JOURNAL OF INVERTEBRATE PATHOLOGY 25, 307–311 (1975)

Observations on Phagocytosis of *Coccomyxa parasitica* (Coccomyxaceae; Chlorococcales) in *Placopecten magellanicus*[1]

R. N. STEVENSON AND G. ROBIN SOUTH

Department of Biology,
Memorial University of Newfoundland,
St. John's, Newfoundland A1C 5S7,
Canada

Received April 15, 1974

Phagocytosis of *Coccomyxa parasitica* in *Placopecten magellanicus* is described. Host phagocytosis occurs throughout the infective process and can also be demonstrated experimentally using cultured algae. Agranular and granular leukocytes of various types are responsible, and the algae are highly resistant to digestion. The greatest concentration of algae occurs in the scallop's mantle region and appears to be related to the circulatory system. The indications are that *C. parasitica* might enter *P. magellanicus* via the normal feeding and digestion processes and that host phagocytosis contributes to the spread of the algal cells.

INTRODUCTION

A parasitic relationship between the alga *Coccomyxa parasitica* and the giant scallop *Placopecten magellanicus* was proposed by Naidu (1971) and discussed by Stevenson (1972) and Stevenson and South (1974). While it may remain questionable whether the relationship is truly parasitic, Naidu (1971) indicated and Stevenson and South (1974) stated that it is, at best, facultative.

Only Naidu (1971) has published a description of possible modes of infection and host response. While not ruling out other means, he favored a direct entry of the algae into the mantle epithelium of the scallop. This occurred, he suggested, when the animals were weakened by various means, but especially by shell damage. Studies by Stevenson (1972), however, demonstrated the importance of phagocytosis as a defense mechanism in *P. magellanicus* infected by *C. parasitica.* The studies, described here, further suggest that the phagocytosis may play a role in bringing about the infection itself.

[1]Requests for reprints should be directed to the second author.

MATERIALS AND METHODS

Methods for the maintenance of scallops and the isolation and culture of *C. parasitica* are described in Stevenson (1972) and Stevenson and South (1974). *Placopecten magellanicus* from Port au Port Bay and Salmonier, Newfoundland, were employed. Regular monitoring of blood, stomach contents, body tissues, and feces for the presence of leukocytes and *C. parasitica* was carried out on infected and uninfected scallops.

Experimental phagocytosis was conducted within the scallop blood and digestive tract, the methods adapted from techniques well-known from work with oysters (Feng, 1958; Stauber, 1950, 1961; Tripp, 1958, 1960, 1963, 1969; Tripp et al., 1966). Cultured cells of *C. parasitica* were injected into the hearts of uninfected scallops and after 24 hr, samples were studied for phagocytic activity. *Coccomyxa parasitica* in seawater were also mixed with freshly collected blood and observed after 24 hr for phagocytosis.

Using cultures of *C. parasitica,* artificial infection of *P. magellanicus* was attempted. Injection of algae into the stomach and other organs was carried out. Starved, uninfected

scallops, after maintenance in filtered seawater in the laboratory, were placed in vessels containing a heavy inoculum of *C. parasitica.* The effects of artificial infection experiments were monitored in the ways described for natural populations.

RESULTS

Various forms of leukocytes were observed in *P. magellanicus* (Fig. 1), and phagocytosis was particularly evident during all stages of *C. parasitica* infection.

Algal cells in the stomach are subjected to digestive phagocytosis. Phagocytic leukocytes of the form shown in Figure 1H were observed in the stomach contents and feces of heavily infected scallops. They usually carried one to four algal cells. Phagocytosed *C. parasitica* are also present in the scallop circulatory system and the body tissues. Haemolymph leukocytes were usually agranular and displayed the greatest degree of pseudopodial development (Fig. 1H, J). They usually carried only one to four algal cells, and contained a single oval nucleus, centrally located.

In infected scallops, the greatest concentration of phagocytosed algae occurs in the host mantle, the principal region of infection (Stevenson and South, 1974). Large round, oval, or irregular leokocytes are prevalent (Figs. 1 A–E, 2), occur in and around the *C. parasitica* colonies, and are instrumental in the ejection of algal cells through the host mantle epithelium. They are characterized by various degrees of granulation, a single large, oval nucleus laterally positioned, and in some instances well-defined vacuoles (Fig. 1B). The smaller examples contained few algal cells (Fig. 1C, D), whereas the larger ones contained up to 35–40 *C. parasitica* (Fig. 2D).

