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Oncorhynchus masou virus: Pathological Changes in Masu Salmon (*Oncorhynchus masou*), Chum Salmon (*O. keta*) and Coho Salmon (*O. kisutch*) Fry Infected with OMV by Immersion Method*[1]

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One and 3-month-old masu salmon *Oncorhynchus masou*, 1-month-old coho salmon *O. kisutch* and 2-month-old chum salmon *O. keta* were infected with *Oncorhynchus masou* virus (OMV) by immersion method. Moribund specimens were then examined histopathologically.

The kidney was found to be the principal target organ for the virus as judged by severity of histopathological changes found in the infected 1-month-old masu salmon. Necrosis of epithelial cells was also observed in the early moribund specimens while partial necrosis of the liver, spleen and pancreas was seen in later moribund specimens from this group.

Necrosis of the kidney haematopoietic tissue was observed in infected 3-month-old masu salmon. Hyperplasia of the haematopoietic cells was simultaneously observed with necrosis in later moribund specimens from this group. While the kidney was considered to be the early target organ for OMV, it gradually became resistant to OMV infection. For this reason, it was considered that the principal target organ changed from the kidney to the liver and marked histopathological changes were observed in the later stages. Foci of necrosis in the liver tended to become more severe with longer incubation period. Hepatocytes showing margination of chromatin were present. Cell degeneration in the spleen, pancreas, cardiac muscle and brain was also observed.

Histopathological changes observed in coho salmon and chum salmon were the same as those of masu salmon.

We have previously described *Oncorhynchus masou* virus (OMV) which was isolated from ovarian fluids of landlocked masu salmon *Oncorhynchus masou* in September 1978 at the Otobe Salmon Hatchery, Hokkaido, Japan.[1,2] This virus was found to be lethal to fry of chum salmon *O. keta*, masu salmon, kokanee salmon *O. nerka*, coho salmon *O. kisutch* and rainbow trout *Salmo gairdneri*.[1,3] In particular, the first 3 species usually exhibited high susceptibility with more than 80% of the fry dying within 4 months after infection. Affected fry frequently became dark and occasionally showed severe exophthalmia and haemorrhage under the jaw before death. Internally, all of the kidney was pale and multiple white spots were observed on the liver.

The histopathologic changes brought about in chum salmon fry infected at 5-month-old in presense to OMV infection have been briefiy described by KIMURA *et al.*[1,2] In this report we describe in more detail the histopathologic changes caused by OMV in salmonids of different ages and species.

Materials and Methods

One hundred frys were used in each age group: 1-month-old and 3-month-old masu salmon, 1-month-old coho salmon and 2-month-old chum salmon. These fishes were immersed for one hour in 10°C water containing 100 $TCID_{50}$/m*l* culture grown OMV and then held in running water at 10–15°C.

Mortality was recorded daily until the end of the 4 month experiment as previously reported.[3]

Frys showing clinical signs were sampled from the following groups: eleven from 1-month and 3-month-old masu salmon; five from 1-month-old coho salmon; ten from 2-month-old chum salmon. Five control fish from each age group were also

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Table 1. List of fish employed

Fish group	Fish No.	Days post-infection
One-month-old masu salmon	1	32
	2	39
	3	46
	4	47
	5	48
	6	48
	7	51
	8	53
	9	54
	10	56
	11	56
Three-month-old masu salmon	1	30
	2	38
	3	39
	4	41
	5	45
	6	46
	7	46
	8	52
	9	52
	10	59
	11	62
One-month-old coho salmon	1	11
	2	13
	3	24
	4	25
	5	26
Two-month-old chum salmon	1	19
	2	25
	3	26
	4	26
	4	26
	6	27
	7	29
	8	29
	9	30
	10	30

taken (Table 1). Specimens were fixed in BOUIN's solution and then transferred into 90% ethanol until processed. Fish were dehydrated in graded alcohol, and embeded in paraffin wax. Sections were cut at 4–6 μm and stained with haematoxylin and eosin stain.

Results

One-month-old Infected Masu Salmon Fry

Mortality of 1-month-old infected masu salmon was 87% in the ensuing 4 months and eleven specimens of them were examined.

