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Author(s)	Oh, Myung-Joo; Yoshimizu, Mamoru; Ueda, Hiroshi; Kimura, Takahisa; Ezura, Yoshio
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Histopathology of the New Virus Infection with Abnormal Swimming in Coho Salmon (*Oncorhynchus kisutch*)

Myung-Joo Oh*¹, Mamoru Yoshimizu*¹, Hiroshi Ueda*²,
Takahisa Kimura*¹ and Yoshio Ezura*¹

*¹Laboratory of Microbiology, Faculty of Fisheries, Hokkaido University,
Minato, Hakodate, Hokkaido 041, Japan

*²Toya Lake Station for Environmental Biology, Faculty of Fisheries,
Hokkaido University, Tsukiura, Abuta, Hokkaido 049-57, Japan

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Histopathological studies were made on a virus-infected coho salmon (*Oncorhynchus kisutch*) characterized by an abnormal swimming. Both naturally and artificially infected coho salmon showed necrosis in the kidney, including the hematopoietic elements and renal tubular epithelial cells, and also degenerative changes of melanomacrophage centers. In the brain, vacuolar degeneration with necrosis of nerve cells, congestion of blood vessels and degenerated nerve fibers were observed. By the EM, viral particles were seen in the cytoplasm of tubular cells and the macrophages in the kidney and in the axons of necrosed nerve cells in the brain.

Key words: virus infection, histopathology, coho salmon, abnormal swimming, artificial infection

Since 1991, a viral disease has been observed among the cultured salmonids; coho salmon (*Oncorhynchus kisutch*), masu salmon (*O. masou*), rainbow trout (*O. mykiss*), iwana (*Salvelinus pluvius*) and ayu (*Plecoglossus altivelis*) in the northern part of Japan. The dominant characteristic of the disease is an abnormal swimming behavior in the infected fry, which included spinning swimming, and lethargic behavior in adult fish. The abnormal swimming behavior is the terminal sign of the disease. A virus was isolated from the brain and kidney of diseased fish and described by Oh *et al.* (1995a).

Pathogenicity of the isolated virus was tested on five species of salmonids; coho salmon, masu salmon, steelhead trout (*O. mykiss*), iwana and ito (*Hucho perryi*). Cumulative mortalities in these fish ranged from 30 to 63% by intramuscular injection and 6 to 34% by waterborne infection. Moribund fish displayed the same behavior as naturally infected fish. The virus and viral antigen were detected in the kidney of infected fish in the early stage, and then the blood and brain (Oh *et al.*, 1995b).

In this paper we describe the histopathological changes in naturally and artificially infected coho salmon observed under light and electron micro-

scopes.

Materials and Methods

Naturally infected fish

Moribund coho salmon (average body weight, 17.5 g) were collected in Miyagi Prefecture in July, 1992.

Experimental infection

The virus strain BrCo-9221 isolated from the brain of an abnormally swimming coho salmon among the fish mentioned above was used. This strain was cloned by plaque method and replicated in CHSE-214 cell line with MEM₁₀ at 15°C for 7 days. Passage number was twelve.

Coho salmon (1.5 g of average body weight) were infected by waterborne and intramuscular injection. Doses were 10^{3.5} TCID₅₀/ml and 10^{3.5}TCID₅₀/fish, respectively.

The details of the experimental infection procedures were described previously (Oh *et al.*, 1995b).

Preparation of specimens

Kidney and brain were examined on 8 naturally

infected fish. Each 5 waterborne-infected, intramuscularly injected and control fish were sampled 15 to 30 days post infection. They were fixed in phosphate buffered 10% formalin solution for light microscopical histopathology. Either tissues were embedded in paraffin, cut at $5\ \mu\text{m}$ in thickness and stained with Meyer's hematoxylin and eosin (H & E). In addition, the brain sections were stained with Kluver-Barrera's stain.

