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VIRUS-LIKE PARTICLES IN PEARL OYSTER *PINCTADA MARGARITIFERA*

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Abstract

Virus-like particles were detected in granulomas associated with focal necrosis within the adductor muscle of the pearl oyster *Pinctada margaritifera* from French Polynesia. About 40 nm in size, these particles were generally found within cytoplasmic vesicles inside heavily degenerated granulocytes. More studies are required to further characterize them.

Introduction

Mass mortality of the pearl oyster *Pinctada margaritifera* has occurred in 1985 in French Polynesia (Cabral, 1990). This mortality was associated with malformations of the shell, lesions of the mantle and additional symptoms, such as necrosis of the adductor muscle. At present, the causes of this disease are not known. A gregarine has been described by Chagot (1993) in the digestive tract of the animals affected by the mortality, but the exact role of this sporozoan was not established. Main data of the investigations relate to perturbations in the mineralization process of the shell (Marin and Dauphin, 1992). Although the mortality has rapidly decreased, returning to a normal level, some symptoms are still persistent. During the last two years, routine examinations have shown that in several atolls, pearl oysters exhibited forms of abscess in the adductor muscle. We report here histological and cytological features of this symptom.

Material and method - Pearl oysters selected for study were characterized by an unusual secretion of mucus and displayed grossly visible abscesses in the adductor muscle. For routine light microscope examination, samples of tissue were preserved in Davidson fixative (Shaw and Battle, 1957) and paraffin sections were stained according to the Mann-Dominici method. Excised muscle tissue and isolated aggregates of granulomatous cells were fixed for electron microscopy in glutaraldehyde (0.4 M cacodylate buffer, pH 7.2) and postfixed in 2 % osmium tetroxide (0.4 M cacodylate buffer, pH 7.2). Tissues, embedded in Epon resin were sectioned and stained with uranyle acetate and lead citrate according to Reynolds (1963).

Results

Extensive necrosis of the adductor muscle was accompanied by disorders in the muscle structure. Sections through the muscle showed a progressive degenerating of the myofibrills. Other changes consisted of diffuse granulomatous inflammatory reaction and occasionally, degraded muscle fibres were interspersed with aggregates of hemocytes. In the centre of the lesion the muscle tissue was entirely necrosed and replaced by a dense granulomatous area (Fig.1a). Electron microscope examination revealed that these granulomas consisted mainly of granulocytes and macrophages which contained lysozomes, and numerous membraneous vesicles and cellular debris (Fig. 1b). In addition pictures of phagocytosis were observed and, in the less affected muscular tissues, glio-interstitial cells proliferated, characterized by the presence of electron dense and membrane bound ovoid bodies (Fig. 1c & 1d). No bacteria or parasites were observed through the lesions of the muscle. However, isometric virus-like particles (VLPs) were found, spread among cellular material (Fig. 2a). It was common to find that internal cellular organization had disintegrated in most of the VLPs-associated cells. Paraspherical or polygonal in shape, VLPs, with a diameter of 40 nm, consisted of a membrane-like envelope coating a central electron dense core which measured 35 nm in diameter (Fig.2b). Some particles

