Vol. 17: 137–143, 1993 | DISEASES OF AQUATIC ORGANISMS Dis. aquat. Org. | Published November 18

Division activities in x-cells of North Sea dab *Limanda limanda*

B. Watermann[1], W. B. Schmidt[2], N. Peters[2]

[1]LimnoMar, Wulfsdorfer Weg 200, D-22926 Ahrensburg, Germany
[2]Zoologisches Institut und Zoologisches Museum, Universität Hamburg, Martin-Luther-King-Platz 3, D-20146 Hamburg, Germany

ABSTRACT: Hitherto undescribed division activities in x-cells of dab *Limanda limanda* are presented. The observed features occurred in a very few samples as clusters in certain regions of the conditions. The results support the view that x-cells are dividing similarly to certain types of amoebas. Additionally found cytoplasmic inclusions were Feulgen-positive. Specific coated granules resemble retro-/lenti-viruses, and are associated with the inclusion bodies.

KEY WORDS: Flatfish · Histology Histopathology Protozoa · Virus division

INTRODUCTION

Since 1979 the presence of x-cells in dab *Limanda limanda* from the North Sea has been well documented with regard to histological, pathological and epizootiological aspects (Watermann 1982, Knust & Dethlefsen 1986, Diamant & McVicar 1987, 1989, McVicar et al. 1987). First described as an inflammatory condition of the subcutaneous tissue, later on x-cells were mainly found in gills and in the pseudobranch. To a very minor extent, small clumps or single x-cells could be found in spleen, head- and trunk kidney and, in 1 case, associated with the ovarian stroma (Diamant & McVicar 1987).

Despite the fact that the histological features of x-cells in dab as well as in other affected species have very often been thoroughly described, the nature and origin of this special cell type still remain doubtful. Since the investigations of Brooks et al. (1969) on dermal 'x-cell papillomas' of Pacific flatfish, it is disputed whether x-cells, which form the major part of these tumour-like conditions, are parasitic protozoans or virus-transformed host cells. Attempts to cultivate x-cells failed, and the majority of the x-cells in the lesions appeared necrotic or in a very inactive stage. Division activities of x-cells could not be detected in lesions of Pacific flatfish up to now but were well documented in pseudobranch tumours of Pacific and Atlantic cod (Dawe 1981, Peters et al. 1984). Likewise x-cells of Pacific flatfish show just a single nucleus, but bi- and multinucleate x-cells are known from x-cell conditions in Atlantic and Pacific cod.

Recent investigations in dab revealed binucleate x-cells with signs of mitotic activities but no clear evidence of nuclear cleavage (Diamant & McVicar 1989). In this report more data concerning division activities in x-cells of dab as well as some peculiar cell structures are presented.

MATERIALS AND METHODS

Dab were collected during several cruises between 1979 and 1984 in the southern North Sea, mainly in the German Bight. The cruises took place in January, May–June, August, October and December.

For light and electron microscopy, tissue samples 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. The standard Feulgen reaction was performed after Burck (1973).

RESULTS

Division activities

Division activities of x-cells could be found in dab with swellings on the trunk and on the fins. These conditions appeared as soft nodules of 1 mm to several cm in size. Yellow to creamy white in colour, they rose from the surrounding fin tissue without a distinct borderline. Swellings on the trunk had a slightly reddish appearance and could reach hazelnut size. In gill swellings no evident mitotic figures could be detected, but binucleate x-cells were regularly present.

In the conditions on the trunk, isolated clusters of x-cells with signs of nuclear division could be observed in the dermal areas. The x-cells in these clusters were surrounded by epitheloid 'envelope cells'. But mitotic activities also occurred in free-floating x-cells in areas with heavy inflammatory reactions and in the presence of masses of neutrophiles and other granulocytes. Tissue samples with indications of division activities were mostly found on cruises in October, some in June and a very few in January.

In light microscopy, mitotic patterns were apparent in x-cells with densely granulated, dark cytoplasm. Division activities were indicated by condensed chromatin particles, or were characterized by polar masses of dark staining material in rod-shape or club-like formation (Fig. 1). (Order of Figs. 1 to 13 reflects the suspected development of the division activities rather than the order of mention in text.)

Nuclear characteristics

The typical appearance of interphase nuclei in x-cells was characterized by a prominent round nucleolus and dispersed chromatin particles in the periphery. Suspicious patterns indicating division activities were revealed by changes in the distribution and granulation of chromatin particles. These particles appeared condensed, more granular and were orientated along an elongated nucleolus (Fig. 2).

