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Changes Induced by Cadmium in the Kidney of Black Sea Bream, *Mylio macrocephalus* (Teleostei)

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Cadmium is known to cause injury to most of the internal organs of higher vertebrates (Felman et al. 1978; Suzuki 1980; Wong et al. 1980; Copius-Peereboom and Copius-Peereboom-Stegeman 1981; Christley and Webster 1983; Phillpotts 1986). Chronic exposure of cadmium produces histopathological changes in the kidney in man and experimental animals (Axelsson et al. 1968; Elinder et al. 1981) and leads to its preferential accumulation in the liver and kidney (Lucis et al. 1969; Kotsonis and Klaassen 1977; Sato and Nagai 1980).

The toxic effects of cadmium on man and experimental animals such as rats, mice and rabbits have been extensively investigated (Friberg et al. 1974; Copius-Peereboom and Copius-Peereboom-Stegeman 1981). However, less is known about injurious effects of cadmium on fish, in particular marine fish species (Tafanelli and Summerfelt 1975). In the present study, the time course of cadmium-induced renal toxicity and histopathological changes of the kidneys of the black sea bream, one of the most common maricultural fish species in Hong Kong, after administration of cadmium chloride are described.

MATERIALS AND METHODS

Young black sea breams, *Mylio macrocephalus*, ranging from 20g to 30g body weight, were obtained from local fish farmers. The fish were kept in the laboratory in plastic tanks containing well aerated, filtered sea water.

A total of 36 fish were separated into four groups, and were given one of the following treatments: a single intraperitoneal injection (IP) of cadmium chloride in 0.9% saline at a dosage of 0.75 mg/100g body weight; two IP injections of cadmium chloride at day 0 and 7; or three IP injections of cadmium chloride at day 0, 7, and 14. Control fish received similar IP injections of 1.0 ml saline/100g body weight. At each time interval, namely 1, 2 or 3 weeks after the last injection, 3 fish of each treatment and control group were sacrificed.

The kidneys of three black sea breams sacrificed at each time period were dissected out and fixed in Bouin's solution. All the specimens were then routinely processed by using paraffin method for light microscopic observations. Sections were cut at a thickness of 8um and were stained with hematoxylin and eosin (H and E) or periodic acid and Schiff's reagent (PAS).

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RESULTS AND DISCUSSION

The basic unit (nephron) of the kidney in fish consists of a renal corpuscle (Bowman's capsule and glomerulus) and various segments of the renal tubule, namely proximal tubule, intermediate segment, distal tubule and collecting duct. The cross-section of a control fish kidney is shown in Fig. 1. Proximal tubules with prominent brush borders (microvilli) bathed in the vascular bed in the interstitial tissue were predominant. Distal tubules and collecting ducts, both devoid of brush borders, were much sparsely distributed. The intermediate segment between proximal and distal tubules was rarely seen. The renal corpuscles were generally located in close vicinity to renal tubules and blood vessels in the interstitial tissue. However, in this marine fish, the black sea bream, only few renal corpuscles were present (Fig. 2). Pigments and leucocytes were very common in the interstitial tissue (Fig. 1).

The proximal tubules appeared to be more sensitive to cadmium exposure than the other segments of tubule and renal corpuscle (Figs. 2 & 3). A considerable degree of histopathological changes was found in the proximal tubules of the kidneys from the specimens one week after single (Fig. 2) or double injections (Figs. 3 & 4). More severe damage of the renal tissues was noted in specimens that had been exposed to cadmium for two weeks or longer (Figs. 5-7). The extent of tissue damage also appeared to increase with the increased number of injections. In fish receiving three injections, the morphological changes in the kidneys were more diffuse and the areas of necrosis were more extensive (Figs. 8-10). Distal tubules and collecting ducts were resistant to a single or double injections of cadmium for one week (Fig. 3). However, in the kidneys of the fish treated with three doses of cadmium, even the distal tubules and collecting ducts were affected (Figs. 8-10).

