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MALPEQUE DISEASE: ISOLATION AND MORPHOLOGY OF A LABYRINTHOMYXA-LIKE ORGANISM FROM DISEASED OYSTERS

by

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Abstract

Malpeque disease is an important shellfish disease in Canada. The susceptibility of oysters to the disease and the associated symptoms have already been studied extensively. The etiology of this disease, however, is little known. A Labyrinthomyxa-like organism was isolated from diseased Malpeque oyster and its morphology in relation to the histopathology of tissues from diseased oysters is discussed.

Résumé

La maladie de Malpeque est une maladie importante chez les crustacés au Canada. La frequence de cette maladie et ses symptomes chez les huêtres ont été étudiees en detail cependant, les causes de cette maladie sont peu connues. Un organisme de type Labyrinthomyxa a été isolé d'une huêtre attante de la maladie de malpeque. Sa morphologie est discustée en relation avec la pathologie des tissus de l'huêtre atteinte de cette maladie.

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Introduction

Repeated epizootics of Malpeque disease in the early part of this century occurred in certain oyster populations in the Maritime provinces, virtually destroying the oyster industry in the affected areas (Needler, 1932; Needler and Logie, 1947; Logie, 1958). The progeny of the small percentage which survived appear to have a degree of resistance to the disease. Oysters growing in Cape Breton (Nova Scotia) have never been found to suffer the disease, although they have consistently been shown to be susceptible to it upon transfer to affected areas (Logie and Drinnan, 1960; Drinnan and Medcof, 1961). The general symptoms associated with Malpeque disease include emaciation of the affected animals, weakness of the adductor muscle, development of pustules, presence of giant spherical cells, poor gonadal development and/or incompleteness of spawning. In our studies on this deaseas we have observed all of the listed symptoms, particularly noting numerous giant spherical cells in certain tissues (Li et al., 1975; Li, 1976). A microparasite with a morphology similar to those giant cells has been isolated, which, in culture, has been seen to undergo endogenous budding as well as binary fission. It has been classified as Labyrinthomyxa species (Li and Clyburne, 1978). The observed, electron dense, buds have been consistently seen in pustules and other tissues of oysters suffering Malpeque disease. It is suggested that this may be an opportunistic organism that affects stressed or weakened animals.

Materials and Methods

Oysters

Oysters from two sources were used in this study. These were oysters transplanted from Cape Breton to Malpeque Bay for their second winter where they suffered 80-90% mortality, and native Malpeque oysters exhibiting weakness of adductor muscle after harvest for market.

Isolation of the organism

The spherical parasitic cells were isolated from gaping Malpeque oysters using small pieces (approximately 1 mm^3) of tissue from the digestive diverticulum, which were placed on a modified Vishniac medium (Booth et al., 1965) containing antibiotics (100 units/ml potassium penicillium G, and 100 mcg/ml of Streptomycin sulphate). The inoculated plates were incubated for 21 days at 15°C.

Histology and electron microscopy

For histological examination oyster tissues were fixed in Davidson fluid (Shaw and Battle, 1957) and the fixed specimens were washed, dehydrated, and sectioned by standard methods. The sections (5-8 µm) were stained with Harris hematoxylin and eosin.

Cells of the isolated organism were harvested for electron microscopy from the culture medium and washed once in sterile sea water. After low centrifugation, the cell pellets were fixed in 2% glutaraldehyde in 0.05 M phosphate buffer, pH 7.0 for 3 hours at 4°C, followed by 1% osmium tetroxide in the same buffer for 1 hour. Pellets were then washed in distilled water and dehydrated in a graded acetone series. A similar double fixation and dehydration procedure was used for infected tissues from oysters that had been transplanted from Cape Breton. The dehydrated specimens were embedded in Durcupan (Fluka). Sections were prepared using an LKB ultratome; thin (1 µm) sections were stained with Azure-II-methylene blue stain, and ultra-thin sections were stained with uranyl acetate and Millonig lead or lead citrate. Specimens were examined with a Hitachi HS-7 or HS-9 electron microscope.

Results

In a number of trials since 1970, oysters transplanted from Cape Breton have consistently suffered 80-90% mortalities in a 2-year exposure to the water of Malpeque Bay. The condition index of these oysters, especially prior the major outbreak of mortality, was significantly lower than that of the native controls (3.04 ± 1.7 versus 8.76 ± 1.2), and the tissues were heavily infected by giant spheres (Fig. 1). Pustules were often observed in the infected tissues (Fig. 2A), and thin sections of pustules indicated the presence of minute granules among aggregated hemocytes (Fig. 2B). These granules are similar to those found in the giant spheres.

