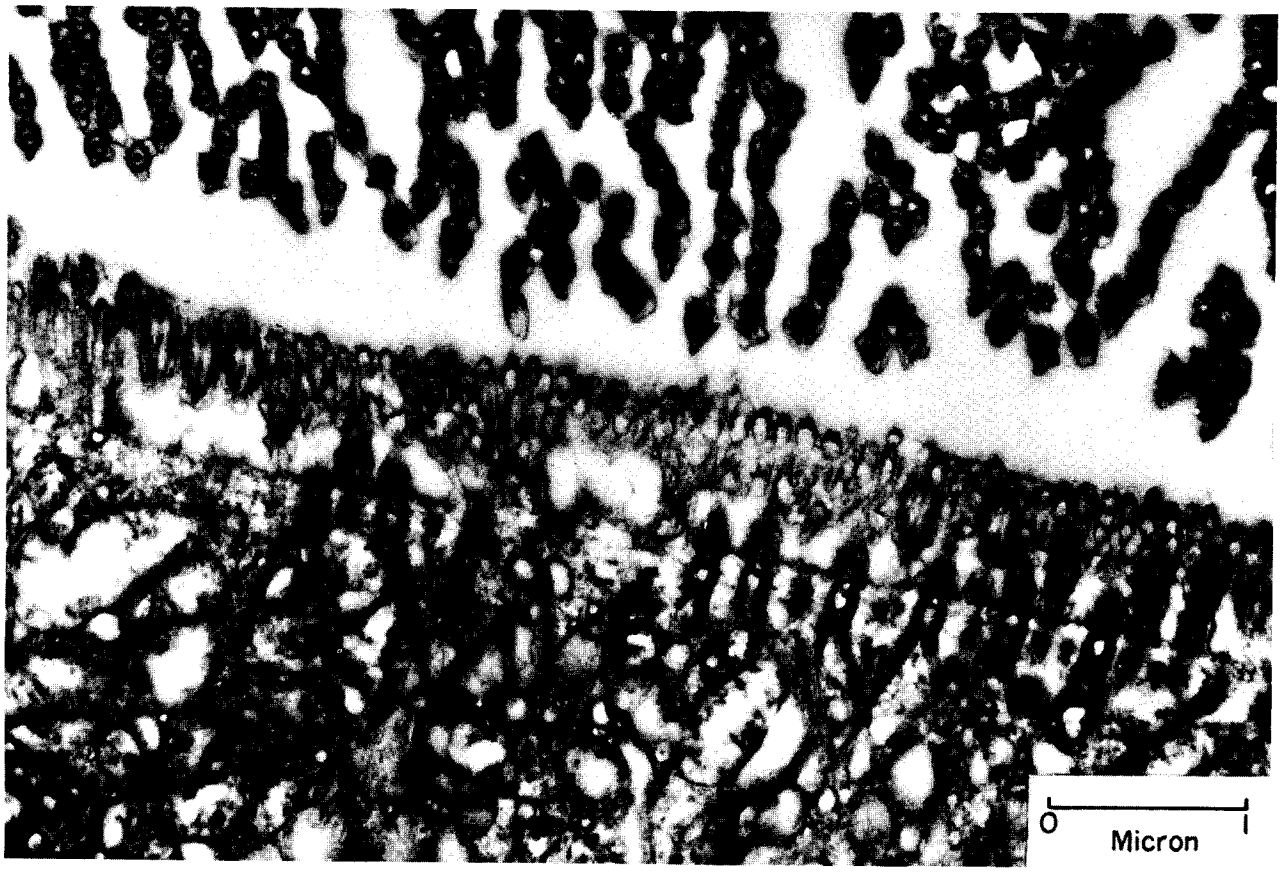

FIGURE 132.—Section perpendicular to the surface of frontal cilia of the filament of the gills of *C. virginica*. The curved frontal cilia are cross sectioned. Note the row of basal corpuscles with rootlets; the sharp line, parallel to the surface of the cell corresponding to plasma membrane; and the 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

