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AN EPIDERMAL PAPILOMA OF THE ATLANTIC SALMON I: EPIZOOTIOLOGY, PATHOLOGY AND IMMUNOLOGY¹

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Abstract: Papillomatosis of Atlantic Salmon (*Salmo salar*) was studied with regard to epizootiology, gross and histologic pathology, and host response. It was found to be a condition of parr in their second summer, but also occasionally of young adult fish (smolts and grilse) which have adapted to salt water.

The lesion was plaque-like to papillomatous and consisted of stratified squamous epithelium with supporting stroma. Immunologic findings tended to support histologic observations that the lesion was ultimately sloughed as a result of a cell mediated immune response.

INTRODUCTION

A benign, proliferative epidermal lesion has been observed in juvenile Atlantic salmon (*Salmo salar*) in Sweden,^{1,9} the United States,¹⁰ Norway (Hastein, T. 1975, Pers. comm.) and Great Britain (Needham, E. A. 1975, Pers. comm.). Although the condition is self-limiting, secondary infection can occur with fatal results. Furthermore, the carcasses of affected fish are of reduced value.

The purpose of the present paper is to report observations on the epizootiology and pathology of this condition, and on the mechanism whereby the lesion is ultimately resolved by the host.

MATERIALS AND METHODS

Salmon studied were from five establishments. Two were hatcheries in Scotland using fresh water; one was an on-growing facility using cages in a sea-loch; one was an experimental rearing establishment using pumped sea water; and the fifth was a sea cage farm on a Norwegian fjord.

Affected salmon were killed by spinal section and blood was collected from the aorta. Tissues, including skin, spleen, kidney, liver, gill, stomach, small and large intestine, pancreas and pyloric caecae were fixed in 10% formalin, embedded in paraffin, and stained with hematoxylin and eosin.² Lendrum's method² was used to demonstrate inclusion bodies, and the MacCallum-Goodpasture method² to demonstrate microorganisms in the tissue.

Cell mediated immunity was assessed by the migration inhibition test.⁶ Fresh blood from affected salmon was centrifuged in microhematocrit tubes which were broken at the buffy coat and placed in a petri dish containing 5 ml of Eagle's minimum essential medium, Glasgow modification.¹² Five grams of a 10% suspension of a minced and sonicated mixture of papilloma, spleen, and kidney was added as an antigen to each test dish. No antigen was added to control petri dishes. The preparations were observed under a dissecting microscope at 3, 5, and 24 h. Lack of migration of the leukocytes into the medium indicated a positive result.

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The Ouchterlony gel diffusion test was done using 2% ion agar on plastic slides with 3 mm wells cut 2 mm apart. One drop of serum from affected salmon was placed in the center well with one drop of antigen suspension from various sources prepared similarly to that used in the migration inhibition test in each of the outer wells. The test was incubated at 4 C and observed daily for one week.

RESULTS

Epizootiology

In each of the freshwater hatcheries, the papillomatosis occurred as an epizootic, beginning in July and reaching a peak in August. In one, it was seen in two tanks of 1+ parr (fresh water salmon in their second summer) of the same parentage. An estimated 15% to 25% of the parr in one tank and 40% to 55% of those in the other were affected. Other tanks, some of which contained parr of the same parentage, and all of which used the same feed and water supplied, were unaffected.

Smaller numbers (5% to 10%) of parr of a similar age group were affected in the other freshwater hatchery. Affected fish were removed and killed, but mortalities were rare. Both hatcheries treated the parr with malachite green to prevent secondary mycotic infection; also, one hatchery used formalin, in case parasites were involved.

By autumn, the lesions began to slough and by early December, nearly all were gone. The period of resolution of the lesions coincided with silvering of the fish, an outward indication of moltification.

Much smaller numbers of fish in sea water (probably less than 1%) were affected, but the papillomata generally persisted longer—up to a year in some cases. The lesions had not been observed at the time the smolts were transferred to sea water, and were assumed to have arisen thereafter.

A grossly indistinguishable condition has been occasionally observed in free

living salmon parr, but not in migrating smolts (Laird, L. M., 1975, and Roberts, R. J., 1975, Pers. comm.).