Other cells besides the nucleolus with granular chromatin had an obviously ellipsoid shape with vaults in the most distant parts, where a horseshoe-like body was present (Fig. 3). In some x-cells, the chromatin particles were arranged in beads within an elongated nucleus. These cells had a rod-shaped form, and the nucleus was invaginated at 1 pole (Fig. 4). These invaginations could extend very deeply into the nucleus without any signs of a parallel cell cleavage (Fig. 7).

In one case, the chromatin particles appeared in the form of an equatorial plate in the centre of the nucleus (Fig. 5).

When the cells had a very stretched form, the nucleolus had faded away whilst the nucleus showed a narrow long extension with round or ovoid accumulations of chromatin material situated at opposite poles of the nucleus [Figs. 3 (lower x-cell) & 6]. In contrast, other cells with polar masses of chromatin exhibited a very distinct nucleolus (Fig. 9). In the stages described above, the nuclear membrane appeared intact.

As shown in Fig. 12, the majority of the x-cells had a prominent nucleolus with lacunae at the periphery. But in some sections, x-cells with 2 nucleoli and a lucent area in between them occurred (Fig. 8).

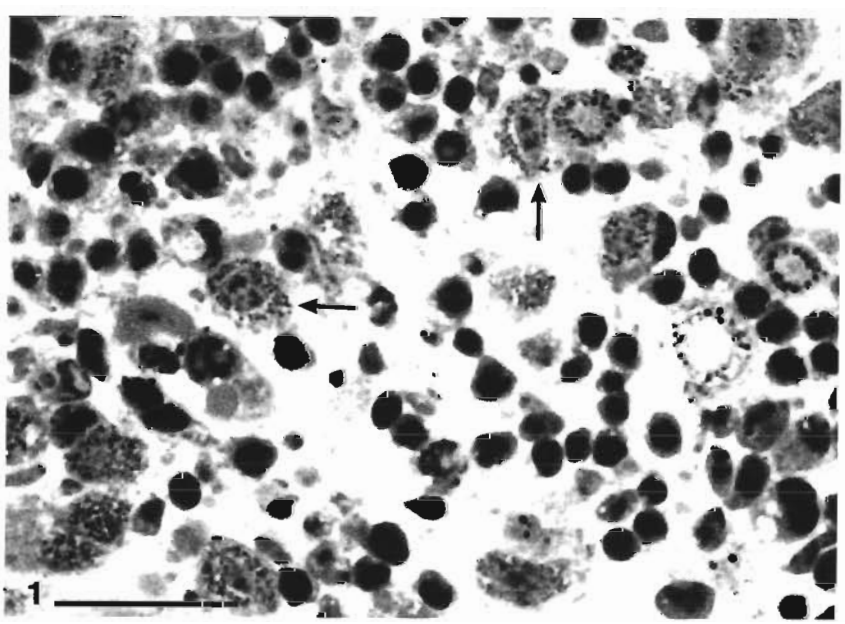
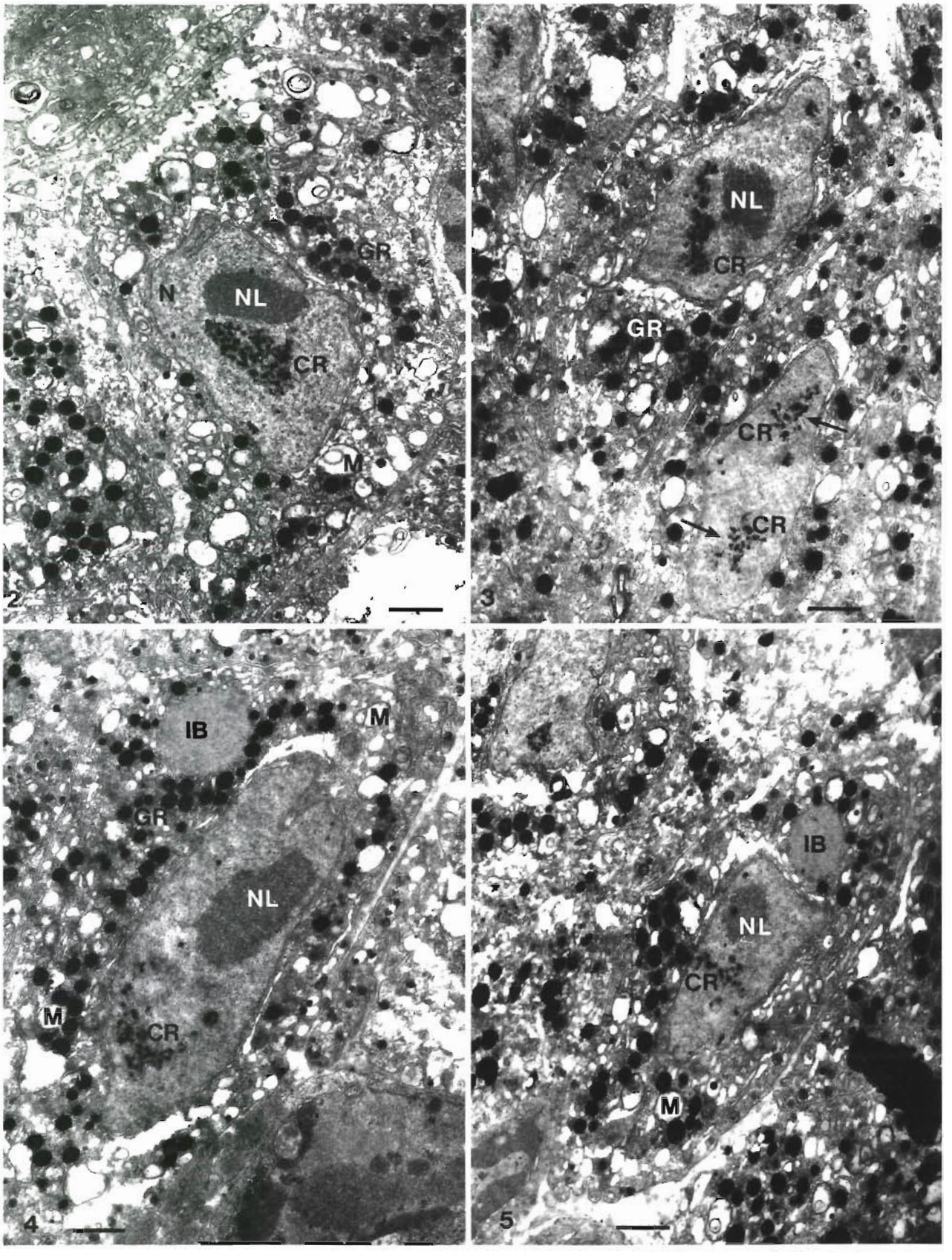
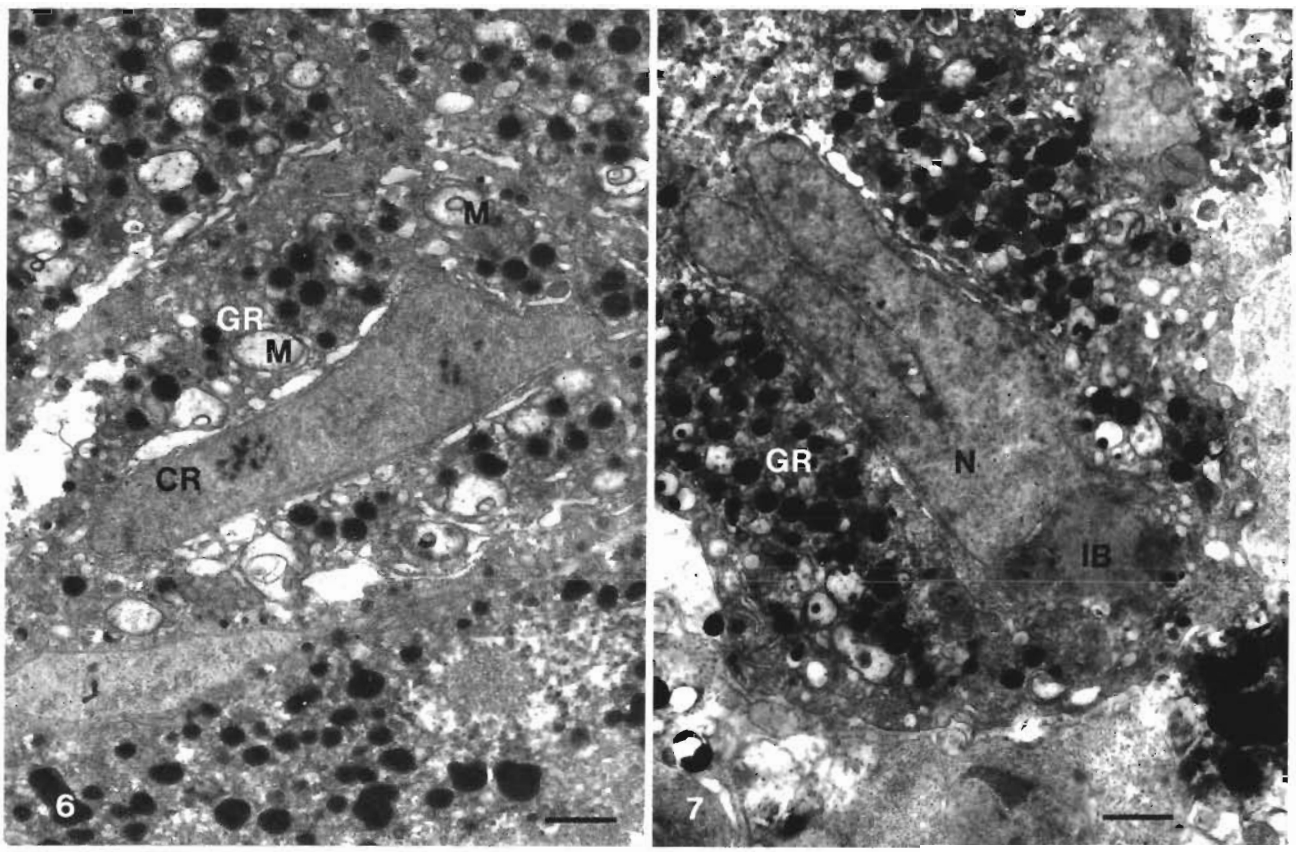


