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Accumulation, Elimination and Cell Response in the Kidney of Juvenile Rockfish (*Sebastes schlegeli*) Exposed to Dietary Cadmium

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Experiments were carried out to investigate Cd accumulation, elimination and cell response in juvenile rockfish (*Sebastes schlegeli*) exposed to sub-chronic dietary Cd (0, 0.5, 5, 25 and 125 mg/kg) for 60 days and depuration periods of 30 days. Cd accumulation in the kidney of rockfish increased with exposure periods and concentrations for the 60 days of dietary Cd exposure. After the end of the dietary Cd exposure, Cd accumulation values in the kidney were 52.9±9.94 μg/g and 90.6±15.7 μg/g for those exposed to 25 mg/kg and 125 mg/kg Cd, respectively. The accumulation factors increased with the exposure period in the kidney. Cd elimination in the kidney of rockfish did not vary significantly and remained constant after the cessation of the dietary Cd exposure. In the primary exposure periods, the effect of kidney tissue in the rockfish exposed to dietary Cd was observed the swelling of capillary of the glomerulus. In addition, there was also hydropic swelling within the pyknotic nuclei, some of hyaline droplet accumulation and the microvilli showed a positive reaction to alcian blue in the tubular cells. While exposure time and concentrations were increased, there was a lot of hyaline droplet accumulation and the microvilli showed a positive reaction to alcian blue in the tubular cells. Fused renal tubule and its necrosis were observed after 60 days at 125 mg/kg.

Key words: Cadmium, *Sebastes schlegeli*, Accumulation, Elimination, Histopathology

Introduction

Toxic heavy metal pollutants are increasingly being released into the environment as a result of industrialization. As a consequence, heavy metals such as Cd have induced toxic effect in the aquatic organisms. Cd is a non-essential element that has severe toxic effects on aquatic animals when present in excessive amounts (Sorensen, 1991). In fish, Cd has adverse effects on growth, reproduction, and osmoregulation (Sorensen, 1991; Lemaire and Lemaire, 1992; Soengas et al., 1996). Moreover, it affects respiratory functions and the composition of plasma by causing hypocalcemia, hypokalemia, and hyperglycemia (Sorensen, 1991). The activity of metabolic enzymes in livers, kidneys, muscles, and other tissues is disturbed following exposure to Cd (Sastry and Subhadra, 1982).

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Although the sub-chronic toxic effects of metals on fish is well documented, that is mostly in fish exposed to waterborne metals, relatively few studied have been conducted on the effects of dietary metals (Handy, 1996). The realization that dietary uptake of metals are a major cause of long-term contamination in wild fish (Dallinger et al., 1987) has renewed interest in the nutritional and toxicological effects of metal in the food of fishes (Handy, 1996). Highly toxic metals such as Cd are easily assimilated from feed, Cd apparently more rapidly in freshwater than in marine fish (Dallinger et al., 1987).

Fish accumulate Cd from polluted environment resulting in accumulation in their tissues, and then redistributed among tissues. Cd accumulation between tissues varies depending on the source of uptake, and whether it is from food or is waterborne. Whatever the exposure method, Cd accumulates significantly in gill, intestine, and mainly liver and kidney (Giles, 1988). Fish are an important food resource and a

major ecosystem component, thus it is important to assess the effect of Cd in fish. Bio-accumulation patterns of metals in fish tissues can be utilized as an effective indicators of environmental metal contamination (Larsson et al., 1985). Moreover, tissues specific accumulation of metal has been proposed as a key indicator of chronic exposure (Bergman and Dorward-King, 1997).

Several factors influence the elimination of metals from the tissues of fish. These include time, temperature, interacting agents, age of fish, metabolic activity of fish and biological half life of metal (Larrson et al., 1985; Douben, 1989; Kargin, 1996; Nielsen and Andersen, 1996). Metal elimination studies are important from the point of view of health protection, allowing for the determination of self-cleansing abilities of contaminated organisms.