Pathology

The papillomata were seen grossly as smooth white plaques or wart-like structures, raised 2-5 mm, and up to 4 cm in diameter. Frequently, they were multiple and were found anywhere on the fish posterior to the head including the opercula, fins and tail (Fig. 1). Many had hyperemic areas.

Microscopically, the lesions were composed of relatively uniform cells resembling those of the middle (malpighian or prickle cell) layer of the normal teleost epidermis. They were 5-15 times thicker than the normal epidermal thickness of 4-12 cell layers. A distinct basal layer usually was not present and it was frequently difficult or impossible to discern a basement membrane. Mucous cells were much less common than in normal epidermis and, when present, usually were in clusters near the surface. Flattening of the more superficial cells to become the outer, squamous layer also was variable. The presence of mucous cells and of a squamous layer could be interpreted as retention of pre-existing normal structures, pushed to the surface by proliferating papilloma cells, or as differentiation of the proliferating cells into these structures (Fig. 2).

Usually a sharp line of demarcation was present between the lesions and the surrounding normal epidermis (Fig. 2). In some instances, several layers of cells, resembling those of the papilloma, could be seen proximal to an apparently normal and complete epidermis, suggesting the transformation was just beginning in such an area, and that, in the early stages, the normal epidermis remained intact over the growing papilloma (Fig. 2). The thicker lesions had supporting stalks of fibrovascular dermis, projecting far up into the mass of epidermal cells. Frequently, these were directly beneath convex portions of the convoluted surface (Fig. 3).

Mitotic figures, infrequent in normal epidermis, were present in moderate

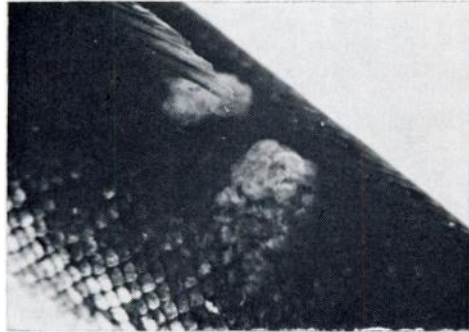


Figure 1

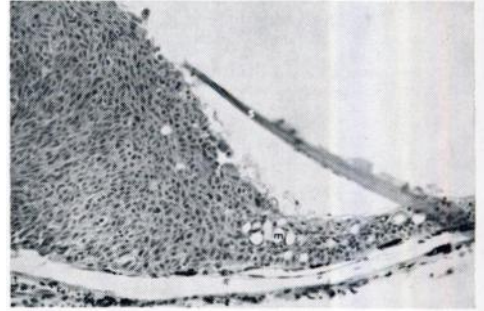


Figure 2

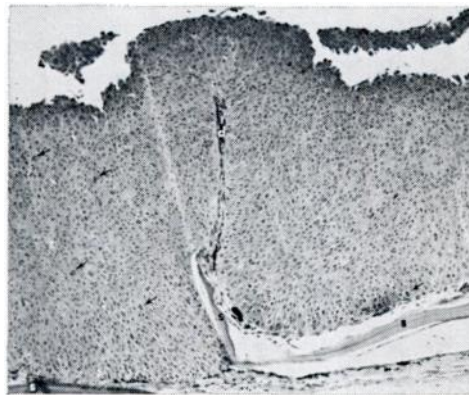


Figure 3

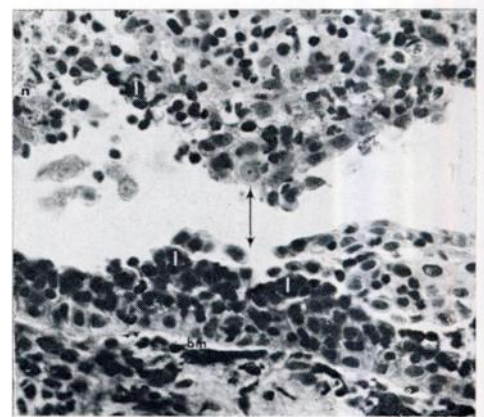


Figure 4



Figure 5

FIGURE 1. Papillomata on a 2½ year smolt.

