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CARDIAC STRUCTURE IN CHRONICALLY STRESSED *OREOCHROMIS NILOTICUS*

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ABSTRACT

After the determination of the 96-hour LC_{50} of lead on one-month-old *Oreochromis niloticus*, the fish were exposed to sublethal concentration of 8 mgs/L lead for 30 days.

The heart of juvenile *O. niloticus*, divided into four regions - bulbus arteriosus, ventricle, atrium and sinus venosus, has a wall of three tissue layers - endocardium, myocardium and epicardium. Comparing the heart of the control and that of the treated fish, the histoarchitecture shows few changes after lead treatment. There is slight degeneration in the muscle fibers in the myocardium.

This study shows that lethality in chronically stressed juvenile *O. niloticus* exposed to sublethal lead for one month is not probably due to heart defect.

Introduction

There is worldwide concern over the impact of environmental pollutants on health and environment. These pollutants have in time progressed to take various forms such as smog toxic chemicals, mutagens and deposition products, radiation, etc. - and seeped through the air, water and soil, and undermined the biota - resulting in an imbalance in the normal activity of the ecosystem.

Heavy metals enter the environment via a variety of routes by (1) surface runoff from rain and (2) waste discharge from sewerage and industrial plants (59).

Industrial effluents discharged into aquatic ecosystem have created problems in water pollution. Heavy metals are among the chemicals present in the effluent that contribute to the problem. Of these heavy metals, lead is the most abundant.

Lead has been recognized as a highly toxic cumulative element in man and animals. In industrialized nations during recent years, long-time exposure of ani-

mals to air-borne lead has been recognized as a health hazard. It has been found to have adverse effects on biochemical and histological conditions (60).

Lead is included in a range of elements which have been called "heavy metals" and "trace metals" alternatively. As part of these trace metals, they enter the ocean and other waters as a result of natural processes and human activities via rivers, land run-off, dumping, the atmosphere and the sea bed. Major natural sources are weathering, degrassing, releases from terrestrial and submarine volcanoes and dissolution of marine sediments. The dominant inputs for most trace metals are through river and land run-off but for a few elements, such as lead and mercury, the atmospheric route is also significant especially in the open ocean. Many industries release trace metals which reach the sea through a variety of routes. Generally, trace metals are discharged together with other wastes, such as sewage detergents and other inorganics. Interaction with these wastes and various components of the sea water alters the original physico-chemical forms of these trace metals. Lead in soluble ion is both mobile and toxic.

It has been established that plants and animals require a variety of elements for their growth and development; however, lead is not one of these. Humans have no way of alienating themselves from these unwanted lead. They are constantly exposed to various forms of products which contain lead. It is present in can solders, in lead sheets used in walls of buildings, in nuclear reactor shielding, in radioactive material containers, in leaded interior paints, in lead shots or ammunition used by duck-hunters, in lead arsenate sprays used in insecticides, and in tobaccos. Its oxides are used in making glass, vitreous enamels in dyes, insecticides and in vulcanizing rubber. It may be used in roofings, water pipes and telephone and telegraph cables. Of course, a great quantity of lead is released by industrial lead smelters and by automobiles, which use lead as an anti-knock additive in gasoline. There seems to be no escape from lead exposure.

The effects of heavy metals should not be studied only in terms of acute toxicity, but also insofar as they affect natural life processes at sublethal levels, just like in this study; as such, levels are commonly encountered in polluted water (62). Sublethal effects of a toxicant have been defined as long-term biological effects on organisms as a result of some manmade changes in the environment which may not necessarily cause death but whose effects may cause some alteration of biological process(es) which would lead to the inability of the organism or their offspring to function normally. Such changes could be the prevention of feeding, a change in behavior which could block some physiological processes, inhibition of reproduction, or the alteration of the ecosystem in such a way that the organism could no longer live there (37, 43, 53, 75).

Lead LC_{50} value in fishes differs between species. The reported 96-hour LC_{50} value ranges from less than seven to about 19 mgs/L lead nitrate (63, 64, 71). Lead-induced toxicity of fishes is more severe in chronic treatment than in acute treatment (64, 63). Sensitivity of fish to lead toxicity has been shown to be affected by age: egg and larval stages being more sensitive than the fingerling stage (32). Furthermore,

lead exposure characterized by wide fluctuations causes greater lead uptake by fishes than the equivalent exposure with less variability (33).

Several studies have been done to assess the histophysiological effects on the development of organs of animals, especially vertebrates.

Lead was found to have deleterious effects on the reproductive organs, causing decrease in reproductive potential (13, 21, 41, 48, 52, 56). Kumar and Pant (1984) showed dilatation in the testicular blood capillaries with necrosis and disintegration of the semeniferous tubules and atresia in the ovary as a result of lead exposure in the fish, *Puntius chonchonius*. This suggests direct action of lead on the gonads of teleost. Renal lesions had also been observed as the primary stress response of the kidney to lead toxicity (4, 8, 15, 18, 30, 36, 44, 60).

On hemopoeitic organs, the following reported destructive effects of lead such as decrease in cell values due to cell death (21, 39, 44, 66, 67, 74).

A research study (35) on primary cultured astrocytes and cerebellar granular neurons of rats using transmission electron microscopy showed that lead is concentrated in nuclear, cytoplasmic and lysosomal inclusions of astrocytes, while the neurons showed lead densities only in the lysosomes. With acute lead exposure, inhibition of maximum respiratory capacity was greater and occurred in lower lead concentration in nuerons than in astrocytes. Similarly, rates were inhibited at lower lead concentration from an eight-day-old pup compared to those of adults. These proved that the in vitro system exhibited responses to lead. In both cases inhibition of energy metabolism is associated with cell damage. They proposed that the capacity of the astrocytes to sequester lead in non-mitochondrial intracellular sites may be critical in the resistance to lead in the mature brain.

