Histochemistry of Copper
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13.1. INTRODUCTION

Copper is an important trace element which becomes toxic in excess and normally it is required for metabolic activities in liver. Copper is essential for metabolic functioning of haemocyanin (White and Rainbow, 1982). Excessive concentration of copper may lead to pathological changes in various tissues and eventually death (Goldfischer et al., 1970). Metallothionein is a readily inducible protein of low molecular weight with a high cysteine content (Haywood et al., 1985a). It can be a major inducible copper binding protein in liver and kidney (Bremner, 1981). Haywood et al. (1985a) reported that excess copper absorbed from the blood is stored in the proximal convoluted tubules as Cu-MT or similar copper associated protein. Copper may be accommodated as free ions or salts or with free aminoacids or dipeptides or nucleic acids or metallothionein (Arumugam and Ravindranath, 1983, Overnell, 1982).

Presence of nuclear metallothionein was demonstrated in rats (Clarkson et al., 1984). Nuclear copper does not stain with conventional copper reactions and it is bound to heterochromatin which may be disruptive (Hardy and Bryan, 1975). Haywood and Comerford (1980) demonstrated high concentration of another important cupro protein caeruloplasmin (CB) which is an important transport protein, in copper loaded rats. Frieden (1980)
recognised caeruloplasmin as a donor protein and Stevens et al. (1984) identified receptor sites for caeruloplasmin in cell membranes.

Haywood (1985) made a detailed study on the toxic effect of copper in rat and the extent of adaptations and the changes that take place in the liver and kidney. McKim and Benoit (1971) studied the effects of long term exposure to copper on survival, growth and reproduction of brook trout Salvelinus fontinalis and reported decreased survival and growth in adult fish and reduced both the number of viable eggs produced and hatchability. Chronic copper poisoning was also reported in sheep (Gopinath et al., 1974 and Gooneratne, 1979).

Bryan (1968) and Ray et al. (1981) reported low concentrations of copper, zinc and cadmium in the abdominal muscle of Palaemon elegans. Lindquist (1968) first reported that lysosomal rupture occurred on a consequence of copper overload which was supported by Gooneratne et al. (1980).

Robinson and Ryan (1988) working on the transport of cadmium and other metals in the blood of the bivalve mollusc Mercenaria mercenaria suggested that metals are transported from the organs that are in immediate contact with the surrounding to the deeper lying tissues. The same authors also determined the distribution of metals in the blood plasma and the circulating haemocytes of
Mercenaria mercenaria. An increase in the copper retained in mucosal cells along with a decrease in copper absorption was observed in rats fed with a high zinc diet, compared to those with a lower level of zinc. (Hall et al., 1979 and Ogiso et al., 1979). Underwood (1977) opined that rats are relatively resistant to copper poisoning.

Histochemical studies for heavy metals are very meagre in amphibia particularly on Rana hexadactyla. Therefore the present investigation was made to histochemically localise copper in liver, intestine, kidney and testis of Rana hexadactyla treated with copper.

13.2. OBSERVATIONS

13.2.1. Accumulation of copper in liver:

In the liver of control frog Rana hexadactyla the presence of copper was seen as a few small black spots over the hepatocytes.

After sublethal treatment of copper for 120 hrs the liver exhibited heavy deposition of copper as aggregated granules in the cytoplasm of hepatocytes.

Median lethal treatment showed heavy aggregation of granules of copper than in sublethal treatment, indicating the high degree of deposition of copper in liver. The damage to the architecture of liver was also heavier in median lethal than sublethal treatment (Plate 7).
13.2.2. **Accumulation of copper in kidney:**

In control frog *Rana hexadactyla*, kidney copper was low as in the case of liver. It was seen as minute granules on the lining cells.

When the frogs were treated with sublethal dose of copper for 120 hrs, heavy granules and globules of copper were observed in the lumen of the proximal tubules indicating a heavy deposition of copper.

After median lethal treatment, more number of granules and globules of copper were observed with a high degree of necrosis (Plate 8).

13.2.3. **Accumulation of copper in intestine:**

A little amount of copper was observed in the intestine of control frog *Rana hexadactyla*.

In the sublethal treatment of copper after 120 hrs, many stainable granules of copper were localised in various cells of villi which caused severe damage to the tissues.

After median lethal treatment of copper for 120 hrs, more number of granules and globules were observed nearly at all villi causing heavy necrosis (Plate 9).

