NEW OCCURRENCE OF EPIZOOTIC SARCOMA IN CHESAPEAKE BAY SOFT SHELL CLAMS, *MYA ARENARIA*

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ABSTRACT

Maryland soft shell clams, *Mya arenaria*, from Chesapeake Bay were sampled from 1969 through January 1983. Four cases of sarcomatous neoplasia were diagnosed histologically [1979 (1), 1982 (2), January 1983 (1)] in 3,584 animals. Hemocytologic sampling between December 1983 and May 1984 revealed peak prevalences of 42-65% in clams from five sites. Sarcomas in laboratory-held clams progressed from early to advanced stages and death. This is the first time epizootic neoplastic disease has been observed in a wild molluscan population which was previously documented to be sarcoma-free. An infectious etiology is implied and data indicate the potential for mass mortality of bay clams.

Neoplastic diseases in soft shell clams, *Mya arenaria*, have been reported from New England populations in both polluted and nonpolluted areas (Barry and Yevich 1975; Farley 1976a; Yevich and Barszcz 1977; Brown et al. 1977, 1979; Brown 1980; Cooper et al. 1982a; Reinisch et al. 1984). Generally, the types of neoplasia noted have been considered as having hemocyte (blood cell) (Yevich and Barszcz 1977; Brown et al. 1977, 1979; Brown 1980; Cooper et al. 1982a; Reinisch et al. 1984) and gonadal (Barry and Yevich 1975; Yevich and Barszcz 1977; Brown et al. 1977, 1979; Brown 1980) origins or have been designated as sarcomatous (Farley 1976a). A single neoplastic clam was reported from Chesapeake Bay with an apparent teratoma composed of nerve and muscle tissue and digestive epithelium (Harshbarger et al. 1977). Chesapeake Bay soft clams collected and examined by several authors between 1971 and 1978 were free of the neoplastic disease (Barry and Yevich 1975; Brown 1980) with the exception of 1 case found in a collection of 3,000 clams used as experimental controls (Brown 1980). Evidence for a viral etiology for hematopoietic neoplasia in clams was reported in a Rhode Island study (Oprandy et al. 1981). Improved techniques such as examination of hemolymph using a combination of histologic and cytologic procedures (Cooper et al. 1982b) and the development of a monoclonal antibody test specific for neoplastic clam cells (Reinisch et al. 1983) have facilitated the identification and diagnosis of the disease. High prevalences of sarcomas have been found repeatedly in populations of Chesapeake Bay clams.

This paper documents the first occurrence of epizootic sarcoma in soft shell clams in Chesapeake Bay, and the first time neoplastic disease has appeared in a wild molluscan population that was previously shown to be free of the disease. Epizootic prevalences of this condition may have a potentially devastating impact on the clam industry of the region.

MATERIALS AND METHODS

Sixty samples of 25 or more soft shell clams (totaling over 3,500 clams) have been collected periodically by the Maryland Department of Natural Resources (DNR) or purchased from seafood outlets from 51 sites in Chesapeake Bay since 1969. Each animal was necropsied and tissues were fixed, processed, and diagnosed histologically via standard methods (Howard and Smith 1983) for diseases and parasites. Recent samples (Table 1) were examined by cytologic methods to determine the percent prevalence and number of abnormal cells. Late spring samples (YCLP, YSWP, YAGH, and YPIS, Table 1) were diagnosed by both histology and histocytology (technique described below).

Hemolymph was drawn via hypodermic syringe into sterile, ambient (15‰), artificial seawater to produce a 1:9 dilution of cells to seawater. One milliliter of this sample was placed on a 25 mm, chambered, poly-L-lysine coated microscope slide and cells were allowed to settle for 1 h (the poly-L-lysine coating improves the adhesiveness of neoplastic cells which in vitro are rounded and do not usually stick to glass [Cooper 1982a]). Fluid and chambers were

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TABLE 1.—Prevalence of *sarcoma in recent* and historic samples diagnosed from Feulgen-stained cytologic and histologic preparations in recent samples and from archived State of Maryland samples. Clams were collected from 1969 through October 1984 from comparable areas.

