

Dermocystidium marinum Infection in Oysters

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A review of knowledge concerning the life cycle, structure, taxonomy, and pathology of *Dermocystidium marinum* is presented. The pathogen causes mortalities of the American oyster, *Crassostrea virginica*, along the Atlantic and Gulf coasts of the United States. The life cycle consists of vegetative reproduction in which uninucleate aplanospores enlarge and undergo successive bipartition (alternating karyokinesis and cytokinesis) to form 4- to 64-cell sporangia from which are liberated uninucleate, coccoid or cuneiform aplanospores which are 2-4 μm in longest axis. Ultrastructure of recently liberated aplanospores includes a pair of centrioles resting in invaginations of the nuclear envelope. The organelles contain a large cylindrical, electron-dense granule in the lumen and have a nine-fold, triplet blade microtubular substructure typical of many eucaryotic cells. A granular wall is continuous around the cell often having host cell phagosome membranes embedded in the wall. One to three lomasomes are found beneath the wall in random locations at the cell surface. Tubulovesicular mitochondria with electron-dense material filling the interior of many segments are found scattered throughout the cytoplasm (Perkins, 1969).

As aplanospores enlarge and mature, a large eccentric vacuole develops which may occupy greater than 50 percent of the total cell volume. At this stage the cells are about 5-10 μm in diameter. From the vacuole membrane is synthesized electron-dense material which accumulates in the vacuole and

forms a free-floating refringent vacuoplast (Fig. 1).

Cytokinesis occurs by plasmalemma invagination after each nuclear division. Daughter cells initiate wall synthesis after the full complement of cells has been reached and before the mother cell wall has ruptured. After rupture, mother cell wall fragments may adhere to the aplanospore.

In moribund oysters uninucleate prezoosporangia may be rarely formed by marked enlargement of aplanospores to about 15-20 μm . The mechanism appears identical to that which occurs in fluid thioglycollate medium (Ray, 1952; Perkins and Menzel, 1966), except that the medium induces enlargement to sizes above 20 μm , cells being observed larger than 100 μm in diameter. Enlargement results in cells with a very large eccentric vacuole which may comprise 90 percent of the cell volume.

Liberation of prezoosporangia from oyster tissue into seawater results in zoospore formation by successive bipartition of the protoplast and subsequent release from the zoosporangia. During zoosporogenesis the large vacuole subdivides as the daughter cells progressively decrease in size. Zoospores initiate infections by establishing themselves or being established by amoebocytes in epithelia. Zoospores then become aplanospores and multiply as described above.

Ultrastructural studies of various cell types in the life cycle reveal numerous characteristics of the Coccidia (Scholtyseck, 1973). *Dermocystidium*

marinum zoospores have a conoid at the anterior end of the cell with several rhoptries terminating in the lumen (Fig. 2). Similar membrane-bound sacs containing electron-dense material, but which do not terminate in the conoid lumen, are found in the anterior half of the cell. Those organelles may be analogous to micronemes. A cytoskeleton of microtubules radiate from a polar ring around the anterior end of the conoid and pass posteriorly through most of the cell length just beneath the plasmalemma (Fig. 2). Micropores have not been found in motile zoospores, but are found in all vegetative cell stages not engaged in zoosporogenesis (Perkins, 1969). The organelle resembles those found in *Eimeria* spp. (Scholtyseck, 1973).

Dermocystidium marinum zoospores are biflagellate as are the microgametes of several Coccidia, but appear to differ in that mastigonemes are present (Fig. 3). Although coccidian microgametes appear to lack mastigonemes, this point has not been adequately studied using the necessary preparations of negatively stained or shadowed whole mounts. There is no evidence that *D. marinum* zoospores are microgametes.

The numerous structures (conoids, polar rings, an ordered cytoskeleton of microtubules beneath the plasmalemma, rhoptries, micronemes, and micropores) found in *D. marinum* and considered by protozoologists to be characteristic of the Coccidia lead me to suggest that the oyster pathogen is a coccidian or at least closely related. In my studies I have found no evidence

Figure 1.—Developing aplanospore of *Dermocystidium marinum*. Eccentric vacuole with vacuoplast (V); lomasome (L); and nucleus (N). 14,000 \times .

