JOURNAL OF BACTERIOLOGY, Oct., 1965
Vol. 90, No. 4

Printed in U.S.A.

Bacillary Necrosis, a Disease of Larval and Juvenile Bivalve Mollusks

I. Etiology and Epizootiology

HASKELL S. TUBIASH,[1] PAUL E. CHANLEY,[2] AND EINAR LEIFSON

Biological Laboratory, U.S. Bureau of Commercial Fisheries, Milford, Connecticut, and Stritch School of Medicine and Graduate School, Loyola University, Chicago, Illinois

Received for publication 26 April 1965

ABSTRACT

TUBIASH, HASKELL S. (U.S. Bureau of Commercial Fisheries, Milford, Conn.), PAUL E. CHANLEY, AND EINAR LEIFSON. Bacillary necrosis, a disease of larval and juvenile bivalve mollusks. I. Etiology and epizootiology. J. Bacteriol. **90**:1036–1044. 1965.—Lethal bacterial infections of a variety of hatchery-spawned bivalve mollusk larvae and juveniles have been studied. The symptoms of the disease and the course of the infection are described. Four biotypes and five antigenic types of bacteria, pathogenic for the larvae of five species of bivalve mollusks, were isolated and described in some detail. All are gram-negative motile rods. Comparative studies were made of a fairly large number of similar bacteria isolated from presumably normal marine fauna. None of these was pathogenic for the bivalve larvae nor did they have antigens in common with the pathogenic group. The four biotypes had a number of characteristics in common that rarely were present in other cultures from marine fauna. Several antibiotic preparations proved to be of value in the treatment and control of the infection.

Marine pelecypods commonly reproduce by releasing gametes into the water, where external fertilization occurs. Usually, shelled larvae develop within 24 hr (Fig. 1). The larvae are planktonic and swim by the ciliary action of the extended velum. The larvae of the hard clam, *Mercenaria* (*Venus*) *mercenaria*, remain in the free-swimming form for approximately 20 days. After their planktonic existence, they metamorphose into relatively sedentary juvenile forms more closely resembling adults. At metamorphosis, most of the more common marine bivalves average 200 to 400 μ in length (Jørgensen, 1946).

Advances in culture techniques have established the feasibility of culturing desirable species of bivalves commercially (Loosanoff and Davis, 1963; Davis and Ukeles, 1961). However, research groups and pilot-plant shellfish hatcheries are hindered by a disturbingly high incidence of fatal epizootics among larval and juvenile stocks. Sporadic fungal infections believed to be caused by members of the genus *Sirolpidium* were reported in early work on methodology of larval culture of Lamellibranchia (Davis et al., 1954). Guillard (1959) isolated a number of bacterial strains from a single moribund hard clam larva. Broth cultures of two of these strains, identified as *Pseudomonas* sp. and *Vibrio* sp., proved pathogenic to *M. mercenaria* larvae in small axenic test-tube cultures. Recurring losses of larval and juvenile bivalve mollusks in the Milford Laboratory prompted a search for causal microbial agents. The outbreaks were characterized by sudden onset and heavy mortality with characteristic "swarming" of bacteria around the dead and moribund larvae. Many ciliated protozoa were also present.

[1] Present address: Biological Laboratory, U.S. Bureau of Commercial Fisheries, Oxford, Md.

[2] Present address: Eastern Shore Laboratory, Virginia Institute of Marine Science, Wachapreague, Va.

MATERIALS AND METHODS

Isolation of pathogens. Moribund larvae of the hard clam, *M. mercenaria*, surrounded by swarming bacteria were collected individually by capillary pipette and washed by threefold centrifugation in sterile seawater. The final washing was decanted, and 1 ml of seawater-Trypticase-glucose-yeast (TGY) broth was added. (TGY broth was composed of 0.4% Trypticase, 0.1% glucose, and 0.1% yeast extract. The medium was adjusted to a final *p*H of 7.4 and autoclaved at 15 psi for 10 min. For solid medium, 1.25% agar was added.) After incubation at 28 C for 4

