Histopathological Effects of Manganese Intoxication

Annabelle A. Herrera and Elena S. Catap
Institute of Biology
College of Science
University of the Philippines
Diliman, Quezon City

ABSTRACT

Manganese, known to be an essential and the least toxic of the trace elements, is released to the aquatic environment by the steel, paint and mining industries, among others. This study was done to investigate the effects of this metal, a potential environmental toxicant, on some organs of juvenile ***Tilapia nilotica.***

Sublethal exposure to 2000 ppm Mn for several days led to histopathological changes in the gills, liver, kidney and intestines. The extent of the damage ranged from formation of cytoplasmic vacuolations, nuclear pyknosis, fatty infiltration and epithelial hypertrophy to severe necrotic conditions.

INTRODUCTION

Manganese (Mn) is one of the metals whose use in industries has increased; the possibility of its hazardous effects has increased as well. Thus, while some metals are classified as essential nutrients, like Mn, they can also serve as environmental hazards if the mechanism which maintains them within their functional limit are unbalanced.

Manganese is among the trace elements least toxic to animals. In fact, it is even involved in many biological processes. But it was discovered that it can affect the respiratory tract (Mn pneumonitis due to acute exposure) and the central nervous system (Chronic Mn poisoning), also known as manganism (**6**).

Those who work in mines (**30**), ore crushing plants, smelters (**13**) and those engaged in occupations where exposure to the metal may be high, are the most vulnerable. Non-occupational cases of manganism have also been reported (**5**). With the introduction of unleaded gasoline, Mn is used as an anti-knock ingredient in automobile fuels (**1**). This means that, for the first time, the general population can be exposed daily to low levels of this essential but potentially toxic metal. Another source of Mn intoxication is the use of fungicide maneb (mn ethylene-bis-dithiocarbamate). Two young agricultural workers who were exposed to maneb developed the Parkinsonian syndrome which characterizes manganism (**20**).

Most studies on Mn toxicity deal with its effects on the central nervous system (CNS). Investigations have shown that the symptoms and signs of Mn encephalopathy share several features in common with Parkinson's disease and they are directly linking the metabolism of catecholamines and the concentration of Mn in the brain (**10, 31, 34, 37, 15**). They observed that it is characterized by psychiatric and neurological symptoms. Rats chronically treated with high oral load of $MnCl_2$ showed decreased concentrations of dopamine and homovanillic acid (HVA) in the brain. A return to normal values was observed after L-DOPA injections (**7**).

Neurochemical and physiological studies of Mn toxicity were mostly performed on mammals. These include lipid peroxidation inhibition in rat brain (**8, 38**); alterations of lipid synthesis (**12**); inhibition of activities of different enzymes like glutamic acid decarboxylase, choline acetyltransferase acetylcholinesterase (**25**) and monoamine oxidase (**27, 26**). It has also been reported that the metal can inhibit Na-K-ATPase and Mg-ATPase (**39, 23**). Hong and co-workers (**22**) also observed that plasma levels of some hormones and neuropeptides can be susceptible to Mn treatment.

Investigations on the toxicity of Mn on aquatic organisms have been done but they are relatively few. According to Cossarini- Dunier (**14**), the metal can be released into the water by industries like steel, mining, paint, textile dyes and fungicides from run-off water. Agrawal and Srivastava (**2**) reported a 96-hr LC_{50} value of 2850 mg/L wherein they used the freshwater fish, *Colisa fasciatus*. They observed hematological abnormalities in the fish exposed to 2500 mg/L $MnSO_4$ for 90 hrs. It was also observed that there were decreased spermatogenic activity and

also hemmorhage in the testes of *Colisa fasciatus* at the same Mn concentration (**40**). Ultrastructural changes in the gills of *Sarotherodon mossambicus* have also been observed. The fish were fed with chicken manure and significantly higher concentrations of Mn and other metals were obtained in the gills (**28**). Nath and Kumar (**32, 33**) also used *Colisa fasciatus* to investigate the impact of Mn on carbohydrate metabolism using 2584 ppm of Mn as treatment. They reported a value of 3230 mg/L as 96-hr LC_{50}. Evtushenko (**19**) studied lipid metabolism and the liver protein synthesizing function in the carp exposed to Mn.

