CHAPTER VII

THE GILLS

	Page
Anatomy of the gills	121
Promyal chamber	123
Gill lamella	125
Skeleton	126
The filaments	126
Ostia	127
Ciliary tracts	128
Terminal groove	129
The muscles of the gills	130
Ciliated cells	132
Fine structure of the cilia	132
Mechanical properties of the cilium	135
Metachronal rhythm	136
Frequency of beat	137
Effect of temperature	138
Composition of sea water and ciliary motion	139
Effects of chemicals on ciliary motion	139
Metallic ions	139
Hydrogen ions	140
Various drugs	140
Inhibition of ciliary movement by antiserum	141
Effect of pressure on ciliary motion	142
Ciliary currents of the gills	142
Mechanical work of the laterial cilia	143
Carmine cone method	144
Effect of temperature	145
Hydrostatic pressure inside the gills	146
Spontaneous inhibition of ciliary motion	146
Bibliography	147

The gills of the oyster and other bivalves perform several important functions. They play a major part in respiration to which the mantle contributes a minor share. They maintain a steady current, filter the water, and collect food particles which are sorted and separated from detritus and other materials in suspension. They serve for the dispersal of sex cells at the time of spawning, and are used for the incubation of fertilized eggs in the larviparous species. The effectiveness of these functions is dependent on coordinated performance of the gill apparatus and on the contractions of the adductor muscle.

ANATOMY OF THE GILLS

Within the class of bivalves the structure of the gills varies in an increasingly complex series of modifications. The simplest of these is one pair of plumlike single gills or ctenidia with two rows of flattened filaments on each gill. This primitive type, present in the order Protobranchia (fig. 115, A), is found in *Nucula, Yoldia, Leda, Solenomya,* and others. More complex structure (fig. 115, B) occurs in the ark shells (Arcidae), scallops (Pectinidae), oysters (Ostreidae), sea mussels (Mytilidae), and other families of Filibranchia. This type is characterized by long and slender filaments kept in place by patches of interlocking cilia. In some of the bivalves of this group, including edible oysters, the gill lamellae are plaited into vertical folds and the reflected plates of the gills are completely united with the mantle and visceral mass. These were formerly designated as a separate order of Pseudolamellibranchia.

The highest degree of complexity is found in the gills of fresh-water mussels (Unionidae), cockles (Cardiidae), clams (Veneridae), and many other mollusks of the order Eulamellibranchia. In these bivalves the lamellae are joined by bars of connective tissue, the filaments are firmly connected by vascular junctions, and the entire gill has the appearance of a perforated, leaflike organ (fig. 115, C).

In the order Septibranchia (*Poromya, Cuspidaria*) the gills are degenerate. They are modified into perforated, muscular partitions between the two pallial chambers, and the gill filaments are greatly reduced (fig. 115, D).

The oyster gills consist of four folds (demibranchs or plates) of tissue suspended from the visceral mass. Two folds on each side of the body arise from a common ridge or gill axis, which is composed of connective tissue and muscles (fig. 73, g.m.). In a cross section made at right angles to the axis of the gill, each demibranch is V-shaped, with the two branches of the V forming its ascending and descending lamellae. The descending lamella arises from the gill axis; its opposite number is the ascending lamella. The two outer ascending lamellae, one on each side of the body, are fused with the mantle. The two inner demibranchs are joined together at

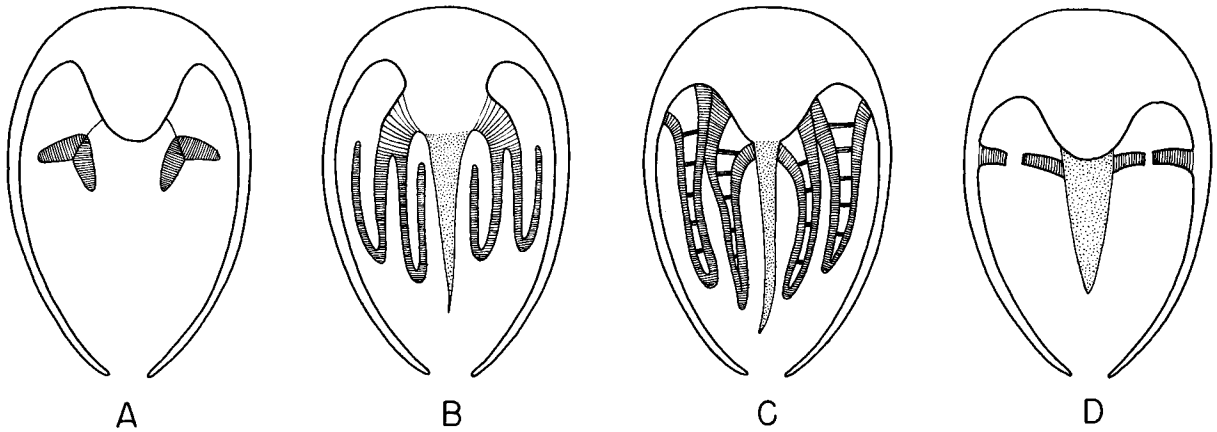

FIGURE 115.—Diagram of the four types of bivalve gills according to A. Lang (1900). A—Protobranchia type (*Nucula, Yoldia, Leda,* and others); B—Filibranchia type (Pectinidae, Ostreidae, Mytilidae, and others); C—Eulamellibranchia type (Unionidae, Cardiidae, Veneridae, and others); D—Septibranchia type (*Poromya, Cuspidaria*).

the central axis of the gills under the common afferent vein (fig. 73, c. af. v.). The section across all four demibranchs resembles two W letters joined together at the center.

Along the entire length of the gill runs the interlamellar space, which in places is divided by the interlamellar partitions or septae into a series of vertical compartments called the water tubes. The septae end close to the junction of the demibranchs and the visceral mass, so that the upper portion of the interlamellar space forms a continuous channel along the horizontal axis of each demibranch. This condition is found only at the upper (dorsal) part of the body at the level of the stomach and above it (fig. 116, *A*). At approximately the level of the heart the entire gill structure has only three points of attachment, in the center at the visceral mass and at each side where the gills fuse with the mantle (fig. 116, B.).

The entire chamber system of the gills can be visualized as four passages which at the level of the heart merge into two epibranchial chambers. The

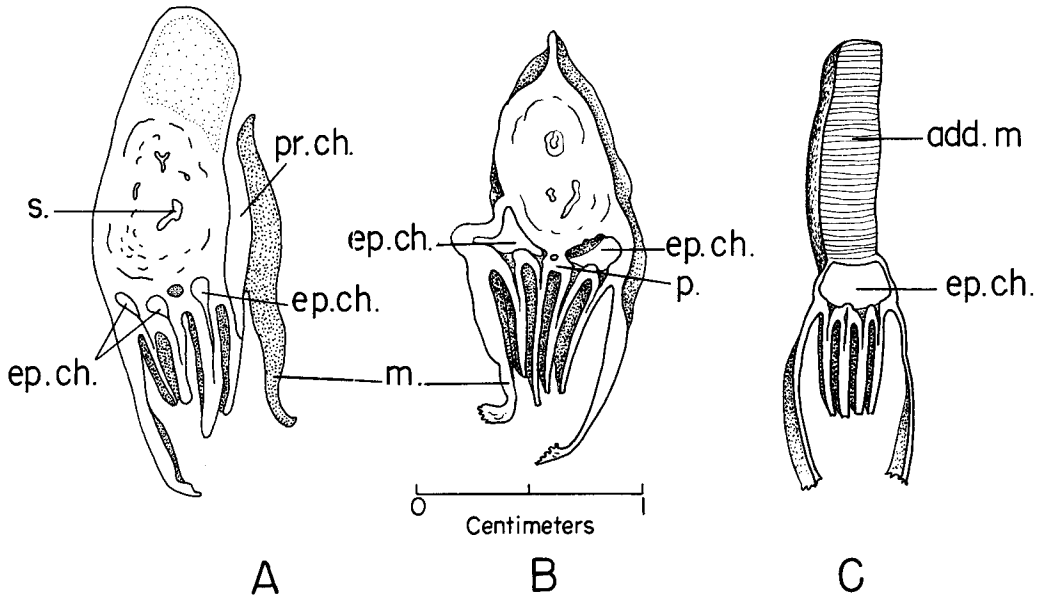

FIGURE 116.—Transverse sections of 1-year-old *C. virginica* (diagram drawn from sectioned and stained preparations). A—Section at the level of the stomach, s.; four epibranchial chambers at the base of the gills, ep.ch., and promyal chamber, pr.ch., at right. B—Section near the level of the heart, two epibranchial chambers, ep.ch., are separated by the pyloric process, p. C—Section at the level of the adductor muscle below the heart, common epibranchial chamber, ep.br.ch. Bouin, hematoxylin-eosin.

latter in turn lead to a common epibranchial chamber which, through the cone-shaped cloaca, opens to the outside.

There are no partitions, valves, or any other features for regulating the flow of water inside the chamber system. The current is maintained solely by the beating of the ciliary epithelium of the gills and of the lining of the chambers. Below the level of the heart the fusion of the gills with the median axis is lost and the two chambers, separated here by the pyloric process of the visceral mass (fig. 72, py.p.), merge together to form a commom epibranchial chamber (figs. 72 and 116, ep.br.ch.). This leads to a wide cone-shaped exhalant chamber or cloaca (fig. 72, cl.) which can be examined by forcing apart the posterior end of the valves and focusing a beam of light on its inner surface. The water tubes appear as large, round holes (fig. 72, w.t.).

PROMYAL CHAMBER

In oysters of the *Ostrea* type all the water collected by the gills is discharged through a single opening of the cloaca. In *Crassostrea*, however, the exhalant system is modified by the presence of an asymmetrical space on the right side of the body called the promyal chamber (fig. 116, pr.ch.). This irregularly shaped pocket between the mantle and the visceral mass extends in a dorsoposterior direction from the level of the pericardium to a wide outside aperture which may be seen by forcing the valves apart and examining the space between them. Openings of the water tubes similar to those found on the inner wall of the cloaca are visible inside the chamber (fig. 75)

The promyal chamber was first observed by Kellogg (1892), who suggested that the water from the gills may be discharged through it. Stafford (1913) showed the chamber in one of the illustrations of his book but did not refer to it in the text. The full anatomical significance of the promyal chamber in *C. virginica* was described by Nelson (1938) and by Elsey (1935) for *C. gigas*.

The position occupied by the promyal chamber makes it apparent that water from the dorsoanterior part of the right demibranch is discharged through this chamber and does not pass through the cloaca. After releasing carmine suspension near the gills of an actively feeding oyster (*C. virginica*), one can observe some of the red discolored water being expelled through the promyal chamber while the principal stream is passing through the cloaca. In spawning males the sperm shed from the right gonoduct is frequently discharged through the promyal chamber.

In assessing the relative importance of the promyal chamber in the movement of water through the gills, Nelson (1938) states that out of 36 water tubes in the right demibranch of *C. virginica* the first 14, comprising more than half the length of the demibranch, are in free communication with the promyal chamber. The remaining 22 water tubes discharge water into the narrowed portion of the epibranchial chamber beneath the adductor muscle or into the cloaca. In the absence of measurements of the amount of water discharged through the promyal chamber, it is impossible to state what percentage of the total output is discharged through the chamber. From anatomical evidence it may be concluded that the greatest part of the water used for ventilation of the gills leaves through the cloaca and only a minor portion through the promyal chamber.

Relative dimensions of the different parts of the exhalant system of the gills can be demonstrated clearly by casts made with plaster of Paris or with latex. Before being injected, the oyster should be completely narcotized. The material used for injection is then forced through both the cloaca and the promyal chamber while the oyster is gently and frequently tapped to permit good penetration to the very ends of the water tubes. When all the passages are full the valves are pressed together and tied with a string. The material is permitted to set for 24 hours before the shells are opened and the soft parts of the oyster removed.

The various parts of the water exhalant system of *C. virginica* are shown in figure 117, which represents the cast of the inner gill chambers viewed from three different angles. The promyal chamber (fig. 117, left, pm. ch.) occupies an irregular area on the right side of the visceral mass and ends in an aperture almost twice as large as the opening of the cloaca (cl.). The relation between the promyal chamber and the two demibranchs of the right side is shown in the central drawing (fig. 117) of the cast viewed from the left side. The right and left demibranchs are separated by a septum (s.) in the upper portion of the posterior side of the gills (fig. 117, right). Most of the inner spaces of the gills are in direct connection with the cloaca; less than one-half of the right demibranch empties into the promyal chamber.

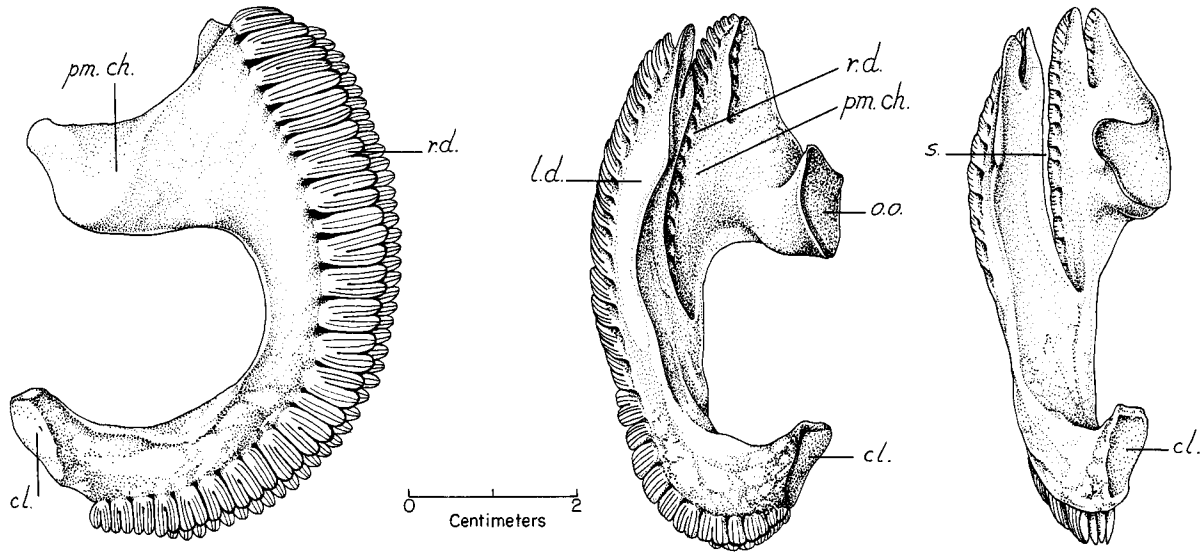

FIGURE 117.—Water exhalant system of the gills of *C. virginica*. Plaster of Paris cast of a large specimen. Left—view from the right side. Center—view at sharp angle from the left. Right—view from the posterior side of the gills. cl.—cloaca; l.d.—left demibranch; o.o.—outside opening (aperture) of the promyal chamber; pm. ch.—promyal chamber; r.d.—right demibranch; s.—septum separating the right and left demibranchs.

The relative size of the promyal chamber is variable. In some specimens it extends over three-fifths of the length of the gills and forms a spacious pocket (fig. 118). The exhalant system of *C. gigas* (fig. 119) is similar to that of *C. virginica*. In the specimens I examined, the promyal chamber extends approximately one-half the length of the gill, and the funnel-shaped cloaca was much wider than in *C. virginica*. In *O. edulis* the promyal chamber is absent and the entire system of water tubes, epibranchial chambers, and cloaca is symmetrical (fig. 120). In comparison with *C. virginica* and *C. gigas*, the cloaca of *O. edulis* is much broader and longer, which is probably the result of the almost circular shape of the body. The water tubes are shorter than in *Crassostrea*.

The promyal chamber also has been described for the Australian rock oyster, *Ostrea* (*Crassostrea*) *cucullata*, Born (*C. comercialis* I. and R.), and in *Ostrea* (*Crassostrea*) *frons* L. from the mangrove roots in Florida (Nelson, 1938).

Oysters of the *Crassostrea* type inhabit and thrive in the brackish and muddy coastal waters, which are less suitable for the mollusks of the *Ostrea* type. It has been implied by some investigators (Elsey, 1935; Nelson, 1938) that the tolerance to muddy waters is due to a superior cleaning mechanism which is somehow associated with the presence of the promyal chamber and prevents

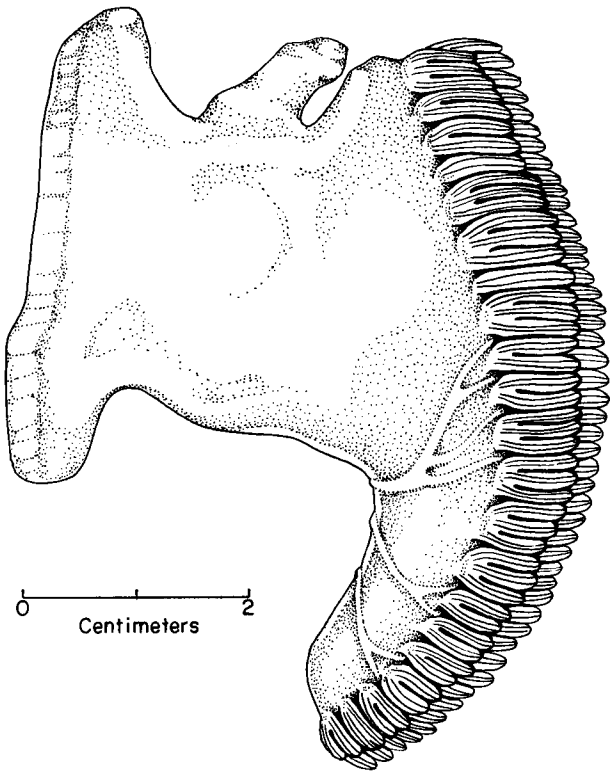

FIGURE 118.—Cast of the water exhalant system of a very large *C. virginica*. Note the extent of the promyal chamber along the gill axis and the impression made by the distended pallial arteries at the lower end of the right demibranch.