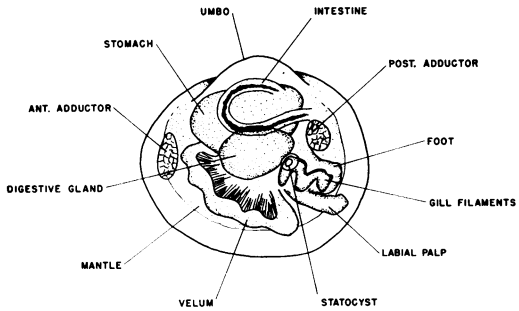

FIG. 1. *Anatomy of a typical bivalve mollusk larva. The velum extends beyond the shell when the animal is swimming. After Jørgensen (1946).*

hr, streak and pour plates were made with TGY agar. The plates were incubated at 28 C for 48 to 72 hr, and colonies were subcultured to agar slants for pathogenicity screening. Tissue was also teased from the gaping shells of moribund juvenile oysters, *Crassostrea virginica*, and juvenile hard clams, *M. mercenaria*, and was cultured in TGY broth as with the larvae. A less tedious technique, adopted subsequently, called for washing dozens to hundreds of affected juveniles or larvae in sterile, round-bottomed, screw-capped centrifuge tubes by fivefold suspension and decantation in sterile seawater. The specimens were ground to a slurry by shaking with sterile glass beads. This homogenate was then plated out in serial dilutions for isolation of predominant bacteria. On occasion, portions of the homogenate were also seeded into healthy larval cultures for rapid determinations of pathogenicity. Isolates which proved pathogenic were preserved by lyophilization in equal volumes of sterile skim milk and seawater.

Pathogenicity screening and range of host susceptibility. A standard test for pathogenicity was performed by inoculating 24-hr suspensions of the test organism into 400-ml cultures of 2- to 7-day-old larvae. Clean, but not sterile, 1-pint (0.47-liter) polyethylene frozen-food containers were employed as convenient culture vessels. Each contained approximately 10,000 larvae in seawater, which had been filtered to remove silt and detritus and ultraviolet-treated to kill protozoa and other plankton. Candidate pathogens were spread on TGY agar slants, incubated for 24 hr at 28 C, washed off with 10 ml of sterile seawater, washed by centrifugation to remove traces of TGY and bacterial metabolites, and diluted to approximately 5×10^8 organisms per milliliter. Larval cultures were seeded with 1- and 2-ml inocula of these bacterial suspensions, incubated at 28 C, and examined for mortality of the larvae after 24 and 28 hr. Suitable pathogenic and saprophytic controls were included, as well as 2-ml seedings of identical bacterial suspensions which had been heated for 30 min at 65 C.

Host susceptibility ranges were determined in a similar manner, except that in addition to *M. mercenaria* and *C. virginica*, larvae of the European oyster (*Ostrea edulis*), bay scallop (*Aequipecten irradians*), and shipworm (*Teredo navalis*) served as hosts for challenge by strain M 17, a pathogen of high virulence. Larvae of *T. navalis* were obtained as the result of a fortuitous single spawning, and incubation was at room temperature (about 20 C) rather than 28 C. Mortality curves were constructed by setting up multiple larval cultures, seeding all simultaneously, and counting viable larvae hourly, one culture per hour, for up to 24 hr. In this manner, an entire larval culture could be sampled and discarded without disturbing the remaining cultures. Differential counts of morbidity and mortality were made by filtering the test larval cultures through a small-mesh (60-μ) stainless-steel wire screen and distributing a representative portion of the larvae thus removed on a Sedgewick-Rafter counting cell. With practice, the dead and moribund larvae, as well as those showing early signs of infection, could be readily differentiated from the normal animals under 100 times magnification. Differential counts were made on 100 evenly distributed larvae. Moribund and dead larvae were counted as "dead"; the rest of the survivors were counted as "alive." Moribund larvae always died within a few hours.

Twenty-seven marine bacterial cultures with morphological and biochemical properties similar to those of the Milford pathogens were screened for pathogenicity and antigenicity. These organisms had been isolated from a variety of normal marine fauna in Long Island Sound and had been studied at Loyola University, Chicago, Ill.

Serological typing. Antigens consisted of live organisms grown for 24 hr on TGY slants, washed, suspended in seawater, and adjusted to a density of approximately 1.5×10^9 organisms per milliliter. Rabbits were immunized by four subcutaneous doses, varying from 0.5 to 2 ml, administered weekly. At 1 week after the final injection, they were bled by cardiac puncture. The sera were separated, inactivated, and tube-tested for agglutinating titer against homologous and heterologous strains of the pathogens. A 0.2-ml volume of the serially diluted serum was mixed with a 0.1-ml suspension of a 24-hr culture of the bacteria, incubated for 4 hr at 15 C, and examined for agglutination. All strains exhibiting pathogenicity for larval bivalves, and the 27 Loyola strains, were tested in this manner.

