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A Histopathological Study on Viral Encephalitis of European Eel

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A viral disease occurred among elvers of European eel, *Anguilla anguilla* transported from France to Mie Prefecture in May of 1977. The diseased fish showed whirling and spiral motion, and white body coloration. On histopathological examinations, some of them showed necrosis in the brain, spinal cord and retina of eyeball. The other fish showed necrosis in the liver, spleen, kidney, heart, digestive tracts and the lateral musculature as well as in the brain. Above all necrotic lesions showed no invasion of bacteria and parasites. A bath with a 0.6% salt solution was effective for the survival of the diseased fish. After the treatment, inflammatory reactions were found to occur in the nectrotic lesions of the brain. A virus was isolated from the diseased fish and showed a cytopathic effect in inoculated RTG–2 cells, similar to that of EVEX(SANO *et al.* 1977). The author proposes to call this disease "Viral Encephalitis of European Eel".

Key words; viral encephalitis, european eel, histopathology,

In Japan some viruses were reported to have been isolated from cultured Japanese eel, *Anguilla japonica* (SANO 1976, SANO *et al.* 1977), imported European eel, *A. anguilla* (SANO 1976; SANO *et al.* 1977), and American eel, *A. rostrata* (SANO 1976). A virus of EVA was associated with an epizootic of American eel, however the histopathological signs confirming the viral infection were not studied (SANO 1976).

A viral disease occurred among elvers of European eel which were transported from France and reared in Mie Prefecture in the spring of 1977. This disease has not occurred since that time. In the present study, a virus was isolated from the diseased fish and the histopathological signs of the diseased fish were studied. This paper describes the histopathological signs of the infected fish and features of the cytopathic effect in inoculated RTG–2 cells.

Materials and Methods

In May of 1977, 48 diseased elvers of European eel were collected from fish-rearing ponds. For a microscopic study, 18 of those were fixed in Bouin's fluid and Herry's fluid,

processed routinely, embedded in paraffin wax, and 3—5 μm sections were stained with Mayer's hematoxylin and eosin, PAS and Giemsa. A treatment by a bath with a 0.6% salt solution was held for 20 diseased elvers in a 10—litter glass aquarium at a mean water temperature of 20 ℃ for four week. During the treatment, 4 or 6 fish were served for a microscopic study at an interval of one week. As the control, 10 diseased elvers were kept in a 10—litter glass aquarium till all fish died. The other 20 diseased elvers were used for the viral isolation*; 5 to 8 of them were homogenized, processed routinely, and inoculated onto RTG—2, FHM and EPC cells at a temperature of 20℃.

Results

Occurrence of the disease; external and internal signs

About 50kg of European eel elvers were imported from France and reared in cement-lined tanks in May of 1977. About 10 days after arrival, abnormal fish with pathological symptoms and mortalities appeared among the eels. The diseased fish showed white body coloration, spiral and whirling motion resulting in death on the bottoms of the tanks. On the tenth day a treatment by a bath with 0.6% salt solution was held, after which the mortality was decreased day by day to zero in one month. About 20% of the elvers were lost during the season as a result of this disease.

Experimental treatment

Untreated control fish all died within five days. On the other hand, fish treated by a bath with 0.6% salt solution survived from over seven days to the end of the treatment. However, during the treatment period most of those fish still showed white body coloration and whirling motion.

Histopathological signs

1. Histopathological examintion on untreated diseased elvers: Eight of eighteen fish observed showed necrosis of various parts of the central nerve system and the retina of the eyeballs. In this case, nerve cells in the olfactory bulb, olfactory lobe (Plate I —1), optic lobe, hypothalamus (Plate I —2), cerebellem, medulla oblongate and spinal cord (Plate I —4) were focally or diffusely necrotized. The granular layers especially around the ventricle of the inferior lobe of the hypothalamus were frequently necrotized (Plate I —3); the necrotized cells separated into the ventricle. On the other hand granular layers of the optic lobe and cerebellum were rarely necrotized. Necrotic nerve cells showed karyopyknosis, karyorrhexis and karyolysis. The focal necrois of nerve cells was followed by degeneration of nerve fibers and edema. In the eyeballs, the brain layer, neuroepithelial layer and pigment epithelium of the retina were extensively necrotized and destroyed, followed by granulation of pigment cells (Plate I —5). In all the necrotized lesions of the brain and retina, no invasion of

*: virological examinations were carried out by Mr. SORIMACHI of Tokyo University in 1977.

bacteria, parasites or inflammatory cells was found. Melanophores in the dermis were pyknotic. In this case, a few fish showed tiny necrotic lesions in the liver, but in most fish livers showed normal-looking hepatocyes with much glycogen or atrophic hepatocytes. Hearts, spleens, kidneys, digestive tracts, gills and lateral musculature of most fish did not show any obvious pathological change.