The objective of this study, therefore, is to investigate the effects of Mn on *Tilapia nilotica,* a known hardy species of fish. Histopathological changes manifested by different tissues will be determined since there has been little information regarding this aspect of Mn toxicity. These findings are significant in understanding fully the mechanism of Mn toxicity.

MATERIALS AND METHODS

Tilapia fry from the Central Luzon State University, Muñoz, Nueva Ecija were acclimated for one week prior to $MnCl_2$ exposure. The 24-hr and 96-hr LC_{50} were determined (4000 mg/L and 3000 mg/L, respectively) after which the fry were exposed to a sublethal concentration of Mn at 2000 mg/L (30 fry/aquarium), while another group served as control. Water spiked with $MnCl_2$ was changed every other day. The fry were fed with commercial fish flakes everyday. The fry, from both the control and treated groups, were then dissected after eight days of exposure. Tissues were fixed with 2.5% glutaraldehyde, washed with 5% sucrose buffer, post-fixed with 1% osmium tetroxide, dehydrated in a series of graded acetone concentrations, infiltrated with propylene oxide and embedded in Araldit resin. Sections were then prepared for light and electron microscope observations. LM sections were stained with toluidine blue while EM sections were stained with uranyl acetate and counterstained with lead citrate. EM sections were observed using a JEOL 100 EM. Photomicrographs and electron micrographs were prepared.

Tilapia fry exposed to 4000 mg/L (24-hr) and 2000 mg/L (96-hr) Mn were processed using the paraffin method. Serial sections prepared were stained with hematoxylin and eosin.

RESULTS AND DISCUSSION

Gills

The main target of the action of pollutants in fishes is considered to be the gills since they are the primary sites for both passive and active exchange of gases and ions (**24**). The normal morphology is shown in Fig. 1. The respiratory lamella is lined by an epithelium that is two squamous cell layers thick. Internal to the epithelium is the lamellar blood sinus, lined and spanned by pillar cells of contractile function. A marginal blood channel, lined by endothelium,occurs within the apex of the lamella. A thick stratified epithelium lines the epithelium between gill lamellae. In this interlamellar epithelium, there occurs scattered cells of two special types: choloride cells and mucous cells (**29**).

Mn-induced gill lesions investigated with light microscope include the following: hyperplasia of the lamellar epithelium which appears to begin at the bases of the lamellae (Fig. 2) and gradually fills up the lamellar troughs which lead to lamellar fusion; epithelial lifting (edematous spaces and sloughing off of branchial epithelium); necrosis and degeneration of the secondary lamellae; and the total collapse of the pillar system.

The most common gill alteration is the lifting of the epithelial cells which represents an infiltration of the epithelia with fluid. Lifting, swelling and hyperplasia of the lamellar epithelium could serve as a defense function, as these alterations increase the distance across which waterborne irritants must diffuse to reach the bloodstream. Lamellar fusion could also be protective in that it diminishes the amount of vulnerable gill surface area (**29**).

Figures 3 and 4 show normal and treated secondary lamella observed using an electron microscope. Observations revealed the sparsity or lack of microvilli, swelling or blebbing of the nuclear envelope, rough endoplasmic reticulum (ER) in the form of vesicles (Fig. 5), damaged mitochondria and widened intercellular spaces. Most of these changes indicate cell damage or death.

Liver

The hepatic parenchymal cells or hepatocytes appear as two-cell layer thick laminae, in anastomosing plates or in clumps or rosettes of several cells (Fig. 6). Each hepatocyte represents an irregular polygonal form and most contain a single spherical nucleus, with a dense nucleolus. The cytoplasm stains irregularly, alternating light and dense regions being located variably in

the cell. The nuclei are frequently masked by the dense regions. The light regions represent areas of glycogen accumulation and the dense regions represent areas of abundant rough endoplasmic reticulum (**11**).