The kidneys and brains of naturally and artificially infected fish were fixed in 1% glutaraldehyde and 4% paraformaldehyde in a 0.1 M cacodylate buffer (pH 7.4) for ultrastructural studies. Fixed samples were cut into cubes not larger than $1\ \text{mm}^3$, and post-fixed in 1.5% osmium tetroxide in the same buffer for 1 h. After rinsing in the same buffer, the materials were serially dehydrated in graded concentrations of ethanol and embedded in Epon. The thick sections were cut at $1\ \mu\text{m}$ and stained with 1% toluidine blue in 1% sodium borate. Ultrathin sections were stained in 5% uranyl acetate in methanol and lead citrate, and observed with a Hitachi H-7000 TEM.

Results

Histopathological changes in naturally infected coho salmon

In coho salmon cultured in Miyagi Prefecture, diseased signs including spinning swimming behavior appeared in first feeding fry to three month old fry.

In most of the fish examined histopathologically, renal tubular epithelial cells showed vacuolar degeneration in their basal areas of cytoplasm (Fig. 1). Diffuse hemorrhage was observed in various particles of the haematopoietic tissue, though not clearly shown in Fig. 1. Sections of the brains revealed encephalopathy. The mesencephalon showed marked empty basket-like cavitations possibly caused by necrosis of Purkinje's cells (Fig. 2).

Disease signs and histopathological changes in artificially infected coho salmon

Most of the artificially infected fish showed spinning swimming prior to death. Mortality started 15 days post infection and continued till 30 days post infection. Some of the moribund fish did not show spinning swimming, but displayed lethargic behavior

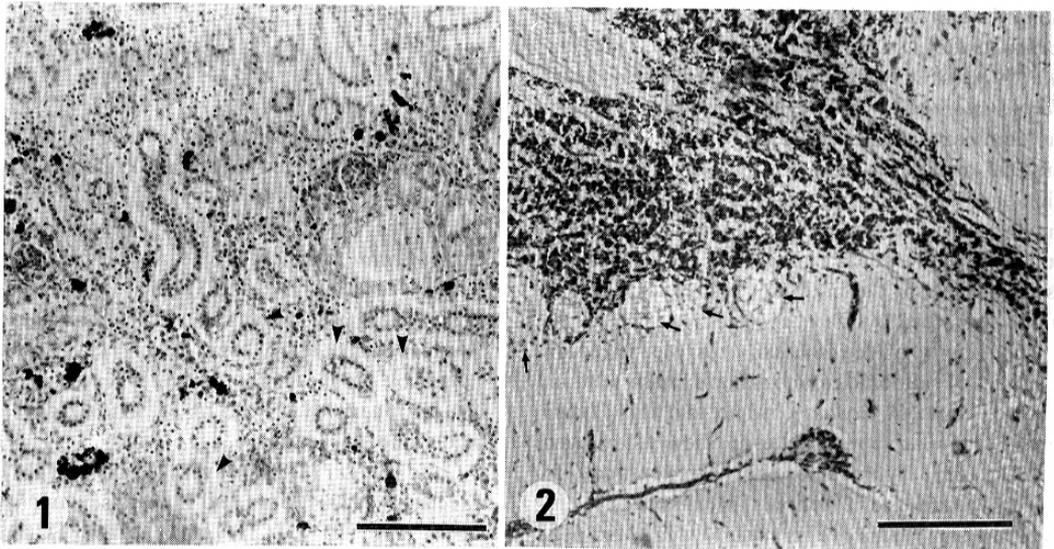


Fig. 1. Kidney of a naturally infected coho salmon. Showing generalized degeneration of the renal tubular epithelial cells (small arrowheads). Hematoxylin-eosin. Bar: $100\ \mu\text{m}$.

Fig. 2. Metencephalon of a naturally infected coho salmon. Showing marked empty basket-like cavitations (arrows) possibly due to necrosis of Purkinje's cell. Hematoxylin-eosin. Bar: $100\ \mu\text{m}$.