Identification of pathogens. A typical strain of each of the five antigenic types of the larval pathogens was studied in some detail. Tests for gelatin liquefaction, nitrate reduction, catalase determination, carbohydrate metabolism, and indole formation were performed as described by Leifson et al. (1964). Artificial seawater (one-half strength, *p*H 7.5 to 8.0) was used for preparing the liquid base medium. Starch-agar was prepared by adding 0.2% soluble starch and 1.5% agar to the base medium. About 1 ml of a 1:10

dilution of Gram's iodine was added to a 2- to 3-day-old slant culture to test for starch utilization. Flagella stains were made according to the method of Leifson (1960), from both agar and slant cultures.

Application and screening of antibacterials. Three proprietary antibacterial formulations were tested, along with chloramphenicol, for therapeutic use against experimental infection with strain M 17: an aqueous preparation containing 250 mg of streptomycin and streptomycin sulfate per ml (Combistrep; Chas. Pfizer & Co., Inc., Brooklyn, N.Y.), sodium sulfamethazine soluble powder (Sulmet; American Cyanamid Co., Wayne, N.J.), and a complex of polyvinylpyrrolidone with 10% free iodine (PVP-iodine).

These formulations had previously been employed empirically to abate shellfish hatchery epizootics. They were evaluated by adding graded levels of the drug, usually 5 to 200 ppm, to 400-ml cultures of clam larvae which had been seeded 4 hr previously with the virulent pathogen. Larval cultures were incubated with the drug for 48 hr at 28 C, and then for 24 hr in new, drug-free water. After the total 72-hr incubation period, counts were made to determine percentage mortality. Controls for drug toxicity were included in all cases.

As the number of pathogenic isolates increased, they were screened for sensitivity to antibiotics by the use of sensitivity discs (Colab Laboratories, Inc., Chicago Heights, Ill.) on TGY agar plates spread with 0.2 ml of 24-hr broth cultures of the test organisms. Antibiotics tested in this manner included: chloramphenicol, kanamycin, neomycin, penicillin, polymyxin B, dihydrostreptomycin, and tetracycline.

RESULTS

Pathology and epizootiology. Two strains of gram-negative bacilli from the isolated moribund clam larvae and three obtained by centrifugation of a group of dead and moribund larvae proved to be highly virulent for larval bivalves. These initial bacterial strains proved to be the etiological agents of a disease we have termed bacillary necrosis. They were typical of many subsequent pathogenic isolates.

The course of this disease in experimentally exposed larvae is swift and dramatic (Fig. 2a). Within 4 to 5 hr after seeding with a virulent pathogen, prodromal signs are a reduction of motility and a tendency for many larvae to lie quiescent with either rudimentary foot or velum extended (Fig. 4b). "Swarms" of bacteria originating from discrete foci on the margins of scattered larvae appear simultaneously. These swarms are a pathognomonic sign of bacillary necrosis, although at this point larvae may seem otherwise normal. The swarms become progressively more dense and ubiquitous, in a manner