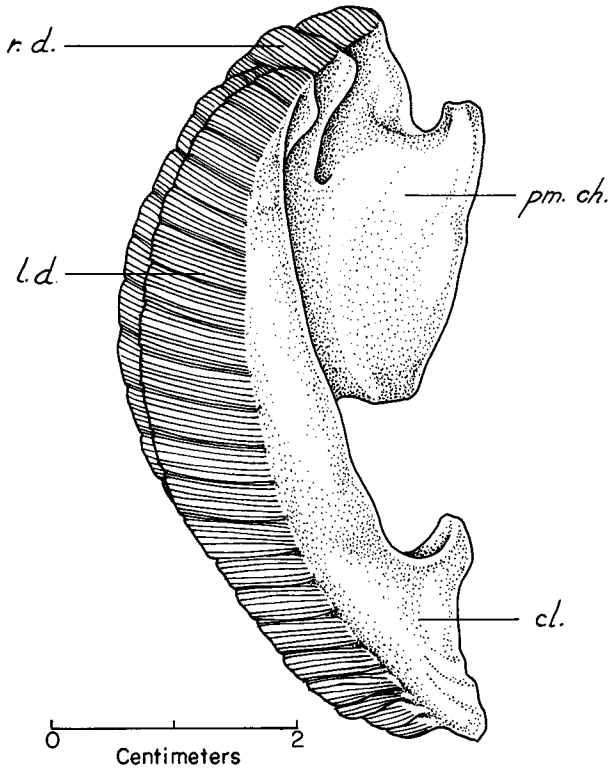

FIGURE 119.—Cast of the water exhalant system of the gill of *C. gigas* viewed from the left side. cl.—cloaca; l.d.—left demibranch; pm. ch.—promyal chamber; r.d.—right demibranch.

the accumulation of sediment in the pallial cavity. No evidence has yet been presented to corroborate this view. The physiological significance of the chamber is not known beyond the fact that it provides an additional outlet for the discharge of water through the gills. Elsey (1935) remarks that in *C. gigas* the promyal chamber allows a free passage of water when the gonads distend before spawning and encroach into the branchial space, partially closing the water tubes. Sections of the visceral mass of *C. virginica* with fully developed gonads do not show any "encroachments" into branchial space. The latter remains unobstructed, and the water tubes fully open. At present it is difficult to see what structural and physiological advantages are gained by the presence of a promyal chamber in *Crassostrea* oysters.

GILL LAMELLA

Each lamella of a gill consists of a great number of tubular filaments arranged at right angles to the axis of the gill. At the edge of the plate the filaments are reflected on themselves and continue upward along the plane of the ascending lamella. The filaments forming the gill-lamella do not lie smoothly on one plane; they are arranged in a series of transverse folds or plicae that give the surface of the gill a plaited appearance noticeable to the naked-eye. A transverse section (fig. 121) shows the arrangement of filaments in alternating grooves and ridges. The number of filaments on a single fold of an adult *C. virginica* is not constant. In my preparations it varied from 10 to 16 per fold.

There are three types of filaments that can be distinguished by their position, shape, and dimensions. The larger, or principal filaments (fig. 121, p.f.), are located at the bottom of the groove between the plicae. In cross section they have a triangular shape with two bulky chitinous rods forming two sides of this triangle. The rods are fused at the apex but are separated at the base, which contains a narrow blood vessel. The two transitional filaments (t.f.), one on each side of the principal one, are smaller and differ in shape from the ordinary filaments (o.f.), which form the rest of the plica. Sometimes the difference is insignificant. In general the ordinary filaments seen on cross section are elongated, club-shaped units.

Throughout their length the filaments of each plica are joined at the bases by regularly spaced interfilamentar junctions (if.j.), which consist of narrow bands of vascular connective tissue. The free portions of the filaments surround the oval-shaped openings, called ostia or fenestrae (o.), through which the water enters the inside passages of the gill. Numerous muscle fibers follow the interfilamentar junction and extend along both sides of the plica to its distal part (if.m.). At intervals, the two lamellae of each demibranch are connected by the partitions (interlamellar septa) made of connective tissue (il.s.) which run across the plate from one lamella to the other. These partitions are more numerous toward the distal (free) edge of the demibranch and diminish toward the base of the gill, where they are found only at about every sixth plica. The interlamellar junctions contain numerous muscle fibers, blood vessels, and nerves. The muscle fibers are arranged in three systems: the longitudinal muscles (l.m.) seen in cross section extend vertically from the proximal to the distal end of the lamellae; the transverse muscles (tr.m.) go from one side of the lamellae to the other; and the tangential

733-851 O—64——9

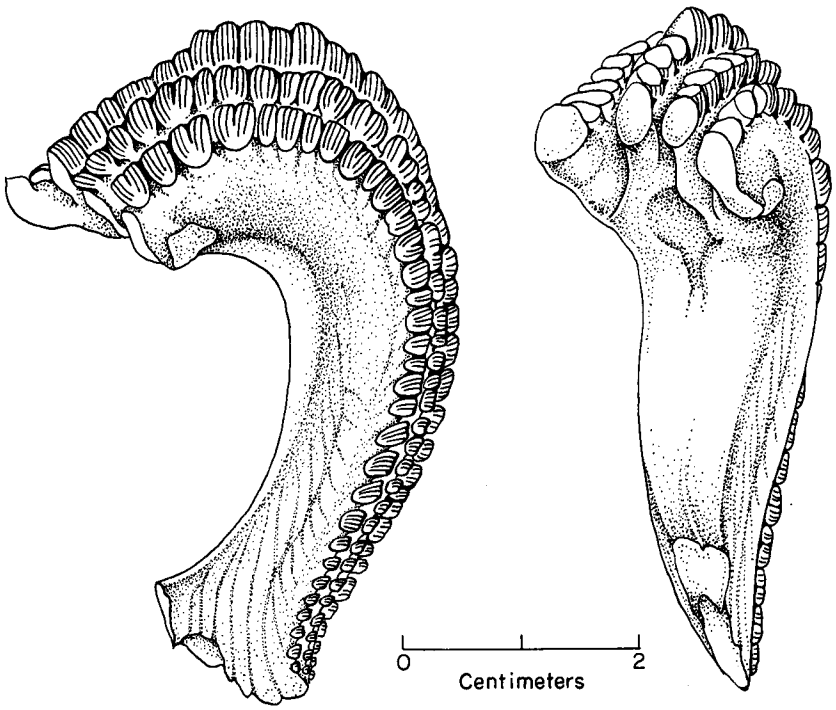

FIGURE 120.—Inner cast of the water exhalant system of the gills of *O. edulis*. Right—viewed from posterior side; Left—viewed from left side.

interlamellar muscles (il.m.) underlie the surface epithelium of the junction between the ascending and descending lamellae. At both ends of the junction near the location of the principal filaments the muscles branch off and form well-pronounced bands underlying the chitinous rods. It can be deduced from the pattern of distribution of the interlamellar muscles that their contractions bring the plicae of the two sides of the demibranch together, constrict the blood vessels, and reduce the diameter of the water tubes.

SKELETON

A framework of chitinous rods forms a scaffolding which supports the entire gill structure. The skeleton can be isolated from the tissues by brief treatment with a weak solution of sodium hydroxide, which does not affect the chitin. Structural elements cleared by this method are shown in figure 122. The skeleton of each filament resembles a ladder with the horizontal rungs slightly curved on one side and joined to the vertical elements by knobs of chitin. On both sides the supporting ladderlike unit of each filament is joined by cross pieces to the next units, forming a continuous framework. The vertical and horizontal bars surround the openings (ostia) between the filaments (o.). Each skeleton unit supports two adjacent filaments. The vertical bars correspond to the walls of the two adjacent filaments, while the horizontal members (the rungs of the ladder) are embedded in the tissue of the interfilamentar junctions. Two purposes are accomplished by such a pattern. The gill acquires rigidity and at the same time provides strong support for the delicate, sievelike membrane through which water passes into the water tubes. Heavy rods support the principal filaments (fig. 123). At the base of the gills the skeleton rods form massive V-shaped arches embedded in fibrous connective tissue (fig. 124).

THE FILAMENTS

The structural unit of the gill is a tubular filament of ciliated epithelium supported by skeleton rods. The central part of the filament is occupied by a space which is periodically filled with blood as the gill plates expand and contract. Connective tissue underlies the proximal part of the filament which consists of nonciliated, almost cuboid cells, tightly packed in a two-cell layer. The distal part of the filament is covered with ciliated cells (fig. 125). Bulky mucous cells occur at irregular intervals and discharge their

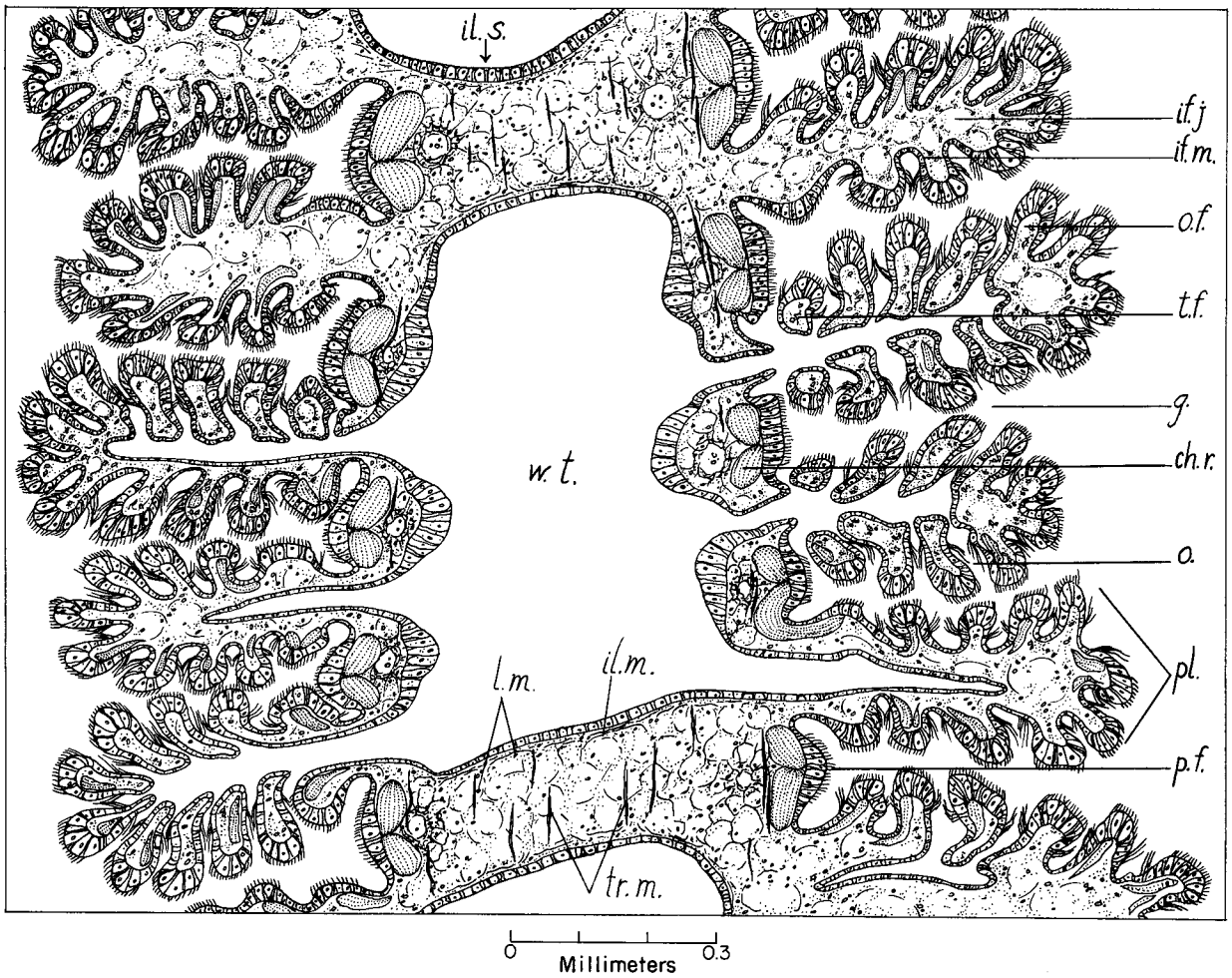

FIGURE 121.—Transverse section of the demibranch of *C. viriginica*. Kahle fixation, hematoxylin-eosin. ch.r.—chitinous rods; g.—groove; if.j.—interfilamentar junction; il.m.—interlamellar muscles; il.s.—interlamellar septum; l.m.—longitudinal muscles of the interlamellar septum; o.—ostium; o.f.—ordinary filament; p.f.—principal filament; pl.—plica; t.f.—transitional filament; tr.m.—transverse muscle of the interlamellar septum; w.t.—water tube.

content to the surface of the gills. The secreted mucus spreads over the gill plates and entangles the particles which settle on them. At the distal edge of the gill the filaments are fused together to form a terminal groove along which food is conveyed toward the mouth. The principal filaments differ from the others by their larger size and nontubular appearance. At the base of each filament there is a blood vessel located in a space between the chitinous rods. The epithelial cells are slightly larger than those of the ordinary filaments and have longer cilia (fig. 126).

OSTIA

Ostia or fenestrae, the oval-shaped open spaces between two adjoining filaments (figs. 121 and 125, o.), are framed by two vertical and two horizontal skeleton bars covered with epithelium. Their configurations and dimensions vary, depending on the degree of contraction of the filamental musculature and the distention of blood spaces. In an actively feeding oyster the contraction and expansion of the ostia regulate the passage of water through the gills. This can be observed on the surface of a gill exposed by cutting off a piece of shell. The dimensions of the ostia are somewhat correlated to the size of the oyster ova which pass through the gills during spawning (see ch. XIV, p. 303). In a viviparous *O. lurida* the ostia are large, varying from 90 by 45 μ in contracted state, to 180 by 60 μ when fully expanded. The ova of this species average 90 μ in diameter. In *C. gigas* and *C. virginica*, which have smaller eggs,

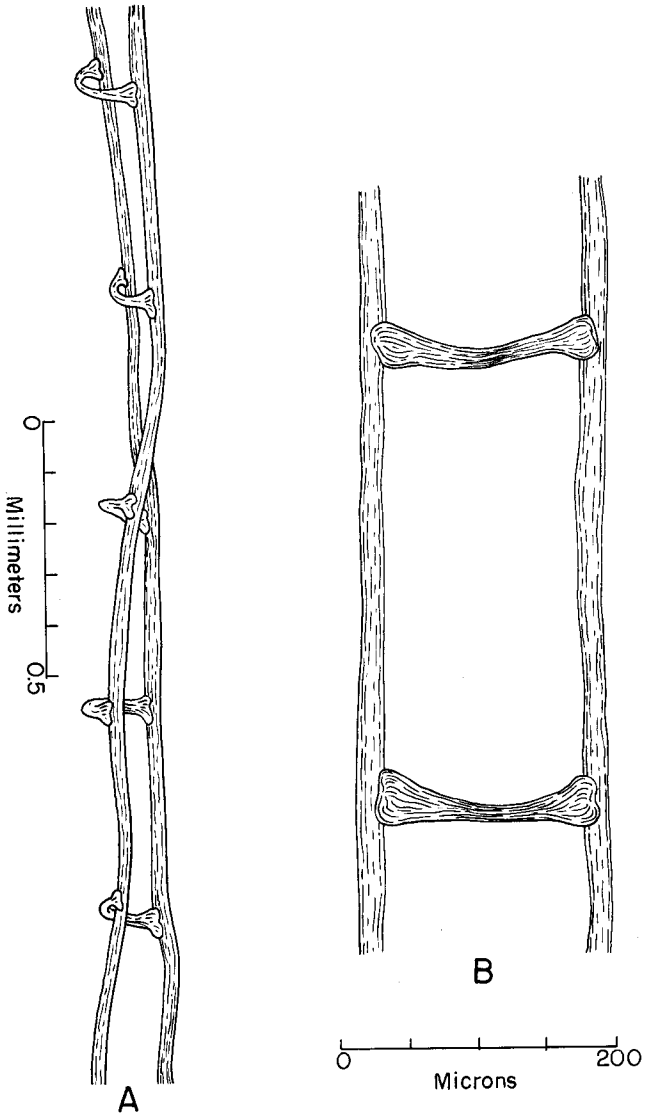

FIGURE 122.—Side view (A) and front view (B) of a skeleton of ordinary gill filaments of *C. virginica*. Soft tissues removed by sodium hydroxide. Unstained preparation, whole mount.

the ostia are approximately only one-third the dimensions of those in *O. lurida*.

CILIARY TRACTS

The surface of the filament is covered by several different ciliary tracts. Cells of uniform size on the outer surface bear the frontal cilia, which are relatively small and beat parallel to the surface of the filament (fig. 125, fr.c.). They are flanked on each side by a single laterofrontal cell (lf.c.) of larger size with a blade-shaped cilium, which according to Atkins (1938) occurs in the family Ostreidae and is somewhat different from the laterofrontal cells of other bivalves. In fixed and stained preparations this wide and curved cilium is frequently frayed. The shape of the cilium and the presence of the basal granules, typical for normal ciliated cells, both indicate that the laterofrontal cilium is formed by the fusion of ordinary filaments, a view which is confirmed by studies made with the electron microscope (see p. 132). The laterofrontal cells of *C. virginica* are fairly large and cone-shaped; their relatively small nuclei are located at the narrow, proximal end of the cell; and the protoplasm is devoid of granules and deeply stained with hematoxylin. The laterofrontal cilia of gills preserved in a relaxed state extend toward their opposing numbers on the adjacent filament and touch their tips. In a contracted gill they are bent and almost undistinguishable from the cila of the frontal cells. The length of the laterofrontal cila on sectioned and stained preparation varies from 11 to 15 μ. Accurate measurements, however, are difficult because of the bending of the cilia which do not remain fully extended even in completely narcotized cells. In *O. edulis*, according to Atkins (1938), the length of the laterofrontal cilia varies from 14 to 25 μ. The

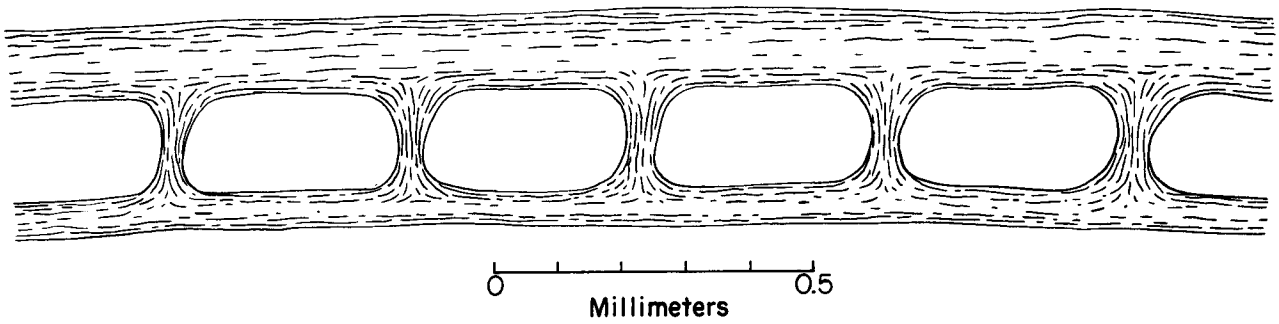

FIGURE 123.—Chitinous rod of the principal filament of *C. virginica*. Soft tissues removed by sodium hydroxide. Unstained preparation, whole mount.

