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IMPACT OF ACUTE COPPER INTOXICATION OF SOME ORGANS OF *CLARIAS MACROCEPHALUS* GUNTHER

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ABSTRACT

Effects of acute copper exposure to *Clarias macrocephalus* at 1.2 mg/L were determined over a period of 48 hours. The gill filaments, kidney and liver were examined for any pathological effects. Histological analysis showed extensive changes in each organ. The gill filaments exhibited hyperplasia of lamellar epithelium, cell proliferation between secondary lamellae and a corresponding reduction of the interlamellar space. Increased vacuolation, widespread necrosis and degeneration of epithelial cells were likewise evident. The tail kidney was characterized by irregularly-shaped cells. Dilation of the Bowman's space with accompanying hypertrophy of the glomerulus was evident. Extrusion of cellular materials characterized the lumen of the tubules. The liver tissue showed general destruction. It was marked by the absence of sinusoids, increased vacuolation and the presence of partially coagulated blood in the central vein.

INTRODUCTION

Copper, like zinc and iron, is considered an essential element for animals. Being a micronutrient, it is present in *Clarias batrachus* at the level of 6.888 + 0.17 ug/g (Daramola and Oladimeji, 1989). However, at high concentrations it may cause poisoning or changes in the organoleptic qualities of food, owing mainly to its role as catalyst in many degradation processes (Galindo et al., 1986).

Copper is a metal water pollutant that may be derived from anthropogenic activities, especially from industrial and agricultural wastes that drain into bodies of water. It tends to accumulate in bottom sediments from which it may be released by various processes of remobilization. Among the heavy metals, copper constitutes an important section of the nonferrous metal industry (Daramola and Oladimeji, 1989). The waste from such industries contains traces of copper which when

discharged into bodies of water are made available to aquatic organisms, particularly fish. These trace amounts may be concentrated in harmful levels (Uthe and Bligh, 1981; Segner, 1987). They can thus move up the food chain in several forms, thereby reaching man in whom they could produce chronic and acute ailments.

Copper accumulation is a function of exposure time and concentration in water. The greater the concentration of copper and the longer the time of exposure, the larger the amount of copper residue found in the fish. The rate of uptake is initially very high and at lower concentrations, although this decreases with time. An increased rate of accumulation over a longer period of exposure implies an impaired capacity to eliminate the heavy metal cupric ions from the fishes' system. This may result in high body burden that may be passed on to consumers (Daramola and Oladimeji, 1989). Investigations of acute effects of copper on fish are therefore an important aspect of environmental pollution control, most especially since human activities progressively increase the concentrations of heavy metals in the aquatic system.

In this study, *Clarias macrocephalus* Gunther are exposed to acute copper contamination. This species has been selected for factors such as its local importance, size, availability, food value and its suitability in the laboratory. Histopathological effects of the metals in the fish are analyzed in vital organs such as the gills, kidney and liver.

The introduction of small amounts of copper ions from natural and enthropogenic sources into the aquatic environment causes multiple changes in freshwater organisms, even at non lethal levels (Khangarot and Ray, 1987a). For instance, exposure of mammalian test animals to heavy metals, even at moderate levels of contact, has been shown to alter immunological responses (Koller, 1980). Sublethal exposure of freshwater fishes to copper on the other hand, have more so reduced their immune responses (Viale and Calamari, 1984). This sublethal toxic effect of heavy metals on the immune system of fish has been pointed out as modification to the outbreak of infections leading to bacterial or viral diseases (Sindermann, 1979). It can be postulated then that the histology of tissues has been altered by exposure to copper.

Vernberg et al. (1974) reported a decrease in metabolic rate in Uca pugilator as induced by heavy metals. Likewise, cupric ions were shown to cause respiratory and cardiovascular depression in *Mytilus edulis* and the effect was attributed to the passive binding of cupric ions with organic ligands (Scott and Major, 1972).

The minimum requirement and maximum tolerable levels for dietary copper remained to be determined for most species of fish. In rainbow trout, the highest admissible concentration is less than 0.005 mg, and for carp less than 0.007 mg. Long-term action of low $CuSO_4 . 5H_2O$ concentration causes extensive pathological changes in the histological structure of parenchymatous organs and in the copper content in the gills and liver of these experimental fish (Svobodova et al., 1982). Murai et al. (1981) observed a toxic effect of supplemental copper at 32 mg Cu/mg

diet in channel catfish. Toxicity was characterized by a reduction in growth, altered blood parameters, an increased feed gain ratio and food refusal.