Other pathological changes have been reported by several workers (50, 54, 55, 58, 70). Renal biopsies from workers who had been chronically exposed to lead showed three types of changes found in the nuclei: (1) lead-induced bodies, (2) clumped granular chromatin and (3) pseudoinclusion or nuclear invagination of cytoplasmic content. Mitochondria in tubular lining cells showed some degree of swelling and distortion of cristae. Endoplasmic reticulum was swollen and in some cells increased in amount. Lysosomes were numerous and contained dense bodies of varying sizes (21).

Similar destructive effects in several species are reported by some authors (39, 54, 52, 56, 73, 75). Renal atrophy is the common observation.

Fish gill structural changes induced by heavy metals have been studied intensively (10, 16, 17, 41). Frequently recorded histopathological lesions include changes in gill epithelium (lifting, hyperplasia, hypertrophy, rupture), bulbing or fusion of gill lamellae, hypersecretion and proliferation of mucocytes and changes in chloride cells and gill vasculature (48, 51, 52, 56).

The digestive system has been studied and analyzed in several studies (4, 11, 22, 26, 28). All other organ systems have been analyzed and among the most significant are studies by various histologists (46, 42, 66, 69, 70). In bone metaphyses, multinucleate cells are abundant.

Related lead-induced physiological alterations have been reported. Protein metabolism in fishes is altered by lead exposure. This has been demonstrated by earlier studies of lead toxicity. Sasty and Gupta (1979) reported inhibition in the activities of these peptidase (aminopeptidase, glycyl-glycine and leucyl-1-glycine dipeptidases) in the digestive system of fish *Channa punctatus*. In the same year, lead-induced reduction in glycogen content in liver, kidney, and brain of fishes was demonstrated (68).

Most often, lead causes significant inhibition of enzyme in the target tissue. Most documented of these enzyme inhibitions is that of delta-aminolevulinic acid dehydratase (delta ALAD) (14, 31, 43, 46, 47). Lead is known to inhibit hemoglobin biosynthesis and shorten the survival of red blood cells. This is because lead strongly inhibits the activity of ALAD, thereby, blocking the formation of porphobilinogen from amino-levulinic acid (74). In fishes, the sensitivity of ALAD to lead poisoning has been demonstrated in the erythrocyte, spleen and renal tissue of rainbow trout, *Salmo gairdneri* (38, 29, 14, 43, 12, 28).

An earlier study has shown that alkaline phosphatase, a key enzyme in the reabsorption of glucose from renal tubules in kidney of the freshwater fish, *Heteropneustus fossilis*, is inhibited by lead (63). This demonstrates an adverse effect on glucose reabsorption and transphosphorylation. Several workers have also shown changes in the cholesterol levels in tissue of fishes exposed to toxic levels of lead. Lately, one study (39) reported a decrease in the cholesterol level in the brain, testis and ovary while the liver showed an elevation in both cholesterol and lipid levels in the teleost *Clarias batrachus* exposed to toxic levels of lead. However, a more recent study (74) revealed a decrease in cholesterol in blood and tissues of *Barbus conchinus* when subjected to the same toxicant. Since cholesterol is an important constituent of the cell membranes and a precursor of steroid hormones, lead-induced changes on this material may be related to either a disruption of plasma membranes and/or altered steroidogenesis (72).

More recent studies on lead-induced physiological alterations include altered immunological response specifically reduced humoral antibody titer (56, 55, 42, 43, 44).

In this study, the effects of chronic lead treatment on cardiac structure of *Oreochromis niloticus* is assessed. This is the first study on the effect of lead on cardiac histology in this species.

Materials and Methods

Oreochromis niloticus were procured from the Bureau of Fisheries and Aquatic Resources, Tanay, Rizal. The juvenile *Oreochromis* were allowed to acclimate for one week before treatment.

LC_{50} was first determined to get the sublethal level for chronic treatment. Experiments were done in glass aquaria for 8 mgs. L^{-} and a control. Water was changed every two days. Test specimens were harvested on the thirtieth day.

Organs were dissected out and immediately fixed in glutaraldehyde in phosphate buffer, pH 7.2, followed by post fixation in 1% osmium tetroxide, the samples were dehydrated in ethanol and propylene oxide, embedded in Araldit, sectioned 1-2 u thick with ultatome, stained with 1% toluidine blue, lead citrate and uranyl acetate and examined under light and electron microscope.

Results and Discussion

Figure 1 shows a diagram of the sagittal section of the heart of *O. niloticus*. Figure 2, the control bulbus arteriosus, is composed of elastic connective tissue and muscle fibers. The thin epicardium covers the thick myocardium of dense bundles. Figure 3 is the treated bulbus arteriosus with the histoarchitecture intact.

Figure 4 shows the control ventricle. The thick myocardium has an outer corticalis and inner spongiosa (3). The outer corticalis consists of densely packed myocardial fibers. The inner spongiosa layer is a network of anastomosing cardiac muscle bundles lined by thin endocardial layer. Thin epicardium is securely attached and covers the entire surface of the ventricular wall.

In the treated ventricle, slightly fewer muscle bundles are found in the myocardium (Figure 5).

The atrium of the control heart is shown in Figure 6. The myocardium is a very thin layer of loose cardiac muscle bundles closely connected to the epicardium. Figure 7 is the treated atrium that still looks essentially the same as the untreated heart.

Electromicrographs of untreated and treated myocardial fibers of the ventricle are presented in Figures 8 and 9.

The primitive heart tube surrounded by unfused splanchnic mesoderm cells develops into the four-heart regions with distinct endocardium, myocardium and epicardium. Rather than fusing into syncytium, the cardiac cells form complex junctions. The heart of the juvenile *Oreochromis niloticus* consists of tightly knit bundles of interwoven fibers (Fig. 8). The cross-striated banding pattern is identical to that of skeletal muscle but each cardiac muscle cell has only one centrally located nucleus. Surrounding the muscle cells is a delicate covering of connective tissue.