Cell response investigations have the capacity to differentiate between organ lesions induced by diseases and other environmental factors from those lesions due to pollutant exposure (Schwaiger et al., 1997). The toxic effects of Cd have been studied in fish (Reid and McDonald, 1988; Thophon et al., 2003). But relatively few investigations have defined the pattern of histopathological alteration in sub-chronic exposure to Cd in kidneys.

The rockfish (*Sebastes schlegeli*) is an economically important food fish in Korea that is commonly cultured in marine based cages (Kim et al., 2004). Despite its importance, relatively little information is available on the effect of Cd, particularly through dietary exposure. Therefore, the aims of the present study were to determine effects on accumulation, elimination and cell response in the kidneys of juvenile rockfish (*S. schlegeli*) after sub-chronic dietary Cd exposure.

Materials and Methods

Experimental fish and treatment

Juvenile rockfish (*S. schlegeli*) were obtained from a rockfish nursery in Koge Island, Korea. Rockfish were acclimated in a 1,000 L aerated running seawater tank for 1 month under laboratory conditions (Table 1). Each tank received a flow of seawater (7 L/min) and was supplied with continuous aeration. Fish were fed Cd-free pellets daily at a rate of 2% body weight. After 1 month in the acclimating tanks, the fish were randomly transferred to 150 L tanks with running water (flow=1.2 L/min) and continuous aeration. After the rockfish were transferred to the exposure tanks, the rockfish were acclimated to experimental conditions. Fish with a total length of 11.61±0.16 cm (mean±SEM, n=600), and a weight of 24.7±0.4 g were selected for the experiment of dietary Cd exposure. Each of the four experimental diets were fed to the rockfish for 60 days and then the fish were fed a Cd-free diet for another 30 days.

Table 1. Physico-chemical parameters of seawater used in the dietary Cd exposure experiment (mean±SEM; n=12).

Parameter	Value
Temperature (℃)	18.00±0.20
pH	8.60±0.20
Salinity (psu)	32.90±0.50
NH_4-N (μg-at/L)	1.41±0.04
NO_2-N (μg-at/L)	0.10±0.01
NO_3-N (μg-at/L)	7.91±0.76
PO_4-P (μg-at/L)	0.63±0.02
SS (mg/L)	5.14±0.32
Dissolved oxygen (mg/L)	6.80±0.04
COD (mg/L)	1.20±0.03
Cd (μg/L)	ND

ND:not detected

Diet preparation

Diets were supplemented with 0 (control), 0.5, 5, 25 and 125 mg Cd/kg feed of $Cd(NO_3)_2 \cdot 4H_2O$ (Aldrich Co., USA). It was dissolved in 1,000 mL acidified water and mixed thoroughly with the other feed ingredients prior to pelleting. All ingredients were mixed and pelleted by a laboratory pellet machine without heating using a 2 mm diameter module (Baokyong Commercial Co., Busan, Korea). After processing, the diets were packed into small bags and stored at 20℃ until they were fed to the fish. Proximate analyses of the diets indicated 48% crude protein, 5% crude lipid, 4% carbohydrate, 15% ash, 1% calcium and 2.7% phosphorous.

Cd analysis

In the experiment, the fish were starved for 24 h prior to sampling to allow all feed to be excreted. Ten fish were removed from each tank every 10 days during the 90 day experiment, and their kidneys were sampled every 10 days for analysis of metal concentration. Tissue samples were dried at 65℃ and kept in desiccators until digestion. Dry tissue was digested with 1:1 HNO_3 (Suprapur grade, Merck) and samples were fumed to near dryness on a hot

plate at 120℃ for overnight. After digestion, the residue was dissolved in 20 mL of 0.2 N HNO_3 and kept in a refrigerator until analysis for Cd. The Cd concentrations in the tissues were measured using a graphite furnace atomic absorption spectrophotometer (AAS, Perkin-Elmer 3300, USA) with a HGA 600 graphite system. The Cd concentrations in the tissues of rockfish were expressed as μg/g dry wt. An accumulation factor (AF) is often used to compare the body burden of an organism with the degree of contamination in the water. The following definition is used here:

$$\text{Accumulation Factor (AF)} = \frac{[Me]_{exp} - [Me]_{control}}{[Me]_{diet}}$$

Where $[Me]_{exp}$, $[Me]_{control}$, $[Me]_{diet}$ are the metal concentration in the experimental group, control group and diet, respectively, in μg/g (Holwerda, 1991). An elimination rate (%) is used as a percentage of decrease from the initial value (60 days).