MATERIALS AND METHODS

A. Test Specimen

The catfish *Clarias macrocephalus* was obtained from a hatchery and used as the test specimen. The juvenile catfish measuring 6.0-8.0 in and weighing about 10.0-20.0 g was acclimated to laboratory conditions three days prior to the experiment.

B. Preparation of Copper Metal

A stock solution of copper was prepared from copper sulfate ($CuSO_4$.20) by dissolution in deionized water and acidification with concentrated HNO_3. The test concentration of 1.2 mg/L was prepared using the stock solution.

C. Copper Treatment

Six aquaria with capacity of 5 l were used in the study. These were divided to make three duplicate set-ups for acute concentrations of copper and one for control. Dechlorinated tap water, which was previously allowed to stand for three days in a tank, was used in all tests.

Three juvenile fish were placed in each aquarium. The fish were fed to satiation twice a day using commercially avialable fish flakes.

D. Paraffin Method

The gill filaments, liver and kidney were processed for histological analysis using the standard paraffin method.

RESULTS

Juvenile catfish exposed to copper were observed to be initially hyperactive following exposure. After approximately three hours, the fishes surfaced, floated for a few minutes and gulped air. At the time of loss of equilibrium, they appeared slanting at the bottom with slight opercular movements. Swimming was sluggish and in abnormal positions. Finally, at the time of death the fishes were found lying on the floor of the aquaria exhibiting feeble opercular movements and labored breathing. Fifty percent mortality was observed over a period of 48 hours.

A distinct response to the physiological stress of copper exposure is the secretion of mucous. Copper induced appreciable mucous secretions, especially at the time of death when the fish were coated with excessive mucous on the body surface. This was evidenced by the change in the clarity and color of water to a murky yellow-brown hue. Other copper-induced responses included reddening of the region surrounding the pectoral fins and barbels, and the limping of barbels.

The fish in the control set-up exhibited normal movements, good reflexes and immediate responses to physical disturbances. A considerable low amount of mucous secretion and less cloudy water were observed.

Histopathology

Gills. Figure 1 shows the untreated gills with normal histological architecture.

Histological alterations of the gill filament were observed in copper-induced *Clarias macrocephalus*. Hyperplasia of the lamellar epithelium was pronounced (Fig. 2). Cell proliferation between the secondary lamellae was apparent. This eventually caused interlamellar space reduction. Loss of interlamellar space was accompanied by sloughing off of the lamellar epithelium.

The secondary lamellae also exhibited a reduction in height and a thickening of cell epithelium. This proliferation of the secondary lamellar epithelia produced a short and stubby appearance (Fig. 1).

A curve characterized the arrangement of pillar cells, as compared to the normal straight orientation (Fig. 1).

Degeneration of pillar cells also occurred.

Cells in the gill filaments exhibited widespread necrosis. Degeneration of cells was evident in the irregularly-shaped, shrunken and unhealthy appearance of cells in the secondary lamellae. Vacuolation (Fig. 4) was noticed.

Degeneration of acidophilic chloride cells near the secondary lamellar base between lamellae was also noted (Fig. 3).

Tail Kidney. Figure 5 is the normal histoarchitecture of the tail kidney. Histological changes characteristic of deformation of cells were observed in the renal capsule (Fig. 6). Irregularly-shaped cells replaced formerly cuboidal Bowman cells. Dilation of the Bowman's space was marked. This was associated with hypertrophy of the glomerulus.

Vacuolation and disintegration of the cells in the epithelium of the renal tubule were observed. Cells of the renal tubule and corpuscle appeared indistinct and largely undemarcated by cell membrane boundaries (Fig. 7). Hydropic degeneration was also manifested in the epithelial cells of the proximal segments of the renal tubule, largely occluding the lumen. The extrusion of cellular materials into the tubular lumen forming cellular casts further gave the lumen a cloudy appearance. Due to hyperplastic growth of epithelial cells and subsequent degeneration (shrinking), sinusoidal spaces were created within the nephron tissue.