Location	Clam bed site	Date	Sample code	No. per sample	Advanced sarcomas (stages 4, 5) (%)	Advanced sarcomas in sample (stages 4, 5) (%)	Prevalence diagnosed by histocytology (%)	Prevalence diagnosed by histology (%)	Combined prevalence
Commercial sample	Site unknown	6 Dec. 1983	OXC	18	13	6	44	—	—
Commercial sample	Site unknown	8 Dec. 1983	EBC	16	100	6	6	—	—
Commercial sample	Site unknown	1 Feb. 1984	KNC	74	18	4	23	—	—
Chester River	Long Point	19 Apr. 1984	YCLP	50	0	0	0	0	0
Chester River	Swan Point	19 Apr. 1984	YSWP	50	24	10	46	32	46
West River	Three Sisters	4 May 1984	YAGH	50	56	28	50	52	57
Eastern Bay	Poplar Island	4 May 1984	YPIS	50	52	24	42	27	42
Total sample data				308				31 (%/200) = 29.5	
Chester River	Swan Point	28 June 1984	YSWP 2	50	0	0	0	—	—
Eastern Bay	Poplar Island	July/Aug. 1984	YPIS	50	0	0	0	—	—
Chester River	Swan Point	July/Aug. 1984	YSWP 3	40	0	0	3	—	—
Eastern Bay	Poplar Island	26 Oct. 1984	YPIS	50	35	12	32	—	—
Chester River	Swan Point	26 Oct. 1984	YSWP 5	50	47	14	25	—	—
Chester River	Swan Point	7 Jan. 1985	YSWP 1	68	40	32	59	—	—
Chester River	Swan Point	29 Mar. 1985	YSWP 2	52	60	39	65	—	—
Chester River	Swan Point	17 May 1985	YSWP 3	52	100	15	15	—	—
					Historic archived samples				
Choptank River, Eastern Bay & Chester River		Dec.-May 1969-78		362				0	
Little Choptank River & West River		1979-Jan. 1983		250				1.6	

removed, while slides were wet-fixed in an aldehyde fixative (1% glutaraldehyde/4% formaldehyde) (McDowell and Trump 1976) in half ambient seawater and stained with Feulgen picromethyl blue (Farley 1969), dehydrated, and mounted with a coverslip using a synthetic mounting medium. We are designating the term "histocytology" to describe this technique. The significance of this method is that the monolayer preparations, which result from treating living cells with histologic procedures, are permanent. Cytologic artifacts are minimal and cases can be accurately staged using cell counting procedures. Since histocytologic preparations contain between 100,000 and 500,000 cells in a monolayer, very early stages of the proliferative process can be diagnosed. Staging is arbitrarily determined by estimating the number and determining the ratio of both normal and neoplastic cells (Table 2). A similar diagnostic and staging method using cytologic techniques was reported by Cooper et al. (1982b); however, our method appears to have better accuracy and increased sensitivity to light cases. Diagnosis of histologic sections is reliable for stages 3-5 (Fig. 1A). As an example, comparison of late spring samples shows that histocytology is the more sensitive method while histology alone clearly demonstrates a massive increase in prevalence from zero in 1969-78 to 29.5% in 1984 (Table 1).

Monoclonal antibody was developed against neoplastic clam cells from Massachusetts clams by techniques described elsewhere (Reinisch et al. 1983). Periodic histocytologic diagnosis and mortality observations were made on clams held in 55 L aquaria with 15‰, 10°C artificial seawater, circulated through floss and charcoal filtering systems.

RESULTS

Sarcomas in clams were diagnosed histologically in 1/25 in November 1979 from Eastern Bay; 1/25 in May 1981 from West River; 1/50 in November 1981 from Little Choptank River; and 1/75 in January 1983 from Chester River. In December 1983, histocytologic diagnoses of clams obtained from a local seafood restaurant showed 8/18 with sarcomas.