Intercalated disks representing the junctional complexes are found at the interface between adjacent cells. The structure of the proteins in the cardiac cells is virtually similar to that in skeletal muscle (Figure 8). The presence of several mitochondria reflects the need for continuous aerobic metabolism in the cardiac muscles.

In other fish species, petroleum compounds (crude oils) chemically induced degeneration of the ventricular myocardium of marine teleost, *Memidis menidia* (1). Hypotonicity in the heart of *Cichlosoma nigrofasciatum* concomitant with poor circulation and hemostatis was a result of intraperitoneal toxicity of lead (4).

In a study on the effect of mercury on tissue proteins, it was found that the levels of total, structural and soluble proteins in muscle tissues decrease significantly (5). The decline in muscle proteins implies intensive proteolysis which contributes to the increase of free amino acids that are fed to the tricarboxylic acid (TCA) as keto acids for energy regeneration.

In the heart, continuous and intensive proteolysis of the structural and soluble proteins of the cardiac muscle result in the disintegration of myocardial fibers. Degeneration of the corticalis results in the lifting off of the epicardium since it cannot securely attach itself to the loose fibers of the corticalis.

Another possible explanation for the slight degeneration of myocardial fibers is the competitive inhibition of Na, K-ATPase by lead. This enzyme is responsible for maintaining the ionic gradient within and without the cell. The inactivation of the functioning of the cell membrane is manifested as change in the architecture of the tissue (2).

The study shows that chronically stressed *O. niloticus* exposed to 8 mgs. L^{-} lead for one month does not have major heart defects. Lethality, therefore, is not probably due to cardiac defect. Abnormalities found in other organs are for worse than changes observed in the heart.

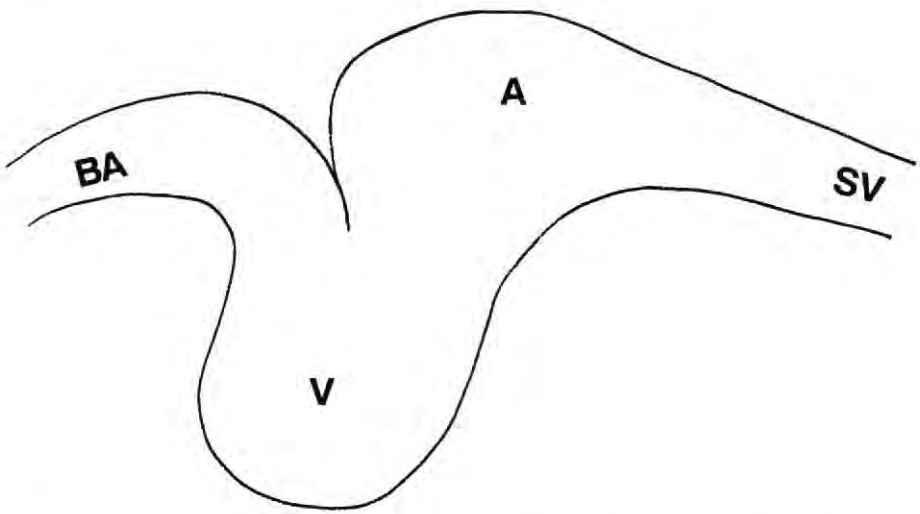

Figure 1. A diagram of the sagittal section of the heart of juvenile *O. niloticus*. BA-bulbus arteriosus, V-ventricle, A-strium, SV-sinus venosus.

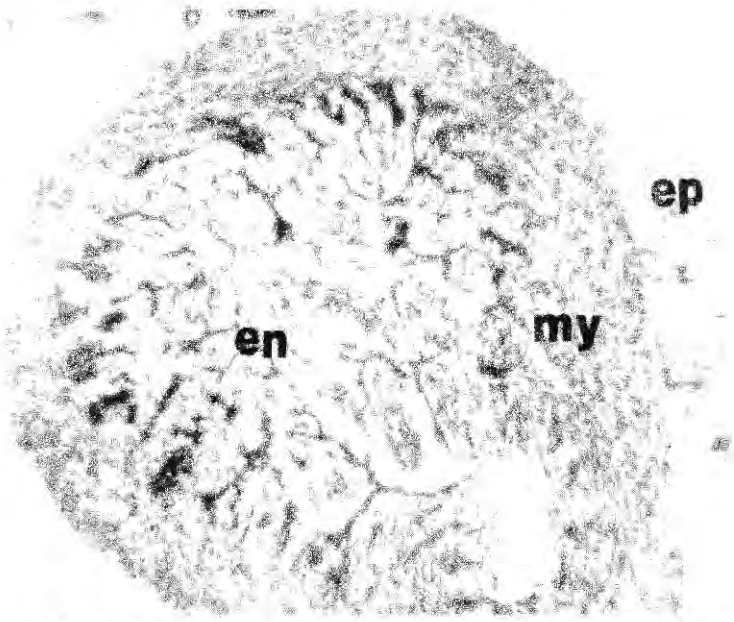

Figure 2. The control bulbus arteriosus has elastic connective tissue and smooth muscle fibers. Ep- epicardium., My- myocardium. en- endocardium. X100