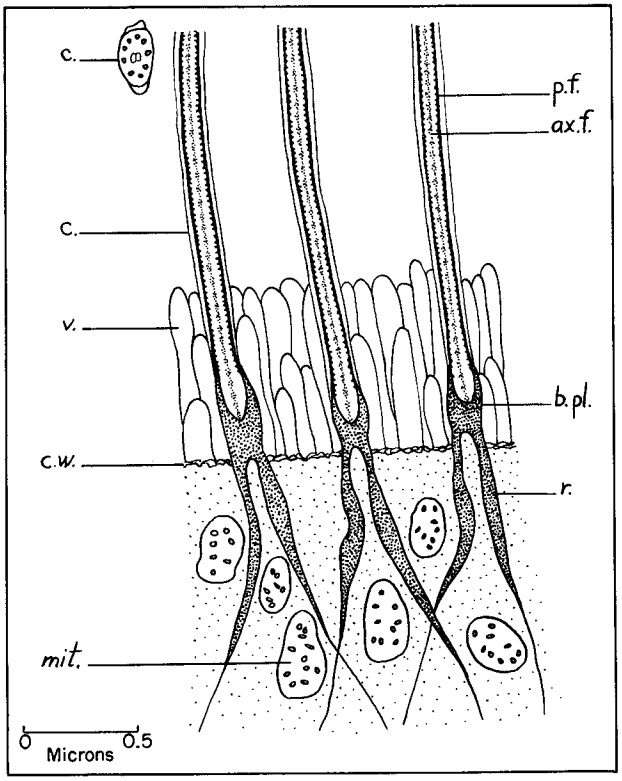

FIGURE 133.—Diagrammatic reconstruction of the distal portion of the ciliated cells of the gill epithelium of *C. virginica*. ax.f.—axial filament; b.pl.—basal plate and basal corpuscle; c.—cilium; cw.—plasma membrane; mit.—mitochondria; r.—rootlet. Cross section of the cilium shown at upper left corner.

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

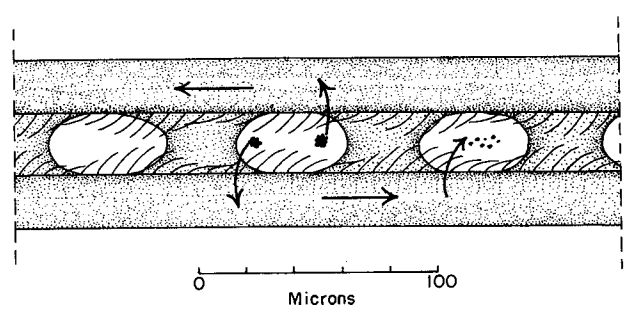

FIGURE 134.—Two tracts of the lateral cilia of *C. virginica* along the two filaments on both sides of the ostia. Small black particles suspended in water are drawn into the ostia while the large ones are discarded by the recovery strokes of the lateral cilia. Drawn from life.

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.

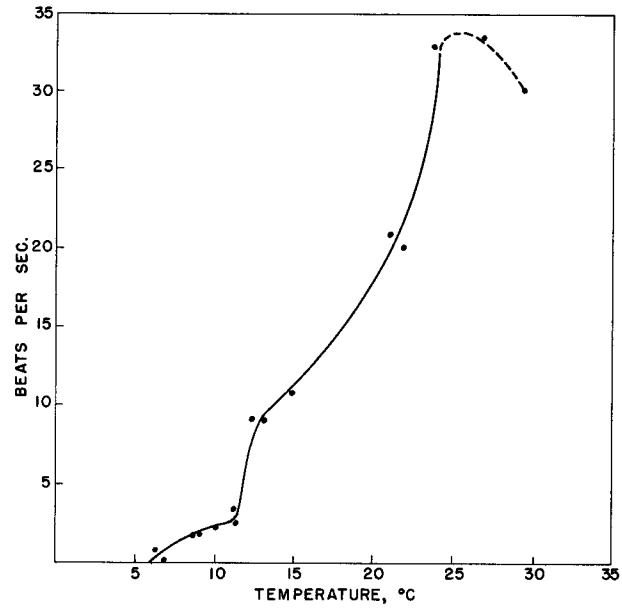

FIGURE 135.—Effect of temperature on the median frequencies of beat in number per second of the lateral cilia of *C. virginica*. Readings were made with Strobotac on a single filament of the gill kept in sea water in a microaquarium. Temperature was changed by circulating warm or cold water in the jacket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

