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Oyster Herpes-Type Virus

Abstract. *A herpes-type virus infection, the first to be found in an invertebrate animal, is reported in the oyster* Crassostrea virginica. *Intranuclear herpes-type viral inclusions were more prevalent in the oyster at elevated water temperatures of 28° to 30°C than at normal ambient temperatures of 18° to 20°C. The inclusions were associated with a lethal disease at the elevated temperatures.*

During a study on the effects of elevated water temperatures on oyster growth and survival, it was discovered that oysters living at an elevated temperature suffered a higher mortality rate than control animals. Histologic examination of the affected oysters revealed a disease in which cells frequently contained intranuclear inclusion bodies comparable to those associated with herpesvirus infections in other animals. Electron microscopy demonstrated typical herpes-type virus particles in the nuclei of cells containing inclusions. This is the first virus disease to be described in oysters (*1*) and, we believe, the first herpes-type virus infection to be recognized in any invertebrate species (*2*).

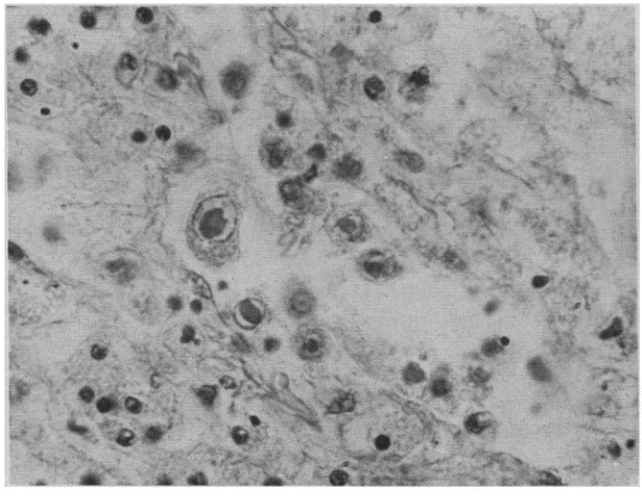
The oysters concerned in this report were all of the species *Crassostrea virginica*. Control and test animals for the initial study of thermal effects were all taken from a single location on the Marsh River, a tributary of the Sheepscot River near Wiscasset, Maine. They had been transplanted from a site in the Piscataqua River near Eliot, Maine, in 1968.

One set of 60 oysters was held in trays receiving water piped directly from the Sheepscot in the area of coolant water discharge from a fossil-fueled generating plant at Wiscasset. The water temperature in these trays was 28° to 30°C, a temperature range which, in the absence of disease, has no adverse effect on oyster survival and growth. A second set of 60 oysters was held under identical conditions except that the water with which the trays were irrigated was taken from the river at a point where there was no temperature elevation from the steam plant's coolant discharge. The water temperature in these trays was 12° to 18°C, and the salinity was the same as for the set held in higher-temperature water. Between June and August 1970, 1 to 2 months after the beginning of the experiment, the mortality in the higher-temperature set was 52 percent (31/60). In the same period, the mortality in the lower-temperature set was 18 percent (11/60).

Ten of the oysters that died in heated water were examined histologically. All were found to have intranuclear inclusions within the cells around the hemolymph sinuses (Fig. 1). The inclusions were consistent with those associated with herpes-type virus infections. The infected oysters had dilated digestive diverticula, cellular infiltrates in the vesicular connective tissue about the hemolymph sinuses, and, in advanced cases, massive cellular aggregates in these sites.

Electron microscopic examination of thin sections demonstrated viral particles within the nuclear inclusions. They were usually hexagonal, 70 to 90 nm in diameter, and had a single coat (Fig. 2). Some particles contained a dense nucleoid, others were empty. Some were seen to have several fine filaments extending through the coat from a dense, eccentrically placed nucleoid; this resulted in a flagellate appearance. Nuclear inclusions sometimes contained tubules with diameters of 45 to 55 nm (Fig. 3). The morphologic features of the virus closely resembled those of the Lucké virus associated with kidney tumors in the frog (*3*), and were characteristic of the structure of herpes-type viruses.

Unfortunately, none of the oysters in the control group grown in unheated water were examined histologically. To remedy this oversight and to deter-



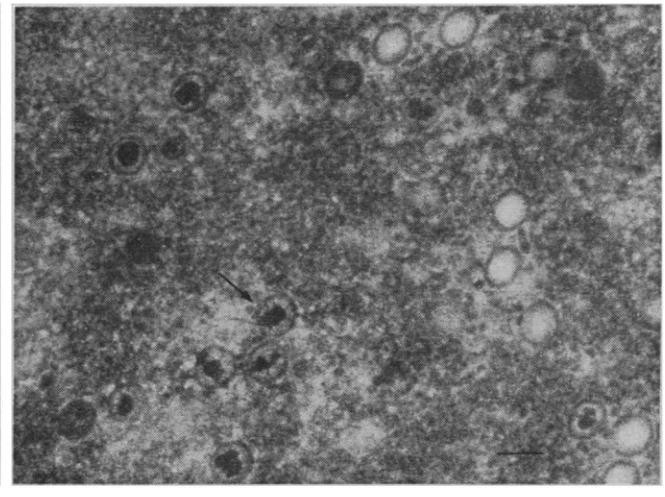


Fig. 1 (left). Intranuclear inclusions. Feulgen-stained section of an oyster infected with herpes-type virus (× 765). Fig. 2 (right). Electron micrograph of a thin section of an infected oyster, showing an intranuclear inclusion with various forms of virus particles, including empty particles and particles that appear flagellate (arrow) (bar 0.5 μm, × 18,600).