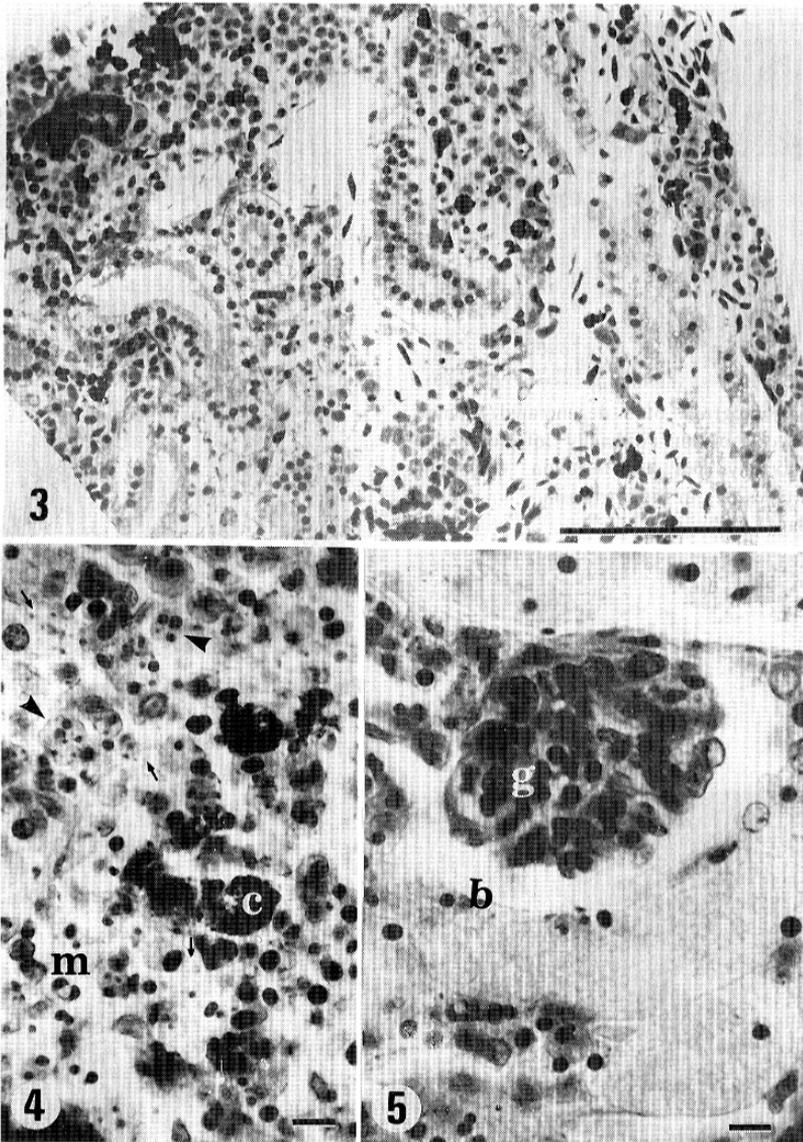


Fig. 3. Mesonephros of an artificially infected coho salmon 21 days post water-borne (WB) infection. Showing various degree of necrosis in tubular cells and appearance of large gaps in the interstitial tissue with degeneration of hematopoietic elements. Toluidine blue. Bar: 100 μ m.

Fig. 4. Pronephros of an artificially infected coho salmon 28 days post intramuscular (IM) injection. Showing many hematopoietic cells undergoing necrosis indicated by karyorrhexis (arrowheads) and karyolysis (small arrows), infiltration of possibly macrophages (m) in the hematopoietic tissues, and degenerative changes of melanomacrophage center (c). Hematoxylin-eosin. Bar: 10 μ m.

Fig. 5. Malpighian corpuscle of an artificially infected coho salmon 28 days post IM injection. Showing an increase in Bowman's space (b) and atrophy of the glomerulus (g). Hematoxylin-eosin. Bar: 10 μ m.