Figure 7 shows some morphological changes in the liver in response to Mn treatment. Vacuolations and fatty infiltrations were evident. Other abnormalities included hepatocyte hypertrophy, nuclear pyknosis and extensive liver cord disarray. Necrosis and degeneration of hepatic tissues were also observed. At the ultrastructural level, mitochondrial damage was the most conspicuous. Some appeared swollen and active while others exhibited cristae which were not clearly outlined (Fig. 8). Vacuoles were present, rough endoplasmic reticulum lost its typical organization and became fragmented. Glycogen tended to accumulate in specific areas rather than disperse evenly. Manifestations of cytopathological changes in the liver clearly suggest impaired metabolic status of this vital organ (**21**). Dickinson and Hart (**16**) reported that sheep liver cytoplasmic aldehyde dehydrogenase is strongly inhibited by Mn ions. Liver glycogen was also reported to be depleted by Mn (**35**). Tolbert et al. (**42**) observed that Mn stimulated gluconeogenesis in enzymatically-isolated rat hepatic parenchymal cells.

Kidney

The normal kidney (Fig. 9) shows coiled uriniferous tubules not arranged in any specific pattern, the Malpighian or renal corpuscles and the parenchyma that form the interstitial hemopoietic tissue. The initial portion of the tubule is the neck segment where numerous cilia are present. The proximal segment that follows is characterized by columnar epithelial brush border. It leads to the distal segment having closely- packed darkly stained cells which in turn end in the collecting duct (**3**).

Renal lesions following Mn exposure included the swelling of the Bowman's capsule or space, vacuolations of the tubular epithelial cells, distended or dilated renal tubules and sometimes nuclear pyknosis (Fig. 10). Degenerative changes such as collapsed glomeruli were also observed. The renal tubules sometimes appeared to be widely separated from one another showing disorganized or hydropic condition. Endoplasmic reticulum of the tubular epithelial cells was destroyed while the mitochondria showed remnants of cristae. Electron microscope observations also revealed the presence of vacuoles and some damage to the

microvilli. Glomeruli capillaries are swollen (Fig. 11). Kramer and co-workers (**23**) determined the potential role of Mn (as trace metal) in the pathogenesis of renal diseases. They investigated the effects of various trace metals on Na-K-ATPase, the biochemical correlate of active cellular transmembrane and sodium-dependent transport. It was found that Mn can inhibit the enzyme and accumulation of this metal may present serious hazards by producing a general defect in cell membrane transport.

Intestine

The intestines show the usual four-layered structure (Fig. 12). The outermost serosa is followed by a muscular coat, consisting of an outer longitudinal and an inner circular layer. The submucosa is divisible into an outer stratum compactum, a dense connective tissue arranged in a wavy pattern and an inner stratum granulosum rich in capillary network. The latter merges with the tunica propria of the underlying mucosal coat, there being no muscular mucosa. The epithelial lining of the mucosa consists of prismatic cells with basal nuclei. The nuclei of the intestinal mucosal cells are round with 2-3 nucleoli. The intestinal mucosal cells show a serrated margin with a number of interspaced goblet cells. The mucosal layer is thrown into folds (**3**).

The intestinal mucosa showed the most severe damage manifesting degenerative changes leading to complete destruction of the cells. Most of the mucosal cells were vacuolated. Some intestinal folds were separated from the mucosal layer. Dense bodies were present in the muscularis.

In severe cases, the goblet cells were considerably enlarged (Fig. 13).

Ultrastructural studies showed the presence of numerous vacuoles and destroyed cytoplasm of mucosal cells.

Other Organs

Ovarian tissue obtained from fry treated with Mn showed decreased number of eggs as compared to the control (Figs. 14 and 15). This resulted in widened spaces between oocytes. Some oocytes even lacked lipid droplets and the granular protein inclusion which were visible in the control.

Other tissues examined did not show alterations in the treated fish.