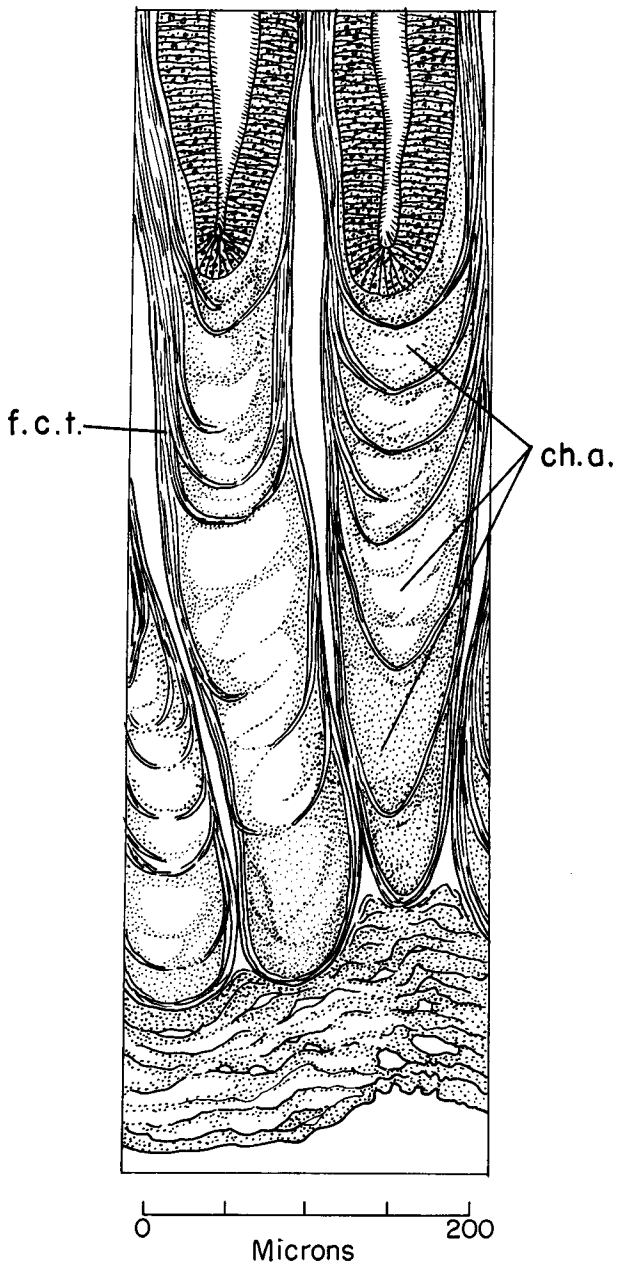

FIGURE 124.—Longitudinal section near the base of the gill of *C. virginica*. Chitinous arches, ch.a., are embedded in fibrous connective tissue, f.c.t. Osmic acid fixation, iron hematoxylin.

frontal and laterofrontal cilia of the principal filaments have the same structure as those of the ordinary filaments, differing only in their greater size. A short distance below the cell surface each cilium terminates in a basal body, a tiny granule from which a pair of rootlets extends deeper into the protoplasm and becomes undistinguishable near the nucleus (figs. 126 and 127).

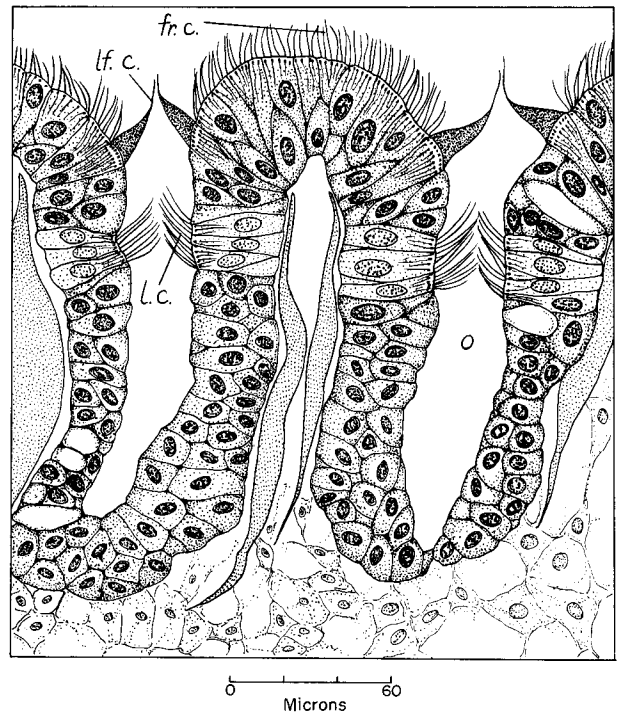

FIGURE 125.— Transverse section through ordinary filament of *C. virginica*. Vertical chitinous rods (stippled areas) and blood space are at the center. fr.c.—frontal cilia; lf.c.—laterofrontal cilia; l.c.—lateral cilia; o.—ostium. Kahle fixation; hematoxylin-eosin.

Besides the frontal and laterofrontal cilia, Atkins (1938) distinguishes in *O. edulis* the "fine frontal" and "paralaterofrontal" cilia, which run on both sides of the central portion of the frontal tract (fig. 127, f.f.c., para l.f.c.). He states (1938, p. 367) that: "Subsidiary laterofrontal cilia are present in Ostreidae, but are very difficult to distinguish even in the living gill." I was unable to identify these cells in sectioned preparations or in the living gill filaments of *C. virginica* examined under high power. The frontal cilia of this species appeared to be of uniform length along the entire cross section of the tract (fig. 125).

Beneath the laterofrontal cilia of the filaments there is a group of six large cells, four of them broad and two narrow, which bear large, stout cilia about 17 to 18 μ in length. These are the lateral cilia (fig. 125 and 127, l.c.), which bend forward slightly toward the outer surface of the filament and touch the cilia of the opposite group.

TERMINAL GROOVE

The free edge of a demibranch formed by the concrescence of the ascending and descending lamellae is a shallow trough called the terminal

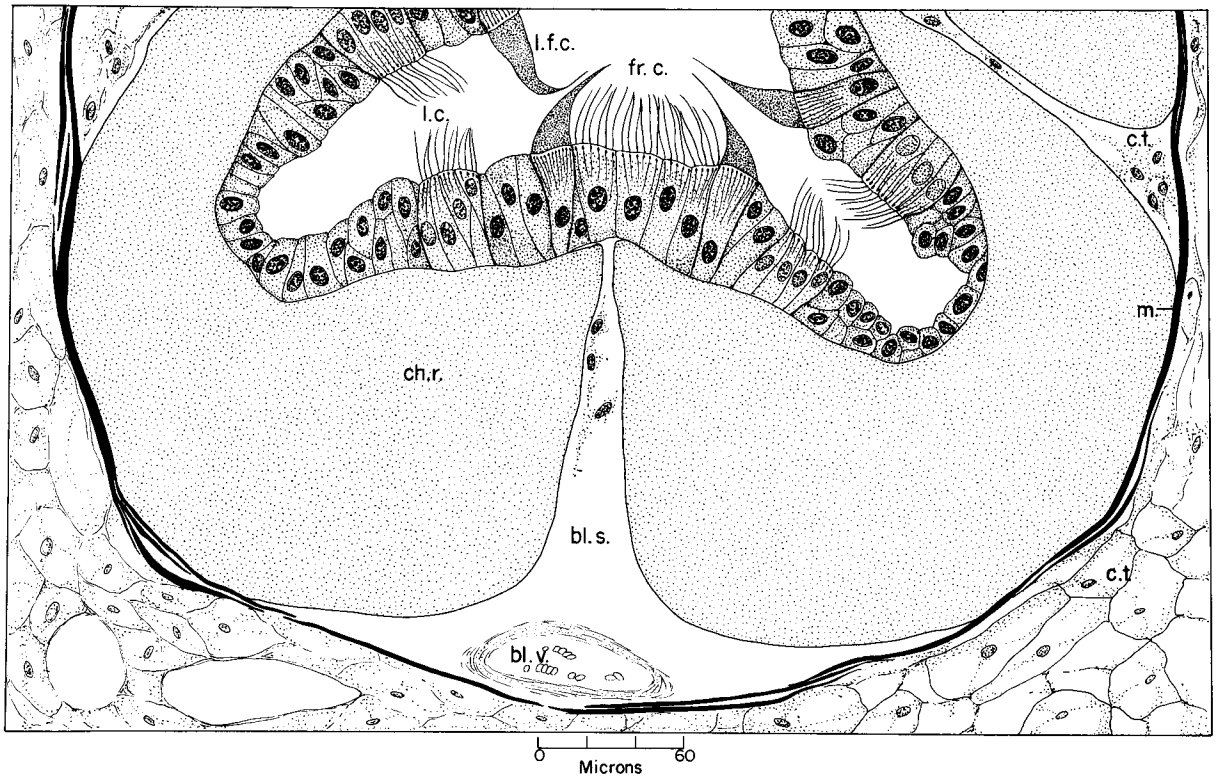

FIGURE 126.—Transverse section of the principal filament of the gill of *C. virginica* drawn from the same preparation as in figure 125. Note the well-developed muscle fibers, m., under the large skeleton bars, ch.r.. c.t.—connective tissue; bl.v.—blood vessel; bl.s.—blood space; fr.c.—frontal cilia; lf.c.—laterofrontal cilia; l.c.—lateral cilia; m.—muscle.

groove. This depression at the border of the gills extends their entire length. The epithelial lining of the terminal groove consists of columnar ciliated cells with large cilia and numerous mucous and eosinophilic cells. The epithelium rests on a basal membrane. Transverse muscle fibers extend between the two sides of the groove. During feeding the grooves are open, the condition which is shown in figure 128. Their contraction brings the edges together and closes the groove. In this way the oyster discards some of the material which was collected by the surface of the gill. The rejected particles entangled in mucus are dropped to the inner surface of the mantle and are discharged. The direction of the ciliary beat along the four terminal grooves is always toward the labial palps and the mouth.

THE MUSCLES OF THE GILLS

The gills of an actively feeding oyster contract and expand at frequent, although irregular, intervals. This behavior is difficult to notice in an intact oyster, but it can be observed in an oyster in which much of the valve has been cut off without injuring the adductor muscle and the gills. The mantle at the exposed area rolls up and leaves the gills in full view, and if carefully performed, the operation has no visible ill effect on the function of the gill.

The most conspicous movements which can be seen with the naked eye are the muscular contractions at the bases of the gills and the corresponding changes in the position occupied by the demibranchs. These four structures may stand apart like stiff leaves of a wide open album or they remain parallel, touching one another like the pages of a closed book. There is also a lateral movement of the filaments which brings them together or pushes them apart. This movement frequently occurs independently of the contractions of the demibranchs and may be limited to a small portion of the plica. Both types of movements affect the opening of the ostia, which are widely stretched when either the four demi-

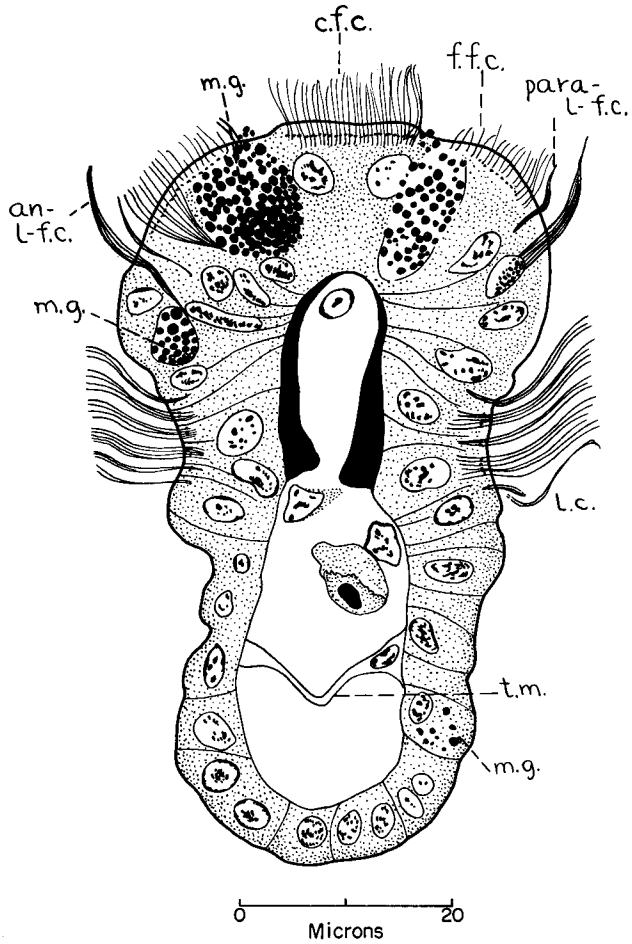

FIGURE 127.—Cross section of the filament of the gill of *O. edulis*, according to Atkins, 1938. an.l.fc.—anterior laterofrontal cilia; c.f.c.—central frontal cilia; f.f.c.—fine frontal cilia; para l.fc.—paralaterofrontal cilia; m.g.—mucous gland; l.—lateral cilia; t.m.—transverse muscle fiber. Chitinous rods are shown as black areas under the epithelium.

branchs or only a group of filaments stand apart and are constricted when the latter are drawn together.

Changes in the position of the demibranchs depend on two distinct systems of muscles located at the gill axis above and below the skeletal arches. In general the muscle fibers follow the configuration of the arches. The larger bands located inside the arches are the flexor muscles, which are attached to the inner sides of the two arms of an arch (fig. 129, f.). Their contraction brings the two adjacent demibranchs together. The smaller bands at the base of the arch (ex.) are the extensor muscles, which cause the demibranchs to stand apart. The action of the two bands shown in the

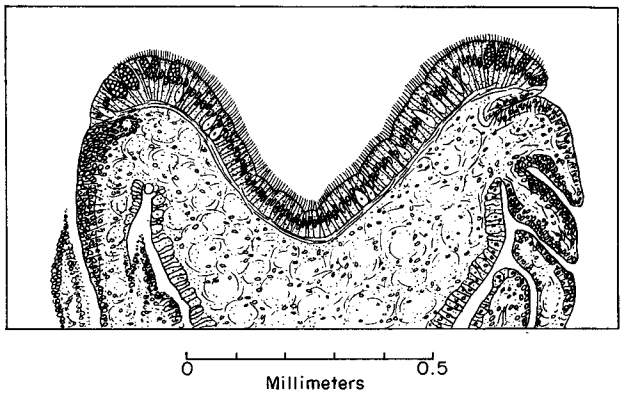

FIGURE 128.—Terminal groove at the edge of a demibranch of *C. virginica*. Longitudinal section of the demibranch. Bouin, hematoxylin-eosin.

figure is antagonistic. The extensor bands are smaller, probably because the elasticity of the chitinous arches pushes the demibranchs apart and this springlike action means that less force is required of the extensor muscles than of the flexor bands.

Other muscle bands of the gills, although less conspicuous than the flexors and extensors of the arches are, nevertheless, of great importance in regulating the transport of water through the complex gill apparatus and in facilitating the exchange of blood inside the gill filament. Water tubes of the gill can be constricted by the contraction of the muscles underlying the epithelium of the interlamellar septa and extending from one lamella to another (fig. 121, il.m.), while the contraction of the transverse muscles of the interlamellar septa compresses the blood vessels. The contraction of the longitudinal muscles of the septa (fig. 121, l.m.) results in the withdrawal and shortening of the entire demibranch. This reaction occurs spontaneously but can also be induced by stimulation. The contraction of the interfilamental muscles (if.m.) brings together the vertical rods of the gill skeleton, causes the curving of the crossbars, and constricts the blood space of the filament, forcing blood into the pallial veins.

Contractions affecting only part of the gill cause the blood to oscillate inside the gills. Because of the open nature of the lamellibranch circulatory system the direct return of blood from the gills to the auricles cannot be accomplished by the pumping action of the heart. Contractions involving the entire gill apparatus are needed to complete the renewal of blood.

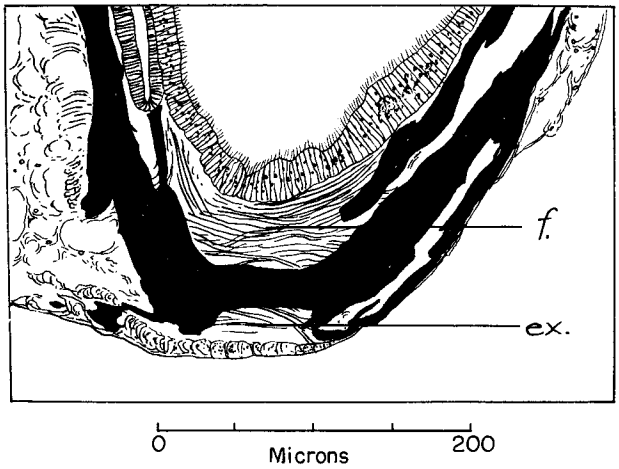

FIGURE 129.—Longitudinal section through the base of a demibranch of *C. virginica*. Kahle, Mallory triple stain. ex.—extensor muscles; f.—flexor muscles. Pieces of the skeleton arch are shown in black.

