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Experimental Infection of Oysters (*Crassostrea gigas*) with Thigmotrichid Ciliates[1,2]

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Ciliates of the order Thigmotrichidia were observed to heavily infect Pacific oysters (*Crassostrea gigas*) that possessed weakened adductor muscles. A description of the ciliates, the method of infection, and the pathology associated with these ciliates is presented.

INTRODUCTION

Bay mussels (*Mytilus edulis*) have been observed in histological preparations to have heavy natural infections of thigmotrichid ciliates (Pauley *et al.*, 1965). Although ciliates are not common in living oysters (Mackin, 1962) and were not found associated with the postmortem decomposition of oysters (Sparks and Pauley, 1964), ciliates of the order Thigmotrichidia were observed in Pacific oysters (*Crassostrea gigas*) injected with turpentine, talc, and sea water in an experiment designed to study the processes of inflammation and wound repair in this mollusc (Pauley, 1965).

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MATERIALS AND METHODS

Six groups of 100 oysters each were maintained in trays at the United States Public Health Service Shellfish Sanitation Laboratory at Purdy, Washington. Details of the experiment have been given by Pauley (1965). Briefly, the six groups utilized were as follows:

Experimental Oysters

(1) One group of oysters was injected with 0.05 ml of turpentine in the Leydig cell area above the palps.

(2) A second group of mollusks received 0.05 ml of turpentine in the adductor muscle.

(3) A third group of animals was injected in the Leydig cell region with 0.05 ml of sea water containing talc.

(4) The fourth group of oysters was challenged in the adductor muscle with 0.05 ml of sea water and talc.

Control Oysters

(5) One group of control animals was injected with 0.05 ml of sea water above the Leydig cell area.

(6) The remaining control oysters received 0.05 ml of sea water in the adductor muscle.

Results

Description of the Ciliates

The ciliates observed in the experimental oysters possessed an oral groove, one or more contractile vacuoles, and a small micronucleus, that varied in location: anterior, posterior, or lateral to the large macronucleus. Many of the ciliates were pear-shaped with a pointed anterior end provided with an unciliated nipple and in appearance they were similar to *Ancistrocoma* sp. observed in the eastern oyster, *Crassostrea virginica* (Burton, 1961; Mackin, 1962; Engle and Rosenfield, 1962). These organisms found in *C. gigas* generally had longer cilia on one side than the other, although some appeared to have cilia of even size on both sides. They were generally found in the gills; in the lumina or chambers, attached to the ciliated epithelium; or in the necrotic debris of this organ (Fig. 1). However, these invaders were also observed in necrotic kidney tubules and the Leydig cells below the rectum.

Pathology Associated with the Ciliates

The attachment to the host was generally by the pointed anterior end of the

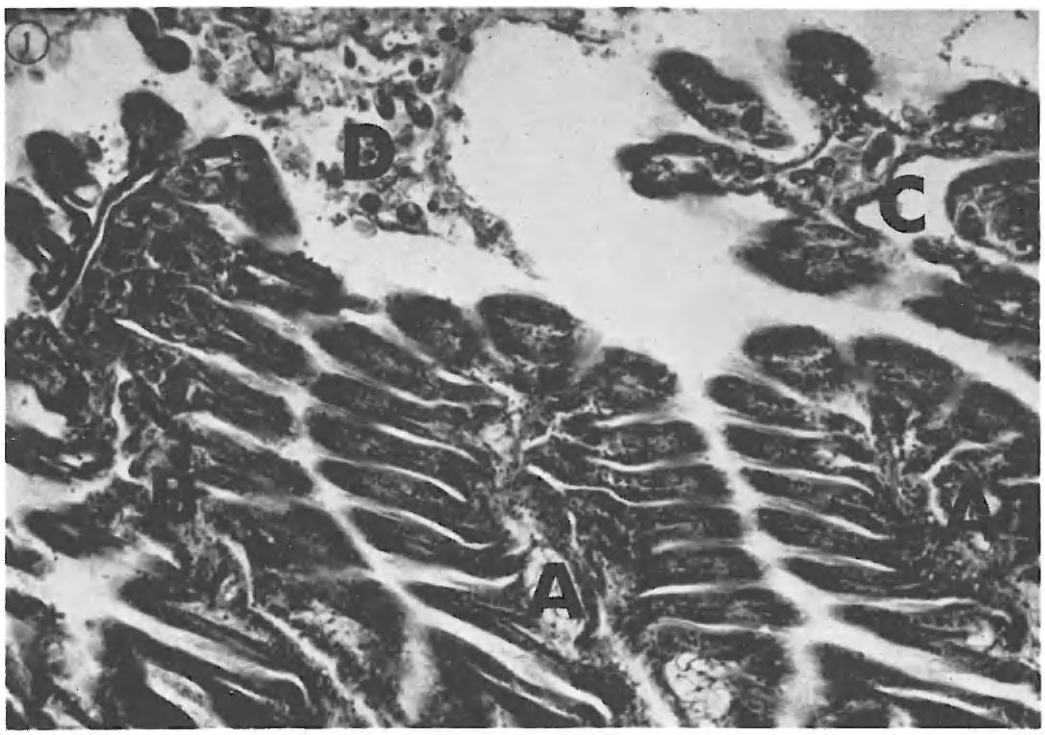

Fig. 1. Gills of oyster infected with ciliates. Note normal gills (a), normal appearing gill infected with ciliates (b), necrotic gill infected with ciliates (c), and liquified gill with ciliates in the debris (d). Mallory's Trichrome. 190 ×.

