Reports

A Protozoon (Dileptus; Ciliata) Predatory upon Metazoa

Abstract. *Dileptus*, a common holotrich, may function as a predator upon a variety of metazoan animals representing such varied phyla as Cnidaria, Platyhelminthes, Aschelminthes, Annelida, and Mollusca. Very young animals and wounded animals are especially vulnerable. Effective predation is directly related to the concentration of *Dileptus* to which the victim is exposed.

Cultures of planarian flatworms (*Dugesia dorotocephala*) have been maintained in our laboratory for several years; they are fed small pieces of beef liver about once a week. On 14 July 1960 there was no sign of a worm in a pan which, a week earlier, had contained several thousand baby worms. Examination of the water revealed it to be swarming with *Dileptus* sp., a common holotrich ciliate. (No attempt is here made to identify the species because the taxonomy of the genus is being revised by Jean Dragesco of Paris.)

Some of the *Dileptus* were removed to Syracuse watchglasses, and fresh baby planarians were added and observed. Contact with the proboscis of *Dileptus* produced an immediate and violent withdrawal response on the part of the worms. Within the first 12 minutes, one worm was seen to be "stung" 27 times. Within 90 minutes, all of the worms under observation had been immobilized and were being devoured by *Dileptus*, which were converging and swarming upon each victim. Within a few hours there was no recognizable trace of the worms other than masses of cells, upon which the *Dileptus* were busily feeding.

Apparently the *Dileptus* thrived upon the beef liver which was supplied for the worms until the population of *Dileptus* reached a critical concentration at or above which they could feed upon the planarians.

Dileptus has long been known to be predatory upon a variety of protozoa (chiefly flagellates and ciliates) and even upon an occasional rotifer (*1*), but no one has previously reported predation upon macroscopic organisms. The offensive weapons of *Dileptus* are the "toxic trichocysts" which line the ventral surface of the proboscis (*1, 2*). Since these trichocysts were seen to be so effective against baby planarians, it seemed logical to test other potential victims. Every small animal tested exhibited a violent response to contact with the ventral surface of a *Dileptus* proboscis.

In general, as might be expected susceptibility to attack is closely correlated with relative amount of exposure to trichocysts. A single *Dileptus* can kill *Stenostomum* (Turbellaria: Catenulida). As few as five *Dileptus* may destroy a baby pond snail (*Physa*), an amphistome cercaria (Trematoda), or a tiny planarian, if the victim happens to make no effective movement at the critical moment. Destruction of *Hydra* requires a larger number of *Dileptus*.

Those animals which are big enough and fast enough or which have impervious coverings (cuticle, shell, mucus) are able to survive. Even these, however, if impeded in their escape or if deprived of their protective coverings, are rendered vulnerable. Thus, for example, a fingernail clam (*Sphaerium*), with a chip of shell removed so that *Dileptus* has access to the tissues, can be destroyed, whereas a similar clam would survive if intact. Wounds in such animals as nematodes (*Cephalobus*) and aquatic oligochaetes (*Nais*, branchiobdellids) greatly increase the likelihood of destruction by *Dileptus*, not only because of exposure of tissues to attack but also because *Dileptus* is apparently "attracted" by chemicals liberated in the vicinity of the wound. A film depicting the destruction of several types of invertebrates by *Dileptus* was shown at the First International Conference on Protozoology, which was held in Prague during August 1961.

The extent to which *Dileptus* functions as a predator upon metazoa under natural conditions is not known, but it has been demonstrated to be a potential predator for a variety of small invertebrates, and it probably acts as a scavenger wherever injured or dead animals are available.

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References

1. J. P. Visscher, *Biol. Bull.* **45**, 113 (1923); A. N. Studitsky, *Arch. Protistenk.* **70**, 155 (1930).
2. M. L. Hayes, *Trans. Am. Microscop. Soc.* **57**, 11 (1938); J. Dragesco and C. Métain, *Bull. soc. zool. France* **73**, 62 (1948); J. Dragesco, *Compt. rend. congr. intern. zool.* 13e Congr., Paris (1948), p. 227; ———, *Bull. Microscop. Appl.* **2**, 92 (1952); J. N. Dumont, *J. Protozool.* **8**, 392 (1961).