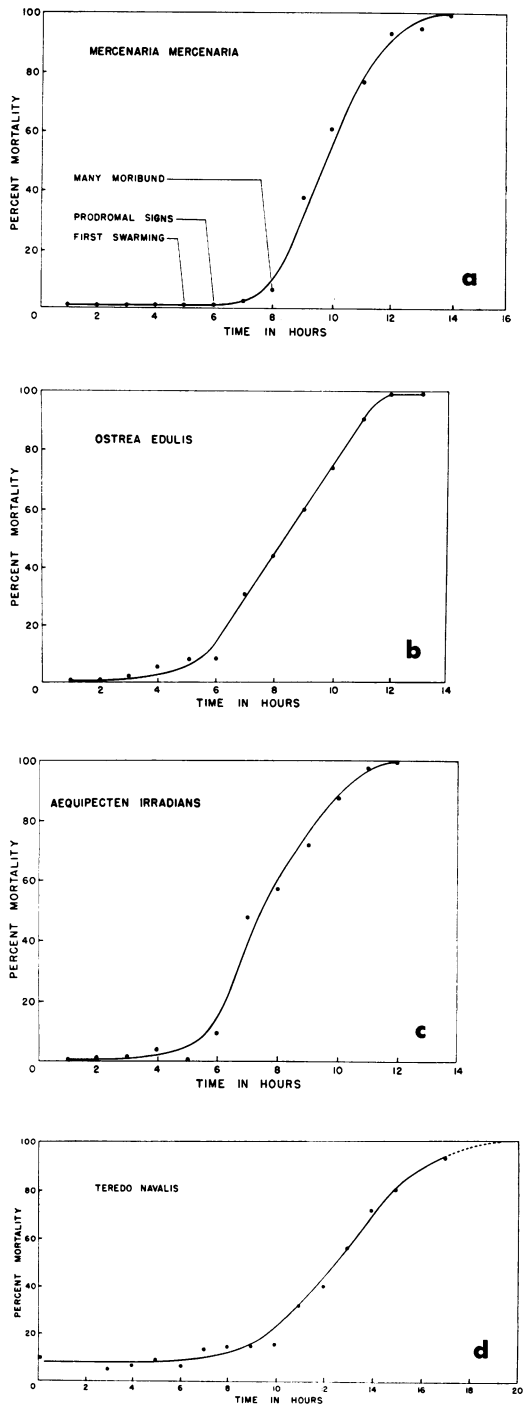

FIG. 2. *Mortality of larvae after challenge with pathogen M 17. (a) Mercenaria mercenaria. (b) Ostrea edulis. (c) Aequipecten irradians. (d) Teredo navalis.*

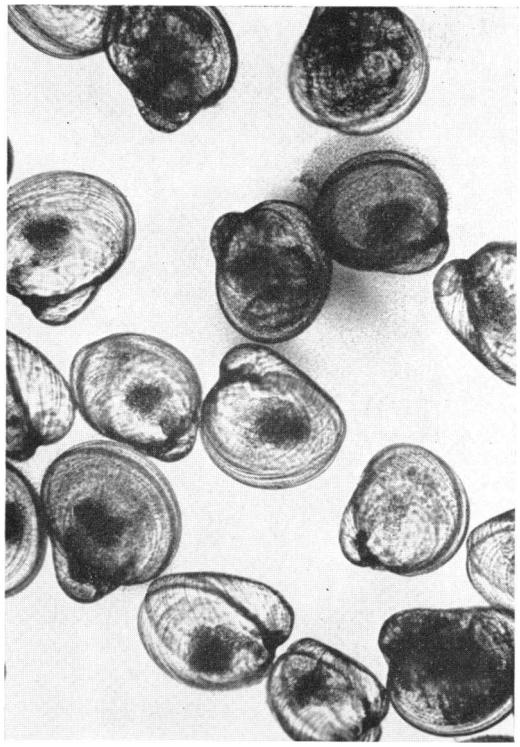

FIG. 3. *Swarms of motile bacteria emanating from heavily parasitized presetting larvae of Crassostrea virginica challenged with pathogen M 17. Swarming proceeds from discrete foci. Soft tissues of most larvae have been or are being necrotized. Viable larvae at top and right corner.* × *140.*

resembling the swarming of bees (Fig. 3). By 8 hr after challenge, death with granular necrosis is widespread. Under low power (100 times), the larval tissues often appear to scintillate from the activity of invading bacteria. In a heavily infected culture, mortality is often complete within 18 hr. Loosely attached or detached portions of the velum frequently continue their ciliary action after all other soft tissues have been destroyed.

The course of an infection induced in straight-hinge larvae of the European oyster, *O. edulis*, by exposure to pathogen M 17 is illustrated in Fig. 4. European oysters are larviparous; fertile ova develop in a brood chamber of the adult for 1 week to 10 days before their release as pelagic larvae. The larvae illustrated were challenged 24 hr after release and experienced an overwhelming, fulminating infection. Ciliated protozoa appeared as scavengers after the height of the bacteria-induced epizootic, and apparently played no part in the primary infection (Fig. 4c). Larvae challenged by pathogenic bacteria that had previously been heated to 65 C for 30 min suffered no mortality and appeared normal in every respect (Fig. 4d).

Histological sections of the larvae, sampled hourly for 24 hr after challenge with pathogen M 17, confirmed a massive bacterial invasion and proliferation, with extensive cellular destruction. These findings support the designation of this disease as bacillary necrosis. A detailed study of the histopathology is in preparation.

Host range. Strain M 17, an early virulent isolate from Milford clam larvae, was pathogenic for all of the lamellibranch larvae challenged (Fig. 2). Subsequently, groups of pathogenic isolates were recovered from moribund juvenile oysters and clams at Milford, as well as from samples of affected juvenile clams from Virginia. The pathogenic strains obtained from oysters were neither as virulent nor as active as those from clams, but each was interchangeably pathogenic for larvae of homologous or heterologous species of all of the lamellibranchs challenged. None of the 27 morphologically and biochemically similar isolates from fauna of Long Island Sound caused appreciable mortality in challenge against larvae of *M. mercenaria*.

