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A Disease of the Freshwater Mussel, *Margaritifera margaritifera*[1]

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Several freshwater mussels (*Margaritifera margaritifera*) were collected from a river in the state of Washington. They possessed large watery lesions on the foot, with a resilience similar to that of a sponge. Some animals possessed scarred wounds that were not pliable or resilient. Histologically, the large watery lesions were edematious areas in which the normal muscle tissue and mucous glands had been replaced by fibrous connective tissue. The epithelial covering over the afflicted area was disorganized, necrotic, lacking, or reduced to a cuboidal or squamous lining. Those animals that possessed scarred wounds had a subacute inflammatory reaction with well-developed granulation tissue and collagen deposits. This disease affected only the foot of the mussels. In many animals 90% of the normal foot tissue was destroyed. The mussels did not appear capable of completely recovering from this pernicious disease. Wound repair was apparently successful in many cases; in these instances a dense collagenous scar was formed.

INTRODUCTION

Margaritifera margaritifera is an ubiquitous species of freshwater mussel in North America. Roscoe and Redlings (1964) have made an excellent ecological study of *M. margaritifera* in Washington. Michelson (1961, 1963) has recently described two infectious diseases in freshwater snails, but there are no previous reports of diseases among freshwater bivalves. In March, 1967, 123 *M. margaritifera* were collected from the Ozette River, Washington, and upon gross examination 75 of these animals (61%) were found to possess resilient watery lesions or scarred wounds. Upon histological examination many of these lesions contained amoeboid cells with inclusions. Because of the spongy resilience of the gross lesions, this disease is tentatively named "spongy" disease. The pathology associated with the disease is described.

[1] This paper is based on work performed under the United States Atomic Energy Commission Contract AT(45-1)-1830.

MATERIALS AND METHODS

All mussels were fixed in methanol, photographed, and then a cross section of the entire body, approximately ¼ inch thick, was taken from the area of each lesion. The tissues were processed by standard methods, embedded in Paraplast and sectioned at 6 μ. Tissue sections were stained in either hematoxylin and eosin, Gridley's protozoa stain (Gridley, 1960), Mallory's trichrome stain (Pauley, 1967), or the MacCallum-Goodpasture bacteria stain (Pauley and Maulsby, 1967).

RESULTS

All *M. margaritifera* collected from the Ozette River were found lying flat upon their sides on the top of the sand. This is unusual since *M. margaritifera* observed in other rivers are buried in the substrate, usually sand, with only their posterior end exposed (Roscoe and Redlings, 1964). Although no accurate numbers were kept, approximately 15–20% of the mussels lying

