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Piscine Erythrocytic Necrosis (PEN): A Viral Infection of the Atlantic Cod and Other Marine Fishes

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Several infections of erythrocytes of poikilothermic vertebrates, previously thought to be protozoan infections, have recently been shown to be associated with viruses (Appy et al., 1976; Johnston and Davies, 1973; Walker, 1971). These infections are distinguished by the presence of characteristic acidophilic cytoplasmic inclusions and/or nuclear degeneration. Electron microscopic examination of infected blood cells revealed that the inclusion bodies were associated with large, hexagonal viruses similar in morphology to the insect iridescent viruses and to lymphocystis virus.

In this study, we have investigated PEN in the Atlantic cod, herring, and other marine and anadromous species. The cytoplasmic inclusions observed in infected erythrocytes of cod, *Gadus morhua,* were 0.3-1.5 μm in diameter, round to ovoid, and Feulgen positive. These inclusions were often surrounded by less acidophilic granules. Electron microscopy showed that these granules were icosahedral virions and that the inclusion body was a viroplasm, presumably the pool of viral precursors: nucleic acid and protein. The nuclei of PEN-infected erythrocytes were irregular in outline, often appearing fragmented. Electron microscopy confirmed that the chromatin was unevenly marginated, thus giving the impression of nuclear fragmentation when viewed by light microscopy.

Electron microscopic examination of thin sections of infected cod erythrocytes revealed icosahedral virions with an average diameter of 300 nm, edge-to-edge, composed of an outer, hexagonal-shaped, electron dense layer approximately 35 nm wide and an inner, less electron dense layer approximately 16 nm wide, surrounding a central electron dense nucleoid approximately 230 nm in diameter. The virions were usually surrounded by an irregular, fibrous, electron translucent zone (Fig. 1).

The overall incidence of PEN-infected cod sampled from several areas in the northwest Atlantic was 15

Figure 1.—Electron micrograph of cod PEN virus. Bar = 0.5 μm.

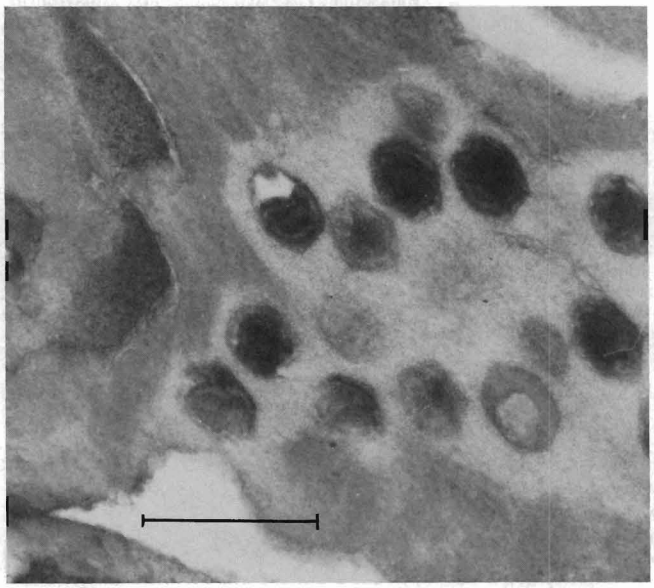
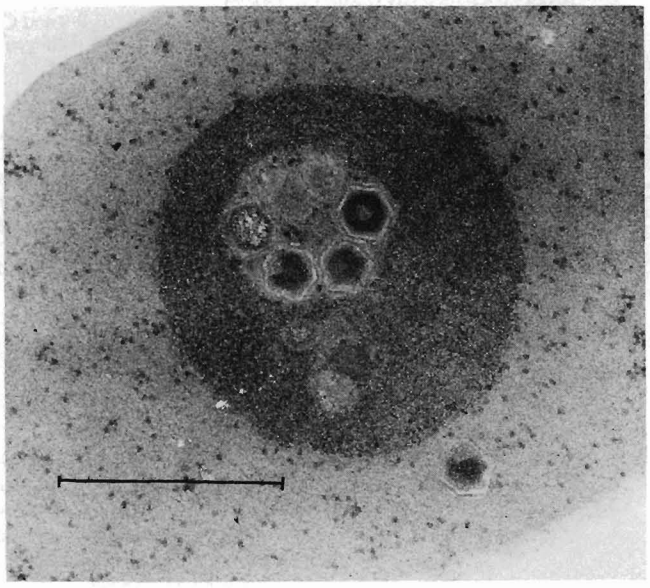


Figure 2.—Electron micrograph of herring erythrocyte showing Type I inclusion with PEN virions at the periphery and in the interior of inclusion. Bar = 0.5 μm.



