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OCCURRENCE AND STRUCTURE OF CYSTS IN X-CELL LESIONS OF ATLANTIC COD (*Gadus morhua L.*)

by

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INTRODUCTION

Despite numerous investigations the nature and origin of x-cells remain unknown. These ovoid, pale-staining cells with a large, round nucleus and prominent nucleolus are known to occur in the form of lesions, termed x-cell tumours. X-cells may produce large aggregations that resemble true neoplasms. They can be found in quite different organs as skin, pseudobranch, gills, ultimobranchial body, kidney, gonad and spleen (Peters et al. 1978; Dawe, 1981; Diamant & McVicar, 1987). X-cell lesions are mainly described in marine species from cold or temperate zones, but a recent publication reported on x-cell tumours in a freshwater species living in warmwater (Diamant et al. 1994).

MATERIALS AND METHODS

Some specimens of cod were collected during several cruises in the North Sea with RV "Anton Dohrn" between 1980 and 1985 with special attention to very early stages of x-cell lesions. Other specimens with very small x-cell lesions and those with large tumorous growth were collected from October 1983 until May 1985 off the Norwegian coast, and kept in tanks for different experiments. Single cod were tagged and treated with metronidazole (Flagyl) which act especially on protozoan organisms. The application of metronidazole was performed by intramuscular, intraperitoneal and direct injections into the tumours. Metronidazole was administered at doses of 5; 10,15 and 20mg/kg fish on five consecutive days. Treated fish were kept for 4 to 8 weeks after the last injection, anaesthetized, killed and organs with x-cell lesions were excised. The tissues were fixed in 4% phosphate-buffered glutaraldehyde, postfixed in osmium tetroxide and embedded in Epon. Semithin sections were stained with toluidine blue, whereas ultrathin sections were stained with uranyl acetate and lead citrate.

RESULTS

In some fish from the North Sea with very small white swellings in the pseudobranch, cysts and untypical multinuclear x-cells were visible on the histological level. The cysts occurred in some specimens with only a couple of x-cells between the pseudobranch filaments. In these lesions, very small parts of the pseudobranch were affected, in which whole cysts and subdivided cysts formed ovoid clusters of x-cells.
The same cystic-structures could be found in some of the metronidazole treated fish. In one cod with large pseudotumours around the ultimobranchial bodies, a very small white

swelling in the pseudobranch, and white spots on heart as well as on the posterior kidney, multitudes of cysts could be detected in the different organs.
Cyst-like x-cells with a clear unstained outer wall occurred in clusters and nests in distinct parts of the lesions. These clusters were situated between the filaments of the pseudobranch, in fibrous tissue near the ultimobranchial body, and between muscle fibers in the heart (Fig. 1)
The x-cell contained several dark staining granules and relatively few pale-staining nuclei. The most striking feature were the arrangements in a few large undivided cysts, and numerous subdivided cysts with different degrees of subdision. The cysts could be subdivided in equal parts or unequal parts with irregular forms which resembled in some stages budding processes (Fig.2).
In several large tumours these cysts were covered by an extremely thick outer layer and exhibited shrinked formations (Fig. 3).
If the subdivision of cysts reached the double-size of normally occurring x-cells, the unstained outer wall disappeared. Instead, a dark staining cell membrane sourrounded the multinucleate and uninucleate x-cells.
The ultrastructure of the x-cell cysts revealed a bilayered fibrous wall. The inner layer appeared more dense with finely fibrous material oriented parallel to the cell surface. The outer layer contained the same fibrous material but the orientation of the fibres was more irregular. The outer membrane of the cysts was rigid or had small protrusions and pores (Fig. 4 + 5). In cysts which were shrinked,these protrusions were very pronounced (Fig. 6). The cytoplasm was mostly filled with smooth ER and free ribosomes interspersed with membrane-bound lacunae filled with fibrillar material. In some x-cells these lacunae filled the whole cytoplasm (Fig. 7). In large cysts with one or two subdivisons nuclei were rare. In more subdivided stages four to two nuclei could be seen in the majority of x-cells (Fig. 8). In most cases of subdivisions of cysts, no nuclear divisions were discernible. Only in one case two nuclei with condensed chromatin appeared (Fig. 9).
In fish with large pseudotumours extensive necrotic areas and the formation of granulomas occurred. These features could be observed in treated and untreated fish. Therefore, the action of metronidazole on x-cells remained inconclusive.

