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PATHOLOGY ASSOCIATED WITH *Cryptobia* INFECTION IN A SUMMER FLOUNDER *(Paralichthys dentatus)*

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Abstract: A laboratory-held summer flounder *(Paralichthys dentatus)* became moribund and presented gross ulcerative and hemorrhagic lesions, concomitant with a space-occupying lesion in the abdominal cavity and a prolapsed rectum. Edema, hemorrhage, and necrosis of the intestine and edema of the stomach wall were noted upon post-mortem examination. Microscopic examination revealed large numbers of *Cryptobia* in the submucosa of the gut and in the liver.

INTRODUCTION

Hemoflagellates of the genera *Trypanosoma* and *Cryptobia* (syn. *Trypanoplasma)* occur in many marine and freshwater fishes.[2,5] The former are especially common in marine fishes but no pathogenic effect on the host has been reported.[4] *Cryptobia,* while rarely reported from marine fishes,[4] often behaves as a pathogen in freshwater and anadromous forms. The following is a description of a moribund laboratory-held summer flounder *(Paralichthys dentatus)* which, upon post-mortem examination, revealed multiple gross and microscopic lesions and a heavy infection of *Cryptobia.*

MATERIALS AND METHODS

During the winter of 1974-75, several summer flounder were being held in concrete tanks of a flow-through water system at the Oxford Laboratory. Because of notable external lesions, general sluggishness and anorexia, a flounder was removed from the tank on 5 January and killed by decapitation. Water temperature at this time was about 4 C and salinity 10 0/00. Fixed tissues were prepared using standard methods, and stained with hematoxylin and eosin, Giemsa's stain and the Grocott modification of Gomorri's methanamine silver stain. Blood smears and kidney imprints also were prepared and stained by Giemsa's technique.

RESULTS

Upon gross examination, the 24 cm fish was found to have a 1 cm length of ruptured intestine protruding from the anus. A swollen area, 2.5 cm in diameter, which was firm on palpation, was present on the left side over the abdominal cavity. Petechiae were present on the anterior dorsal fin, scattered over the caudal fin and in a small 0.5 by 2 cm area over the hypaxial muscles of the right side. An ulcer, 1.2 cm in diameter, was present on the left side adjacent to the hemorrhagic area of the dorsal fin.

When the viscera were examined, it was found that the prolapsed portion of the intestine had become separated from the remainder of the gut about 1.2 cm from the anus. The distal end of the intestine which remained in the body cavity was necrotic and hemorrhagic. The stomach, much enlarged by an increase in thickness of its wall, was found to be the cause of the grossly distended abdomen. The spleen appeared larger than normal for a fish of this length, being 7 mm in its longest dimension. The liver was of normal size and of pale yellow-orange coloration. The gall bladder was distended with pale, amber-colored fluid. Neither the body cavity nor

stomach appeared to contain excess fluid.

Microscopically, sections through the skin and muscle of the ulcerated area showed necrosis and sloughing of the epidermis, loss of nuclear detail in the stratum compactum of the dermis, edema of the stratum spongiosum and necrosis and edema of the muscle of the hypodermis (Figure 1). A few bacteria of various morphologic types were seen in the ulcerated area. The mucosa of the intestine, in the area of rupture, was necrotic and had detached from the basement membrane in many areas. Submucosal blood vessels were congested, lymphatics were dilated, and a diffuse leucocytic infiltrate was present (Figure 2). The stomach wall was edematous (Figure 3), and the spleen was congested.

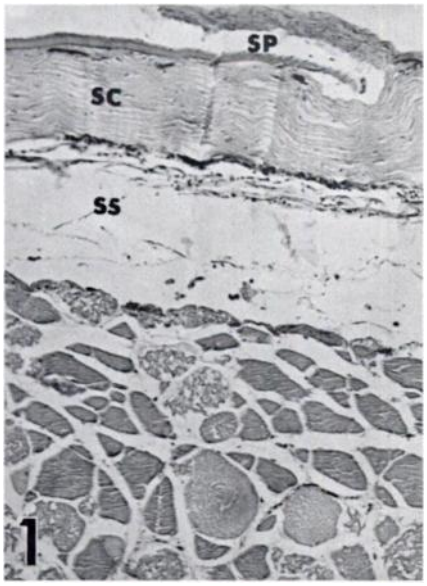

FIGURE 1. Area at edge of ulcer showing loss of epidermis, necrotic dermal tissue over scale pocket (SP), loss of nuclear detail of stratum compactum (SC), edema of stratum spongeosum (SS), and necrotic muscle bundles of hypodermis. (H&E)

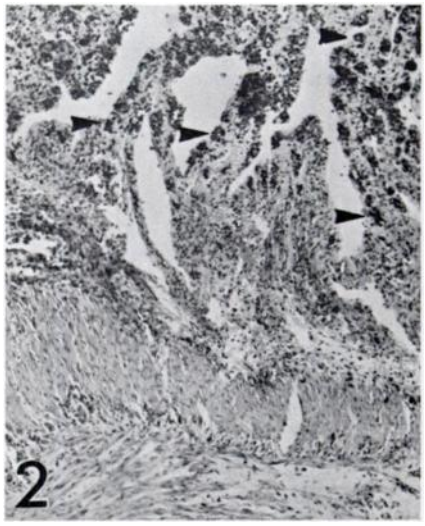

FIGURE 2. Small intestine showing loss of mucosa, large spaces probably representing dilatated lymphatics, abundant petechiae (arrowheads) in submucosa and diffuse leucocytic infiltrate. (H&E)