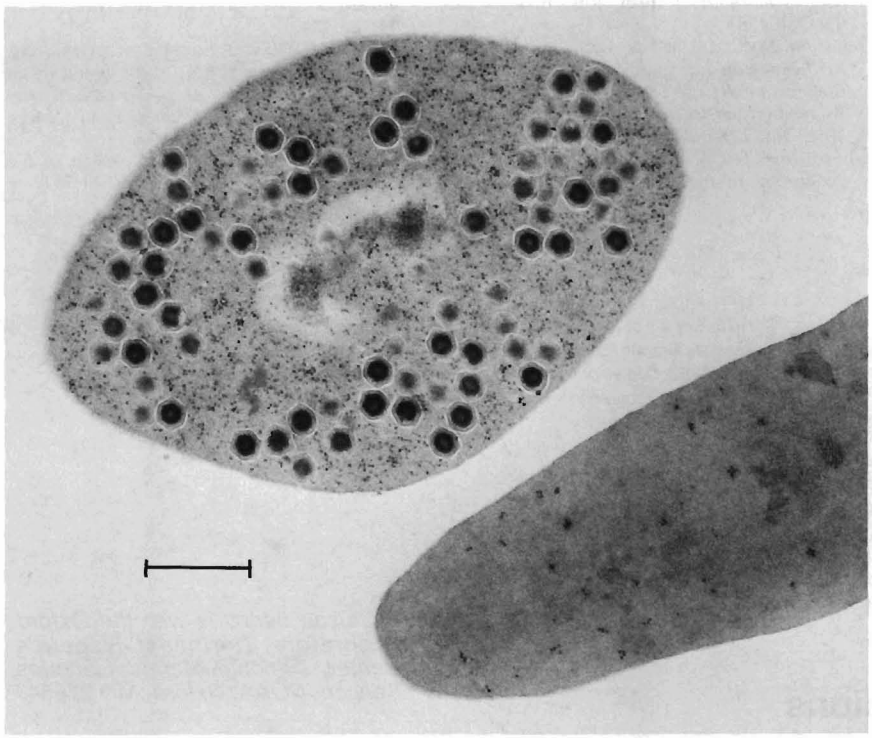


Figure 3.—Electron micrograph of herring erythrocyte showing typical PEN virions. Bar = 0.5 μm.

percent (59/390), and the intensity of infection ranged from 0.01 percent to more than 99 percent.

Cytoplasmic inclusions similar to those of PEN in cod have been observed in erythrocytes of herring, *Clupea harengus harengus*, sampled from the Maine coast; nuclear degeneration was also frequently observed (Sherburne, 1973). Infection has been observed in all developmental stages of red blood cells, from erythroblasts to mature erythrocytes. Nuclear chromatin condensation with formation of pseudovacuoles was evident, especially in mature cells. Many infected cells showed only nuclear degeneration without a visible cytoplasmic inclusion.

In this investigation, electron microscopy indicated that infected cells generally lost their characteristic elliptical shape. Also, the nucleus usually appeared irregular in shape with a distinct margination of the chromatin. Two apparently distinct types of inclusions were observed in infected cells. The first type was similar to the viroplasm observed in infected cod cells. These were roughly circular in shape and up to 1.5 μm in diameter. In all instances, viruslike particles were found in close association with the inclusions, either at the periphery or occasionally within the interior of the inclusion (Fig. 2). The second type of inclusion did not have virus associated with it, and appeared morphologically distinct from the first type. These inclusions were variable in size, ranging from 0.5 μm to 3 μm in diameter. They were usually less electron dense than the surrounding cytoplasm, and were surrounded by multiple membranes.

The virions present in infected herring erythrocytes were hexagonal in shape with an average diameter of 145 nm (Fig. 3). The virion was composed of an outer electron dense layer 8 nm in width, a less electron dense layer 16 nm wide, and a densely staining core approximately 100 nm in diameter containing a 40-nm central, electron translucent area. Thus, the virus infecting herring was distinguishable from the virus of cod by its size and ultrastructure.