Marked histopathologic changes were observed in the kidney of all specimens examined. The pathology was characterized by syncytium formation (Fig. 1), typical of cells infected with *Herpesvirus*, and necrosis of the haematopoietic tissue (Fig. 2). Haematopoietic and reticular cells showed hypertrophy, pyknosis and lysis. The anterior kidney was characterized by severe degeneration and the haematopoietic cells of the middle kidney were reduced in number. Glomeruli and tubules were frequently not affected. Some pyknosis was observed in the renal epithelial cells surrounding the degenerated haematopoietic tissue.

Necrosis of the epidermal cells which sometimes extended into the connective tissue was observed in the mouth, jaw, operculum and head of the five early moribund specimens (Fig. 3). Numerous granules were present in the degenerated cells.

The liver was atrophied and necrosis was observed in the later moribund specimens (Fig. 4). The lesions tended to become more severe with longer incubation period.

Partial necrosis of the pancreas and spleen was observed in the terminal case of the disease (Fig. 5, 6). Changes were found in the intestinal tract. It was found to be relaxed and the epithelial cells were atrophied (Fig. 7).

Three-month-old Infected Masu Salmon Fry

The mortality of 3-month-old infected masu salmon was 65% in the ensuing 4 months and eleven specimens were examined histopathologically.

In these fish, marked histopathologic changes were observed in the liver. As shown in Fig. 8, multiple foci of severe necrosis accompanied by pyknosis and karyorrhexis were present in all specimens examined. Syncytium formation was observed in the foci but the number of nuclei was less than that observed in RTG-2 cells infected with OMV.[1,2] Nuclei of hepatocyte surrounding the foci showed margination and aggregation of chromatin (Fig. 9). The foci was small in early moribund specimens but became severe in the later moribund specimens. In addition, last two specimens showed degeneration of all parenchyma. One specimen exhibited vacuolar degeneration of all parenchyma accompanied by nuclear swelling (Fig. 10).

Necrosis was present in the kidney of the nine early moribund specimens (Fig. 11); while hyperplasia of the haematopoietic cells was observed

in six later moribund specimens (Fig. 12). Hyperplasia and necrosis were simultaneously present in some of them. In the last two specimens, only hyperplasia of haematopoietic cells and no necrosis was observed. Thus, the kidney of 3-month-old infected masu salmon was considered to be previously infected but became resistant to OMV infection after a period of time. In the spleen, reticular cells were necrotic and lymphoid cells were necrotic and reduced in number. Haemorrhages were also observed (Fig. 13).

In the brain, mainly corpus cerebelli, necrosis and haemorhages were observed. This was considered to be related to the abnormal swimming behavior observed in the moribund fish (Fig. 14).

Partial necrosis was also observed in the pancreas.

Two-month-old Infected Chum Salmon Fry

Haematopoietic tissues in the kidney of the early moribund specimens were necrotic. In the epithelial cells of the tubule, hyaline droplet degeneration and pyknosis were observed (Fig. 15). The glomeruli were not affected.

Partial necrosis was observed in the spleen, liver, adipose tissue, pancreas and stomach. The intestine showed necrosis and frank sloughing of the mucosa into the lumen (Fig. 16).

In the later moribund specimens, necrosis of the liver was more severe than that of the kidney and all cells of the liver were necrotic.

One-month-old Infected Coho Salmon Fry

Marked histopathologic changes were observed in the liver and kidney. In the liver, vacuolation and necrosis were seen. Necrosis of the haematopoietic tissue and hyaline droplet degeneration of the tubule epithelial cells occurred in the kidney. In some of the diseased fish necrosis of the pancreas was also observed.

Discussion

One-month and 3-month-old masu salmon infected with OMV were examined histopathologically. With respect to 1-month-old masu salmon, necrosis of haematopoietic tissue in the kidney was observed in all moribund specimens. The kidney was considered to be a target organ as judged by the extensive tissue involvement. Necrosis was observed in the epithelial cells of the mouth, jaw, operculum and head of the early moribund specimens. Partial necrosis of the liver, spleen and pancreas were seen in the later moribund specimens. Because focal necrosis was first observed in the epithelial cells of early moribund specimens, the epithelial cells were presumed to be the initial target tissue. These cells were also the most likely site of initial viral infection and it was interesting that the lesions in these cells correspond to the location where tumore appeared later.[4]

In the 3-month-old infected masu salmon, haematopoietic tissue of the kidney of nine early moribund specimens were necrotic. In six specimens of long incubation (more than 46 days after infection), hyperplasia of haematopoietic cells was simultaneously observed with necrosis. In the haematopoietic cells of last two specimens, hyperplasia was observed but necrosis was absent. The kidney is therefore considered to play an important part in early OMV infection. On the other hand, histopathological foci in the liver tended to become gradually more severe with longer incubation period. There was an inverse relation between the liver and kidney, and the major target organ appeared to change from the kidney to the liver. This shift of target organs may be due to the development of the immune system in the kidney of the fry.