Ten of the eighteen elvers observed showed necrotic lesions in the gills, heart, liver, spleen, kidney, stomach, intestine and lateral musculature. In the liver, hepatic cells extensively showed cloudy swelling and necrosis. Necrotic ares extended around blood vessels showing coagulation and destruction of necrotized cells (Plate II−1). Kidney showed necrosis of epithelia of tubules and hematopoietic tissue (Plate II−2). In the affected renal tubules, necrotized epithelial cells separated into the lumen and hemorrhage also occurred. The necrotic areas of the hematopoietic tissue were occupied by necrotized cells and debries, and became edematous. Glomeruli did not show any obvious pathological change. Spleen showed necrosis of the pulps (Plate II−3). Heart showed degeneration of the cardiac muscle and necrosis of the endocardium, in which necrotic cells separated into the lumen (Plate II−4). Stomach showed focal necrosis in the muscle layers. Lateral musculature showed focal necrosis, followed by edema and hemorrhage (Plate II−5). Melanophores in the dermis were pyknotic. In this case, small focal necrosis were found to occur in the brain and retina of the eyeball of some fish. No bacterium, parasite or inflammatory cell was found to have penetrated into any of the above necrotic lesions.

2. Elvers treated with salt solution: The brain and retina of a total of eighteen fish were affected. In the first week the brain, medulla oblongata and spinal cord showed confined necrosis of nerve cells without any inflammatory reaction (Plate III−1). However in the eyeballs, many inflammatory cells were found to have infiltrated into the necrotized retina and orbital tissue (Plate III−2). Neutrophils outnumbered other inflammatory cells. Hepatic cells were atrophic in some fish or looked like normal. Other visceral organs did not show any obvious pathological change. In the second week, neutrophils were found to infiltrate into necrotic areas of the brain (Plate III−3,4), although necrotized cells and the debris still remained in the necrotic lesions. In the affected eyes, inflammatory cells infiltrated into the necrotic and edematous retina and orbital tissue. In the retina, debris of necrotized cells disappeared and unaffected cells remained (Plate III−5). Hepatic cells were markedly atrophic.

Two fish with systemic affection were found in the first week. Their livers showed the appearance of regenerating cells with basophilic cytoplasms and mitotic features, and infiltration of inflammatory cells among debris in necrotic lesions (Plate IV−1). Unaffected hepatic cells showed cloudy swelling. In the kidney, renal tubules lined by flat cells with basophilic cytoplasms and mitotic feture were found (Plate IV−2). These tubules still contained debris of necrotized cells in the lumens. The affected hematopoietic tissue became edematous and debris of necrotized cells almost disappeared. The spleen of one fish showed edema, in which necrotized cells disappeared and spared cells remained (Plate IV−3). The spleen of the other

fish still contained debris of necrotized cells in the edematous pulps. In the heart, the endocardium was lined with swollen cells with basophilic cytoplasms (Plate Ⅳ−4). The peritoneum was infiltrated by many inflammatory cells.

Cytopathic effects on cells

Cytopathic effect (CPE) appeared in RTG−2, FHM and EPC cells after 2−3 days inoculaton at 20℃. The CPE observed in inoculated RTG−2 cells was spherical pyknosis of cells accompanying karyopyknosis. Severely affected cells resulted in cell-lysis (Plate Ⅳ−5).