The algae are highly resistant to leukocyte digestive processes and occurred as healthy-looking, undivided cells in all types of situations. Only a few instances of cell damage or lysis were observed. In some examples, algal cell division progressed within the leukocytes.

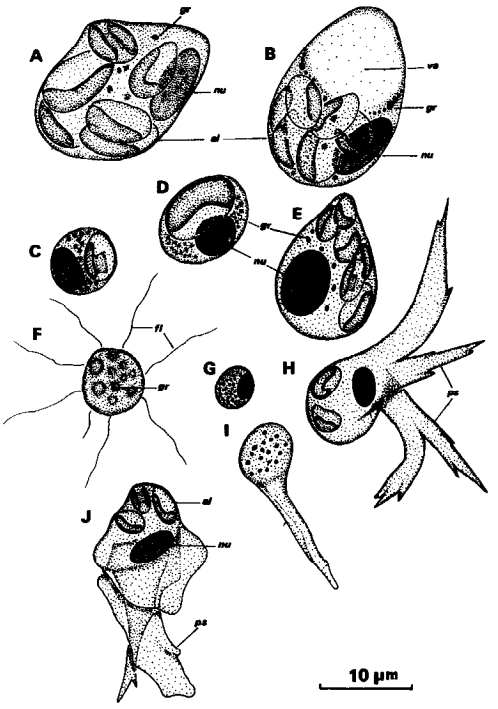
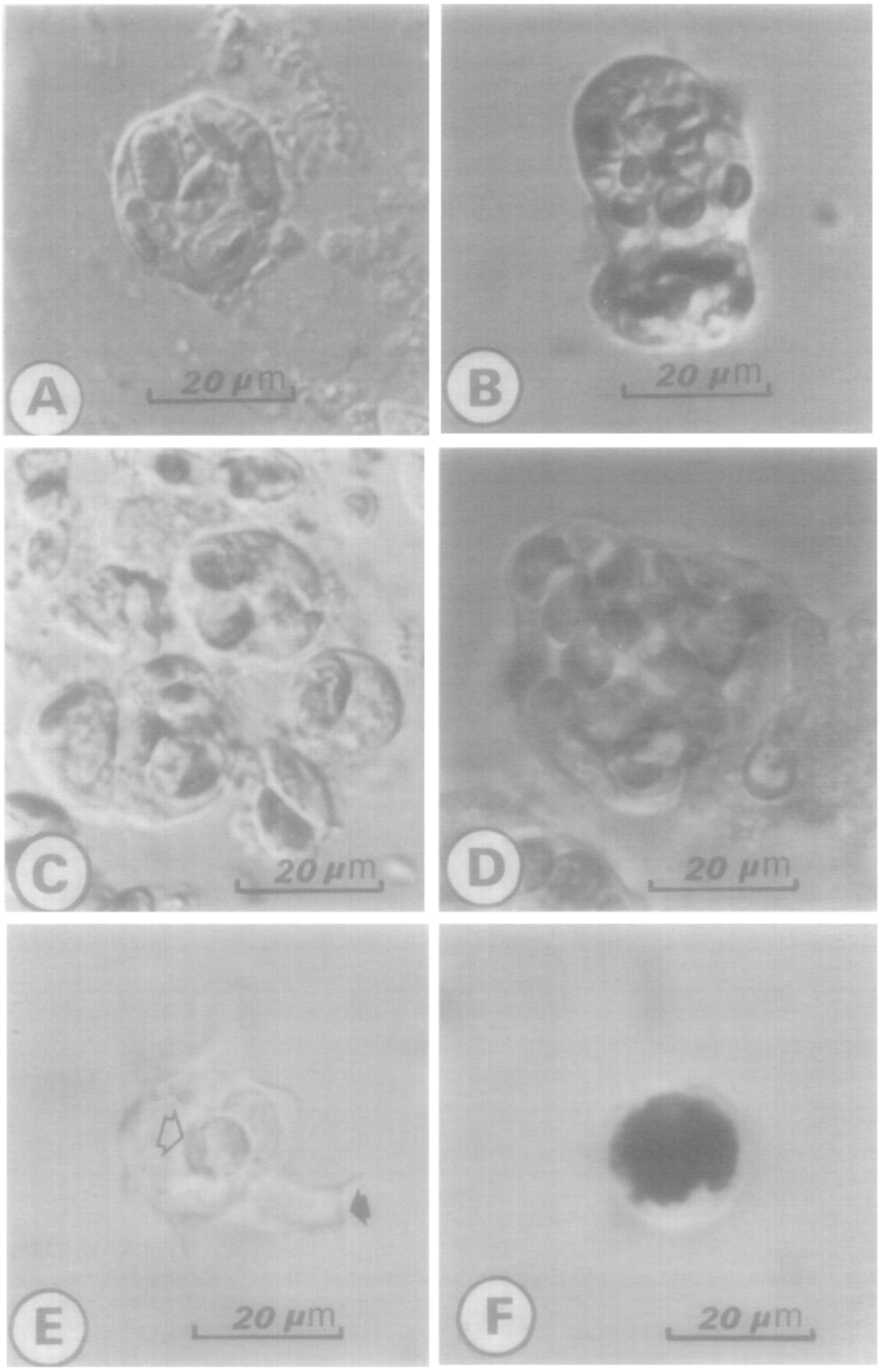