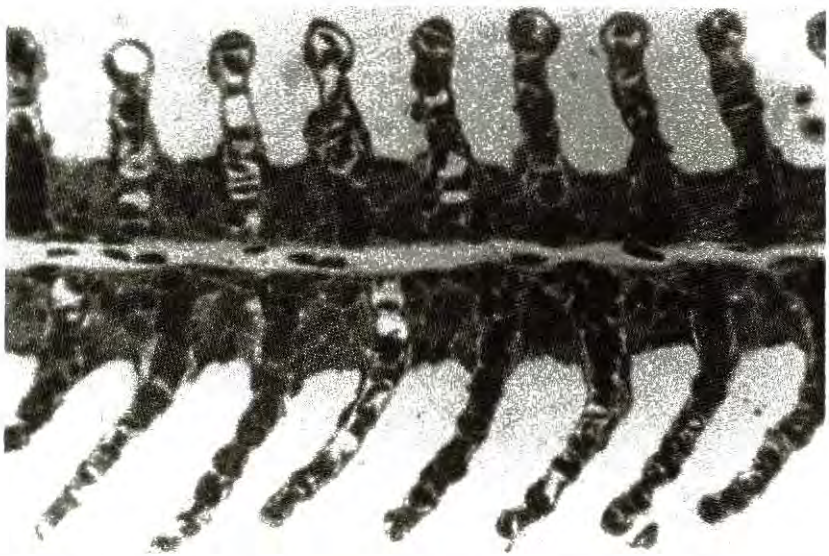

Figure 1. Respiratory lamellae of untreated fish [SG (Secondary lamella); PC (Pillar cell); MBS (Marginal blood sinus); LBS (Lamellar blood sinus); IE (Interlamellar epithelium)]

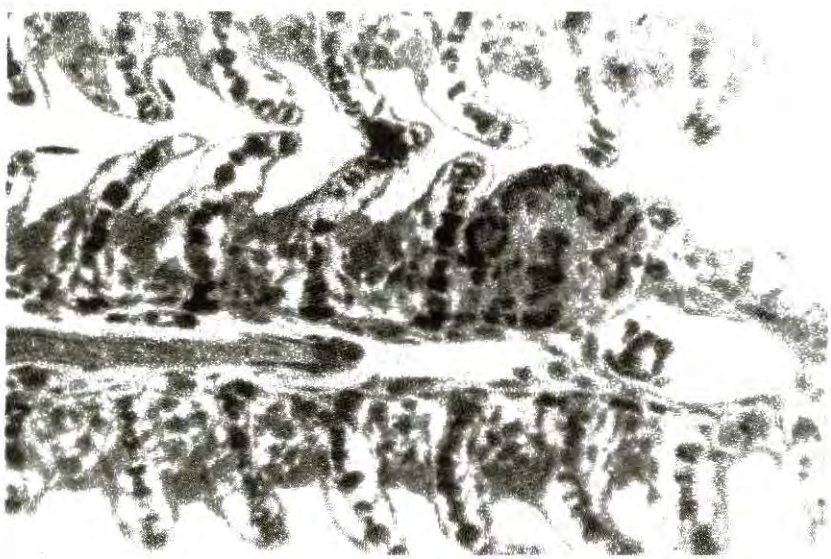

Figure 2. Respiratoy lamellae of treated fish showing hyperplastic growth of epithelial cells along the bases of the lamellae and epithelial lifting (edematous spaces)