Cell response and kidney tissue processing

Ten fish were sampled from each tank every 10 days throughout the experiment. The kidney tissues were rapidly removed and fixed with Bouin's solution. After dehydration in graded concentrations of ethanol, the kidney tissues were embedded in paraffin blocks. Four to five micron sections were stained with Mayer hematoxylin and 0.5% eosin, and alcian blue-periodic acid-Schiff's solution (pH 2.5).

Statistical analysis

Data are expressed as means ± standard error. Statistical analysis was done using a one-way analysis of variance (ANOVA) followed by Duncan's multiple comparisons test of mean values if significant differences were found ($P<0.05$).

Results

Cd accumulation and elimination

Cd accumulation and elimination in the kidney of *S. schlegeli*, as a function of exposure time and exposure concentration are shown in Fig. 1. The Cd accumulation in the kidneys of rockfish were increased with exposure periods and concentrations for the 60 days of dietary Cd exposure. During Cd exposure periods, Cd accumulation in kidneys at high exposure concentration (25 and 125 mg/kg) was significantly elevated above the Cd concentration in the control. After 60 days of dietary Cd exposure, Cd

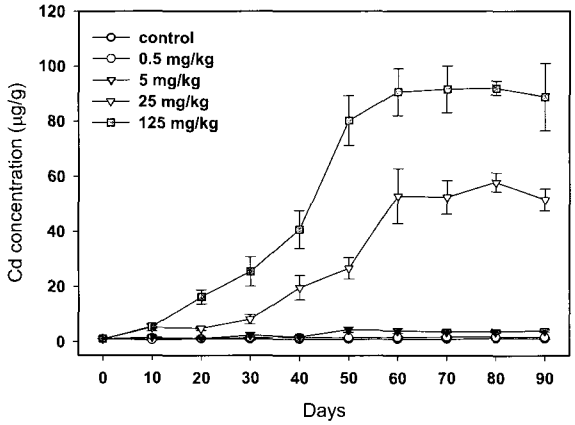

Fig. 1. Changes of Cd concentration in the kidney of the juvenile rockfish, *Sebastes schlegeli* exposed to dietary Cd for 60 days, followed by a depuration period of 30 days (mean±SEM; n=10).

accumulation values in kidneys were 52.9±9.94 μg/g (a 60-fold increase compare to the control) and 90.6± 15.7 μg/g (102-fold) for those exposed to 25 mg/kg and 125 mg/kg-Cd, respectively. For rockfish exposed to a 25 mg/kg and a 125 mg/kg-Cd diet, the following linear relations were obtained: Cd (25 mg/kg)=7.621 days - 13.649 (r^2=0.808, $P<0.05$) and Cd (125 mg/kg)=15.825 days - 26.251 (r^2=0.920, $P<0.05$). On the other hand, Cd accumulation was not significantly different from that in the control at 0.5 mg/kg and 5 mg/kg-Cd diet during 40 days of dietary Cd exposure. The Cd accumulation in kidney was significantly increased after 50 and 60 days at 5, 0.5 mg/kg. After 60 days of Cd dietary exposure, the Cd concentrations were 3.94±0.79 and 1.65±0.38 μg/g, and was approximately 2-fold and 4-fold higher than in the control diet group at 0.5 and 5 mg/kg Cd diet group, respectively. The accumulation factors are presented for the kidney at 0.5, 5, 25 and 125 mg/kg-Cd dietary exposure in Fig. 2. The accumulation factors increased based on the exposure period in the kidneys.

Cd elimination in the kidney of rockfish did not vary significantly and remained constant after the cessation of the dietary Cd exposure (Fig. 1). At the end of depuration, the Cd concentration value was 1.70±0.46, 3.98±0.42, 51.50±3.95 and 88.80± 12.30 μg/g at the 0.5, 5, 25, 125 mg/kg-Cd, and the Cd concentration was similar to that of the 60 days value, respectively.