FIG. 1. Leukocytes of *Placopecten magellanicus.* Cells involved in phagocytosis of *Coccomyxa parasitica* are shown in A–E and H–J, while cells never observed containing *C. parasitica* are shown in F and G. Leukocytes varied in shape and size, depending on the number of engulfed algal cells. gr = granules; nu = nucleus; va = vacuole; fl = flagella; al = cell of *C. parasitica;* ps = pseudopodia

Cells of *C. parasitica* ejected from the mantle epithelium or with the feces were viable, and the latter could be cultured in the laboratory. In other experimental observations, phagocytic activity was demonstrated with leukocytes from the hemolymph and from host tissues (Fig. 2). Cultured *C. parasitica* and India ink injected into the

FIG. 2. Natural (A–D) and induced (E, F) phagocytosis in *Placopecten magellanicus.* A–D. Large leukocytes from the tissues of infected *P. magellanicus* containing variable numbers of cells of *Coccomyxa parasitica.* The distortion and plasticity of the leukocyte membrane is evident in D. E shows a leukocyte containing a cultured cell of *C. parasitica* (open arrow), introduced artificially into the scallop. Characteristic pseudopodium is present (solid arrow). F. Engulfed particle of India ink. For details consult text.


A
20 µm
B
20 µm
C
20 µm
D
20 µm
E
20 µm
F
20 µm

ventricles of uninfected scallops were engulfed by numerous leukocytes (Fig. 2E), which displayed characteristic pseudopodia. India ink was clearly resolved in the leukocytes which, in many instances, assumed the general shape of the ink particles (Fig. 2F).

Cultured *C. parasitica* injected into the adductor muscle and mantle tissues of uninfected scallops were engulfed by large, generally spherical leukocytes. When injected into the mantle region, the cultured algae became established as colonies. Ultimately encapsulation, a normal host reaction to infection occurred (Naidu, 1971) and the algae became morphologically modified (Stevenson and South, 1974). Active phagocytosis occurred throughout the artifically induced infection process. In many scallops in which the introduction had apparently not "taken," cultured *C. parasitica* was observed in the leukocytes.

Attempts to induce infection of uninfected scallops via the feeding and digestive mechanisms met with failure under laboratory conditions, although phagocytosis of ingested algae was observed in the stomach of experimental animals.

DISCUSSION

Several apparently different types of leukocytes seem to be involved in the phagocytosis of *C. parasitica* in *P. magellanicus*. Studies on the oyster *Crassostrea virginica* by Galtsoff (1964) revealed two general types of leukocytes: hyaline cells and granular cells. Both forms occur in *P. magellanicus*, as described here, and both types phagocytize *C. parasitica* in nature and under experimental conditions. As described by Cheng and Rifkin (1970), there is a wide form-range of granular leukocytes in *C. virginica*, and there seems to be no basis for the distinction of definite types within the series. A similar situation seems to occur in *P. magellanicus*, with a wide variation in shape and size of granular leukocytes.

Phagocytosis in *P. magellanicus* compares in part with the same phenomenon in other molluscs (Cheng and Rifkin, 1970). While some degradation of *C. parasitica* may occur, however, the inherent algal resistance to host digestion results in an emphasis on phagocytic transport and elimination of algal cells.

The distribution of *C. parasitica* colonies in infected *P. magellanicus* is nonrandom, and the importance of the shell-fold areas has been recognized (Naidu, 1971; Stevenson and South, 1974). The distribution is hypothetically related to the scallop's circulatory system. Understanding of the circulation of *P. magellanicus* is at present very incomplete; nevertheless, the proximity of the circumpallial blood vessels to the shell-fold tissue where *C. parasitica* incidence is highest is very suggestive of a relationship between infection sites and circulation.