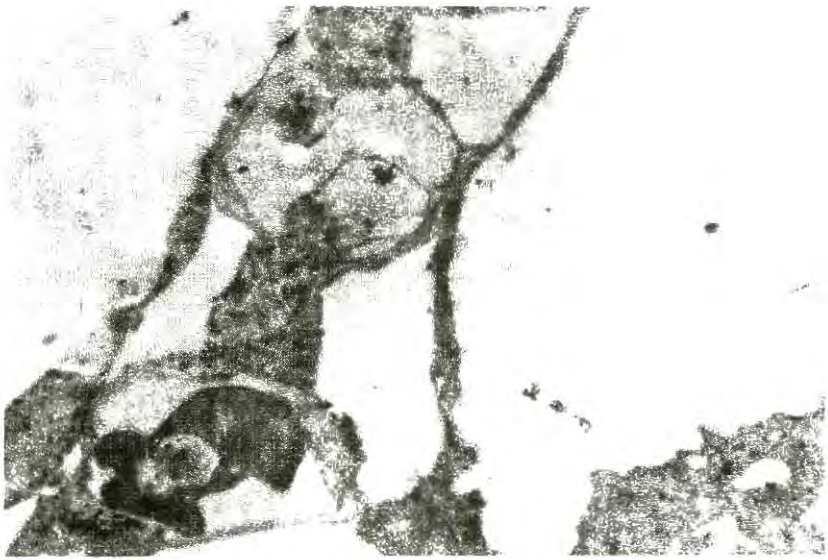

Figure 3. Electronmicrograph of secondary lamella showing intact pillar cell system [E (epithelium); PC (Pillar cell); RBC (Red blood cell); LBS (Lamellar blood sinus)]

Figure 4. Electronmicrograph of treated gill lamellae with damaged microvilli

Figure 5. Electronmicrograph of epithelial cell of treated fish with blebbing of nuclear envelope and the endoplasmic reticulum in fragments or vesicles [B (Blebs); N (Nucleus); M (Mitochondria); ER (Endoplastic reticulum)]

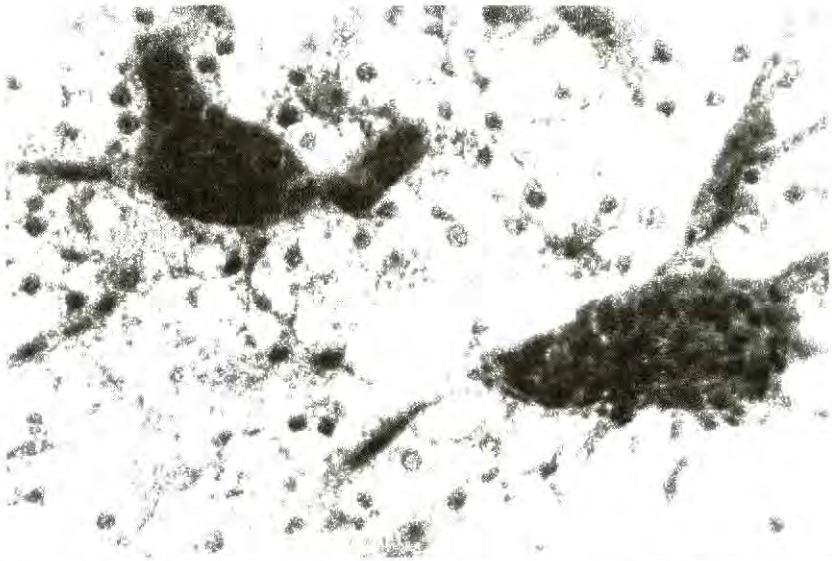

Figure 6. Liver from control fish [H (Hepatocyte); CV (Central vein); S (Sinusoids); G (Glycogen)]

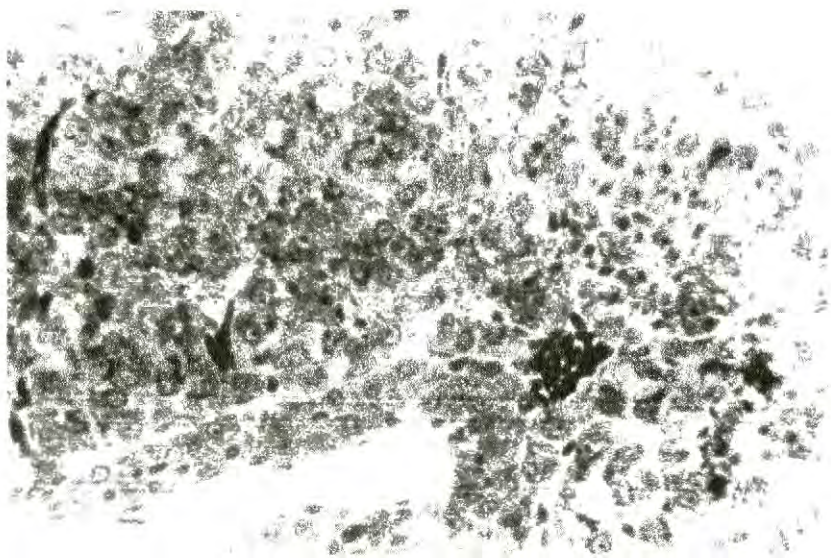

Figure 7. Liver from treated fish showing nuclear pyknosis and liver cord disarray

