CHAPTER VIII

THE ADDUCTOR MUSCLE

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ANATOMY

The adductor muscle of the oyster is a massive organ that controls the opening and closing of the valves. It occupies a slightly asymmetrical position at the ventroposterior part of the body and is surrounded by the following internal organs: the visceral mass, pericardium, epibranchial chamber of the gills, and cloaca (fig. 72). The rectum adheres to the posterior side of the muscle. The protrusion of the visceral mass, containing the crystalline style sac and the lowermost part of the gonad, covers the anterior side of the muscle. A wedge-shaped visceral ganglion located inside the epibranchial chamber rests in a slight depression on the side of the muscle under the visceral protrusion. The ganglion can be exposed by cutting through the wall of the epibranchial chamber and lifting the tip of the visceral mass.

The adductor muscle of the monomyarian mollusks, i.e., those which have only one muscle (such as edible oysters, pearl oysters, scallops, and *Spondylus*), corresponds to the posterior adductor of other bivalves. The anterior adductor, present in larvae, disappears during metamorphosis shortly after the attachment of the larva.

Shortly after the metamorphosis of the larva the posterior adductor muscle develops into the most conspicuous and the heaviest organ of the oyster. In valves of *C. virginica* and in some other species of edible oysters the muscle scar where the adductor is attached to the shell is darkly pigmented. The shape and dimensions of this area are variable (see p. 30 ch. II).

The weight of the muscle of *C. virginica* accounts for 20 to 40 percent of the total weight of the tissues. After spawning, when other parts of the body are watery and poor in solids, the relative weight of the adductor increases. Examples of this condition, usually encountered after the discharge of a large number of sex cells and before the accumulation of the reserve materials (glycogen) in the connective tissue, are given in table 18. It may be deduced from these data that the weight of the adductor muscle is not affected by the changes in the chemical composition which take place in other organs. For further discussion of this problem the reader is referred to chapter XVII of this book.

The adductor is comprised of two distinct parts. About two-thirds of the total bulk of the muscle is translucent, oval-shaped, and slightly concave at

TABLE 18.—*Relative weight of the adductor muscle of six adult* C. virginica *(4 to 5 inches in height) during the spawning season (August) in Woods Hole, Mass. (fresh basis), 1951*

Oyster	Weight		Adductor muscle (total weight)
	Meat	Adductor muscle	
	Grams	*Grams*	*Percent*
Ripe male	17.8	3.5	19.7
Ripe male	15.0	3.7	21.8
Ripe female	18.7	4.4	23.5
Ripe female	6.5	2.1	32.3
Partially spawned female	6.5	2.3	35.3
Spawned out, sex undetermined	5.2	2.2	42.3