In the fall of 1974 a serious mortality occurred in harvested native Malpeque oysters prior marketing. The condition index of these oysters was low (2.66 ± 0.7) compared with apparently healthy oysters (5.89 ± 0.8), and they exhibited weakness in the adductor muscle evidenced as gaping. The tissues of these oysters were massively infected with giant spheres in all 20 specimens examined. An organism was isolated on the modified Vishniac medium, which, as seen by phase contrast microscopy, ranged from less than 1 µm to over 20 µm in diameter (Fig. 3). A movement of the granular inclusions in the cells was observed occasionally in the freshly mounted preparations. These granular inclusions apparently were released from the cell upon rupture of the cell membrane. Electron microscopy of the cultured cells revealed that the cells might contain eccentric nuclei and numerous buds in cytoplasmic vacuoles (Fig. 4). The electron dense buds appeared to be covered by filamentous structures, but their detailed internal structure could not be discerned. Figure 5 shows a group of bud enclosed within a membrane, however, no nucleus was observable. The released buds appeared to develop nuclei preparatory to undergoing binary fission. Under certain growth conditions the organism formed cysts or sporangia (Li and Clyburne, 1978). Both electron dense buds and cysts were commonly observed in the infected tissues of the transplanted Cape Breton oysters (Figs. 6 and 7). They appear to be identical to the organism isolated from the diseased native Malpeque oysters, although the parasite in the transplanted oysters was not isolated.

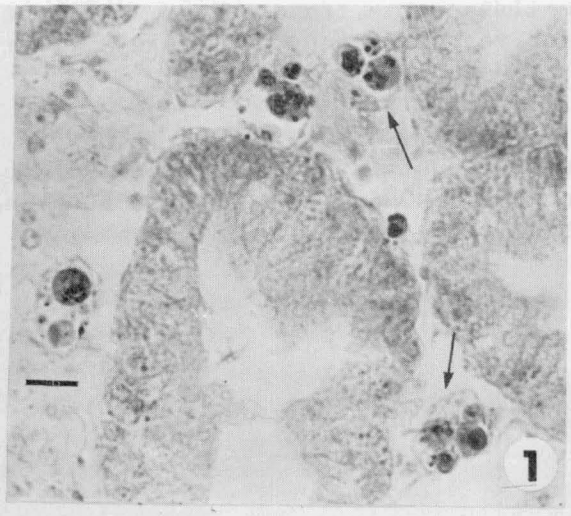
Figure 1: Section of the transplanted Cape Breton oyster after 2 years in Malpeque Bay. Note infection of giant spheres with minute granular inclusions in the vicinity of digestive diverticulum (see arrows). Harris hematoxylin and eosin. Bar = 10 µm.

Figure 2: Sections of mantle pustules of the transplanted Cape Breton oyster.
2A: the developing mantle pustules. H and E. Bar = 150 µm.
2B: thin section of the pustule showing minute granules among the aggregated hemocytes (see arrow). Azure-II-methylene blue stain. Bar = 10 µm.

Figure 3: Micrograph of phase contrast microscopy of a freshly mounted preparation of Labyrinthomyxa-like organisms grown on the modified Vishniac medium at 15°C. Note the cells contain granular inclusions of various sizes. Bar = 20 µm.

Figure 4: Electron microscopy of the Labyrinthomyxa-like organism on Vishniac medium. Note the cell with eccentric nucleus and numerous electron dense endogenous buds in the cytoplasmic vacuoles. V = vacuoles; B = buds. Bar = 1 µm.

