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A Tumorlike Growth on the Foot of a Freshwater Mussel (*Anodonta californiensis*)[1,2]

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ABSTRACT

A freshwater mussel, *Anodonta californiensis*, was found which possessed a small tumorlike growth on the foot that measured 3 mm in diameter and 2 mm in height. This abnormal growth, thought to be an adenomyoma, was located laterally near the ventral edge of the foot. The growth consisted of dark basophilic glandular cells and muscle fibers with a deeply convoluted epithelial covering. The cause of the lesion is not known.

INTRODUCTION

Tumors are a rare occurrence among bivalve molluscs. Williams (1890) reported a pedunculated tumor, composed of glandular and muscle cells, on a freshwater mussel, *Anodonta cygnea*. Collinge (1891) also observed two tumors from the same species of freshwater mussel, but gave no microscopic description. These three tumors were all apparently outgrowths of the mantle. A connective tissue tumor arising from the palps of *A. implicata* was described by Butros (1948). Mesenchymal tumors have been reported originating from the pericardial cavity of eastern oysters, *Crassostrea virginica*, (Ryder, 1887; Smith, 1934) and the rectum of a Pacific oyster, *C. gigas* (Sparks et al., 1964a). Hueper (1963) noted several papillary tumors around the rectum of soft-shelled clams, *Mya arenaria*, and presented excellent photographs of the gross lesions, but gave no microscopic discussion. A tumorlike mass, which was the rectum expanded due to a fecal impaction, was also reported by Sparks et al. (1964b).

During a routine monthly sampling of a population of Columbia River freshwater mussels (*A. californiensis* and *A. oregonensis*) for glycogen analysis, a mature male *A. californiensis* was observed to possess a small tumorlike growth, located laterally near the ventral edge of the foot. A description of this lesion seems appropriate due to its unique location and the dearth of histochemical observations about molluscan neoplasms.

MATERIALS AND METHODS

The freshwater mussel was examined grossly and fixed in 10% calcium-formal fixative. The tissues were embedded in Paraplast and sections were made through the abnormal growth to determine its microscopic structure.

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The tissue sections were stained with Harris' hematoxylin and eosin or Clarke's PAS reagent. To help localize the glycogen, tissue sections treated with 1% diastase were utilized as controls, since diastase-labile portions of PAS-positive cells are interpreted to represent glycogen. Clarke's procedure (Clarke, 1965) was modified for the freshwater mussels by under-staining in the PAS reagent and over-treating in the diastase because of the high affinity of this animal's tissue for the PAS solution.

RESULTS

Upon gross examination, the tumorlike growth was a small, firm papilliform projection of the foot. The abnormal growth was located laterally near the ventral edge of the foot and measured 3 mm in diameter and 2 mm in height.

The lesion was covered by a convoluted, vacuolated epithelium with faded cytoplasm (Fig. 1) that was strikingly different from the normally tall columnar epithelium of the foot (Fig. 3). The stroma of the tumor consisted of deeply basophilic glandular cells and muscle fibers (Fig. 4). The epithelial covering was conspicuously convoluted with deep crypts that penetrated into the base of the lesion (Fig. 5). The basophilic cells of the tumor appeared similar to those found just beneath the normal epithelium of the foot, but differed from the normal glandular cells in that they were arranged irregularly. The muscle fibers of the abnormal growth also differed from the normal muscle of the foot by not being arranged in large orderly muscle bundles. Among the muscle and gland cells of the tumor, a few small, yellow cells, classified as leucocytes, were present, but no heavy infiltration of leucocytes was observed. The neoplasm was apparently benign since it was well delimited and did not appear to be invasive into the normal tissue.

The distribution of glycogen in the tumorlike growth showed no apparent abnormality (Fig. 2 and 6). The muscle fibers of the lesion had fine granular deposits of glycogen (Fig. 6 and 7) similar to the normal foot muscles. The basophilic glandular cells in the tumor were highly PAS-positive (Fig. 6 and 7), like the glandular cells beneath the normal epithelium. Since these highly PAS-positive cells did not possess glycogen, it is presumed that they contain a mucopolysaccaride.

