Vol. 15: 171–174, 1993 | DISEASES OF AQUATIC ORGANISMS Dis. aquat. Org. | Published April 29

X-cell lesions in the liver of coho salmon *Oncorhynchus kisutch*

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ABSTRACT: A coho salmon *Oncorhynchus kisutch* (Walbaum) was found to develop lesions associated with so called X-cells propagating in the liver parenchyma. The ultrastructural features, insufficient to determine the origin of X-cells, corresponded to those found in pseudobranchial tumours of Atlantic cod by Watermann & Dethlefsen (Helgoländer Meeresunters. 35: 231–242, 1982).

INTRODUCTION

The most important data on X-cells summarized recently from case reports and long term studies on disease conditions caused by X-cells (Diamant & McVicar 1990) suggest that X-cells are in fact parasites, possibly related to amoebae. Conclusive evidence as to the protistan nature of X-cells has yet to be found. The presence of X-cells in more than 20 species of fishes from different families (mainly pleuronectids and gadoids), suggested a single species of parasite with cosmopolitan distribution, though the possibility of several different species was also mentioned (Kent et al. 1988). Yet another possibility, that X-cells are developmental stages of some of the phyla of protistan parasites, has not been considered. At present, the available information on ultrastructure is insufficient for a comparative study of all the agents termed X-cells during the last decade. Since the presence of X-cells has been associated with mortalities, which could be significant in the culture of flatfish and cod (Diamant & McVicar 1986), this agent should be considered a potential pathogen.