Figure 8. Electronmicrographs of hepatocyte with mitochondria devoid of cristae and disorganized endoplasmic reticulum

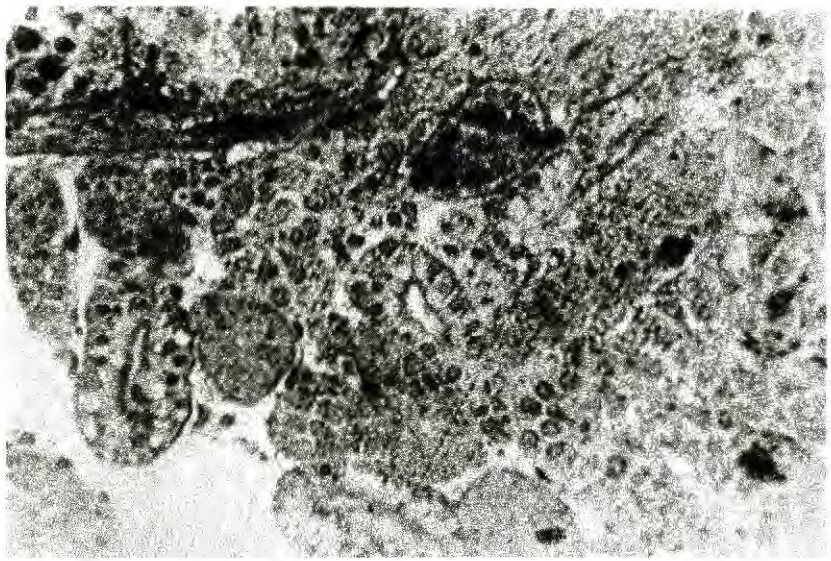

Figure 9. Renal tissue from untreated fish [G (Glomerulus); D (distal tubule); P (Proximal tubule); HT (Hemapoietic tissue)]

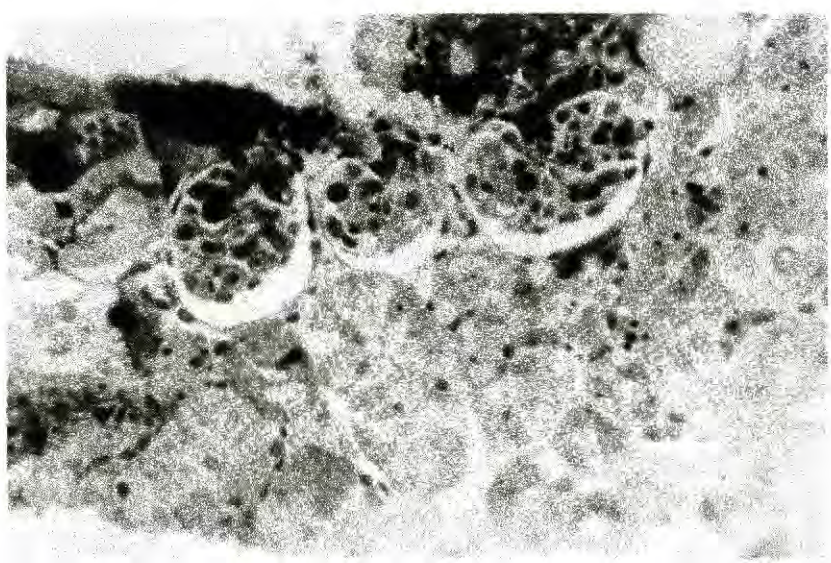

Figure 10. Treated kidney showing Bowman's capsule (BC) and hypertrophied tubular cells (HP)