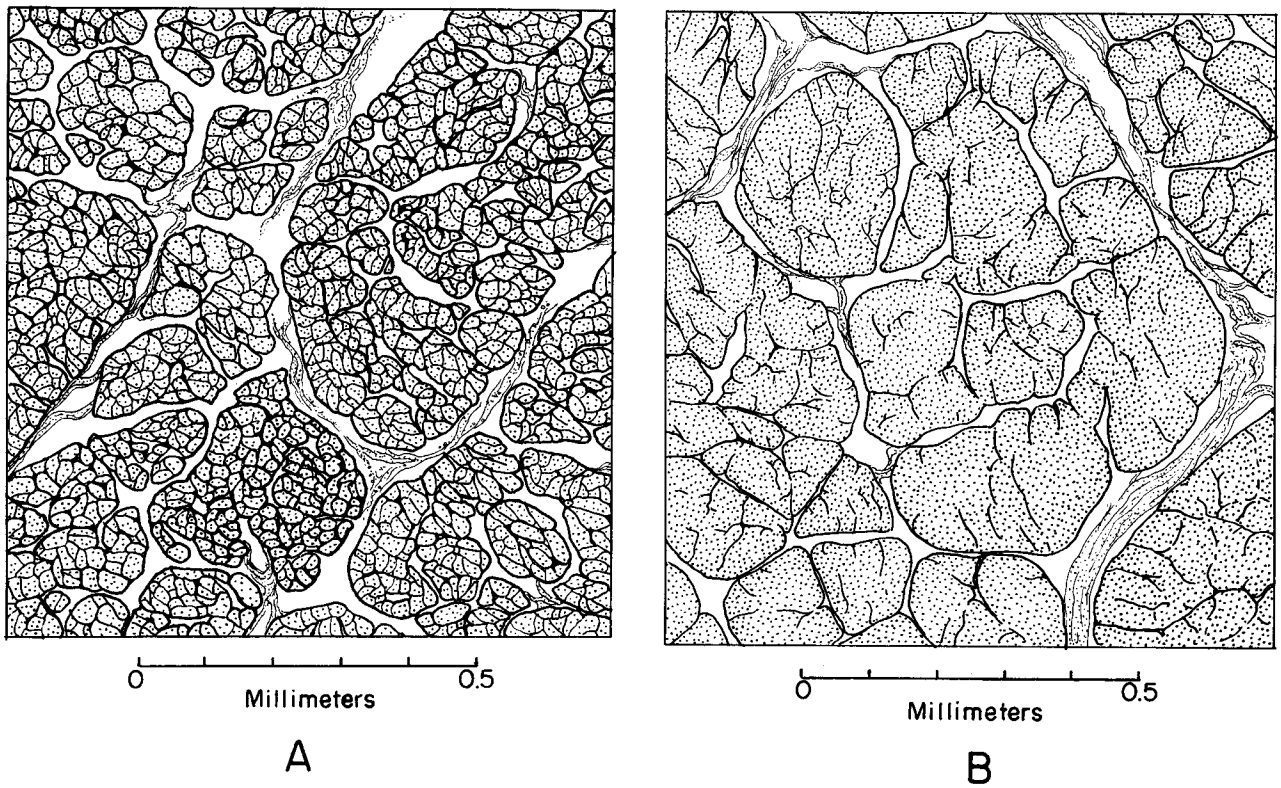

FIGURE 141.—Cross sections of the two portions of the adductor muscle of *C. virginica*. A—white or opaque part. B—translucent part. The muscle bands of the white part are more compact and are surrounded by tougher connective tissue than those of the translucent part (right). Bouin, with formalin hematoxylin-eosin.,

the dorsal side adjacent to the pericardium. This portion is frequently called the vitreous or dark part. The remainder is crescent-shaped and an opaque milky-white. The fibers of this part are tougher than those of the translucent portion; the difference shows clearly when the muscle is being cut or teased.

The fibers of the adductor muscle form dense bands surrounded by connective tissue. In a cross section examined under a low-power microscope (fig. 141) the bands appear as separate units packed more or less parallel to one another. This arrangement is less pronounced in the translucent part (fig. 141, right). The tissue that surrounds the muscle bands is better developed in the opaque section. A layer of connective tissue separates these two major parts of the adductor.

Connective tissue provides a framework for the muscle. Individual fibers do not run the full muscle distance between the two valves; they are anchored at one or both ends in the sheets of tissue which surround the bands. A very thin membrane, called endomysium, invests each muscle cell; the sheathing around the bands of cells is epimysium; the septa which radiate from the latter form perimysium.

The cross-sectional areas and the weight of the two portions vary in different specimens. It was reported by Hopkins (1930) that the ratio of weight of the translucent to the white part of the muscles of oysters growing near Beaufort, N.C., depends on ecological conditions. In the oysters found at the upper limit of their vertical distribution near the high-water level the ratio was 1.26, while in the oysters taken at a level 2.5 feet lower, where they were submerged during about three-quarters of the time, the ratio was 2.51.

The entire adductor muscle is well supplied with blood; wandering leucocytes are usually seen between the fibers and in the connective tissue. Both parts of the adductor muscle are abundantly supplied with nerves. The innervation of the muscle is discussed in Chapter XII.