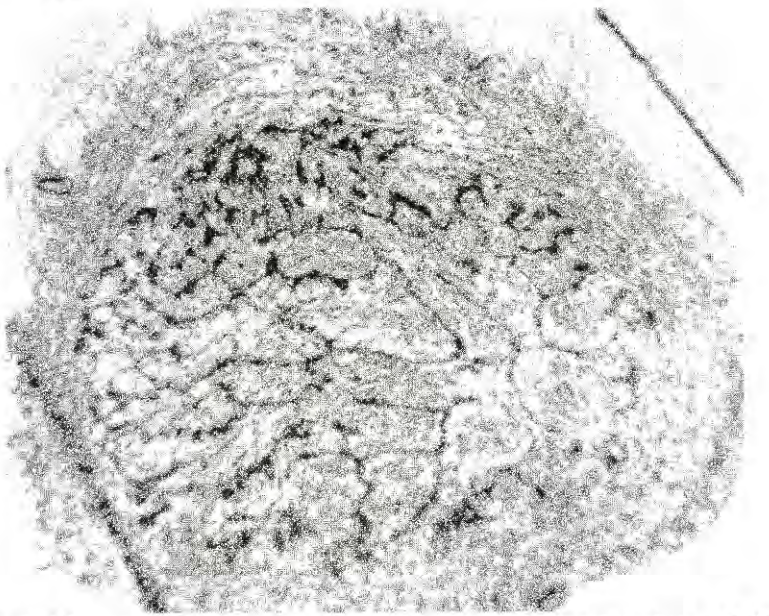

Figure 3. The treated bulbus arteriosus with intact histoarchitecture. X100

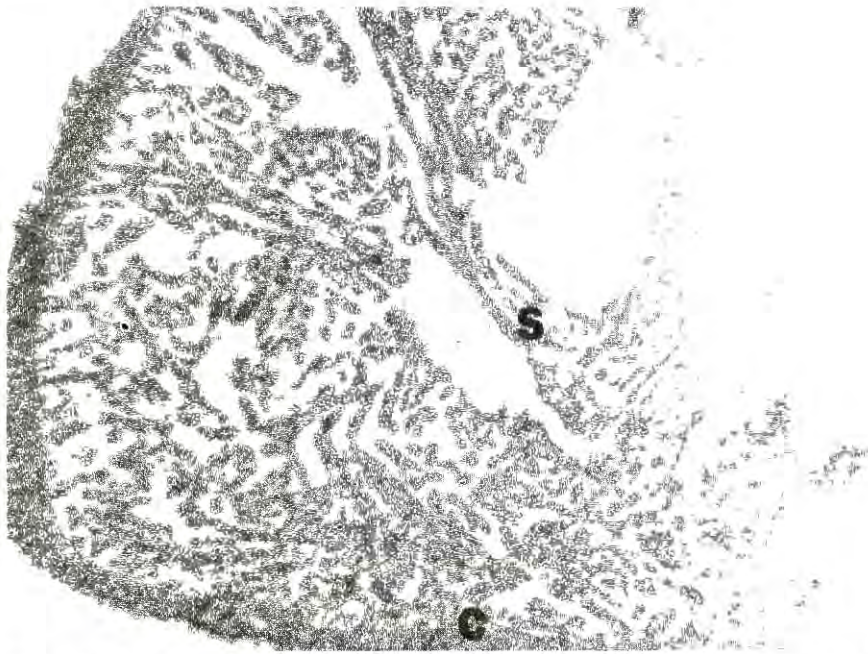

Figure 4. The normal ventricle shows thick myocardium of outer C- corticalis and S- inner apongiosa. X100

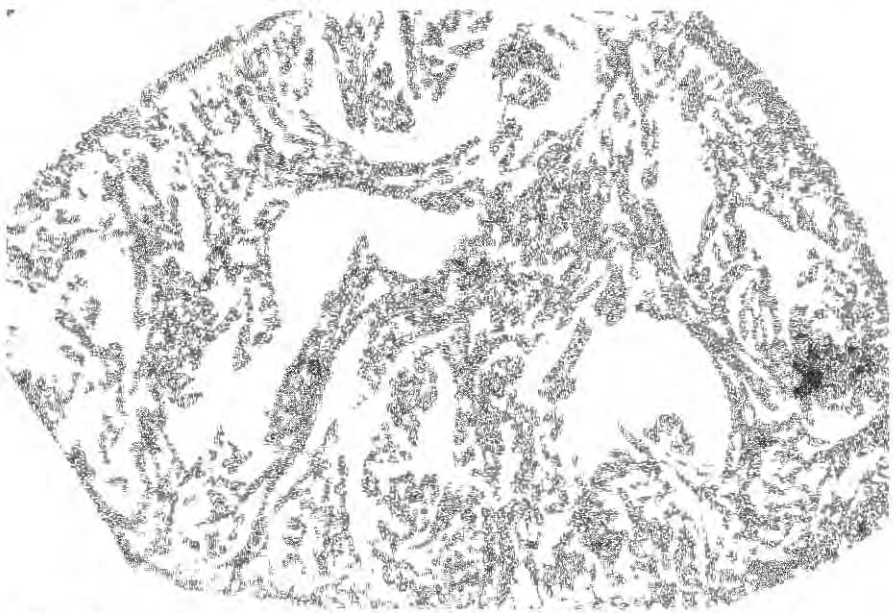

Figure 5. The treated ventricle with slightly fewer muscle fibers. X100

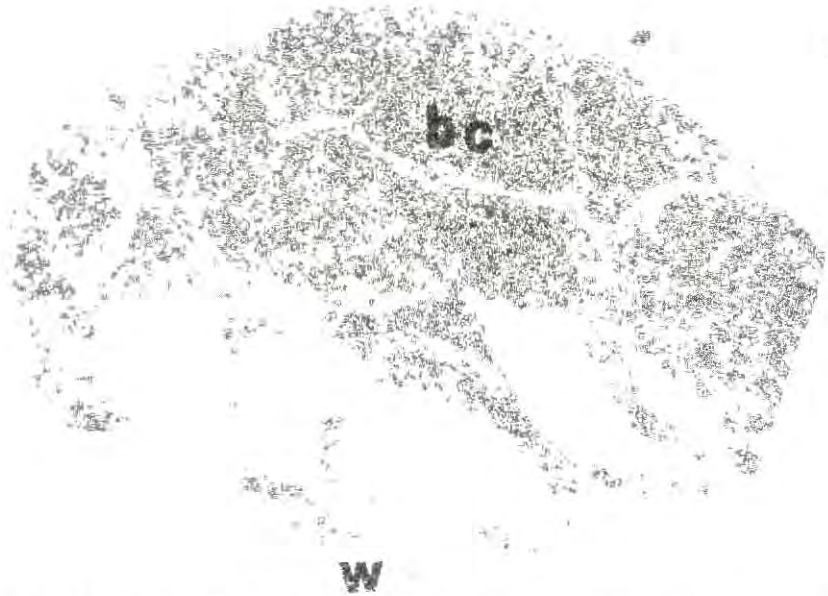

Figure 6. The control atrium with very thin layer of loose cardiac muscle. bc- blood cells, w- heart wall. X100