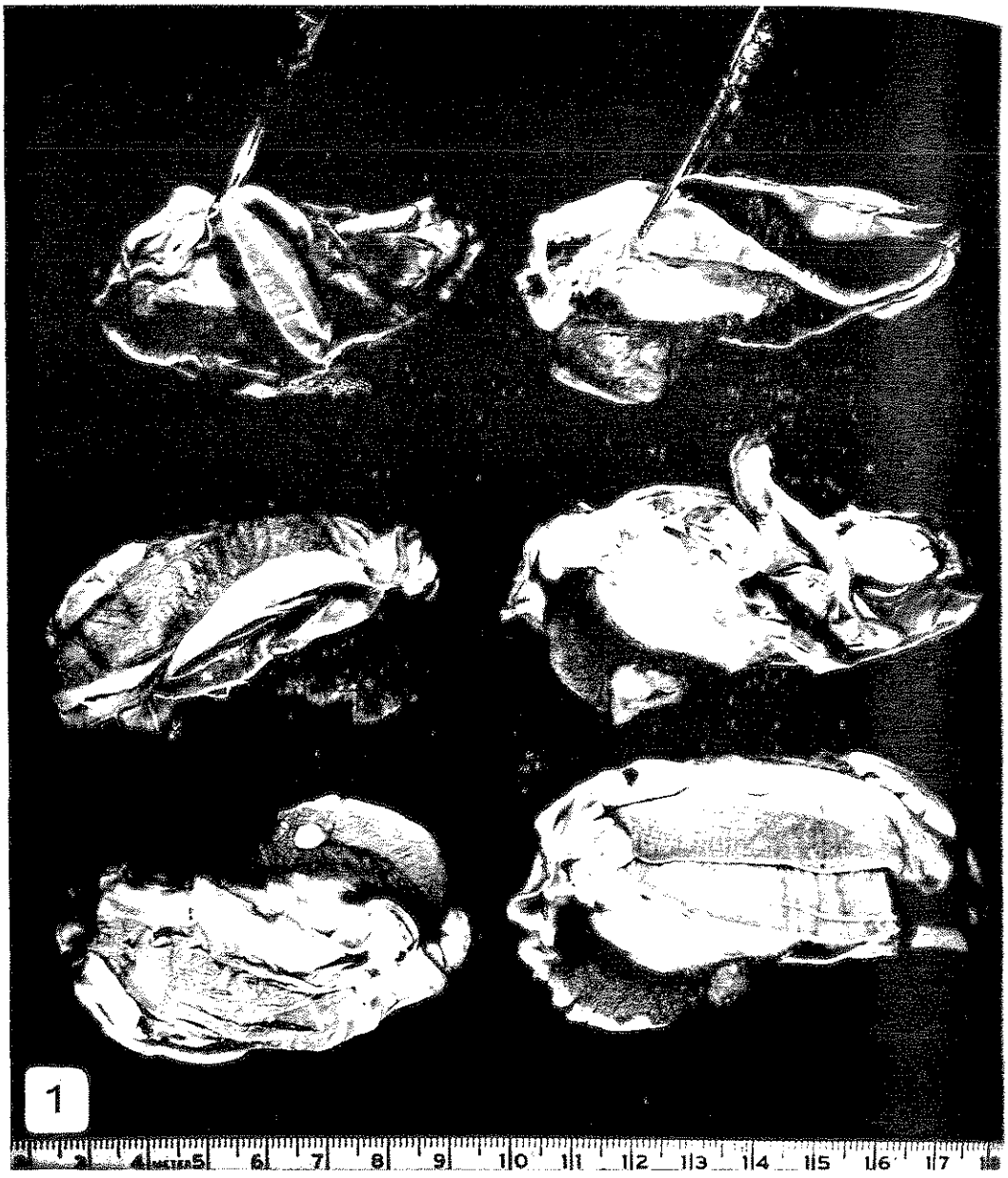

FIG. 1. Freshwater mussels (*Margaritifera margaritifera*) exhibiting various gross lesions characteristic of "spongy" disease.

on the surface were "gapers" in various stages of decomposition. Many of these mussels had a putrid smell, which would indicate they had been dead at least 112 hr, if freshwater mussels have a postmortem decomposition pattern similar to marine oysters, as described by Sparks and Pauley (1964). At the time of collection, it seemed strange that so many shells were present with soft parts still in them. However, the significance of these dead *M. margaritifera* was not apparent until the animals were examined in the laboratory and found to possess a very high incidence of abnorma

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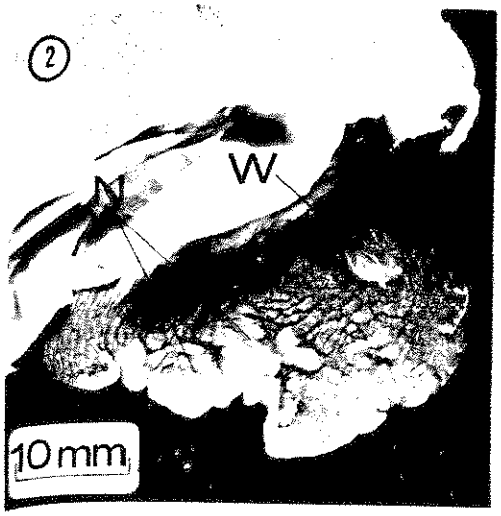


FIG. 2. Higher magnification of a mussel (left-center in Fig. 1) that possesses both a circular watery cyst (W) and a firm, scarred, nodular area (N).

lesions, such as small polypoid growths, ulcers, nodular wounds, and watery cysts (Figs. 1, 2, and 3). The most prevalent type of lesion was the watery cyst. After examining the animals grossly and histologically, it became apparent that all of the abnormal lesions were various stages of the same disease, with the probable exception of several small black polypoid growths (Fig. 3), which were all about 2 × 2 × 2 mm. The watery cysts varied greatly in size from 3 × 3 × 3 mm to 27 × 14 × 4 mm, and were observed as single or multiple lesions on one or both sides of the animals. These lesions evidently interfered with the animals' ability to dig and position in the sand. The most noticeable feature of the lesions was the resilience of the watery