In the 1-month-old infected coho salmon, the prime target organ was the kidney as was the case of 1-month-old infected masu salmon. Similar to the 3-month-old masu salmon the major target organ of 2-month-old infected chum salmon was the kidney in the early moribund specimens and changed to the liver in longer incubation.

We have reported previously that the primary target organ in the 5-month-old infected chum salmon with OMV was the liver and that the kidney was not affected.[2] These results are in agreement with the later specimens of the 3-month-old masu salmon examined in this study.

Comparisons of foregoing findings with those of WOLF and SMITH[5] and SANO[6] revealed interesting similarities and some differences. WOLF and SMITH worked with rainbow trout fry parenterally infected with *Herpesvirus salmonis* and reported that the kidney was the primary target organ for the virus and that hyperplasia of the haematopoietic tissue in the kidney was observed in half of the specimens examined. There were similarities between OMV and *H. salmonis* infection in these points. The liver was one of the more important target organs for *H. salmonis*, while in the 3-month-old infected masu salmon

with OMV, the liver was the primary target organ. Some differences between OMV and *H. salmonis* in syncytium formation and gill pathology were observed. In the case of rainbow trout infected with *H. salmonis*, syncytia judged to be pathognomonic were formed in the pancreas while syncytia of salmonids infected with OMV occurred in the kidney and liver. In contrast, SANO reported leakage of a protein-like fluid into the BOWMAN'S capsule in *O. nerka* fry naturally infected with NeVTA (Nerka virus in Towada lake, Akita and Aomori Pref.). In the kokanee salmon infected with NeVTA, syncytia were formed in the kidney. While *H. salmonis* and NeVTA infection produced pathologic changes in the gill, fish infected with OMV did not develop such changes. Although *H. salmonis* and NeVTA have been regarded as the same or similar viruses,[7] OMV has the oncogenic nature which has not been reported in these two viruses.[8] More research will be necessary to determine the serological and biochemical relationships among these three viruses.

Acknowledgements

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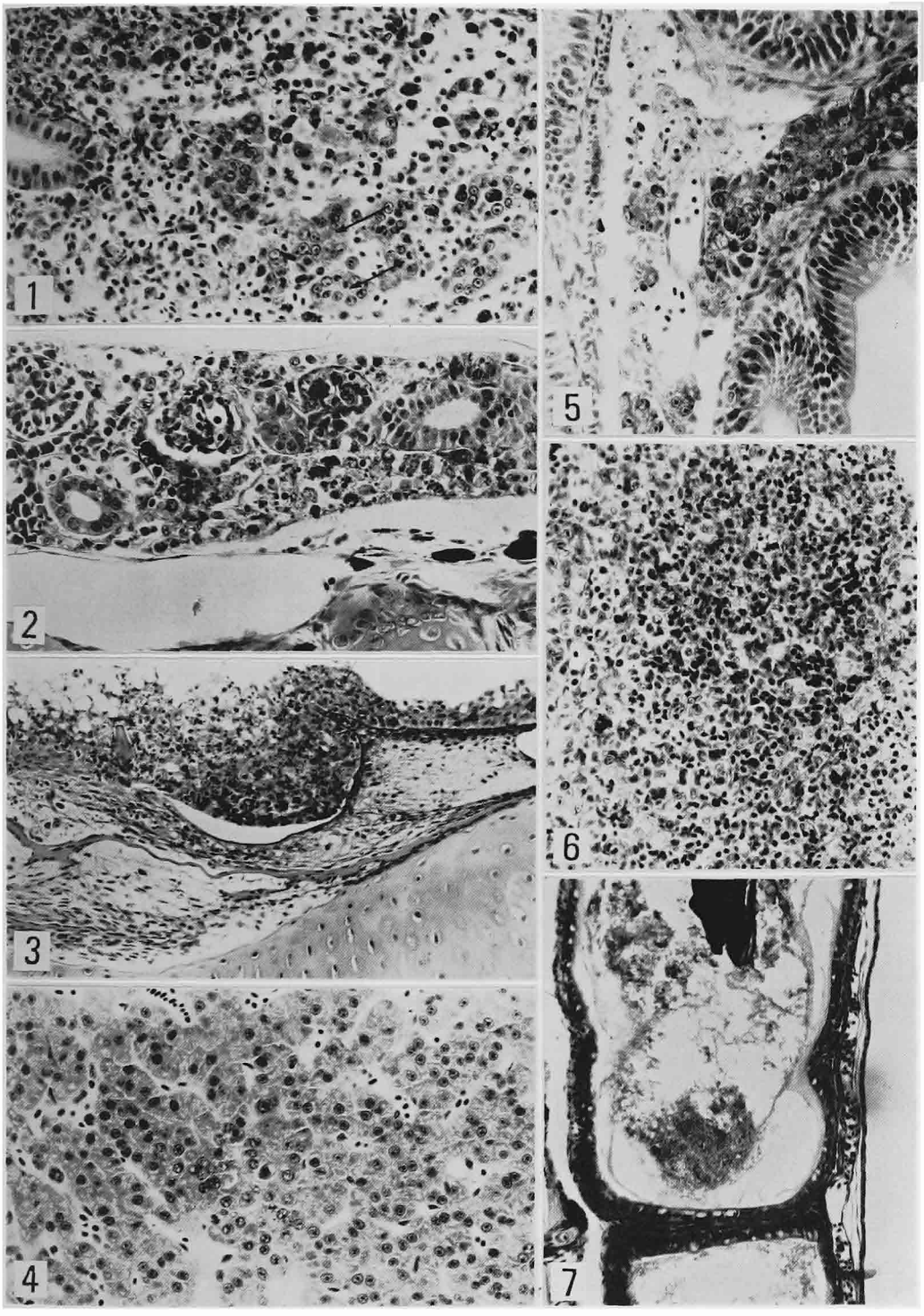
Explanation of ōgures