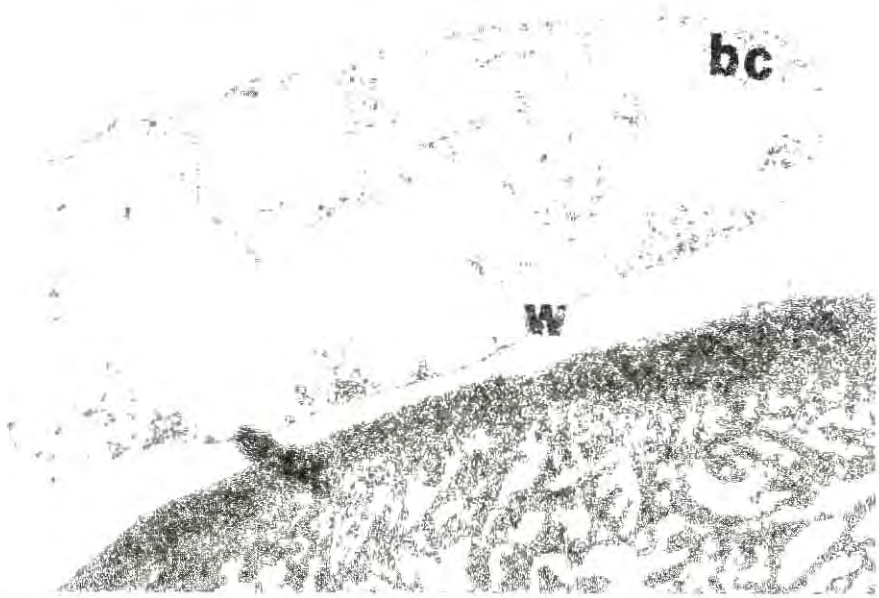

Figure 7. The treated atrium looks essentially similar to the control. bc- blood cells, w- heart wall. X100

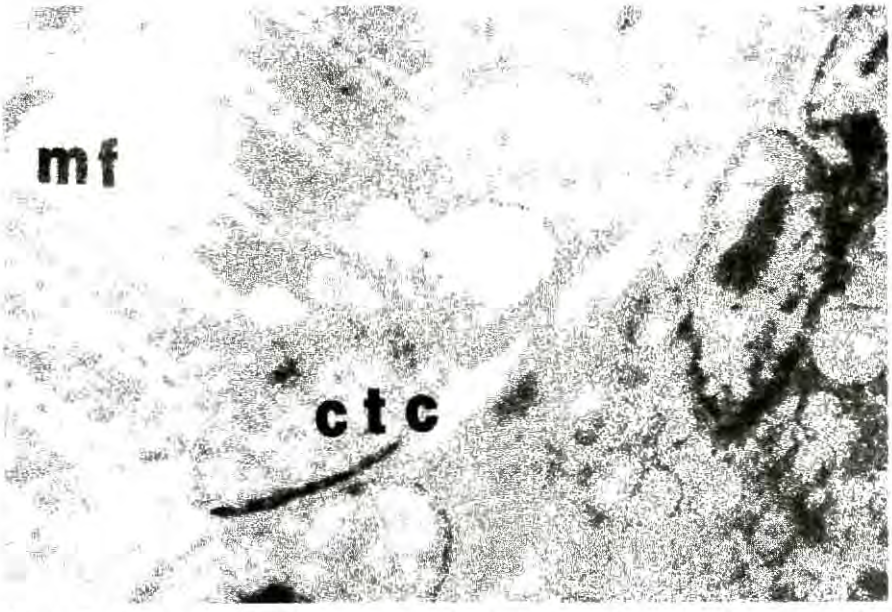

Figure 8. Low-power electronmicrograph of untreated ventricular myocardial fibers, CTC- connective cells, MF- myofilaments. X4,000

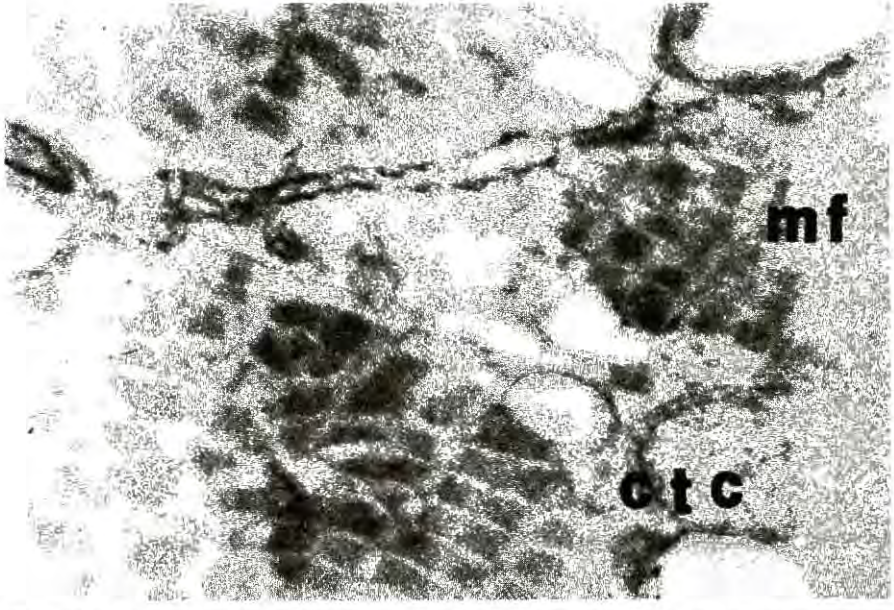

Figure 9. Low-power electronmicrograph of treated ventricular myocardial fibers. CTC-connective tissue cells, MF- myofilaments. X4,000

