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A Histopathological Study of Streptococcal Disease in Tilapia

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A histopathological study was made on an infection of *Streptococcus* sp. in cultured tilapia, *Sarotherodon niloticus*, which occurred in warm water ponds during the winter of 1980. The external signs were dermal hemorrhage and exophthalmia. The internal signs were dropsy, epicarditis, peritonitis, pale-colored liver, splenomegary and nodule formation in gonads. On histopathological examination, the bacterial dissemination was systemic. Abscesses and granulomas were found to develope in the infected orbital adipose tissue of the exophthalmic eyes. Infiltration of bacteria-laden macrophages and granuloma formation were observed in the infected lesions of the epicardium, capsules of the liver and spleen, peritoneum, stomach, intestine, brain, ovary and testis.

Introduction

Streptococcal infections of fishes have attracted attention of fisheries biologists in Japan and abroad in recent years (KUSUDA *et al.*, 1976; KITAO *et al.*, 1981; KITAO *et al.*, 1982; MIYAZAKI 1980, 1982; RASEAD 1983; KAIGE *et al.*, 1984). The studies on streptococcal infections are divided into two fields, bacteriology of the causative agents and the histopathology of infected fishes. The present authors have studied mainly of the histopathological aspects of the disease. The object of this paper is to describe the histopathology of streptococcal infection of cultured tilapia, *Sarotherodon niloticus*.

Materials and Methods

Thirteen infected fish (three from Nara Prefecture and ten from Osaka Prefecture) were collected from warm water ponds inside houses covered with plastic sheats during the winter of 1980. Sampled fish were one year old, 15–20 cm in body length. After external and internal examination and bacterial isolation with heart infusion agar plates, eye lesions, brain, heart, liver, spleen, kidney, digestive tracts, gonads and gills were fixed in 15% formalin solution, embedded in paraffin wax with routine histological procedures. Tissue sections were stained with Mayer's hematoxylin and eosin (H-E), PAS, Giemsa, Goodpasture, Berlin blue, Azan, Sudan black B and Weigert's fibrin method. The isolates were tested with sheep blood-agar plates (Nissui) and the rabbit antiserum against *Streptococcus* sp. (MGT-7754) from diseased yellowtail.

Results

I. External and internal signs and bacteriological tests

The majority of the infected fish showed corneal opacity of eyes, exophthalmic eyes, destruction of eye balls, dark body coloration and dermal hemorrhage in the lower jaw, abdomen and opercles (Fig. 1). Internally, they exhibited dropsy, epicarditis, peritonitis, pale colored liver and splenomegary. Some diseased fish moreover showed nodules in the ovary or testis.

The isolates displayed γ-hemolysis on blood agar plates after 24 hour incubation at 25°C and were agglutinated by the rabbit antiserum to

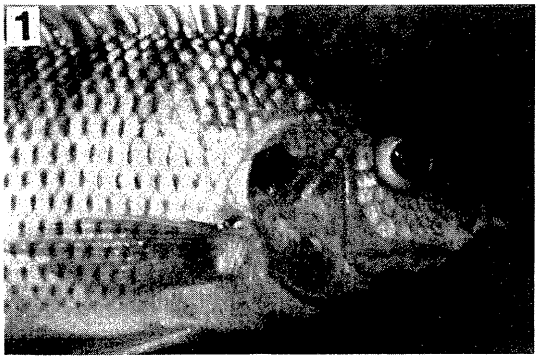

Fig. 1. Tilapia exhibiting exophthalmic eyes and hemorrhagic opercles.

Streptococcus sp.