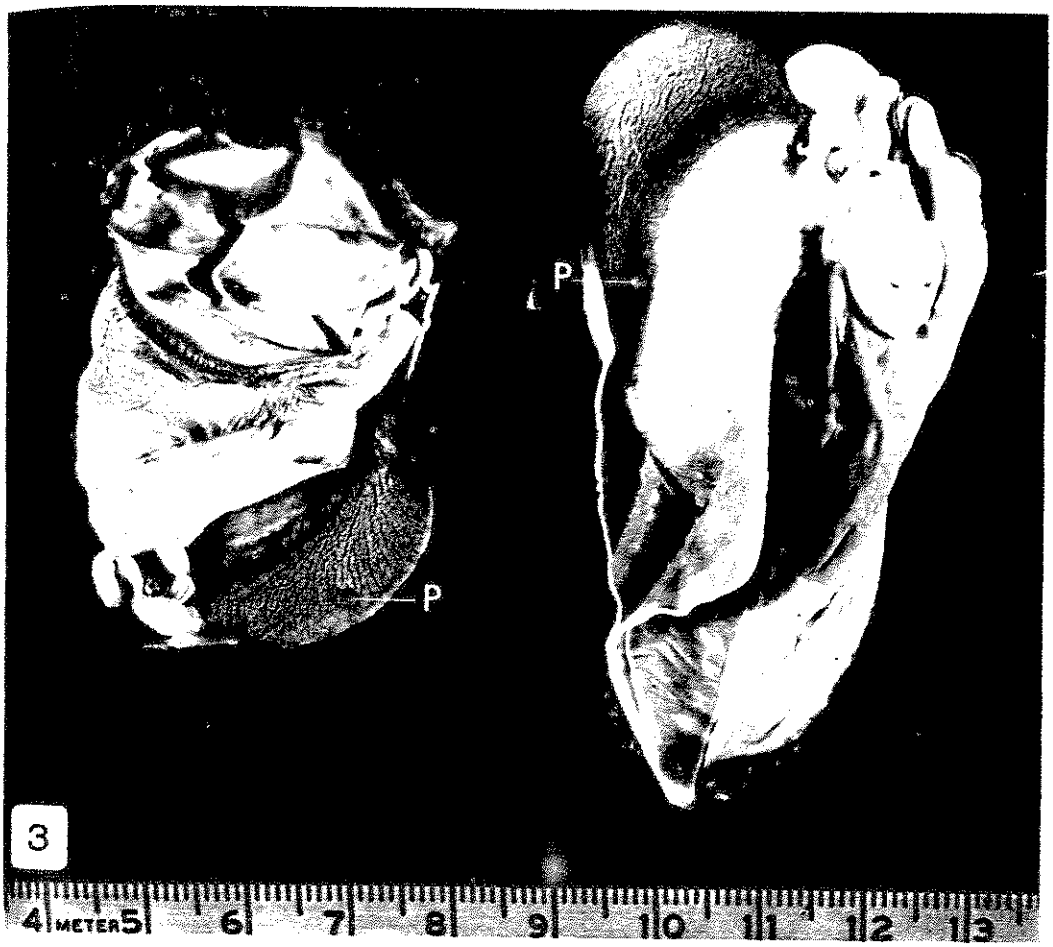


FIG. 3. Gross appearance of small polypoid growths (P) on the foot of mussels.

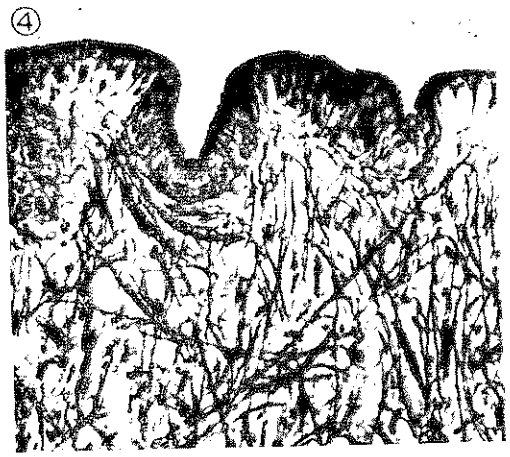


FIG. 4. Normal foot of *M. margaritifera* showing the relationship of the muscles and basophilic gland cells beneath the tall columnar epithelium that is ciliated. Mallory's trichrome. 255 ×.