9 January 1962

Haplosporidium costale (Sporozoa) Associated with a Disease of Virginia Oysters

Abstract. A new species of *Haplosporidium* is reported as the cause of a previously unrecognized disease of *Crassostrea virginica* (Gmelin) on the Eastern Shore of Virginia. The species is characterized by small (3.1 by 2.6 μ) operculate spores without projections. Infections are first seen in late winter, and mortality reaches its peak in early June.

In 1959 plans were made to follow possible invasion of Chesapeake Bay by the agent "MSX," which destroyed the oyster industry in Delaware Bay (*1*). The patterns of mortality associated with *Dermocystidium marinum* and certain less serious diseases had been well worked out (*2*). During studies in 1959, 1960, and 1961 certain deviations from expected mortality patterns were noted at Seaside, on the Eastern Shore of Virginia. Oysters began to die abruptly in mid-

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Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to contributors" [*Science* **125**, 16 (1957)].

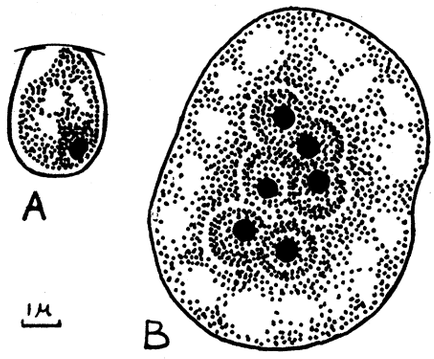

Fig. 1. *Haplosporidium costale*. *A*, Mature spore; *B*, early plasmodium.

May and stopped dying early in July. Numerous dead and dying oysters were collected and fixed in formol–acetic acid–alcohol for subsequent microscopic examination. Sections were stained with Harris or iron-alum hematoxylin and eosin.

As evidence of a previously unrecognized disease accumulated, it also became apparent that there was an undescribed parasite associated with the mortality. For convenience the parasite was first given the name SSO (for Seaside organism). The presence of SSO in live oysters prior to the mortality and in a large proportion of dead oysters implicated it as the etiological agent, here described as a new species of *Haplosporidium*.

Haplosporidium costale n. sp.: Endoparasite of connective tissues of *Crassostrea virginica*. Spores obovate, truncate, operculate, uninucleate, 2.42 to 4.20 μ (mean 3.09 μ) by 2.14 to 3.26 μ (mean 2.58 μ), lacking projections (Fig. 1*A*). Sporocysts: 7 to 14 μ (mean 9 μ) in diameter, containing 20 to 50 spores per cyst. These measurements, made on stained sections, are approximately 25 percent less than those made on spores in fixed material mounted in lactophenol (that is, before dehydration). Host: *Crassostrea virginica* (Gmelin). Type locality: Hog Island Bay, Virginia. Range: Bays and inlets of Seaside, Virginia, from Chincoteague to Cape Charles, occasionally in Chincoteague Bay and on Bayside in The Gulf and Cherrystone Inlet. The species name refers to its coastal distribution.

The earliest stage observed, cytozoic and histozoic in connective tissue, is a small, multinucleate (4 to 12 usually) plasmodium, 6.1 by 7.8 μ in size; essentially isodiametric, at times irregular in outline; cell membrane at first definite, later inconspicuous; nucleus vesicular, essentially isodiametric with a rather indefinite nuclear membrane and tiny but readily apparent endosome; nucleus 1.6 μ in diameter; endosome 0.6 μ in diameter (Fig. 1*B*).

The early plasmodia (trophozoites) enlarge, the nuclei multiply in number and the cytoplasm becomes vacuolated. A definite central vacuole is sometimes visible. The multinucleate plasmodium finally cleaves into uninucleate portions, each of which becomes a characteristic operculate spore (Fig. 1*A*).

