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Proliferative Disorders in Bivalve Mollusks

C. AUSTIN FARLEY

Farley

C. Austin Farley is with the Oxford Laboratory, Middle Atlantic Coastal Fisheries Center, National Marine Fisheries Service, NOAA, Oxford, MD 21654.

INTRODUCTION

The purposes of this report are to describe briefly the types of neoplasms and related disorders seen in mollusks, to attempt characterization of their tissue origins, and to compare these diseases to vertebrate neoplasms.

METHODS

A collection of proliferative disorders in mollusks has been assembled over a period of years through pathologic studies of epizootic neoplastic disease (Christensen, Farley, and Kern, 1974; Farley, 1969b; Farley and Sparks, 1970) and routine histologic studies from many locations (Farley, 1968, 1969b; Farley and Sparks, 1970).

Diagnosis and characterization of the neoplastic diseases in mollusks discussed here were accomplished primarily by light microscopy and standard staining methods. Recently, the traditional methods have been supplemented by electron microscopy (Farley, 1976).

RESULTS

Invasive disorders have been found in 5 genera and 8 species of bivalve mollusks. Five basic types of neoplasms have been recognized, and all are thought to be invasive. Several non-neoplastic proliferative conditions have also been seen.

Non-Neoplastic Proliferative Disorders

Cellular response to the oyster pathogen *Minchinia nelsoni* is characterized by infiltration of infected tissue by hyaline hemocytes which resemble lymphocytes morphologically and produce a lesion similar to that caused by chronic inflammation in mammals (Farley, 1968, 1975). The cells involved in this parasite infection are morphologically similar to the cells involved in hematopoietic neoplasms which have been described previously (Farley, 1969a). However, in neoplasias of mollusks, evidence of parasitic involvement was lacking and anaplasia and mitotic activity was much more evident in the hemic neoplasms.

An epizootic disease was found in 6 percent of 50 clams, *Mya arenaria*, collected from Umpqua Bay, Oreg. Histologic lesions resembled hematopoietic neoplasms (Fig. 1) but appeared to be associated with the presence of small, difficult to see, protistan parasites (Fig. 2). The parasites were similar to the labyrinthuloid organisms described by Mackin and Schlicht (1976). This condition appeared to be similar to the leukemoid response seen in mammals.

Focal hyperplastic lesions of oysters, *Ostrea lurida*, were found in gill epithelia that had been introduced into Yaquina Bay, Oreg., from Washington, held in trays, and monitored for 1½ years. Lesions were seen in less than 5 percent in the base sample and in native Yaquina Bay oysters, but high prevalences (48 percent) developed in the introduced population within 3 months and continued throughout the 1½-year study. Lesions were characterized by elongation and increased cellularity of gill filaments, increased nuclear size, and increased cytoplasmic basophilia (Fig. 3). The cause of this condition was not determined.

Neoplastic Lesions

Epizootic neoplasia occurred in populations of mollusks from three areas.

Yaquina Bay Studies

The first epizootic was discovered in mussels (*Mytilus edulis*) and oysters (*O. lurida*) from Yaquina Bay, Oreg. (Farley, 1969b; Farley and Sparks, 1970). In *M. edulis*, the lesions consisted of enlarged anaplastic cells with dense lobed nuclei containing from one to five large acidophilic nucleoli. Numerous mitotic figures, many of them abnormal, were present. Lesions appeared to originate in the connective tissue spaces of the plicate organ in the mantle. Diffuse invasion of all connective tissues and blood spaces resulted in an apparently fatal outcome. The epizootic occurred locally in the lower region of Yaquina Bay with a peak prevalence of 14 percent. Lesions were seen first in August, becoming common by November, and disappearing with presumed mortality in May. A similar epizootic sarcomatoid disease was seen in *O. lurida* from Yaquina Bay (Farley and Sparks, 1970). This disease had an epizootic pattern identical seasonally and geographically to the one occurring in *Mytilus*. Lesions appeared to originate multifocally in the connective tissues of oysters. Neoplastic cells were more spherical than in *Mytilus* but had similar patterns of nuclear enlargement and density. Most contained only one nucleolus. Mitotic activity was intense only in certain phases of development. Prevalences were found to peak at 12 percent.

Other species of mollusks (*Macoma irus* and *Macoma nasuta*) from Yaquina Bay had similar anaplastic neoplastic diseases of indeterminate cytologic origin, with prevalence of 5 percent in May (Kern, personal commun.[1]). Introduced populations of mollusks (*Crassostrea gigas* and *O. lurida*) showed no evidence of neoplasia over the 1½-year study period.