Cell responses

The kidney of the control fish had normal appea-

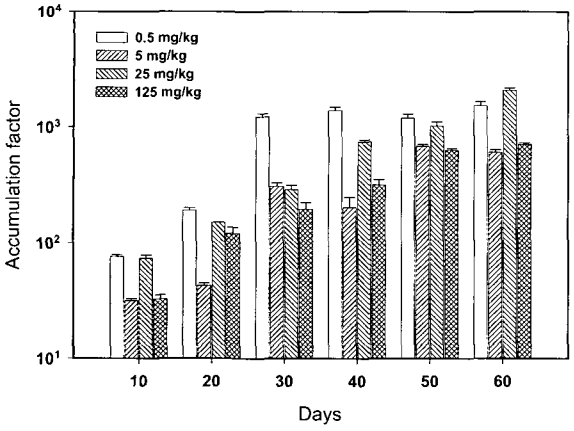

Fig. 2. Accumulation factor in kidney of the juvenile rockfish (*Sebastes schlegeli*) exposed to dietary Cd (mean±SEM; n=10).

rance at all times (Fig. 3A). In Cd dietary exposed fish at 5 mg/kg (10 days), the tissue showed swelling of the capillary of glomerulus. Also there was hydropic swelling of the pyknotic nuclei, some hyaline droplet accumulation and microvilli with positive reactions to alcian blue in tubular cells (Fig. 3B). In addition, there were a lot of hyline droplets and the microvilli displayed a positive reaction to the alcian blue in tubular cells at 25 mg/kg (30 days), and this was observed in many cells (Fig. 3C). After 40 days of the Cd dietary exposure, kidney changes were characterized by glomerular alternation and vacuolar accumulation in tubules at 25 mg/kg (Fig. 3D, E). At 250 mg/kg (60 days) and 125 mg/kg (50 days), there were abnormal glomerulus and fused renal tubules (Fig. 3F). After 60 days of exposure, the kidneys of the rockfish occurred a distinct necrosis of the renal tubule within the 125 mg/kg Cd diet group (Fig. 3G).

Discussion

Metal accumulation in tissues of aquatic animals is dependent upon exposure concentration and periods as well as some other factors such as salinity, temperature, interacting agents and metabolic activity of tissue (Heath, 1995; Ay et al., 1999). Moreover, it is also known that metal accumulation in the tissues of fish is dependent upon the rate of uptake, storage and elimination (Roesijadi and Robinson, 1994; Heath, 1995). In the present experiment, Cd accumulation in the kidney of juvenile rockfish were increased with exposure periods and concentrations during 60 days of exposed to dietary Cd. Similar patterns of Cd accumulation in kidney were also shown in other studies carried out with aquatic animals (Kumada et al., 1980; Harrison and Klaverkamp, 1989; Kraal et al., 1995; Berntssen et al., 2001).

Generally, kidney in the fish tends to concentrate metals, and plays major role in detoxification and excretion of metals through induction metal-binding proteins, such as metallothioneins (MTs). This is closely related to heavy metal exposure and metal taken up from the environment can be detoxificated by binding on these proteins (Roesijadi and Robinson, 1994). In rockfish, Cd accumulation in the kidney was significantly increased with exposure periods and concentrations for 60 days. Kraal et al. (1995) found thatthe Cd accumulation in the kidney of carp (*Cyprinus carpio*) was 10-fold higher than in the liver, and more effective than in the liver. Theses same results were observed the other studies of Cd dietary exposure (Kumada et al., 1980; Harrison and Klaverkamp, 1989; Handy, 1993; Berntssen et al., 2001). However, other studies show contrasting results, where Cd accumulation in the kidney was lower than that of liver (Woodworth and Pascoe, 1983; Handy, 1993). Kraal et al. (1995) suggested that the relative importance of the different target organs for Cd accumulation varies strongly with species. Although little literature has explained this difference, it can be assumed that the kidney of rock fish are an important storage organ for dietary Cd. However, more effective storage organs than the kidney, with regard to dietary Cd are necessary when considering various factors such as fish species, life cycle and habitat.