Fig. 1–7. One-month-old infected masu salmon fry.
Fig. 1. Syncytia (arrows) observed in the head kidney. (HE, ×300)
Fig. 2. Necrosis of haematopoietic cells in the kidney. (HE, ×300)
Fig. 3. Necrosis of epithelial cells in the mouth. (HE, ×150)
Fig. 4. Necrosis observed in the liver. (HE, ×300)
Fig. 5. Necrosis observed in the pancreas. (HE, ×300)
Fig. 6. Necrosis observed in the spleen. (HE, ×300)
Fig. 7. Change in the intestinal tract. Epithelial cells were atrophied. (HE, ×150)
Fig. 8–14. Three-month-old infected masu salmon fry.
Fig. 8. Multiple foci of severe necrosis in the liver. (HE, ×60)
Fig. 9. Necrosis of the liver. Margination (arrow A) and aggregation (arrow B) of chromatin were observed around the necrotic cells. (HE, ×300)
Fig. 10. Vacuolation in the liver. (HE, ×150)
Fig. 11. Necrosis of haematopoietic tissue in the kidney. (HE, ×300)
Fig. 12. Hyperplasia of haematopoietic cells observed at later moribund specimens. (HE, ×150)
Fig. 13. Necrosis of the spleen. (HE, ×300)
Fig. 14. Haemorrhages of the brain. (HE, ×150)
Fig. 15, 16. Two-month-old infected chum salmon.
Fig. 15. Hyaline droplet degeneration of the tubule epithelial cells in the kidney. (HE, ×300)
Fig. 16. Necrosis of the epithelial cells in the intestine. (HE, ×300)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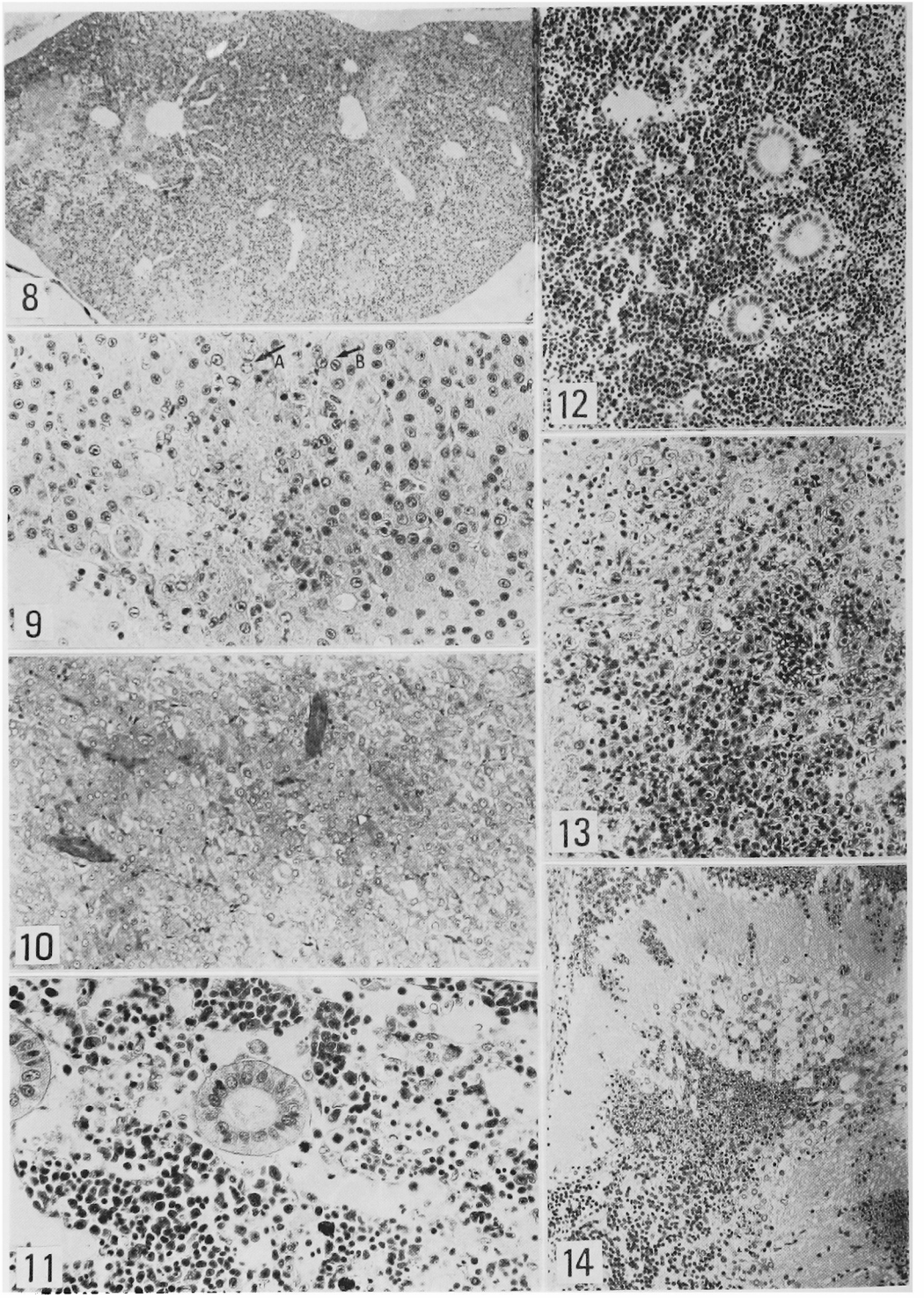
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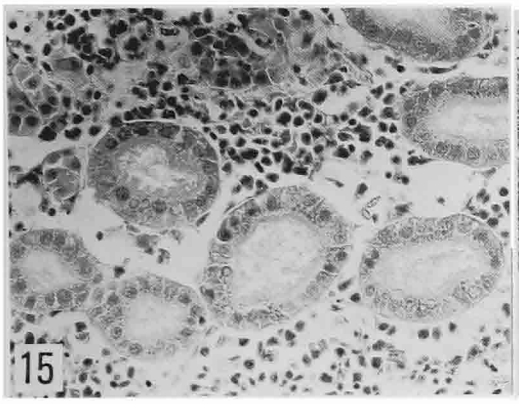
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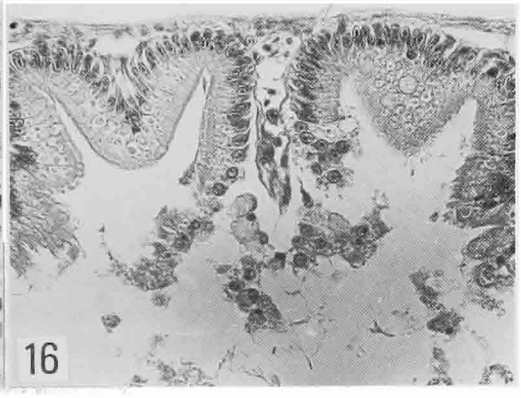
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