Serology. All rabbit antisera agglutinated homologous antigens in dilutions from 1:128 to 1:512 and were tested for cross-agglutination against heterologous antigens. In this manner, five serological strains of pathogens were typed and labeled after their geographical origins: Milford A, J, and O, and Virginia C and D (Table 1). Type A includes four Milford and two Virginia isolates and types C and D each include two Virginia isolates. The three strains comprising type O originated from outbreaks in *C. virginica* larvae. Type J is the latest established, and consists entirely of pathogens isolated from diseased juvenile clams, *M. mercenaria*, at the Milford Laboratory hatchery.

None of the 27 Loyola strains, originating from normal fauna of Long Island Sound, was agglutinated to a significant titer by the antisera for the Milford and Virginia strains (Table 2), nor were they pathogenic for any of the bivalve larvae tested.

Characteristics. The characteristics of the larval pathogens are listed in Table 3.

Morphology. All five antigenic types of larval pathogens, Milford 17 (type A), Milford 27 (type O), Milford 74 (type J), Virginia 65 (type D), and Virginia 67 (type C), were composed of gram-negative small rods, measuring about 0.5 by 1.5 μ. Some individuals of strains M 17 and V 65 showed slight somatic curvature. Members of the other strains were quite straight. All strains

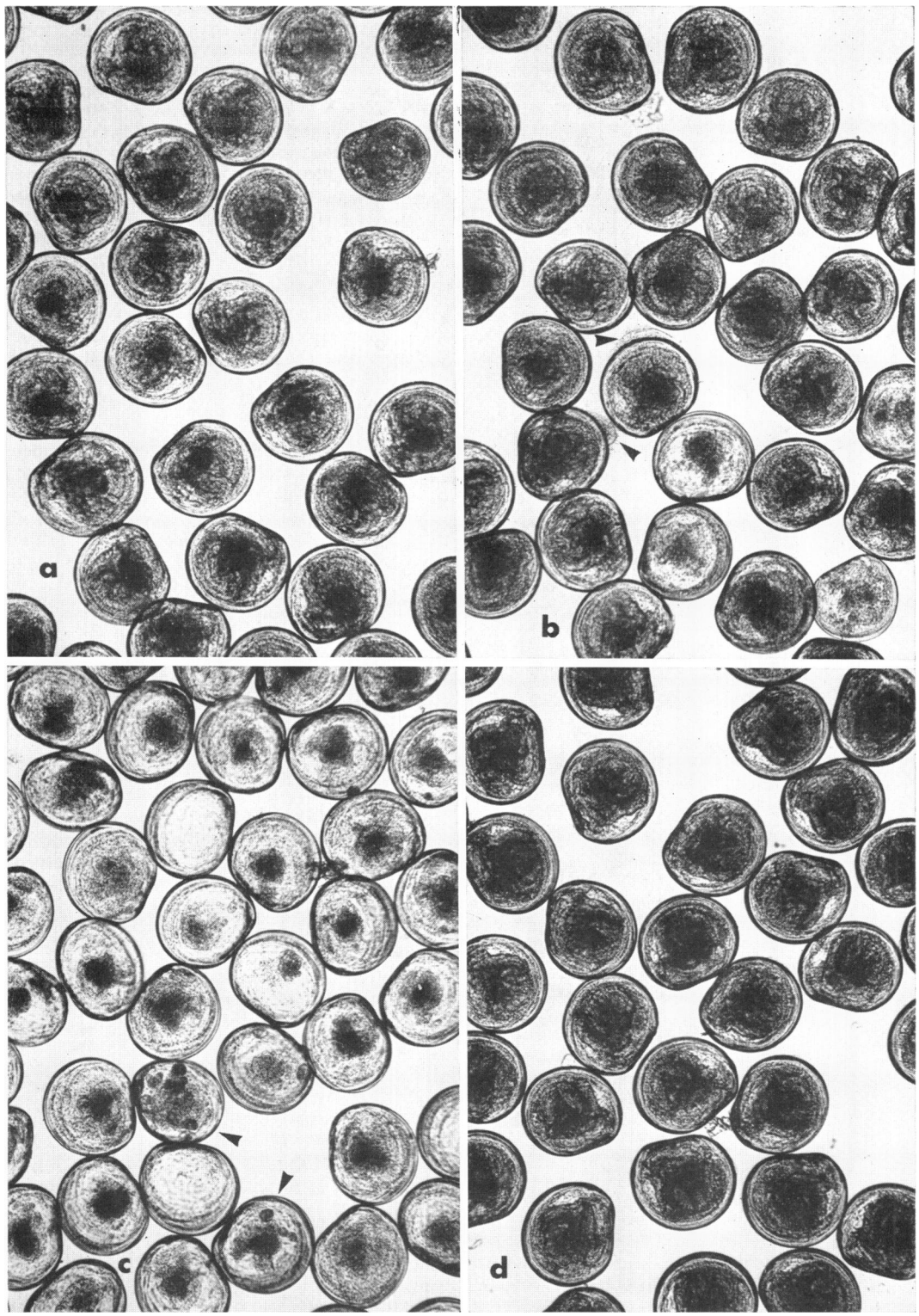

FIG. 4

TABLE 1. *Serological typing of bivalve pathogens*

Type	Strain	Isolate
Milford	A	17
		20A
		20C
		63VA
		64VA
		70
	J	71
		72
		73
		74
		76
	O	27
		30
		32
Virginia	C	61VA
		67VA
	D	65VA
		66VA

TABLE 2. *Agglutination titers of Loyola strains of marine bacteria*

Antigen	Antiserum				
	A	J	O	C	D
Loyola strains					
23 strains	—	—	—	—	—
LA 7	8*	—	4	—	—
LO 20	4	—	—	—	—
LO 23	—	8	—	—	—
LC 26	—	—	8	—	—
Controls					
A 17	128	—	—	—	—
J 71	—	128	—	—	—
O 27	—	—	256	—	—
C 61	—	—	—	256	—
D 65	—	—	—	—	128

* Agglutination titer equals reciprocal of serum dilution causing agglutination. Loyola series showed no significant agglutination with Milford antisera.

showed polar monotrichous flagellation when stained from liquid cultures (Fig. 5a and b).

