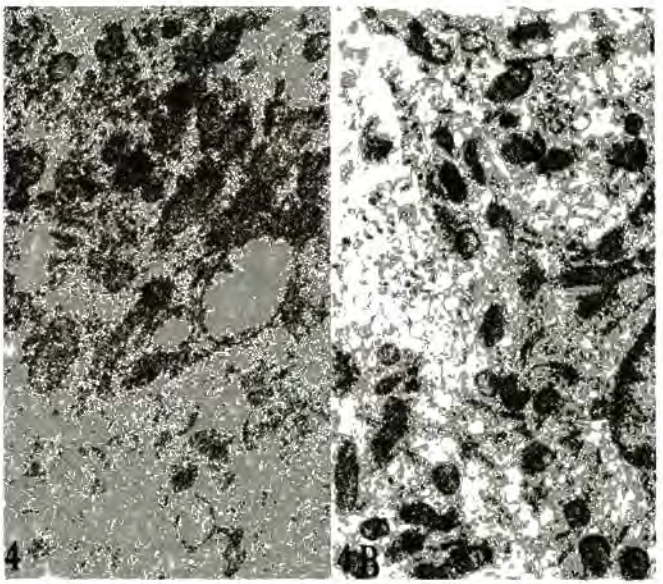

Fig. 4. Chloride cells have extensive vacuolations. x10,000. B. untreated. x5,000.

et al., 1985) all contribute to this. Also, the epithelial layer exhibits an incresed fraction of mucous, chloride and rodlet cells (Part and Svanberg, 1981; Oronsaye and Brafield, 1984; Karlsson-Norrgren et al., 1985), leading to the proximal distance between secondary lamellae and the complete fusion of some. Karlsson-Norrgren et al. (1985) identified the balloon-like secondary lamellae in Cd-treated zebrafish and rainbow trout to be teleangiectasia or the total breakdown of the pillar cells resulting to the reduction in height of the lamellae.

Gills are affected by Cd poisoning because they are specialized for gas and ion exchange with the water and is also the main uptake site for other dissolved compounds. The increase in mucous cells is a natural reaction to the presence of foreign substances. However, the thickening of the mucous layer increases the diffusion distance between blood and water (Part and Svanberg, 1981), thereby reducing the diffusion capacity. The increase in the volume of nontissue spaces (Karlsson-Norrgren et al., 1985) also leads to inadequate gas exchange. Teleangiectasia adversely affects blood circulation and subsequently leads to respiratory impairment. Observed changes in the chloride cells of the gills upon metal toxicity is connected with its function of ejecting absorbed heavy metals such as Cd (Oronsaye & Brafield, 1984).

The degeneration of the intestinal epithelium is observed as a result of Cd exposure at a concentration of 0.1 ppm $CdCl_2$. Apical microvilli are destroyed and debris of cytoplasmic particles are scattered in the intestinal lumen (Fig. 5). However, smooth muscle cells of the intestinal wall are intact.

The gastrointestinal tract comes in direct contact with dissolved Cd in water which explains the damage observed. Tokumaru et al., (1980 b) undertook the study of the effects of Cd in the intestines of fishes, among other organisms. Histological responses to Cd include the slight enlargement of blood capillaries, the lymphocytic infiltration of the epithelial mucosa and the great quantity of blood and epithelial cells in the intestinal lumen. Cd effects on the gut of other aquatic animals such as *Daphnia* are the shrinkage of the diverticula, emptying of the chlorophyll and paralysis (Griffiths, 1980). EM studies revealed the formation of electron dense deposits o basal and lateral membranes and on the mitochondria and microvilli of the epithelial cells.

The brain is also affected by Cd as manifested by pycnosis of neurons and neuroglia. Numerous vacuolations, dilation of the ER and the lifting off of the secondary nuclear envelope are observed. (Fig. 6).

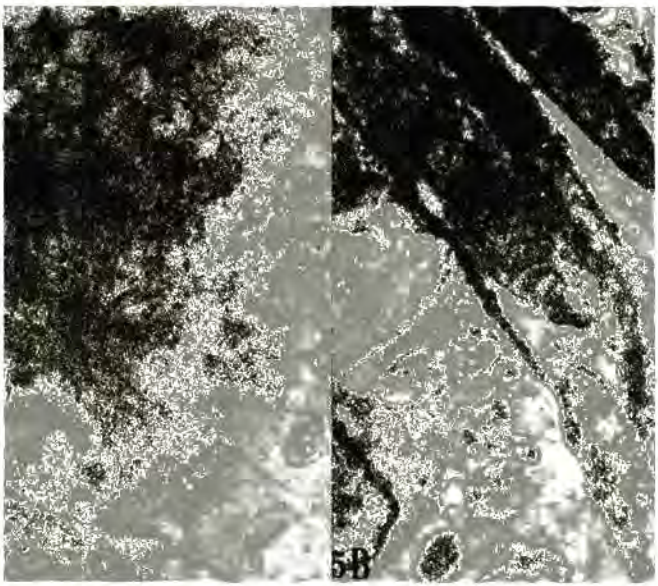

Fig. 5. Intestinal apical brush border is damaged and necrotic cells mucosa are scattered in the lumen. x6,000. B. untreated. x6,000.