Figure 5: Electron micrograph of a modified spore or sporocyst of the Labyrinthomyxa-like organism grown on the Vishniac medium. Note numerous buds without distinct nucleus and vacuole. B = buds. Bar = 1 µm.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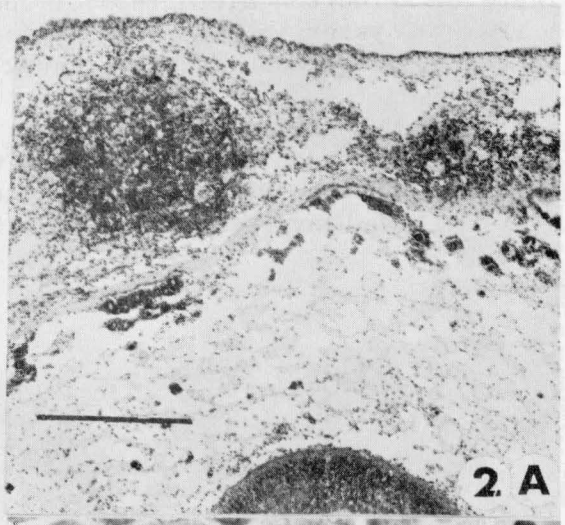
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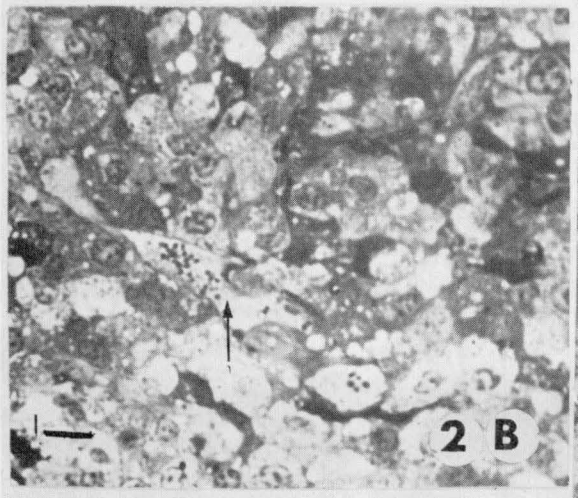
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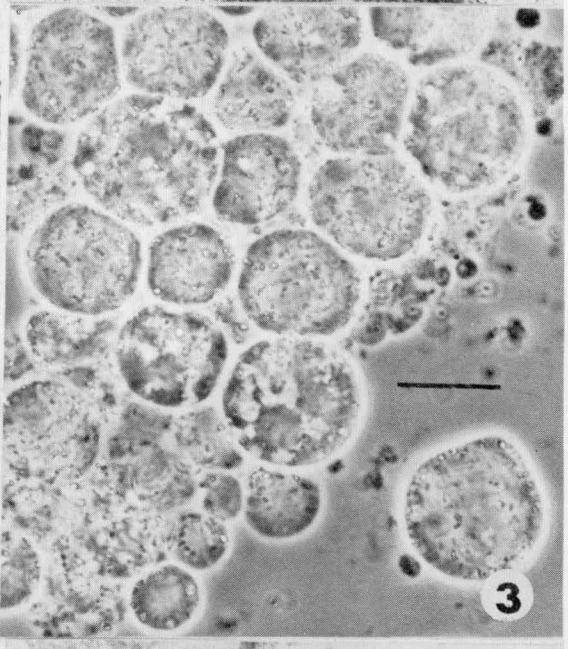
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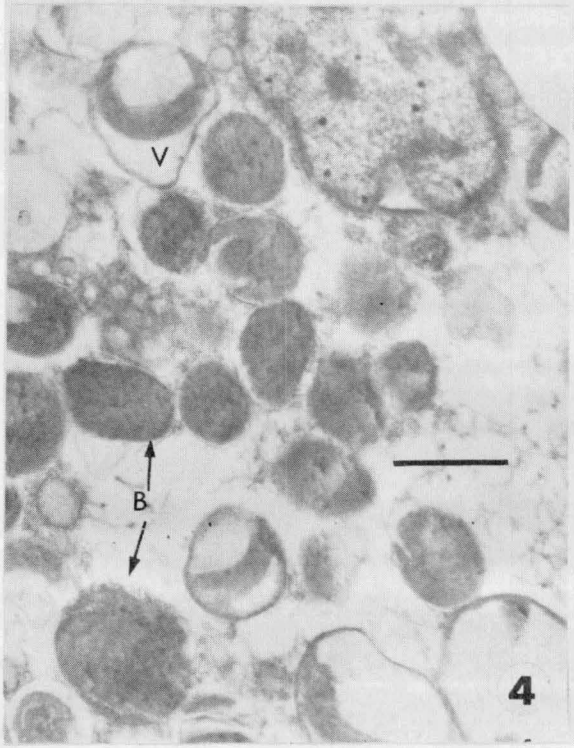
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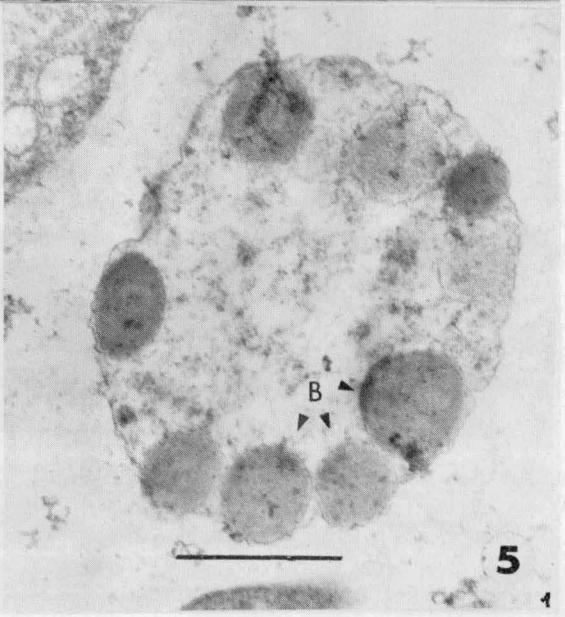
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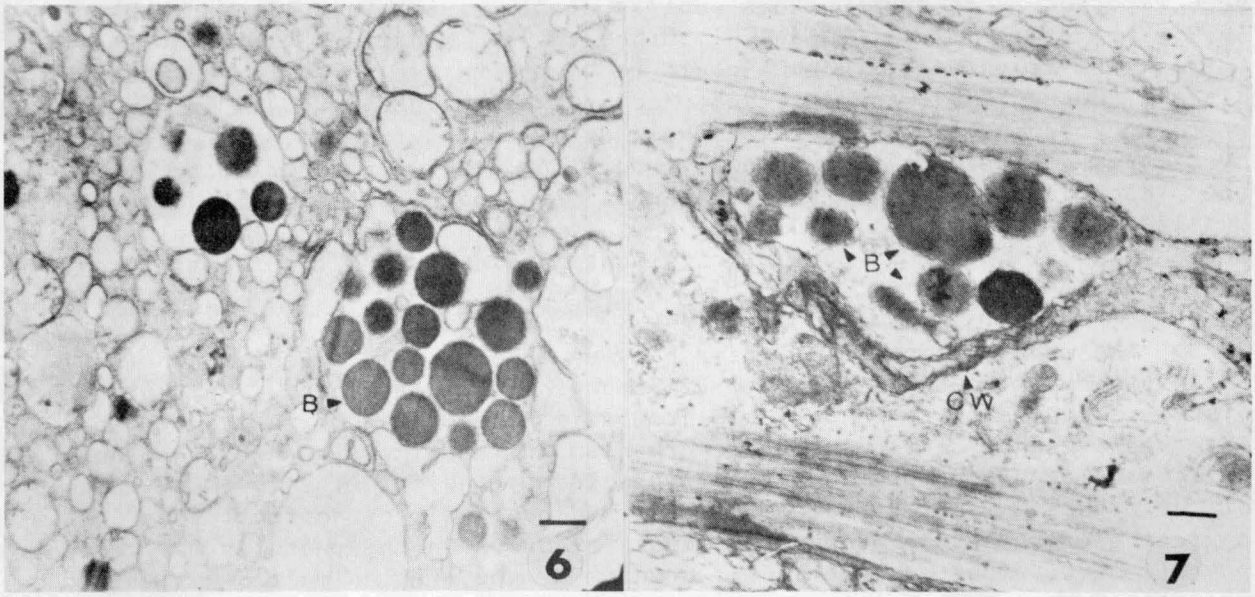
Figure 6: Electron micrograph of the tissue of a transplanted Cape Breton oyster in Malpeque Bay for 2 years showing the numerous electron dense buds in the tissue. B = bud. Bar = 1 µm.