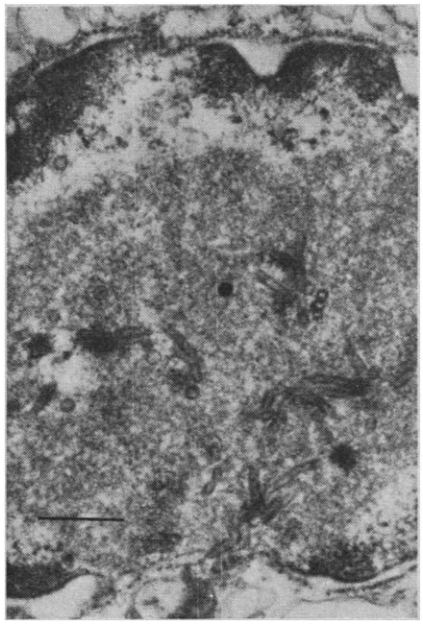


Fig. 3. Electron micrograph of a thin section of an infected oyster, showing tubules within an intranuclear inclusion (bar 0.1 μm, × 85,000).

mine whether the disease was present in the oysters when transplanted to Marsh River, we collected 200 oysters from the Piscataqua River near Eliot on 2 September 1970. Of these, one had pallor of the digestive gland. Light microscopy revealed herpes-type intranuclear inclusions in this specimen, but not in any of 50 other oysters selected randomly from the 200. Electron microscopy (Fig. 2) confirmed the presence of inclusion bodies and virus particles in the single grossly diseased animal. There were no infections in 50 oysters collected from the Marsh River on the same date. However, in 50 oysters collected from the Marsh River in June 1970, there were intranuclear herpes-type inclusions in 5. This is probably the result of a seasonal variation in the prevalence of overt infections. These findings also indicate that the virus infection was present in the Marsh River samples used for the temperature-effect study, and in the Piscataqua River population from which the Marsh River oysters originated.

The evidence suggests that the herpes-type virus infection is enzootic under ambient temperature conditions, and that it was introduced to the Marsh River by importation of infected oysters from the location near Eliot, on the Piscataqua River. The higher mortality of oysters held at higher temperatures correlates well with the high prevalence of herpes-type virus inclusions in that group. The lower mortality among oysters held at lower temperatures likewise correlates with the lower prevalence of inclusion bodies in oysters found where water temperatures were known to be lower. Elevated water temperatures appear to favor spread of the infection or activation of the infection from an occult to an overt phase, or both.

Detailed comparisons of the oyster herpes-type virus infection with herpesvirus in other animals must await further study. However, it is already evident that the virus itself is strikingly similar in fine structure to the Lucké virus, which has been implicated as a cause of renal adenocarcinoma in *Rana pipiens* (*3*). The disease in oysters associated with the herpes-type virus may have a proliferative component, manifest in the cellular aggregates around hemolymph sinuses and in the vesicular tissues. The origin of these aggregates is not clear, but they appear to be derived from hemocytes. It is also not clear whether the cell aggregates represent a self-limited reactive response, or a neoplasm. Herpesvirus infection has been associated with lymphoproliferative disease in monkeys (*4*), fowl (*5*), and man (*6*). Whether there is a relation between the herpes-type virus in the oyster and herpesvirus diseases in other animals, especially man, should be explored.

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Paramyxovirus-Like Particles Associated with Acute Demyelination in Chronic Relapsing Multiple Sclerosis

Abstract. *Electron microscopy of small perivenous demyelinating lesions in a formalin-fixed brain of a multiple sclerosis subject revealed nuclear and cytoplasmic particles resembling paramyxovirus nucleocapsids. These particles, 18 to 20 nanometers in diameter, were found in mononuclear cells related to the central vein and infiltrating the zone of active demyelination. It is suggested that multiple sclerosis lesions may be initiated by seeding of lymphocytes bearing latent paramyxovirus to white matter of the central nervous system.*

Adams and Imagawa reported that titers of antibody against measles are slightly higher in multiple sclerosis (MS) subjects than in healthy subjects of the same age (*1*). Subsequent studies have confirmed this, and also that antibodies against measles are found more frequently in the spinal fluid of MS subjects as compared to healthy subjects and patients with other neurological disorders. The relatively slight elevation in antibody titer and the fact that this is not a constant finding in MS led Brown *et al.* (*2*) to suggest that if measles virus is involved in the pathogenesis of MS, it is present in an unusually masked, latent, or defective form, or that different latent agents may cause the same MS syndrome. Ter Meulen *et al.* recovered an infectious agent related to a group 1 parainfluenza virus from cell cultures established from brain tissue from two MS subjects (*3*). Other efforts to implicate a myxovirus or other virus as an etiologic agent in MS, including electron microscopy of brain tissues, have been unsuccessful (*4*). Acute lesions suitable for electron microscopy have been difficult to obtain, and previous electron microscopic studies in this and in other laboratories have dealt with chronic, subacute, and shadow plaques and white matter remote from plaques (*5*). In the present ultrastructural study of a formalin-fixed MS brain, a sampling procedure with