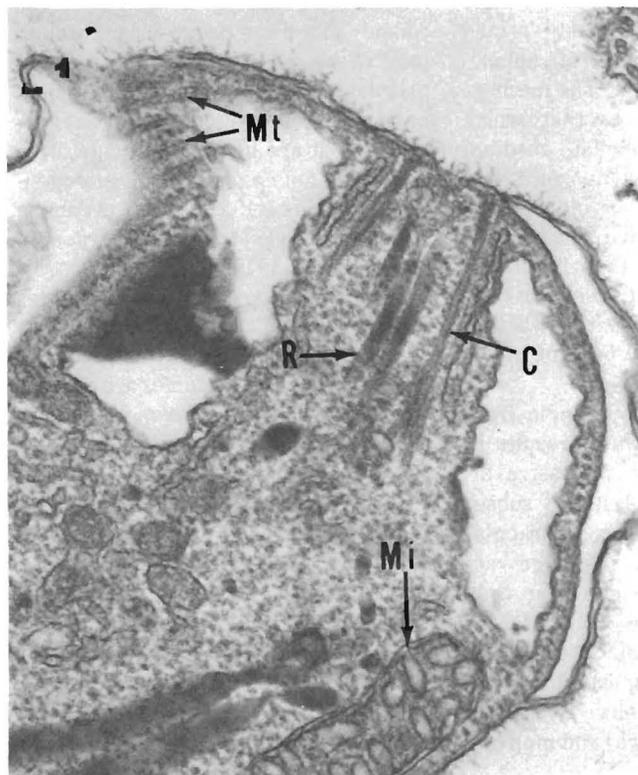
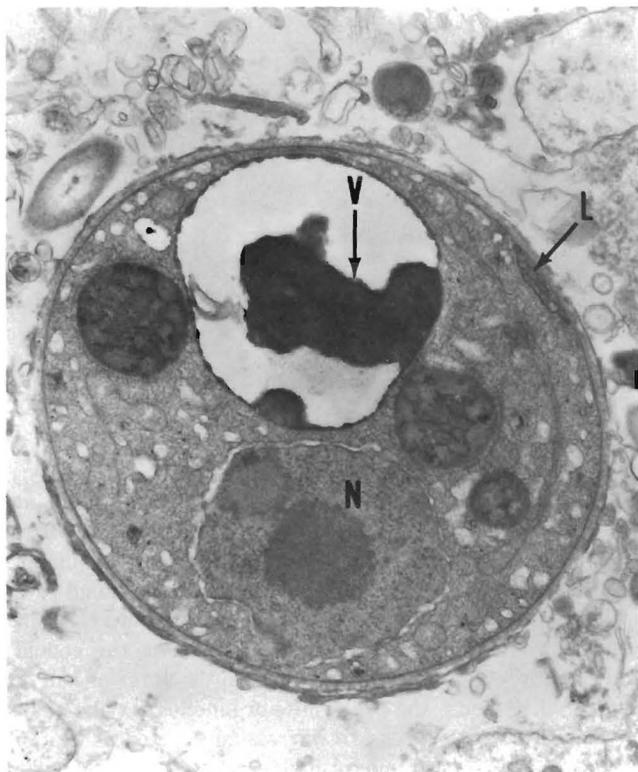


Figure 2.—Anterior end of zoospore showing conoid (C), microtubules (Mt) of cytoskeleton, mitochondrion (Mi), and rhoptries (R) within conoid. 64,000 \times .

Figure 3.—Whole amount of *D. marinum* zoospore. Mastigonemes (M) along one side of anterior flagellum. 11,000 \times .

