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Histopathological Changes in the Gastrointestinal Tract of Fish, *Puntius gonionotus*, Fed on Dietary Cadmium

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Cadmium is a well-known heavy metal pollutant. The important food sources of fish, phytoplanktons or invertebrates accumulate cadmium through the food chain. Fish also accumulate heavy metals in their tissues by absorption along the gill surface and gut tract wall to higher concentration levels than in their environment (Handy 1993). The adverse effects of cadmium have been studied in many fish species and in numerous target organs such as the liver, kidney and gills, (Shukla and Pandey 1988). But there is little information in the literature on what is possibly the main target organ of toxic dietary cadmium, namely the gastrointestinal tract (GI). Maage and Julshamn (1993) hypothesized that the intestine is the main organ of regulation in the uptake of dietary metal in Atlantic salmon. The strong regulatory capacity of the intestine in relation to dietary metals makes this organ valuable in assessing toxic responses to dietary cadmium. *Puntius gonionotus* are commonly found throughout Thailand, in rivers, streams, floodplains, and reservoirs. They are also common market fish and they feed on both plant and animal matter. They have been developed as a laboratory test model in Thailand for many years. Therefore, the aim of the present work was to study the histopathological changes in the intestine of the common silver barb, *P. gonionotus*, fed cadmium-contaminated cyanobacteria, *Spirulina platensis*.

MATERIALS AND METHODS

P. gonionotus with an average body weight of 28.96±4.53 g, were acclimatized under laboratory conditions for 7 days prior to use. They were maintained in an aquarium with aerated and dechlorinated tap water with pH 7.7-8.2, DO 7.2 ± 0.5 mg/L, total hardness 80 mg/L (as $CaCO_3$), alkalinity 85-93 mg/L, temperature 26.0 ± 2°C, under a static system. The acclimatized fish were transferred from the stock tank into two experimental aquaria (120x60x60 cm in dimensions). The experiment consisted of two groups : control group which were fed on normal *S. platensis* and experimental group which were fed on metal-laden cyanobacteria. There were three replicates per group, with 10 fish in each replicate. Fish were starved for 48 hr, then fed daily on a single meal of either a control diet consisting

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of normal cyanobacterial cells, *S. platensis*, or a cadmium-containing diet consisting of metal-laden cyanobacterial cells.

A stock solution of 1000 mg/L Cd was prepared by dissolving 1,792 g of analytical grade $CdCl_2.H_2O$ (Mallinkrodt Chemical Works) in 1000 mL distilled water. The metal-laden *S. platensis* were prepared by placing cells from the linear growth phase into cadmium solution at 96-h IC_{50} value (18.35 mg Cd/L; Rangsayathorn et al. 2002). Cadmium concentration in the metal-laden cyanobacteria was 10.41 mg Cd/g biomass.

After feeding, half of the volume of water was removed and replaced with new water. Fifteen fish were removed from each experimental aquarium after two and four weeks of feeding, before the daily meal. They were anaesthetized using MS-222 at a concentration of 10 mg/L. *P. gonionotus* does not possess a true stomach but instead shows an expansion at the anterior part of intestine, called the intestinal bulb. For light microscopic study, the gastrointestinal tracts (intestinal bulb and intestine) were dissected out and fixed in Bouin's fixative for 48 hr. They were then dehydrated in a graded series of alcohols. Finally, they were embedded in paraffin, sectioned at 5-7 μm thickness, and stained with PAS and hematoxylin, and observed under an Olympus Vanox light microscope. For electron microscopic study, samples were fixed in 2% glutaraldehyde, in sodium cacodylate buffer pH 7.4 at 4°C for 24 hr and post-fixed in 1% OsO_4 in sodium cacodylate buffer pH 7.4 at 4°C for 1 hr. They were dehydrated in a graded series of ethanols and embedded in araldite. Sections were cut with a diamond knife and double-stained with uranyl acetate (30 min) and lead citrate (30 min). They were examined under a JEX-100SX transmission electron microscope, operating at 75 KV.

