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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MFR PAPER 1337

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)

[1]Farley, C. A., Oxford Laboratory, Northeast Fisheries Center, NMFS, NOAA, Oxford, MD 21654. Unpublished data.
[2]Kern, F. G., Oxford Laboratory, Northeast Fisheries Center, NMFS, NOAA, Oxford, MD 21654. Pers. commun.
[3]Wolf, P. H., New South Wales State Fisheries, 211 Kent St., Sydney, 2000, New South Wales, Australia. Pers. commun.
[4]Bonami, J.-R., Universite des Sciences et Techniques du Languedoc, Laboratoire de Pathologie Comparee, Place Eugene Bataillon, 34- Montpellier, France. Pers. commun.

Symmetry - icosahedral (6- and 5-sided particles)
Size - 40- to 45-nm, nonenveloped virion
Developmental features - replicates and assembles in nucleus producing Feulgen positive, intranuclear inclusion and some hypertrophy of cell. Most closely resembles *Polyomavirus* (Harshbarger[5]).

Herpetoviridae

Host - *Crassostrea virginica*
Tissue - hemocytes
Nucleic acid - DNA (Feulgen positive, intranuclear inclusions)
Symmetry - icosahedral (6- and 5-sided particles)
Size - 90-nm capsid; 200 × 250-nm, enveloped virion
Morphology and development - virus replicates in nucleus, assembles in nucleus and cytoplasm. Envelopment is in cytoplasm de novo in virogenic stromal areas. Cytoplasm assemblage activity is associated with microtubules. Toroidal structure is seen in nucleoids. A lateral bodylike organelle is present in virions. Internal membranes occur as inner envelopes of the capsid (Farley et al., 1972).

Other possible herpes infections in mollusks based on similarity of intranuclear inclusions have been seen in *Ostrea edulis* (Alderman[6]) and *Mercenaria mercenaria*. Recent observations by Farley (footnote 1) and Kern (footnote 2) of gonadal "tumors" in hard clams (Barry and Yevich, 1972) indicate the presence of herpes-type intranuclear inclusions in affected cells.

Iridoviridae

Host - *Octopus vulgaris*
Tissue - muscle
Nucleic acid - DNA, presumptive (malachite green)
Symmetry - icosahedral (6- and 5-sided particles)
Size - 110-120 nm (envelope not readily apparent)
Development - assembly in cytoplasm (no nuclear involvement apparent). Size, morphology, and location in cytoplasm is similar to insect iridovirus features (Rungger et al., 1971).

Host - *Crassostrea angulata*
Tissue - connective tissue cells
Nucleic acid - DNA (basophilic cytoplasmic inclusions which stain similar to nuclear chromatin with standard nuclear stains)
Symmetry - 350-nm, enveloped virion
Development - no obvious nuclear involvement. Assembles in cytoplasmic inclusions by budding through de novo membrane at edge of virogenic stroma. Most similar to lymphocystis virus of fish and other vertebrates (Comps et al., 1976)

Togaviridae

Host - *Ostrea lurida*
Tissue - hemocyte
Nucleic acid - RNA presumptive (no Feulgen positive material seen in cytoplasm of cells)
Symmetry - icosahedral? (6-sided virions)
Size- 50-nm, enveloped virion
Development - viruslike particles bud through plasma membrane (Fig. 1). Infected cells and virions rare, seen in one animal. Most similar to *Alphavirus*. Evidence too scant to definitively identify as virus (Farley, footnote 1).

Retroviridae

Host - *Crassostrea virginica*
Tissue - epithelia of digestive diverticula
Nucleic acid - RNA (presumptive) (no Feulgen positive material in cytoplasm of cells)
Symmetry - anisometric
Size - 100- to 110-nm, enveloped virion
Development - immature particles, with electron lucent centers and electron dense capsid material closely apposed to membrane, bud through the apical microvillar plasma membrane of epithelial cells in the digestive gland (Fig. 2). Peplomeres are evident in the virion envelope (Farley, 1975). Most similar to C type particles from baboon and human placenta.

Paramyxoviridae (viruslike inclusions)

Host - *Mya arenaria*
Tissue - teratomatous glandular tissue
Nucleic acid - RNA, presumptive (Feulgen negative, intranuclear and cytoplasmic inclusions)
Cytology - inclusions characteristic of measles or distemper lesions where seen in histologic sections of this tumor (Otto[7]; Farley, footnote 1). Electron miscoscopy of deparaffinized material is planned.

Reoviridae

Host - *Sepia officinalus*
Tissue - stomach epithelium?
Nucleic acid - 2 RNA, presumptive on basis of other characteristics
Symmetry - icosahedral (6- and 5-sided particles)
Size- 75-nm, nonenveloped capsid
Development - assembly at edge if virogenic stromal inclusions in the cytoplasm. Microtubular arrays were seen in association (Devauchelle and Vago, 1971).

Host - *Telina tenuis, C. gigas,* and other bivalve mollusks
Nucleic acid - 2 RNA (preliminary biochemical characterization)
Symmetry - icosahedral (negative stain)
Size - 55-nm, nonenveloped virion
Other features - immunologically related to IPN virus of fish. Increased titer recorded in infection experiments. No histopathology or thin section ultrastructural characterization (Hill, 1976).