The following sequence of changes in the kidney tissues could be detected after exposure to cadmium. The first sign of morphological changes in the kidney after injection of cadmium was found in the proximal tubule (Fig. 2). Initial changes of these tubules included: deformation of brush border, gradual atrophy of basal cytoplasm and condensation of nuclear material (Figs. 2 & 3). Following these initial changes, there was focal necrosis of tubular cells and pyknosis of nuclei (Fig. 4). Degenerated cells were frequently seen extruding into the lumina of tubules which were filled with fragments of cellular components (Figs. 5-7). Focal degeneration of tubular cells was usually followed by more extensive necrosis of the whole nephron (Figs. 9 & 10). As the focal areas of necrosis became more widespread, the tubules were surrounded by more and more leucocytes and macrophages (Figs. 6-9). Thus the area of interstitial tissue containing leucocytes and macrophages seemed to be increased as the tubules became reduced (Fig. 10).

Renal corpuscles were less sensitive to cadmium exposure than proximal tubules (Fig. 2), but some changes of the renal corpuscle occurred in the kidneys subjected to two injections of cadmium (Fig. 4). The alterations in the renal corpuscles were detected firstly by the widening of the space between the capsule

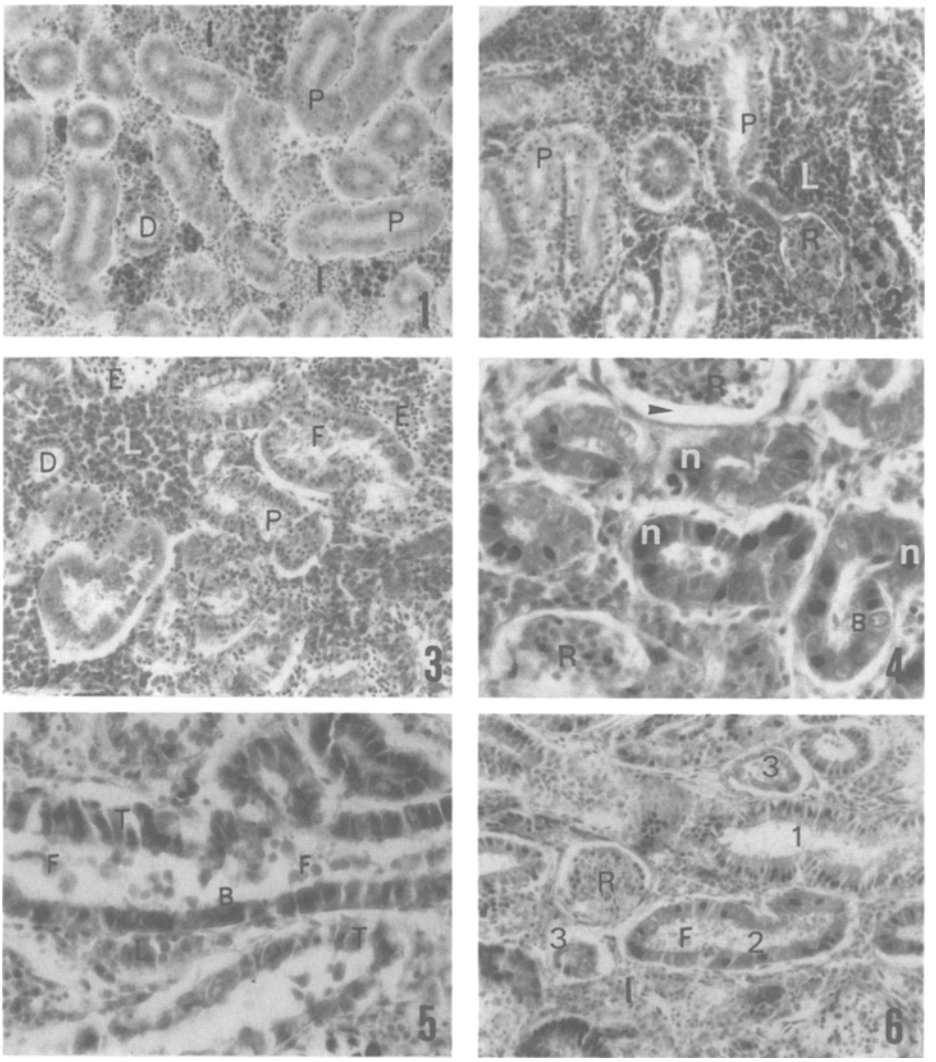

Figure 1. Photomicrograph of a kidney from a control fish. H & E. X460

Figure 2. Section of kidney of fish sacrificed one week after one injection of cadmium. H & E. X460