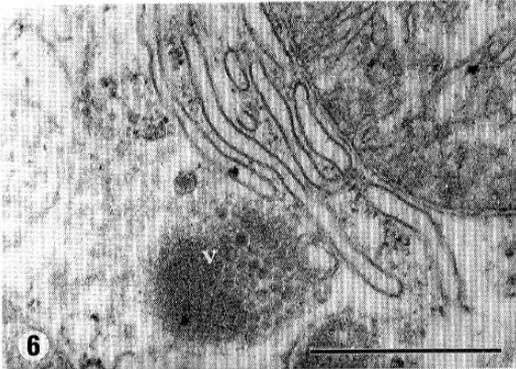


Fig. 6. Electron micrograph of a degenerated tubular epithelial cell of an artificially infected coho salmon 15 days post IM injection. Showing virus particles (V) in the cytoplasm. TEM. Bar: 1 μ m.



Fig. 8. High magnification of Fig. 7. Showing virus particles (arrowhead) in the cytoplasmic vesicles of a macrophage. TEM. Bar: 1 μ m.

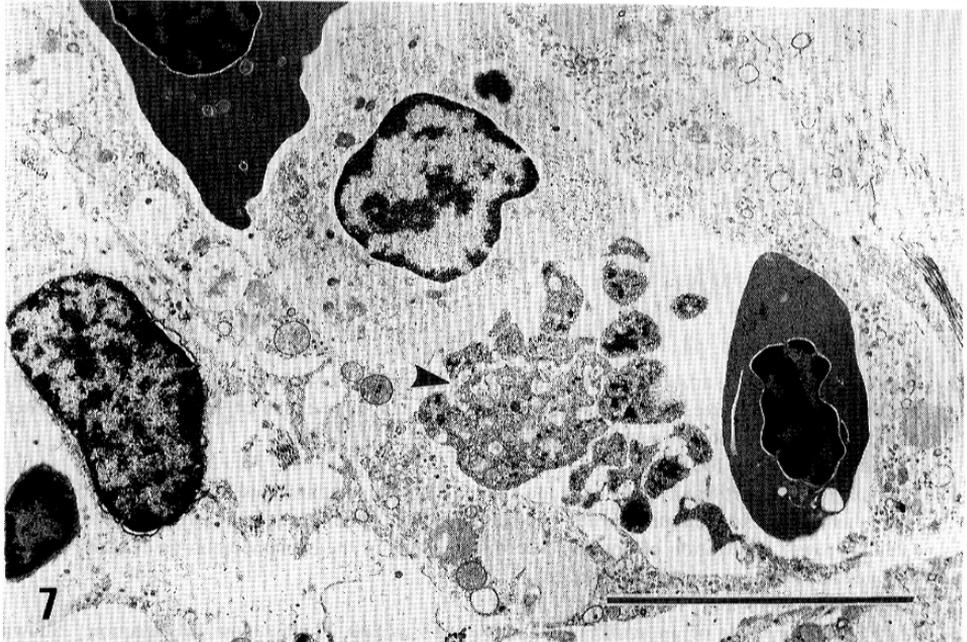


Fig. 7. Electron micrograph of a macrophage in a capillary of kidney of an artificially infected coho salmon 21 days post WB infection. Showing numerous vesicles (arrowhead) in the cytoplasm. TEM. Bar: 10 μ m.

with dark skin and soon died. Exophthalmia was generally observed and some fish showed hemorrhage in the dorsal and caudal fins.