Naidu's (1971) contention that the availability of light within the scallop is important in determining the distribution of the algae is not easily disregarded. In particular, a high concentration of algae in the vicinity of shell damage areas, and hence of higher light penetration, was noted by him. The shell-fold region is, however, a highly active area metabolically during shell deposition processes. The high correlation between shell deformity and the incidence of infection may not be because the scallop has become enfeebled. An acceleration of shell deposition resulting from shell damage would result in a marked increase in phagocytic activity. A greater availability of *C. parasitica*-carrying leukocytes could result, with a higher possibility of infection.

Our demonstration of the importance of phagocytosis in the scallop–alga relationship provides an alternative to Naidu's (1971) theory that infection occurs by direct penetration of the host epithelium. No substantiating evidence was provided to support the suggestion that *C. parasitica* bores its way into the scallop tissue. Tripp (1969) has pointed out that the viscid mucous layer covering exposed molluscan tissue acts as a physical, and perhaps chemical, barrier to

penetration: the mucous net traps potential parasites and the entangled material is ejected. In *P. magellanicus* it could be postulated that *C. parasitica* gains entry via the normal feeding and digestive processes, and that the spread and ultimate establishment of the algae are dependent on phagocytic activity. Supporting histopathological evidence is required if the infection mechanism is to be fully understood.

ACKNOWLEDGMENTS

We are grateful for the assistance of the technical staff of the Marine Sciences Research Laboratory and the Department of Biology, Memorial University of Newfoundland. Financial aid was provided by a Memorial University student bursary to the first author, and NRCC Grant A-4648 to the second author. We are especially thankful to Professor Marshall Laird and Dr. William Threlfall for their critical review of the manuscript.

REFERENCES

CHENG, T. C. AND RIFKIN, E. 1970. Cellular reactions in marine molluscs in response to helminth parasitism. *In* "Symposium on Disease of Fish and Shellfishes." Snieszko, S. F., (ed.), pp. 443–496. *Am. Fisher. Soc.*, Spec. Publ. No. 5, *Washington, D.C.*

FENG, S. Y. 1958. Observations on distribution and elimination of spores of *Nematopsis ostrearum* in oysters. *Proc. Natl. Shellfish. Ass.*, **48**, 162–173.

GALTSOFF, P. S. 1964. The American oyster *Crassostrea virginica* Gmelin. *Fish. Bull., Fish Wildlife Serv.*, **64**, 1–480.

NAIDU, K. S. 1971. Infection of the Giant Scallop *Placopecten magellanicus* from Newfoundland with an endozoic alga. *J. Invertebr. Pathol.*, **17**, 145–157.

STAUBER, L. A. 1950. The fate of India ink injected intracardially into the oyster, *Ostrea virginica* Gmelin. *Biol. Bull.*, **98**, 227–241.

STAUBER, L. A. 1961. Immunity of invertebrates, with special reference to the oyster. *Proc. Nat. Shellfish Ass.*, **50**, 7–20.

STEVENSON, R. N. 1972. "*In vivo* and *in vitro* Studies on an Endozoic Alga from the Giant Scallop *Placopecten magellanicus* (Gmelin)." M.Sc. Thesis, Memorial University of Newfoundland.

STEVENSON, R. N. AND SOUTH, G. R. 1974. *Coccomyxa parasitica* sp. nov., (Coccomyxaceae, Chlorococcales) a parasite of Giant Scallops *Placopecten magellanicus* (Gmelin) in Newfoundland. *Br. Phycol. J.*, **9**, 319–329.

TRIPP, M. R. 1958. Disposal by the oyster of intracardially injected red blood cells of vertebrates. *Proc. Nat. Shellfish. Ass.*, **48**, 143–147.

TRIPP, M. R. 1960. Mechanisms of removal of injected microorganisms from the American oyster *Crassostrea virginica* (Gmelin). *Biol. Bull.*, **119**, 273–282.

TRIPP, M. R. 1963. Cellular responses of mollusks. *Ann. N.Y. Acad. Sci.*, **113**, 467–474.

TRIPP, M. R. 1969. General mechanisms and principles of invertebrate immunity. *In* "Immunity to Parasitic Animals" (G. J. Jackson, R. Herman and I. Sinder, eds.), Vol. 1, pp. 111–128. Appleton-Century-Crofts, Meredith Corp., N.Y.

TRIPP, M. R., BISIGNANI, L. A., AND KENNY, M. T. 1966. Oyster amoebocytes in vitro. *J. Invertebr. Pathol.*, **8**, 137–140.