Chesapeake Bay Epizootic

Epizootic gill carcinoma was found in *Macoma balthica* populations in the lower reaches of two tributaries which empty into the Choptank River (Christensen, Farley, and Kern, 1974). The disease was extremely localized geographically and had a seasonal pattern and prevalence similar to the Yaquina Bay epizootic (12 percent, October through early June). Disease was confined to *M. balthica* even though *M.*

[1]Kern, F. G. Oxford Laboratory, Middle Atlantic Coastal Fisheries Center, NMFS, NOAA, Oxford, MD 21654. Personal commun.

Figure 1.—Leukemoid response in *Mya arenaria*. HHE stain, blue stain (FPM); 312 ×.

Figure 2.—Protistan parasitelike cells (arrows) which stain differentially with Giemsa. 1,250 ×.

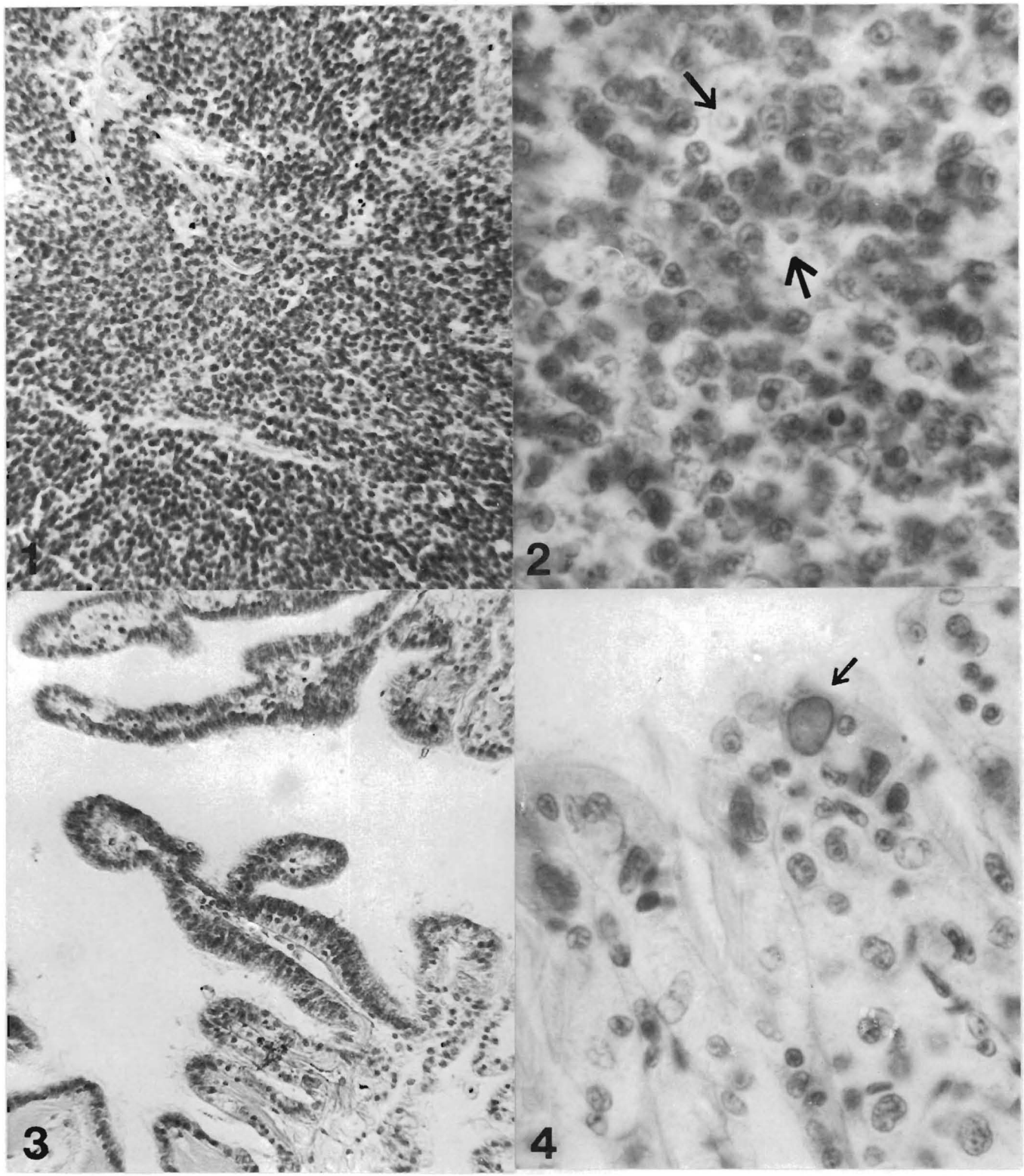

Figure 3.—Hyperplastic gill lamellae in *Ostrea lurida*; note elongation, basophilia, and increased cellularity in lesion. HHE; 312×.