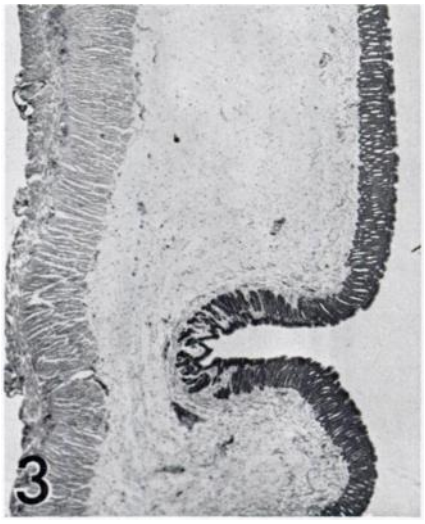

FIGURE 3. Section through stomach wall showing edematous submucosa. (H&E)

Sections of the liver were remarkably free of erythrocytes in the hepatic sinusiods. In the sub-capsular area of the liver, there was some necrosis and edema of the acinar tissue of the pancreas (Figure 4). The alveolar connective tissue in this area appeared to be hypercellular. At higher magnification, the increased cellularity was found to be caused by necrotic pancreatic exocrine cells, phagocytes and abundant protozoa (Figure 5). These organisms were also found in the submucosa of the inflamed portion of the intestine.

Kidney tissue had normal appearing interstitium. No necrosis or dilation of the tubules was evident, but erythrocytes were found in the tubular lumina in several areas. Some glomeruli were congested and there were focal glomerular

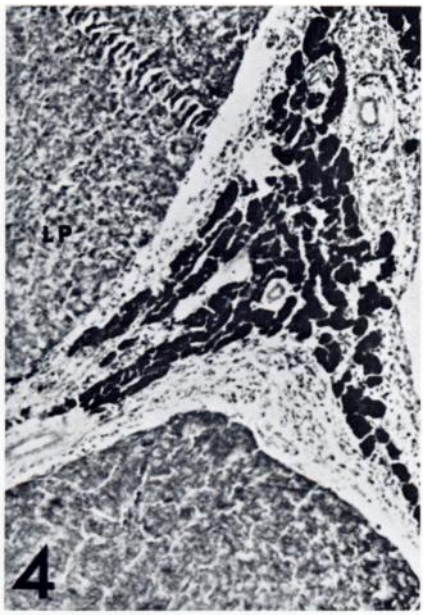

FIGURE 4. Section through liver surface showing pancreatic acinar tissue (dark staining cells) surrounded by hypercellular loose connective tissue between pancreas and liver parenchyma (LP). (Giemsa)

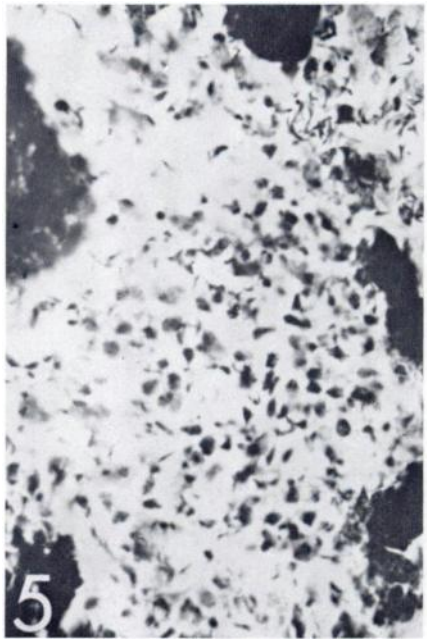

FIGURE 5. Higher magnification of a portion of Figure 4 showing abundant protozoa, phagocytes and necrotic debris (Giemsa).

lesions, which appeared to be fibrin thrombi, in the capillary tufts and the afferent arteriole often appeared dilated (Figure 6). Some necrosis of hemopoietic tissue was apparent and imprints contained numerous *Cryptobia* sp. (Figure 7). Flagellates were rare in peripheral blood smears.

Examination of Giemsa-stained tissue sections did not reveal any bacteria associated with the visceral lesions. Because of the heavy *Cryptobia* infection associated with the visceral lesions, serial sections of liver and gut were examined for parasitic emboli in vessels of the viscera. Although none were found, some venous thrombi were noted in the subcapsular areas of the liver. In addition, several similar small thrombi were seen in the mesentery in sections of the gut.