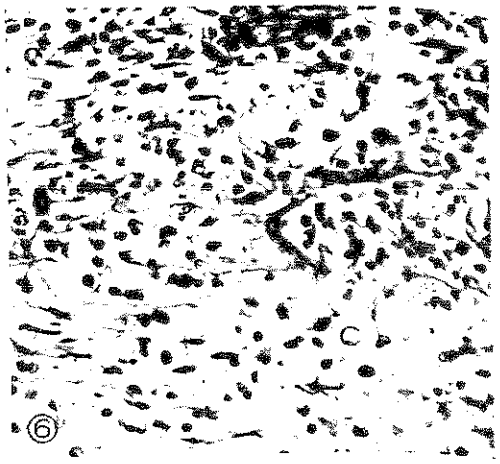


FIG. 6. Edematous lesion being infiltrated by leukocytes and fibroblasts. Note that a few collagen fibers are present (C). Mallory's trichrome. 225 ×.

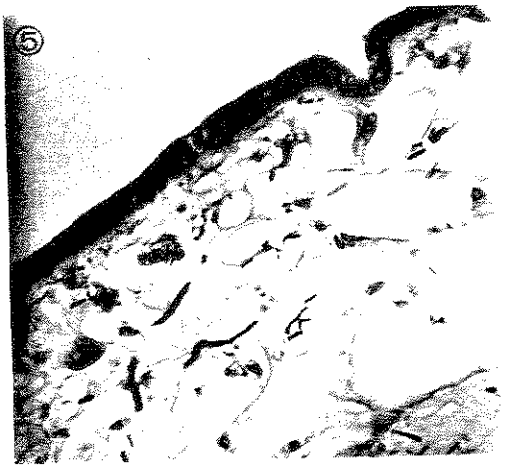
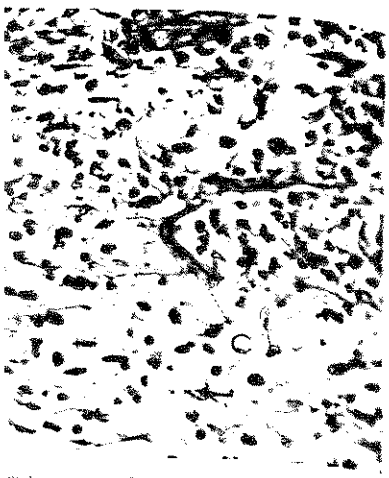


FIG. 5. Edematous watery cyst. Note the stroma of loose connective tissue with channels and the cuboidal epithelial covering that lacks cilia. Mallory's trichrome. 255 ×.

cysts, when pressure was applied to them. Hence, the name "spongy" disease.

The disease was confined entirely to the tissues in the foot of *M. margaritifera*, and up to 90% of the foot was found to be involved. Several stages in the pathogenesis were often observed in a single lesion. Careful study indicated a possible relationship between the texture and color of the lesion and the histopathology. The first noticeable feature of the disease was the destruction of the normal architecture of the muscle fibers and the basophilic gland cells by edema. A loose edematous connective tissue developed and the gross lesion became spongy. At this time, the normally tall columnar epithelium became more cuboidal and the normal cilia was lacking. There were channels present in this loose connective tissue bounded by a membrane that was probably formed by the connective tissue (Fig. 5, compared to Fig. 4). Broken remnants of muscle fibers and basophilic gland cells were still observable in the loose connective tissue.

As the loose connective tissue became infiltrated by leukocytes and fibroblasts, a few collagen fibers were noticeable (Fig. 6). At this stage, cells with cytoplasmic inclusions were first observed and they were present in all subsequent stages of the disease (Figs. 9–12). The epithelium was reduced to a squamous or necrotic cuboidal epithelium. Although broken muscle fibers