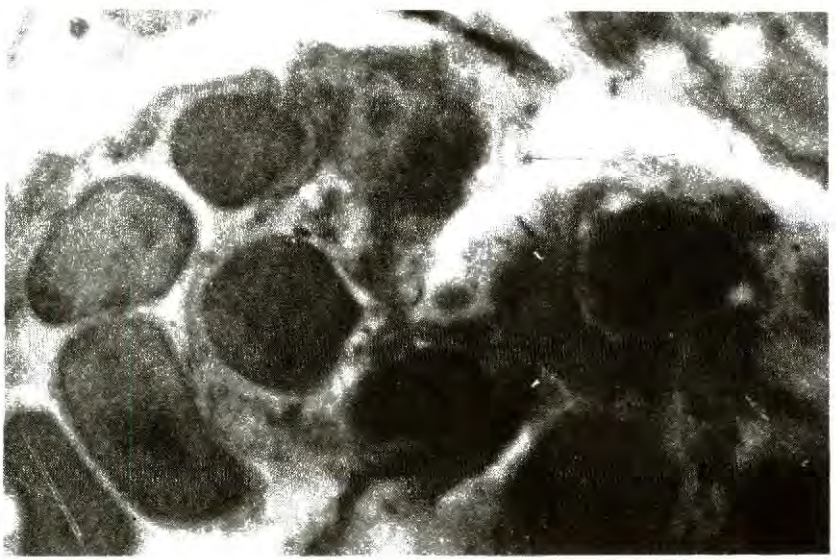

Figure 11. Electronmicrograph of glomerulus showing swollen capillaries (C); M (Mesangial cells); P (Podocyte)

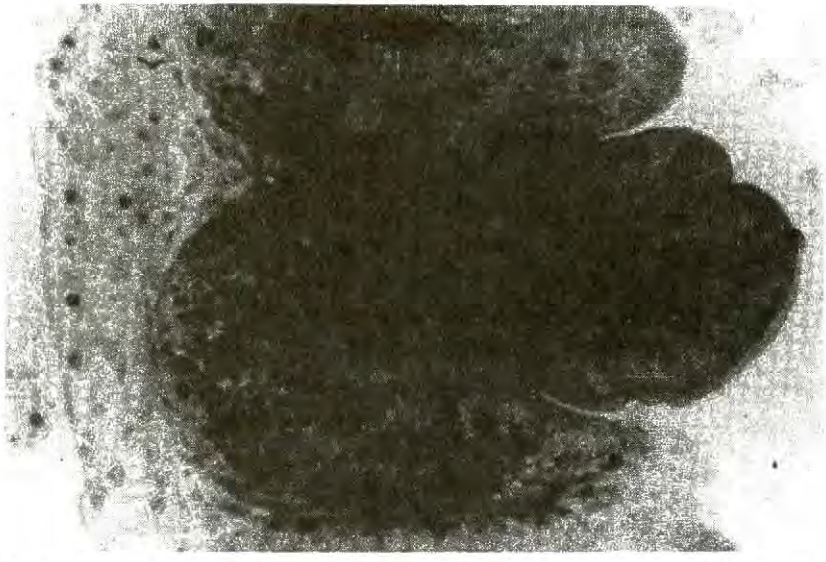

Figure 12. Normal fish intestinal structure [S (Serosa); M (Muscularis); SM (Submucosa); M (mucosa); G (Goblet cell)]