CILIATED CELLS

The structure and function of vibratile elements of the cells have been the object of numerous investigations beyond the scope of this book. The reader is, therefore, referred to comprehensive reviews of the problem of ciliary motion made by Gray (1928) and more recently by Atkins (1938) and Brown (1950). Several theories based on studies of the structure and action of cilia fail to give a satisfactory explanation of ciliary motion, which at present still remains a biological mystery.

Cilia examined in transmitted light or viewed on a dark background in reflected light appear to be optically homogenous. In polarized light they are birefringent (Schmidt, 1937). Observations with the light microscope disclose the presence of an axial filament (axoneme) surrounded by a thin sheet of cytoplasm (Wenyon, 1926). As a rule, the cilia emerge from tiny basal granules near the cell surface and penetrate through the cuticle, which under the light microscope appears as a thin homogenous membrane. Studies of the role and origin of basal bodies in various ciliated cells have resulted in a great number of speculations. Experiments by Peter (1899) showed that in small fragments of a crushed protozoan the cilia continued to beat as long as they were in organic connection with the adjacent pieces of cytoplasm. He deduced from this observation that the ciliary mechanism is located near the surface of the cell. Similar results were obtained with lateral cells stripped away from the filaments of *Mytilus* gills. The cilia that were removed from the basal granules remained motionless while those connected with them continued to beat (Gray, 1928). The microdissection technique in more recent years supports these findings. It was demonstrated that in the ciliated cells of the gills of *Anodonta* the motion of the cilia ceases when the cell is cut transversely in the immediate region of the basal granules. Transverse cuts made at any level within the proximal two-thirds of the cell had no effect on ciliary motion, but if the cut was made across the zone occupied by the fibrillae or rootlets in the distal third of the cell, the coordination of the ciliary motion was destroyed although the cilia continued to beat. These observations seem to support the validity of the theory, advanced independently by Henneguy (1897) and Lenhossék (1898), that the basal granule, homologous and sometimes identical with the centrosome of the mitotic figure, is the center which controls the activity of the cilium.

FINE STRUCTURE OF THE CILIA

With the advance of electron microscopy considerable progress has been made in the study of the fine structure of cilia. It has been discovered that throughout the plant and animal kingdoms, regardless of the position of the organism on the evolutionary level and irrespective of the organs studied, cilia have a common structural pattern. The cilia of the gill epithelium of the oyster are no exception to this rule. Thin sections of the frontal and lateral cells of the filaments fixed in buffered osmic acid and examined under the electron microscope show a structure which is undistinguishable from that of the cilia of vertebrates, protozoa, or the tails of spermatozoa. The cilium consists of a protoplasmic matrix in which are embedded 11 filaments; 2 single filaments are at the center and 9 double ones are arranged in a ring on the periphery. The central pair is connected to the peripheral ring by radial trabeculae or spokes. Short pieces of dense material join the outer filaments to the membrane (fig. 130), which binds more osmium and is, therefore, darker than their interior, making the cilia appear tubular (Fawcett, 1958). The two central filaments are oval shaped in cross section. The plane in which these filaments are oriented is similar for all the cilia of the cell and is thought to be perpendicular to the direction of the ciliary beat (Fawcett, 1958).

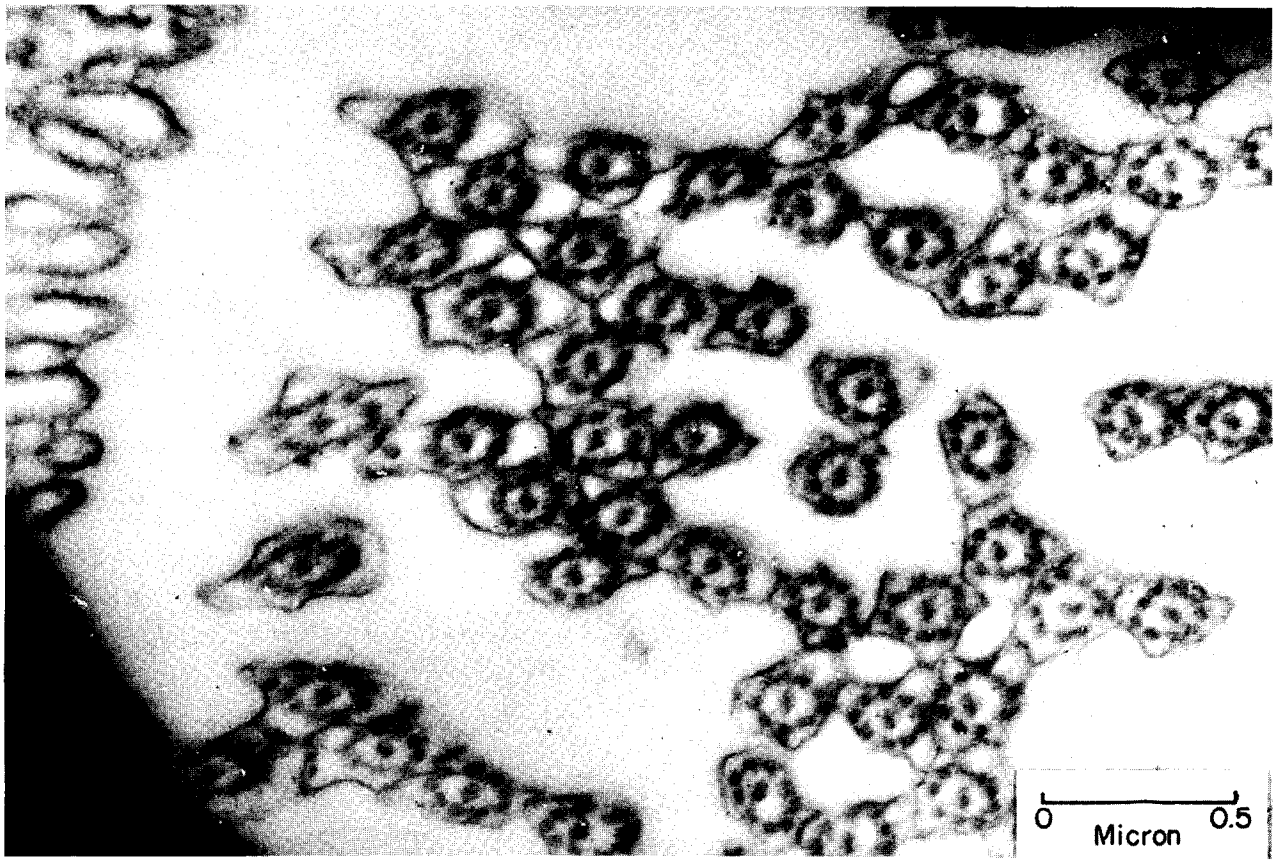

FIGURE 130.—Cross section of the group of frontal cilia of the gill of *C. virginica*. Microvilli of the cell surface are seen at the bottom. Electron micrograph. Buffered osmic acid 1 percent.

The orientation is apparent in the electron micrograph (fig. 131) of a longitudinal section of the distal part of the lateral cell of the filament of *C. virginica* and on transverse sections of the frontal cilia (fig. 130). Because the latter cilia are curved in the direction of the beat, they were cut transversely and appear in the micrograph a short distance above the cell surface. Their oval-shaped axial filaments are oriented parallel to the surface of the cell, i.e., in the direction of ciliary beat. The membranelike laterofrontal cilia consist of several individual cilia embedded in a protoplasmic membrane, but each element retains the typical structure of a single cilium (fig. 131).

The basal corpuscles of cilia are arranged in rows (fig. 132), and the central part of each is surrounded by denser cortex, giving the appearance of an empty central cavity. In the longitudinal section (figs. 131 and 132) they are elongated with a pair of rootlets arising from each proximal end. Rootlets of the cilia of the clam, *Elliptio complanatus*, have a periodic striation of about 750 Å. Similar periodicity appears in electron micrographs of oyster cilia made in the course of my studies, but the picture is not as clear as that published by Porter and Fawcett (see DeRobertis, Nowinski, and Saez, 1954, p. 382).

The distribution of rootlets follows a precise pattern. Each rootlet of a pair turns at an acute angle and crosses over the rootlet of the adjacent corpuscle. The rootlets may be followed further down the cytoplasm toward the nucleus (not shown in the micrograph); some of them cross the second rootlet emerging from the other side of the same corpuscle as can be seen at the center and left side of figure 132. The crossed rootlets are in close contact with each other, but it is not clear whether or not they are fused. Apparently direct communication between the basal corpuscles is lacking.

The question of whether the rootlets are simply the anchoring structures of the cilia or play an active part in its movement remains unanswered.

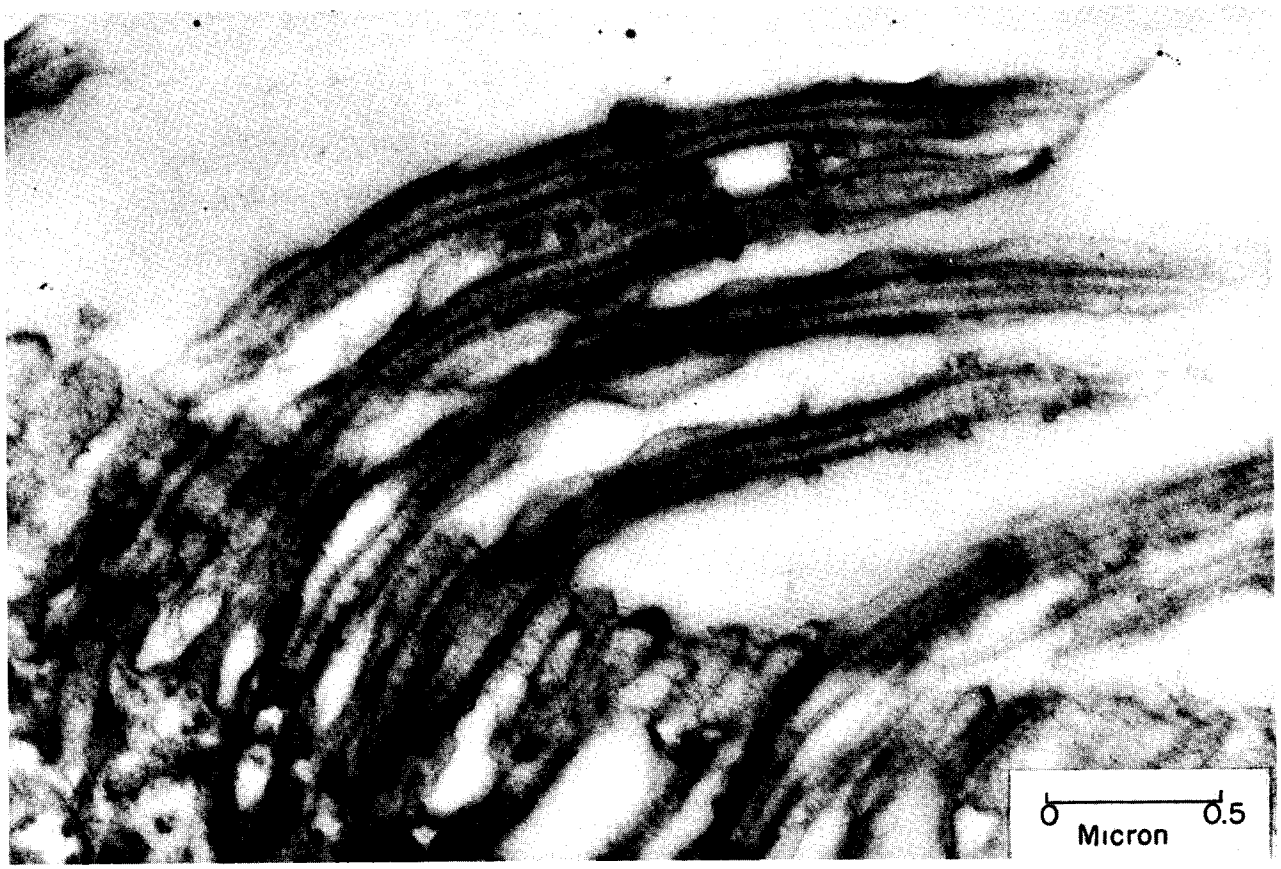

FIGURE 131.—Longitudinal section of the distal portion of laterofrontal cell of the gill of *C. virginica*. Since the plane of section passes at the middle of the cilium only single axial and two peripheral filaments can be seen. The basal corpuscle and the beginning of rootlets are at the lower part of the micrograph. Electron micrograph. Buffered osmic acid 1 percent.

There is the possibility that they may represent a coordinating mechanism of the ciliary epithelium. The fact that the rootlets of the two adjacent corpuscles cross each other is in favor of this view, which was advanced by Grave and Schmitt (1925) on the basis of their observation of the cilia of fresh-water mussels made with the light microscope. Exploration with the electron microscope gives additional support to their hypothesis which, however, requires further corroboration.

The free surface of the ciliated cell appears as a thin homogenous layer, devoid of visible structure, when examined in the light microscope. In reality this layer consists of fingerlike processes called microvilli (figs. 130 and 132), which are found in various tissues; they are considered a device to increase the surface of the cell. Their number has been estimated as high as 3,000 per single cell of intestinal mucosa, and there is no doubt that numerous fingerlike processes greatly increase the surface area of the gill and facilitate the exchange of gases and ions. In figure 132 the layer of microvilli, about 0.5 μ in thickness, rests upon the plasma membrane of the cells. The cytoplasm under the membrane contains numerous mitochondria.

The complex ultrastructure of the ciliated cell of the oyster gill is shown diagrammatically in figure 133, which represents a reconstruction of the principal features seen on electron micrographs. The diagram is based on a large number of micrographs and summarizes our present knowledge of the dimensions and arrangement of the various parts which comprise the ciliated apparatus of the oyster gill.

Although the mechanism of ciliary motion is not known, studies of the ultrastructure of the cilia suggest that the molecular organization of both cilia and myofibrillae of the muscle cells are homologous and that the mechanism of their contraction is similar. This conclusion gains further support from biochemical studies which

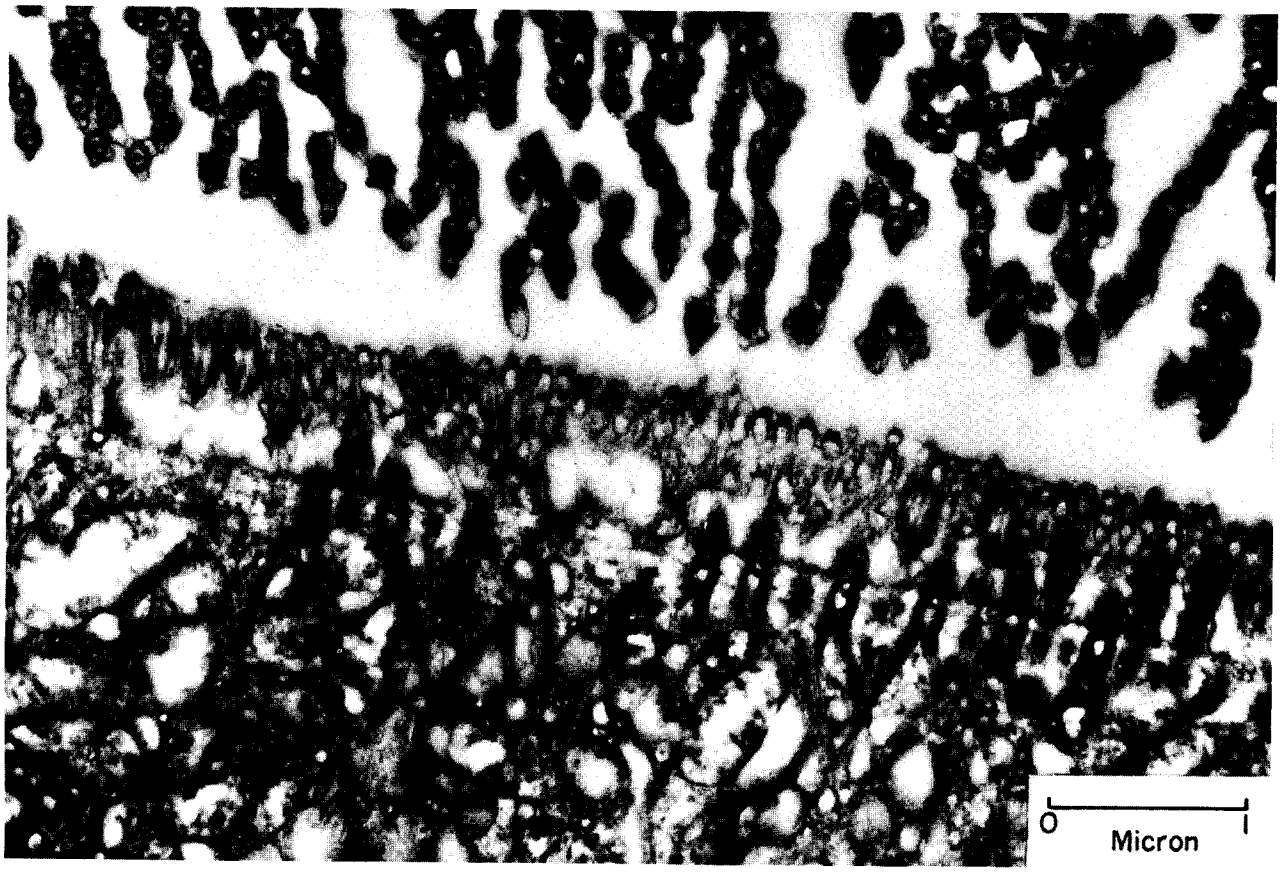

FIGURE 132.—Section perpendicular to the surface of frontal cilia of the filament of the gills of *C. virginica*. The curved frontal cilia are cross sectioned. Note the row of basal corpuscles with rootlets; the sharp line, parallel to the surface of the cell corresponding to plasma membrane; and the microvilli above it. Electron micrograph. Osmium fixation. Buffered osmic acid 1 percent.

show that both the contraction of the muscles and the movement of bacterial cilia is stimulated by adenosinetriphosphate (DeRobertis, Nowinski, and Saez, 1954, pp. 389).