DISCUSSION

The cysts reported here, resemble those described earlier from single cod caught in the North Sea (Watermann & Dethlefsen, 1982). But in the material presented here, more different stages which may represent subdivisions of cysts to typical x-cells, could be identified. The most striking feature of the cysts were the typical forms of subdivision which may be equal or unequal. Sometimes the subdivisions had the appearance of budding, but in all cases where cysts could be found, identical features occurred. The different stages resembled well those of division and cyst stages in the Hartmannellid amoeba *Dobellina mesnili* as well as in *Schizamoeba salmonis* (Bishop & Tate, 1939; Davis, 1926).
Additionally, the ultrastructure of cysts as described by Page (1985) resembled those for some Hartmannellidae and Vahkampfiidae. The double-layered fibrous cyst walls were in structure and thickness comparable to those found in limax amoebae. The protrusions of the cysts may reflect different stages of shrinking due to fixation and stage of the cysts.
As some of the specimens were treated with metronidazole, the shrinkage of cysts as well as the formation of lacunae may be attributed to the action of this compound (Buchner & Edwards, 1975; Ings & Constable, 1975). But these features occurred in untreated cod as well. It might be that the treatment with metronidazole favour the formation of cysts, so it can be used for following-up studies on cysts.
The cysts and multinuclear stages in x-cell lesions in Atlantic cod (*Gadus morhua L.*) resemble those, described as singular events in an earlier paper (Watermann & Dethlefsen, 1982). The material presented here, shows that the cystic structures and subdivision figures were not accidental cases.

The rare events of nuclear divisions in cystic cells resemble those described by Dawe (1981) for cod, and those described by Watermann et al. (1993) for dab. In both papers mitotic figures are presented in uninucleate and multinucleate x-cells. Especially the arrangement of condensed chromatin bodies as parallel strands insight a contact nucleus are significant.

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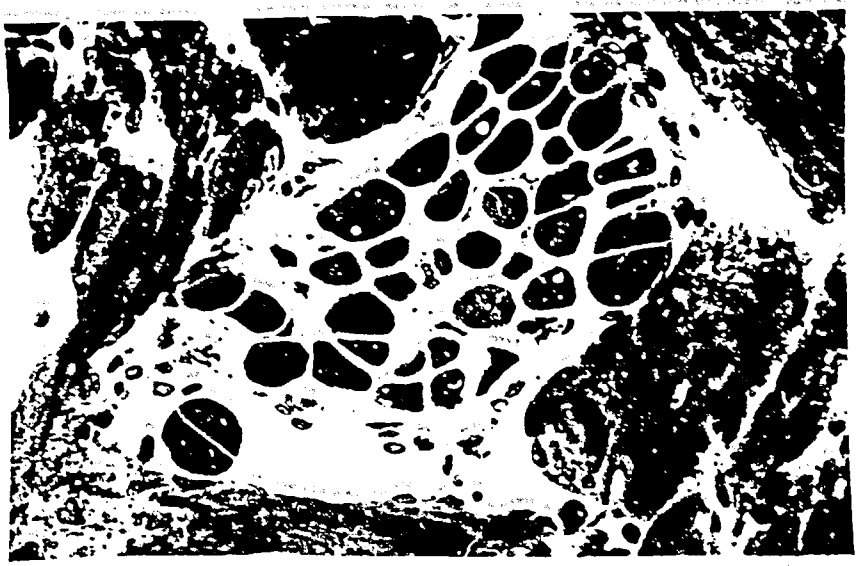

Fig. 1: Cysts in the heart of cod with numerous dark-staining granules and unstained cyst walls. 2.5 cm correspond to 10 μm. Toluidine-blue.

Fig. 2: Numerous cysts of x-cells with equal and unequal subdivisions of the cyst cytoplasm. 2.5cm correspond to 10 μm.