RESULTS AND DISCUSSION

P. gonionotus, as in many other species of cyprinid, does not possess a true stomach but instead shows an expansion at the anterior part of intestine, called the intestinal bulb. The histological structure of the intestinal bulb (Figure 1A) was similar to that of the intestine. There were four layers of intestine: an outer serosa, a muscularis, submucosa, and mucosa (Figure 1A). The serosa covered the outer surface and was composed of a single layer of simple squamous epithelial cells. The muscularis was composed of a muscle layer. The submucosa was made up of loose areolar blood vessels containing eosinophilic granule cells. It folded to form a lamina propria, which supported the mucosa epithelium. The mucosa epithelium consisted of enterocytes lined with lamina propria, and mucous-secreting goblet cells thrown into villi (Figure 1B).

The typical enterocytes of the intestine were high columnar cells with median to basally located oval nuclei. (Figure 3A). The apex of the cell was covered with numerous long microvilli forming a striated border towards the lumen. Numerous mitochondria were distributed in the cytoplasm, particularly in the apical and basal portions of the cell. Golgi apparatus occurred in the vicinity of the nucleus.

Lamellae of rough endoplasmic reticulum (RER) and ribosomes were found throughout the cytoplasm.

After two-week feeding, the mucosal area of the intestine was affected. Several lesions were discernible in the villar region. Vacuolation in some enterocytes was observed (Figure 1C). Blood vessels in the lamina propria were dilated and infiltrated with numerous lymphocytes (Figure 1C). A slight vacuolation was observed in the submucosa (Figure 1D). There were a loss of cell outline, accumulation of large cytoplasmic vacuoles and karyolysis (Figure 2A). The mucous cells were filled with mucus and the excess was secreted into the lumen of the intestine (Figure 2B). Many cells showed sloughing off of cell apices into the lumen (Figure 2C).

The ultrastructural study showed that the enterocytes accumulated numerous apical vacuoles and lipid droplets (Figure 3B). Lysosomes, secondary lysosomes and myelin bodies were also found in the cytoplasm (Figure 3C). Dilation of the nuclear envelope was seen (Figure 3C). In addition, dilation and vesiculation of the RER were observed (Figure 3D), and the Golgi apparatus was also dilated (Figure 3E). The nucleus showed an irregular shape (Figure 3D).

Mucosal and submucosal areas demonstrated a high degree of cell necrosis after four-week feeding. Large vacuoles were observed in the cytoplasm and the nuclei showed karyolysis (Figure 2A). The enterocytes had lost the cell outline (Figure 2A). The areas between villi were filled with mucus (Figure 2B). The enterocytes showed cell rupture near the tip and appeared to be sloughing off into the lumen (Figure 2C). Blood vessels of the lamina propria were highly dilated (Figure 2C). Vacuolation in the submucosa and a discrete submucosal layer was observed (Figure 2D).

The ultrastructure of enterocytes showed an increase in diameter of lipid droplets (Figure 4A). Numerous vacuoles and lysosomes were distributed throughout the cytoplasm (Figure 4A). Mucous cells were increased in number and mucus granules filled the cell. Cell necrosis was indicated by the loss of cytoplasmic organelles and rupture of the cell membrane (Figures 4B, 4C). The nucleus showed an irregular shape and the nuclear envelope was dilated (Figure 4C). The RER and the Golgi apparatus were noticeable and highly dilated (Figure 4C). Heterophagosomes and myelin bodies were only found in the enterocytes of the intestine (Figure 4D). Mitochondria appeared swollen and had lost their cristae (Figure 4E).

Normally, in fish exposed to waterborne pollutants, there is a chance of toxic pollutants entering the GI tract causing a deterioration of tissue structures along the tract. In the case of dietary cadmium, the GI tract seems to be the main target organ (Banerjee and Bhattacharya 1995). In the present study, the mucus secretion from the goblet cells of the GI tract after feeding was believed to have been stimulated by cadmium taken up by fish. This mucus may serve to dilute toxic substances and possibly also has the capacity to detoxify them (Anderozzi et