MECHANICAL PROPERTIES OF THE CILIUM

Most of the observations on the structure and and movements of lamellibranch cilia were made on the gills of *Mytilus*. There is no reason to think, however, that the cilia of the oyster gill are fundamentally different from those of the mussel.

The gill cilium is a flexible and elastic rod which can be deformed by mechanical pressure applied with a microdissection needle. The deformity is repaired rapidly when the pressure is removed. Gray (1928) interprets these observations on *Mytilus* cilia as an evidence of transverse elasticity of the cilium.

The movement of the cilium consists of two distinct phases, the forward effective stroke and the much slower recovery stroke which brings the cilium to its initial position. The velocity of the effective stroke is considered to be five times that of the recovery stroke (Kraft, 1890). The effective stroke begins with the curving at the tip and extends down to the base, bending the entire cilium into an arch of 180°; throughout this period the cilium behaves as a rigid rod mounted to the cell by its end. During the recovery stroke the cilium straightens from the base to the tip and moves backward as a limp thread. Both the effective and the recovery strokes take place in the same plane, which remains constant (Gray, 1922a; Carter, 1924).

The movement of a cilium results from contraction of its filaments. It is not clear, however, whether all 11 filaments are equally involved in the effective and recovery strokes. Furthermore, it appears probable, although definite proof is

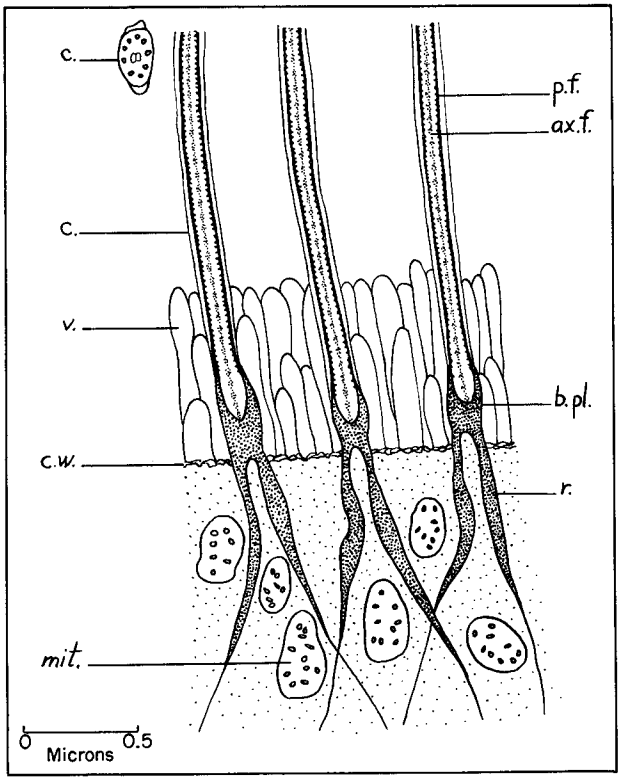

FIGURE 133.—Diagrammatic reconstruction of the distal portion of the ciliated cells of the gill epithelium of *C. virginica*. ax.f.—axial filament; b.pl.—basal plate and basal corpuscle; c.—cilium; cw.—plasma membrane; mit.—mitochondria; r.—rootlet. Cross section of the cilium shown at upper left corner.

wanting, that the pair of axial filaments gives the cilium the necessary rigidity but does not participate in the movement.

METACHRONAL RHYTHM

Automatism is a general characteristic of ciliary motion. This typical property of ciliated epithelium, common to all animals which have ciliated cells, is a fundamental characteristic of the ciliary motion of lamellibranch gills. As Gray (1928, p. 4) stated: "There can be little doubt that all cilia are fundamentally automatic in their movement and that the power possessed by organisms to inhibit the locomotion of their cilia is of extraneous nature."

In any ciliated surface there is some sort of coordinating mechanism that manifests itself in the metachronal rhythm of the beat. The term metachronal rhythm or metachronism denotes the regular sequence of ciliary motion in which any cilium in a given series is slightly out of phase with the cilium behind and in front of it. Since the cilia in one row of the epithelium beat at the same rate but are in different phase, their combined movement gives the optical appearance of a wave passing over a wheat field on a windy day. The beating of the lateral cilia along the isolated filament of an oyster gill is an excellent object in which to observe the metachronal wave. In the drawing of an exposed surface of the gills of a live oyster examined under a compound microscope (fig. 134) the metachronal waves along the two rows of the lateral cilia move in opposite directions. The effective stroke of the lateral cilia in this case is at right angles to the direction of the metachronal wave (i.e., perpendicular to the plane of the drawing). The crest of the wave includes the cilia that are ready to begin their effective stroke; in the troughs are the cilia that are about to start the recovery stroke.

The direction of the metachronic wave is not disturbed by the temporary cessations caused by such extraneous agents as narcotics or cold. Upon recovery the metachronic wave proceeds in the same direction as when the motion was artificially stopped. In the ciliated epithelium of the roof of a frog's mouth the metachronic wave is not disturbed even if a piece of epithelium is cut off and then placed back after rotating it 180° (Brücke, 1917). Transplantation of the gill epithelium of an oyster was tried in the Bureau's shellfish laboratory without success. Copious discharge of mucus, continuous bleeding of the wound area, and the curling up of the filaments interfered with the implantation of the excised pieces. In all my experiments the host animals discarded the implants in a short time.

The fact that small pieces of ciliated surface

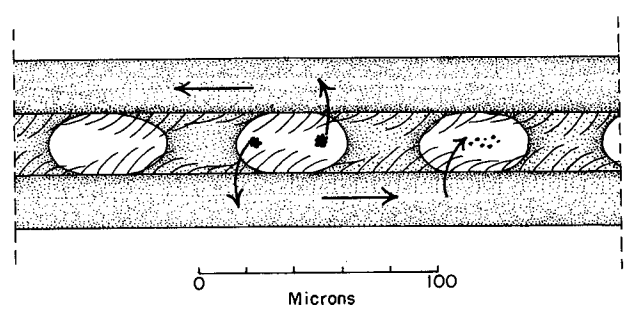

FIGURE 134.—Two tracts of the lateral cilia of *C. virginica* along the two filaments on both sides of the ostia. Small black particles suspended in water are drawn into the ostia while the large ones are discarded by the recovery strokes of the lateral cilia. Drawn from life.

or even single ciliated cells removed from the organism continue to beat for a long time leads to the conclusion that in the majority of cases the ciliary motion is independent of nervous control of the organism. This is, however, not a general rule since the ciliary motion on small fragments of the lips of the snail, *Physa*, removed with the attached nerve, soon ceases unless the nerve is stimulated (Merton, 1923b). Numerous investigations give support to the concept that in many invertebrates and vertebrates the nervous system is an effective agent in the control of coordinated activity of ciliary tracts (Babák, 1913; Carter, 1927; Göthlin, 1920; Lucas, 1935; McDonald, Leisure, and Lenneman, 1927; Seo, 1931).

Bipolar cells and nervelike fibers immediately below the ciliated epithelium of the gills of fresh-water mussels, *Lampsilis* and *Quadrula*, described by Grave and Schmitt (1925), were supposed by these authors to serve as conduction paths for stimuli which they claim regulate and coordinate ciliary movements of the gills of these mollusks. According to their point of view, the ciliated cells of the bivalve gills have a dual control. They may be perfectly autonomous and continue to beat in the complete absence of neural connections; on the other hand, the automatic beat of the cilia may be regulated through supplementary nervous connections in conformity with the state of the organism as a whole. These authors assume that ciliated tissues of fresh-water mussels are both autonomous and under the control of the nervous system.

Intracellular fibrillae of the gills of *Mya*, *Lampsilis*, and *Quadrula* were considered by Grave and Schmitt (1925) to be the conductive paths for coordinating and regulating ciliary movement. A complex system of interconnecting rootlets of the ciliated cells of oyster gills described above (fig. 132) gives additional support to this view. Grave and Schmitt (1925) described also the nervelike apparatus of bipolar cells and fibers. Reinvestigation of the tissues of fresh-water mussels by Bhatia (1926) did not support these findings. No such structures were found in my preparations of the gills of *C. virginica*, or, according to Lucas (1931), in the gills of *Mytilus edulis*. Their existence in the gills of fresh-water mussels seems to be doubtful.

FREQUENCY OF BEAT

The rate of ciliary beat can be observed easily on lateral cilia because of their relatively large size and well-pronounced metachronic wave. Observations must be made on small excised pieces of gill since the position of the lateral cilia on the sides of the filaments makes it impossible to watch their activity on an intact demibranch. In my preparations the filament or a group of them was separated by using fine needles, and kept in a micro-aquarium filled with sea water. The temperature was controlled by circulating cold or warm water in the outside jacket of the microaquarium.

The frequency of beat was determined by using a stroboscope of the type manufactured by R.C.A. and sold under the name "Strobotac". The reddish flickering light given off by this instrument is sufficient to observe cilia under a magnification of about 250 ×. Readings are made directly on the panel of the instrument by rotating the knobs controlling the frequencies. The instrument must be adjusted to the zero point and frequently checked.

Gradual decline in the frequency of beat on the excised filament becomes apparent after several hours; the disturbance of the metachronism in the preparations kept for more than 24 hours is a sign of pathological conditions. Such preparations should be discarded.

The frequency of beat varies greatly in different oysters of the same age, origin, and environment. For instance, among the 12 large adult specimens from New England waters tested in August 1956, the range of variation at room temperature of 22° to 23° C. was from 16 to 27 beats per second. All the specimens were in excellent condition and appeared normal in every respect.

In addition, there are sometimes wide variations in the frequencies of ciliary beat in the adjacent filaments of the excised gills. In studies of the effect of temperature and other environmental factors on the rate of beat, therefore, all the readings must be made over the same portion of the ciliary tract. This is sometimes difficult because of the mobility of the excised pieces and copious secretion of mucus which interferes with the observations.

In the data summarized in table 14 the beat frequencies were recorded in a selected locus of the tract of lateral cilia kept at nearly constant temperature. The filaments were taken from the 14 different oysters listed in the first column of the table. Observations lasted from 10 to 30 minutes. The maximum range of variation recorded during

each test was from 16.6 to 20.5 beats per second. The greatest difference between the individual oysters was recorded in two ripe males; one had the median frequency of 15.5 per second (at 23.3° C.) while in the other the cilia beat at the rate of 24.8 per second (at 25.1° C.). In the majority of the oysters the median rate of cilia beat varied between 18 and 22 per second.

TABLE 14.—*Frequency of beat of lateral cilia of 14 adult* C. virginica *recorded at nearly constant temperatures*

[Readings were made at intervals of 1 or 2 minutes]

Oyster	Duration	Temperature range	Beats per second			Recordings
			Max.	Min.	Median	
	Minutes	*° C.*	*No.*	*No.*	*No.*	*No.*
Spawned out, sex undetermined	21	23.8–23.8	20.7	18.3	19.6	20
Two-year-old	10	24.1–24.4	17.3	15.7	16.3	10
Ripe male	15	23.3–23.3	15.3	16.0	15.5	15
Spawned out male	10	23.2–23.6	21.5	20.5	21.2	10
Spawned out male	10	24.0–24.2	21.3	19.3	20.0	10
Ripe male	12	25.1–25.2	25.2	23.5	24.8	10
Spawned out female	30	25.2–25.4	23.7	21.1	22.1	15
Spawned out female	15	24.2–24.3	20.5	16.6	18.5	15
Spawned out female	10	25.1–25.1	20.6	17.3	19.0	10
Ripe female	10	24.5–24.5	18.8.	15.6	17.3	10
Ripe female	20	26.5–26.5	18.0	15.5	17.3	20
Spawned out female	10	23.0–23.1	21.5	19.3	20.7	10
Spawned out female	10	23.0–23.1	19.6.	18.8	19.3	10
Spawned out female	30	22.4–23.2	20.7	19.7	20.6	15

EFFECT OF TEMPERATURE

In evaluating the biological significance of the experimental data of the effect of temperature on beat frequencies, one should remember that the pieces of isolated tissue used were in an abnormal situation. They were deprived of blood supply, separated from close association with other structural elements of the gill, and subjected to increased concentrations of metabolites. It is conceivable that under normal conditions the lateral cilia of an intact gill may react somewhat differently.

Stroboscope observations fully confirm the fact that temperature controls the ciliary beat. This effect was observed in a series of determinations made during the summer using small pieces of filaments taken from 39 adult New England oysters kept in water at various temperatures. At the start of each series of readings 10 minutes were allowed for adjustment to the desired temperature which was kept constant within plus or minus 1° C. Ten stroboscope readings were made at 1-minute intervals and repeated at higher or lower temperature. No more than three different temperature levels were used on one preparation. Careful precautions were taken to prevent the movement of the excised filaments in the microaquarium so that all the readings would be made on exactly the same locus of the ciliary tract. This was necessary because of the considerable differences in the rate of beating which occasionally occur along the adjacent filaments.

The results, summarized in table 15, show the maximum median frequency of 27.7 beats per second at temperatures of 25° to 27° C. The ciliary activity became irregular at about 35° C., and the movement ceased at 37° to 38° C. Whether these limits are applicable to oysters from warm southern waters is not known, since all the observations were made only on the New England oysters. Between 35° and 37° C. the motion was so irregular that its frequency could not be recorded with certainty. Irregular beating at the rate of about two beats per second was observed in some specimens during short exposure to the temperature of 45.6° C. Judging by the median values of the beat frequencies, the optimum temperature is between 23° and 27° C. (see fourth column, table 15). The ciliary activity declines rapidly below 21° C. and ceases completely at 5° to 7° C.

Individual variations in the frequency of beat among oysters of a single population suggest differences in their physiological states and different requirements for food and water for respiration. As a rule, spawned-out females remained inactive for some time in late August and early September. During this period the gonads containing unspawned sex cells were reabsorbed and tissues became watery because of the reduction in solids content. The adductor muscles remained contracted, and the shells were closed for unusually long periods, lasting from 3 to 4 days, or opened

TABLE 15.—*Frequencies of beat of lateral cilia of the gills of adult* C. virginica *at different temperatures*

[Stroboscope readings made on excised filaments kept in sea water]

Temperature range	Frequency of beats per second			Preparations used	Oysters used
	Minimum	Maximum	Median		
° C.	*Number*	*Number*	*Number*	*Number*	*Number*
36–37	Irregular	Irregular	Irregular	2	1
33–35	----------	24.2	----------	1	----------
31–33	17.3	27.7	22.5	5	2
29–31	23.8	23.8	23.8	1	1
27–29	19.3	27.7	23.3	3	2
25–27	20.8	33.3	27.7	7	3
23–25	21.3	33.3	25.6	7	3
21–23	16.6	27.7	20.8	13	5
19–21	16.7	20.3	17.2	3	2
17–19	13.3	16.7	13.7	6	2
15–17	10.0	11.6	10.5	6	2
13–15	3.6	11.9	10.2	7	3
11–13	2.2	8.9	5.6	8	3
9–11	1.6	2.6	2.1	8	2
7–9	1.5	1.9	1.8	5	2
5–7	----------	----------	----------	5	5

only for a short time. Even when the valves opened, the gills produced a weak and unsteady current interrupted by frequent cessations of ciliary motion.

The effect of temperature on ciliary activity can be seen more clearly in the experiments in which only a single gill filament was used. The results are shown in figure 135 in which the median frequencies of the beat are plotted against the temperature. As in previous observations 10 readings were made at each temperature step and the entire experiment was completed in about 2½ hours. The frequency of beat rapidly increased between 10° and 25° C. The slowing down of ciliary motion below 10° C. was gradual until all movements ceased at about 6° C. The curve shown in figure 135 has four distinct slopes that indicate the differences in the response of the lateral cilia to temperature changes: a) a very slow increase between 6° and 11° C.; b) a more rapid acceleration between 11° and 15° C.; c) a gradual increase between 15° and 25° to 26° C.; and d) a decline as the temperature rises toward the 30° C. mark.

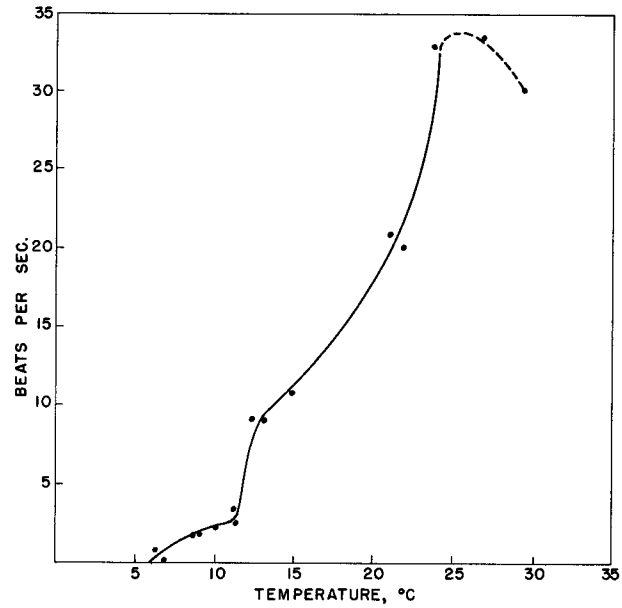

FIGURE 135.—Effect of temperature on the median frequencies of beat in number per second of the lateral cilia of *C. virginica*. Readings were made with Strobotac on a single filament of the gill kept in sea water in a microaquarium. Temperature was changed by circulating warm or cold water in the jacket of the microaquarium. Duration of the experiment 2½ hours.

COMPOSITION OF SEA WATER AND CILIARY MOTION

Ciliary motion may be affected by changes in the chemical composition of sea water and by various drugs. Ionic balance of the outside medium is one of the principal conditions for continuous ciliary activity of the gill. The most important ions are sodium, potassium, calcium, and magnesium; the increase in concentration of one without a corresponding compensation in the concentration of another or the withdrawal of one of the ions may completely disrupt the ciliary motion.

EFFECTS OF CHEMICALS ON CILIARY MOTION

METALLIC IONS

The most favored object for study of the effect of ions on ciliary motion of bivalve gills has been the frontal cilia of the excised pieces of *Mytilus* gills (Lillie, 1906; Gray, 1922b). Only occasionally were the lateral cilia used in these observations.