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Literature Cited

1. Altmann, L., H. Lohmann and H. Wiegand. 1988. Acute lead exposure transiently inhibits hippocampal neuronal activities in vitro. Brain Res. **455** (2): 254-261.
2. Angle, C. R. and M. S. McIntire. 1979. Air-borne lead and children: The Omaha study. Toxicol. Appl. Pharmacol. **48** (1): A178.
3. _____, S. J. Stohs, M. S. McIntire, M. S. Swanson and K.S. Rovang. 1980. Lead-induced accumulation of erythrocyte pyrimidine nucleotides in rabbit. Toxicol. Appl. Pharmacol. **54** (1): 161-167.
4. Antonio. G., M. Teresa and I. C. Vasquez. 1988. Effect of atmospheric lead upon the livers and kidneys of pigeons from the city of Madrid (Spain). Environ. Toxicol. Lett. **9** (3): 227-238.
5. Araki, S., H. Aono, and K. Murata. 1986. Adjustment of urinary concentration to urinary volume in relation to the erythrocyte and plasma concentration: An evaluation of urinary heavy metal and organic substances. Arch. Environ. Health. **41** (3): 171-177.
6. _____, T. Honma, S. Yanagihara and K. Ushio. 1980. Recovery of slowed nerve conduction velocity in lead-exposed workers. Int. Arch. Occup. Environ. Health **46** (2): 151-158.
7. Arnvig, E., Randjean and J. Beckmann. 1980. Neurotoxic effect of heavy lead exposure determine with psychological tests. Toxicol. Lett. (Amst.), 5 (6): 399-404.
8. Avia, A., E. John, J. Bernstein, D. I. Goldsmith and A. Spitzer. 1980. Lead intoxication during development: Its late effect on kidney function and blood pressure. Kidney Int. **17** (4): 430-437.
9. Bayle, P., F. Dhermain and G. Keck. 1986. Three cases of lead poisoning in the greater flamingo *(Phoenicopterus ruter)* in the Marseilles region (France). Bull. Soc. Linn. Provence **38** (0): 95-98.
10. Bengeri, K. V. and H. S. Patil. 1987. Histopathological changes in the gill of *Puntius arulius* induced by lead. J. Anim. Morphol. Physiol. **34** (1/2): 113-116.
11. Bengeri, K. V. and H. S. Patil. 1986. Lead induced histological changes in the liver of *Puntius arulius*. J. Anim. Morphol. Physiol. **33** (1/2): 147-150.
12. Ranica, M. and Z. Konrad. 1980. Some morphological and biochemical haematological parameters of abnormal lead absorption in fish. In Lead in the Marine Environment. Pergamon Press: Oxford, pp. 263-270.
13. Bronish, H., E. Glusa and G. Nowal. 1987. Investigations on the effects of experimental lead intoxication. Z. Grsamte Hyg. Grenger. **33** (9): 430-439.
14. Canpvas, M. 1984. Effect of concentration of Pb^{2+} ion on the enzyme (d-ALAD) activity in *Mugil auratus*. Bol. Inst. Esp. Ovangh. **1** (2): 155-156.
15. Cramer, K. R. A. Goyer, K. Vageburg and M. Wilson. 1974. Renal ultrastructure, renal function and parameter of lead toxicity in workers with period of lead exposure. Brit. J. Indust. Med. 31: 113-127.
16. Crespo, S. 1982. Surface morphology of dogfish *(Scyliorhinus canicula)* gill epithelium, and surface morphological changes following treatment with zinc sulfate: A scanning electron microcopy. 67: 159-166.
17. _____ and K. J. Karnaky. 1983. Copper and zinc inhibit chloride transport across the opercular epithelium of seawater adopted killfish, *Fundulus heteroclitus*. J. Exp. Biol. 102: 337-341.
18. _____, F. Soriano, C. Sampera and J. Balasch. 1981. Zinc. and copper distribution in excretory organs of the dogfish, *Scyliorhinus canicula* and chloride cell response following treatment with zinc sulphate. Marine Biol. 65: 117-123.