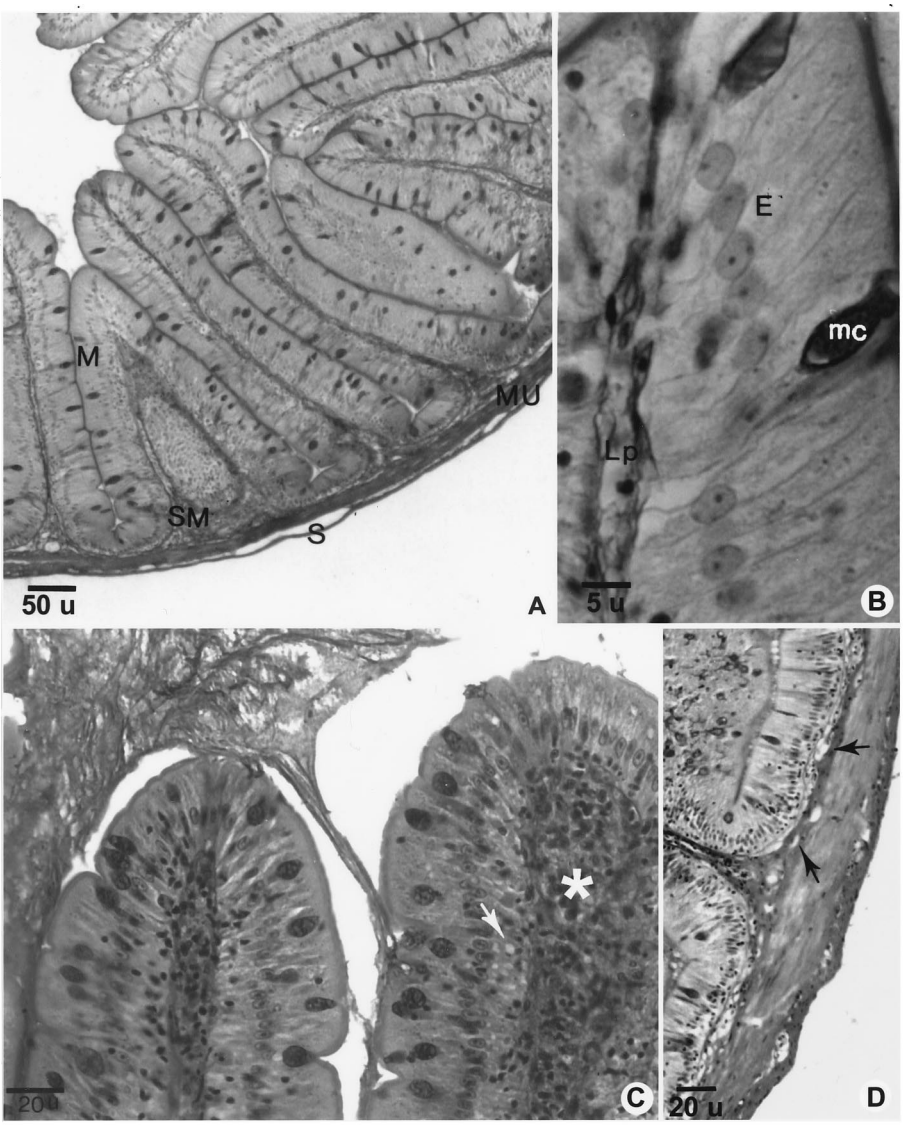

Figure 1. Light micrographs of intestine of fish. A) Transverse section showing four layers of normal intestine: mucosa (M), submucosa (SM), muscularis (MU) and serosa (S). B) High magnification of normal enterocyte (E) of intestine, lamina propria (Lp), mucous cell (mc). C) Low magnification showing vacuolation in enterocytes (arrow) and dilation of blood vessel and infiltration of lymphocytes in lamina propria (*). D) Low magnification showing a slight vacuolation in submucosa (arrows).