The order *Haplosporidia* Caullery and Mesnil is an artificial assemblage of spore-producing organisms that are not assignable to other groups. The characteristics of the genus are: a large plasmodium which divides into uninucleate bodies, each of which develops into a truncate spore with a lid at one end; envelope may be prolonged into processes; in aquatic annelids and mollusks.

Haplosporidium costale falls into the group of species characterized by spores with an overhanging lid and no appendages, and it has the smallest spores in the group. Division of the sporoblast nucleus and fusion of nuclei or binucleate spores have been described for some species (*3*). The spores of *H. costale* are uninucleate, and in the sporoblasts neither division nor fusion of nuclei was observed.

The fate of ripe spores is not known and it is not clear how oysters become infected with this organism. Uninucleate amoebulae are described for certain species of *Haplosporidium* and not for others (*3*). We have not observed uninucleate trophozoites in the new species.

The disease has been followed closely for 3 years now, and its sharp seasonality is noteworthy. *Haplosporidium costale* is first evident in live oysters in February. Prevalence of infections in mid-May may run as high as 39 percent. During 2 weeks in June 1960, the death rate caused by the organism exceeded anything ever experienced in Chesapeake Bay with other pathogens. The mortality is sharp but of short duration, and the parasite lapses into obscurity for another year. Certain other species of *Haplosporidium* exhibit a similar seasonality (*3*, *4*).

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References and Notes

1. H. H. Haskin, L. Stauber, J. G. Mackin, personal communication (1959).
2. W. G. Hewatt and J. D. Andrews, *Texas J. Sci.* **6**, 121 (1954); J. D. Andrews and W. G. Hewatt, *Ecol. Monographs* **27**, 1 (1957).
3. L. Granata, *Arch. Protistenk.* **35**, 48 (1914); O. Jirovic, *ibid.* **86**, 500 (1936); M. Caullery, *Traité de Zoologie, Tome I, Fasc.* 2 (Masson, Paris, 1953); P. N. Ganapati and C. C. Narasimhamurti, *Parasitol.* **50**, 581 (1960); J. H. Barrow, *Trans. Am. Microscop. Soc.* **80**, 319 (1961); P. Debaisieux, *Cellule* **30**, 293 (1920).
4. We acknowledge the invaluable aid and criticism of Dr. Harold H. Haskin, Rutgers University, Dr. John G. Mackin, Texas A.&M. College, and Dr. Victor Sprague, Chesapeake Biological Laboratory. Mrs. Dorothy K. Emory, with the assistance of Miss Patricia Turner, prepared the slides. This report is contribution No. 102 from the Virginia Institute of Marine Science. Detailed results of our study of the mortality are in preparation.

21 December 1961

Cell Membrane Fusion and the Fertilization Mechanism in Plants and Animals

Abstract. Plasmatic fusion in both plant and animal fertilization seems to start with the coalescence of cell membranes of the gametes. In male gametes, the area of fusion is predetermined: in some algae it is the flagellar tip, while in animal organisms it is the acrosome filament. These organelles thus fulfill comparable roles in the mechanism of fertilization.

Recent publications on animal fertilization in *Hydroides* and the rat (*1*, *2*) have revealed the central role of the coalescence of egg cell and spermium membranes during the fusion of gametes. This mechanism of fertilization resembles strikingly the oogamous fertilization of the green alga *Prasiola stipitata*, as described by Friedmann (*3*) and Manton and Friedmann (*4*). It seems appropriate, therefore, to point out some facts which seem to be analogous in these phenomena and which might well be characteristic of the fertilization process in both plants and animals.

A diagrammatic representation of some aspects of the fertilization in *Prasiola* is given (Fig. 1), based on light- and electron-microscopic observations already published (*3*, *4*).

The process of plasmatic fusion in *Prasiola* can be summarized as follows. The spermatozoid bears two equal flagella. The fibrillar core of the flagellum is ensheathed by a membrane which is continuous with the cell body membrane (Fig. 1*A*). The basal bodies of the flagella are held close to the spermatozoid nucleus by fibrous roots. (The