An intensive survey and study of local populations was initiated in December 1983 to evaluate the extent of this apparently new epizootic in Chesapeake Bay soft shell clams. Table 1 presents epizootiology of field collections while Table 2 shows comparable information on laboratory-held clams. Field prevalences were found to be high in most samples from December 1983 through April 1985. At the same time, disease intensities which were light in Decem-

TABLE 2.—Sarcoma progression in individual, laboratory-held soft shell clams: Dec. 1983-June 1984. Whole numbers = percent ratios of neoplastic cells to hemocytes and numbers in parentheses = stages of disease. Stages: Early (1) = 0.01-0.09% and (2) = 0.1-0.9%; Intermediate (3) = 1-49%; Advanced (4) = 50-89% and (5) = 90-100%. (Diagnosed from Feulgen-stained cytologic preparations.)

Date	Specimen data										Sample data			
											Neoplastic clams		Normal clams	
	OXC 1	OXC 2	OXC 4	OXC 8	OXC 10	OXC 11	OXC 13	OXC 1	OXC 18	EBC 6	Advanced cases (%) (N = 10)	Cumulative mortality (%) (N = 10)	Cumulative mortality (%) (N = 20)	
6 Dec. 1983	70(4)	0.5(2)	0.01(1)	(0)	0.02(1)	12(3)	0.5 (2)	0.1(2)	(0)	—	14	0	0	
12 Dec. 1983	76(4)	6 (3)	—	0.03(1)	—	10(3)	0.96(2)	—	—	5(3)	11	0	0	
19 Dec. 1983	Died	11 (3)	29 (3)	0.9 (2)	0.1 (2)	Died	1 (3)	0.8(2)	(0)	62(4)	14	20	5	
3 Jan. 1984		33 (3)	16 (3)	2 (3)	8 (3)		33 (3)	0.9(2)	(0)	50(4)	14	20	15	
16 Jan. 1984		40 (3)	50 (4)	8 (3)	8 (3)		25 (3)	0.1(2)	0.1(2)	67(4)	25	20	15	
30 Jan. 1984		80 (4)	50 (4)	10 (3)	2 (3)		17 (3)	0.8(2)	0.1(2)	57(4)	38	20	20	
22 Feb. 1984		90 (5)	80 (4)	53 (4)	2 (3)		70 (4)	5 (3)	0.1(2)	95(5)	63	20	25	
3 Apr. 1984		95 (5)	90 (5)	85 (4)	40 (3)		92 (5)	8 (3)	10 (3)	95(5)	63	20	25	
13 Apr. 1984		Died	98 (5)	99 (5)	Died		98 (5)	12 (3)	62 (4)	99(5)	83	40	40	
May 1984			Died	—	—		Died	—	—	Died	—	70	50	
June 1984				Died				Died	Died		—	100	55	