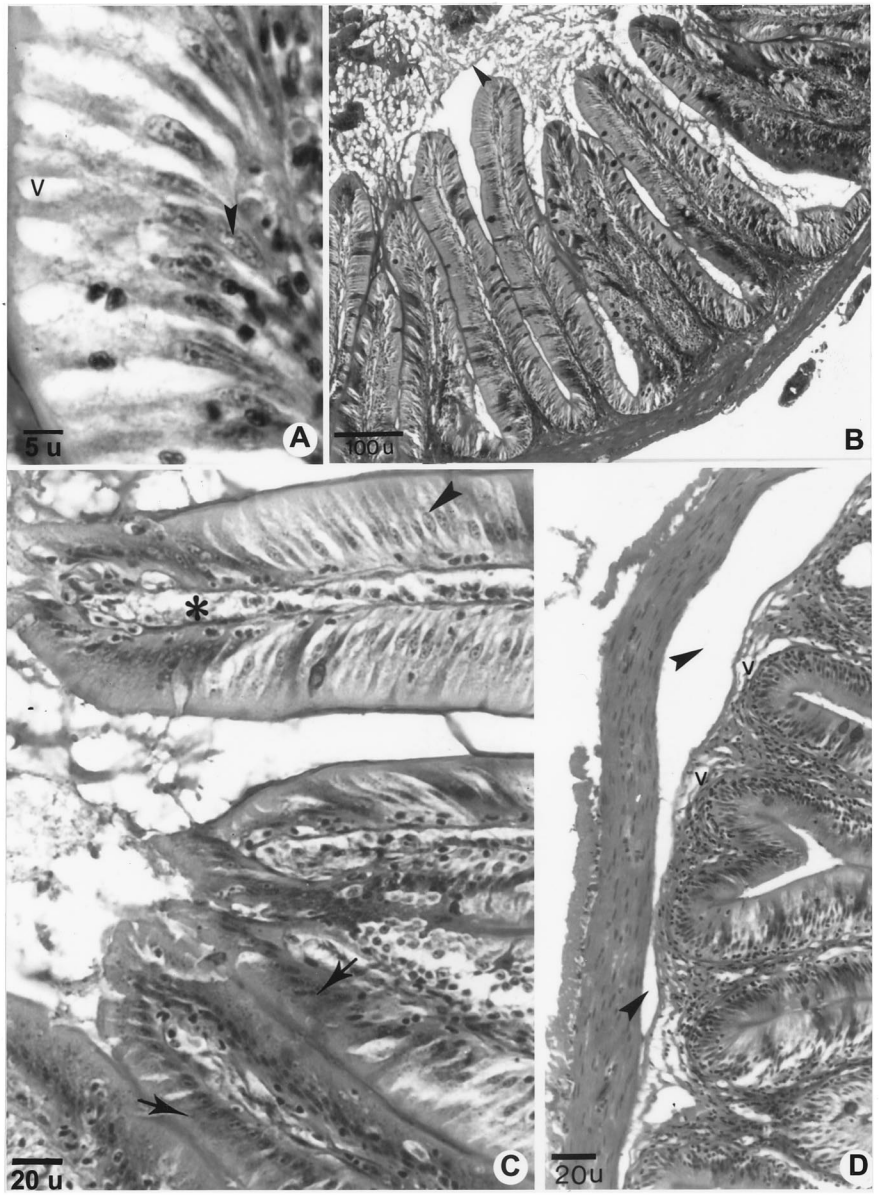

Figure 2. Light micrographs of intestine of fish. A) High magnification of enterocytes showing increase in number of vacuole (V), and karyolysis (arrowhead). B) Low magnification showing excess mucus which fills the lumen (arrowhead). C) Low magnification of enterocytes showing sloughing off of cell apices, loss of cell outine (arrows), karyolysis (arrowhead) and dilated blood vessels (*). D) Low magnification of intestine showing vacuolation (V) in submucosa and discretion of submucosa (arrowheads).

