Delta J. Sci. 16 (3) 1992

ELECTRON MICROSCOPICAL OBSERVATIONS ON THE HEPATOTOXIC EFFECTS OF ZINC ON THE NILE FISH OREOCHROMIS NILOTICUS

BY

[*]Fouad, A. Abou-Zaid [**]Tharwat, S. Shenouda

[**]Ahmed, E. Abada

[*]Faculty of Science, Tanta, Tanta Univ.

[**]Faculty of Education, Kafr El-Sheikh, Tanta Univ.

Received: 17-9-1992

ABSTRACT

The Nile fish Oreochromis niloticus was subjected to a sublethal concentration of 3 ppm. Zn solution for 32 days. Investigation of liver ultra-structure demonstrated a congruent modification of hepatocellular structure. The most prominent changes were concerned with the nucleus, rER, mitochondria and lysosomes.

INTRODUCTION

Pollution and its effects on aquatic life are considered as one of man's greatest crimes against himself. Metals are well known pollutants causing disorders in population-function of some, if not all, living organisms. Ecosystems which are frequently exposed to metal pollution are known to be biologically non-productive.

Zinc is an essential trace element in living organisms. However, traces of this element are frequently toxic and can remove all forms of animal life from streams (Michael, 1984). Defining maximum safe level of any particular element is difficult, as much ancillary informations such as : PH, temperature, dissolved oxygen content, presence of other elements which act synergistically (La Roche, 1972), length of exposure, species and age of exposed fish, are required.

In Egypt, the toxicity of heavy metals on fish has been studied by many authors (Hilmy *et al.*, 1980 & 1984 ; El-Sarha, 1986; Mazhar *et al.*, 1986 & 1987; Abd El-Rahman, 1987; Abou-Zaid *et al.*, 1988 a & b and Shenouda *et al.*, 1992).

Normal background values of zinc in natural inland surface waters may vary from 0.001 to 0.2 mg/L. However, the concentration of zinc was found to reach 0.7 mg/L., during spring, in the Nile water near Kafr El-Zayat (Shenouda *et al.*, 1992).

As the liver tissues of the fish represent the main target for polutant (Abou-Zaid *et al.*, 1988 a), the present work is planned to evaluate the hepatotoxic effects of zinc on the liver of the common Nile fish *Oreochromis niloticus*.

MATERIAL AND METHODS

Individuals of the Nile fish *Oreochromis niloticus* were caught from Kafr El-Zayat region (about 110 Km north of Cairo), during Spring. The physico-chemical properties of water at this region were: PH 7.2, total hardness 16.4 mg/L., conductivity 400, heavy metal contents- Cr 0.02, Cu 0.007, Cd 0.004, Ti 0.001, Zn 0.30 and Pb 1.01 mg/L (Shenouda *et al.*, 1992).

A stock solution was prepared by dissolving 100 mg of zinc acetate in one liter of distilled water. In order to avoid metal precipitation, 1-2 drops of HCl were added to the stock solution (PH 6.2 ± 0.2). The stock solution was weekly prepared and fresh dilutions were almost prepared for the experiment.

Fishes were acclimated to laboratory conditions for 96 hs and to acidic media for 72 hs before the experiment. A series of four glass aquaria (60 x 30 x 40 cm) were prepared, ten healthy individuals were put in each aquarium. Twenty individuals were exposed to 5.0 ppm zinc solution for 32 days. the remaining number of fishes was kept as control.

After the treatment period (32 days) both of the tested and control fish were dissected, the liver was taken, cut into small pieces, fixed in cold cacodylate-buffered, 3% glutaraldehyde at PH 7.1 for 2 hs, rinsed several times in the buffer, then post fixed in cold 1.0% OsO_4 in 0.1 M cacodylate buffer for 2 hs. Samples were dehydrated in a series of graded ethanols, placed into propylene oxide and embeded in araldite. Ultra thin sections were stained with uranyl acetate and lead citrate prior to examination under the electron microscope (Zeiss E M 9 S2).