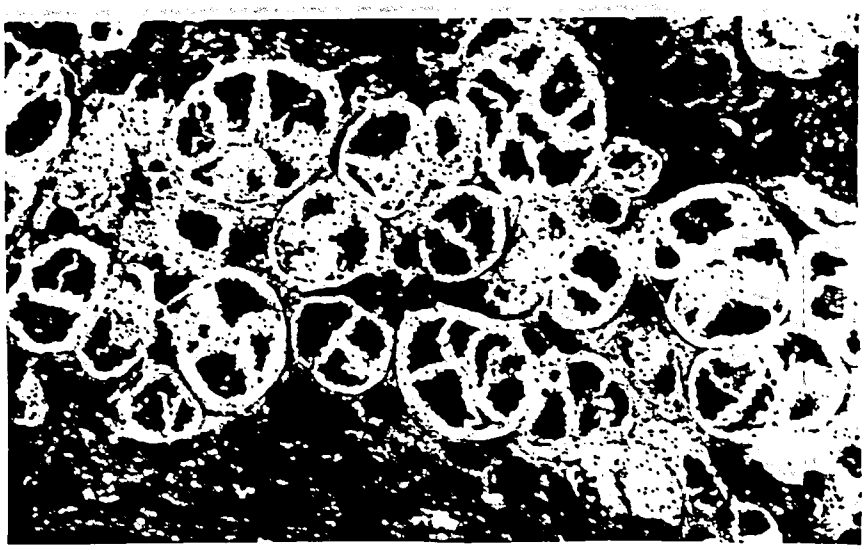

Fig. 3 Shrinked cysts with very thick outer walls. Several subdivisions and shape/size of non-subdivided cysts. 2.5cm correspond to 10 μm.

Fig. 4: Ultrastructure of cysts with a doublet (left) and two unequally subdivided stages. The outer wall is composed of fibrous material arranged in a double-layer. 1cm corresponds to 1 μm.

Fig. 5: Close view of an encysted x-cell with protrusions and pores at the cell membrane. Between the ER membrane-bound lacunae are visible, filled with fibrous material. 2cm correspond to 1 μm.

Fig. 6: Several shrinked cysts with extensive protrusions of the cell membrane. 1cm corresponds to 1μm.

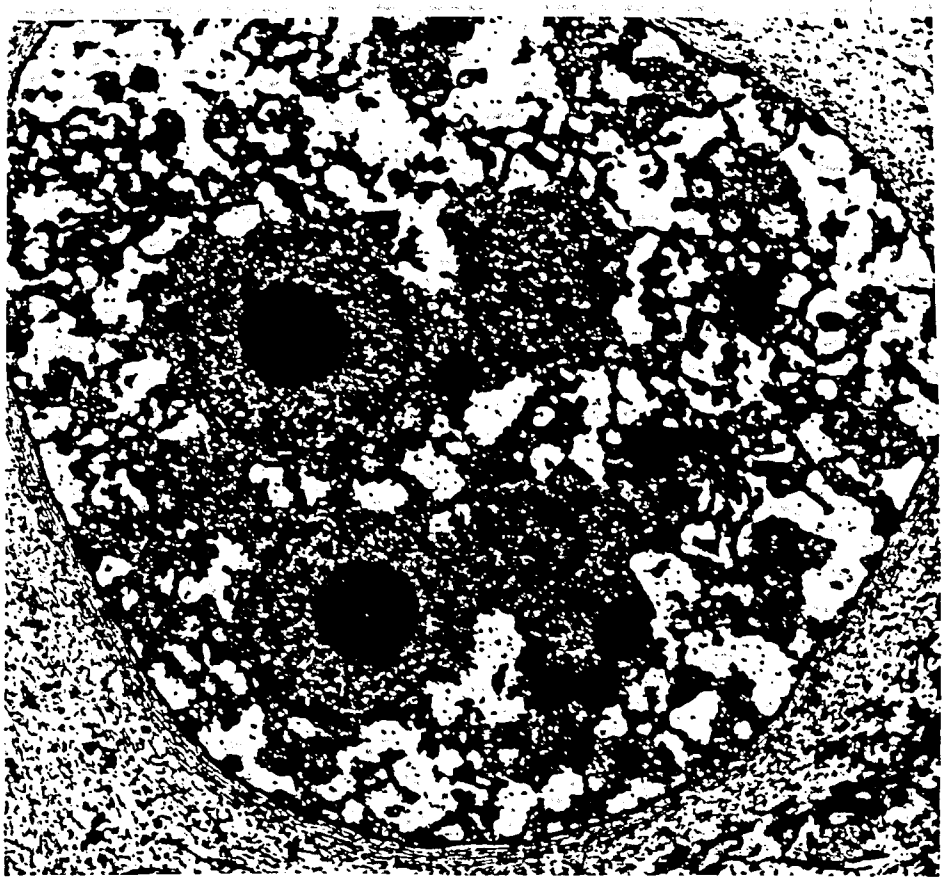

Fig. 7: X-cell cyst with a dissolved outer layer of the cyst wall. The whole cytoplasm is composed of lacunae with sparse fibrillar material. Four nuclei are visible. 1cm corresponds to 1μm.

