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HERPES-LIKE VIRUS INFECTING JAPANESE OYSTER (*CRASSOSTREA GIGAS*) SPAT

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Introduction

During the summer of 1993, abnormal sporadic mass mortalities (80-90%) and morbidities occurred among four batches of young Japanese oyster (*Crassostrea gigas*) spat from the French Atlantic coast. These mortalities were only observed in July. Samples of affected animals were examined by electron microscopy. This paper reports the presence and appearance of herpes viruses in these samples.

Material and Methods

Samples of cultured 3-7 month-old oyster spat were collected on the French Atlantic coast. These spat were survivors of populations in which mortalities of 80-90% had occurred.

For light microscopy, oysters were fixed in Davidson's fluid. For transmission electron microscopy, tissue pieces were fixed for 1h in cold 2.5% glutaraldehyde in cacodylate buffer and post-fixed in 1% osmium tetroxide in the same buffer. Tissues embedded in Epon were cut on a LKB ultramicrotome and were stained with uranyl acetate and lead citrate, and examined with a JEOL JEM 1200 EX transmission electron microscope at 60 kV.

Results and Discussion

The main histological changes in the diseased spat consisted essentially of the presence of enlarged nuclei that showed abnormal shape and abnormal chromatin pattern throughout the connective tissue in the gills, the mantle and around the digestive tubules. The inflammatory reaction in these areas was reduced.

By electron microscopy interstitial cells of gills and mantle exhibited intranuclear and intracytoplasmic virus-like particles. In infected cells, the nucleus contained spherical or polygonal particles, 80 nm in diameter (Fig. 1). Some particles appeared empty and consisted of structures assumed to be capsids (Fig. 2), others contained an electron-dense core and were interpreted as being nucleocapsids (Fig. 2). Unenveloped particles were observed in the cytoplasm of myocytes in the mantle connective tissue (Fig. 3). Enveloped single virions were observed in cytoplasmic vesicles into others cells (Fig. 4). In cytolytic cells and in extracellular spaces, enveloped viruses were also seen (Fig. 5). These particles consisted of a capsid with an electron-dense core that was in turn surrounded by a unit-membrane like structure (Fig. 6). Envelope and capsid were separated by a reduced electron-lucent gap. Fine filaments passed from the toroidal core to the inside of the capsid (Fig. 6). The enveloped particles had spike-like protrusions on the surface .

Ultrastructural changes were found to be related to the presence of the virus in the oyster spat. Abnormal accumulations of granular endoplasmic reticulum associated with swollen mitochondria (Fig. 7) and condensed nuclei with electron-lucent centre (Fig. 1) were often observed. Degenerating and lysing infected nuclei were frequently present too.

The virions described resemble herpes viruses in morphological characteristics, in cellular locations and in size range (Roizman et *al.*, 1981; Murphy and Kingsbury, 1990; Roizman, 1990). In addition, the fibrils spanning the space from the core to the inner surface of the capsid are similar to the arrangement in herpes viruses (Furlong et *al.*, 1972; Roizman, 1990). It seems capsids and nucleocapsids are formed in the nucleus and then pass through the nuclear membranes into the cytoplasm.