Fig. 1. *Limanda limanda*. Free-floating x-cells with mitotic-like nuclear patterns (arrows). Toluidine blue. Scale bar = 10 µm



Figs. 2 to 5. *Limanda limanda*. Fig. 2. Ovoid nucleus of an x-cell with lateral situated granular chromatin particles (CR). Scale bar = 1 µm. Fig. 3. Elongated nuclei of x-cells with granulated chromatin particles beside the nucleolus, and a horseshoe-like body at 1 pole of the upper x-cell. In the lower x-cell chromatin particles are distributed at opposite poles of the cell (arrows) Scale bar = 1 µm. Fig. 4. Elongated nucleus of an x-cell with an invagination at 1 pole. Chromatin particles and nucleolus present (Bar = 1µm). Fig. 5. Elongated nucleus of an x-cell with chromatin particles arranged as in an equatorial plate Scale bar = 1 µm

Abbreviations used in figures. N: nucleus, NL nucleolus, M. mitochondrium, GR. granules, CR. chromatin particles, IB· inclusion body; OC: outer coating; SG special granules; CY. crystalloid structures



Figs. 6 & 7 *Limanda limanda*. Fig. 6. x-cell with very elongated nucleus and chromatin particles situated at the poles. Scale bar = 1 µm. Fig. 7. Deeply invaginated nucleus of an x-cell. Scale bar = 1 µm

