Technical Papers

Preliminary Note on the Occurrence of a New Protistan Parasite, *Dermocystidium marinum* n. sp. in *Crassostrea virginica* (Gmelin)[1]

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During the course of extensive investigations on oyster physiology, ecology, mortality, and histology in Louisiana and other Gulf states the authors, separately and independently, discovered a hitherto undescribed parasite of oysters. Comparison of notes and materials has confirmed that we have been observing the same parasite, and presentation of this preliminary report and a description of the parasite now seem desirable. In spite of the fact that studies have indicated a wide distribution of the organism, the authors are not aware of any previous report by investigators in the Gulf of Mexico or other regions.

The genus to which this parasite belongs was described (*8*) by Perez (1907) as *Dermocystis*. This name being preoccupied by *Dermocystis* Stafford (1905), the new name, *Dermocystidium*, was published by Perez (*9*) in 1908. In 1913 Perez (*10*) gave an extended description of the genus. Because of certain variations in morphology, the authors feel that the parasite found in the oyster is specifically distinct from any previously described species.

Inasmuch as Perez (1913), in his study of the type species, *D. pusula*, purposely abstained from designation of those characters of generic rank, as opposed to those of specific value only, there exists today no clear-cut generic diagnosis. Also it is obvious from a study of the descriptions of subsequent additions to the genus that many obscurities of cyclic development still exist—a fact which makes an attempt to designate generic characters still undesirable. The following description, therefore, omits any such analysis.

Dermocystidium marinum n. sp.

Studies of numerous stained sections and fresh preparations show that the most characteristic stage is a nearly spherical spore, 3 μ to 10 μ in diameter (Fig. 1A); the majority are 5 μ to 7 μ. Characteristically the cell contains a very large, slightly eccentric vacuole containing a large polymorphic refringent inclusion body, hereafter referred to as a vacuoplast. The cytoplasm of the cell forms a rather thin peripheral layer of alveolar nature, which is thicker on the side containing the nucleus. Occasionally the cytoplasm presents a slightly fibrous appearance and often contains aggregations of deep staining granules which are identical in staining reaction to the vacuoplast. The nucleus in the spore stage consists of a compact endosome surrounded by a clear zone usually free of chromatin material. The entire nucleus is ordinarily oval, the membrane often indistinct.

The vacuoplast, when well formed, stains in shades of gray to black with Heidenhain's iron hematoxylin and stains a very light rose or diffuse pink with Delafield's hematoxylin and eosin. Because the vacuoplast stains heavily at the surface and less deeply internally, it often has the appearance of being hollow. Morphologically it may be a nearly prefect sphere, a lobular body, branched, or beaded. Often it is represented by several separate bodies or is broken into a large number of small granules, many of which may be distributed in small subvacuoles of the cytoplasmic layer (Fig. 1A and 1D).

Occasionally binucleate stages are seen (Fig. 1C), the significance of which is not yet clear. Developmental stages of the spores usually have a vesicular type of nucleus, with some distributed chromatin and a less definite vacuole and vacuoplast (Fig. 1D).

In reproduction, segmentation of the nucleus occurs with subsequent distribution of the daughter nuclei, followed by condensations of cytoplasm around each (Fig. 1B), and this in turn is followed by cytoplasmic cleavage and final liberation of sporelike cells by rupture of the thin containing membrane. The number of such cells produced is indeterminate, but occasionally as many as thirty may result from division of a single mother cell. Their size at liberation varies greatly.

There are indications that endogenous budding occurs by a process of successive delaminations, producing spores of unequal size in an enveloping membrane formed from the parent cell. Any tissue of the host may be infected, common localizations being in the intestinal epithelium, adductor muscle, gills, mantle, and heart, or in cases of heavy infestation all tissues may be invaded. Development of the parasites is often intracellular in phagocytic or connective tissue cells.

Type host: *Crassostrea virginica*, the commercial oyster of the Atlantic and Gulf coasts.

Type locality: Sugar House Bend in the southern part of Barataria Bay, Jefferson Parish, Louisiana.

Type slides: In the collections of the authors.

The most closely related species is probably *Dermocystidium salmonis* Davis 1947 (*3*). *D. marinum* differs specifically from the latter species in (1) the extreme polymorphism of the vacuoplast, (2) in the apparent lack of a definite reproductive cycle involving simple

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binary division of the protoplasm, (3) in the possession of stages in multiple cleavage not described by Davis for *D. salmonis*, and (4) by the difference in host, *D. salmonis* being parasitic on gills of the chinook salmon.