Figure 7: Electron micrograph of a mantle pustule of a transplanted Cape Breton oyster demonstrating a group of electron dense buds enclosed in heavy wall. B = bud; CW = wall. Bar = 1 µm.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Discussion and Conclusion

Labyrinthomyxa marinum has been one of the oyster pathogens most studied over the past decades (Mackin et al., 1950; Ray, 1966; Perkins, 1969). Recently a Labyrinthomyxa-like organism related to shellfish mortalities has been reported by several workers (Sparks et al., 1968; Valiulis and Mackin, 1969; Kern et al., 1973; Li and Clyburne, 1979). Mackin et al. suggested that Labyrinthomyxa patuxent (Hogue) could be the causative agent for Malpeque disease (Hogue, 1921; Mackin, 1968; Mackin and Schlicht, 1976). It can reproduce by endogenous budding as well as by binary fission, and will undergo cyst formation under certain growth conditions, etc. In contrast to L. marinum, the present isolate showed no increase in size and atypical reaction with Lugol's solution (unpublished data) when it was incubated in a thioglycollate medium (Ray, 1966). Our morphological data indicate that the isolate greatly resembles L. patuxent (Hogue).

The morphological similarity between the isolated organism and the parasitic cells found in infected tissues of the transplanted Cape Breton oysters suggests that it could be involved in the oyster mortalities. The observed presence of the parasitic organism in the digestive diverticulum, gill, mantle and in the vicinity of vessels may indicate the route of infection of the organism via the digestive tract and/or by direct contact. The aggregate of homocytes around the minute granules in the pustules may reflect pustule development as a defence mechanism of the host animal to wall off the infective agent. The native Malpeque oysters were harvested after a major storm (Woo, personal communication) when silting had occurred and salinity was abnormal for an extended period. This supports Laird's suggestion that stress may have a significant effect on the susceptibility of the oyster to this disease (Laird, 1961). The organism appears to exist in contaminated waters for opportunistic attack of stressed host.

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