Cytoplasmatic structures in the x-cells

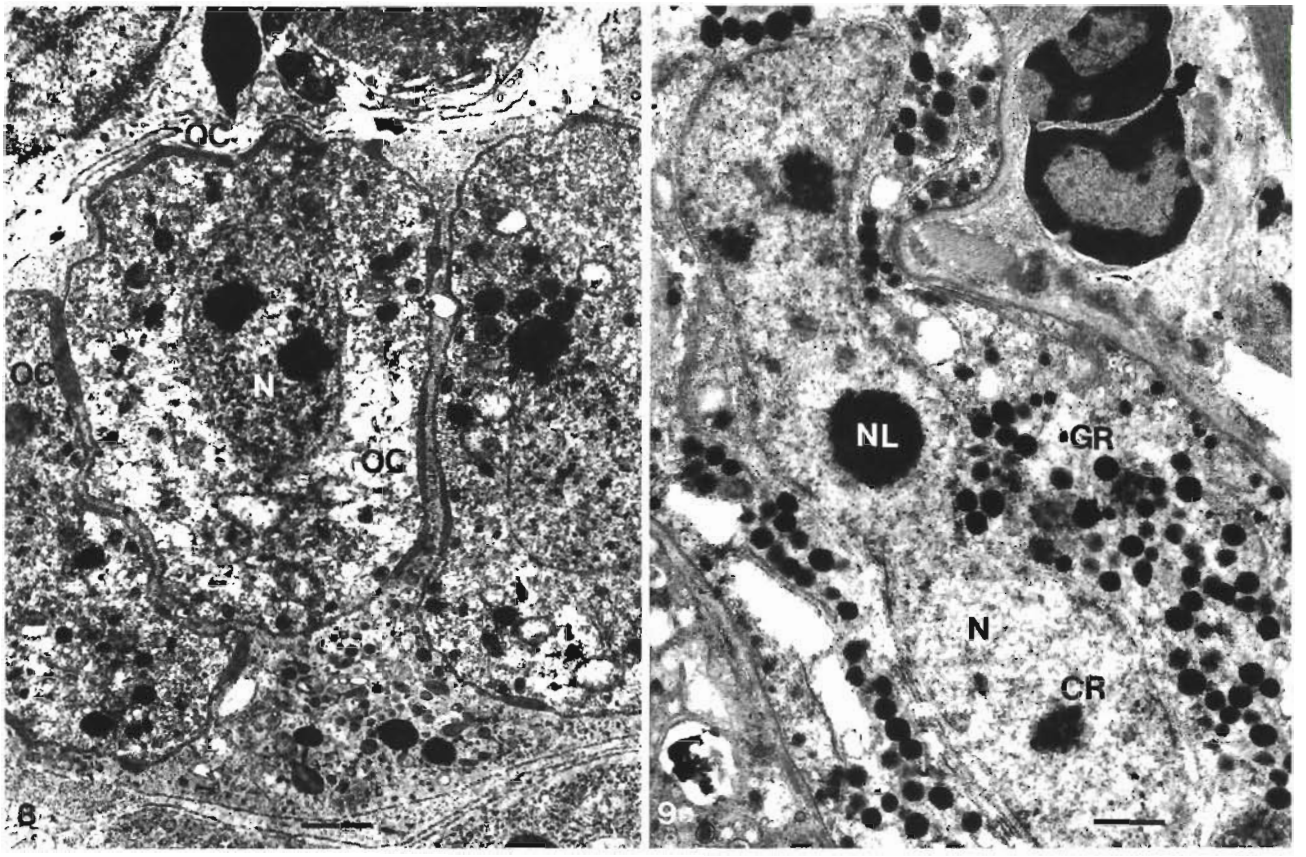
Besides the described division patterns, x-cells with peculiar structures in the cytoplasm occurred in the sections with clusters of dividing x-cells. In a number of x-cells, large bodies of finely dispersed material could be found in the neighbourhood of the nucleus. These round to oval bodies were not membrane bound and exhibited 4 or 5 smaller dark circles at their periphery in several cases (Figs. 10 & 11). In cells with division activities, these inclusion bodies were often situated directly next to the nucleus but did not show any cleavage (see also Figs. 4, 5 & 7). The inclusion bodies gave a Feulgen-positive reaction.

Some x-cells possessed an irregularly undulated outer membrane with an obvious glycocalyx on the outer borderline (Fig. 10). Moreover, other cells exhibited dense coatings of varying thickness at their surface (Fig. 12), and in these cases several membrane-bound particles with rod-shaped or irregularly formed inclusions different from the majority of granules in x-cells, lay closely attached to the cell membrane. The border of those granules stained darker, and was evidently thicker than those of the normal granules (Figs. 10 & 12).

In addition, some structures were present in a number of x-cells which appeared either in asteroid form or as parallel packed needles. There was no distinct membrane around these structures (Fig. 13).

DISCUSSION

The changes in the nuclei of the x-cells from inflamed dab papillomas described here are a significant departure from the normal condition, the interphase stage, and appear to represent various phases of nucleus division. In this context, the concentration of chromatin particles in the immediate vicinity of the nucleolus can be interpreted as prophase, the arrangement in something like an equatorial plate as metaphase, and the distribution of chromatin packets at both poles of the elongated nucleus as late anaphase. Strangely enough, a complete dissolution of the nuclear membrane occurs at no time, and in case the nucleolus also appears to remain intact. Occasionally, 2 nucleolar bodies are observed in the same nucleus. The sometimes deep invaginations observed on 1 side of the stretched nucleus can hardly be regarded as