Liver. Copper-induced effects disrupted the normal architectural patterns of the liver (Fig. 8). The integrity of the liver tissue was destroyed leaving large gaps and an appearance of torn tissue. Absence of sinusoidal spaces was marked, emphasizing the swelling of the cytoplasm. The plates of liver tissue exhibited many cellular spaces devoid of nuclear content or with significantly reduced nuclei, indicative of edematous hepatocytes (Fig. 9).

The tail kidney exhibited several histopathological changes. Defense mechanisms similar to the gills were apparent. These included dilation and hypertrophy of the Bowman's cells as well as vacuolation.

Degeneration was evident in different forms, the most pronounced of which was exsanguination. Loss of blood was due to the destruction of the red blood cells by hydropic distension due to water uptake, leaving the cells anemic (Khangarot et al., 1987b). Hydropic degeneration of the cells in the renal tubule contributed largely to blood loss. Extrusion of cellular casts in the tubular lumen was also observed by Chang and Spreacher (1976). These cellular masses are believed to be discharged by the kidney during cell injury, thereby occluding the lumen.

Sinusoidal spaces were created within the nephron tissue due to the initial hyperplastic growth of epithelial cells and subsequent degeneration or shrinking.

The liver is the organ responsible for the detoxification proccesses. Fish liver contains enzyme systems necessary for the detoxification of toxic materials such as heavy metals, pesticides and petroleum hydrocarbons (Haensly et al., 1982). It is therefore the main organ for accumulation of the toxicant copper. Several studies serve as evidence that the liver accumulates the greatest amount of copper residues as compared to muscle, gills, brain and blood (El-Domiaty, 1987; Radhakrishnaiah, 1988).

Extensive histopathological changes in the liver were observed due to increased copper content (Svobodova et al., 1985). Among these are extensive vacuolations, destruction of the integrity of the hepatocyte, absence of sinusoids, presence of cellular spaces without nuclear material and proliferation of partially coagulated blood in the central vein.

Studies by Lanno et al. (1987) show that rainbow trout has the ability to sequester dietary copper in discrete granules in the cytoplasm of the hepatocytes. The toxicant is acted upon by liver superoxide dismutase which stabilizes the system by binding to radicals (Gaitlin and Wilson, 1986). However, increased copper intake reduces the enzyme activity, thereby allowing prevalence of copper toxicity in the hepatocytes. The advanced effect of copper toxicity is evidenced by destruction of the integrity of liver tissue.

Water intake brings about the swelling of cytoplasm thus narrowing eventually closing sinusoidal space. Swelling of the cytoplasm of hepatocyte leads to its subsequent destruction (Sultan and Khan, 1981). Edema, which refers to the increase in the volume of tissue fluid (Leeson, 1985), also results in the presence of cellular spaces with greatly reduced nuclei.

Partial coagulation of the central vein blood is also indicative of copper toxicity as seen in the liver of *Mollinenesia* (Sultan and Khan, 1981).

Extensive vacuolation is a prominent feature of hepatocytes induced by copper. This indicates an increase in fat and glycogen content (Grizzle and Rogers, 1976). Lipid accumulation is a common hepatic response to toxic agents such as heavy metals, carbon tetrachloride, phosphorous and chlorinated hydrocarbon insecticides (Anthony et al., 1986).

SUMMARY

Histopathological effects of 1.2 mg/L copper exposure on *Clarias macrocephalus* over a period of 48 hours were analyzed. The gill filaments, tail kidney and liver showed disruption of the normal histological pattern. Copper at 1.2 mg/L is toxic to *Clarias macrocephalus* G.

Figure 1. The control gills with normal histological pattern (x 100)