19. Chowdhury, A. Roy, R. V. Rao, A. K. Gautam and S. K. Kasayap. 1987. Functional changes of testes in lead intoxicated rats. Ind. Health **25** (20): 55-62.
20. Cholewa, M., A. L. Hanson, K. W. Jones, W. P. McNally and I. Fand. 1986. Regional distribution of lead in the brains of lead intoxicated rats. Neuro. Toxicology (Little Rock). 7 (1): 9-18.
21. Coughlan, D. J., S. P. Gloss and J. Kubota. 1986. Acute and subchronic toxicity of lead to the early life stages of smallmouth bass *(Micopterus dolomieui)*. Water Air Soil Pollut. **28** (3/4): 265-276.
22. Crespo, S., G. Nonette, D. A. Colin, C. Leray, L. Nonette and A. Aubree. 1986. Morphological and functional alterations induced in trout intestine by dietary cadmium and lead. J. Fish. Biol. **28** (1): 64-80.
23. Cupo, M. A. and W. E. Donaldson. 1988. Effects of lead and niacin on growth and serotonin metabolism in chicks. J. Nutr. **118** (1): 107-113.
24. Donald, J. M., M. G. Cutlernad and M. R. Moore. 1987. Effects of lead in the laboratory mouse: Development and social behavior after lifelong exposure to 12 micromolar lead in drinking fluid. Neuropharmacology **20** (4): 391-399.
25. Egle, P. M., and K. P. Shelton. 1986. Chronic lead intoxication causes a brain-specific nuclear protein to accumulate in the nuclei of cells lining kidney tubules. J. Biol. Chem. **261** (5): 2294-2298.
26. Gabor, S., V. M. Magna and S. Vladov. 1987. Lead poisoning of mallards. *Anas platyrynchos*, caused by lead shot. Magy Allatov Lapja. **42** (10): 621-626.
27. Gautam, A. K. and A. R. Chowdhury. 1987. Effects of lead on erythropoietic system of intact and splenectomized rats. Indian J. Physiol. Pharmacol. **31** (2): 117-124.
28. Haux, C., A. Larsson, G. Lither and M. Skobeck. 1986. A field study of physiological effects on fish in lead contaminated lakes. Environ. Toxicol. Chem. **5** (3): 283-288.
29. Helmy, M. M., A. E. Lemke, P. G. Jacob and Y. Y. Al-Sultan. 1979. Hematological changes in Kuwait mullet, *Liza macrolepsis* (Smith), induced by heavy metals. Indian J. Mar. Sci. **8** (4): 278-281.
30. Herbertson, B. M., A. J. King and J. Allen. 1987. Epithelial cell proliferation in the rat urinary system induced by parenteral injection of lead salts. Br. J. Exp. Pathol. **68** (2): 167-178.
31. Hodson, P. V. B. R. Blunt and D. J. Spry. 1978. Chronic toxicity of water-borne and dietary lead to rainbow trout *(Salmo gairdneri)* in Lake Ontario water. Water Res. **12** (11): 869-878.
32. Hodson, P. V., E. R. Blunt, U Borginan, C. K. Minne and S. McGaw. 1983. Effect of fluctuating lead exposures on lead accumulations by rainbow trout *(Salmo gairdneri)*. Environ. Toxicol. Chem. **2** (2): 235-238.
33. Hodson, P. V. E. R. Blunt, D. Jensen and S. Morgan. 1979. Effect of fish age on predicted chronic toxicity of lead to rainbow trout in Lake Ontario water. J. Great Lakes Res. **5** (1): 84-89.
34. _____, J. W. Hilton, B. R. Blunt and S. V. Slinger. 1980. Effects of dietary ascorbic acid in chronic lead toxicity to young rainbow trout. Can. J. Fish. Aquat. Sci. **37** (2): 170-176.
35. Hozman, D., J. E. Olson, C. Devries and K. Benson. 1987. Lead toxicity in primary cultured cerebral astrocytes and cerebellar granular neurons. Toxicol Appl. Pharmacol **89** (2): 211-225.
36. Imai, J. 1987. Toxic effects on lead chromate exposure on renal tubules. Acta Sch. Med. Univ. Sifu. **35** (4): 620-628.
37. Ivanitskii, A. M., K. P. Stasenkova, A. B. Sokolov, V. A. Konyshev, N. B. Maganova, I. N. Gazdarova, T. Z. Rysina and L. M. Piskun. 1985. Study of the toxic action of lead after uptake of food in model experiments. Vopr. Pitan. **0** (2): 63-66.
38. Johansson-Sjobeck, M. L. and A. Larson, 1979. Effects of organic lead on delta aminolevulinic acid dehydratase activity and haematological variables in rainbow trout, *Salmo gairdneri*. Arch. Environ. Contam. Toxicol. **8** (4): 419-431.
39. Katti, S. R. and A. G. Sathyanesan. 1983. Lead nitrate induced changes in lipid and cholesterol levels in the freshwater fish *Clarias batrachus*. Toxicol. Lett. **19** (1-2): 93-96.
40. _____, 1984. Effect of lead, cadmium and combination on the ascorbic acid content of the brain, liver and ovary of the fish *Clarias batrachus*. Environ. Evol. **3** (4): 596-598.
41. _____, 1985. Chronic effects of lead and cadmium on the testis of the catfish, *Clarias batrachus*. Environ. Toxicol. **3** (4): 596-598.