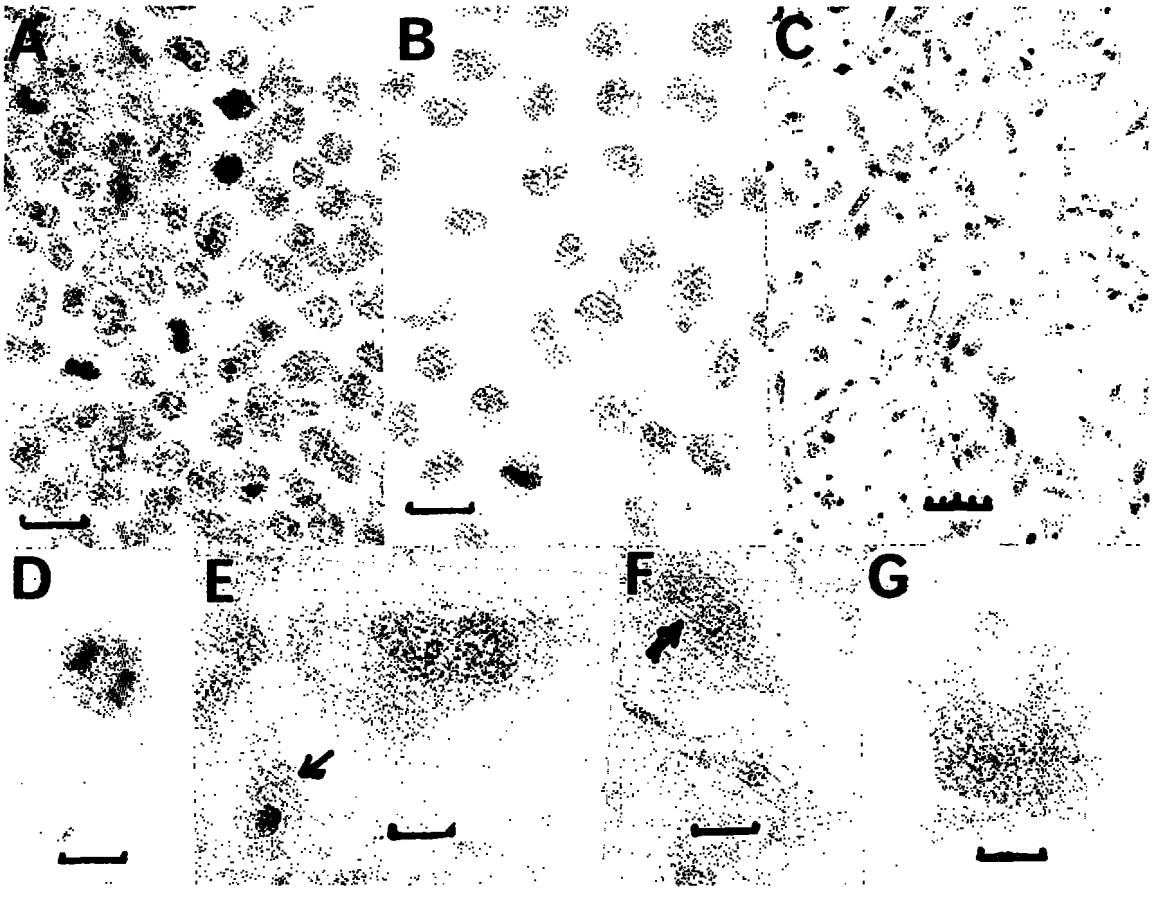

FIGURE 1.—Cytology of clam sarcoma, 1 unit = 10 μm. (A) Histologic section: Note large, hyperchromatic nuclei, abundant mitotic figures and metaphase with laggard chromosomes. (B-G) Histocytologic preparations: (B) Stage 5 (all cells neoplastic); rounded cells show mitosis and large, reniform, hyperchromatic nuclei. (C) Stage 3 sarcoma; about 10% of the cells are neoplastic. Compare sizes of normal (small) and neoplastic (large) nuclei. (D) Mitotic figure in anaphase. (E) Binucleate neoplastic cell with prominent, multiple nucleoli (normal hemocyte, arrow). (F) Neoplastic cell with intranuclear inclusion (arrow). (G) Very large neoplastic cell with nucleus and prominent Golgi zone.

ber progressed to advanced and terminal stages by April in laboratory-held animals. This situation was reflected in the field by an increase in the prevalence of advanced cases as the season progressed. The higher histologic prevalence in the YAGH sample was due to four positive cases from sections of dead animals which were not diagnosable by histocytology. This information provides additional evidence of mortality in feral populations. Cooper et al. (1982a) demonstrated in laboratory experiments the lethal nature of this disease in animals with advanced cases and noted similar implications in field monitored populations. A chronic phase with remission was reported by Cooper, but these features were not evident in the Chesapeake Bay epizootic. It is conceivable that some resistance has developed in the long-term occurrence of this disease over generations of clams in New England. Selection has not, as yet, had a chance to develop resistant animals in Chesapeake Bay. The mortality which began in laboratory-held animals in April was 100% by the end of June (Table 2). Field prevalences also dropped to zero in June. Sarcomas reappeared in the population in October.