ciliate. With one exception, there was no apparent cellular defense reaction to the ciliates by the host, although the tissue around them was generally necrotic and in many cases only liquefied tissue debris remained. The one exception in *C. gigas* was a control oyster that possessed ciliates in one area of the gonadal tubules in which no sperm was present, while the unparasitized portions of this oyster's gonad contained sperm of normal appearance. In this control oyster, the invading organisms caused a general inflammatory reaction (Pauley, 1965; Pauley and Sparks, 1965) with a thick band of leucocytes surrounding the ciliates, possibly as an attempt to prevent further invasion of the host (Fig. 2). Burton (1961) observed unusual concentrations of leucocytes and abnormal tissue associated with *Ancistrocoma* in *C. virginica*.

When the parasites found in *C. gigas* enter the gill ostia and attach to the epithelium of the water tubes, they appear to occlude these chambers (Fig. 3). This occlusion undoubtedly interferes with normal respiration in the oyster and may cause anoxia of large areas of host tissue.

Some ciliates were attached to the normally tall, ciliated, columnar epithelium of the palps and in many instances were associated with a metaplastic change of this epithelium into a low cuboidal epithelium. It could not be determined with certainty whether or not there was any loss of cilia from this metaplastic epithelium.

The two groups of oysters injected with turpentine harbored by far the largest number of ciliates per individual and, as shown in Table 1, had the highest frequency of infection. Those oysters receiv-

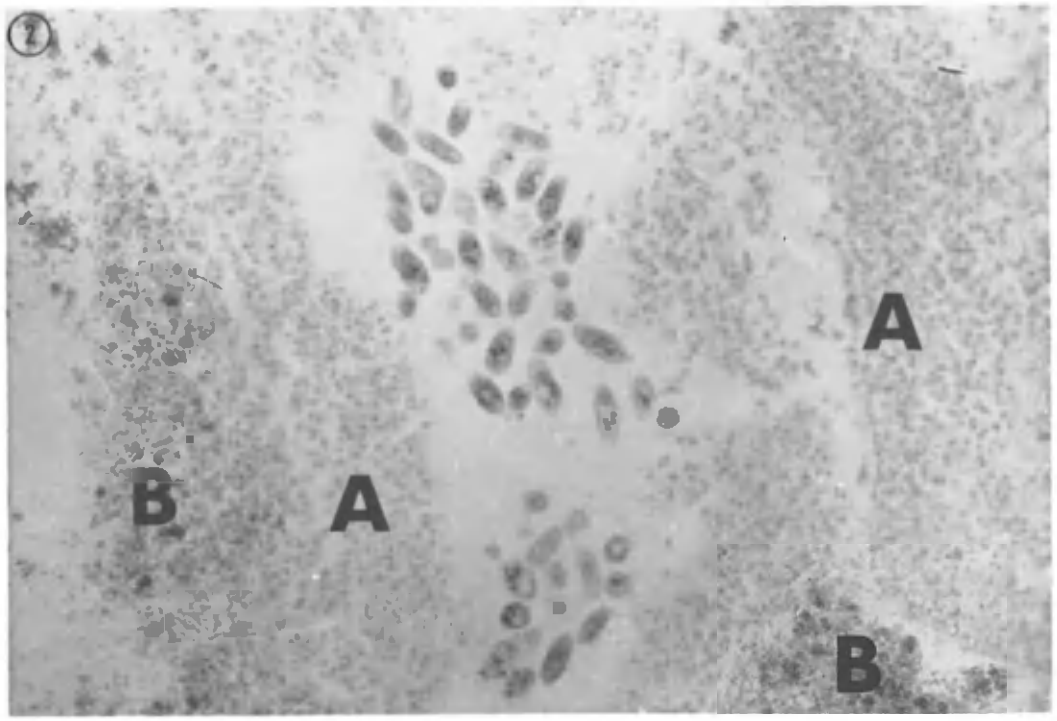

FIG. 2. Ciliates in the gonad of control oyster. Note heavy leucocytic infiltration into the gonadal tubule as an apparent host reaction to these invaders (a), and Necrotic sperm (b). Hematoxylin and eosin. 150 ×.