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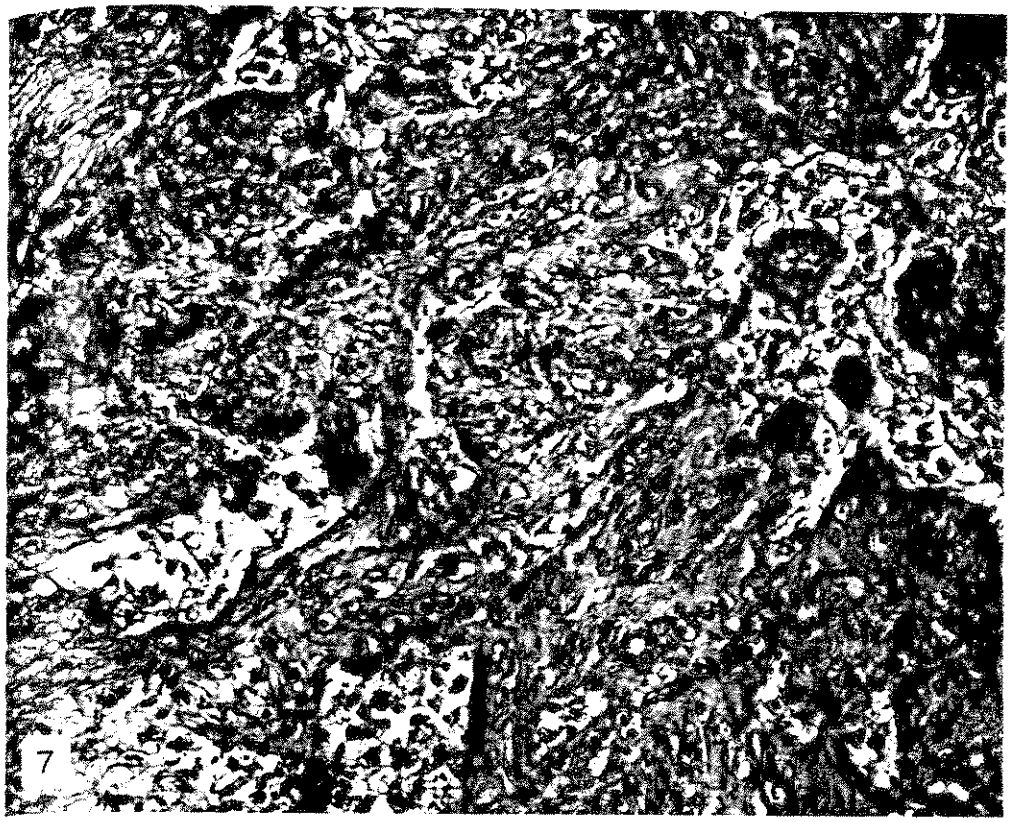

FIG. 7. Granulation tissue with compact collagen surrounding channels (at right and lower left) that are filled with leukocytes, fibroblasts, and connective tissue cells. Mallory's trichrome, 285 ×.

were still observable, the basophilic gland cells had been completely destroyed.

As the number of leukocytes, fibroblasts, and collagen fibers increased, there was a corresponding reduction of edema. The gross lesions were yellowish or whitish and, although still pliable, they had lost much of their resilient characteristic. The epithelium was either a necrotic squamous layer or completely lacking.

As the amount of collagen increased, granulation tissue formed, with compact collagen surrounding channels filled with leukocytes, fibroblasts, and connective tissue cells (Fig. 7). The epithelium was still lacking in some cases, but often a stratified squamous epithelium had formed over the area of the lesion. This stage and the subsequent one appeared grossly as firm scarred nodules.

Eventually collagen completely filled the damaged area, forming a dense scar (Fig. 8) that contained only a few leukocytes, fibroblasts, and necrotic muscle fibers. The epithelium was either a stratified squamous or a ciliated cuboidal layer.

The inclusion-containing cells had a large basophilic nuclei and one or more inclusions of various sizes in the cytoplasm (Figs. 9–12). The inclusions possessed characteristic clear halos around them, which varied in size.

Eight *M. margaritifera* possessed small polypoid growths on the foot (Fig. 3) that were firm, normal in color, and appeared to be attached to the foot by a small stalk.