The average wavelength of the polar flagella was 1.8 to 2.0 μ for all five cultures. When stained from either broth or agar slant cultures, strains M 27 and M 74 were polar monotrichous flagellate. Strain M 17 showed a few individuals with one or more lateral flagella of relatively short wavelength (about 1.2 μ). In addition to the polar flagellum of normal wavelength, strains V 65 and V 67 showed numerous loose flagella of short wavelength. This phenomenon was described by Leifson (1964) and is characteristic of polar flagellate marine bacteria which "swarm" on agar media.

Cultural characteristics. Growth was uniformly turbid, colorless, and dense in the liquid base medium. On agar, growth was smooth and colorless; strains M 17 and V 65 were very mucoid, and V 67 swarmed on solid media. All strains were halophilic and grew luxuriantly in simple peptone-yeast extract medium with the addition of 3% sodium chloride. All strains were more or less psychrophilic, and at 37 C either failed to grow or grew very slowly as compared with growth at 20 and 30 C. All grew well in media of *p*H 7 to 9. A characteristic of all five strains was their brief longevity when cultures were stored at 4 C. Most strains of fermentative marine bacteria in the Leifson collection could be maintained in a refrigerator by transfer every 2 months, whereas the larval pathogens invariably were dead after this period of storage. The same situation was found with cultures of *Pseudomonas piscicida*, a fish pathogen, which also died after a few weeks of storage. However, larval pathogens proved to be viable after more than 2 years of lyophilized storage.

Physiological characteristics. Strains M 27 and M 74 were morphologically and physiologically indistinguishable. The remaining strains had many characteristics in common but also exhibited differences (Table 3).

Perhaps the most definitive characteristics of the pathogenic group are: polar monotrichous flagellation in liquid media, fermentative anaerogenic metabolism of carbohydrates, gelatin liquefaction, starch hydrolysis, and a negative or weak catalase reaction. Few cultures of bacteria isolated from presumably normal Long Island Sound animals had all of the characteristics common to the larval pathogens.