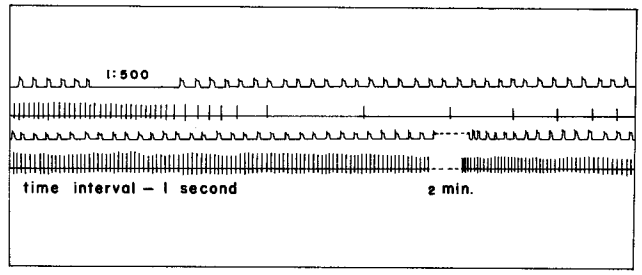

FIGURE 136.—Kymograph record of the effect of digitalin (1:500) on the rate of ciliary activity of the gill of *C. virginica*. Electric drop counting method. First and third line indicate time intervals of 1 second; dotted line marks the 2 minute interruption in recording. Second and fourth lines show 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.

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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

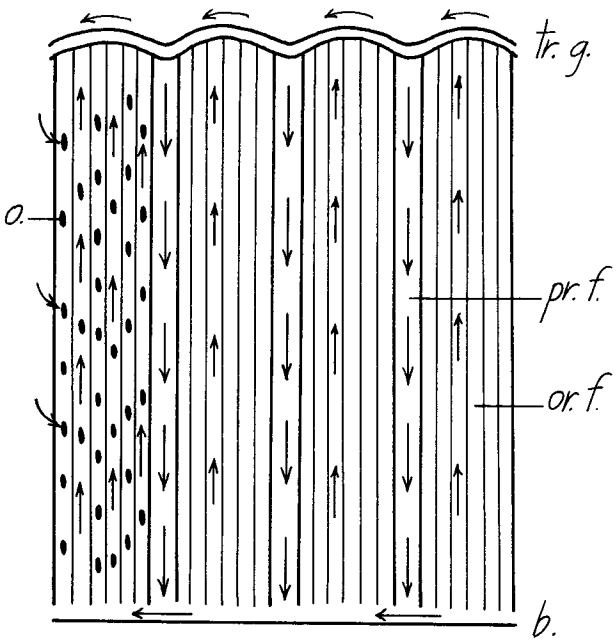

FIGURE 137.—Diagram of the system of ciliary currents on the surface of the demibranch of *C. virginica*. The four plicae are shown slightly pulled apart to indicate the principal (wide) filaments at the bottom of the grooves. Open ostia, o., are shown only on the left plicae; the mouth is toward the 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-