Figs. 8 & 9. *Limanda limanda*. Fig. 8 x-cell with thick outer coatings at several sites of the cell surface. Scale bar = 1 µm Fig. 9. Elongated nucleus of an x-cell with prominent nucleolus, and clumped chromatin particles at the polarsites Scale bar = 1 µm

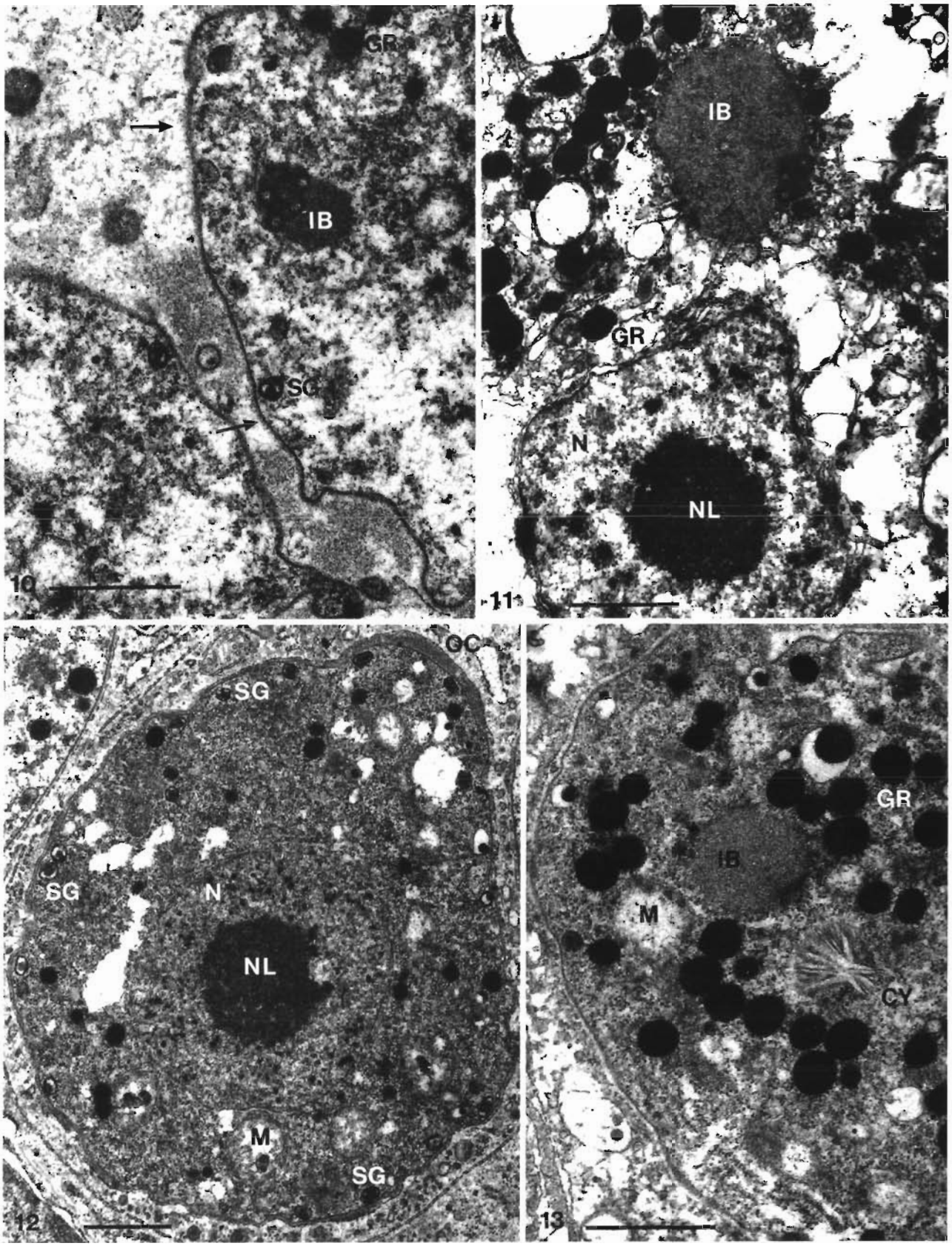
signs of division, since they run parallel and not perpendicular to the division plane of the chromatin. On the other hand, the above-mentioned observations of Diamant & McVicar (1989), that binucleate x-cells occasionally occur in gill lesions of dab, suggest that a complete division of the nuclei after the division processes of the chromatin given here is probable.