Figure 3. Section of kidney from fish sacrificed at day 14 after receiving two cadmium injections at day 0 and 7. Note population of leucocytes and red blood cells (erythrocytes) in interstitial tissue. H & E. X460

Figure 4 Section of kidney from fish treated as in Fig. 3. Note the widened space (arrow) between the capsule and glomerulus. PAS. X800

Figure 5. Tubular degeneration in fish kidney treated as in Fig. 3. PAS. X920

Figure 6. Section of kidney from fish sacrificed at day 21 after receiving two injections of cadmium at day 0 and 7 showing various stages of tubular degeneration. 1 to 3: indicating degree of necrosis. H & E. X460

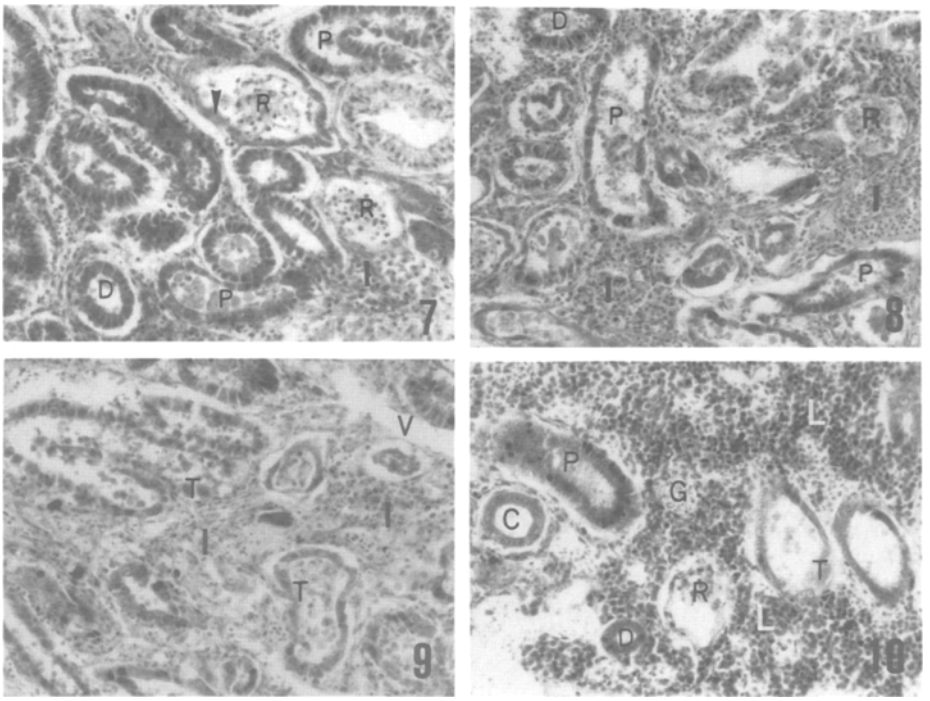

Figure 7. Section of kidney from fish sacrificed at day 28 after two injections of of cadmium at day 0 and 7 showing advanced stage of regression of renal corpuscles. Note the thickening of capsular wall (arrow heads) and shrinkage of glomerular capillary knots. H & E. X460

Figure 8. Extensive degeneration of kidney tissue in fish sacrificed at day 28 after receiving three injections of cadmium at day 0, 7 and 14. H & E. X460

Figure 9. Advanced stage of kidney degeneration in fish sacrificed at day 35 after receiving three cadmium injections. H & E. X460

Figure 10. Section of kidney from fish sacrificed at day 42 after receiving three injections of cadmium. H & E. X460

B: deformed brush border of proximal tubule; C: collecting duct; D: distal tubule; E: erythrocytes; F: cell debris in the lumen; G: granuloma-like structure; I: interstitial tissue; L: leucocytes and macrophages in interstitial tissue; N: pyknotic nuclei; P: proximal tubule; R: renal corpuscle (glomerulus); T: degenerated tubular cells; V: blood vessel.

and the capillary network (glomerulus) after exposure to cadmium (Figs. 4 & 6).