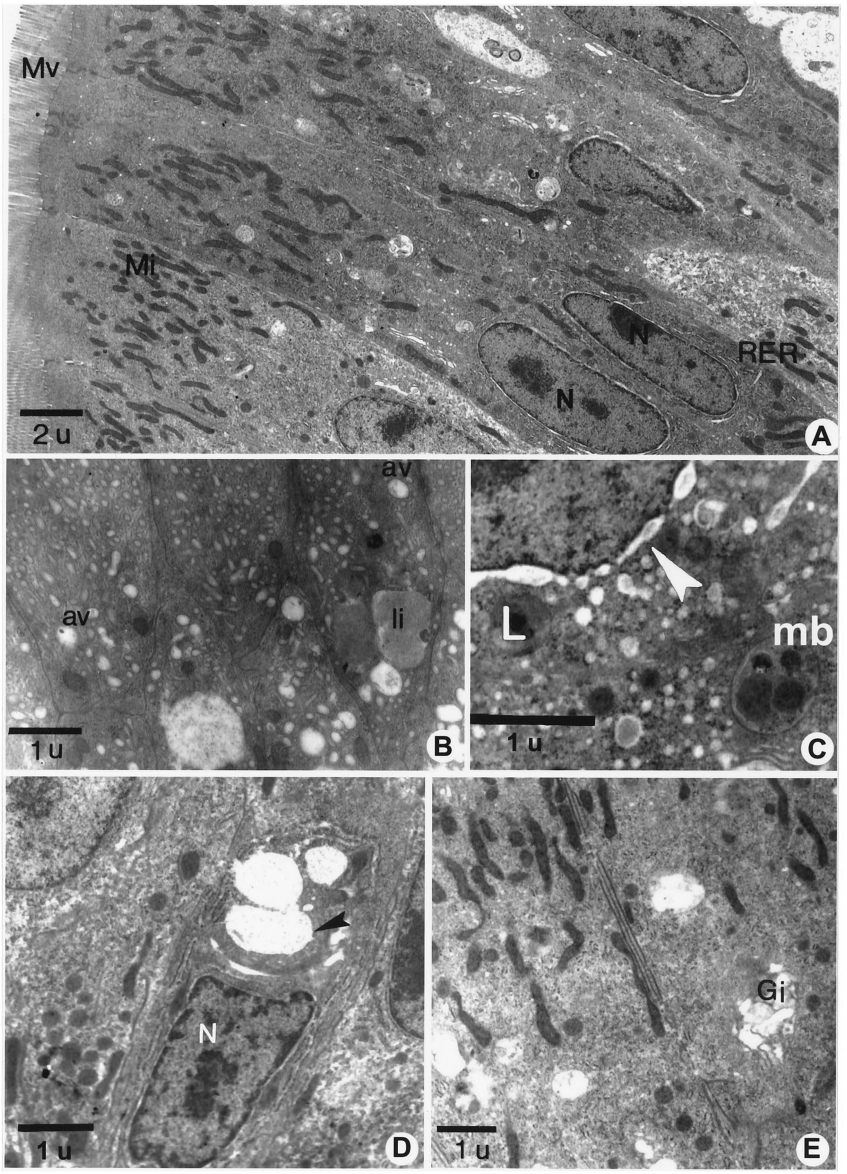

Figure 3. Transmission electron micrographs of intestinal enterocyte of fish. A) Low magnification of control fish showing columnar cell with oval nucleus (N), numerous mitochondria (Mi), microvilli (Mv), and RER. B) High magnification showing apical vacuoles (av) and lipid droplets (li). C) High magnification showing lysosome (L), myelin body (mb), and dilation of nuclear envelope (arrowhead). D) High magnification showing irregular nucleus (N), dilated and vesiculated RER (arrowhead). E) High magnification showing dilation of Golgi apparatus (Gi).