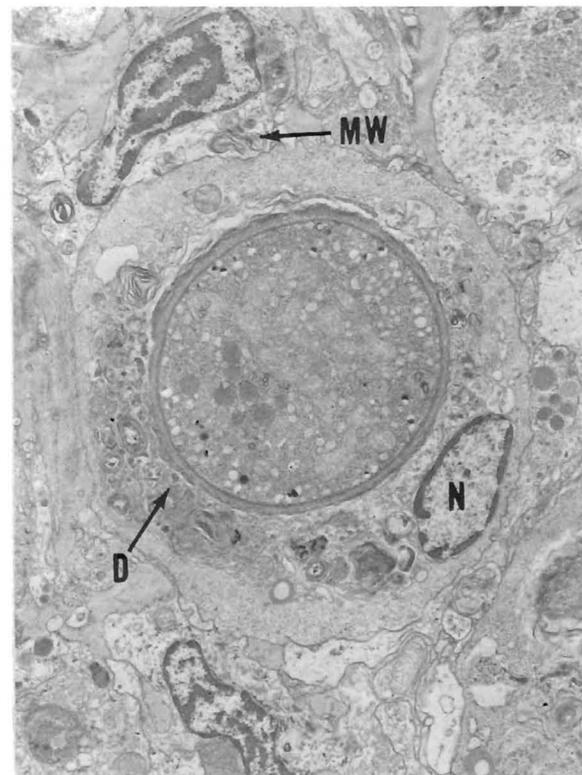
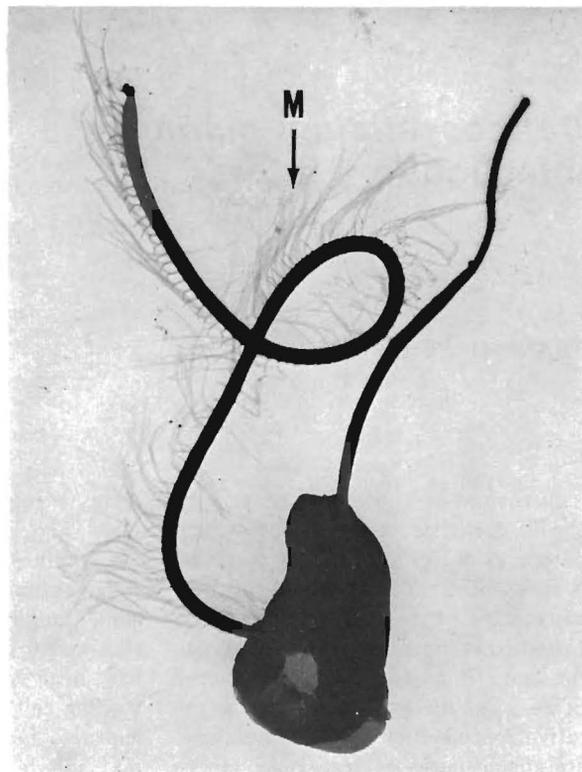


Figure 4.—Aplanospore in oyster amoebocyte of mantle connective tissue showing degenerative changes in host cell. Amoebocyte nucleus (N) is pycnotic and cell debris (D) is found around the aplanospore. Connective tissue and muscle cells of mantle appear less severely affected; however, myelin whorls (MW) are present in cells nearest the amoebocyte. 9,000 \times .

that *D. marinum* is a labyrinthulid (see Olive, 1975, for review of the group) as suggested by Mackin and Ray (1966), the assumption being made that *Labyrinthomyxa* spp. are labyrinthulids. The oyster pathogen lacks labyrinthulid characteristics such as a bilateral row of mastigonemes on the anterior flagellum and formation of ectoplasmic nets from sagenogenetosomes (Perkins, 1972). Labyrinthulids lack the coccidian characteristics mentioned above. One unusual ultrastructure which *D. marinum* and labyrinthulids have in common is the large cylindrical kinetosome and centriole granule; however, such an inclusion is also found in ciliates (Allen, 1969; Didier, Iftode, and Versavel, 1970).

The histopathology of *D. marinum* infections is essentially as already described by Mackin (1951). Any cell stage appears capable of initiating infections except possibly prezoosporangia. Infections are initiated in the gill, mantle, or gut epithelia or in the connective tissue near the basement membrane. Cells of the pathogen may lie between or within host cells (Fig. 4). Multiplication results in a cluster of uninucleate aplanospores which spread through the host via amoebocytes. Extensive leucocytosis, noticeable at the tissue level, may or may not occur.

Foci of pathogen cells are established in the connective tissue and epithelia in which multiplication proceeds as de-

scribed above resulting in progressive degeneration among juxtaposed host cells. Tissue response in the early stages often consists of an attempt at "encapsulation" by several layers of host amoebocytes; however, as the pathogenic cells increase in number the "capsule" disappears. In later stages of infection foci may attain several hundred micrometers in diameter and contain thousands of pathogen cells mixed with host cell debris.

In addition to dense accumulations in foci or abscesses, the pathogen disperses throughout the connective tissue and vascular system often occluding the hemolymph sinuses. Although the epithelia are invaded and abscesses may form, epithelia are among the least damaged tissues in well-advanced infections. Adductor and smooth muscle are also invaded and extensive multiplication of the pathogen often occurs therein; however, since very heavy infections often occur, the adductor is probably not heavily damaged until late in the infection, otherwise predators would enter and kill the oyster as soon as shell closure was no longer possible.

Probably a lethal toxin is not formed by the pathogen since very large cell numbers may accumulate in all tissues before host death results. Destruction of host cells appears to be limited to those cells in the immediate vicinity of the pathogen.

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