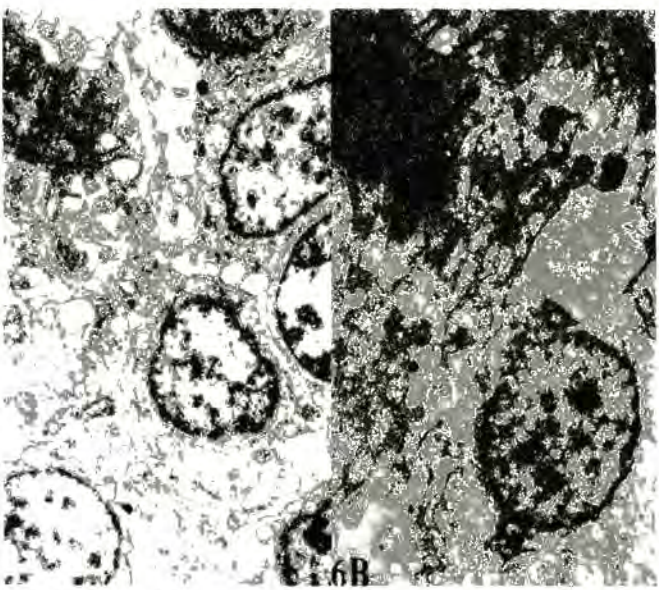

Fig. 6. Damaged neurons have abundant vacuoles and dilated endoplasmic reticulum. x6,000. B. untreated. x6,000.

Reproductive structures and endocrine organs are also damaged and the results have been presented elsewhere (Herrera, 1988; Herrera, 1989; Herrera and Baylosis, 1989; Herrera, 1990).

Generally, muscles are relatively unaffected by Cd exposure. Ultrastructure study of fins exposed to 0.1 mg Cd/L show muscle filaments to be intact (Fig. 7).

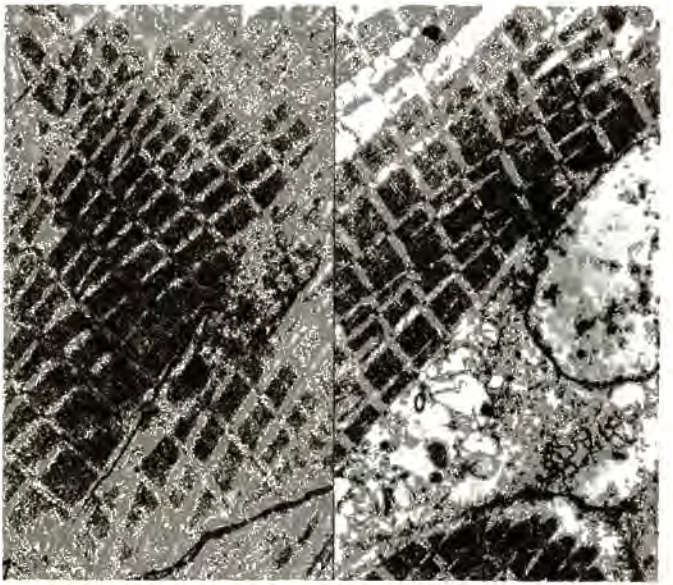

Fig. 7. Muscles of fish exposed to 0.1 ppm Cd are relatively unaffected. x5,000. B. untreated. x5,000.

Morphological changes undergone by most tissues due to Cd toxicity are explained by the theory of Cherian et al. (1983), that Cd may not all be filtered by the glomerulus and are instead taken up by other tissues with reticuloendothelial cells. Chronic exposure to Cd may lead to an excess of toxic free Cd ions, which react with the PO_4^{-3} and Sh groups of membranes and enzymes leading to impaired membrane active transport and enzyme activity in organogenesis.

SUMMARY

Exposure to cadmium at the concentration found in Philippine waterways causes histological defects in organogenesis in T. nilotica. As a whole, the juvenile fish exhibit erratic behavior and retarded growth.

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