RESULTS AND DISCUSSION

The ultrastructure of hepatocytes of the normal fish is characterized by regularly arranged polygonal cells which are subdivided into areas some predominantly containing organelles and others - storage products. A rounded to ovoid nucleus is usually located in the center of the cell. It possesses a prominent nucleolus and small amounts of condensed heterochromatin. Chromatin aggregates occur in certain preferable sites in the nucleus forming certain familiar chromatin pattern. Such pattern of chromatin aggregates may be designated as follows (Fig. 1):

1- Peripheral or marginal chromatin: Irregular-shaped masses of chromatin adjacent to the nuclear envelope, between the nuclear pores. There is a clear area devoid of hetero-chromatin immediately behind the nuclear pores.
2- Chromatin centers: Randomly distributed chromatin aggregates within the nuclear matrix.
3- Nucleolus-associated chromatin : Focal aggregates of chromatin granules along the periphery of the nucleolus.

Although the nucleolus contains mainly protein and RNA, it has been shown that it also contains a small amount of DNA (Miller and Beatty, 1969).

Around the nucleus, an extended layer of organelles are arranged consisting of rough endoplasmic reticulum "rER", mitochondria, peroxisomes and few lysosomes.

The membrane-bound passage of rER was found to be continuous with the perinuclear cisternae and the outer membrane of the nuclear envelope was frequently studded with some polyribosomes. One or two cisternae of the rER may also run along the cell membrane and few short or vesicular cisternae were found in a peribilar position (Fig. 1).

The smooth endoplasmic reticulum "sER" occupies focal areas of cytoplasm, usually accompanied by glycogen rosettes. Because of the abundance of glycogen, the sER was difficult to visualize.

The mitochondria appear as elongated bodies 2.5x1.0 Um.

They contain numerous small cristae and a higher number of electron dense granules. They are always accompanied by membrane of rER.

The lysosomes constitute a group of membrane-bound particles which contain acid hydrolases. These organelles are considered to be the main part of the intracellular digestive system, (sometimes referred to as the vascume or vacuolar apparatus), capable of digesting or degrading a variety of endogenous and exogenous substances (De Duve and Wattiaux, 1966). Among lysosomal elements found in the normal hepatocyte of the fish are small, rounded and single-membrane-bound bodies with electron-dense contents. Larger lysosomes are rare.

Large parts of the hepatocytes are occupied by stored products. Glycogen is deposited in distinct field. The number and size of fat inclusions show individual variations both between the liver parenchymal cells of the same fish or between the liver cells of different individuals.

The exposure of *Oreochromis niloticus* for 32 days to 3.0 ppm zinc solution results in many cytological alterations. Cell size is slightly reduced, the intercellular compartmentation is deteriorated, as evidenced by reduction and sometimes disorganization of rER and by dissolution of the distinct glycogen fields. In general, the most prominent changes are concerned with the nucleus, rER, mitochondria and lysosomes.

Electron microscopic picture of the hepatocytes of the exposed fish shows that they have passed through massive necrosis including pyknosis and, to some degree, karyorrhexis. Pyknosis involves a shrinkage of the nucleus and condensation of the chromatin. In karyorrhexis, the nuclear chromatin is aggregated or clumped into numerous masses, but the nuclear envelope remains reasonably intact.

The present electron microscopic studies have, in fact, focused the attention to a much common and consistent pattern of nuclear change in the necrotic cell, which is spoken off as chromatin margination. Here, a condensation of the chromatin occurs along or adjacent to the inner membrane of the nuclear envelope, while chromatin is highly decreased than other part of the nucleus (Fig. 2). Many authors reported such changes in necrosis resulted from the action of various noxious agents (Ghadially, 1988).

Margination of chromatin appears to be a fairly early change that occurs in the nucleus after irreversible injury leading to cell death. Trump *et al.* (1963 & 1965) studied this phenomenon in slices of mouse liver incubated in a septic condition for various periods of time, prior to fixation.

Parts of the rER appears to be sparsely populated by ribosomes. Since numerous ribosomes are often found lying in the cytoplasm, it looks as if the ribosomes had dropped off the rER into the cytoplasmic matrix (Fig. 2). This phenomenon is called "degranulation" of rER, which is associated with a loss of polyribosome configuration so that solitary ribosomes are seen in the cytoplasmic matrix.