Several factors influence the elimination of metal from the tissues of fish. These include time, temperature, interacting agents, age of fish, metabolic activity of fish and biological half-life of the metal (Larsson et al., 1985; Douben, 1989; Heath, 1995; Kargin, 1996; Nielsen and Andersen, 1996). A Cd elimination route may include the renal pathway, and digestive system through elimination with feces. In addition, diapedesis (one-way migration of molluscan hemocytes from internal tissue through the epithelial layer, and into either the gut lumen or the surrounding water), may also be principle Cd excretion routes in aquatic animal (Roesijadi and Robinson, 1994). In this study, Cd elimination in the kidney slowly decreased or remained constant. Harrison and Klaverkamp (1989) found that Cd elimination in the kidney and the liver of rainbow trout (*Salmo gairdneri*) and whitefish (*Coregonus clupeaformis*) remained fairly constant

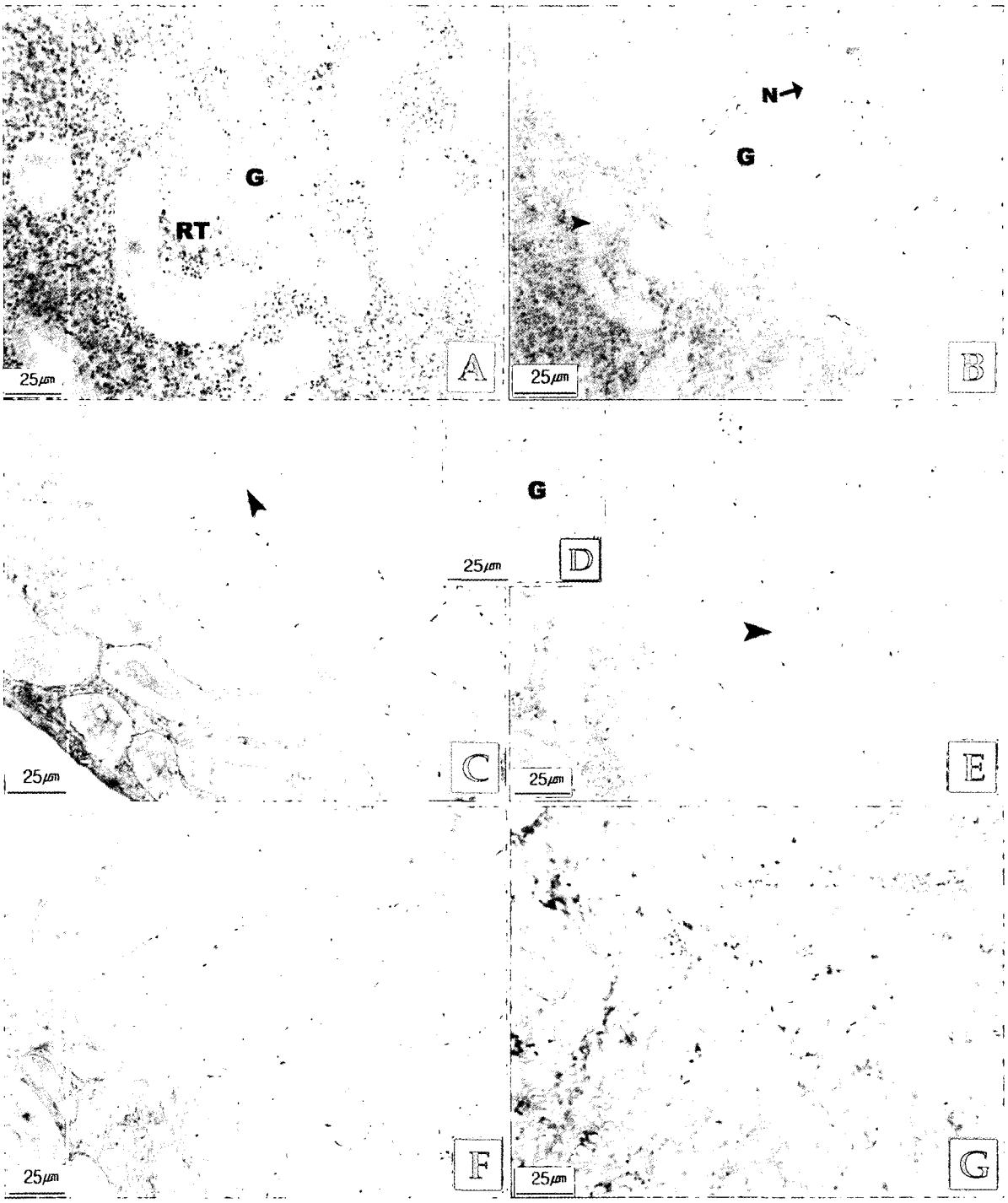

Fig. 3. Histopathological changes of kidney of the rockfish (*Sebastes schlegeli*) exposed to dietary Cd. A, Control. Note normal glomerulus and renal tubule; B, Cd 5 mg/kg (10 days). Note the swelling of capillary of glomerulus. And hydropic swelling with pyknotic nuclei (arrow), some of hyaline droplet accumulation (arrowhead) and the microvilli with positive reaction by alcian blue in tubular cells; C, Cd 5 mg/kg (30 days). Note a lot of hyline droplet (arrowhead) and the microvilli with positive reaction by alcian blue in tubular cells; D, Cd 25 mg/kg (40 days). Note the glomerular alternation; E, Cd 25 mg/kg (40 days). Note the vacuolar accumulation in tubules (arrowhead); F, Cd 25 mg/kg (50 days). Note abnormal glomerulus and the fused renal tubule; G, Cd 125 mg/kg (60 days). Note the necrosis of renal tubule. Abbreviations: G, glomerulus; N, nuclei; RT, renal tubule.

