Attempt to Induce a Bactericidal Response in the Oyster

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Received for publication 12 September 1968

The oyster *Crassostrea virginica* can clear injections of antigen quite readily. We recently showed that this invertebrate is capable of clearing secondary injections of T2 coliphage more rapidly than primary (1, 2). Others have shown that oyster hemolymph contains natural hemagglutinin which enhance the phagocytosis of erythrocytes by oyster leukocytes in vitro (6). Tripp and Kent also reported that bacteria are rapidly phagocytized, and 90% are destroyed within 24 hr. Although antimicrobial substances have not been extensively investigated in the oyster, McDade and Tripp (4) have identified lysozyme in the hemolymph of the oyster which lyse intact *Bacillus megaterium* and *Micrococcus lysodeikticus*. Results such as these have led us to investigate the bactericidal response of the oyster to bacteria isolated from its gut flora. Since attempts to induce a humoral response in the oyster by injections of T2, STH, and sheep red blood cells were not successful, it was felt that, perhaps, antigens that are more closely associated with the animal's environment should be tried. If the oyster was to show any response at all it would most likely be to antigens with which it has had to deal during its evolutionary development, for example, normal gut flora. This approach led to the successful demonstration of an induced bactericidin in the spiny lobster *Panulirus argus* (3).

Various bacterial antigens were prepared with oysters taken directly from their natural habitat. The animals were removed from their shells and placed in a Virtis no. 45 homogenizer for 10 min at slow speed. Care was taken to prevent any outside contamination. The resulting homogenates were streaked onto various types of growth media, including Nutrient Agar (Difco), blood-agar, and E M B Agar (Difco). All plates were incubated for 16 hr at 37 C. Isolation procedures provided a number of pure colony types. From these, two of the most numerous were chosen and designated OY-1 and OY-2. Partial identification showed OY-1 to be gram-negative coccoid rods characteristically giving large gray colonies on Nutrient Agar. OY-2, a pleomorphic gram-negative rod, was seen as small yellow colonies on Nutrient Agar. Each of the organisms was grown up in quantity overnight in BHI (Difco) and again checked for purity. Vaccines were then prepared by killing the organisms in 0.3% formalinized saline, centrifuging, and resuspending in 0.3% formalinized saline to a concentration of 2.0 × 10^9 cells per ml.

Oysters measuring 7 cm or greater in length were collected and divided into two groups of 80 animals each. These animals were held in hardware cloth cages and kept in their natural environment (1). One group of oysters was given an intramuscular injection of 0.1 ml of OY-1; the other group received the OY-2 vaccine in a similar fashion. These oysters were bled at 0, 12, 48, 72, and 96 hr. At each interval, 12 animals were bled from the heart and sacrificed after bleeding. This hemolymph was assayed for bactericidal activity against both the OY-1 and OY-2 organisms by the method described by Schwab and Reeves (5).

Results of these assays showed no bactericidal activity to be present for either isolate OY-1 or OY-2 at any time from 0 to 96 hr, despite the fact that a similar approach in another invertebrate, the spiny lobster, resulted in the demonstration of an inducible bactericidin in lobster hemolymph. During primary immunization, the lobster bactericidin was detectable within 12 hr and reached its highest level between 24 and 48 hr (3).

Thus, a procedure which was capable of inducing a type of immune response in a crustacean was ineffective in a mollusk. Since the mollusk is considered to be more primitive than the crustacean, it is tempting to speculate that this observation may have evolutionary significance, but a final generalization will have to await additional experiments with other species from these two groups.

It appears from our data and those of others (reviewed by S. T. Feng, Federation Proc. p. 1685, 1967) that the oyster is incapable of a humoral response to foreign material. Thus, the defense mechanism of the oyster is characterized by phagocytosis and intracellular digestion. Whether the natural hemagglutinin or other yet unidentified hemolymph substances play a role in the oyster's defense system remains to be determined.
This investigation was supported by Public Health Service research grant AI-02693 and training grant AI-00293 from the National Institute of Allergy and Infectious Diseases.

LITERATURE CITED