Discussion

On histopathological examinations, pathological changes found in diseased elvers were characterized by necrosis of tissue and organs. Among the fish observed, those with necrosis of the brain and retina outnumbered those with systemic necrosis. As to necrosis of the brain and retina, infiltration of inflammatory cells was confirmed in the necrotic lesions after the treatment with 0.6% salt solution. This indicates that necrosis of the brain developed to encephalitis. Viral isolation from diseased elvers was confirmed with virological technics. No viral disease showing the histopathological characteristics described above has been found among European eel nor other species of eels. Based on the results on this study, the author proposes that this disease is called "Viral Encephalitis of European Eel". Virological examinations of the "Encephalitis Virus" were carried out by Mr.SORIMACHI of Tokyo University (in 1977). According to his information, the isolated virus was RNA type and inactivated with ether, chloroform and heat (50℃, 30min.). The size was 100−220nm. The pathogenicity was tested with intraperitoneal injection of medium containing inoculated RTG−2 cells to anguillettes of Japanese eel. No mortality resulted. On the other hand, SANO *et al.*(1977) reported the isolation of a virus from elvers of European eel transported from France and named the virus EVEX but did not confirm whether the virus was associated with a disease of the European eel. The feature of CPE and the biological characteristics of EVEX were very similar to those of the "Encephalitis Virus" of the present study. The pathogenicity of EVEX has been examined in Japanese eel elvers, ayu, carp, rainbow trout fry and rainbow trout yearling (NISHIMURA *et al.* 1981). EVEX was confirmed tobe pathogenic in only rainbow trout fry but not in other fishes. The results indicated EVEX required severe conditions to evoke the pathogenicity in elvers. As described above, the result of the examination of the pathogenicity of "Encepalitis Virus " in elvers was negative. Further studies on the pathogenicity of this virus in elvers are necessary under different conditions.

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References

NISHIMURA T., M. TOBA, F. BAN, N. OKAMOTO and T. SANO, 1981. Eel rhabdovirus, EVA, EVEX and their infectivity to fishes. *Fish Pathol.* 15(3/4): 173–184.

SANO T., 1976. Viral disease of cultured fishes in Japan. *Fish Pathol.*, 10(2): 221–226.

SANO T., T. NISHIMURA, N. OKAMOTO and H. FUKUDA, 1977. Studies on viral diseases of Japanese fishes. VII. A rhabdovirus isolated from European eel. *Anguilla anguilla*. *Bull. Jap. Soc. Sci. Fish.*, 43 (5): 491–495.

Explanation of Plate I

Fig. 1 Olfactory lobe showing extensive necrosis of nerve cells. H-E stain, X320.

Fig. 2 A detail of severely necrotized hypothalamus. Necrotized nerve cells show karyopyknosis, karyorrhexis and karyolysis without invasions of bacteria and parasites. H-E stain, X400.

Fig. 3 Necrotized granular layer of inferior lobe of hypothalamus. Necrotized cells are separated into the ventricle. H-E stain, X160.

Fig. 4 Spinal cord showing extensive necrosis of nerve cells and edema. H-E stain, X200.

Fig. 5 Extensively necrotized retina of eye ball. The brain layer and neuroepithelial layer are extensively necrotized. Necrotized pigment cells are granulated. L: lens. H-E stain, X160.

Explanation of Plate II

Fig. 1 Necrotized liver. Necrosis of hepatocytes extends around blood vessels. Necrotized hepatocytes are coagulated and destroyed to debris. H-E stain, X200.

Fig. 2 Necrotized kidney. Necrosis involves renal tubules and hematopoietic tissue. Glomeruli spared of necrosis. H-E stain, X200.

Fig. 3 Spleen showing extensive necrosis. H-E stain, X200.

Fig. 4 Heart showing extensive necrosis of the endocardium and muscle fibers. H-E stain, X200.

Fig. 5 Lateral musculature showing necrosis. H-E stain, X100.

Explanation of Plate III

Fig. 1 Inferior lobe of hypothalamus of treated fish with salt solution for one week. Necrosis of nerve cells in the granular layer is confined. H-E stain, X200.

Fig. 2 Affected retina of treated fish with salt solution for one week. Many inflammatory cells infiltrate into necrotized retina and the surrounding orbital tissue. Necrotized retina still contains necrotic cells and granulated pigment cells. C: choroid membrane, R: retina, H-E stain, X160.

Fig. 3 Olfactry lobe of treated fish with salt solution for two weeks. Many inflammatory cells infiltrate into necrotic lesions, in which necrotized cells still remain. H-E stain, X200.

Fig. 4 A detail of the above olfactory lobe. H-E stain, X400.

Fig. 5 Affected retina of treated fish with salt solution for four weeks. Excepting granulated pigment cells, necrotized cells mostly disappear from retina showing edema. H-E stain, X200.