Kidney: Hematopoietic tissues and tubules of the kidney were severely affected. Both showed marked

degeneration and various degrees of necrosis. In the mesonephros of the fish observed 21 days post infection, tubular epithelial cells showed necrosis, and large gaps were found in the interstitial tissues due to partial disappearance of hematopoietic elements

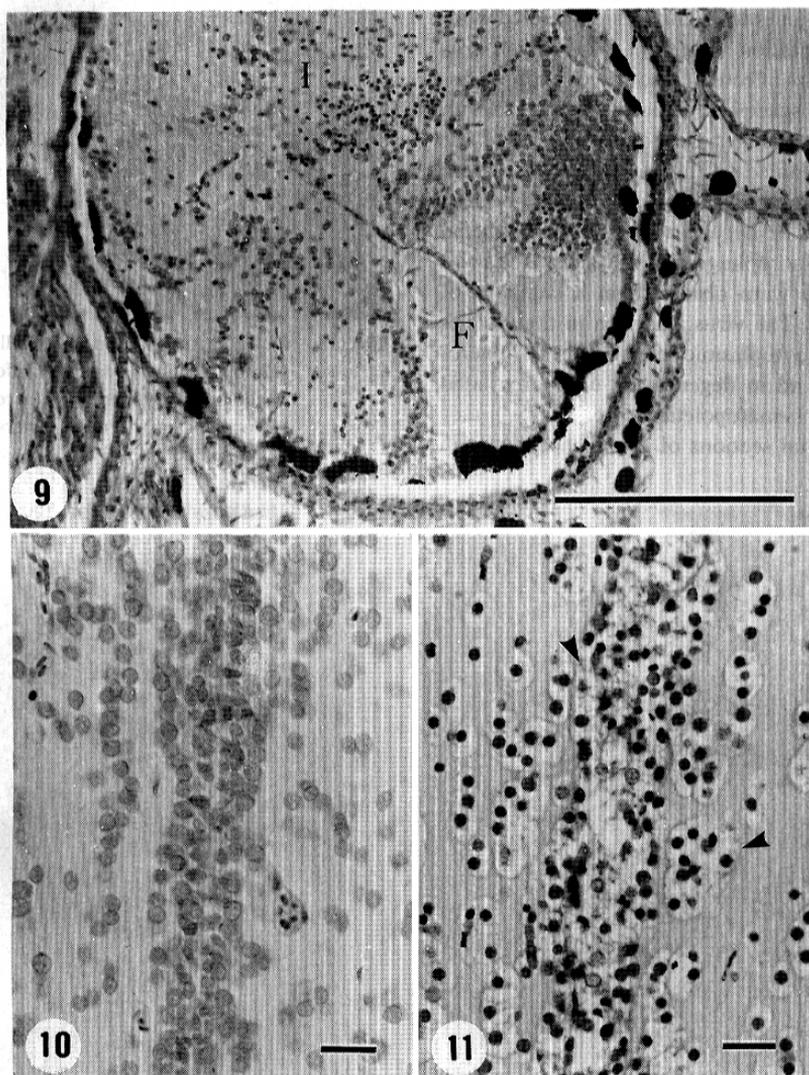


Fig. 9. Telencephalon of an artificially infected coho salmon 21 days post WB infection. Showing a liquefactive focus (F) and inflammatory cells (I). Hematoxylin-eosin. Bar: 100 μ m.

Fig. 10. Molecular layer of the optic tectum of a control coho salmon. Showing normal molecular layer. Hematoxylin-eosin. Bar: 10 μ m.

Fig. 11. Molecular layer of the optic tectum of an artificially infected coho salmon 28 days post WB infection. Showing necrosis of the nerve cells in the molecular layer with formation of formy area (arrowheads) due to loss of cytoplasm, leaving distinct, condensed pyknotic nuclei. Hematoxylin-eosin. Bar: 10 μ m.

(Fig. 3). Hematopoietic tissues of pronephros were extensively affected, and karyorrhexis and karyolysis were found in non-differentiated blast cells. Infiltrate of mononuclear inflammatory cells, morphologically indistinguishable from macrophages was observed in the lesions. Melanomacrophage centers in the lesions showed degenerative changes associated with slightly depigmentation (Fig. 4). Malpighian corpuscula were also affected, associated with an increase Bowman's space and atrophy of the glomeruli (Fig. 5). These changes were observed all of the moribund fish obtained on the day 28 post infection.