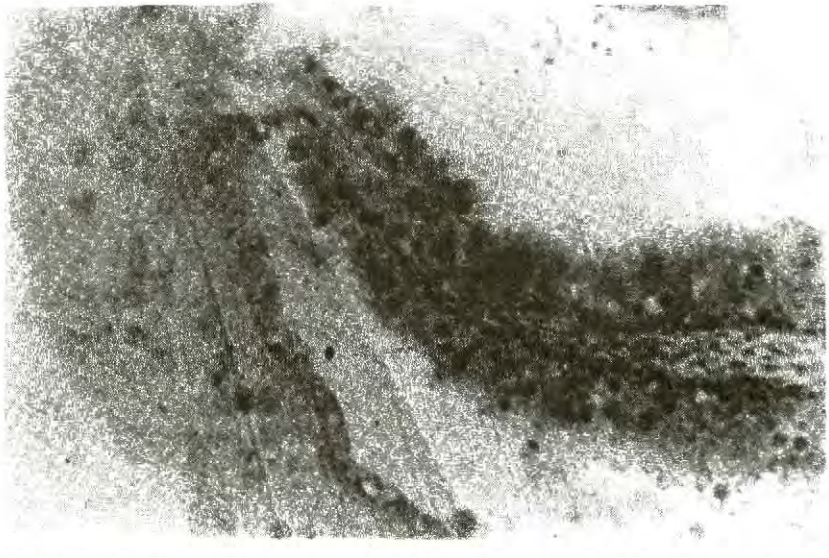

Figure 13. Intestinal mucosal folds from treated fish exhibiting degenerated epithelial lining of the mucosa (Goblet cells are enlarged.)

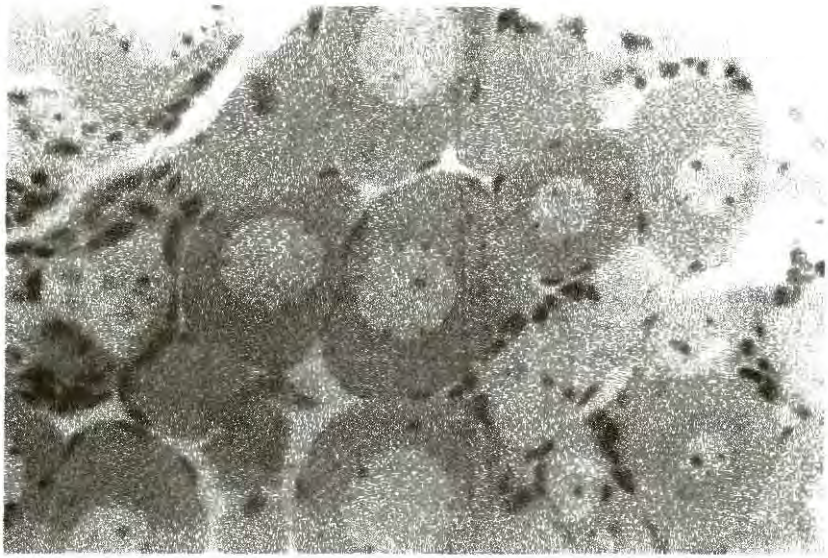

Figure 14. Oocyte from control fish (They appear closely packed.)

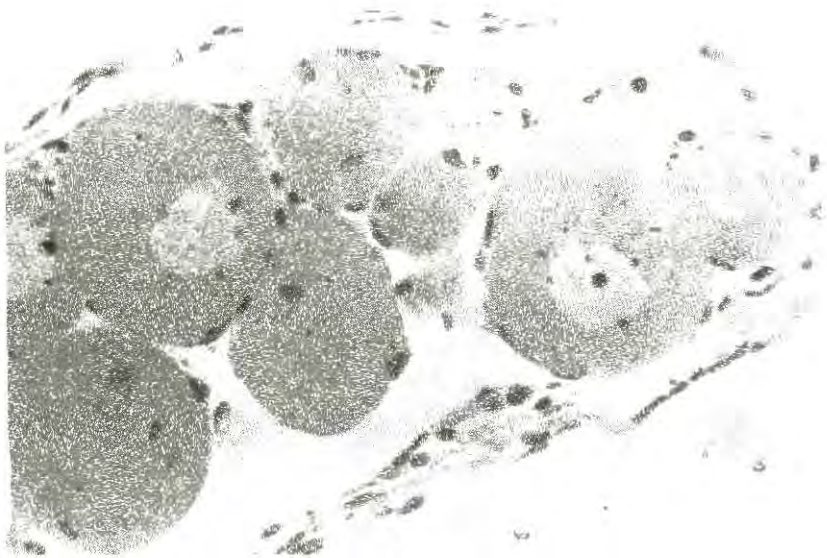

Figure 15. Ovary from a treated fish with loosely arranged oocytes and separated with thicker connective tissue

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