FIG. 4. *Larvae of European oyster challenged with M 17. × 140. (a) At 1 hr after infection. Larvae appear normal. (b) At 7 hr after infection. Several heavily infected larvae show necrosis of internal tissues; arrows indicate two exhibiting characteristic extension of the velum after infection. Larvae have been fixed in formalin and do not show swarming bacteria which were seen in the living preparation. (c) At 13 hr after infection. Almost all have been infected and necrotized by the pathogen. Arrows indicate secondary invasion by ciliated protozoa. (d) At 24 hr after seeding with a suspension of pathogen which had been heated at 65 C for 30 min. Larvae appear healthy and identical to normal controls.*

TABLE 3. *Characteristics of larval bivalve pathogens**

Characteristic	M27	M74	M17	V65	V67
NaCl-free base broth	−	−	−	−	−
Base broth plus 3% NaCl	+++	+++	+++	+++	+++
Growth at 37 C	−	$+_w$	$+_w$	+	−
Gelatin liquefaction	++	++	++	++	++
Dextrose fermentation	+++	+++	+++	+++	+++
Sucrose fermentation	+++	+++	+++	+++	+++
Maltose fermentation	+++	+++	+++	+++	+++
Starch hydrolysis	+++	+++	+++	+++	+++
Lactose fermentation	−	−	−	−	−
Xylose fermentation	−	−	−	−	−
Catalase reaction	+/−	+/−	+/−	+/−	+/−
Nitrate to nitrite	+++	+++	+++	+++	−
Indole formation	+++	+++	−	+++	−
Mannitol fermentation	+++	+++	+++	−	−
Phenylalanine deaminase	+++	+++	−	+++	−
Swarming on agar	−	−	−	+	+++
Lateral flagella on agar	−	−	+	+++	+++
Wavelength of polar flagella	1.9	1.9	2.0	1.7	1.9
Somatic curvature	−	−	+	+	−

* Symbols: − = lack of growth, acid production, or any negative reaction; + = degree of growth, acid production, or any positive reaction; $+_w$ = slight or weak growth.

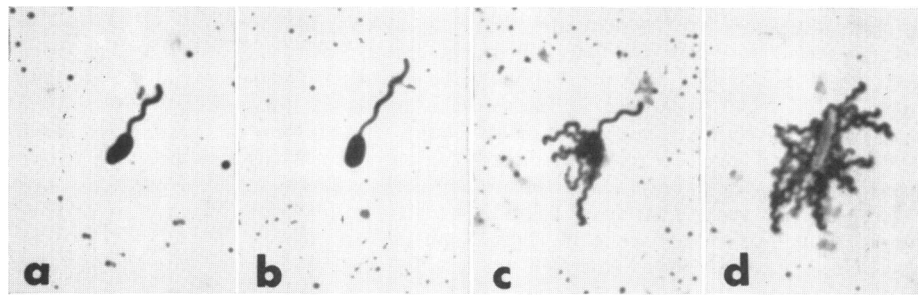

FIG. 5. *Flagellation of pathogenic strains. Leifson stain. (a) Strain V 65 showing polar monotrichous flagellation and slight somatic curvature. Stained from liquid culture. Strain M 17 had the same morphology. (b) Strain V 67 stained from liquid medium, showing polar monotrichous flagellation and straight soma. Strains M 27, M 74, and V 67 showed the same morphology when stained from liquid media. (c) Strain V 67 stained from agar slant culture. The polar flagellum is present along with the lateral flagella of shorter wavelength. (d) Strain V 67 stained from agar slant culture. Only lateral flagella are seen. Strain V 65 showed this same morphology when stained from slant culture. Strain M 17 also showed an occasional lateral flagellum.*

Control of infection with antibacterials. In vitro assays with antibiotic sensitivity discs against type cultures of the pathogenic serotypes revealed that all were in some degree sensitive to four antibiotics: chloramphenicol, polymyxin B, erythromycin, and neomycin (Table 4).

Combistrep added to the seawater in larval cultures proved very effective as a therapeutic agent against challenge with organism M 17 (Table 5). We found that 50 to 100 ppm of the antibiotic (200 to 400 ppm of Combistrep) were useful, experimentally, for therapeutic application. This veterinary preparation, developed for treatment of poultry, also enhanced larval growth in concentrations up to 2,000 ppm (Hidu and Tubiash, Proc. Natl. Shellfisheries Assoc., *in press*). Chloramphenicol was equally effective against pathogen M 17. Although Sulmet was well tolerated by larvae at very high levels, it demonstrated no protective properties. PVP-

TABLE 4. *Antibiotic sensitivity of bivalve pathogens*

Antibiotic	Serotype cultures				
	M17 (A)	M27 (O)	V67 (C)	V65 (D)	M74 (J)
Chloramphenicol	+*	+	+	+	+
Erythromycin	+	+	+	+	+
Kanamycin	+	0	0	+	0
Neomycin	+	+	+	+	+
Penicillin	0	+	0	0	+
Polymyxin B	+	+	+	+	+
Streptomycin	+	0	0	+	0
Tetracycline	0	0	+	+	+

* Plus indicates zone of inhibition with high-concentration Colab Multidisks.