SUMMARY AND DISCUSSION

Viruses and viruslike lesions have been found in over 13 species of mollusks, and represent several major orders. The absence of viruses in gastropods is probably due to lack of routine investigation rather than a real difference in occurence.

All of the viruses which have been found infecting mollusks can be tentatively placed in families previously associated primarily with mammalian diseases. This surprising revelation suggests two tentative conclusions: 1) most, if not all, of the major groups of

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[6]Alderman, D. J., Ministry of Agriculture, Fisheries and Food, Fish Diseases Laboratory, The Nothe, Weymouth, Dorset DT4 8UB. England. Pers. commun.
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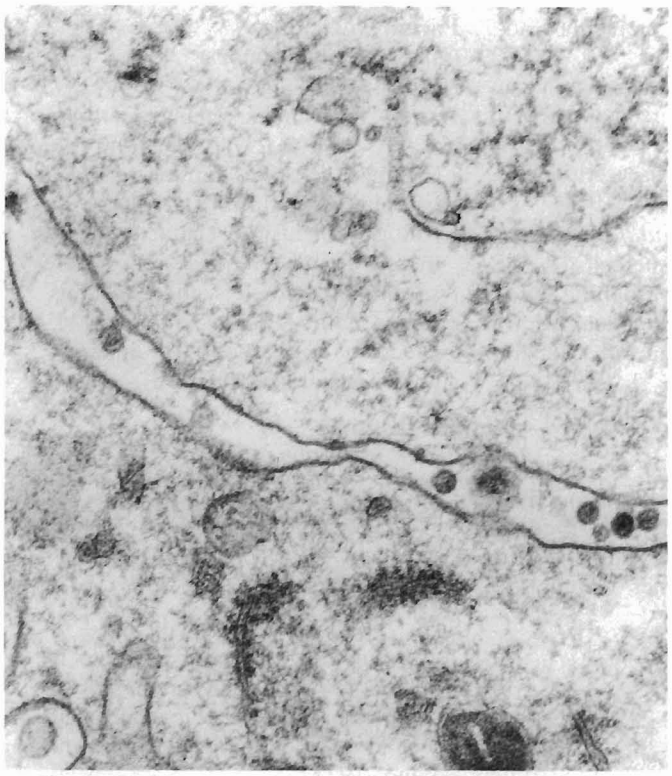

Figure 1.—Viruslike particles budding from plasma membrane of *Ostrea lurida* hemocyte. Size, morphology, and development resembles *Togavirus*. 54,000 ×.

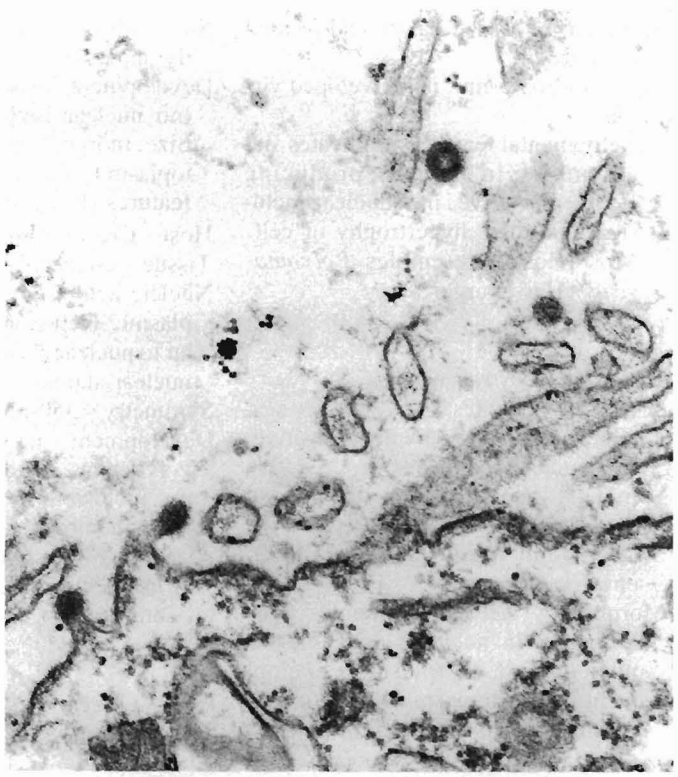

Figure 2.—C type viruslike particles budding from plasma membrane of digestive gland epithelium in *Crassostrea virginica*. Note peplomerelike structures adjacent to membrane and electron lucent center of free particle. 54,000 ×.

viruses have been described, and 2) the evolutionary development of these groups occurred much earlier than thought previously, primarily taking place in the sea within marine invertebrates prior to the evolution of vertebrates.

Major diversity is seen in the oyster herpes-type virus which shows relationships with more highly developed groups such as the Iridoviridae and the Poxviridae.

The comparative virology of marine invertebrates appears to be providing information which may lead to a much closer understanding of virus phylogeny and host parasite and vector relationships.

The study of marine invertebrate viruses is hindered by lack of experimental systems such as tissue culture methodology and marine animal husbandry. The use of histopathology, ultrastructural morphology, and cytologic development has provided information which can be used to at least identify viruses to family. Knowledge of this type can be used to infer other relationships concerning the biological aspects of viruses and virus diseases in marine forms.

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