DISCUSSION

Reviews of molluscan neoplasia show no definite malignant growths have been reported among these animals (Scharrer and Lochhead, 1950; Wautier and Wautier, 1953; Kenton, 1964). Carcinogens have been used on a variety of invertebrates with negative results (Scharrer and Lochhead, 1950), but have induced tumors in cephalopods, *Sepia* sp., (Jaquemain et al., 1947). Since the area occupied by the tumor of *A. californiensis* was well defined with no evidence of invasion into the surrounding normal tissue, this lesion was considered to be a benign growth. Therefore, following vertebrate terminology, this benign tumor consisting of glandular and muscle cells could be termed an adenomyoma.

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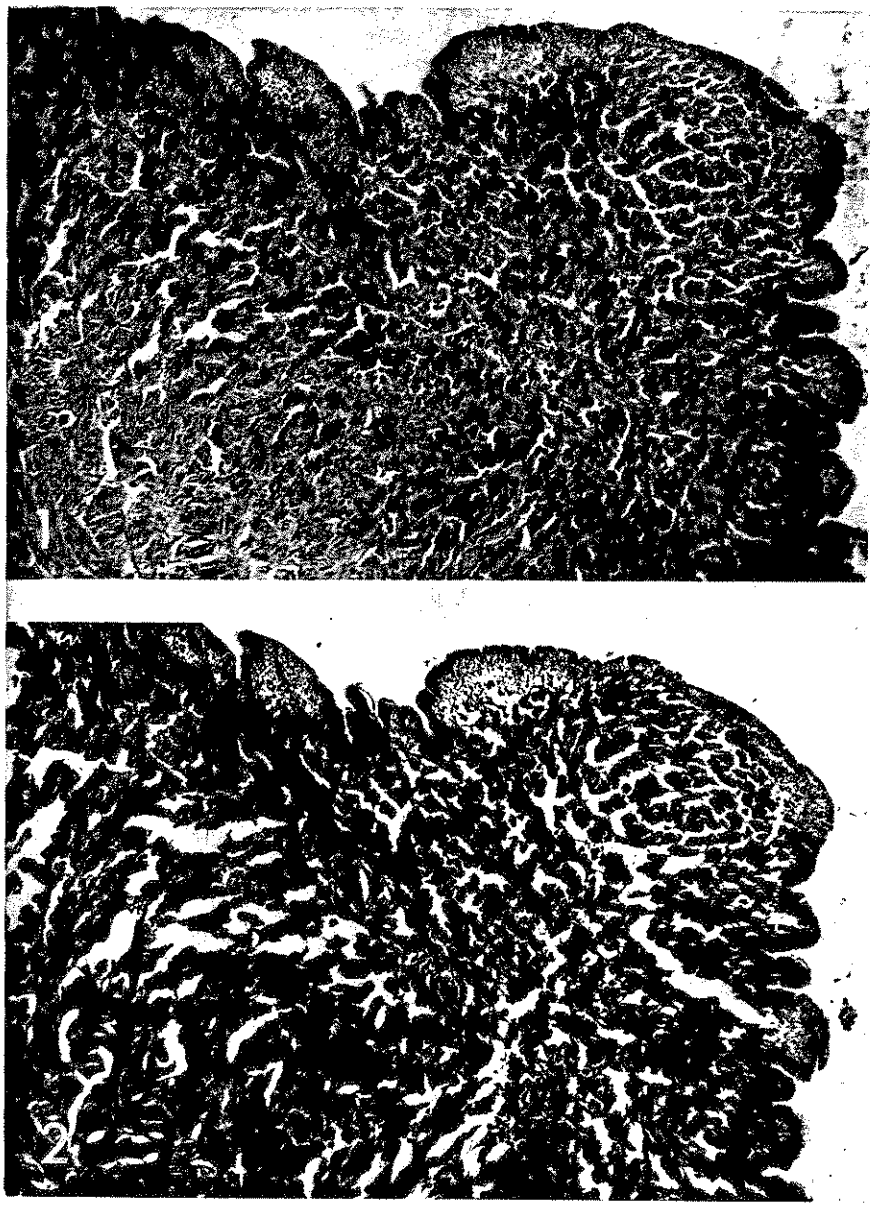

FIG. 1. Benign turmorlike growth on the foot of a freshwater mussel. Normal muscles of the foot are visible at lower left. Hematoxylin and eosin. 30×.