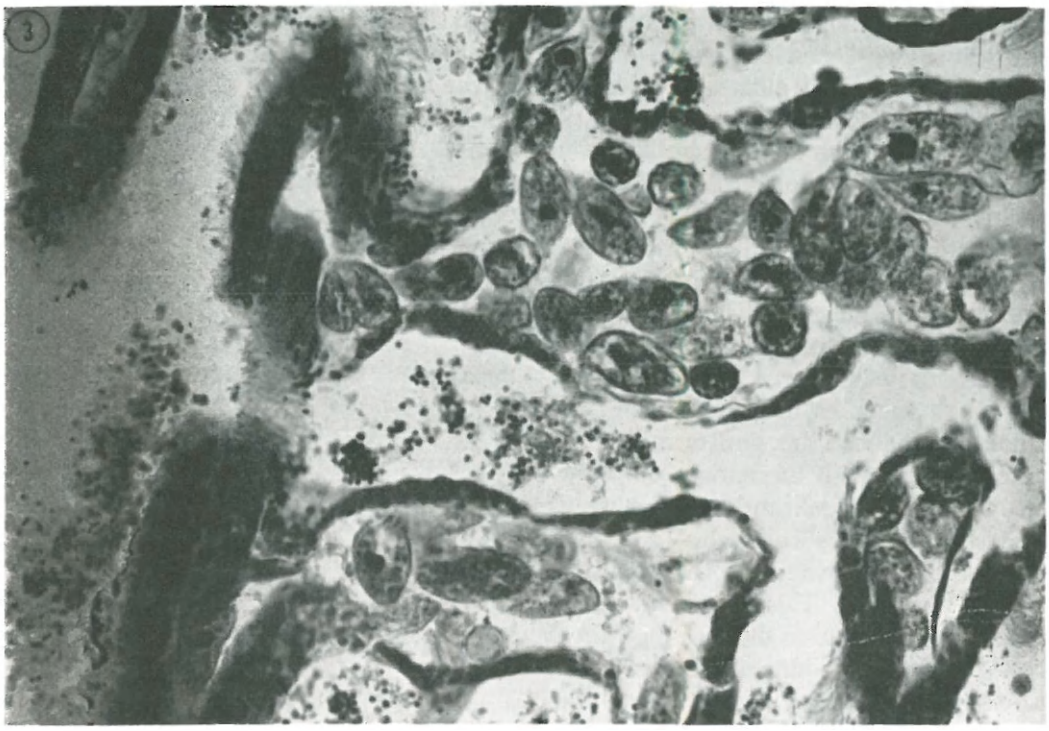

FIG. 3. Occlusion of the gill chambers of a Pacific oyster by ciliates. Small dark black spots are sperm. Hematoxylin and eosin. 360 ×.

ing turpentine directly into the adductor muscle were first found to be infected 112 hours postinjection and subsequently in almost all oysters sacrificed after 168 hours.

TABLE 1

CILIATE INFECTION RATES OF EXPERIMENTAL AND CONTROL OYSTERS

Treatment of group of oysters infected	Number of oysters in group	Percent infected with ciliates
Muscle with turpentine	100	42%
Connective tissue with turpentine	100	25%
Muscle with talc	100	0%
Connective tissue with talc	100	2%
Muscle with seawater (Control)	100	2%
Connective tissue with seawater (Control)	100	3%

The oysters receiving turpentine in the connective tissue did not contain any observable ciliates until 128 hours and they were present in about half of the oysters sacrificed after 168 hours. The adductor muscles of all turpentine-injected oysters harboring ciliates were swollen, edematous, heavily congested, and had hypertrophied muscle fibers. However, all oysters possessing swollen adductor muscles were not infected with ciliates.

DISCUSSION

Whether or not these ciliates are true parasites of the oyster or only secondary invaders is not known, but the latter is probably the case. The heavy infections in the two groups injected with turpentine appear to be related to the length of time the adductor muscle was swollen and im-

properly functioning. This indicates that oysters in a weakened condition are susceptible to invasion by ciliates and possibly other protozoans which normally present little or no problem to the shellfish, but which may cause pathological changes and possibly death of the host during a weakened condition. Mackin (1962) noted that ciliates are not commonly found infecting healthy oysters, but that they may become a complicating factor in diseased oysters as a secondary invader.

Although the sites of infection and the pathology produced are similar in the two groups injected with turpentine, the infection rates are significantly different, as demonstrated statistically by the chi-square test with a value of 4.32 with one degree of freedom. This significant difference is probably due to the fact that direct injection into the adductor muscle of an irritant, such as turpentine, initiates the improper functioning of this organ much more quickly than if the inflammatory substance is injected at a site distant from the muscle.

The oysters in the control and talc groups harboring ciliates did not have swollen adductor muscles as did the oysters injected with turpentine and the percentage of infection was quite small (0% to 3%) in these groups, which indicates that there is possibly a small natural infection of ciliates present in oyster populations (Table 1). The only pathology observed in these two groups of oysters was the metaplastic change of the epithelium of the palps from tall columnar to a low cuboidal condition. Although talc causes a wound response in oysters (Pauley, 1965), it does not appear capable of significantly weakening this mollusc as does the injection of turpentine.

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