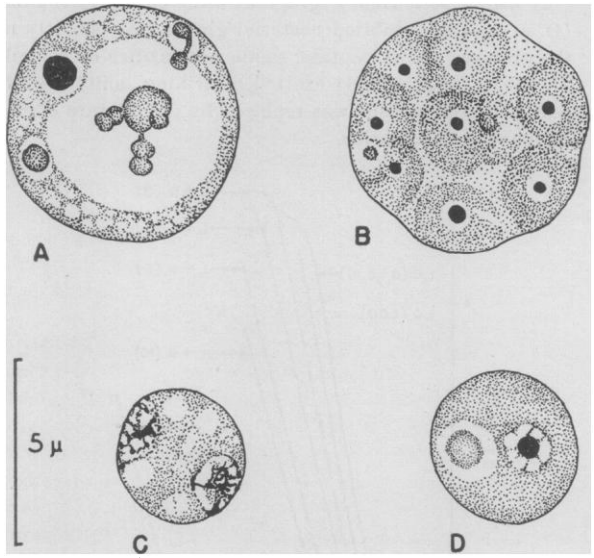

FIG. 1. Drawings from specimens stained with Heidenhain's iron haematoxylin and eosin. A—A mature spore with markedly irregular vacuoplast, cytoplasmic inclusions, and very large vacuole. B—Multiple fission resulting in several daughter cells. C—A binucleate stage with chromatin in diffuse condition, and showing beginning vacuolation of the cytoplasm. D—An immature spore with small vacuole and vesicular nucleus. The radiating fibers from the endosome are not commonly seen.

All other described species of *Dermocystidium* have hosts confined to fresh-water environments, the estuarine environment of *D. marinum* being unique.

A small body of literature has been built up on *Dermocystidium*. At the present time the known species include *D. pusula* (Pérez) 1907, *D. branchialis* Léger (*7*), 1914, *D. ranae* Guyénot and Naville (*4*), 1921–22, *D. daphniae* Jirovec (*5*), 1939, *D. vejdovskyi* Jirovec (*6*), 1939, and *D. salmonis* Davis (*3*), 1947. Hosts include various species of salamanders of the genus *Triturus*, frogs of the genus *Rana*, several fishes including trout, pike, and salmon, and one invertebrate, *Daphnia magna*. All species excepting one have been reported from localities in France, Germany, and Ireland. One species only is recorded in the United States, *D. salmonis* Davis, from the Sacramento River, California.

Several closely related genera are known, but the evaluation of most of the relationships is a problem for the future. At present it seems certain that *Blastocystis* Alexeieff 1911 is the nearest well-established genus. The relationship is based on certain parallels of staining reaction of the nucleus and the vacuoplast. For an exhaustive treatment of staining reactions of *Blastocystis* see Bach and Kiefer (*2*), 1923, and for *Dermocystidium* see Perez (*10*), 1913. Also, there are definite parallels of developmental cycle including production of what Alexeieff (*1*) considered to be ascospores.

Data acquired thus far independently by each author show the association of this organism with dead or dying oysters under certain environmental conditions, the limits of which can be reasonably well defined. The chief controlling factors appear to be temperature and salinity, low temperature and low salinity evidently retarding the development of the infestation.

Oysters in all conditions of health are susceptible to infestation, but factors resulting in fatigue, such as spawning, and miscellaneous adverse environmental conditions accelerate the intensity and spread of the organism.

Investigations of the taxonomy, physiology, ecology, and distribution of this parasite covering the geographical range of *Crassostrea virginica* are still in progress. A full report will be published elsewhere at a later date.

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Action of Penicillin on Bacterial Utilization of Amino Acids and Peptides[1]

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In a previous communication (*5*), there was reported the isolation of a rodlike, Gram-negative bacillus from a contaminated solution of L-leucylglycine in 0.9% NaCl. This organism, termed strain SF, grew satisfactorily in a solution of 0.1 M L-leucylglycine in physiological saline, but did not grow when the dipeptide was replaced by a mixture of 0.1 M L-leucine and 0.1 M glycine. Only poor growth was obtained in the presence of 0.1 M L-leucine. Addition of glucose to these media did not alter appreciably the extent of bacterial growth.

The growth response of strain SF has now been studied in a more complex basal medium which contains, per liter, 5 g NaCl, 0.2 g $MgSO_4 \cdot 7H_2O$, 2 g KH_2PO_4, 2 g K_2HPO_4, and 1 ml of the trace element solution described in reference *3*. Maximal growth in the presence of L-leucylglycine, dissolved in this medium, was attained at a dipeptide concentration of 0.005 M, and subsequent growth studies with the dipeptide or its component amino acids were conducted at this molar concentration for each test compound. In these experiments, Evelyn colorimeter tubes containing the test solutions (final volume, 10 cc) were inoculated with approximately 10^8 organisms, and the slanted tubes were shaken (120 oscillations per min) at 30° C. The extent of bacterial growth was measured

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