FIG. 2. Tumorlike growth on the foot of a freshwater mussel, stained for glycogen. Note intense PAS-positive reaction of both the tumor and the normal muscles of the foot. Clarke's PAS. 30×.

FIG. 3. Foot of freshwater mussel, showing part of the abnormal growth at left and normal epithelium of the foot at right. Hematoxylin and eosin. 30×.

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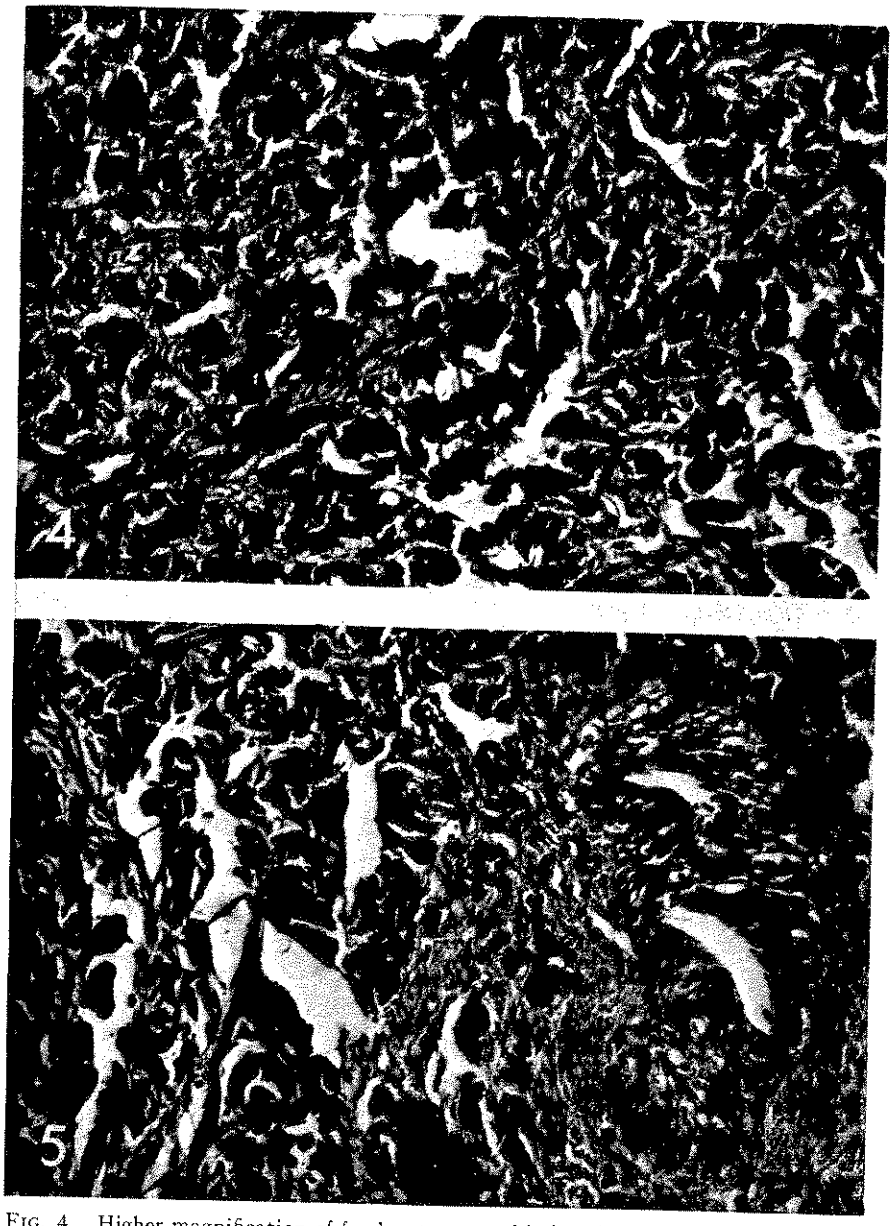