II. Histopathological aspects

Eye: In eyes with corneal opacity, bacterial invasion and infiltration of bacteria-laden macrophages were observed in the conjanctiva. The cornea was erosious and invaded by bacteria. In exophthalmic eyes bacteria were disseminated into the cornea, conjanctiva, orbital adipose tissue and oculo-motor musculature which resulted in either exudative or granulomatous inflammation with the progress of the infection. In eyes with exudative inflammation, many neutrophils and macrophages infiltrated into the infected tissue (Fig. 2). In the infected adipose tissue, many neutrophils were accumulated producing abscesses with bacterial multiplication. The infected oculo-motor musculature was infiltrated by bacteria-laden macrophages, and observed to be necrotic and hemorrhagic. The optic nerve involved by the inflammation, which resulted in necrosis. Choroidal capillaries were engorged with blood and neutrophils. On the other hand, in eyes with granulomatous inflammation, the infected cornea, conjanctiva and the tissue in the orbit were infiltrated predominantly by macrophages and walled off by layered epithelioid cells (Fig. 3). In most cases, granulomas contained inflammatory cells showing coagulative necrosis, in which bacteria remained inside inflammatory cells and amorphous matter.

Heart: The epicardium of the heart and the tunica adventitia of the bulbus arteriosus were often invaded by bacteria. Inflammation occurred in the infected tissue was varied from specimen to specimen. Some specimen showel, infiltration of bacteria-laden macrophages and precipitation of fibrin which was histochemically confirmed by a positive reaction to Weigert's fibrin method and red coloration with Azan stain (Fig. 4). Most of the bacteria-laden macrophages were packed by bacterial cells and necrotized. The other specimens showed the production of granulomas with a thin layer of epithelioid cells in the epicardium. Granulomas in most cases contained caseous matter and bacterial cells.

Liver: Bacteria were sometimes disseminated in the parenchyma and the capsule of the liver. Bacterial dissemination was more dominant in the capsule than in the parenchyma. The infected capsule showed fibrin precipitation and infiltration of bacteria-laden macrophages (Fig. 5). In the parenchyma, Kupffers' stellate cells phagocytized bacterial cells and hepatocytes showed atrophy, cloudy swelling, vacuolization and necrosis.

Spleen: Spleen was sometimes invaded by bacteria in the capsule, pulps and sheathed arteries. Fibrin precipitation and infiltration of bacteria-laden macrophages occurred in the capsule. Splenocytes phagocytized bacterial cells and sinuses were engorged with blood or oligoemic in pulps (Fig. 6). In the spleen spared of the infection, macropages phagocytized ceroid and hemosiderin increased in number.

Kidney: In kidney with marked bacterial dissemination, glomeruli were invaded by bacterial cells without obvious damage and reticuloendothelial cells phagocytized bacterial cells in the hemopoietic tissue. Tubular epithelial cells were spared of the infection, though they showed cloudy swelling, vacuolization and hyaline droplet degeneration.

Digestive tracts: Stomach and intestine were sometimes invaded by bacteria. Bacteria-laden macrophages infiltrated in the tunica propria, submucosa and serosa of the stomach (Fig. 7) and the intestine. Peritoneum was often infiltrated by bacteria-laden macrophages.

Gonad: Testes and ovaries were often invaded by bacteria. In those organs, bacteria more markedly multiplied than in other organs. Infected lesions of the testis and ovary showed either exudative or granulomatous inflammation with the progress of the infection. The infected testis with exudative inflammation extensively showed marked

bacterial multiplication, tissue necrosis, congestion of capillaries, edema and infiltration of bacteria-laden macrophages and neutrophils (Fig. 8). Bacteria-laden inflammatory cells were packed by bacterial cells and necrotized. The testis with granulomatous inflammation showed the production of granulomas enclosing affected tissue, inflammatory exudates and bacteria (Fig. 9). In granulomas, bacteria remained in amorphous matter and inflammatory cells showing coagulative necrosis (Fig. 10).

In the infected ovaries with exudative inflammation, bacteria-laden inflammatory cells infiltrated into the ovarian lamellae. On the other hand, the ovaries with granulomatous inflammation showed the production of granulomas enclosing bacteria and coagulated or caseous matter.

Brain: The infected brain was infiltrated by bacteria-laden macrophages or showed the production of granulomas in the ventricles and meninges (Fig. 11).