13.2.4. **Accumulation of copper in testis:**

A few spots of copper were located in the control testis of *Rana hexadactyla* as in the case of liver, kidney and intestine.
After the treatment of sublethal and median lethal doses of copper, increased amount of copper was seen as small round spots over the wall of the seminiferous tubule with disorganisation of cell nests and scattered sperm bundles which were severe in median lethal treatment. The accumulation of copper in testis was found to be lower than other test organs such as liver, kidney and intestine of both treatments (Plate 10).

After the treatment of copper, the deposition of copper was in the following order in the organs.

Liver > kidney > Intestine > Testis.

13.3. DISCUSSION

Accumulation of copper in liver:

*Anas hexadactyla* treated with both sublethal and median lethal doses of copper, produced heavy deposition of copper in the liver. The probable reason for the heavy deposition in the liver may be due to the fact that liver is the site of all metabolic activities and the centre for detoxification. This observation gains supports from the findings of Goldfischer et al. (1970) and Haywood et al. (1985b). Clausen and Wolstrup (1978) demonstrated that mute swans from an industrial area (olsemagle) which was polluted with copper had twice the hepatic copper content of swans from other parts of Denmark. Simp sen et
al. (1979) also made a similar observation in lead poisoned mute swans. Knoll and Fromm (1960) demonstrated that liver takes part in the elimination of excess metals from the body of fish.

According to Djangmah and Groves (1970) and Martin (1975) the hepatopancreas may act as a store for body copper. The hepatopancreas of *Palaemon elegans* contains disproportionately large amounts of the total body levels of copper, zinc and cadmium (White and Rainbow, 1986). Ishmael *et al.* (1971) and Soli (1980) reported that copper poisoning in sheep is associated with high liver copper concentrations and hepatocellular necrosis. Such copper granules correspond ultrastructurally to dense membrane bound bodies (McNary, 1963). Panemangalore *et al.* (1983) have demonstrated metallothionein bound to copper in the hepatic nuclei of foetal and neonatal rat.

Rainbow trout continued to accumulate copper in liver for 107 weeks (Goettl *et al.*, 1974). Muller and Prosi (1975) also reported high copper accumulation in the liver of roaches (*Rutilus rutilus*). The onset of liver necrosis varied with the magnitude of copper loading and necrosis first occurred as randomly distributed necrotic foci progressing to a more diffuse form which occasionally involved whole tubules (Haywood and Loughran, 1985). Martin and Flegal (1975) have hypothesized that squid actively accumulated copper for synthesis of their major respiratory pigment haemocyanin.
Haywood et al. (1985b) demonstrated that copper was apparent in the hepatic parenchymal cells after 1 week of copper supplementation and deposition became very heavy at 2 weeks when it was present in particulate form in hepatocytes in all zones of all the tubules.

Cytotoxic effects of copper have been attributed to spillover of copper from the lysosomes to other subcellular compartments resulting in damage to lysosomal and mitochondrial membranes (Lindquist, 1968). Boone and Schoffeniels (1979) suggested a possible mobilization of stored copper from hepatopancreas under physiologically stressful conditions.

**Accumulation of copper in kidney:**

*Rana hexadactyla* treated with sublethal level of copper exhibited high deposition of copper in kidney with tissue damage. Deposition of copper was more in median lethal treatment than in sublethal treatment. This finding agrees with the findings of Wolff (1964) and Porter (1950). The amount of copper in kidney was lower than that of deposition of copper in liver and the excess copper might have been excreted through urine. In this context, Underwood (1977) reported that urinary excretion of copper is marked in cholestatic disease and Wilson's disease. Haywood (1985) also supported the renal excretion of excess liver copper in rat. Kidney is found to be involved in the mechanism of elimination of metals from fish (Knoll and Fromm, 1960).
Haywood et al. (1985a) demonstrated that excess copper absorbed from the blood by active receptor sites on the tubule cells is stored in the proximal convoluted tubules as Cu-MT or a similar copper associated protein. Copper albuminate given parenterally to mice passes first into the glomerular filtrate and then into the proximal tubule cells with associated necrosis (Vogel, 1960).

Metallothionein can be an inducible copper binding protein in kidney (Bremner, 1981) and its presence has been associated with resistance to copper poisoning in salmon (McCarter and Roch, 1983). Copper has also been demonstrated in the kidneys of copper poisoned sheep (Gopinath et al., 1974) and rats (Wolf, 1960).

