Soft-Shell Clam, \textit{Mya arenaria}, a Convenient Laboratory Animal for Screening Pathogens of Bivalve Mollusks

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Received for publication 30 April 1971

Attempts to introduce infectious or foreign material into oysters and other bivalve mollusks usually involve force or trauma because of immediate, prolonged adduction of the tightly closing valves. The soft-shell clam, \textit{Mya arenaria}, is unable to seal its valves completely and relaxes readily, exposing soft tissue and a siphon. This species is free from fouling organisms and is readily available at all seasons in the New England and mid-Atlantic areas. Suspensions of five strains of \textit{Vibrio} sp. that cause bacular necrosis in larval and juvenile bivalve mollusks were injected into the heart, siphon tissue, and the incumbent and excurrent siphon lumina of soft-shell clams. All vibrio strains caused significant mortality, usually within 2 days. Heaviest losses resulted from heart and excurrent siphon injections. No mortality occurred in control clams injected with seawater, broth, \textit{Serratia} sp., and \textit{Escherichia coli}. The soft-shell clam appears to be a useful animal for testing the pathogenicity of marine microorganisms for bivalve mollusks.

The American or eastern oyster, \textit{Crassostrea virginica}, is the most widely studied and economically valuable bivalve mollusk of the Western Hemisphere (4). During an investigation of the microflora and fauna associated with extensive oyster mortalities in Delaware and Chesapeake Bays (2, 6), numerous bacterial strains were isolated from diseased oysters and enzootic environments (7), and a practical method for evaluating their pathogenicity was sought. Because of the oyster’s heavy, tightly closing valves secured by powerful, persistent adductor muscles, attempts to introduce infectious or other foreign material usually involve force and tedious manipulations resulting in trauma to the animal (1, 3, 8–10). Additionally, oyster shells provide habitats for diverse populations of commensal, competitive, and parasitic flora and fauna which may tend to obscure reactions to the agent or substance under test.

The soft-shell clam, \textit{Mya arenaria}, is unable to seal its valves completely, relaxes readily, and when relaxed it extends a large, fleshy siphon. The shells are comparatively smooth, chalky-white, and free from fouling organisms, and they can be readily labeled with ordinary lead pencil or common marking inks. This cosmopolitan inhabitant of sandy coves of the Northern Hemisphere is originally native to the coasts of the North Atlantic. However, as the result of fortuitous introduction into San Francisco Bay around 1880, its geographic range now extends from Alaska to Monterey, Calif. This species is also found along the Asiatic coast from Kamchatka to the southern reaches of the Japanese Islands (5). It is readily available during all seasons in the New England and mid-Atlantic coastal areas. It was, therefore, believed that this clam might serve as a useful laboratory animal for screening potential pathogens of bivalve mollusks.

MATERIALS AND METHODS

A group of marine vibrios have been demonstrated to be the etiological agents causing fatal epizootics of bacular necrosis among larval and juvenile bivalve mollusks (11, 12). These bacteria were, therefore, utilized as test pathogens, and other species were used as presumably nonpathogenic controls.

Bacterial strains. Five type cultures of the etiological agents of bacular necrosis, including three strains of \textit{Vibrio anguillarum} (ATCC 19105, 19106, and 19109), one of \textit{V. alginolyticus} (ATCC 19108), and one \textit{Vibrio} sp. (ATCC 19107) were grown on Trypticase-glucose-yeast extract (TGY) agar prepared with filtered Chesapeake Bay water and autoclaved at 15 psi for 10 min (11). The salinity of the water was 12%. Cultures were incubated for 24 hr at 26 C, washed from the agar surface, centrifuged, and resuspended in sterile Chesapeake Bay water to turbidimetrically adjusted concentrations of approximately 10^8 organisms per ml.

To determine whether pathology was caused by ac-
clams, as well as eastern oysters, hard clams (Mercenaria mercenaria), and blue mussels (Mytilus edulis), are refractory to 24 hr of exposure in large concentrations of these vibrios (11). Other inoculation sites chosen were the siphon tissue and the primitive heart lying directly below the shell hinges (Fig. 1).

Acclimatized clams were removed from the water, dried, and labeled with a marking pen. The points of 18-gauge needles were ground off and smoothed to form 33 mm long cannulas. Clams were challenged by injection with 1 ml of bacterial suspensions into the incumbent and excurrent siphons (Fig. 2). With a little practice, the cannulas could be readily inserted about 25 mm into the siphon lumina even though the animals retracted completely. Handling the clams and insertion of the cannulas induced voiding of excess water, and very little of the inocula was expelled. Separate groups of animals were injected in the heart and siphon tissue (Fig. 3 and 4) with 0.2-ml inocula by means of a 25-gauge needle (2 × 10⁶ cells). Intracardiac injection alone was used for challenge with filtrates and heat-inactivated cells. Fifty clams were injected with each organism by each route, except in the case of the inactivation studies and the controls, for which 10 rather than 50 animals were used for each test point. In total, 1,300 clams were utilized: 1,000 for exposure to the

Fig. 1. Stylized anatomical drawing of the soft-shell clam, Mya arenaria. The siphons are encased in a muscular, retractable organ which is shown extended. After Hanks (5).

Fig. 2. Clam is exposed by injection of 1 ml of bacterial suspension into the excurrent siphon, deposited about 25 mm into the lumen. Cannula is blunt 25-gauge hypodermic needle. Arrow points to orifice of the incurrent siphon.

Fig. 3. Intercardiac injection of 0.2 ml is made with 25-gauge needle. Stroking tissue on the left (anterior) side of the hinge causes the animal to retract that side and expose soft tissue nearest the heart.

Fig. 4. Siphon tissue is injected with 0.2 ml by use of a 25-gauge needle. Caution was exercised not to puncture the lumina of the siphons.
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and probably effective manner for introducing or “force-feeding” experimental substances into Mya. However, for economy of inoculum, technical simplicity, and optimal response to bacterial pathogens, routine challenge via the cardiac or siphon tissue routes, or even concurrently by both, is recommended.

An open, flowing seawater system is essential for holding clams where mortalities are anticipated, since dead animals decompose quickly, producing massive fouling in closed systems, with ancillary pathology unrelated to the primary challenge. Recirculated systems or closed aquaria should only be used for small populations under constant observation.

The fact that not one of the control animals succumbed testifies to the innate resistance of adult mollusks to bacterial infection and trauma. By contrast, it was found in a current study that blue crabs (Callinectes sapidus) experienced high mortalities after parenteral challenge with smaller doses of S. marcescens and A. liquefaciens. It had been noted previously that soft-shell clams as well as eastern oysters, hard clams (Mercenaria mercenaria), and blue mussels (Mytilus edulis) were refractory to 24 hr of immersion in heavy concentrations of all five vibrios (11).

Although the idiosyncrasies of individual tolerances in Mya do not permit the expression of tidy quantitative ID$_{50}$ or LD$_{50}$ values, the method described does offer a practical qualitative procedure for estimating microbial pathogenicity in a phy-

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