Discussion

On bacteriological tests, the causative bacterium of streptococcal disease in pond-cultured tilapia, *Sarotherodon noloticus* was identified as *Streptococcus* sp., the causative bacterium of streptococcal disease of cultured marine fishes (KUSUDA *et al.*, 1976; KITAO *et al.*, 1982). MIYAZAKI (1980, 1982) has studied the clinical and histopathological aspects of *Streptococcus* sp. infection in yellowtail, *Seriola quinqueradiata,* striped beakperch, *Oplegnathus fasciatus,* Japanese horse mackerel, *Trachurus japonica* and striped jack, *Caranx delicatissimus*. External and internal signs of these infected fishes were common to these fishes and characterized by exophthalmia, ulcerative or nodular lesions on the body surface, epicarditis and discolored liver. The histopathological aspects of diseased fishes were characterized commonly by the occurrence of suppurative inflammation, infiltration of bacteria-laden macrophages and the formation of granulomas in the infected areas. The present study revealed that the pathology of diseased tilapia was similar to that of above marine fishes infected with *Streptococcus* sp., excepting that septic condition of the diseased tilapia was severer than that of the marine fishes. It may be concluded that *Streptococcus* sp. evokes similar histopathological changes in different species of fish susceptible to the bacterium.

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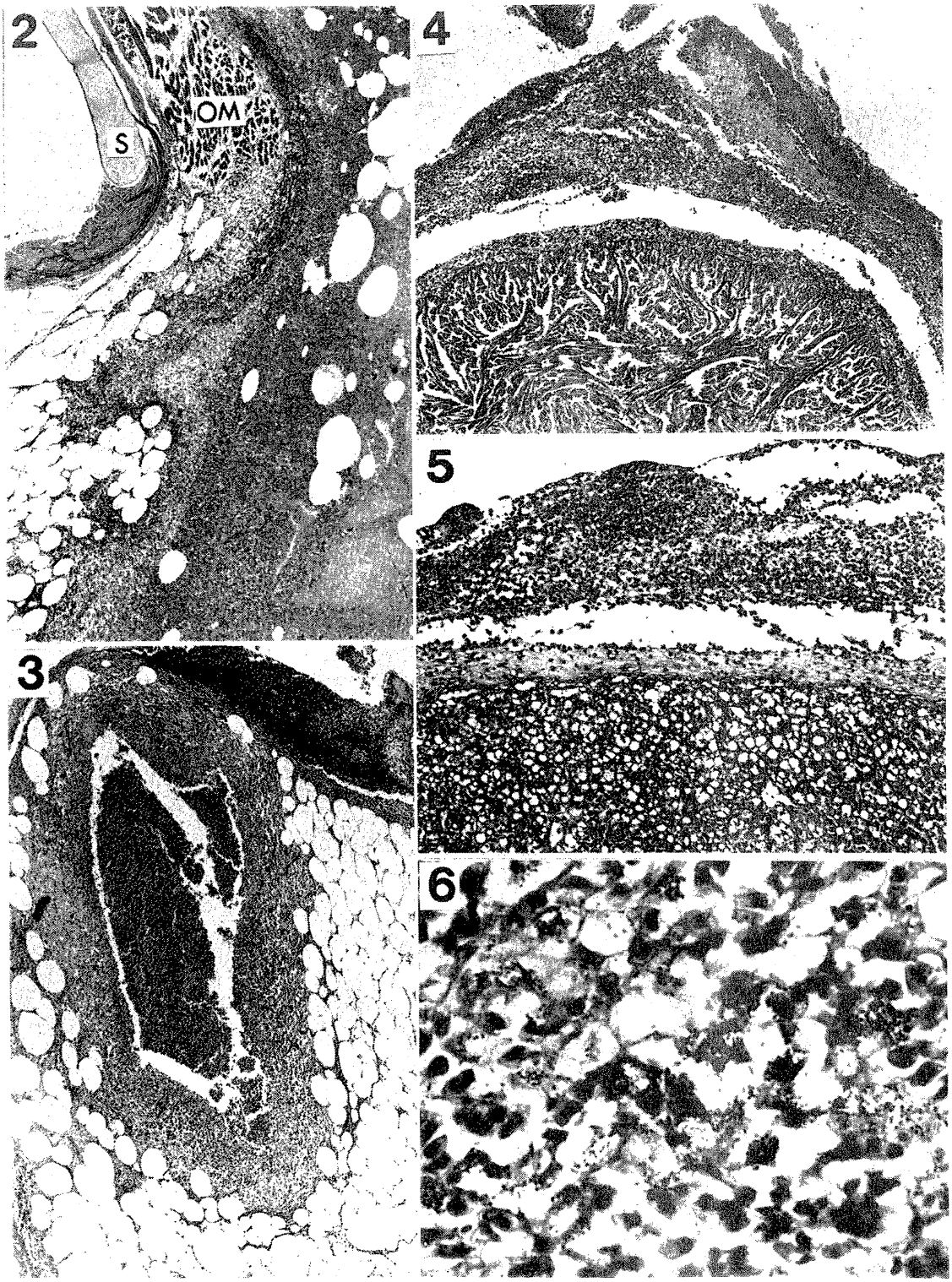
Explanation of Figures