The monovalent metallic ions are important in the stability of the ciliated cells and maintenance of ciliary motion. By using a series of samples of artificially varied sea water it can be shown experimentally that the replacement of sodium by other monovalent cations rapidly affects the rate of ciliary beat. The effect is the greatest with lithium and smallest with potassium. In the order of their effectiveness the ions can be placed as follows: $Li < Na < NH_3 < K$. There is, however, a marked difference between the effects produced by sodium and potassium. The frontal cilia beat more rapidly in solutions containing greater concentrations of potassium and are less affected by changes in the concentration of sodium. The laterofrontal cilia of *Mytilus* are affected by potassium in a manner not observed in other cilia. The first reaction to the increased concentration of this ion is an increase in the rate of beating. With further addition of potassium the recovery stroke becomes incomplete and the cilia vibrate very rapidly with greatly reduced amplitude and impaired efficiency.

Magnesium inhibits the beat of the lateral cilia of the excised pieces when the concentration of this metal in the surrounding water exceeds its concentration in the blood. Potassium antagonizes the action of magnesium while sodium produces no such effect.

The difference between the effects of magnesium and potassium is also apparent in the way these

ions act on the stability of the intercellular matrix. Under normal conditions magnesium is essential for the maintenance of stability. If the gill preparation is placed in a medium containing sodium and magnesium, the cells remain stable; these deteriorate rapidly in a mixture of magnesium and potassium. It is probable that the potassium ion drives away magnesium from certain areas inside the cell and sodium ions do not (Gray, 1922b). In the absence of calcium the rate of ciliary beat is gradually decreased and eventually ceases (Gray, 1924), but the increase of calcium in the surrounding water produces no marked effect on ciliary motion.

As long as the normal equilibrium of the cations sodium, potassium, calcium, and magnesium is maintained in the surrounding medium, the ciliated cells (of *Mytilus*) are insensitive to changes in the concentration of anions (Cl^-, NO_3^-, Br^{--}, I^{--}, acetate, and SO_4^{--}).

It may be assumed that the results of observations on *Mytilus* gills are applicable to the oyster and that changes in the ionic equilibria in sea water may have a similar effect on the efficiency of the ciliated mechanism of oysters.

HYDROGEN IONS

The effect of variations in the concentration of hydrogen ions on the rate of ciliary motion in bivalve gills is greater than that caused by changes in the concentrations of any other ions. This has been demonstrated on the gills of *Anodonta*, *Mytilus*, *Mya*, and *Ostrea* (Chase and Glaser, 1930; Gray, 1928; Haywood, 1925; Nomura, 1934; Yonge, 1925). The greatest effect is produced by those acids which, like carbonic acid, penetrate the cell surface most rapidly. Nomura (1934) found the following order of efficiency of acids in arresting the ciliary motion of *Pecten:* $H_2CO_3 > CH_3COOH > H_3PO_4 > HCl$. Ciliary activity ceases in 1 minute at pH 3.8 when HCl has been added, but with CH_3COOH or H_2CO_3 the stoppage would occur in the same time at the much higher pH of 5.5. A decrease in the pH values of sea water from 8.1 to 6.1 reduces the ciliary motion of the gills of *C. virginica* to about 37 percent of their normal rate. In these observations by Galtsoff and Whipple (1931) the pH of sea water was changed by bubbling carbon dioxide, and measurements were made of the rate of flow of water produced by the lateral cilia. Ciliary motion stops completely over the entire gill surface of the oyster when the pH of water is reduced to 5.3 to 5.6. Minimum pH in which the cilia can function depends on the concentration with which they are normally at equilibrium. This was demonstrated clearly by Yonge (1925) on the cilia of *Mya*. Thus the average pH inside the style sac of this clam is 4.45 and the cilia of the sac stop functioning below pH 3.5 to 4.0, while the gill cilia normally surrounded by sea water of about pH 7.2 come to a standstill at pH 5.2 to 5.8.

VARIOUS DRUGS

The effects of various drugs on ciliary motion of the gill epithelium of *Anodonta*, *Pecten*, *Mytilus*, and *Ostrea* have been observed by various investigators.

The reaction to any effective drug usually takes place in four consecutive stages: (1) retardation of the frequency of beat, (2) disappearance of metachronism along the ciliary tract and its perseverance within the individual cells (unicellular metachronism), (3) synchronous beating of the cilia of a single cell (disappearance of unicellular metachronism), and (4) cessation of beat.

The degree of depression depends on the concentration of the drug used and the duration of its action. Cessation of beat in the gills of *Anodonta* was observed in the following compounds (Bethell, 1956): 0.5 percent chloral hydrate (in 4 to 5 minutes); 1 percent novocaine (9 minutes); 1.5 percent pilocarpine hydrochloride (in 10 minutes). In 1 to 1.5 percent veratrine sulfate the metachronal wave slows until movement ceases. Caffeine (2 percent solution) accelerates the ciliary motion for 3 minutes and in 6 minutes completely depresses it. The effect of adrenaline on the gills of *C. gigas* was studied by Nomura (1937). The rate of ciliary motion was observed on excised oblong pieces of the gill that were placed in a graduated, narrow glass tubing. They crawled along the glass surface of the tubing, and their advance during 1 minute was recorded. The crawling velocity in various concentrations of adrenaline also was recorded, and the degree of depression of ciliary motion was expressed in percentage of the velocity attained in natural sea water. The results show that the ciliary movement is depressed in proportion to the concentrations, which varied from 10^{-10} to 10^{-5}.

Observations made in the Bureau's shellfish

laboratory at Woods Hole using adult *virginica* showed that 5 ml. of 1 percent solution of chloral hydrate applied to the mantle cavity of an oyster kept in a 4 l. tank of slowly changing water depressed the beating of the lateral cilia by 50 to 87 percent. Twenty-five minutes after the removal of the drug the effect disappeared and normal (i.e., preceding) rate of ciliary motion was reestablished. Application of 1 ml. of 0.1 percent chloral hydrate to the mantle and gills had no visible effect, but 4 ml. of the same concentration injected in the vicinity of the gills increased the ciliary motion by 15 percent. The effect lasted only a few minutes.

In the above experiments the duration of the drug action was brief since the oysters were kept in running sea water. Different results were obtained when the test oysters were left in stagnant water. No appreciable effect was noticed in 0.015 percent solution of chloral hydrate, a slight decrease (about 12 percent) was recorded in 0.019 percent, and the ciliary action stopped in 0.03 percent solution.

Slight depression of ciliary motion (from 11 to 13 percent) was obtained by a single 1 ml. dose of nembutal solution (concentration 0.02 g. per l.) injected directly into the mantle cavity. No decrease in ciliary motion appeared in the control tests in which 1 ml. of sea water was injected. Ciliary activity in all these tests was measured by determining the velocity of the cloacal current.

Introduction of 3 ml. of digitalin (1:500) into the pallial cavity results in an immediate, 90 percent depression of ciliary activity. Figure 136 represents part of the record obtained by using the electric drop counting method described in chapter IX, p. 190. The effect is dissipated in about 2 minutes.

A solution of pilocarpine of 1:10,000 in sea water applied directly to the excised pieces of *C. virginica* gills has no effect on lateral cilia. In the test made in the Woods Hole laboratory the frequency of beat in natural sea water varied in this experiment from 10.5 to 11.4 per second, and from 10.3 to 10.9 per second after addition of the drug. The concentration of 0.5 percent slowed down the frequency by approximately 40 percent (6.5 to 6.8 per second). All observations were made at 23.5° C. Atropine sulfate solution of 1:1,000 had only a slight effect on the frequency of beat of the lateral cilia, reducing it by about 17 percent at 22.3° C.

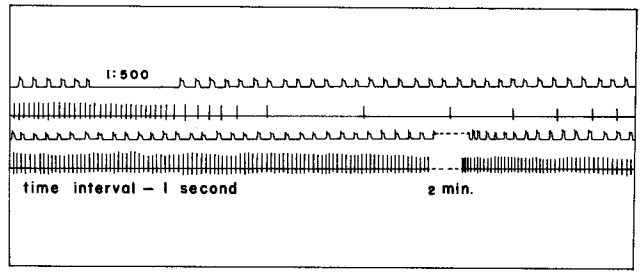

FIGURE 136.—Kymograph record of the effect of digitalin (1:500) on the rate of ciliary activity of the gill of *C. virginica*. Electric drop counting method. First and third line indicate time intervals of 1 second; dotted line marks the 2 minute interruption in recording. Second and fourth lines show the contacts made by each drop of water discharged through the cloaca. Two ml. of digitalin solution were injected into the pallial cavity in 5 seconds, which are indicated at the top by the straight line which interrupts the beat recording. A signal key was depressed for 6 seconds (upper line) when the digitalin was being added.

The effects of acetylcholine and eserine are of particular interest because of their importance to the functioning of the neuromuscular mechanism. Eserine inhibits the action of choline esterase, the enzyme which hydrolizes acetylcholine and prevents its accumulation. The latter would cause an excessive neuromuscular activity. Nomura and Kagawa (1950) found that at concentrations higher than 10^{-6} both acetylcholine chloride and eserine inhibit ciliary movement of the gills of *C. gigas*. These investigators deduced from their observations and from the experiments of Nomura (1937) that acetylcholine and adrenaline, while inhibiting ciliary motion in the oyster, have the opposite effect on the heart of this mollusk.

INHIBITION OF CILIARY MOVEMENT BY ANTISERUM

Antiserum produced in rabbits by the injection of minced gill tissue of *Anodonta* inhibits ciliary motion of the gills of this species. This observation of Galli-Valerio (1916) was confirmed by Makino (1934) for *C. gigas*, *Meretrix*, and other bivalves.

The problem was further studied by Tomita (1954, 1955), who improved the technique of preparation of the antisera by eliminating the preservatives (merthiolate and phenol) which are known to depress the ciliary motion in the concentrations commonly used for this purpose.

733-851 O—64——10

The antigens were prepared by Tomita in the following manner. The gills of *C. gigas, Anadara inflata,* and *Pecten yessoensis* were minced in 0.85 percent saline and homogenized in a blendor. The protein content of the homogenate was estimated from the determination of nitrogen made by microKjeldahl method, and the preparation was diluted with saline to give the final protein content of 1 mg. per ml. Merthiolate in the concentration of 1:10,000 was added as a preservative. On alternate days a quantity of antigens containing 2.5, 5.0, and 7.5 mg. of protein per kg. of body weight were injected into healthy rabbits. After 2 weeks the animals were bled and the antisera were placed aseptically in sterile ampules without any preservatives and stored in a refrigerator.

Small pieces of gill tissues, 3 to 4 mm. long and 3 mm. wide were cut from the free margin of the middle demibranch and placed in sea water in a glass tubing about 12 mm. in diameter. The relative speed of crawling estimated by Nomura's method (1937) was taken as a measure of ciliary activity in normal sea water (100 percent efficiency) and in various dilutions of the antiserum. Complete stoppage of crawling was recorded in the dilution 1:40 after 32 minutes. Considerable depression of ciliary motion was noticed in the dilution 1:320 after 77 minutes of exposure. It is regrettable that no observations were made on the ciliary motion of an intact gill or that the frequency of ciliary beat in the excised pieces was not measured by a stroboscope or by any other technique more reliable than the "crawling" method.

The antisera of the two other species of bivalves (*Anadara* and *Pecten*) have an inhibitory effect on the gills of *Ostrea*. The inhibition was, however, less pronounced than that caused by the anti-*Ostrea* serum. The anti-muscle serum tested on the gills of all three species was less effective than the anti-gill serum. The author deduced from these observations that both "tissue-specificity" and "species-specificity" are involved in the inhibitory effect of the antisera.

EFFECT OF PRESSURE ON CILIARY MOTION

Observations of the effects of increased hydrostatic pressure on ciliary motion were made by Pease and Kitching (1939) using the gills of *Mytilus edulis*. Part of an excised gill plate was placed inside the glass chamber of a pressure bomb designed by Marsland, and the surrounding sea water was saturated with veratrine, which according to Gray (1928) considerably prolongs the activity of the cilia.

Under normal pressure the rate of beating, measured stroboscopically, was about 9 to 10 times per second, considerably slower than the normal rate of 15 to 17 per second that one expects at the temperatures of 21° to 24° C. at which the tests were conducted. Apparently the use of veratrine was unnecessary because the duration of the experiments did not exceed 90 minutes and some of them were completed within 8 to 16 minutes. The tests show that a sudden increase in the hydrostatic pressure by 1,000 pounds per square inch or more immediately increases the frequency of beat of the lateral cilia. Decompression results in a reduction in frequency below the normal level and slow recovery. Pressure in excess of 5,000 pounds per square inch decreases the frequency and causes permanent injury. The authors claim that the change in temperature due to compression or decompression is too small to account for the observed effects, because, on theoretical grounds, it may be expected that the temperature increases by 0.6° C. when the water is compressed adiabatically to 5,000 pounds. The actual temperature in chamber of the pressure bomb was not observed.

It would be of interest to repeat these experiments using pieces of gill epithelium kept in normal sea water not poisoned by veratrine.

CILIARY CURRENTS OF THE GILLS

The ciliary currents at the surface of the gills of an intact organ can be observed by dropping small particles (carmine, carborundum, colloidal carbon, and willemite) on the surface of the demibranchs and following under the binocular microscope their movement and direction. The most important contributions to the studies of this subject were made by Wallengren (1905a), Orton (1912), Kellogg (1900, 1915), Yonge (1926), and Atkins (1937, 1938).

There are five major tracts on the surface of the gill of *C. virginica* (fig. 137). The frontal cilia beat parallel to the surface of the demibranch from the base toward its free margin. This current, maintained along all ordinary filaments (or.f.), carries the particles settled on the surface of the gill to the terminal groove (tr.g.). This is lined with ciliated cells that beat parallel to the edge of the gill and push the particles entangled in mucus toward the mouth. Between the plicae the current caused

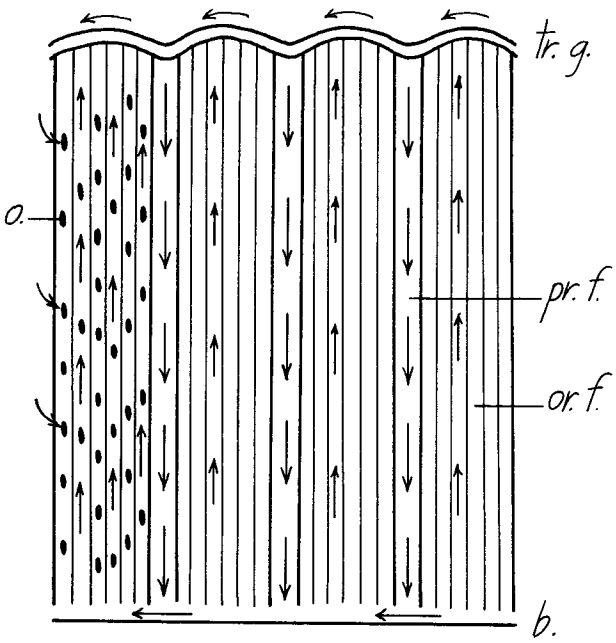

FIGURE 137.—Diagram of the system of ciliary currents on the surface of the demibranch of *C. virginica*. The four plicae are shown slightly pulled apart to indicate the principal (wide) filaments at the bottom of the grooves. Open ostia, o., are shown only on the left plicae; the mouth is toward the left; b.—base of the gills; or.f.—ordinary filaments; o.—ostia; pr.f.—principal filament; tr.g.—terminal groove.

by the frontal cilia of the principal filaments (pr.f.) runs in the opposite direction, i.e., from the free edge of the gill toward the base. Particles carried by this current enter the track along the base of the gills (b.), which runs parallel to the direction of the current in the terminal groove and carries food particles toward the mouth. The lateral cilia (not shown in the diagram) beat at right angles to the surface of the gill and create a current that forces water inside the water tubes and into the epibranchial chamber.

Small single particles fall into the grooves and eventually are carried by the principal filaments toward the mouth while the larger particles or a mass of small ones entangled in mucus are pushed by the frontal cilia toward the free edge of the demibranch and may be dropped from the gill before entering the terminal groove. Frequently a group of particles is passed from the edge of one demibranch to the surface of the underlying one before it is discarded. The complex system of ciliary currents in the gill constitutes an efficient selective mechanism for the sorting of food. Final selection is made along the surface of the labial palps, which reject a large portion of the material brought in by the gills (see p. 115)

The ciliary tracts of the gills of *O. edulis* described by Atkins (1937), in general resemble those observed on the gill of *C. virginica* (fig. 138). In the three species of oysters *C. virginica*, *O. edulis*, and *C. angulata*, the ciliation is essentially the same.

MECHANICAL WORK OF THE LATERAL CILIA

The lateral cilia function principally as movers of water. They force water through the ostia into the water tubes of the gills and maintain inside the gill a current that passes through the branchial chambers to the outside. The hydrostatic pres-

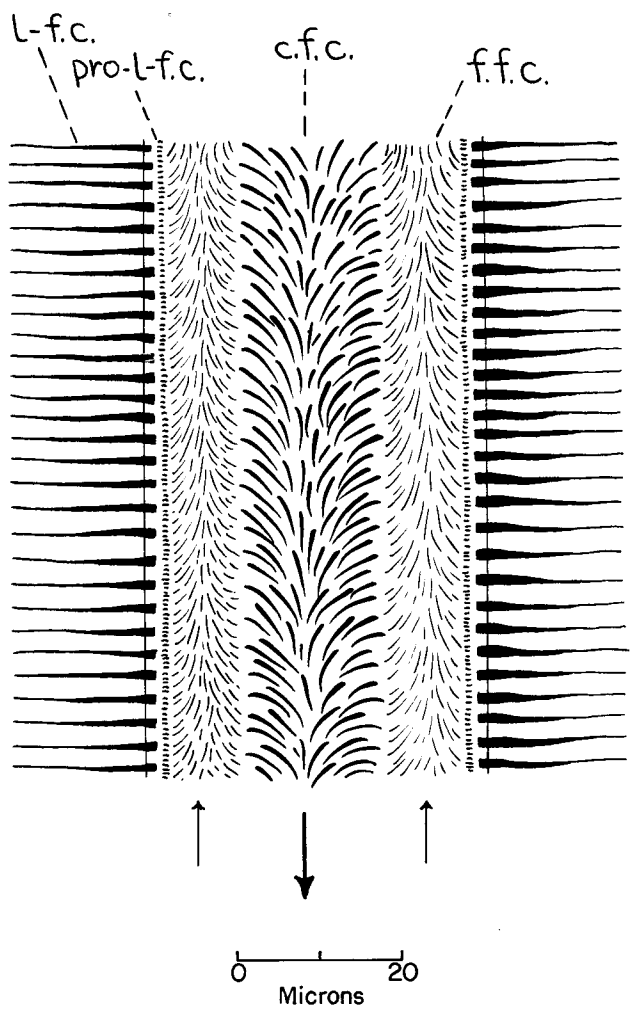

FIGURE 138.—Frontal view of a living filament of *O. edulis*. c.f.c.—coarse frontal cilia; f.f.c.—fine frontal cilia; lf.c.—laterofrontal cilia; pro.lf.c.—subsidiary laterofrontal cilia. From Atkins, 1937, figure 1.

sure inside the gill chamber is maintained solely by these lateral cilia, which form a pumping mechanism with their synchronized beating over the entire gill surface.