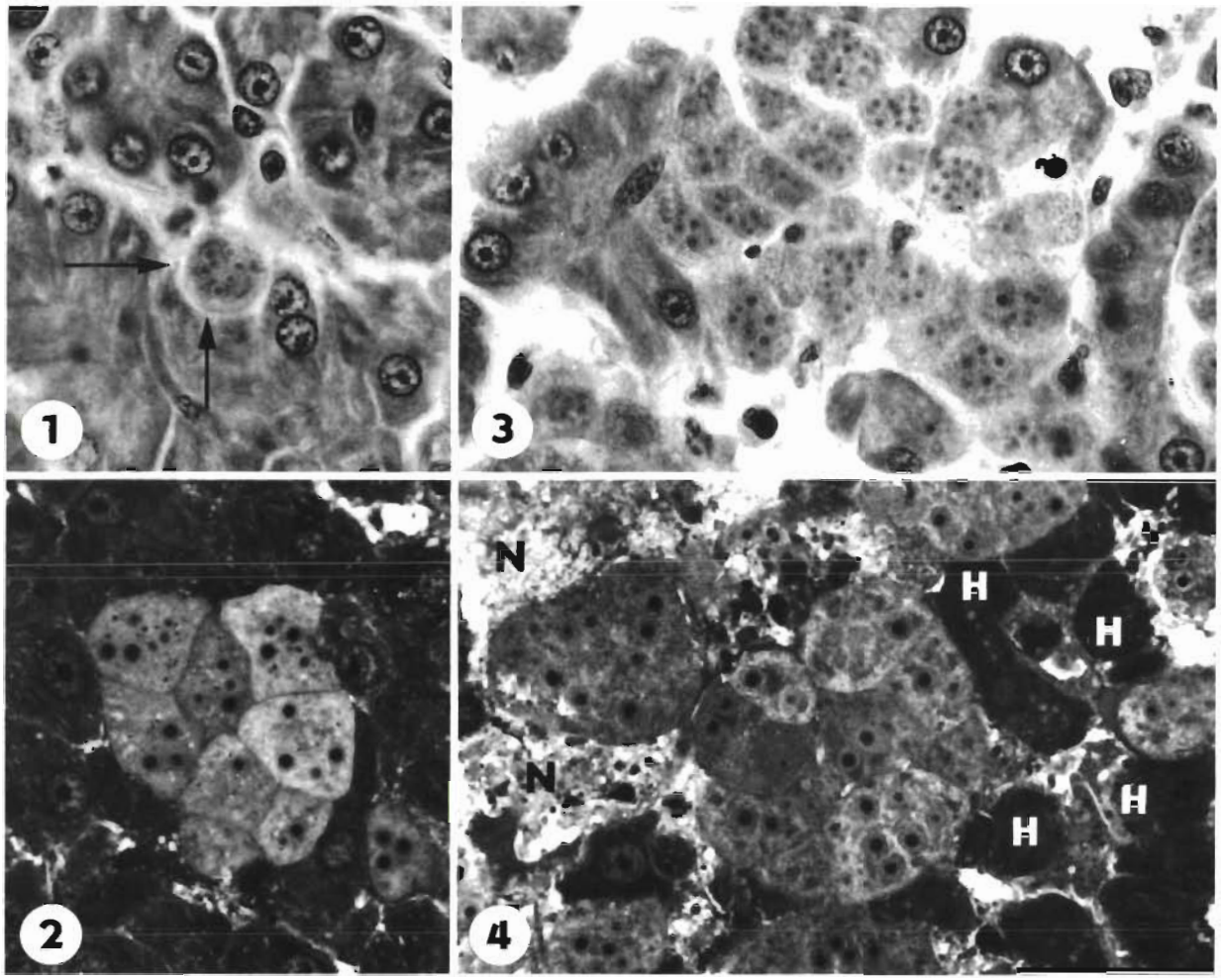
METHODS

Moribund specimens of coho salmon *Oncorhynchus kisutch* (Walbaum) cultured in cages stationed in the Atlantic near the northwest coast of Galicia were sampled in March 1991. The nutritional condition corresponded to the size category of fishes measuring 25 to 30 cm in length.

Samples of liver parenchyma with irregular foci of altered colouration were fixed in 10 % buffered formalin and in Bouin's solution. Routine histological examination of tissue samples embedded in paraffin was supplemented by transmission electron microscopic observations. Small tissue samples previously fixed in formalin and Bouin's solution were postfixed in cacodylate buffered osmium tetroxide and reembedded in Epon-Araldite. Semi-thin sections stained with toluidine blue were used for light microscopy. Ultra-thin sections double-stained with uranyl acetate and lead citrate were examined with a Philips 420 B transmission electron microscope.

RESULTS

Histological examination of the liver parenchyma revealed massive alterations in the liver architecture, i.e. tubular arrangement of hepatocytes disappeared due to the presence of numerous aggregations of large multinucleate cells. These multinucleate cells, predominant in large areas, measured up to 30 µm. The liver parenchyma adjacent to aggregations of multinucleate cells was compressed and atrophic, and in many places appeared necrotic (Figs. 1 to 4). There was no evidence of inflammatory response in and around the damaged areas. Aggregates of polynuclear cells were



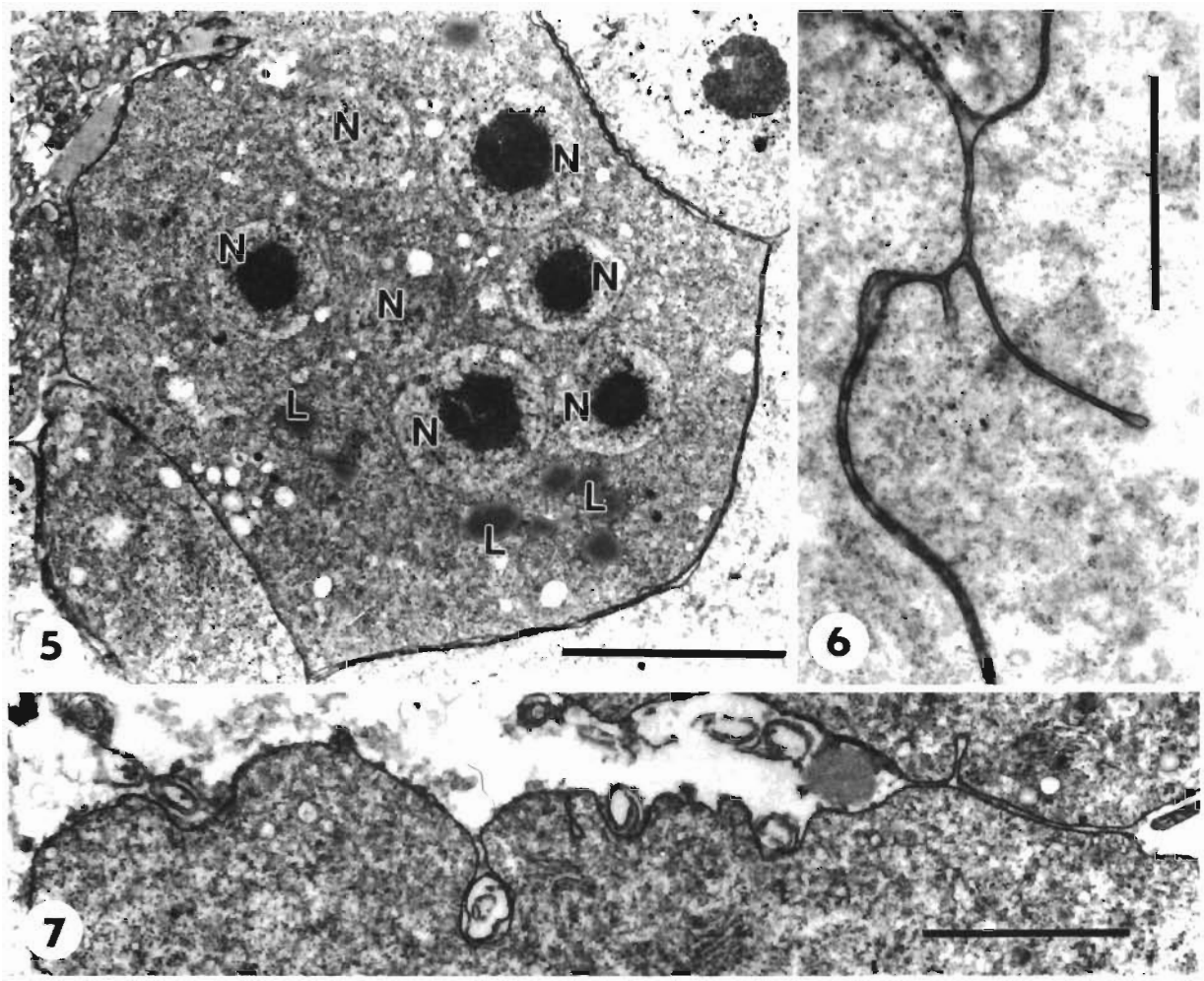
Figs. 1 to 4. *Oncorhynchus kisutch*. Fig. 1. An isolated multinucleate X-cell (arrows) localized in the blood channel causes atrophy of surrounding hepatocytes. HE, × 880. Fig. 2. A group of multinucleate X-cells surrounded by altered hepatocytes. Semithin section, toluidine blue, × 880. Fig. 3. Large agglomeration of multinucleate X-cells associated with atrophy and necrosis of the liver parenchyma. HE, × 880. Fig. 4. Multinucleate X-cells predominate in large areas of liver parenchyma leaving only small islets of hepatocytes. H: hepatocytes; N: necrotic debris. Semithin section, toluidine blue, × 900