Degranulation of the rER is a distinct morphological change and is accompanied by other morphological alterations, such as dilatation, vesiculation of the rER and disaggregation of polyribosomes. These changes are referred to as disorganization of rER.

Examples of degranulation of the hepatocyte rER suggesting a dropping off of ribosomes were seen in rat liver after ethionine administration (Villa-Trevino *et al.*, 1964; Baglio & Farber, 1965 and Wood, 1965) or carbon tetrachloride administration (Smuckler *et al.*, 1962; Renolds, 1963 and Krishan & Stenger, 1966).

One would expect that changes of the type mentioned above would disturb protein synthesis and there is an ample biochemical evidence that protein synthesis is, in fact, impaired by pollutants which produce this change. For example, it has been shown that after carbon tetrachloride poisoning the synthesis of several export proteins was depressed (Smuckler *et al.*, 1962). The cell fractionation studies showed that this defect was related to polyribosome disaggregation and decreased capacity to incorporate amino acids (Smuckler & Benditt, 1963).

Examination of the mitochondria in hepatocytes of the exposed fish indicates the presence of mitochondrial involution or dissolution in pathological situations. The greater frequency may be looked at as an activation of normal mechanisms geared to the removal of organelles that are damaged by zinc influence. Here, the way in which the mitochondria appeared to be got rid off by the cell is mitochondrial pyknosis (Figs 2&3).

The term "mitochondrial pyknosis",which so succinctly describe this change was first employed by Wachstein & Besen (1964) who demonstrated mitochondrial pyknosis in coagulative necrosis produced in renal tissue with DL-serine.

Many mitochondria have quite large intramitochondrial lipidic inclusions; each is characterized by a rounded or irregular form, an amorphous appearance and medium to high electron density (Figs 2 & 3). Such inclusions have been noted in a great number of hepatocyte mitochondria of ethionine-fed rats by Minick *et al.*, (1965).

The lysosomes of the exposed fish hepatocytes show a significant increase in number and most of them possess altered morphology. They are larger in size and consist of moderately dark matrix containing fibrillar and globular inclusions.

A close relationship between lysosomes and heavy metals was detected by Sternlieb & Goldfischer (1976). Moreover, George (1982) stated that the accumulation of metals in lysosomes upon exposure to environmental pollutants has been documented in a wide variety of aquatic organisms. It was proposed that metals released by lysosomal degradation of metal-binding proteins are detoxified by binding to lipofuscin granules. The granules of lipofuscin represent residual bodies left behind in the cell after lysosomal activity. Lipofuscin accumulation is originated by various influences (Harris, 1966 and Wolman, 1975).

In the ultrathin sections of the present study lipofuscin granules are found as rounded or irregular bodies limited by a single membrane. The contents are variable but,

Delta J. Sci. 16 (3) 1992

mainly they consist of highly electron-dense granular material and one or more medium-dense or lucent lipid droplets (Figs 2 & 3).

The view that lipofuscin granules are lysosomal bodies is supported by many studies which have demonstrated the presence of acid phosphatase and other acid hydrolase in them (Ghadially, 1988).

CONCLUSIONS

Liver tissue is a target organ for heavy metal toxicity in fish (Chernoff & Dooley, 1979). Although some aspects of accumulation kinetics are still under discussion, there is an agreement that the liver shows a significant zinc uptake (Reynolds, 1963; Smuckler & Benditt, 1963; Baglio & Farber, 1965; Trump *et al.*, 1965 and Abou-Zaid *et al.*, 1988 b). Results of the present study fit well into that scheme of zinc accumulation.