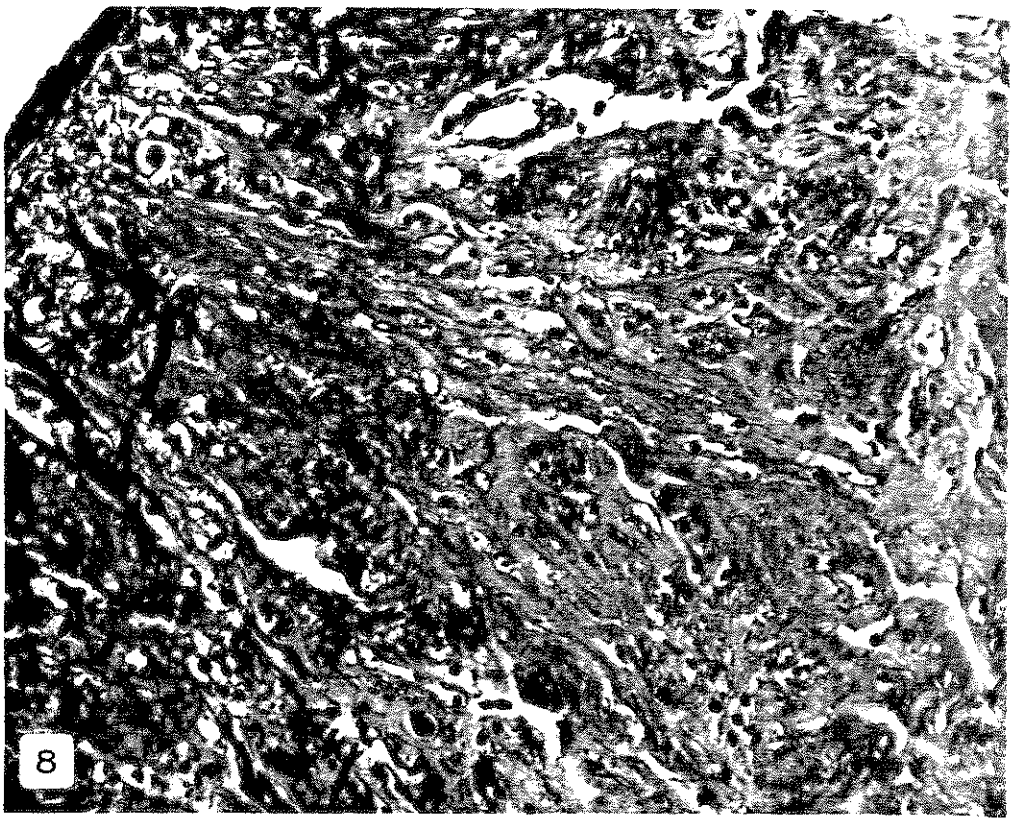

FIG. 8. Dense collagenous scar. Note the regenerating epithelial covering at upper left. Mallory's trichrome. 285 ×.