42. _____. 1986. Lead nitrate induced changes in the brain constituents of freshwater fish, *Clarias batrachus*. Neurotoxicol. 7 (3): 47-52.
43. Mrajnovic-Ozretic M., and B. Ozretic. 1980. The ALAD activity test in lead exposed grey mullet, *Mugil auratus*. Mar. Ecol. **3** (3): 187-191.
44. Kramer, H. J., H. C. Gonick and B. Lu. 1986. In vitro inhibition of sodium, potassium ATPase by trace metals: Relation to renal and cardiovascular damage. Nephron. **44** (4): 329-336.
45. Kim, M. and K. Cho. 1986. Metabolic changes in growing rats fed diets with different levels of lead and protein. Korean J. Nutri. **19** (5): 323-332.
46. Kitchen, I. and J. McDowell. 1985. Impairment of ketocyclazocine antinociception in rats by perinatal lead exposure. Toxicol. Lett. (Amst.). **26** (2/3): 101-106.
47. Mucharz, E. J. and B. Stawiarska-Pieta. 1986. The effects of lead on protein and lactate dehydrogenase activities in hepatic sliced cultured in vitro. Arch. Hig. Rad. Toksicol. **37** (2): 225-230.
48. Kumar, S. and S. C. Kant. 1984. Comparative effects of the sublethal poisoning of Zn, Cu and Pb on the gonads of the teleost *Puntius conchonius* Ham. Toxicol. Lett. 23 (2): 184-194.
49. Liu, W. K. and M. H. Wong. 1986. Ultrastructural changes in gills of *Sarotherodon mossambicus* treated with chicken manure. Environ. Res. **30** (1): 164-171.
50. Lorton, D. and W. J. Anderson. 1986. Altered pyramidal cell dendritic development in the motor cortex of lead intoxicated neonatal rats: A Golgi study. Neurobehav. Toxicol. Teratol. **8** (1): 45-50.
51. Mallatt, J. 1985. Fish gill structural changes induced by toxicants and other irritants: A statistical review. Can. J. Fish. Aquat. Sci. 42: 630-648.
52. Marn, C. M., R. E. Mirarchi and M. E. Lisano. 1988. Effects of diet and cold exposure on captive female mourning doves dosed with lead shot. Arch. Environ. Contam. Toxicol. **17** : 589-594.
53. Mautino, M. and J. U. Bell. 1986. Experimental lead toxicity in the ring-necked duck. Environ. Res. **41** (2): 538-545.
54. Maxwell, K., H.V. Vinters, J. A. Berlinger, J. V. Beady and P. A. Cancilla. 1986. Effects of inorganic lead on some functions of the cerebral microvessel endothelium. Toxicol. Appl. Pharmacol. **84** (2): 389-399.
55. Oskarsson, A., L. Olson, M. R. Palmer, B. Lind, H. Bjork-Lund and B. Hoffer. 1986. Increased lead concentration in brain and potentiation of lead-induced neuronal depression in rats after combined treatment with lead and disulfiram. Environ. Res. **41** (2): 623-632.
56. Perez-Coll, S. 1988. Embryotoxicity of lead on *Bufo arenarus*. Bull. Environ. Contam. Toxicol. 41: 241-252.
67. Primor, N., R. Sabnay, V. Lavie and E. Zentkin. 1980. Toxicity to fish, effects on gill ATPase and gill ultrastural changes induced by pardachirus secretion and its derived toxin pardaxin. J. Expt. Zool. 211: 33-43.
58. Pond, W. G., J. T. Yen and L. Krook. 1985. Responses of rat to dietary lead in the presence or absence of natural or synthetic zeolites. Nutr. Rep. Int. **32** (4): 815-826.
59. Reish, D. and R. Carr. 1978. Effects of heavy metal on survival, reproduction, development and life cycles. Marine Pollution Bull. **9** (1): 24-27.
60. Russo, M. A., S. C. Kapoor and G. D. V. van Rossum. 1988. Localization of lead in the kidney and liver of rats treated in vivo with lead acetate: Ultrastructural studies of unstained sections. Br. J. Exp. Pathol. **69** (2): 221-234.
61. Ryden, E. B. and C. T. Walsh. 1987. The effects of lead on cholinergic contractile function in the rat forestomach. Toxicol. **45** (1): 65-78.
62. Saliba, L. and R. Kryz. 1976. Effect of heavy metal on hatching of egg. Marine Pollut. Bull. **9** (10): 181-182.
63. Sastry K. V. and M. K. Agrawal. 1979. Effects of lead nitrate on the activities of a few enzymes in the kidney and ovary of *Heteropneustes fossilis*. Bull. Environ. Contam. Toxicol. **22** (1-2): 55-59.
64. _____ and A. K. Gupta. 1988. Alterations in the activities of a few dehydrogenesis in the digestive system of the teleost fishes exposed to lead nitrate. Ecotoxicol. Environ. Saf. **4** (3): 232-239.

65. Satchell, G. H. 1984. Respiratory toxicology of fishes. Aquatic Toxicol. 2: 1-50.
66. Savic, G., S. Zivkovic and I. Hajraktari. 1987. Cytogenetic effect of lead nitrate on mice *(Mus musculus)* bone marrow cells. Acta Biol. Med. Exp. **12** (1): 39-40.
67. Schmidt. P. F., R. R. Lehmann, K. Ilsemann and A. H. Wilhelm. 1985. Distribution patterns of lead in the aortic wall determined by lase microprobe mass analysis. Artery **12** (5): 277-285.
68. Shaffi, M. M. A. Qayyum and R. Goyal. 1979. Lead intoxication (effects) on the tissue glycogen content in freshwater fish *Heteropneustes fossilis.* Zool. Jahrh. Abt. Anat. Ontog. Tiere. **101** (3): 402-406.
69. Sharma, S. and K. C. Kanwar. 1985. Reproductive performance in mice following lead administration. Res. Bull. Panjab Univ. Sci. **36** (3/4). 389-394.
70. Sokol, R. Z., C. E. Madding and R. S. Swerdloff. 1985. Lead toxicity and the hypothalamic-pituitary-testicular axis. Biol. Reprod. **33** (3) 722-723.
71. Srivastava, A. K. 1987. Changes induced by lead on fish testis. J. Environ. Biol. **8** (4): 329-332.
72. _____ and S. Mishra. 1979. Blood dyscrasia in a teleost *Colisa fasciatus* after acute exposure to sublethal concentrations of lead. J. Fish. Bio. **14** (2): 199-203.
73. Tejani, A., I. Lancman and S. Rajkumar. 1986. Progressive renal damage due to lead intoxication in early life. Int. J. Pediatr. Nephrol. **7** (1): 9-12.
74. Tewari, H., T. S. Gill and J. Pant. 1987. Impact of chronic lead poisoning on the haematological and biochemical profiles of a fish, *Barbus conchonius.* Bull. Environ. Contam. Toxicol. **37:** 748-752.
75. Tsalev, D. L. and Z. K. Zaprianov. 1981. Atomic absorption spectrometry in occupational and environmental health practice. Vol. 1. ORC Press. Florida.
76. Walsh, C. T. and K. M. Harnett. 1986. Inhibitory effects of lead on contractility of longitudinal smooth muscle from rat ilium. Toxicol. Appl. Pharmacol. **83** (1): 62-68.
77. Wide, M. 1985. Lead exposure on critical days of fetal life affects fertility of the fetal mouse. Teratology **32** (3): 375-380.