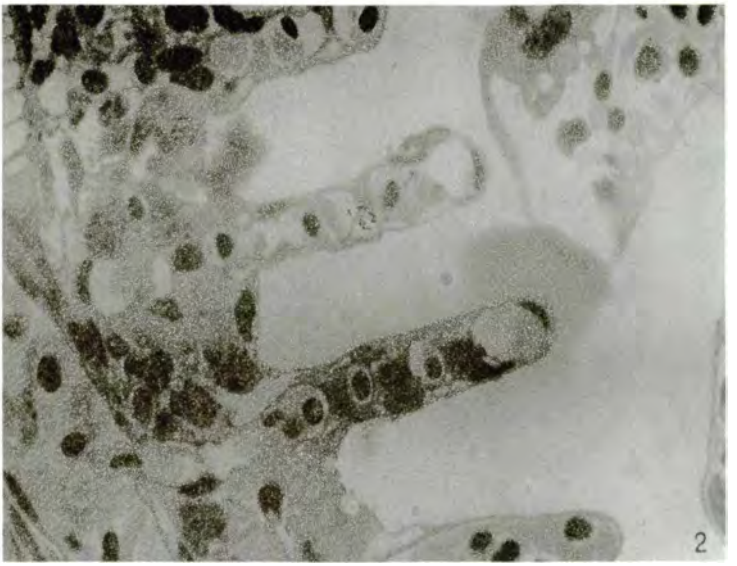

Figure 2. Copper-treated gills have pronounced hyperplasia of the lamellar epithelium (x 100).

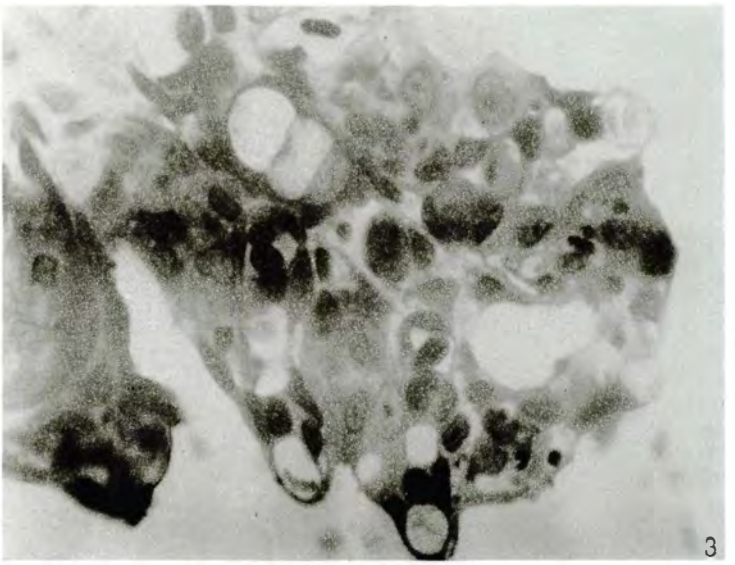

Figure 3. Some lamellae of the treated gills have short stubby appearance (x 100).

Figure 4. Vacuolation of cells is evident in the treated gills (x 100).

Figure 5. The normal histoarchitecture of the tail kidney

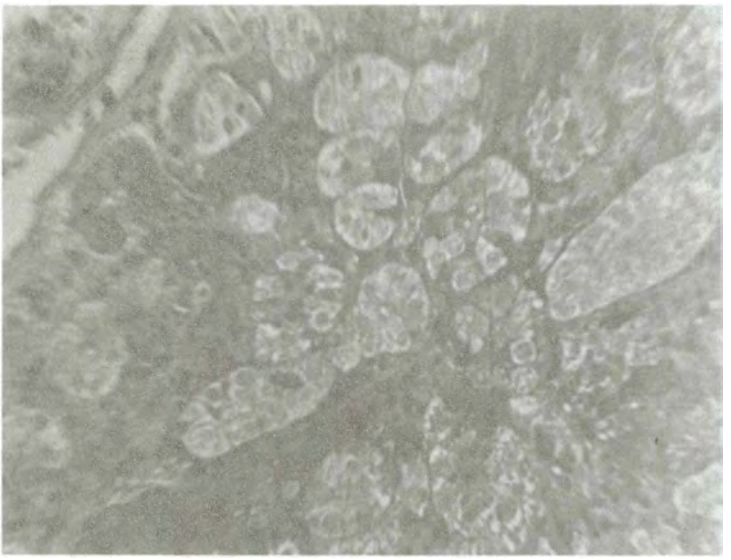

Figure 6. Irregularly shaped cells appear in the treated kidney.