during the depuration phase. Kuroshima (1987) also reported that Cd level in kidney of girella (*Girella punctata*) remained constant or slowly increased after the end of exposure, and he suggested that Cd once taken up in a body is hardly excreted but is redistributed among tissues. Moreover, marine teleosts have fewer glomeruli so must function exclusively as a secretory kidney than freshwater teleosts and probably would have little ability to excrete metals via the routes (Heath, 1995). Therefore, it can be concluded that the capability for the elimination of Cd in urine may be insufficient, and hepatic-biliary excretory route is a less important elimination route. Clearly, more information is required on the elimination of Cd when fish are exposed to Cd pollution.

The principal function of the teleost kidney is the maintenance of a stable internal environment with respect to water and salts. Studies concerning the histopathological effects of Cd toxicity in teleost fish have been limited to investigations involving waterborne Cd. You et al. (1978) observed thickening of the basal lamina of tubule cells in the crucian carp (*Carassius carassius*) treated with Cd. In the olive flounder (*Paralichthys olivaceus*) treated with PCBs (Kim et al., 2003) and Cu (Lee et al, 2001), they observed glomerular alternation and a microvilli with positive reaction to the alcian blue of the tubular cells. Also, histological changes observed in various studies on kidneys exposed to pollutants include the dilation of the capillary of the glomerulus, swelling, hyaline droplets and vacuole in tubular cells. Besides tubular degeneration and necrosis were observed (Thophon et al., 2003). In this study, increased dietary Cd to all exposure groups for 60 days showed that kidney tissue has a more distinct glomerular alternation, hydropic swelling of the tubular cell within the pyknotic nuclei, and vacuole, the microvilli with a positive reaction to the alcian blue and necrosis of the tubular cells. Lee et al. (2001) reported that the microvilli with a positive reaction to the alcian blue of the tubular cells is presumed to be linked to the malfunction of the renal tubule. Also, thickening of the basal lamina in the renal tubule promote metabolic anomalies. Finally, extensive damage of the renal tubule or glomerulus can lead to replacement of the tubule by interstitial lymphoid tissue (Hibiya, 1982).

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