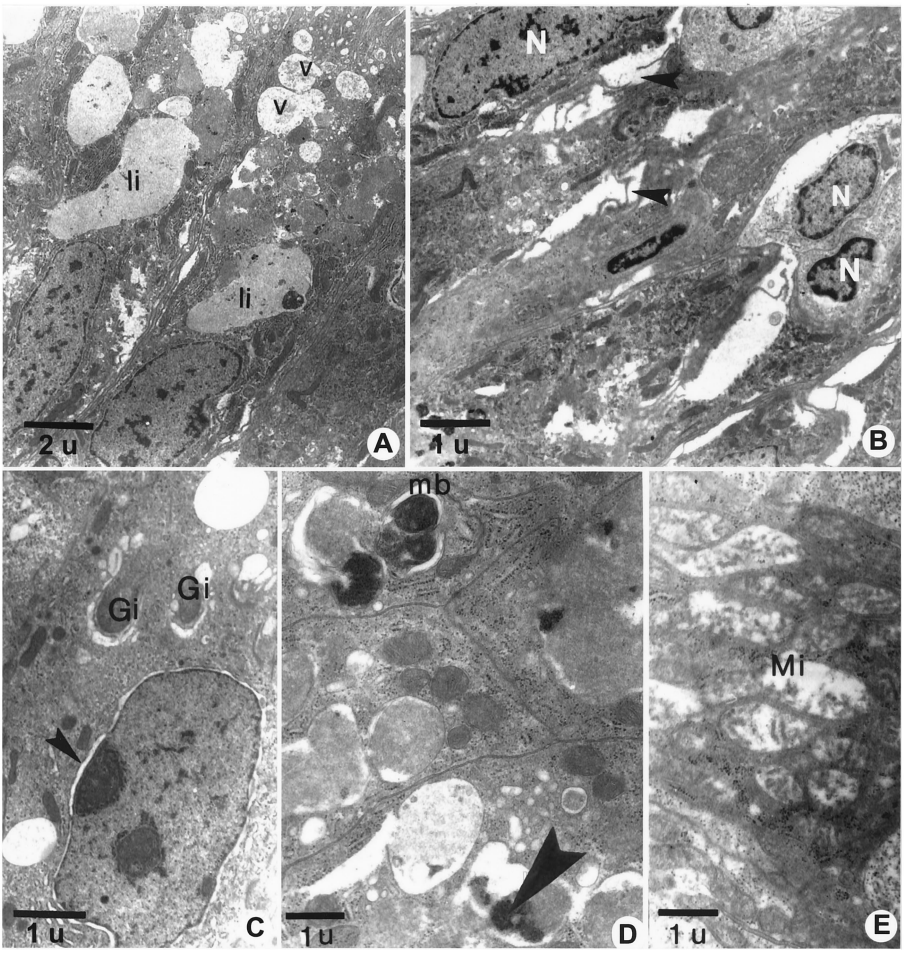

Figure 4. Transmission electron micrographs of intestinal enterocyte of fish. A) Low magnification showing lipid (li) and vacuole (V) accumulation. B) High magnification showing irregular nucleus (N) in necrotic cell and rupture of cell membrane (arrowheads). C) High magnification showing dilation of Golgi apparatus (Gi) and nuclear envelope (arrowhead). D) High magnification showing heterophagosome (arrowhead) and myelin body (mb). E) High magnification showing swollen mitochondria (Mi) which had lost their cristae.

al. 1994). The vacuolation in the submucosal area observed in this study was similar to that observed by Banerjee and Bhattacharya (1995) who found vacuolation in the submucosal area of *Channa punctatus* after exposure to mercury for 7 days.

Increase in number of mucous cells and ultrastructural alterations observed in the enterocytes of the intestine such as increase in number of vacuoles, lysosomes and lipid droplets, and myelin bodies are often considered as nonspecific stress responses. These responses also include dilation and fragmentation of the RER. The accumulation of lipid droplets in the cytoplasm results from the decline of protein synthesis that accompanies cellular injury, whereas the dilation and fragmentation of RER might result from an intensified productivity of mucus in the lumen of the intestine, in response to the ingestion of toxic substance (Sastry and Gupta 1998). When heavy metals enter a cell, they stimulate the cell to produce defensive stress-induced lysosomes (Wang et al. 1998). Heavy metals can also destroy other organelles, such as mitochondria and SER. Lysosomes digest these destroyed membranous structures in the cell through the action of autophagosomes resulting in the presence of myelin bodies which were found in more destroyed cells. Mitochondria are the organelles most sensitive to toxic substances in the cell. Cadmium can bind with a negative charge present on the organelle membrane leading to a change in cell membrane permeability. These changes result in an imbalance of ions in the organelle, in turn disturbing and inhibiting respiration in the mitochondria. When the structure of the mitochondrion is destroyed and its function is lost, it leads to, a loss in the energy necessary for life activities, finally causing cell necrosis (Mallatt et al. 1985).

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