Neoplastic clam cells from OXC 1 and EBC 6 (Table 2) were incubated with the murine monoclonal antibody IE7 which is specifically reactive with Massachusetts *Mya* neoplastic cells (Reinisch et al. 1983). Upon fluorescence activated cell sorter analyses, neoplastic cells from OXC 1 (Fig. 2) and EBC 6 were positive when stained with IE7.

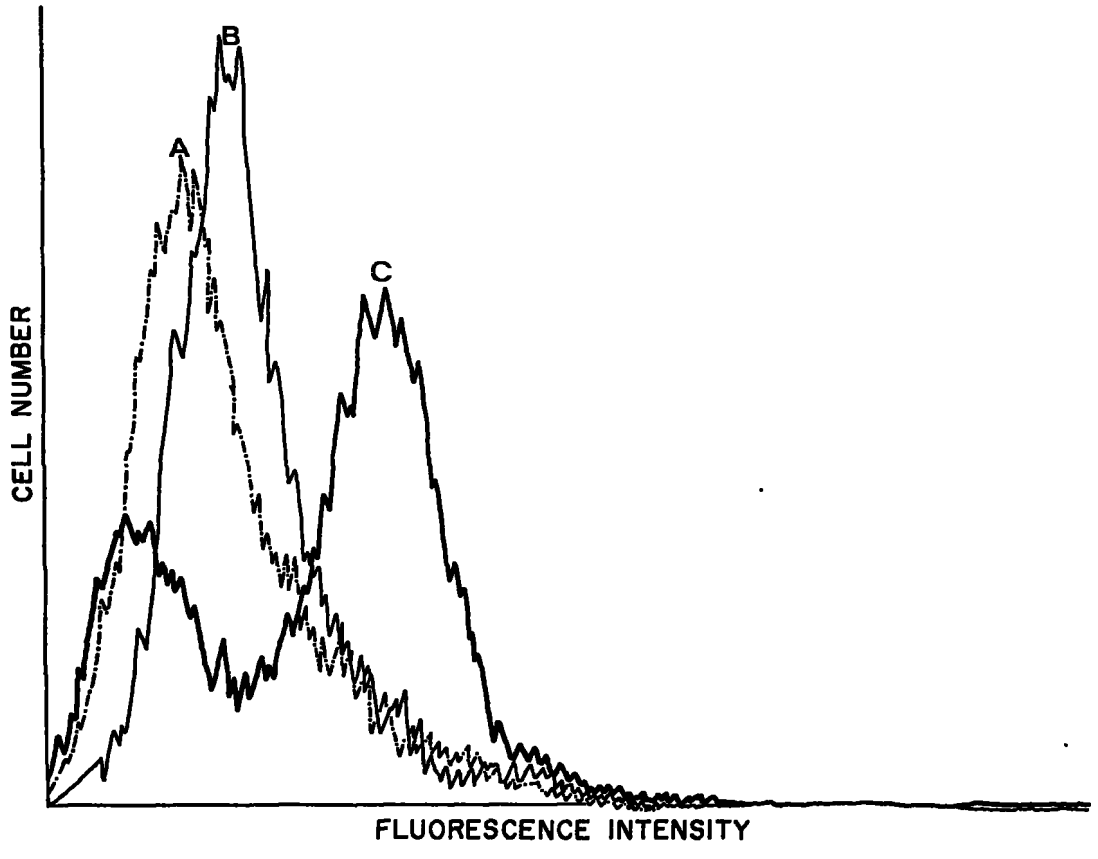

FIGURE 2.—OXC 1 cells were fixed in 0.1% neutral formaldehyde. Following three washes in sterile seawater, the cells were then incubated with: (A) a 1:50 dilution of fluoresceinated (FITC) goat and antimouse IgG antibody (-·-·-·-), (B) a 1:100 dilution of heat-inactivated normal mouse serum, and subsequently with a 1:50 dilution of FITC-goat antimouse IgG antibody (——), or (C) monoclonal antibody IE7, and subsequently with a 1:50 dilution of FITC-goat antimouse IgG antibody (——). All the antisera were diluted in sterile seawater immediately prior to use. The samples were then evaluated by a Becton-Dickinson Fluorescence Activated Cell Sorter IV (Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA).