Under EM, numerous viral particles could be seen in the cytoplasm of degenerated tubular epithelial cells (Fig. 6). Macrophages with numerous vesicles in the cytoplasm were observed on a capillary of kidney (Fig. 7). The virus was seen in the vesicles and outside of cytoplasmic membrane of macrophage (Fig. 8) and in degenerated undifferentiated blast cells of the hematopoietic tissue.

Brain: Horizontal sections of the telencephalon in

the moribund fish showed liquefactive foci (Fig. 9). In contrast to control fish (Fig. 10), infected fish on day 28 post infection showed necrosis of the cells in the part of the molecular layer of the mesencephalon,



Fig. 13. Optic tectum of an artificially infected coho salmon 28 days post WB infection. Showing vacuolated degeneration (arrowheads) in the molecular and granular layers and congestion of a blood vessel in the cerebella valvula (arrows). Hematoxylin-eosin. Bar: 100 μ m.

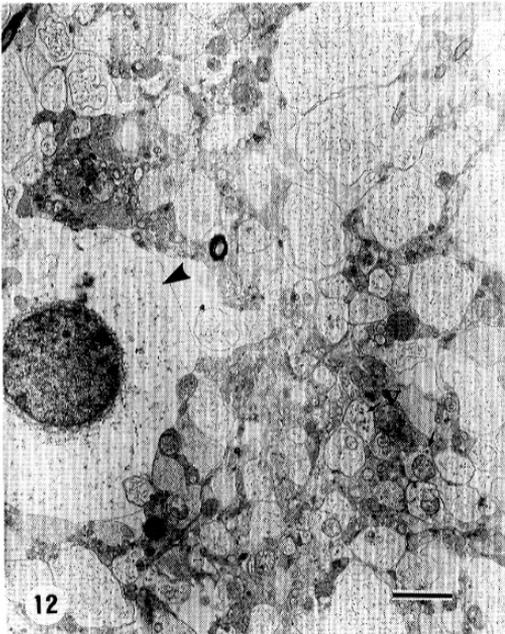


Fig. 12. Electron micrograph of a degenerated nerve cell (arrowhead) of the molecular layer of optic tectum of an artificially infected coho salmon 28 days post WB infection. Showing loss of cytoplasm and denser nuclei and virus particles (V) in the axons. TEM. Bar: 1 μ m.

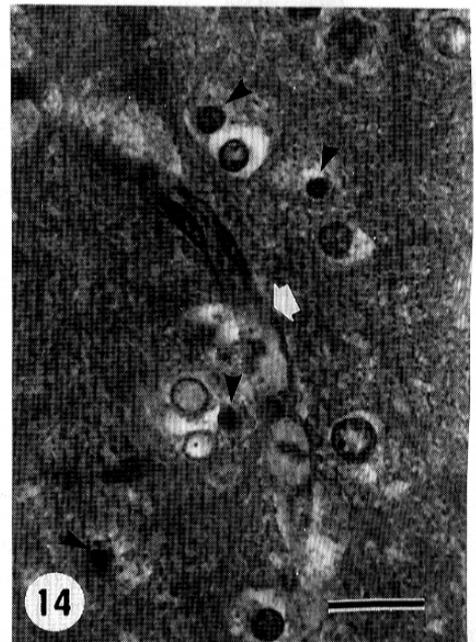


Fig. 14. Optic tectum of an artificially infected coho salmon 28 days post WB infection. Showing neural phagocytic glia-like cells (arrowheads) around the blood vessel (arrow). Kluver-Barrera. Bar: 10 μ m.