After exposure to sublethal concentration of zinc acetate solution for 32 days the hepatocytes of the Nile fish *Oreochromis niloticus* show massive necrosis, this includes pyknosis and, to some degree, karyorrhexis. Chromatin margination is common in exposed liver cells. Degranulation of the rER is a distinct morphological change, it is accompanied by other morphological alterations, such as dilatation and vesiculation of the rER and disaggregation of polyribosomes. These changes are referred to as disorganization of the rER. Mitochondrial pyknosis is frequantly found in the exposed hepatocytes. Lysosomal lipofuscin granules are found as rounded or irregular bodies containing highly electron- dense granular material and one more medium -densy or lucent lipid droplets.

REFERENCES

Abd El-Rahman, S.I.A. (1987): Pathological effects of wastes of some factories on some water fauna in Alexandria M. Sc. Thesis, Fac. Sci. Alex. Univ. Egypt.

Abou-Zaid, F.A.; Elserafy, S. and El-Shourbagi, I.K. (1988 a):

---------------; Salem, S.B. and El-Shourbagi, I.K. (1988 b): The toxic effect of copper and zinc on the liver of *Tilapia nilolica*, as revealed by an acute test. Delta J. Sci., 12 (3): 1167-1194.

Baglio, C. M. and Farber, E. (1965): Reversal by adenine of the ethionine-induced lipid accumulation in the endoplasmic reticulum of the rat liver. J. Cell Biol. 27, 591.

Chernoff, B. and Dooley, J.K. (1979): Heavy metals in relation to the biology of the mummichog, *Fundulus heteroclitus*. J. Fish Biol. 24, 309-328.

De Duve, C. and Wattiaux, R (1966): Function of lysosomes. A. Rev. Physiol. 28-435.

El-Sarha, A. I. (1986): Effect of paired combination of zinc and copper on some physiological parameters of *Clarias lazera*. M.Sc. Thesis Alex. Univ. Egypt.

George, S. G. (1982): Subcellular accumulation and detoxication of metals in aquatic animals. In

Delta J. Sci. 16 (3) 1992

physiologicl Mechanisms of Marine pollutant Toxicity (eds W.B. Vernberg, A. Calabrese, F.P. Thurbery and F.J. Vernberg). pp. 3-52. Academic Press, New Yourk.

Ghadially, F. N. (1988): Ultrastructural pathology of the cell and matrix. 3 rd ed. Butterworths, London.

Harris, C. (1966): A lipofuscin-like pigment in the kidneys of ostrogen-treated rats. Arch. Path. 82, 353.

Hilmy, A. M. and Saied, M. M. (1980): Blood chemistry levels after acute and chronic exposure to HgCl in Killifish, *Aphanius dispar*. Water, air and soil pollution, 14: 34-49.

Hilmy, A. M.; Shabana, M.B. and Daabees, A.Y. (1984): Bioaccumulation of cadmium: Toxicity ***Mugil cephalus***. Comp. Biochem. Physiol. 18: 139-143.

Krishan, A. and Stenger, R.J. (1966): Effects of starvation on the hepatotoxicity of carbon tetrachloride. A light and electron microscopic study. Am. J. Path. 49, 239.

La Roche, G. (1972): Biological effects of short-term exposure to hazardous materials. In Control of Hazardous Material Spill: 199-206.

Mazhar, F. M.; Ashry, M.A. and Kadry, S.M. (1986): Effects of environmental pollution on the histological features of the major organs of the Nil catfish *Clarias lazera*. Proc. Zool. Soc. A.R., Egypt, 12: 75-89.

-- (1987): Effect of environmental pollution by mercury on blood parameters of the Nile catfish *Clarias lazera*.

IBID, 13: 247-256.

Michael, P. (1984): Ecological methods for field and laboratory investigations. Tata McGraw-Hill Pub.Comp. Limit. New-Delhi. pp. 400.

Miller, O.L.Jr. and Beatty, B.R. (1969): Nuclear structure and function. In Handbook of Molecular cytology. Ed. by A. Lima-de-Faria. Amsterdam and London. NorthHolland Publ. Company.

Minick, O.T.; Kent, G.; Orfei, E. and Violin, F.I. (1965): Non-membrane enclosed intramitochondrial dense bodies. Expl. Molec. Path. 4, 311.

Reynolds, E.S. (1963): Liver parenchymal cell injury. 1- Initial alteration of the cell following poisoning with carbon tetrachloride. J.Cell Biol. 19, 139.