FIG. 4. Higher magnification of freshwater mussel lesion. Note the deeply basophilic cells and the muscle fibers that make up the majority of the tumor. Hematoxylin and eosin. 155×.

FIG. 5. Tumorlike growth on freshwater mussel, showing deep epithelial crypt at right and located near the base of the tumor. Hematoxylin and eosin. 155×.

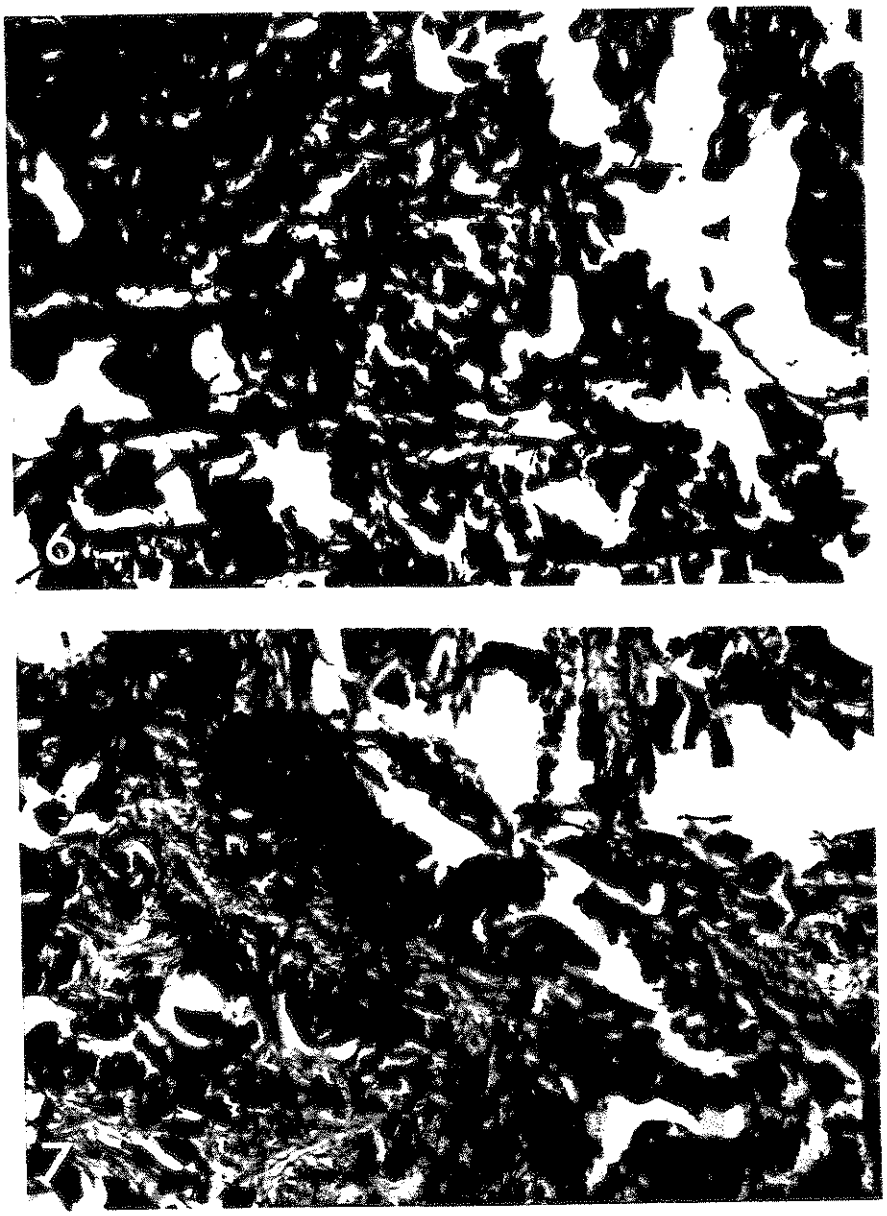

FIG. 6. Freshwater mussel lesion stained for glycogen. Note intense PAS-positive reaction of both the glandular cells and the muscle fibers. Clarke's PAS. 155×.