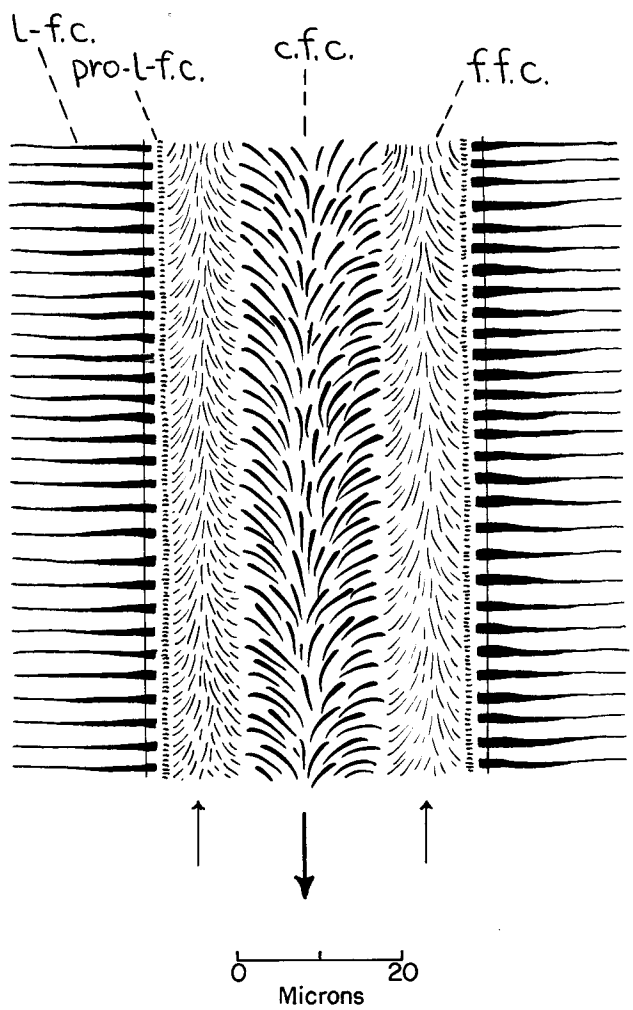

FIGURE 138.—Frontal view of a living filament of *O. edulis*. c.f.c.—coarse frontal cilia; f.f.c.—fine frontal cilia; lf.c.—laterofrontal cilia; pro.lf.c.—subsidiary laterofrontal cilia. From Atkins, 1937, figure 1.

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

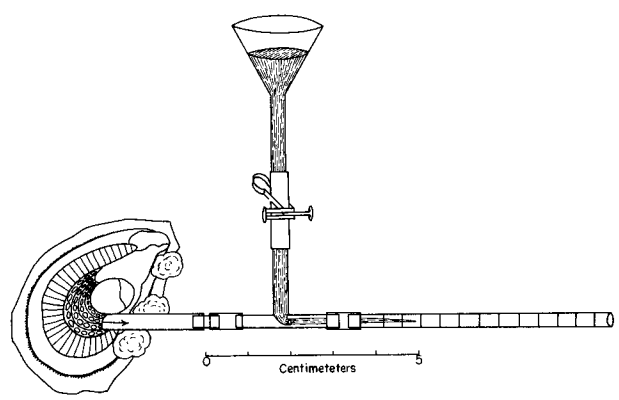

FIGURE 139.—Diagram of the carmine cone method for the study of the efficiency of the lateral cilia of the oyster gill. In order to indicate the position of rubber tubing inside the cloaca, the right valve is not shown; the tank in which the oyster is kept is omitted from the diagram. The funnel with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

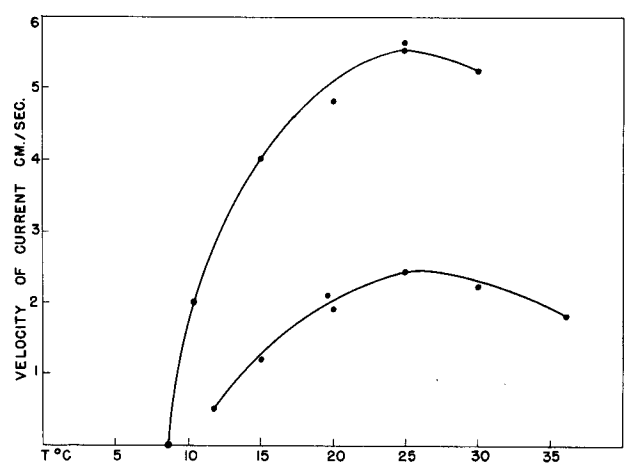

FIGURE 140.—Effect of temperature on the velocity of the cloacal current produced by slow (lower curve) and fast (upper curve) adult oyster, *C. virginica*, of about 4 inches in height. Each dot represents the mean velocity of the current of 10 consecutive readings made at intervals of 2 to 3 minutes.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