One group of herring, which upon capture had an 11 percent incidence of PEN infection, was held in captivity at 14°-15°C. Six days after capture 57 percent of the fish sampled had typical inclusions, and 13 days after capture 95 percent of the fish sampled were PEN positive. It is not known if this increase in PEN incidence after being held in captivity was the result of horizontal transmission of the infection, or reflects the development of previously inapparent infections as a result of stress.

PEN also has been found in the blood of anadromous alewives, *Alosa pseudoharengus* (Sherburne, 1977). Fifty-six percent of prespawning and 10 percent of the postspawning adults sampled exhibited PEN. However, the highest intensity of infection in any noncaptive individual was approximately 0.2 percent. PEN has not been found in juvenile alewives. The infected erythrocytes from alewives resembled the characteristic patterns of PEN in cod, but characteristic nuclear distortion was generally present without a visible cytoplasmic inclusion. Low levels of natural infection have prevented elucidation of the virus in wild fish by electron microscopy.

One alewife, held in captivity with PEN-infected herring as well as other species which were uninfected, exhibited PEN inclusions in approximately 90 percent of its erythrocytes. Nuclear degeneration patterns characteristic of both the cod and herring PEN infection were observed in this fish. Examination by electron microscopy revealed virions similar in size and ultrastructure to the PEN virus of herring. Both types of inclusions described for the herring infection were observed in this fish. It is not known whether this fish became infected as a result of exposure to infected herring or represents a natural infection. Consequently, the relationship of this virus to the infection observed in wild fish is not known.

Light microscopic evidence of PEN infections has been obtained for several additional species, including longhorn sculpin, sea raven, flounder, rock

gunnel, and smelt. Confirmation of a viral involvement in infection of these species awaits the capture of fish with sufficiently high numbers of infected erythrocytes to warrant electron microscopic examination.

At least two viruses associated with PEN have been described; studies on the transmission of these viruses as well as their effects on infected fish are in progress.

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Viruses and Viruslike Lesions in Marine Mollusks

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A new and exciting phase of virological research has developed recently with the discovery of virus diseases in marine invertebrate organisms, particularly those found in mollusks and crustaceans. This brief report summarizes the known characteristics of molluscan viruses and attempts to systematically categorize them into appropriate families. Hopefully, this review will provide some understanding of virus classification and will prove useful for identifying viruses in marine organisms.

DESCRIPTION OF VIRUSES BY TENTATIVE GROUP

Pedoviridae

Host - chlamydial parasite of *Mercenaria mercenaria* (Harshbarger et al., 1977)
Nucleic acid - unknown, presumptively 2 DNA linear
Symmetry - octahedral on the basis of 2- 3- 4-sided rotational planes in paracrystalline array
Size - 50-nm, nonenveloped virion
Morphology and development - short tails visible in 4-sided plane of array at 45° angle from the square
A phage has also been found in a mycoplasma which was infecting *Telina tenuis*, and formed paracrystalline arrays of 70-nm hexagonal particles (Buchanan, 1973).

Papovaviridae

Host - *Crassostrea virginica*
Tissue - gametogenic epithelium
Nucleic acid - DNA (presumed 2 circular) (Feulgen positive, intranuclear inclusions)
Symmetry - icosahedral (6- and 5-sided particles), 2-3 symmetry in paracrystalline array
Size - 53-nm, nonenveloped virion
Morphology and development - replicates and assembles in nucleus, sometimes associated with filaments and microtubules. Massive hypertrophy of cell pathognomic. Most similar to *Papillomavirus* (Farley, 1976a).
Similar histologic lesions have been seen in *Crassostrea gigas* (Farley[1] and Kern[2]), *C. commercialis* (Wolf[3]), *Ostrea lurida*, and *O. edulis* (Bonami[4]). Smaller cells with similar inclusions have been seen in *Mya arenaria* gill epithelium and in *Macoma balthica* hemocytes.

Host - *Mya arenaria*
Tissue - connective tissues, hemocytes, gill epithelium
Nucleic acid - DNA (Feulgen positive, intranuclear inclusions) (Farley, 1976b)

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[4]Bonami, J.-R., Universite des Sciences et Techniques du Languedoc, Laboratoire de Pathologie Comparee, Place Eugene Bataillon, 34- Montpellier, France. Pers. commun.