FIG. 7. Abnormal growth stained for glycogen and treated with diastase. Note that the muscle fibers possessed PAS-positive material (Fig. 6) that was diastase labile, whereas the glandular cells remained PAS-positive after diastase treatment. Clarke's PAS–1% diastase. 155×.

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However, Scharrer and Lochhead (1950) state that terminology developed almost exclusively for use in mammalian pathology should not be applied to invertebrate animals.

The tumor on *A. californiensis* differed from previous tumor reports among bivalves by its unique location and small size, its lack of a stalk, and its microscopic structure. Collinge (1891) did report an unstalked tumor, but gave no microscopic description. Williams (1890) reported an adenomyoma in *A. cygnea*, with the glandular cells just beneath the epithelium and entirely separated from the muscular stalk or core. The growth reported by Williams (1890) would appear to closely resemble the pedunculated adenoma, or polyp, commonly found in the human colon (Robbins, 1962). The lesion on *A. californiensis* reported here in no way resembles a polyp; hence the name adenomyoma, rather than pedunculated adenoma.

The large tumors reported in *A. implicata* (Butros, 1948), *C. virginica* (Smith, 1934), and *C. gigas* (Sparks et al., 1964a) possessed a marked inflammation. A few cells resembling leucocytes were found throughout the tumor in *A. californiensis*, but these cells differed from any of the blood cell descriptions for freshwater mussels given by Dundee (1953). Shallow convolutions on the surface of molluscan tumors have been reported by Butros (1948) and Smith (1934). Sparks et al. (1964a) noted deep epithelial folds or crypts on a molluscan neoplasm. However, none of these crypts penetrated into the base of the tumor as deeply as those in the lesion on *A. californiensis*. The microscopical comparisons of these tumors indicate that the adenomyoma found on the foot of *A. californiensis* is uniquely different from molluscan neoplams reported previously.

Most of the tumors recorded from molluscs have been large, plainly visible neoplasms. The small size of the growth on the foot of *A. californiensis* suggests that neoplasms might be observed more commonly among molluscs if more effort and time were spent examining shellfish populations for tumorous growths. It is possible that neoplasms are more common than the literature indicates, since Hueper (1963) found 2% of a population of *M. arenaria* possessing rectal papillomas. However, Williams (1890) found only one tumor after examining 700 freshwater mussels, *A. cygnea*, and no neoplasms were observed by Taylor (1966) after examining 1114 California mussels, *Mytilus californianus*, and 1000 bay mussels, *M. edulis*, for possible tumorous growths.

This tumor may be considered as a spontaneous growth (Scharrer and Lochhead, 1950) since its cause is unknown. As pointed out by Sparks et al. (1964), it is possible that tumors in molluscs are not neoplasms as the word is used in vertebrate pathology, and they have resulted as a response to injury or infection. There is a possibility that the tumorlike growth on the foot of *A. californiensis* is not a true neoplasm, but a hyperplasia of the basophilic and muscle cells beneath the epithelium of the foot, since the cells within this lesion all appeared normal with both hematoxylin and eosin stain and Clarke's PAS reagent. If this abnormal growth is a hyperplasia, it may have been produced

by some type of irritation or injury. Many investigators have reported invertebrate tumors which are in reality wound responses (Scharrer and Lochhead, 1950), and tumefactions (swellings) occur in molluscs due to infection (Taylor, 1966). The origin and classification of molluscan tumors will remain uncertain until more of these abnormal growths are studied and the processes of injury and infection among molluscs are thoroughly understood.

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