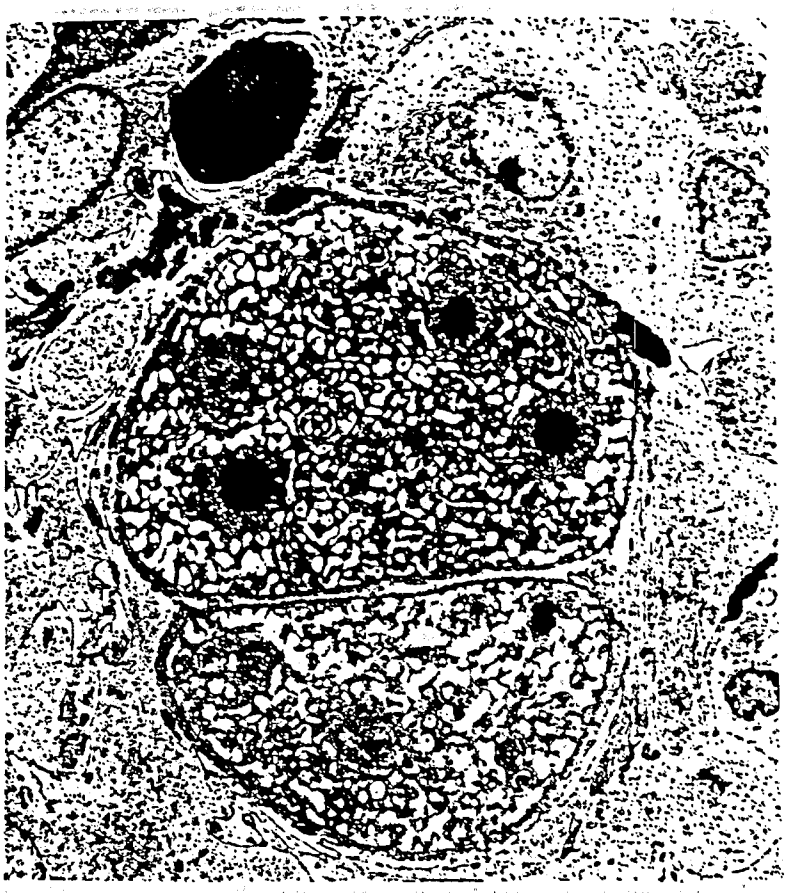

Fig. 8: A doublet of cysts with four nuclei in the upper part, and a dense contrasting cyst wall. 1cm corresponds to 1μm.

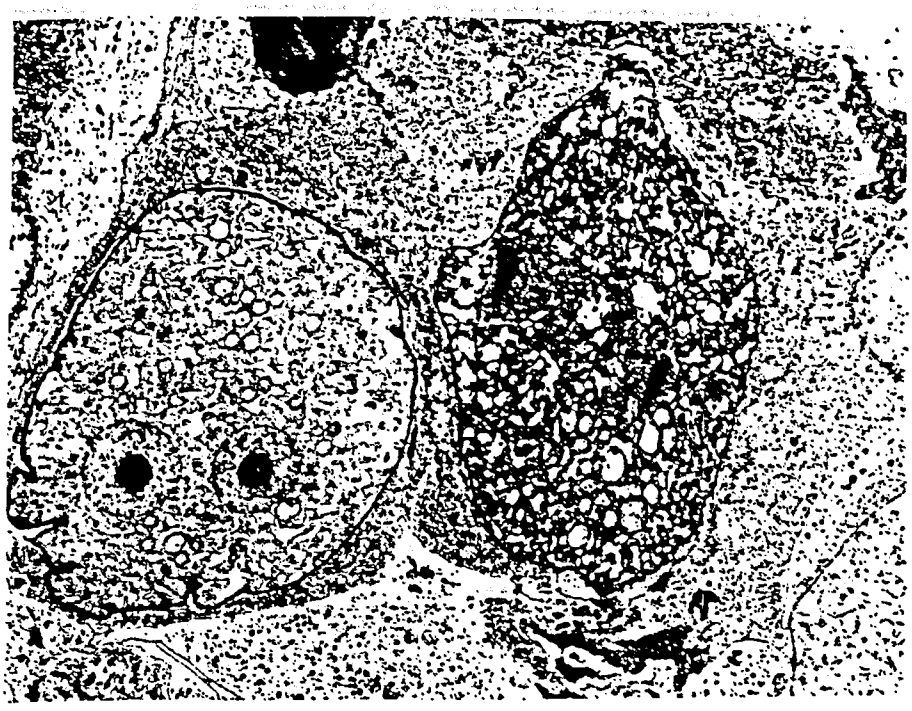

Fig.9: Condensed chromatin bodies (right cyst). 1cm corresponds to 1μm