Histochemical and subcellular fraction studies of kidney have shown changes in the intracellular localisation of copper. Granules and globules of stainable copper in the lining cells and lumen of the proximal tubules have been reported (Haywood, 1985). Kidney copper was detected in globular droplets and granules in the cytoplasm of the proximal convoluted tubules from 2 weeks onwards and frequently appeared to be extruded into the lumen of the renal tubule in rat (Haywood et al., 1985a).

From the above discussion it is enlightened that copper accumulates as stainable granules after treatment. It
accumulates more in liver and less in kidney. As the liver is the seat of all metabolic activities, more accumulation was seen in liver. Since kidney is the site of elimination, it is bound with less copper than the liver.

**Accumulation of copper in intestine:**

Intestinal copper content of *Rana hexadactyla* was found to be lower than that of liver and kidney. Since intestinal elimination of copper through faecal matter is poor and it is not the target organ in eliminating the toxicants, intestine showed comparatively less deposition of copper than liver and kidney. But the deposition of copper content in median lethal treatment is greater than the sublethal treatment. This may be due to the higher concentration of copper in median lethal dose than in sublethal dose. Generally the bioconcentration of copper and zinc was found to be more in the gill followed by mantle and then by alimentary canal (Krishnaveni, 1989) in *Crassostrea madrasiensis*. A similar trend was reported in oyster from Cuddalore backwaters (Rajendran et al., 1988). Metallothionein has been found in the gastrointestine of many fish species (Kito et al., 1982b). Metallothioneins are important metal binding proteins. Klaverkamp et al. (1984) reported that white suckers from a contaminated Flin Flon lake had 4.5 fold increase in intestine MTN concentration when compared to suckers from a relatively uncontaminated lake.
Accumulation of copper in testes:

In the present investigation when Rana hexadactyla was treated with copper, testis showed lower deposition of copper content than that of other test organs such as kidney, liver and intestine. Since testis has no role in the detoxification or elimination of excess metal, poor amount of copper was found in testis at both treatments. But in median lethal treatment more copper contents were localized on the walls of the seminiferous tubule as black spots than in the sublethal treatment. More concentration of copper in the median lethal dose might be attributed to the high copper content in the median lethal treatment which had resulted in severe cellular damage in the testicular structure and scattered sperm bundles. These changes agree with the abnormalities observed in the copper sulphate and cadmium chloride exposed testis of viviparous teleost Labistes reticulatus (Rekha Segal and Panday, 1984). Ahsan and Ahsan (1974) also inferred that cadmium chloride blocked the spermatogenetic activity at the secondary spermatogenetic level in Clarias batrachus with pycnosis. Sangalang and Halloran (1974) studied the effect of cadmium on brook trout testis. Benoit (1975) observed chronic effects of copper on reproduction of blue gills.
1. All the tissues analysed showed higher copper accumulation in median lethal treatment than in sublethal treatment.

2. The liver recorded the maximum and testis minimum amount of accumulation of copper. The accumulation was in the following order.

   Liver > Kidney > Intestine > Testis.
A. Section of the liver of the control frog *Rana hexadactyla* showing scattered presence of copper as black globes.

B. Section of the liver of the frog *Rana hexadactyla* treated with sublethal dose of copper showing deposition of copper as black patches.

C. Section of the liver of the frog *Rana hexadactyla* treated with median lethal dose of copper showing heavy deposition of copper as big black patches.
A. Section of the kidney of the control frog *Rana hexadactyla* showing very little presence of copper as small round black spots.

B. Section of the kidney of the frog *Rana hexadactyla* treated with sublethal dose of copper showing deposition of copper as small black globes.

C. Section of the kidney of the frog *Rana hexadactyla* treated with median lethal dose of copper showing heavy deposition of copper as black globes and spots.
A. Section of the intestine of the control frog *Rana hexadactyla* showing very little presence of copper as a few small black spots.

B. Section of the intestine of the frog *Rana hexadactyla* treated with sublethal dose of copper showing deposition of copper as black granules.

C. Section of the intestine of the frog *Rana hexadactyla* treated with median lethal dose of copper showing more deposition of copper as black granules.
Section of the testis of the control frog *Rana hexadactyla* showing very poor amount of copper as black spots.

Section of the testis of the frog *Rana hexadactyla* treated with sublethal dose of copper showing accumulation of copper as black spots at the boundaries of the seminiferous tubule.

Section of the testis of the frog *Rana hexadactyla* showing more accumulation of copper as black spots on the boundaries of the seminiferous tubule.