Shenouda, Th.S.; Abou-Zaid, F.A.; Al-Assiuty, A.I. and Abada, A.E.(1992): Water pollution and bioaccumulation of the highly pollutant agents in different organs of *Oreochromis niloticus* Near Kafr El-Zayat. Proc. Zool. Soc., A.R.Egypt. (In Press).

Smuckler, E.A. and Benditt, E.P. (1963): Carbon tetrachloride poisoning in rats: Alteration in ribosomes of the liver. Science, 140, 308.

Smuckler, E.A.; Iseri, O.A. and Benditt, E.P. (1962): An intracellular defect in protein synthesis induced by carbon tetrachloride. J.Exp. Med. 116, 55.

Sternlieb, I. and Goldfischer, S. (1976): Heavy metals and lysosomes. In lysosomes in Biologyand Pathology. (J.T. Dingle and R.T. Dean, eds),pp. 185-200.

Amsterdam: North Holland.

Trump, B.F.; Goldblatt, P.J. and Stowell, R.E. (1963): Nuclea and cytoplasmic changes during necrosis in vitro (autolysis): an electron microscopic study. Am.J.Path.43, 23a.

Trump, B.F.; Goldblatt, P.J. and Stowell, R.E. (1965): Studie on necrosis of mouse liver in vitro. Ultrastructura alterations in the mitochondria of hepatic parenchymal cells. Lab. Invest. 14, 343.

Villa-Trevino, S.; Farber, R.; Staehelin, T.; Wettstein, F.O and Noll, H. (1964): Breakdown and reassembly of ra liver ergosomes after administration of ethionine o puromycin. J.Biol. Chem. 239, 3826.

Wachstein, M. and Besen, M. (1964): Electron microscopy of renal coagulative necrosis due to dl-serine, with special reference to mitochondrial pyknosis. Am.J.Path. 44. 383.

Wolman, M. (1975): Biological peroxidation of lipids and membranes. Israel J.Med. Sci. 11 Supplement.

Wood, R.L. (1965): The fine structure of hepatic cells in chronic ethionine poisoning and during recovery. Am.J.Path..46, 307.

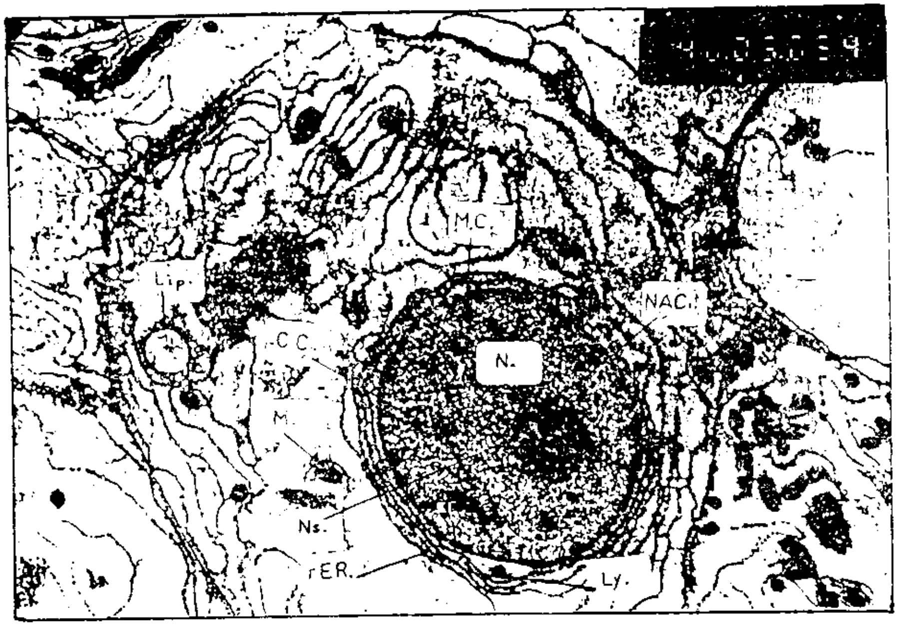

Fig. (1) : The ultrastructure of normal hepatocytes of Oreochromis niloticus , showing the nucleolus (Ns.) inside the nucleus (N.) , nucleolus-associated chromatin (NAC.) , chromatin centers (CC.) , marginal chromatin (MC.), Lysosomes (Ly.) , rough endoplasmic reticulum (rER.), mitochondria (M.) and a limited amount of lipids (Lip.) .