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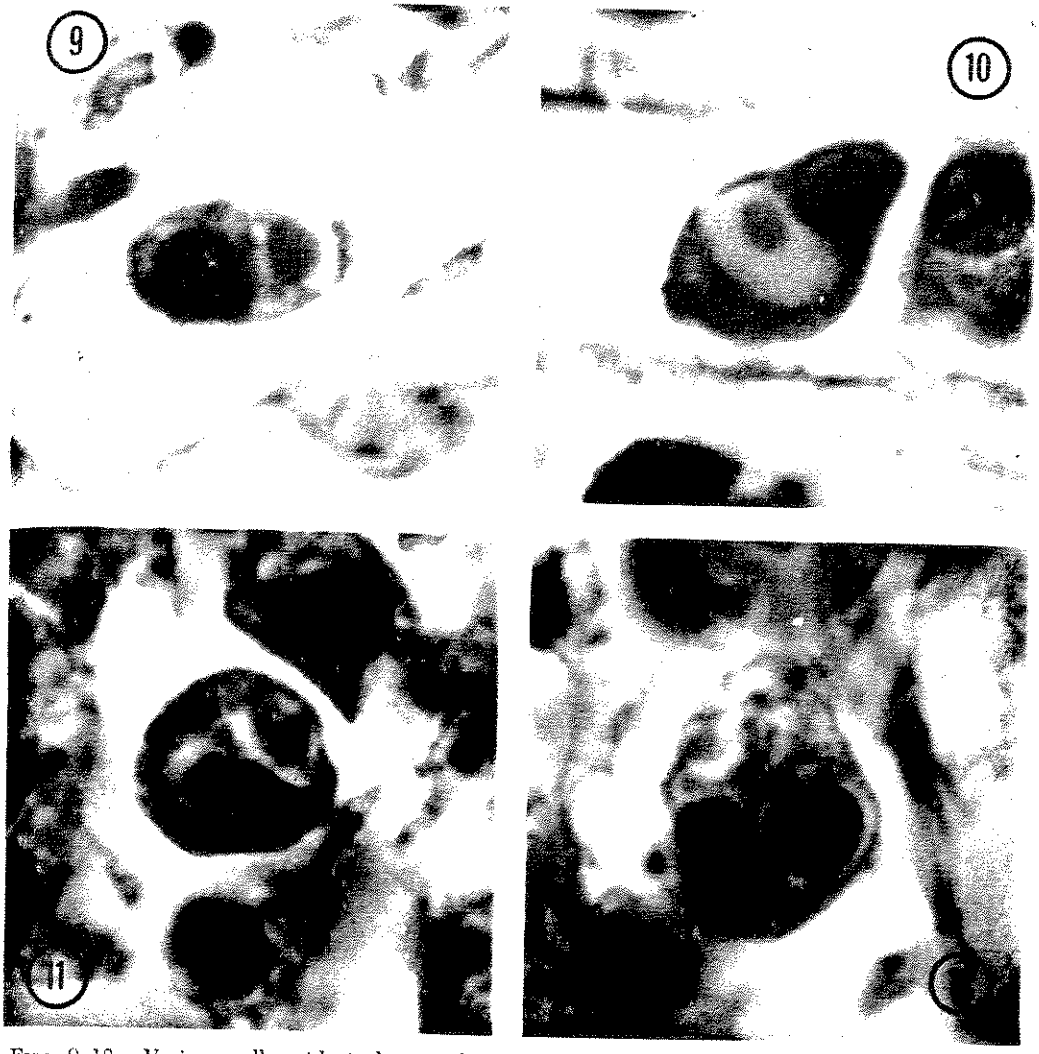

FIGS. 9–12. Various cells with inclusions found in many of the lesions and thought to be the possible cause of "spongy" disease. Note the characteristic halos around the inclusions. Mallory's *trichrome*. 3200 ×.

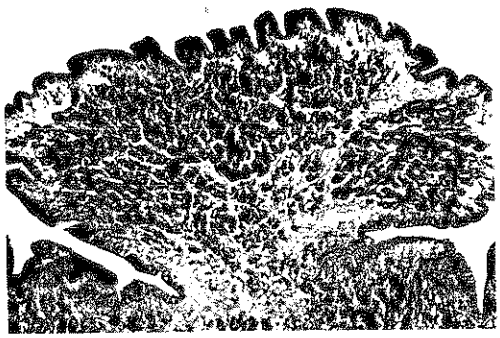

FIG. 13. Polypoid hyperplasia of the basophilic gland cells in the foot of *M. margaritifera*. Hematoxylin and eosin. 48 ×.

Microscopically, these growths were mushroom- (Fig. 13) or dome-shaped. Each growth was completely covered by a normal tall, ciliated columnar epithelium. The growths were composed almost entirely of hyperplastic basophilic gland cells, supported by a few muscle and collagen fibers. No inflammatory reaction was associated with any of these growths. All of the cells present in the lesions were normal and no mitotic figures were present. No invasion into the foot by these growths was evident.

DISCUSSION

Watery cysts have been described from the oysters *Crassotrea virginica* (Mackin, 1962) and *Crassotrea gigas* (Pauley and Sayce, 1967; Pauley et al., 1968), but the histopatholgy associated with the "spongy" disease in *M. margaritifera* has not been described in mollusks.

The later stages of the disease in *M. margaritifera* indicated that these mollusks were successful in repairing the damage in the foot by the formation of a dense collagenous scar. However, it seemed apparent that in many, if not all, cases this scar formation inimically altered the normal physiology of these animals, since they were unable to dig and orient themselves in the sand properly with only the posterior portion of the animal exposed. The unusual postion of the *M. margaritifera*, with the entire animal on the surface of the substrate, was similar to that observed by Sparks and Chew (1966) in littleneck clams (*Venerupis staminca*) infested with larval cestodes.

The cause of "spongy" disease appeared histologically to be the amoeboid inclusion cells. A positive taxonomic identification of this parasite and the elucidation of its life cycle cannot be made without fresh living material. If more diseased mussels can be obtained a postive identification of the causative organism will be attempted.

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