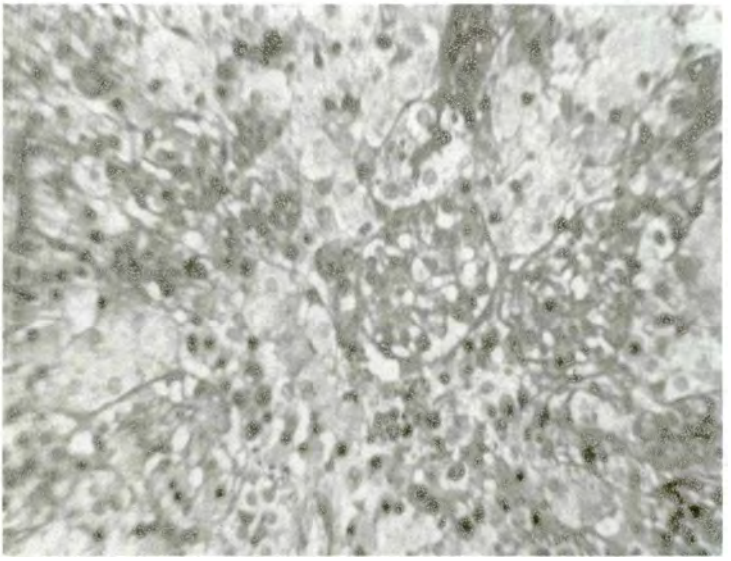

Figure 7. Cell membrane boundaries are not distinct in the renal tubules of treated fish (x 400).

Figure 8. The normal architectural pattern of the control liver (x 400)

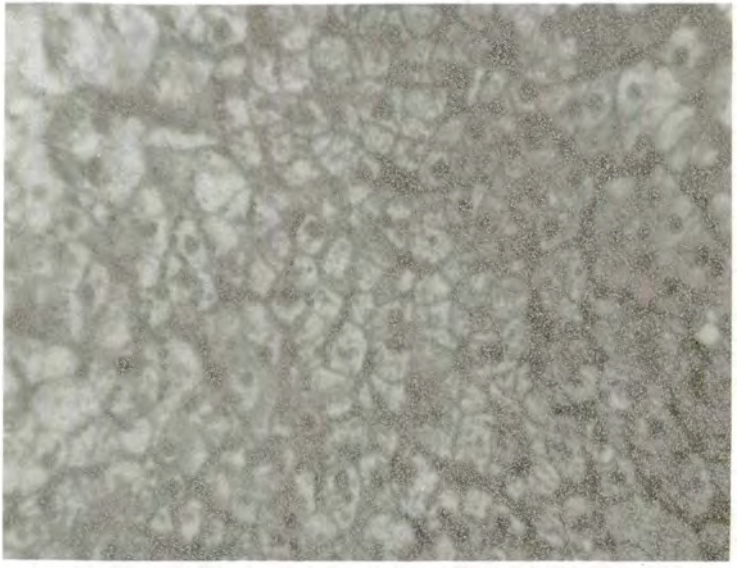

Figure 9. Edematous hepatocytes are formed in the treated liver (x 400).

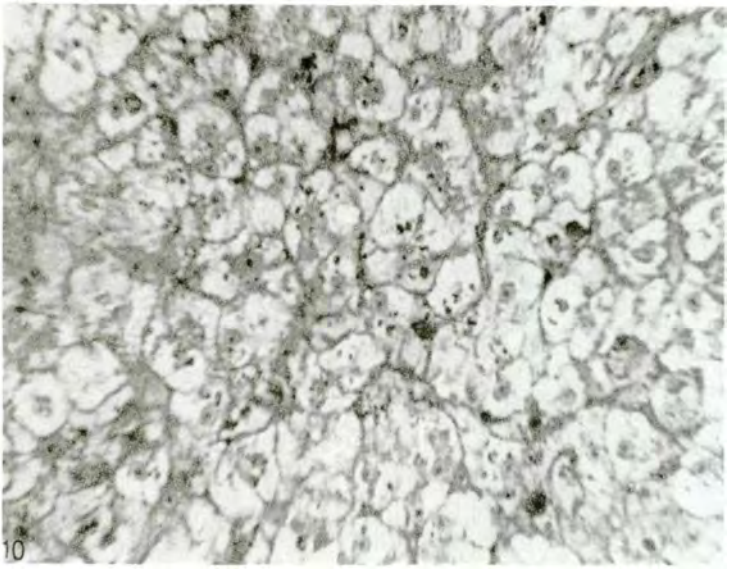

Figure 10. Widespread vacuolation of hypertrophied hepatocytes is evident in treated fish (x 400).

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