Fig. (2)

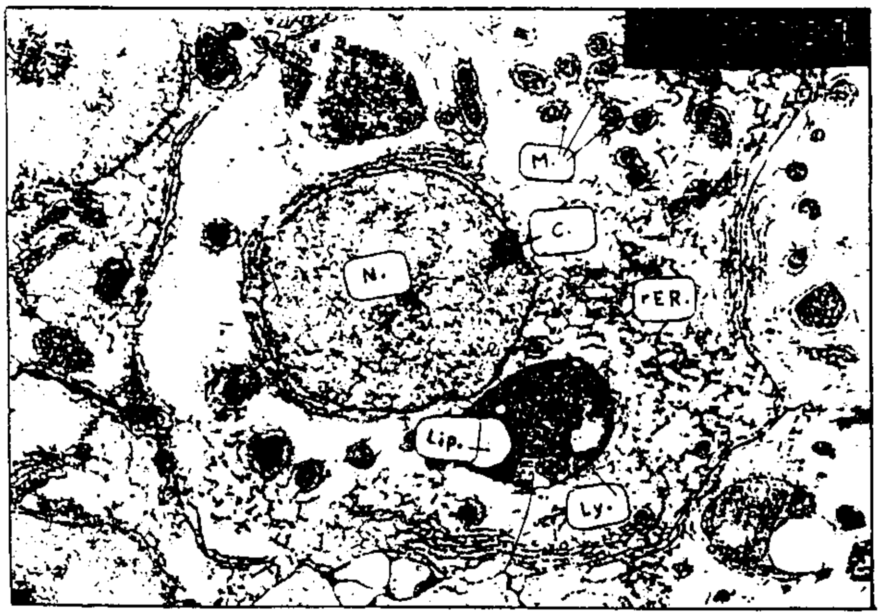

Fig. (3)

Figures (2) and (3) show the ultrastructure of hepatocytes of the exposed Oreochromis niloticus fishes to 3 ppm. zinc solution for 32 days , in which there are : mitochondrial pyknosis (M) , enlarged lysosomes (Ly) , chromatin margination (C) and degranulation of the rough endoplasmic reticulum (rER) .

Delta J. Sci. 16 (3) 1992

مشاهدات بالميكروسكوب الإلكترونى عن التأثيرات السامه للزنك على الكبد فى أسماك البلطى النيلى

* فؤاد عفيفى أبو زيد
** ثروت صادق شنوده أحمد السيد عبادة
* كلية العلوم - طنطا - جامعة طنطا
** كلية التربيه - كفر الشيخ - جامعة طنطا

بعد تعرض أسماك البلطى النيلى لمحلول من خلات الزنك بتركيز ٣ أجزاء فى المليون لمدة ٣٢ يوما تبين حدوث الكثير من التغيرات فى تركيب الخليه الكبديه ٠

أهم هذه التغيرات كانت تخص النواه الشبكه الكروماتينيه المحبيه ؛ الميوكوندريا والليبوسومات ٠ فقد حدث إنكماش فى حجم النواه وتجمعت كميات من الكروماتين بداخلها فى كتل ثم ترسبت بالقرب من السطح الداخلى لغلافها - كما تفككت بعض أجزاء من الشبكه الكروماتينيه وتناثرت حبيبات الرايبوسوم فى سيتوبلازم الخليه ٠

وبالإضافة إلى ذلك ؛ فقد تفتتت ودمرت أعداد كبيرة من الميتوكوندريا وتضخمت الليسوسومات وإزداد عددها بطريقة واضحه وإحتوت بداخلها على بعض الألياف والحبيبات الدهنيه ٠