DISCUSSION

Epizootiology

Laboratory and field observations complement each other and confirm the suspicion that affected animals die from the disease. Individual diseased clams monitored in aquaria from early December 1983 to May 1984 had progressed from early stages 1 and 2 to advanced stages 4 and 5 with 100% mortality. The high prevalences and advancing stages seen in natural populations may signal significant, impending mortalities. Samples collected from Swan Point (YSWP) in July and August 1984 (Table 1) showed 1/15 and 0/25 sarcomas, respectively. Samples from Poplar Island in July and August were 0/25 and 0/25. High sarcoma prevalences reappeared in the fall in smaller clams at Swan Point (25%) and Poplar Island (32%) in October. The decrease in prevalence to zero corresponds with observations of laboratory-held animals, suggesting that the disease was also 100% fatal in field populations. The experiments of Brown (1980) and others (Oprandy et al. 1981) indicate an infectious etiology for the disease. The nature of the new situation in Chesapeake Bay suggests that an infectious agent may have been established in clams by introduction from New England, since previous information indicated that the disease was confined to sites north of New Jersey (Barry and Yevich 1975; Yevich and Barszcz 1977; Brown et al. 1977, 1979; Brown 1980; Koepp 1984). Introductions of clams from New England to

Maryland have been documented in the past (post-tropical storm Agnes in 1972) (S. V. Otto unpubl. data). Antigenic similarity between neoplastic clams in New England and Maryland suggests that target cells in the disease are the same in both areas. Additionally, the sudden appearance of isolated occurrences of the disease in widespread areas of the Bay and the apparent tenfold increase in frequencies since its appearance in 1978 in populations occurring over most of the geographic range of soft clams in the Bay suggest an infectious etiology rather than point source chemical oncogen activity or pollution (Barry and Yevich 1975; Yevich and Barszcz 1977; Cooper et al. 1982a; Reinisch et al. 1984) as has been implied in some New England studies.

Classification

Histologically, the clam sarcomas (Fig. 1A) consist of diffusely disseminated round cells with a large, 6-10 μm, hyperchromatic, often lobed nucleus containing one or more prominent nucleoli. Cytoplasm is sparse, mitosis is common, and nuclei are more than twice as large as normal hemocyte nuclei. Histocytologic preparations (Fig. 1B-G) reveal sarcoma cells with identical characteristics and which can be definitively recognized on the basis of their morphology.

Other authors (Yevich and Barszcz 1977; Brown et al. 1977; Reinisch et al. 1983) have called this disease a "hematopoietic neoplasm" because of the general similarity of neoplastic cells and hemocytes, and because of its occurrence in vascular spaces. While this is the most probable origin for these cells, previous studies in other species have shown that these criteria can be misleading. The neoplasm in *Macoma balthica* (Christensen et al. 1974), which was characterized by anaplastic cells inhabiting the vascular spaces, was shown ultrastructurally to be of epithelial origin and, therefore, diagnosable as a carcinoma (Farley 1976b). Since no specific identifying organelles have been seen in the soft clam neoplasm (Brown et al. 1977) and since some monoclonal antibodies developed against neoplastic cells do not cross react with normal hemocytes, we prefer the more conservative term "sarcoma" which identifies the disease by behavior and cytology but does not imply a particular cell origin. These data indicate disease irreversibility and satisfy most of the other criteria for sarcoma or carcinoma, namely: 1) loss of cell specialization (anaplasia); 2) cell proliferation; 3) invasiveness (diffuse infiltration of connective tissue and muscle); 4) clonal alteration of genetic material (probable polyploidy evidenced by enlarged, hyperchromatic nuclei); and 5) clinical features such as progression and malignancy.

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