TABLE 5. *Therapeutic effect of antibacterials challenged with pathogen M17*

Drug level in water of larval culture	Drug	Per cent larval survival* (72 hr)
ppm		
0	Unprotected (positive) control	0
5	Chloramphenicol	78
10	Chloramphenicol	91
20	Chloramphenicol	91
50	Chloramphenicol	98
	Combistrep	99
	PVP-iodine	0
100	Chloramphenicol	100
	Combistrep	99
	PVP-iodine	0
	Sulmet	0
200	Combistrep	100
	Sulmet	0
300	Sulmet	0

* All uninoculated drug controls were satisfactory except PVP-iodine, which was toxic to larvae at 100 ppm.

iodine proved toxic to larvae at potentially useful concentrations. Preliminary tests with polymyxin B sulfate, erythromycin, and neomycin sulfate indicate that these drugs are tolerated by larvae at concentrations which may be therapeutically useful.

DISCUSSION

The bacilli found to be pathogenic for the various bivalve larvae were of the type commonly classified as either *Aeromonas* sp. or *Vibrio* sp. The slight somatic curvature of some individuals of strains M 17 and V 65 probably has little taxonomic significance. The five larval pathogens had many characteristics in common (Table 3), but could be classified into four biotypes, as well as into the five antigenic types. A multiplicity of serological types is no surprise in view of similar situations among bacterial and viral disease agents of higher animals.

None of the cultures isolated from presumably normal marine fauna, and with generally similar morphological and physiological characteristics, had antigens in common with the larval pathogens, and none was pathogenic for bivalve larvae. Despite these results, it is felt that, in all probability, the etiological agents of bacillary necrosis normally exist as widely distributed saprophytes or commensals of marine forms, since typable pathogens were frequently isolated from cultures maintained in unsanitary or otherwise unfavorable physical environment.

The route of infection has not yet been defined but, as a rule, the few malformed larvae found in both experimentally and spontaneously infected cultures were the last to show signs of infection and survived the longest. Since, unlike healthy larvae, these abnormal forms seldom feed, it is probable that the initial invasion of the pathogen is through the larval alimentary tract. The question of the route of entry should be resolved by current histopathological studies. On the other hand, adult hard clams, American oysters, blue mussels (*Mytilus edulis*), and soft clams (*Mya arenaria*), exposed to massive concentrations of all pathogenic serotypes for 24 hr in standing seawater cultures, showed no ill effects, although these filter-feeders ingested vast numbers of the test bacteria.

Antibiotic preparations apparently can be used effectively in the prevention and treatment of larval hatchery infections and may offer a practical short-term means of control. Discovery of the sources of the infectious agents, of conditions that encourage their proliferation, and of environmental factors which predispose bivalve larvae to invasion by these bacteria might serve to clarify the epizootiology of bacillary necrosis and establish a rational basis for hatchery sanitation procedures.

ACKNOWLEDGMENTS

We thank Manton Botsford for preparing the figures and photomicrographs, and Rudolph Hugh and Graham L. Bullock for examining the pathogens. Chloramphenicol was supplied through the courtesy of Parke, Davis & Co., Detroit, Mich.

LITERATURE CITED

DAVIS, H. C., V. L. LOOSANOFF, W. H. WESTON, AND C. MARTIN. 1954. A fungus disease in clam and oyster larvae. Science **120**:36-38.

DAVIS, H. C., AND R. UKELES. 1961. Mass culture

of phytoplankton as foods for metazoans. Science **134**:562–564.

GUILLARD, R. R. L. 1959. Further evidence of the destruction of bivalve larvae by bacteria. Biol. Bull. **117**:258–266.

JØRGENSEN, C. B. 1946. Lamellibranchia, p. 277–311. *In* Gunnar Thorson [ed.], Reproduction and larval development of Danish marine bottom invertebrates. C. A. Reitzels Forlag, Copenhagen.

LEIFSON, E. 1960. Atlas of bacterial flagellation, p. 3–7. Academic Press, Inc., New York.

LEIFSON, E., B. J. COSENZA, R. MURCHELANO, AND R. C. CLEVERDON. 1964. Motile marine bacteria. I. Techniques, ecology, and general characteristics. J. Bacteriol. **87**:652–666.

LOOSANOFF, V. L., AND H. C. DAVIS. 1963. Rearing of bivalve mollusks, p. 1–128. *In* F. S. Russell [ed.], Advances in marine biology, vol. 1. Academic Press, Inc., New York.