Local disturbance in the coordination of ciliary motion caused by the change in the ratio between the effective and recovery strokes or by the changes in the phase of beat results in a drop of pressure and decrease in the current velocity. In the absence of valves or any other regulatory devices, the synchronous beat of the lateral cilia over the entire surface of the gills is an essential condition for the effective functioning of the gill.

One can see under the microscope that slight mechanical disturbances, such as tapping of the dish in which the gill fragments are kept, disorganize the metachronal wave of the lateral cilia and affect the frequency of their beat. The gill may be compared to a folded tubular sieve, with the meshes of the sieve corresponding to the ostia surrounded by the lateral cilia. The contraction of the gill muscles brings the filaments together, constricts the ostia, and reduces the spaces between the filaments. In this way the passage of water through the gill may be restricted.

CARMINE CONE METHOD

The efficiency of the lateral cilia can be measured with a simple device known as the carmine cone method (Galtsoff, 1926). The method is based on measurements of the velocity of the gill's current in a horizontal glass tubing introduced into the cloaca. The valves of the oyster are gently forced apart until they are wide enough to allow the insertion of soft rubber tubing into the cloaca. A wooden wedge is placed between the valves to keep them from closing. The insertion of rubber tubing of a suitable diameter is made by gently rotating it counterclockwise until the rubber is slightly pressed against the outside wall of the cloaca. The tubing is then secured in its position by packing the space around it with cotton. A cotton plug is inserted into the opening of the promyal chamber and is covered with plastic clay. The entire operation can be performed within 2 or 3 minutes and is greatly facilitated by narcotizing the oyster in an 8 to 10 percent solution of magnesium sulfate in sea water.

The oyster with rubber tubing in the cloaca is then placed in a shallow white enamel tray filled with sea water and gently tilted back and forth to remove any air bubbles that may have remained under the valves. A small balloon pipette is introduced into the rubber tubing to suck out the air bubbles that may be trapped in the epibranchial chamber. The presence of the cloacal current is checked by placing a drop of fine carmine suspension against the end of the tubing. The suspension may be added to the gills as well, and in a few seconds a fine carmine cone appears in the cloacal current.

The end of the rubber tubing is now connected to one arm of an inverted T tube which has a slightly curved glass funnel sealed inside the other arm. This arm is joined to a horizontal glass tubing of known diameter, not less than 15 cm. long and graduated in 0.5 cm. (fig. 139). A thistle funnel filled with fine carmine suspension is attached so the vertical arm of the inverted T tube, and the tube and the funnel are held by two clamps mounted on a heavy stand (not shown in the diagram). The carmine suspension must be released by a pinchcock without disturbing the rubber tubing inserted in the cloaca, and the amount released must be very small in order to avoid back pressure of water in the gills. Because of the frictional resistance of water moving inside a circular tube, the highest current velocity is at the center of the cross sectional area of the horizontal tube. A minute quantity of carmine suspension or of a solution of nontoxic dye in sea water released from the funnel forms a sharply defined cone inside the tube, the tip of which moves from zero to 10 or 15 cm. mark; the time of its

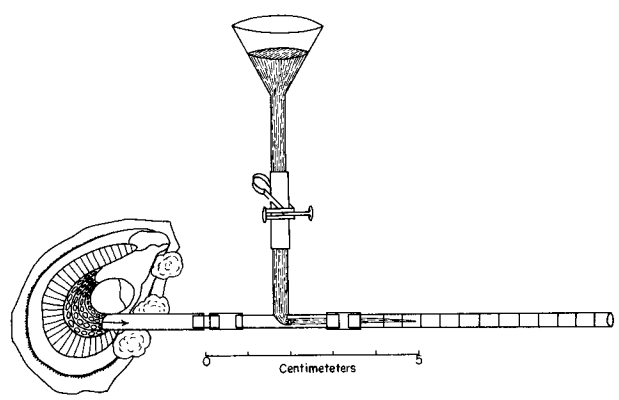

FIGURE 139.—Diagram of the carmine cone method for the study of the efficiency of the lateral cilia of the oyster gill. In order to indicate the position of rubber tubing inside the cloaca, the right valve is not shown; the tank in which the oyster is kept is omitted from the diagram. The funnel with carmine suspension is perpendicular to the plane of the drawing.

passage is recorded by using a stop watch graduated to one-tenth of a second.

Glass tubing of sufficiently wide diameter should be used to avoid turbulent flow. For large *C. virginica* tubing of 5 to 6 mm. in diameter was satisfactory.

The efficiency of the lateral cilia can be expressed either in terms of the velocity of the cloacal current or by computing the mechanical work they perform. The fact that a distinct cone forms at the center of the tube through which the current is running indicates that we are dealing with a viscous flow for which the velocity can be expressed by the Poisseul's formula:

$$S=\frac{D^2\Delta p}{16\mu l}$$

In this formula S is the velocity at the axis of the tube in cm./sec.; D is the diameter; and l the length of the tube in cm.; $\Delta\ p$ is pressure drop between the two marks along the tube in dynes/cm.^{2}; and μ is viscosity of sea water in poises (C.G.S. unit).

The mean velocity (Sm) of the current of the entire cross sectional area of the tube is one-half the velocity at the axis. The rate of discharge, V, in cc. per second is computed by using the following formula:

$$V=\frac{\pi D^2 0.5S}{4}$$

The rate of mechanical work W (in ergs per second) can be determined from the formula: $W=2\pi l\mu S^2$. For a detailed discussion of the mechanical activity of oyster gills, the reader is referred to the original publication of Galtsoff (1928b).

The cone method is simple and requires no elaborate equipment. It can be used in any field or temporary laboratory and is particularly useful for rapid toxicity tests in tracing the physiologically active components of various pollutants. The method has, however, several limitations that should be kept in mind. First, the volume of water passing through the cloaca does not represent the total amount transported by the gills because a certain portion of the water is discharged through the promyal chamber. Second, the tests should be completed in 1 day because the prolonged presence of tubing inside the cloaca and of the wedge between the valves may produce pathological conditions. Because of the sensitivity of the cilia to mechanical disturbance great care should be exercised to avoid jarring, shaking, and vibrating when preparing a test. The oysters usually recover within 12 hours after being placed in running sea water and show no ill effects of the narcosis and handling.

EFFECT OF TEMPERATURE

The cone method proved satisfactory in a study of the effect of temperature on the efficiency of the lateral cilia. The results of many tests performed in the Woods Hole laboratory show considerable variability in the velocity of the cloacal current of oysters of the same size and origin. At a given temperature and under identical conditions the lateral cilia of some oysters work faster than those of others. Consequently, no definite rate of work maintained by the gill epithelium at a specified temperature might be considered as typical or normal for an oyster of a stated size and type.

An example of the effect of temperature on current velocity produced by the lateral cilia of oysters of identical size transporting water at different rates is shown in figure 140. In both experiments the water was agitated by an electric stirrer and its temperature was changed by using heating or cooling units placed at the end of the tank farthest from the oyster. Not less than 15 minutes for adjustment was allowed at each temperature step. Readings were made starting at 20° C. and decreasing to the extreme low temperature at which no current was produced. Then the water

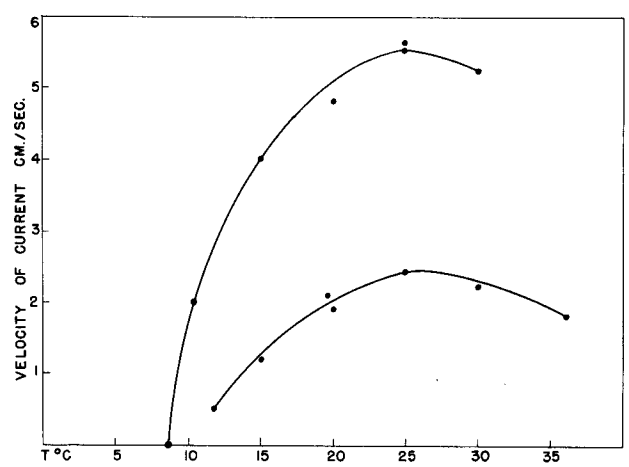

FIGURE 140.—Effect of temperature on the velocity of the cloacal current produced by slow (lower curve) and fast (upper curve) adult oyster, *C. virginica*, of about 4 inches in height. Each dot represents the mean velocity of the current of 10 consecutive readings made at intervals of 2 to 3 minutes. Carmine cone method.

was warmed to the extreme high and cooled again to 20° C. for the last observation. Each circle represents a mean of 10 consecutive readings made at intervals of 2 to 3 minutes. The lower curve represents the activity of an oyster in which slow ciliary motion started only at 11.3° C. The upper curve is typical for an oyster which maintains a rapid transport of water. In both curves the maximum activity occurred at 20° to 25° C. Rapid acceleration in the rate of current took place between 10° (or 11.3°) and 15° C. Essentially the relationship between the temperature and current velocity is similar to the effect of temperature on the frequency of beat of lateral cilia shown in figure 135, although the slope of the latter curve is steeper than in the two curves shown in figure 140. Within the range of the temperature used in these tests, the action of the cilia was completely reversible.

The increased rate of activity induced by temperature may be expressed by temperature coefficients determined at 10° intervals. These values, calculated from a large number of observations with the cone method and given in table 16, show considerable difference in Q_{10} based on the determinations of current velocity and on the rate of work.

TABLE 16.—*Temperature of coefficients (Q_{10}), of the rate of ciliary activity of lateral cells of C. virginica*

Temperature range	Temperature coefficient based on velocity of current	Temperature coefficient based on rate of work performed by the cilia
°C.		
5–10	6.0	4.8
10–20	2.5	4.4
15–25	1.8	2.4
20–30	1.5	1.8
25–35	1.3	1.2

The current velocity is not a true measure of the work performed by cilia because the viscosity of the water changes with temperature. In the formula $W=2\pi l\mu S^2$ the work required to maintain a current at a constant speed is proportional to viscosity, μ. Since at a lowerte mperature the viscosity of sea water is greater than at higher temperatures, more energy is required to propel cold water. As the work needed to produce current of a given velocity is proportional to the square of the velocity at the axis of the current, it is apparent that the decrease in the frictional resistance due to lesser viscosity of water is not sufficient to compensate for the additional energy required for maintaining faster current. Temperature coefficients computed on the basis of the rate of work performed are, therefore, more significant than the Q_{10} based on the velocity of current.

HYDROSTATIC PRESSURE INSIDE THE GILLS

The velocity of the cloacal current is proportional to the difference in hydrostatic pressure inside the gill chambers and at the opening of the cloaca. The pressure can be measured by introducing an L-shaped glass tubing into the free end of the rubber tubing inserted into the cloaca and recording the difference between the level of sea water in the tube and the level in the container in which the oyster is kept. Correction should be made for the position of the meniscus in the tube due to surface tension. Using this simple device I found that in an actively feeding adult *C. virginica* the pressure inside the epibranchial chamber may be as high as 7 to 8 mm. of seawater column. If the temperature and salinity of water are known, the pressure may be calculated in grams per unit area.

SPONTANEOUS INHIBITION OF CILIARY MOTION

When the bivalve mollusks close their shells and cut off their access to outside water, they enter a state of suspended animation during which their normal functions are greatly slowed down or completely cease. This state of diminished activity observed in *Anodonta* and *Sphaerium* (*Cyclas*) was regarded by Gartkiewicz (1926) as sleep. Through the transparent shell of *Sphaerium* he was able to see that the ciliary motion of the gills and the beating of the heart were at a complete standstill when the shells were closed. This observation corrected the erroneous opinion of earlier investigators (Wallengren, 1905a, 1905b) that ciliary activity persists when the valves are closed.

The cessation of ciliary motion after the closing of shells was attributed to the accumulation of carbon dioxide and the decrease of pH. A pH of less than 6.0 probably does not occur in the body fluids of bivalves after they close their valves because of the buffering action of carbonates of the shell substance.

In the gills of *C. virginica* ciliary motion ceases shortly after the closing of the valves and is re-

newed after they open. It is probable that in these cases the depression of ciliary activity is due primarily to the accumulation of metabolites There exists, however, another type of inhibition of ciliary motion that is not associated with changes in the outside environment. It can be observed on gills exposed by the removal of a portion of the valve. The oyster is placed in a suitable container supplied with slowly running sea water, and the gills are strongly illuminated and examined under a dissecting microscope.

The time required for a small inert particle (carmine, or powered oyster shell) to be moved along the terminal groove between the two selected points in the microscope's field of view is recorded with a stopwatch. Copious discharge of mucus that impedes the transport of particles along the groove was avoided by adding only minute quantities of material in suspension. Readings were repeated every minute, and the degree of expansion of the gill lamellae and ostia were recorded. The observations lasted from 10 to 30 minutes. Ciliary motion over the terminal groove of the gill frequently slowed down as the adductor contracted, but previous rhythm was resumed within a few seconds after relaxation of the muscle. The most spectacular were the instances of complete cessations of ciliary motion over the surface of the entire gill following strong contraction of the adductor muscle and complete closure of the valves. Since a portion of the shell was removed the surface of the gill remained in contact with fresh sea water and the cessation of ciliary activity could not be attributed to the accumulation of carbon dioxide or other metabolites.

The association of the inhibition of ciliary motion with the contracted state of the adductor muscle is shown in table 17, which contains excerpts of the records of observations made on two male and two female adult oysters. Temporary depression and sometime stoppage of ciliary motion were often observed after occasional contractions of the gill muscles. In these cases the inhibitory impulses seem to be less pronounced than in the case of the contraction of the adductor muscles. Electric shock applied from the DuBois inductorium direct to the gill epithelium or to the edge of the mantle had no effect on ciliary beat of the frontal and terminal cilia. Only in the case of a shock sufficiently strong to cause contraction of the adductor muscle was there a cessation of ciliary activity.

TABLE 17.—*Association of the velocity of ciliary current along the terminal groove of the external right demibranch and the state of contraction of the adductor muscle*

[Excerpts of the protocols of the four experiments with adult and sexually mature C. virginica made in August at Woods Hole, Mass.]

Sex	Time [1]	Temperature	Adductor	Time needed to move a particle over a distance of 1 cm.	
				Min.	Max.
		°C.		Seconds	Seconds
Female	7:42- 7:44 a.m.	21.6	Relaxed	13.6	13.8
	7:45- 7:47 a.m.	21.6	Contracted	22.0	22.0
	7:48- 8:10 a.m.	21.6	Relaxed	12.0	12.5
Male	10:20- 10:22 a.m.	21.0	Relaxed	22.0	23.0
	10:24-10:27 a.m.	22.0	Partially contracted.	26.6	29.2
	10:30-10:35 a.m.	22.0	Relaxed	22.3	22.6
Female	4:58- 5:08 p.m.	21.5	Relaxed	20.6	22.2
	5:25- 5:33 p.m.	22.0	Contracted [2]	no movement	
Male	3:21- 3:28 p.m.	22.8	Relaxed	14.4	16.6
	3:31- 3:41 p.m.	22.8	Contracted	no movement	

[1] Readings made every minute within time shown in this column.
[2] Complete contraction. Ciliary motion stopped along the entire terminal groove and on the surface of the gill.

Extirpation of the visceral ganglion or its burning with an electric needle had no effect on ciliary motion of the gill, indicating that inhibition does not originate in the ganglion. The frequent coincidence of the cessation of ciliary motion with the contraction of the adductor muscle and the subsequent resumption of ciliary activity after its relaxation suggests the possibility of a neuroid transmission of the inhibitory impulse which may originate during muscular activity and spread over the ciliated surface of the gill.

Since the problem of the impulses causing inhibition of ciliary motion has not been studied sufficiently, it is impossible at this time to present a reasonable explanation of this puzzling phenomenon.

The transport of water by the gills during feeding and respiration is discussed in chapter IX since this function is controlled jointly by the mantle and adductor muscle.

BIBLIOGRAPHY

ALDER, JOSHUA.

1854. A reply to some statements of Dr. Williams on the controversy respecting the branchial currents in the Lamellibranchiata. Annals and Magazine of Natural History including Zoology, Botany, and Geology, series 2, vol. 14, pp. 177–182.

ATKINS, DAPHNE.

1937. On the ciliary mechanisms and interrelationships of lamellibranches. Part II. Sorting devices on the gills. Quarterly Journal of Microscopical Science, vol. 79, No. 315, pp. 339–373.

1938. On the ciliary mechanisms and interrelationships of lamellibranches. Part VII. Latero-

frontal cilia of the gill filaments and their phylogenetic value. Quarterly Journal of Microscopical Science, vol. 80, No. 319, pp. 345–436.

BABÁK, EDWARD.
1913. Zur Regulation des Atemstromes bei den Lamellibranchiaten. Zugleich ein Beitrag zur Physiologie der Flimmerbewegung. Zeitschrift für Allgemeine Physiologie, Band 15, pp. 184–198.

BETHELL, FRANK H.
1956. Factors affecting ciliary activity. *In* William S. Spector (editor), Handbook of biological data, pp. 331–332. W. B. Saunders Company, Philadelphia, Pa.