Despite intensive scrutiny, it was only possible to find the mitotic figures described here in a few afflicted dab. The mitotic phenomena were restricted to a few clear-cut zones in the inflammations, but always appeared in groups. This would suggest that long phases of mitotic inactivity are occasionally interrupted by simultaneous mitosis in limited areas of the x-cell lesions. The material presented here was collected during 11 cruises, but division activities could be found only in a very few dab. Taking into account a possible seasonal cycle for the reproduction of the x-cells, from our material an increased frequency of division activities could be observed in autumn.

Dawe (1981) found similar mitotic figures in x-cells from pseudobranchial tumours of Pacific cod as well as double and even multiple nucleated x-cells. However, Dawe described the dissolution of the nucleolus during prophase and the absence of the nuclear membrane during meta- and anaphase. As in our case, Dawe was not able to complete the cell division.

The mitotic figures observed in the x-cells of dab and cod show a certain similarity to cell division of some amoebae. Dawe (1981) mentioned *Dobellina mesnili* from the Hartmannellidae family in this connection. Singh (1975) described the Hartmannellidae as having 1 or 2 nucleoli, which disappear in the course of mitosis, and the chromosomes as being arranged in an equatorial plate, whereas our observations seem to correspond better to those of Gläser (1912) on *Amoeba tachypodia* and of Fizer & Wilhelm (1978) on *Vahlkampfia lobospinosa*.

Without question, the thickening of the outer cell membrane of x-cells can become extended to a 'precystic' layer and have amoeba-glycocalyx type characteristics (Page 1979, 1985, Cann & Page 1982). Similar thickenings of the outer cell membrane were also observed in x-cells of the teleosteans *Gadus morhua* and *Pagothenia borchgrevinki* (Watermann &



Figs 10 to 13 *Limanda limanda*. Fig. 10. Cytoplasm of an x-cell with inclusion body which contains several dark-staining circles in addition to its normal fine structure. Along the outer cell surface special granules are situated with distinct membranes including dark-staining irregular bodies. The cell surface shows a glycocalyx (arrows). Scale bar = 1 µm. Fig. 11. Inclusion body in the cytoplasm of an x-cell with irregular coarsed material and some circles at the periphery. Scale bar = 1 µm. Fig. 12. x-cell with several distinct granules situated just below the outer membrane. Note as well the thick coating on the outside of the cell. (N) Nucleus. (NL) Nucleolus. (M) mitochondrium. Scale bar = 1 µm. Fig. 13. Part of the cytoplasm of an x-cell with inclusion body and crystalloid structures. Scale bar = 1 µm

Dethlefsen 1982, Franklin & Davison 1988). At the margins of early pseudobranchial tumours of cod we have even found regular cyst formation, whereby the cysts sometimes contained multiply nucleated plasmodia of the x-cell type but were sometimes empty, multiply nucleated x-cells then being present between the more or less collapsed cyst cases (Watermann & Dethlefsen 1982).

The finely structured Feulgen-positive reacting cytoplasmic inclusion bodies observed by us in the x-cells of dab look like viral DNA, as can be seen in quite similar form in cases of well-known viral infections of fish, e.g. viral hematopoietic necrosis (VHN). The packets of round, hollow bodies of about 200 nm diameter frequently associated with the inclusion bodies could represent immature virions. The round to oval cyst-like particles of the same size situated at intervals under the cell membrane, which have irregular, sometimes rod-like inclusions and were described by Diamant & McVicar (1989) as mucocysts, might also be interpreted as being mature virions which have morphological similarities to lentiviruses from the retrovirus group (Coffin 1990).

Crystalloid inclusions are not infrequent in protozoans, especially in hartmannellids (Page 1987), although they are generally enveloped in a membrane. Crystalloid inclusions which are not membrane bound, as mentioned above for dab, indicate virus infection and are regarded as a typical cytopathological effect of such an infection (Grafe 1977).

Our results for dab give some evidence, on the one hand, that the x-cells are of protozoan nature, but they also support the contention that the x-cells might be virus-transformed host cells. An explanation consistent with all findings is that x-cells might be virally infected parasitic protozoans. In this connection it should be noted that free-living protozoans frequently resort to a parasitic way of life, then becoming hardly indentifiable in the host organism (Page 1974, Larsson et al. 1992). However, the true nature of x-cells can only be revealed by molecular biological DNA analysis of the x-cell nucleus.