FIGURE 2. Photomicrograph showing the abrupt transition from normal epidermis, with numerous mucous cells (m), to papilloma, with few. A scale (s) has been dislodged in sectioning and there is slight artifactual separation of epidermis from the dermis. 100X

FIGURE 3. Photomicrograph showing convoluted surface and dermal stalk (d). Occasional mitotic figures (arrows) are seen. Scale (s) are at the bottom. 100X

FIGURE 4. Photomicrograph showing necrosis (n) and separation (arrow) of epidermis along a line of heavy lymphocyte (l) infiltration. Boundary (bm) between dermis and epidermis is near the bottom. 350X

FIGURE 5. Photograph of the ulcer which remains when the papillomata are sloughed.

numbers (1-6 per low power field). Individual cell necrosis was present, especially in areas most distant from the dermal blood supply. Although such premalignant characteristics as anaplasia, pleomorphism, bizarre mitotic figures (Fig. 3) and the formation of cords and whorls occasionally were seen, downward invasion through the basement membranes was not seen.

Eosinophilic intracytoplasmic inclusion bodies were present in some of the lesions, especially in degenerating cells. They were single or multiple spherical hyaline bodies which stained by Lendrum's method. Rarely, similar intranuclear bodies were present.

The host response to the lesions varied widely. Some lesions showed only hyperemia and a few leukocytes migrating out of the vessels, while others were massively infiltrated with lymphocytes (Fig. 4). In other lesions, which appeared to be the most advanced in terms of host response, large necrotic areas with significant numbers of neutrophils and macrophages were present. The final stage was ulceration (Fig. 5) followed, in uncomplicated cases, by rapid epithelialization.

The most frequent complication was secondary infection of the ulcers which interfered with epithelialization. Fungi identified as *Saprolegnia* sp. were most frequently incriminated. Bacteria could not be identified in the lesions by the Goodpasture method.² The cause of death in these cases probably was the ionic imbalance caused by the loss of the osmoregulatory function of the skin. A similar mechanism may have caused the death of fish with uninfected ulcers which were too large to be epithelialized before ionic deregulation.

Immunology

Serum from affected and convalescent salmon failed to produce lines of precipitation on the agar gel diffusion test.

Macrophage migration inhibiting activity was demonstrable in buffy coats from affected salmon of Scottish origin in the presence of antigen obtained from fish on Scottish farms. Macrophage mi-

gration was normal in tests using frozen tumor material from Norwegian salmon as the antigen, as well as in controls.

DISCUSSION

The results closely agree with those of Needham (1975, Pers. comm.), who observed up to 50% prevalence of the papilloma in parr, up to 10% in smolts at sea, and up to 1.5% in returning grilse.

The lesion was grossly and microscopically similar to that reported in *Mustelus canis*¹¹ and *Stizostedion vitreum*.⁸

Morgan *et al.*³ have shown that temperature and exercise dramatically affect wound healing in Salmon. Some of the variability in host response encountered in this study may be explained on that basis. However, since the fish were all maintained at relatively similar temperature (14-17 C) and water flow rates, the principal factor to which the observed variation is attributed is the stage in the pathogenesis at which the lesions were sampled.

Discussions with hatcherymen revealed that all papillomata affecting freshwater salmon were sloughed, leading to the recovery or occasionally the death of the fish. No papillomata remained on fish parr through the winter. We therefore believe that the Salmon's immune mechanism at some point recognized the papilloma as antigenically foreign and mounted a graft rejection type response. Wales and Sinnhuber⁷ reported lymphocytosis in rainbow trout hepatomas and viewed these "lymphoid invasions as immune reactions to a foreign substance". Roberts⁵ reported regression of lymphocystic lesions following lymphocytic invasion in plaice.

In the absence of complications, the papillomata caused little apparent harm to the fish, although the lesions did mechanically impede locomotion. In free living fish, the lesions would be expected to defeat the protective coloration and render the fish more subject to predation, but most death of farmed fish associated with the papilloma probably result from infection of the resulting ulcer and/or osmotic imbalance.

Acknowledgements

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