Figure 4.—Feulgen positive intranuclear inclusion in gill epithelial cell from *Mya arenaria*. 1,250 ×.

Figure 5.—Sarcoma in *Mya arenaria* in connective tissue of gill. Feulgen picromethyl blue stain (FPM); 312×.

Figure 6.—Higher magnification of *Mya* sarcoma cells; note anaplastic appearance and mitotic figures. FPM stain; 1,250×.

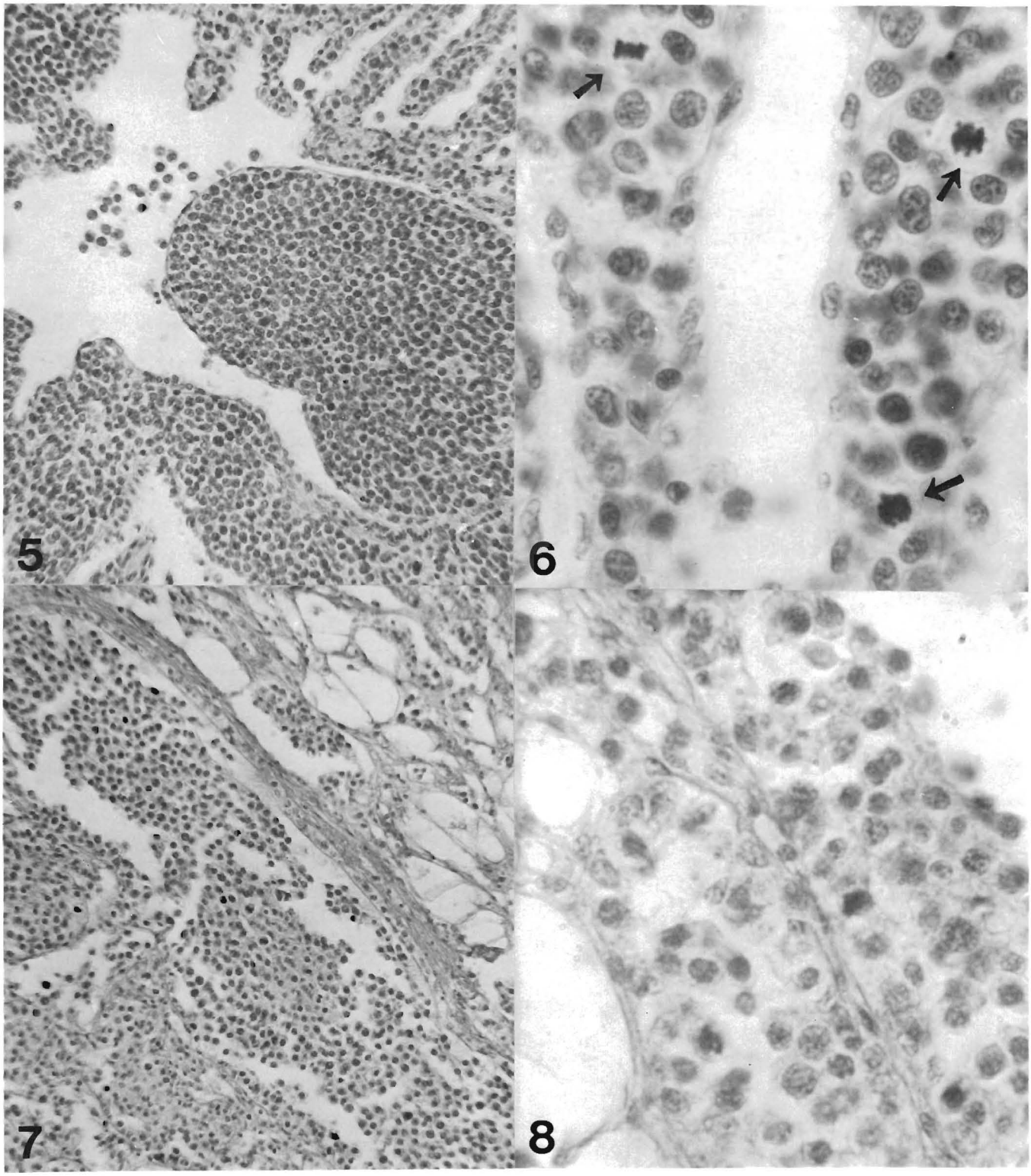

Figure 7.—Germ cell sarcoma in *Crassostrea virginica*. Neoplastic cells are in oviduct, tubules, and connective tissue spaces. FPM stain; 312×.