scattered randomly throughout the liver parenchyma. Because of the limited number of samples, no conclusions about the genesis of lesions could be made.

Histopathological examination suggested a non-fish origin of the multinucleate cell agglomerations. The nature of this agent, however, was impossible to determine at the light microscopical level.

DISCUSSION

Under the electron microscope, the whole multinucleate X-cell population found in the liver of coho salmon was of the same type. Individual cells revealed variations in shape, size and in the number of nuclei. There was also some variation in the quality of preservation of the cytoplasm. The ultrastructural features were identical with those of X-cells described in previous reports. The most striking similarity was found with the X-cells described in pseudobranchial tumours in Atlantic cod by Watermann & Dethlefsen (1982). The multinucleate X-cells from the liver of coho salmon (Fig. 5) with deep invaginations of the outer membrane (Figs. 6 & 7), finely granulated cytoplasm and aggregates of endoplasmic reticulum closely apposed to the nuclei resemble the type documented in Figs. 8 and 9 of Watermann & Dethlefsen's (1982) paper. Contrary to other descriptions (Diamant & McVicar 1986, McVicar et al. 1987, Kent et al. 1988, Diamant & McVicar 1990), the X-cells found in coho salmon were all multinucleate, and lacked electron-dense bodies, either single or in aggregates, and vacuoles. No mitotic activity was observed. There were also differences in the associated lesion. Contrary to many other findings



Figs. 5 to 7 *Oncorhynchus kisutch*. Fig. 5. X-cell with finely granular cytoplasm and 7 nuclei (N) at the level of section, lipid inclusions (L) and several small vacuoles. Scale bar = 5 µm. Fig. 6. Deep invaginations of the cell membrane of an X-cell. Scale bar = 3 µm. Fig. 7. Invaginations (food uptake?) at the cell surface of an X-cell. Scale bar = 3 µm

(Dawe 1981, Myers 1981, Watermann & Dethlefsen 1982, Peters et al. 1983, Kent et al. 1988), no tumour-like structures were observed in the liver of coho salmon, and tissue damage was more or less of an infiltrative character, i.e. there were no spherical or nodular formations with zonal differences between the center and the periphery of lesions. So called cysts with multinucleate cells (described by Watermann & Dethlefsen 1982) were absent. Enveloped stages mentioned by McVicar et al. (1987) were not observed.

Although no new features of X-cells could be recognized in coho salmon infection, this case-report was prepared in order to draw attention to their occurrence in a hitherto unrecorded host and to increase the probability of future recognition of the agent.

Although coho salmon is an essentially freshwater fish, it spends most of its adult life in the sea. This may present a distributional link with findings of X-cells in marine fish. Increased attention should be paid to examination of other hosts, both freshwater and marine, which could shed more light on the nature of these cells.

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Responsible Subject Editor: W. Körting, Hannover, Germany

Manuscript first received: December 3, 1992
Revised version accepted: January 21, 1993