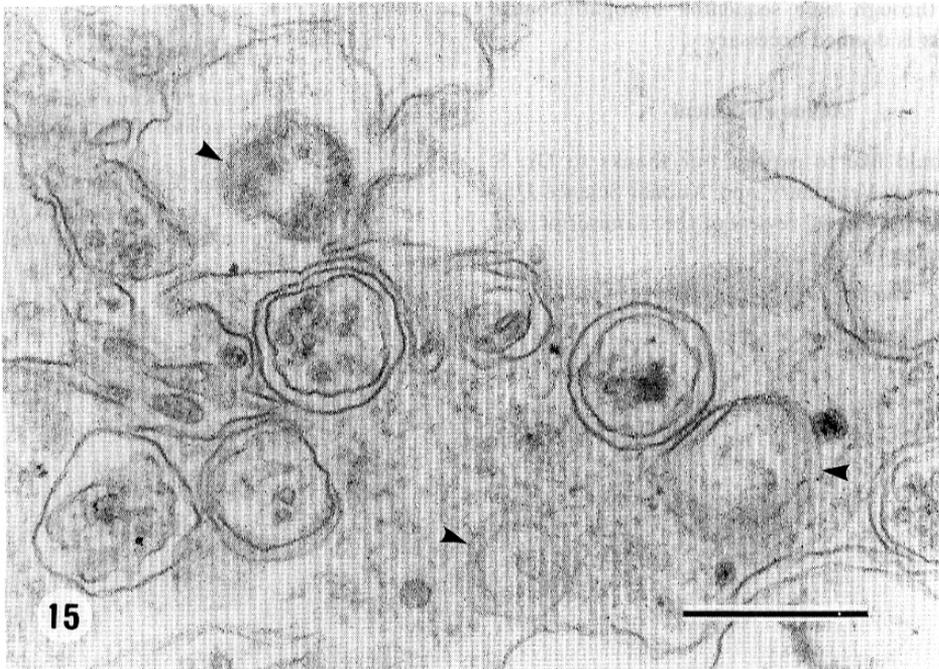


Fig. 15. Electron micrograph of molecular layer of the optic tectum of an artificially infected coho salmon 28 days post WB infection. Showing degenerative changes (arrowheads) of nerve fibers. TEM. Bar: 1 μ m.

with the formation of formy areas due to loss of cytoplasm, leaving condensed pyknotic nuclei (Figs. 11 and 12). Virus particles were detected in axons of necrosed nerve cell under EM observation (Fig. 12). Examinations of the molecular and granular layers of the optic tectum showed vacuolation (Fig. 13). Phagocytic glia-like cells were observed around the vacuolated areas (Fig. 14). Congestion of blood vessel was observed in various parts of the brain and some part of the brain were undergoing weak hemolysis. Most of the nerve fibers in the optic tectum indicated degenerative changes when observed under EM (Fig. 15).

Discussion

In a previous report (Oh *et al.*, 1995b), we suggested that the primary target organ of the new virus, i.e. the causative agent of the disease characterized by an abnormal swimming was the kidney, and the replicated virus spreaded through the circulatory system from the kidney to other organs and attacked the brain seriously, which probably resulted

in the observed abnormal swimming behavior.

In this experiment, the kidney were shown to be most severely affected by the virus. Infected cells showed various stages of necrosis, with marked nuclear karyorrhexis and karyolysis. Virus particles were found in the cytoplasm of renal tubular epithelial cells, and in the cytoplasm of macrophages and in degenerated undifferentiated blast cells of the hematopoietic tissue.

The brain showed focal liquefactive degeneration, and the cells in the molecular layer of the optic tectum seemed to have lost their cytoplasm, forming a halo area with a densed nucleus. This specific changes were described as vacuolar degeneration. Macrophages, i.e., glia cells, were found around necrotic sites. Congestion of blood vessels was observed and some parts of the brain were undergoing weak hemolysis. Virus particles were observed in the axon.

From these features, it is apparent that the kidney and brain were significantly affected by the virus infection. It is able to mention that the mechanism of abnormal swimming and lethargic behaviors are related to the virus infection of the brain. However,

a more through time sequential histopathological study thus is deemed necessary.

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