Fig. 2. Exophthalmic eye showing suppurative inflammation in the orbital adipose tissue. OM: oculomotor musculature, S: sclera, H-E, ×20.
Fig. 3. Exophthalmic eye showing the production of granulomas in the orbital adipose tissue. H-E, ×40
Fig. 4. Heart showing epicarditis with infiltration of bacteria-laden macrophages and fibrin precipitation. H-E, ×32.
Fig. 5. Hepatic capsule showing infiltration of bacteria-laden macrophages and fibrin precipitation. Giemsa, ×80
Fig. 6. Spleen showing splenocytes phagocytizing bacteria. Giemsa, ×600.
Fig. 7. Stomach showing infiltration of bacteria-laden macrophages in the submucosa. H-E, ×100.
Fig. 8. Testis showing extensive bacterial multiplication, necrosis and edema. Giemsa, ×480.
Fig. 9. Testis showing the production of granulomas. Azan, ×32
Fig. 10. A detail of the granuloma, in which bacteria remained in great numbers. Giemsa, ×320.
Fig. 11. Brain showing the production of granulomas in the menings. H-E, ×50.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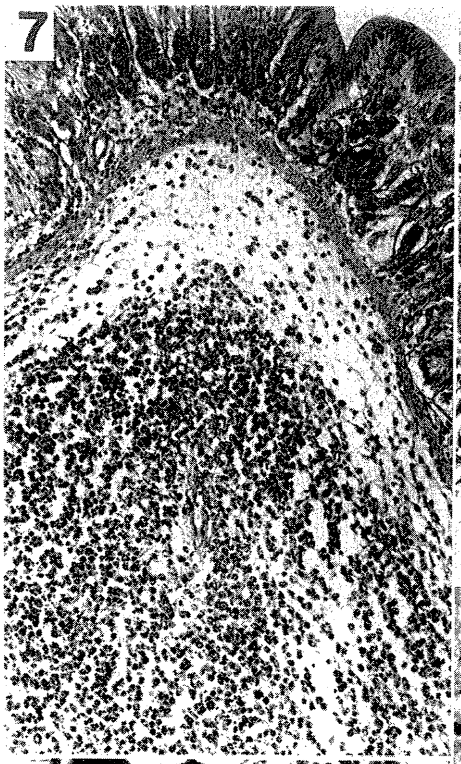
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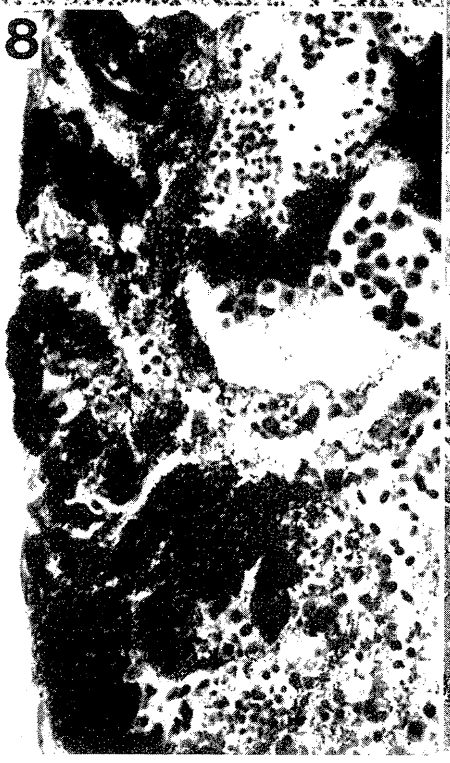
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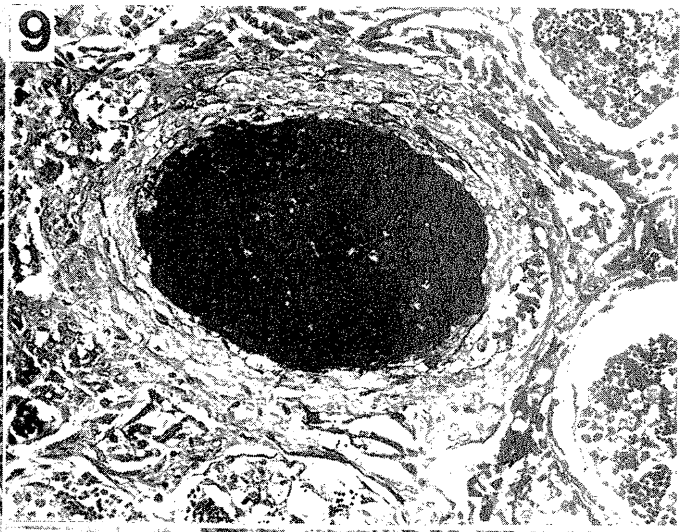
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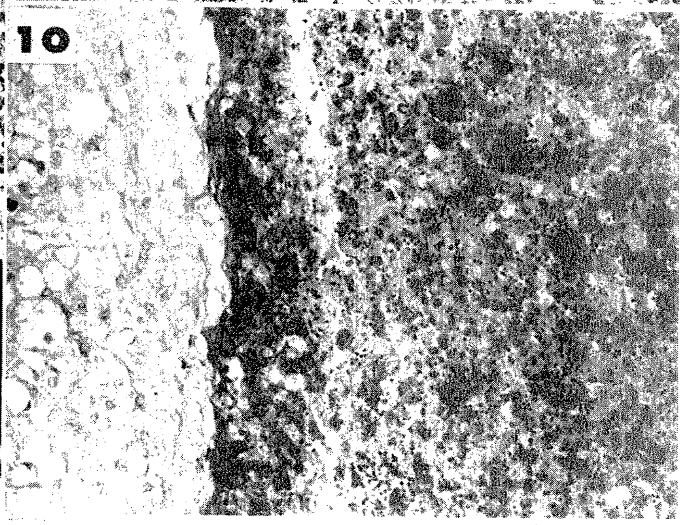
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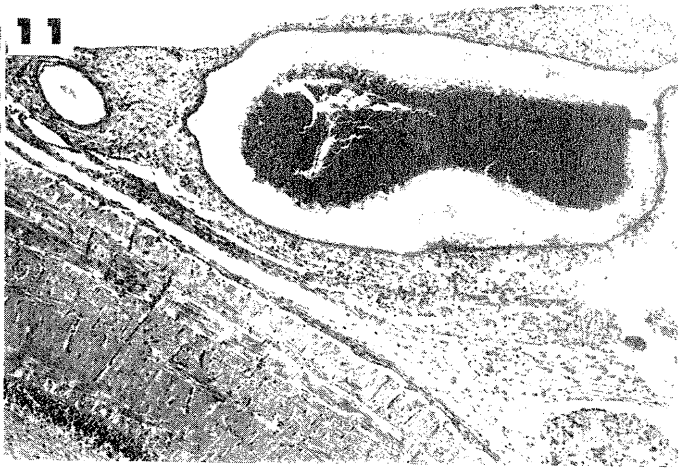
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