LITERATURE CITED

Brooks, R. E., McArn, G. E., Wellings, S. R. (1969). Ultrastructural observations on an unidentified cell type found in epidermal tumors of flounders. J. natn. Cancer Inst. 43: 97–109

Burck, H.-C. (1973). Histologische Technik. Thieme, Stuttgart

Cann, J. P., Page, F. C. (1982). Fine structure of small living paramoeba (Amoebida) and taxonomy of the genus. J. mar. biol. Ass. U.K. 62: 25–43

Coffin, J. M. (1990). Retroviridae and their replication. In: Field, B. N., Knipe, D. M. (eds.) Fields virology, Vol. 2. Raven Press, New York, p. 1437–1500

Dawe, C. J. (1981). Polyoma tumors in mice and x-cell tumors in fish, viewed through telescope and microscope. In: Dawe, C. J., Harshbarger, J. C., Kondo, S., Sugimura, T., Takayama, S. (eds.). Phyletic approaches to cancer. Japan Scientific Societies Press, Tokyo, p. 19–49

Diamant, A., McVicar, A. H. (1987). The effect of internal and external x-cell lesions on common dab, *Limanda limanda* L. Aquaculture 67: 127–133

Diamant, A., McVicar, A. H. (1989). Distribution of x-cell disease in common dab, *Limanda limanda* L, in the North Sea, and ultrastructural observations of previously undescribed developmental stages. J. Fish Dis. 12: 25–37

Fizer, E. R., Wilhelm, W. E. (1978). Promitosis and rod occurrence in the Limax ameba *Vahlkampfia lobospinosa*. J. Protozool. 25 (1): 34–38

Franklin, C. E., Davison, W. (1988). X-cells in the gills of an Antarctic teleost, *Pagothenia borchgrevinki*. J. Fish. Biol. 32: 341–353

Gläser, H. (1912). Untersuchungen über die Teilung einiger Amöben, zugleich ein Beitrag zur Phylogenie des Centrosoms. Arch. Protistenk. 25: 27–152

Grafe, A. (1977). Viren. Parasiten unseres Lebensraumes. Springer, Berlin

Knust, R., Dethlefsen, V. (1986). X-cells in gills of North Sea dab (*Limanda limanda* L.), epizootiology and impact on condition. Arch. FischWiss. 37: 11–24

Larsson, J. I. R., Bach de Roca, C., Gaju-Ricart, M. (1992). Fine structure of an amoeba of the genus *Vahlkampfia* (Rhizopoda, Vahlkampfiidae), a parasite of the gut epithelium of the bristletail *Promesomachilis hispanica* (Microcoryphia, Machilidae). J. Invertebr. Pathol. 59: 81–89

McVicar, A. H., Bucke, D., Watermann, B., Dethlefsen, V (1987). Gill x-cell lesions of dab *Limanda limanda* in the North Sea. Dis. aquat. Org. 2: 197–204

Page, F. C. (1974). *Rosculus ithacus* Hawes, 1963 (Amoebidae, Flabelluidae) and the amphizoic tendency in amoebae. Acta Protozool. 13 (12): 143–156

Page, F. C. (1979). The fine structure of *Vexillifera bacillipedes* (Amoebida, Paramoebidae). Arch. Protistenk. 122: 9–19

Page, F. C. (1985). The limax amoebae: comparative fine structure of the Hartmannellidae (Lobosea) and further comparisons with the Vahlkampfiidae (Heterolobosea). Protistologica 21(3): 361–383

Page, F. C. (1987). The classification of 'naked' amoebae (phylum Rhizopoda). Arch. Protistenk. 133: 199–217

Peters, N., Schmidt, W., Kranz, H., Watermann, B., Stich, H. F. (1984). Das X-Zellen-Problem. Mitt. hamb. zool. Mus. Inst. 80: 53–65

Singh, B. N. (1975). Pathogenic and non-pathogenic amoebae. Wiley & Sons, New York

Watermann, B. (1982). An unidentified cell type associated with an inflammatory condition of the subcutaneous connective tissue in dab, *Limanda limanda* L. J. Fish Dis. 5: 257–261

Watermann, B., Dethlefsen, V (1982). Histology of pseudobranchial tumours in Atlantic cod (*Gadus morhua*) from the North Sea and the Baltic Sea. Helgoländer Meeresunters. 35: 231–242

This article was submitted to the editor

Manuscript first received: December 27, 1992
Revised version accepted: August 4, 1993