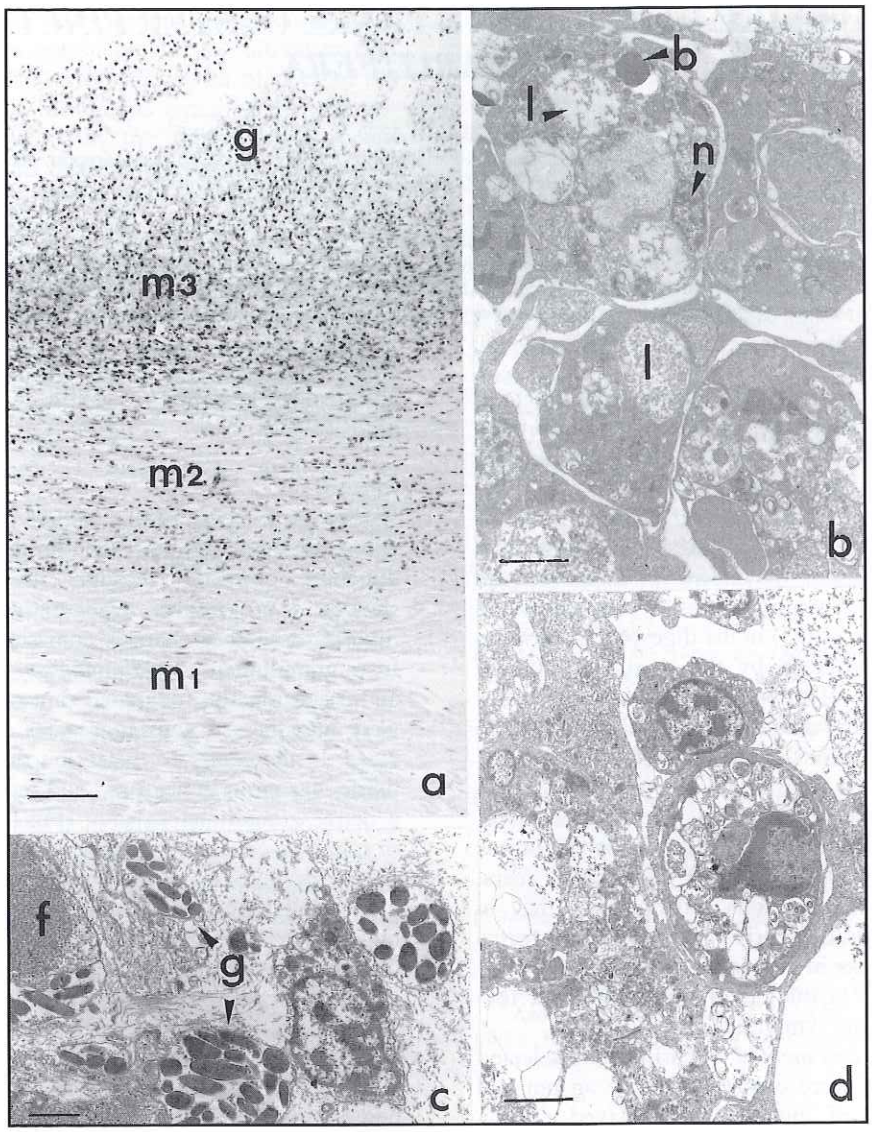

Figure 1. **a** - Section through a muscle lesion showing different stages of alteration of the muscular tissues. (m1): sound tissues; (m2): intermediate area exhibiting hemocytes infiltrated muscular tissues; (m3): inner layer consisting of debris of muscle fibres associated with numerous hemocytes; (g): granuloma. Mann-Dominici. Bar=100 µm. **b** - Inside the granuloma, the hemocytes are strongly aggregated; nucleus (n); lysosome (l); electron-dense bodies (b). TEM. Bar=2 µm. **c** - The intermediate area of the lesion contains numerous glio-interstitial cells (g); muscle fibres (f). TEM. Bar=2 µm. **d** - Electron micrograph of a granular blood cell that has ingested a second granulocyte. TEM. Bar=1 µm.

were seen in membrane bound vacuoles (Fig. 2c).

Discussion

Symptoms similar to those reported here have been previously recognized in scallop

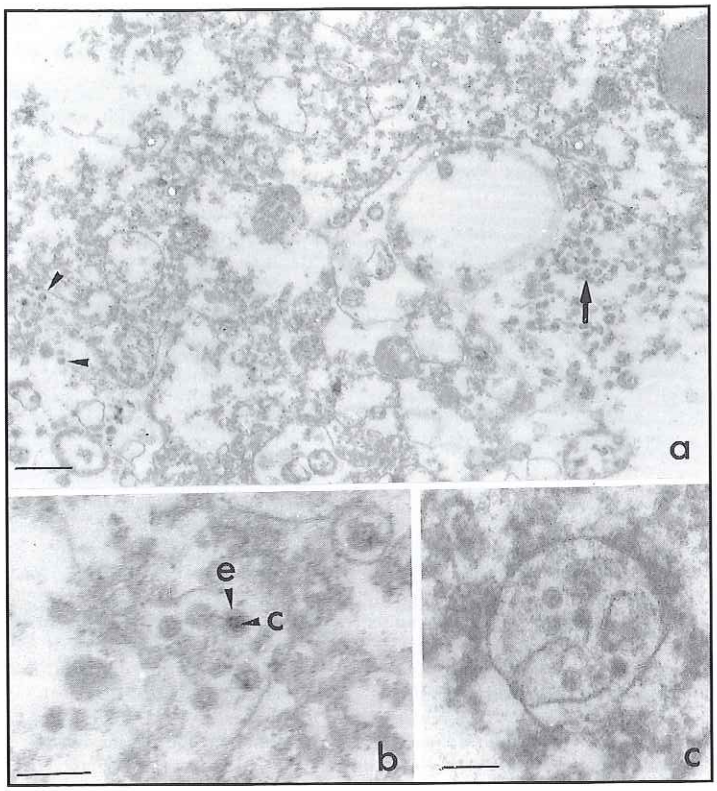

Figure 2. a - Whithin the hemocytes the VLPs appear spread (arrows) or grouped (head of arrow) through the cytoplasm. TEM. Bar=500 nm. **b** - Electron micrograph showing the morphology of the VLPs. Central core (c); outer envelope (e). Bar=100 nm. **c** - Membrane bound vesicle containing VLPs. TEM. Bar=100 nm.

Patinopecten yessoensis, which has been affected by abnormal mortality (Mori, 1975). As seen in the pearl oyster, degeneration of the muscle fibres was associated with an inflammatory reaction. However, light microscope examinations did not reveal bacterial or parasitic infection. We might also emphasise that the lesions found in the muscle of *Pinctada margaritifera* display some similarities to granulocytomas which developed in the mussel *Mytilus edulis* infected by a picornalike virus (Rasmussen, 1986). This virus replicates in cytoplasmic vesicles within granulocytes which could aggregate to form the granulocytomas. Although the significance of the VLPs found in *P. margaritifera* is presently unknown, perhaps the occurrence of an infection similar to the one previously described in the mussel, could be hypothesised to explain the formation of granulomas in the muscle of the pearl oyster. We could also emphasise the resemblence of the VLPs here reported with the virus-like particles found in scallop *Pecten novaezelandiae* by Hine and Wesney (1997).To date, few numerous viruses are known as pathogens for marines bivalves. Herpes viruses and Iridoviruses were reported in *Crassostrea virginica* and *C. gigas* (Farley *et al.*, 1972; Nicolas *et al.,* 1992); Hine *et al.*, 1992) and in *C. angulata* and *C. gigas* (Comps, 1988; Elston, 1979), respectively. Some information on the presence of other virus-like particles in marine lesions was summarised by Farley (1978) and Laukner

(1983). In the genus *Pinctada,* the first virus infection was described by Norton *et al.* (1993). The virus, probably belonging to the papovaviridae, has been observed in the enlarged nucleus of labial palp epithelial cells of the golden-lipped pearl oyster *Pinctada maxima.*

Further studies are planned to isolate and further characterise the particles described.

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