Figure 8.—Cytologic appearance of germinal sarcoma cells. Cells are uniform inside and anaplastic. FPM stain; 1,250×.

arenaria and *Crassostrea virginica* both occupied the same location. Histologically, the neoplastic cells were similar to those seen in *M. edulis* from Yaquina Bay. However, the primary lesions in gill epithelia suggested that epithelial cells were being transformed into anaplastic neoplastic cells. Electron microscopy confirmed that cells in the gill epithelium were transforming into frankly neoplastic cells. Fully developed neoplastic cells exhibited plical folds on the plasma membrane. The plical folding is a pathognomonic feature of these cells (Farley, 1976).

Massachusetts Epizootic

A sample of 50 clams, *M. arenaria*, was received on 19 September 1972, from Jones Creek, Annisquam River, Mass., an area where paralytic shellfish poisoning had occurred. Ten percent of the clams had gill hyperplasia; 20 percent had finely granular intranuclear Feulgen positive inclusions in gill epithelial cells (Fig. 4); and 12 percent had invasive sarcomatoid neoplasms (Kern, pers. commun., footnote one). Lesions (Fig. 5) were diffusely disseminated in connective tissue, blood spaces, and muscle. Neoplastic cells (Fig. 6) were similar to those seen in the *M. balthica* from Chesapeake Bay, with enlarged, lobed, densely granular nuclei with sparse to moderate amounts of cytoplasm. Mitotic figures were abundant.

Nonepizootic Neoplasms in Mollusks

An oyster from the Tred Avon River, Chesapeake Bay, Md., had a local lesion in the mantle consisting of anastomosing patterns of cells that were similar to cells comprising the walls of molluscan blood vessels. There were metastatic lesions in walls of blood vessels remote from the primary site. Lesions had cells with reticulin-positive external fibers surrounding them. This lesion was tentatively identified as a hemangiosarcoma by Farley and Sparks (1970).

One of 50 oysters collected on 19 September 1969, from the Mispillion River, Delaware Bay, had an advanced invasive neoplasm consisting of cells that resembled undifferentiated gametocytes and apparently were originating from the germinal epithelium (Fig. 7, 8). Diffuse invasion was seen in walls of blood vessels, connective tissue, and gastrointestinal tract epithelia. The neoplasm was diagnosed as a dysgerminoma or seminoma (Farley, unpubl. report[2]).

Newman (1972) described an undifferentiated sarcoma in an oyster (*C. virginica*) from New Haven Harbor, Conn. The sarcoma originated from a local lesion in the connective tissue of the gill. Neoplastic cells had disseminated sparsely to sites throughout the connective tissue and hemolymph spaces of the oyster. Tumor cells were enlarged and had nuclei 8-9 $m\mu$ in diamater that were characterized by dense chromatin, large multiple nucleoli, and irregular nuclear membranes. Mitotic activity was intense, with abnormal (tripolar) figures evident.

DISCUSSION

All adequately studied epizootics had several features in common. The diseases developed in the fall and continued through spring. All neoplasms were invasive, all consisted of anaplastic appearing cells, and, within a single species, the appearance of neoplastic cells was identical in each epizootic. No environmental relationships were evident nor was an etiology readily apparent. All the epizootics appeared to have a fatal outcome and prevalences within populations (roughly 12 percent) were comparable in all populations.

Comparing molluscan neoplasms, histologically, to vertebrate neoplasms demonstrates some very strong similarities as well as some distinctive differences. At this stage, it is much easier to diagnose molluscan tumors than those of vertebrates since known molluscan lesions are highly anaplastic and thus very similar to each other. Molluscan lesions closely resemble anaplastic lesions of vertebrates. It is conceivable that subtler lesions exist in mollusks but have not yet been recognized. The occurrence of lesions originating from different tissues is common to both groups, suggesting that they are biologically similar disorders. The invasive process in mollusks is different from vertebrates in that invasion is diffuse instead of metastatic. This difference is probably due to the lack of dense tissues, the semiclosed nature of the circulatory system, and the highly anaplastic nature of neoplastic cells in mollusks.

[2]Farley, C. A. Oxford Laboratory, Middle Atlantic Coastal Fisheries Center, NMFS, NOAA, Oxford, MD 21654. Unpubl. report.

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