Explanation of Plate Ⅳ

Fig. 1 Liver of treated fish with salt solution for one week. In the necrotic lesions, regenerating hepatocytes with basophilic cytoplasms and mitotic feature (arrow) appear, inflammatory cells infiltrate into and necrotized hepatocytes remain. H-E stain, X200.

Fig. 2 Kidney of the above fish. Renal tubules are lined by flat epithelial cells with basophilic cytoplasms and mitotic feature although the lumens contain debris of necrotized cells. The affected hematopoietic tissue is edematous and necrotized cells mostly disappear. H-E stain, X200.

Fig. 3 Spleen of the above fish. Pulps become to edematous, in which necrotized cells disappear and unaffected cells remain, H-E stain, X200.

Fig. 4 Heart of treated fish with salt solution for one week. The endocardium is lined by swollen basophilic cells. H-E stain, X200.

Fig. 5 Cytopathic effect of inoculated RTG−2 cells. Infected cells are pyknotic. Giemsa stain, X400.

Plate I

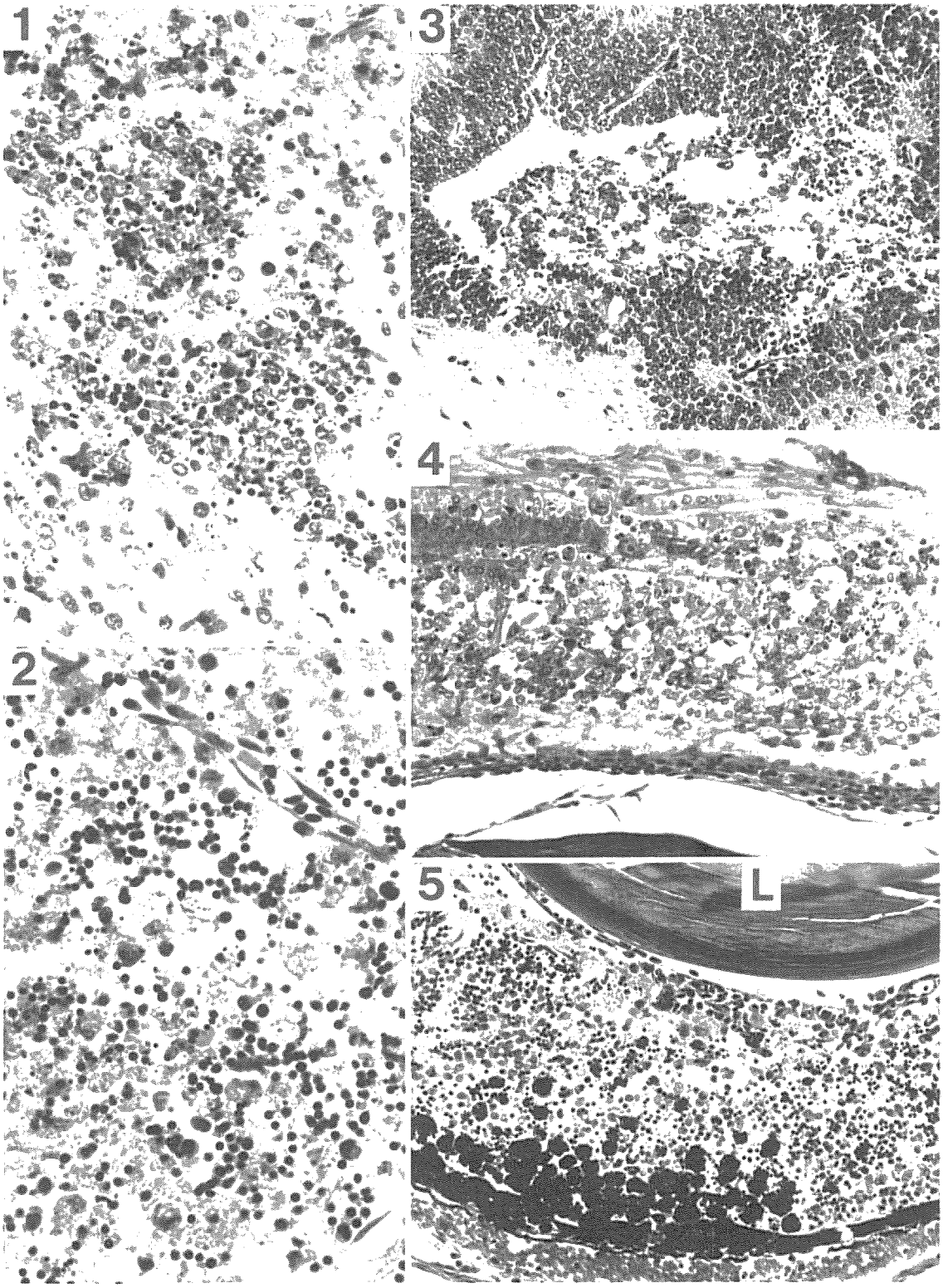

Plate II

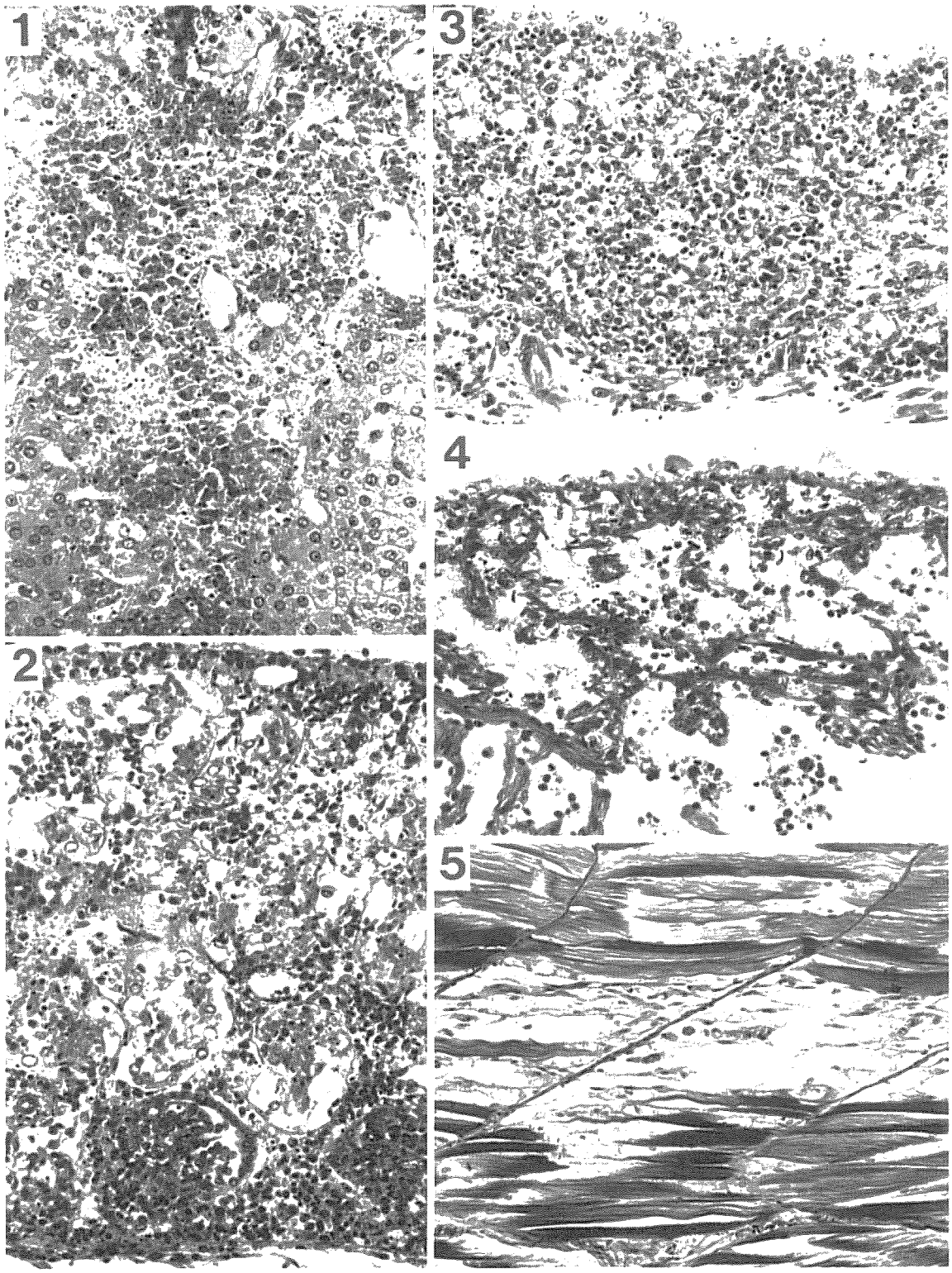

Plate III

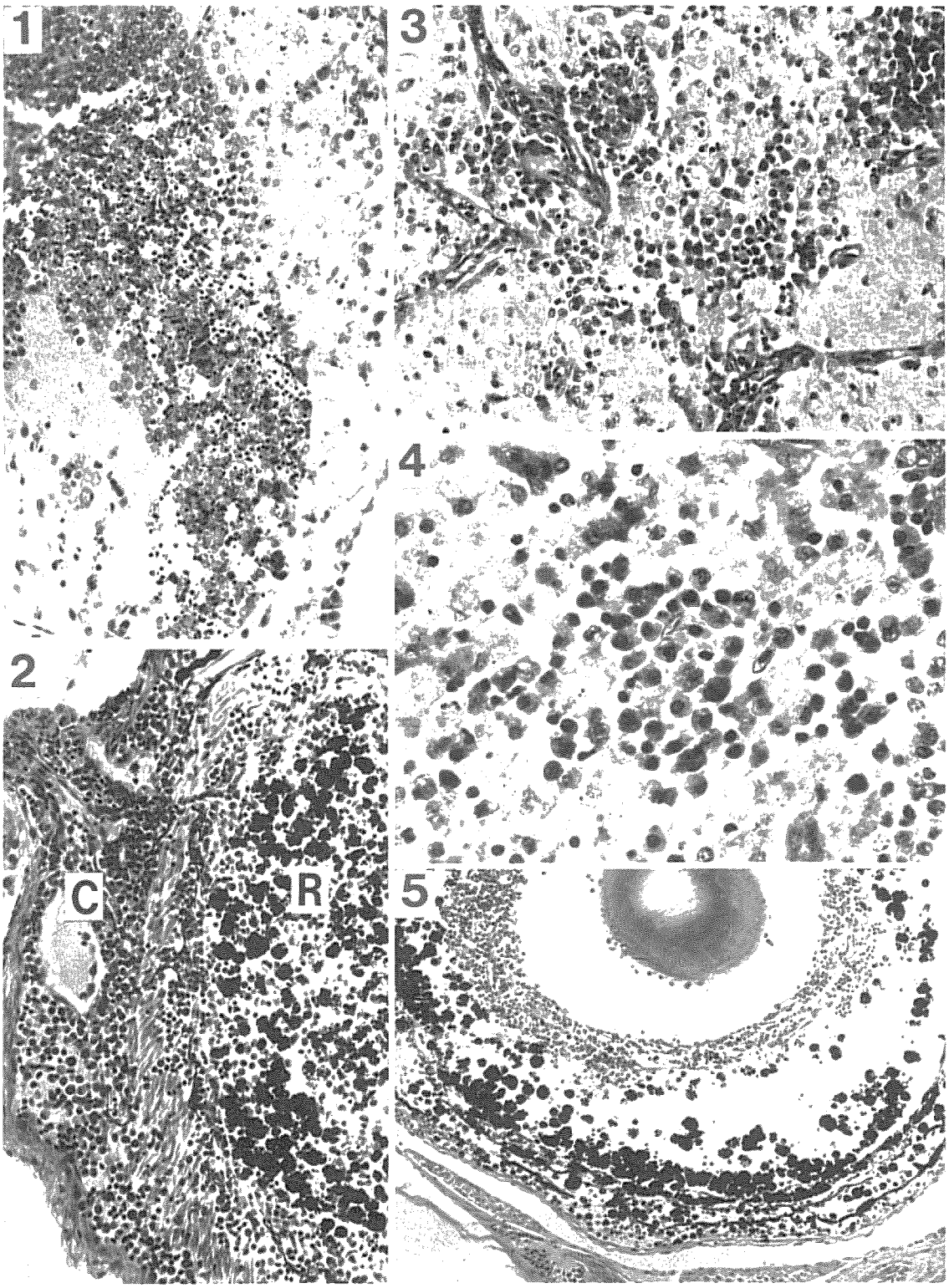

Plate Ⅳ