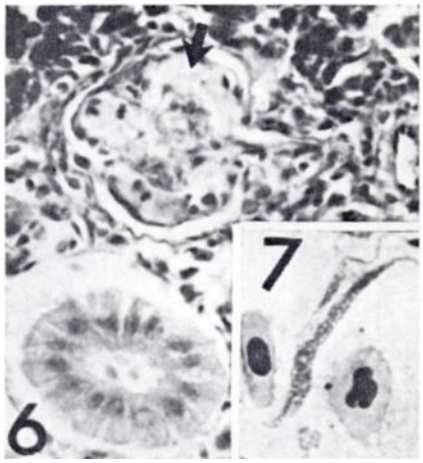

FIGURE 6. Fibrin thrombus in glomerular capillary tuft. Note dilatated afferent arteriole at right (H&E).

FIGURE. 7 A single *Cryptobia* as seen in a kidney imprint. Nucleated RBC's are 10-11 μm in length (Giemsa).

DISCUSSION

Disease associated with *Cryptobia* infection has been reported in tench *(Tinca tinca)*, carp *(Cyprinus carpio)*, crucian carp *(Carassius carassius)*, goldfish *(Carassius auratus)*, king salmon *(Oncorhynchus tshawytscha)*, and Coho salmon *(Oncorhynchus kisutch)*.[2,6,7,8,10] Signs of *Cryptobia* disease are given as listlessness, emaciation and sunken eyes.[7] Anemia has been reported in infected tench, rainbow trout, and salmon.[8,10] Ascites and exophthalmia have been reported in trout.[10]

Wales and Wolf[10] described the pathology of king salmon and rainbow trout with heavy *Cryptobia* infections in a California hatchery. The parasites were found in the skin, blood and ascitic fluid of yearling salmon, and were found in tissue sections of muscle and kidney. Anemia and pale gills were noted in the most severe salmon infections. Rainbow trout with heavy infections had exophthalmia, distended abdomen, raised scales and anemia. Putz[5] found that *C. salmonicida* injected into Coho salmon caused 100% mortality after ten days. Signs in these moribund fish included exophthalmia, ascites and paleness of gills and liver. In histologic sections of experimentally infected fish, flagellates were found causing partial occlusion of many capillaries in the dermis and gills.

Becker and Katz[1] have demonstrated experimentally that the rhynchcobdellid leech, *Piscicola salmositica*, can act as a vector of *C. salmonicida*. These authors also have suggested that other mechanisms of transmission may be involved in some cases, because leeches have never been observed on infected rainbow trout in the hatcheries at Mt. Shasta, California, and Reston, Washington, where *Cryptobia* infections are common.

In the marine environment, *Cryptobia* infections have been reported from winter flounder *(Pseudopleuronectes americanus)*, smooth flounder *(Liopsetta puttnami*, Gill) and European flounder *(Pleuronectes flesus)*.[5,9] The author also has found windowpane flounder *(Scopthalmus aquosus)* to be infected. Disease has not been associated with *Cryptobia* infections in any of these flatfishes, or in any strictly marine species, even though in some areas the prevalence of infection is very high (66% of winter flounder between 97 and 155 mm in length near St. Andrews, New Brunswick[3]). The organism is reported to be abundant in peripheral blood only during the warmer months, being located in the deep tissues during winter.[5,8]

In the present case, it is not certain that *Cryptobia* was the cause of the observed lesions in the summer flounder. It is conceivable that parasitic emboli or venous thrombi, which may have formed subsequent to partial occlusion of vessels by the parasites, may have caused localized ischemic tissue injury, resulting in hemorrhage and necrosis.

The pattern of small venous thrombi in the mesenteries, combined with the pale liver and congested spleen could suggest a pre-portal blockage of venous return. The edema of stomach and intestinal submucosa may have been lymphedema caused by partial occlusion of lymphatics by the parasites. Because the fish had not been feeding for some time and because of its heavy parasite burden, hypoproteinemia may have been a factor. Osmotic stress, due to the low salinity of the environment in which the fish was being kept, also may have contributed to the diseased condition. Because of the absence of ascitic fluid, increased portal venous pressure probably was not involved.

Several bacterial pathogens of fishes may cause dermal ulceration, pettechiae in the intestine and mesenteries, splenomegaly and anal prolapse. These include *Aeromonas hydrophila, A. salmonicida* and *Vibrio anguillarum*. In most cases, these diseases occur during periods of high or rising water temperatures. *A. salmonicida* and *V. anguillarum* generally have much hemorrhage associated with the dermal ulceration.

The renal tissue did not show the tubular necrosis and increase in erythroblastic activity which commonly accompanies vibriosis.[11] The chronic form of *V. anguillarum* disease usually causes gross lesions on the ventral or unpigmented side of flatfish.[11] It is unfortunate that no attempt could be made to culture bacteria from the lesions. The lesions observed may have had multiple etiologies. The summer flounder in the present case was subjected to temperatures and salinities below those normally experienced by this species. The relation of heavy cryptobial infection to disease of fishes needs additional experimental study. Nowicki[5] was unable to experimentally induce disease in infected carp even after splenectomy. Perusal of the existing literature leaves the impression that disease may occur only under hatchery conditions. Whether this is due to facilitated transmission during confinement or additional stresses under these conditions is uncertain.

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