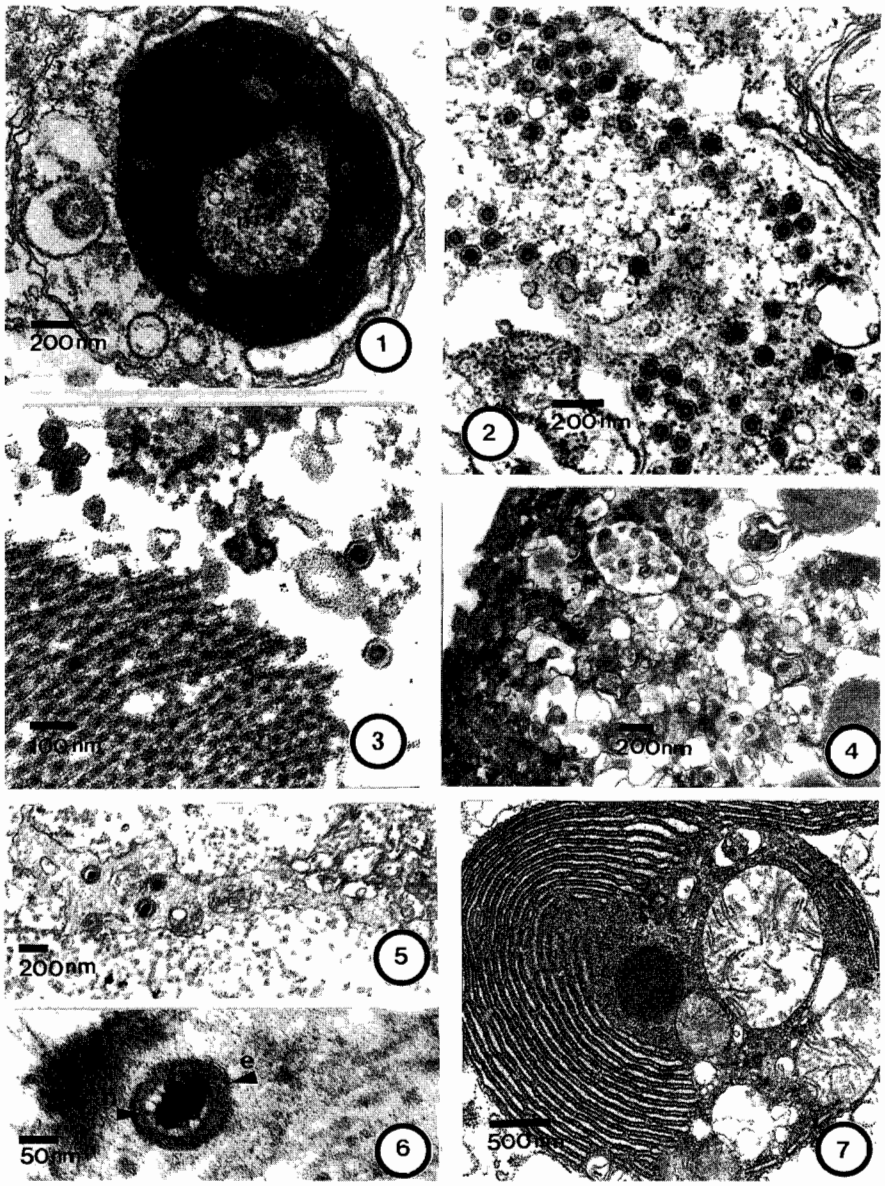

Fig. 1 Infected cell showing empty capsids (c) and nucleocapsids (n); capsids can be also found in the cytoplasm. EM (bar = 200 nm). **Fig. 2** Structure of empty capsids and particles with brick-shaped electron dense core. EM (bar = 200 nm). **Fig. 3** Unenveloped particles in myocyte cell cytoplasm. EM (bar = 100 nm). **Fig. 4** Enveloped virions within cytoplasmic vesicle. EM (bar = 200 nm). **Fig. 5** Enveloped particles found in extracellular spaces. EM (bar = 200 nm). **Fig. 6** Fine structure of an extracellular enveloped virus : the nucleocapsid (n) is surrounded by an envelope (e). EM (bar = 50 nm). **Fig. 7** Ultrastructural changes in infected cell : accumulation of granular endoplasmic reticulum. EM (bar = 500).

Enveloped virions are released at the cell surface or by cytolysis. Extracellular naked nucleocapsids may derive from lysed infected cells. However the nature of the virus nucleic acid must still be confirmed by biophysical characterisation .The literature contains several reports of herpes-like viruses from marine vertebrates or invertebrates (Buchanan and Richards, 1982; Hedrick and Sano, 1989; Sano *et al.*, 1985; Hedrick *et al.*, 1990), including herpes-type viruses in oysters (Farley *et al.*, 1972; Alderman, 1980; Hine *et al.*, 1992; Nicolas *et al.*, 1992; Comps and Cochennec, 1993).

The morphological characteristics, and the size of the nucleocapsid of the virus described in this study are close to those of the virus reported in Pacific oyster larvae, *Crassostrea gigas*, in French hatcheries (Nicolas *et al.*, 1992) and in New Zealand (Hine *et al.*, 1992). To determine the exact relationship between these viruses and to develop sensitive diagnostic methods for detection, purification and characterisation are needed. Indeed, considering the economic importance of the Japanese oyster to the French shellfish industry, a study of experimental infection is needed to confirm the pathogenicity of this virus.

Summary

Mass mortalities were reported in the summer of 1993 in cultured 3-7 month-old Pacific oysters, *Crassostrea gigas*, from the French Atlantic coast. Ohservations with a transmission electron microscope showed the presence of herpes-like virus particles in gills and mantle connective tissucs in surviving young spat.

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