After two to three weeks, the capsular wall became much thickened and the glomerular capillary knot became shrunken (Fig. 7). The cells of glomeruli also appeared to undergo nuclear pyknosis with cytoplasmic degeneration, leading to disorganization of its fenestration structural arrangement and filtering mechanism (Figs. 4 & 7). After three to four weeks, the glomeruli of renal corpuscles were highly disorganized in a more advanced stage of degeneration (Figs. 8-10).

A tumour-like structure was occassionally observed in the kidneys of treated fish three weeks or more after receiving three injections of cadmium (Fig. 10). This structure appeared to be a large cyst consisting of a central core of necrotic cells and debris surrounded by a zone of macrophages and epitheloid cells. It was circumscribed by fibrous connective tissue and was stained positively with PAS.

The present report shows that the extent of histological changes in the kidney of a marine fish after administration of cadmium is related to the number of injections and exposure time. The kidney seems to be a very vulnerable organ for exposure to cadmium. A single dose of 7.5mg/kg body weight administered intraperitoneally in our experiment was strong enough to produce severe acute renal damage in the fish. The microscopic findings of kidney injury in the black sea bream include: early deformation of brush border, atrophy of basal cytoplasm and nuclear pyknosis in the proximal tubules, and necrotic changes in renal corpuscles. These microscopic observations are quite similar to the findings in renal damage seen after chronic low-level cadmium poisoning in rats and rabbits (Axelsson et al. 1968; Kotsonis and Klaassen 1978). Cadmium accumulates preferentially in kidney tissues (Kotsonis and Klaassen 1977). When the body burden of cadmium increases, new proteins such as metallothionein are synthesized in the liver and kidney. Metallothionein is thought to be able to detoxify cadmium through complexing and covalent binding. Most of the cadmium that has accumulated in the tissues is detected in metallothionein (Sato and Nagai 1980). Nevertheless, once the accumulated cadmium in the kidney exceeds the binding capacity of metallothionein, free cadmium ions will bind to other cell components causing cellular damage (Yoshiki et al. 1975). The membranous organelles, such as mitochondria, endoplasmic reticulum and nuclear envelope, are most easily affected by cadmium in which disorganization, rearrangement and malfunction may occur. Thus, the proximal tubules which possess numerous mitochondria rather than the distal tubules are easier damaged by cadmium. The collecting ducts are usually more resistant to cadmium exposure. The injury to collecting ducts are only obvious in the fish receiving three injections.

The appearance of atrophic or pyknotic nuclei in fish kidney increases with the increase of time course and dose of cadmium exposure. The phenomenon of nuclear changes in fish is probably similar to that found in other animals (Copius-Peereboom and Copius-Peereboom-Stegeman 1981). It has been suggested that a nuclear and nucleolar change are induced preceding atrophy and necrosis of cells in other animals. At the beginning, the change may probably form part of a defense mechanism, leading to an activation of synthetic or other activities in the cell, such as synthesis of metallothionein. But for prolonged treatment, further accumulation of cadmium causes a condensation of nuclear material to form darkly stained pyknotic nuclei. The synthetic ability of metallothionein is then repressed and free Cd ions may thus exert its toxic effects (Copius-Peereboom-Stegeman and Jongstra-Spaapen 1979).

Leucocytes are common in the interstitial space of control fish, but they are rarely aggregated so densely and abundantly as in the cadmium-treated renal tissue. The increase of leucocytes may have been an inflammatory response to

cadmium. Leucocytes may either remove or engulf injured and non-functional cells. They sequester insoluble and colloidal particles of the metal compounds as well as cadmium ions, and then excrete them as residual vacuoles (Loose et al. 1978). In the present study, tumor-like structures which may have been granulomas were occassionally found in the kidneys of fish receiving three weekly injections of cadmium. It was probably a proliferative chronic inflammatory response induced by cadmium exposure. Cadmium has shown to be carcinogenic in ducks (Brown and Chatel 1978), and able to cause formation of tumor-like structure in certain internal organs. Tafanelli and Summerfelt (1975) reported that granulomas containing numerous macrophages and mononuclear inflammatory cells could be induced in the testis of fish exposed to cadmium.

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