BHATIA, D.
1926. The structure of the latero-frontal cells of the gills of *Mytilus edulis*. Quarterly Journal of Microscopical Science, vol. 70, No. 280, pp. 681–691.

BROWN, FRANK A., Jr.
1950. Cilia. *In* C. Ladd Prosser (editor), Comparative animal physiology, ch. 18, pp. 640–653. W. B. Saunders Company, Philadelphia, Pa.

BRÜCKE, E. T. VON.
1917. Versuche an ausgeschnittenen und nach einer Drehung um 180° reimplantierten Flimmerschleimhaut-Stücken. Pflüger's Archiv für die gesamte Physiologie des Menschen und der Tiere, Band 166, pp. 45–54.

BUSH, HIRAM J.
1922. The histological structure of the gills of the Najades with special reference to the histology of the groove along the edge of the inner gill. American Midland Naturalist, vol. 8, Nos. 4, 5, pp. 89–104.

CARTER, G. S.
1924. On the structure and movements of the latero-frontal cilia of the gills of *Mytilus*. Proceedings of the Royal Society of London, series B, vol. 96, pp. 115–122.
1927. On the nervous control of velar cilia of the nudibranch veliger. British Journal of Experimental Biology, vol. 4, No. 1, pp. 1–26. (Appeared September 1926).

CHASE, AURIN M., and OTTO GLASER.
1930. Forward movement of Paramecium as a function of the hydrogen ion concentration. Journal of General Physiology, vol. 13, No. 6, pp. 627–636.

CLARK, WILLIAM.
1853. On the branchial currents in the bivalves. Annals and Magazine of Natural History including Zoology, Botany, and Geology, series 2, vol. 12, No. 71, pp. 303–318.

DEROBERTIS, E. D. P., W. W. NOWINSKI, and FRANCISCO A. SAEZ.
1954. General cytology. 2d ed. W. B. Saunders Company, Philadelphia, Pa., 456 pp.

DUBUISSON, M., and J. VAN HEUVERSWYN.
1931. Recherches histologiques et chimiques sur les branchies d'*Anodonta cygnaea* Lin. Archives de Biologie, Paris, tome 41, pp. 37–74.

ELSEY, C. R.
1935. On the structure and function of the mantle and gill of *Ostrea gigas* (Thunberg) and *Ostrea urida* (Carpenter). Transactions of the Royal Society of Canada, third series sec. 5, Biological Sciences, vol. 29, pp. 131–160.

ERHARD, HUBERT.
1910. Studien über Flimmerzellen. Archiv für Zellforschung, Band 4, pp. 309–442.
1928. Methoden zur Untersuchung der Flimmer-, Geissel-und Spermatozoenbewegung. *In* Abderhaldens Handbuch der biologischen Arbeitsmethoden. Abteilung V, Methoden zum Studium der Funktionen der einzelnen Organe des tierischen Organismus. Teil 2, Hälfte 1, pp. 213–280. Urban und Schwarzenberg, Berlin.

FAWCETT, DON W.
1958. Structural specializations of the cell surface. *In* Sanford L. Palay (editor), Frontiers in cytology, ch. 3, pp. 19–41. Yale University Press, New Haven, Conn.

FAWCETT, DON W., and KEITH R. PORTER.
1954. A study of the fine structure of ciliated epithelia. Journal of Morphology, vol. 94, No. 2, pp. 221–281.

FOSTER, ELIZABETH, MARTHA BARNES BAYLOR, NORMAN A. MEINKOTH, and G. L. CLARK.
1947. An electron microscope study of protozoan flagella. Biological Bulletin, vol. 93, No. 2, pp. 114–121.

GALLI-VALERIO, B.
1916. Präzipitine und Trichotoxine für Albumine und Flimmerepithel von *Anodonta anatina* L. Zeitschrift für Immunitätsforschung und experimentelle Therapie, Band 24, pp. 311–314.

GALTSOFF, PAUL S.
1926. New methods to measure the rate of flow produced by the gills of oyster and other molluscs. Science, vol. 63, No. 1626, pp. 233–234.
1928a. Experimental study of the function of the oyster gills and its bearing on the problems of oyster culture and sanitary control of the oyster industry. Bulletin of the U.S. Bureau of Fisheries, vol. 44, for 1928, pp. 1–39. (Document 1035.)
1928b. The effect of temperature on the mechanical activity of the gills of the oyster (*Ostrea virginica* Gm.). Journal of General Physiology, vol. 11, No. 4, pp. 415–431.
1958. Ultrastructure of the ciliated epithelium and the correlation between the ciliary motion and muscular contractions in the gills of *Crassostrea virginica*. [Abstract.] Anatomical Record, vol. 132, No. 3, pp. 440–441.

GALTSOFF, PAUL S., and DOROTHY V. WHIPPLE.
1931. Oxygen consumption of normal and green oysters. Bulletin of the U.S. Bureau of Fisheries, vol. 46, for 1930, pp. 489–508. (Document 1094.)

GARTKIEWICZ, ST.
1926. Contributions a la caractéristique du "sommeil" des Lamellibranches. (Suite). Rythme cardiaque et mouvements de l'epithelium ciliaire. Archives Internationales de Physiologie, vol. 26, pp. 229–236.

GÖTHLIN, GUSTAF FR.
1920. Experimental studies on primary inhibition of the ciliary movement in *Beroë cucumis*. Journal of Experimental Zoology, vol. 31, No. 4, pp. 403–441.

GRAVE, CASWELL, and FRANCIS O. SCHMITT.
1925. A mechanism for the coordination and regulation of ciliary movement as revealed by microdissection and cytological studies of ciliated cells of mollusks. Journal of Morphology, vol. 40, No. 3, pp. 479–515.

GRAY, J.
1922a. The mechanism of ciliary movement. Proceedings of the Royal Society of London, series B, vol. 93, pp. 104–121.
1922b. The mechanism of ciliary movement. II. The effect of ions on the cell membrane. Proceedings of the Royal Society of London, series B, vol. 93, pp. 122–131.
1924. The mechanism of ciliary movement. IV. The relation of ciliary activity to oxygen consumption. Proceedings of the Royal Society of London, series B, vol. 96, pp. 95–114.
1928. Ciliary movement. The Macmillan Company, New York, 162 pp.

HAYWOOD, CHARLOTTE.
1925. The relative importance of pH and carbon dioxide tension in determining the cessation of ciliary movement in acidi ed sea water. Journal of General Physiology, vol. 7, No. 6, pp. 693–697.

HENNEGUY, L. F.
1897. Sur les rapports des cils vibratiles avec les centrosomes. Archives d'Anatomie Microscopique, tome 1, pp. 481–496.

HERDMAN, W. A.
1905. Note on some points in the structure of the gill of the Ceylon pearl-oyster. Journal of the Linnean Society, Zoology, vol. 29, No. 191, pp. 226–229.

JAKUS, M. A., and C. E. HALL.
1946. Electron microscope observations of the trichocysts and cilia of paramecium. Biological Bulletin, vol. 91, No. 2, pp. 141–144.

JØRGENSEN, C. BARKER.
1943. On the water transport through the gills of bivalves. Acta Physiologica Scandinavica, vol. 5, fascicule 4, pp. 297–304.

KELLOGG, JAMES L.
1892. A contribution to our knowledge of the morphology of lamellibranchiate mollusks. Bulletin of the U.S. Fish Commission, vol. 10, for 1890, pp. 389–436.
1900. The ciliary mechanism in the branchial chamber of the Pelecypoda. Science, vol. 11, No. 266, pp. 172–173.
1915. Ciliary mechanisms of lamellibranchs with descriptions of anatomy. Journal of Morphology, vol. 26, No. 4, pp. 625–701.

KRAFT, HEINRICH.
1890. Zur Physiologie des Flimmerepithels bei Wirbelthieren. Archiv für die gesammte Physiologie des Menschen und der Theire, Band 47, pp. 196–235.

LACAZE-DUTHIERS, H.
1856. Mémoire sur le développement des branchies des mollusques acéphales lamellibranches. Annales des Sciences Naturelles, quatrième série, Zoologie, tome 5, pp. 1–47.

LANG, ARNOLD.
1900. Lehrbuch der Vergleichenden Anatomie der Wirbellosen Thiere. Erste Lieferung: Mollusca. Gustav Fischer, Jena, 509 pp.

LENHOSSÉK, M. V.
1898. Ueber Flimmerzellen. Verhandlungen der Anatomischen Gesellschaft auf der zwölflen Versammlung in Kiel, vom 17–20 April, 1898, Band 12, pp. 106–128.

LILLIE, RALPH S.
1906. The relation of ions to contractile processes. I. The action of salt solutions on the ciliated epithelium of *Mytilus edulis*. American Journal of Physiology, vol. 17, No. 1, pp. 89–141.

LUCAS, ALFRED M.
1931. The distribution of the branchial nerve in *Mytilus edulis* and its relation to the problem of nervous control of ciliary activity. Journal of Morphology, vol. 51, No. 1, pp. 195–205.
1932. Coordination of ciliary movement. I. Methods of study and the relation of ciliary coordination to ciliary inhibition. Journal of Morphology, vol. 53, No. 2, pp. 243–263.

LUCAS, ALFRED M.
1935. Neurogenous activation of ciliated epithelium. American Journal of Physiology, vol. 122, No. 3, pp. 468–476.

MAKINO, KATASHI.
1934. Beobachtungen über die Immunitätsreaktionen bei Molluskenarten. Zeitschrift für Immunitätsforschung und experimentelle Therapie, Band 81, pp. 316–335.

M'ALPINE, D.
1888. Observations on the movements of the entire detached animal, and of detached ciliated parts of bivalve molluscs, viz., gills, mantle-lobes, labial palps, and foot. Proceedings of the Royal Society of Edinburgh, vol. 15, pp. 173–204.
1889a. Continued observations on the progression and rotation of bivalve molluscs and of detached ciliated portions of them. Proceedings of the Royal Society of Edinburgh, vol. 16, pp. 725–743.
1889b. Observations on the movements of detached gills, mantle-lobes, labial palps, and foot in bivalve molluscs. Transactions and Proceedings of the Royal Society of Victoria, vol. 24, part 2, pp. 139–149.

MCDONALD, JAMES F., C. E. LEISURE, and E. E. LENNEMAN.
1927. Neural and chemical control of ciliated epithelium. Proceedings of the Society for Experimental Biology and Medicine, vol. 24, No. 9, pp. 968–970.

MENEGAUX, A.
1889. Sur la branchie des Lamellibranches et sur la comparaison avec celle des Scutibranches. Bulletin de la Société Philomathique de Paris, série 8, tome 1, pp. 137–144.

1890. Sur l'endothélium dans les branchies des Pélécypodes. Bulletin de la Société Philomathique de Paris, série 8, tome 2, p. 47.

MERTON, H.

1923a. Studien über Flimmerbewegung. Pflügers Archiv für die gesamte Physiologie des Menschen und der Tiere, Band 198, pp. 1-28.

1923b. "Willkürliche" Flimmerbewegung bei Metazoen. Biologisches Zentralblatt, Band 43, pp. 157-162.

MITSUKURI, K.

1881. On the structure and significance of some aberrant forms of Lamellibranchiate gills. Quarterly Journal of Microscopical Science, vol. 21, pp. 595-608.

1883. On the structure and significance of some aberrant forms of Lamellibranchiate gills. Studies from the Biological Laboratory, Johns Hopkins University, vol. 2, pp. 257-270.

NELSON, THURLOW C.

1938. The feeding mechanism of the oyster. I. On the pallium and the branchial chambers of *Ostrea virginica*, *O. edulis* and *O. angulata*, with comparisons with other species of the genus. Journal of Morphology, vol. 63, No. 1, pp. 1-61.

1941a. On the locus of action of diantlin upon the oyster's gills as revealed by the effects of acetylcholine, eserine, and adrenalin. [Abstract.] Anatomical Record, vol. 81, No. 4, suppl., p. 88.

1941b. Stimulation by the sperm of *O. cristata* of water pumpage in *O.* (*Gryphaea*) *virginica*. [Abstract.] Anatomical Record, vol. 81, No. 4, suppl., p. 88.

NELSON, THURLOW C., and C. M. YONGE

1947. On the early development of the gill of the oyster, *Gryphaea virginica*, and its bearing upon the phylogeny of the ctenidia of Lamellibranchia. [Abstract.] Anatomical Record, vol. 99, No. 4, p. 607.

NOMURA, SHICHIROKU.

1928. Influence of narcotics on ciliary movement of the gill of the oyster. Proceedings of the Society for Experimental Biology and Medicine, vol. 25, No. 4, pp. 252-254.

1934. Studies on the physiology of ciliary movement. II. Intracellular oxidation-reduction potential limiting the ciliary movement. Protoplasma, Band 20, pp. 85-89.

1937. Studies on the physiology of ciliary movement. III. Effect of adrenaline. Science Reports of the Tôhoku Imperial University, series 4, Biology, vol. 12. No. 2, pp. 277-309.

NOMURA, SHICHIROKU, and KEN-ICHI KAGAWA.

1950. Studies on the physiology of ciliary movement. IV. Effect of acetylcholine and eserine. Science Reports of the Tôhoku University, series 4, Biology, vol. 18, No. 4, pp. 437-445.

ORTON, J. H.

1912. An account of the natural history of the slipper-limpet (*Crepidula fornicata*), with some remarks on its occurrence on the oyster grounds on the Essex coast. Journal of the Marine Biological Association of the United Kingdom, vol. 9, No. 3, pp. 437-443.

OSBORN, H. L.

1884. On the molluscan gill. [Abstract.] Johns Hopkins University, Circulars, vol. 3, No. 32, p. 128.

PEASE, DANIEL C., and J. A. KITCHING.

1939. The influence of hydrostatic pressure upon ciliary frequency. Journal of Cellular and Comparative Physiology, vol. 14, No. 1, pp. 135-142.

PECK, R. HOLMAN.

1877. The minute structure of the gills of the lamellibranch Mollusca. Quarterly Journal of Microscopical Science, vol. 17, pp. 43-66.

PETER, KARL.

1899. Das Centrum für die Flimmer-und Geisselbewegung. Anatomischer Anzeiger, Band 15, pp. 271-283.

POSNER, CARL.

1877. Histiologische Studien über die Kiemen der acephalen Mollusken. Archiv für mikroskopische Anatomie, Band 14, pp. 132-157.

PRENANT, A.

1914a. Les appareiles ciliés et leurs dérivés. Chapitre VI. Le mouvement ciliare et flagellaire et ses causes. Journal de l'Anatomie et de la Physiologie Normales et Pathologiques de l'Homme et des Animaux, Cinquantième Année, 1914-1919, pp. 150-204.

1914b. Les appareils ciliés et leurs dérivés. Chapitre VII. Appareils ciliés dérivés directs. Journal de l'Anatomie et de la Physiologie Normales et Pathologiques de l'Homme et des Animaux, Cinquantième Année, 1914-1919, pp. 424-528.

RIDEWOOD, W. G.

1903. On the structure of the gills of the Lamellibranchia. Philosophical Transactions of the Royal Society of London, series B, vol. 195, pp. 147-284.

SATÔ, SYÔITI.

1936. Ciliary movement of the gill of an oyster, *Ostrea gigas*, in relation to temperature. Bulletin of the Japanese Society of Scientific Fisheries, vol. 4, No. 6, pp. 409-410. [In Japanese with English synopsis.]

SCHÄFER, E. A.

1891. On the structure of amoeboid protoplasm, with a comparison between the nature of the contractile process in amoeboid cells and in muscular tissue, and a suggestion regarding the mechanism of ciliary action. Proceedings of the Royal Society of London, vol. 49, pp. 193-198.

SCHMIDT, W. J.

1937. Zur Doppelbrechung der Cilien. Protoplasma, Band 28, pp. 18-22.

SEO, AISABURŌ.

1931. Studies on the nervous regulation of the ciliary movement. Japanese Journal of Medical Sciences, III, Biophysics, vol. 2, No. 1, pp. 47-75.

STAFFORD, JOSEPH.

1913. The Canadian oyster, its development, environment and culture. Committee on Fisheries,

Game and Fur-bearing Animals, Commission of Conservation, Canada. The Mortimer Company, Ltd., Ottawa, Canada, 159 pp.

TOMITA, GUNJI.

1954. Inhibitory effects on ciliary movement of antisera against gill and muscle of some bivalves. Science Reports of the Tôhoku University, series 4, Biology, vol. 20, No. 3, pp. 237–243.

1955. Studies of ciliary antigens of the oyster by absorption experiments. Science Reports of the Tôhoku University, series 4, Biology, vol 21, Nos. 3–4, pp. 219–225.

VERWORN, MAX.

1891. Studien sur Physiologie der Flimmerbewegung. Archiv für die gesammte Physiologie des Menschen und der Thiere, Band, 48, pp. 149–180.

WALLENGREN, HANS.

1905a. Zur Biologie der Muscheln. I. Die Wasserströmungen. Lunds Universitets Årsskrift, Ny Följd, Afdelningen 2, Band 1, No. 2, pp. 1–64.

1905b. Zur Biologie der Muscheln. Die Nahrungraufnahme. Lunds Universitets Årsskrift, Ny Följd, Afdelningen 2, Band 1, No. 3, pp. 1–59.

WENYON, C. M.

1926. Protozoology. A manual for medical men, veterinarians and zoologists. Vol. 1, 778 pp. William Wood and Company, New York.

WORLEY, LEONARD G.

1934. The dual nature of metachronism in ciliated epithelium. Journal of Experimental Zoology, vol 69, No. 1, pp. 105–121.

1941. Microdissection studies of the ciliated depithelial cell. Journal of Cellular and Comparative Physiology, vol. 18, No. 2, pp. 187–197.

WYMAN, JEFFERIES, JR.

1925. Neuroid transmission in ciliated epithelium. Journal of General Physiology, vol. 7, No. 4, pp. 545–559.

YONGE, C. M.

1925. The hydrogen ion concentration in the gut of certain lamellibranchs and gastropods. Journal of the Marine Biological Association of the United Kingdom, vol. 13, pp. 938–952.

1926. Structure and physiology of the organs of feeding and digestion in *Ostrea edulis*. Journal of the Marine Biological Association of the United Kingdom, vol. 14, No. 2, pp. 295–386.