MICROSCOPIC STRUCTURE

The muscle fibers of the two parts of the adductor differ in both size and structure. The white muscles are smooth and wide, while the dark

(translucent) fibers are thinner and have a peculiar striation which has been described as oblique, double-oblique, helicoidal, and spiral. Some investigators (Kellogg, 1892; Orton, 1935; Hopkins, 1936) and authors of biology textbooks (Borradaile and Potts, 1961) refer to the translucent part as consisting of striated muscles.

Both types of fibers appear under the light microscope as long cylindrical cells, slightly thickened in the middle and tapering toward the ends. An oval-shaped nucleus with one or several nucleoli is near the surface, outside the contracting elements which make up the bulk of the cell. Clear homogenous cytoplasm (sarcoplasm) which can be seen under high magnification forms a very thin surface layer of the cell and around the nucleus. The major part of the cell is made of slender fibrils that differ in their orientation in the two types of muscle cells.

The principal structural elements appear in unstained, isolated fibers examined with phase contrast oil immersion lens under high magnification. Whole mounts can be made after pieces of muscle are macerated in 20 percent nitric acid and then placed in glycerol. Treatment with nitric acid apparently does not affect the visible structure of the fibers. Preparations should be made from fibers which have been taken from both a fully relaxed and a completely contracted adductor. The desirable state of relaxation is obtained by narcotizing the oyster in 5 to 10 percent magnesium sulfate solution for 48 hours; treating the mantle with a strong solution of hydrochloric acid causes long-lasting contraction. In opening the oyster, care should be exercised not to damage the visceral ganglion, since injury to this nerve center may cause relaxation of the adductor.

WHITE MUSCLE FIBERS

White muscle fibers isolated from a completely relaxed adductor of a fully narcotized *C. virginica* are from 2 to 3 mm. long and about 10 μ in diameter. The fibers are too short to stretch from one valve to the other and, with the exception of those attached to the shell, end in connective tissue. Occasionally they bifurcate but do not anastomose. The body of the fiber consists of many fibrils of variable length and a diameter of only a fraction of a micron. The fibrils are oriented parallel to the long axis of the cell and those close to the surface appear to be darker. The arrangement of the fibrils changes somewhat, depending on the state of contraction. Figure 142, A–D, represents four camera lucida drawings made of a white muscle fiber; (A) the fiber is in a completely relaxed state, (B) it is strongly contracted, (C) it is partially contracted, and (D) a noncontracted fiber is folded by the contraction of the surrounding fibers. All drawings were made from glycerin-mounted preparations examined with phase contrast lens. The difference between the relaxed and contracted fiber is primarily in the thickness of the fiber, which in B is about three times greater than in A. In both cases the orientation of fibrils is the same. In a partially contracted and slightly twisted fiber, C, some of the fibrils are at an angle to the long axis of the cell while others retain their original orientation. The fiber D, found in the same preparation with C, is folded but not contracted. Its surface layer of transparent cytoplasm was wider than in the others and the fibrils followed the zig-zag outlines of the fiber. Although the sample was isolated from a contracted adductor, only a few fibers were found in highly contracted state B. The fiber A was separated from a completely relaxed muscle.

DARK MUSCLE FIBERS

The fibers of the dark (translucent) part of the adductor are from 1 to 2 mm. long and in a relaxed state are about 5μ in diameter. When isolated in teased preparations, the fibers have a tendency to twist and coil. The connective tissue around them is less tenacious than in the white muscle, and the fibers can be separated easily by fine needles. As early as 1869 Schwalbe showed that the fast adductor muscle of *Ostrea* is composed of fibers which exhibit a clearly defined diamond lattice pattern. Marceau (1909) maintained that double obliquely striated muscles are widely distributed in the fast parts of the shell closing muscles of bivalves, and Anthony (1918) advanced a theory that oblique striations are a stage in the evolutionary development of transverse striation. The fact that true cross striation occurs in the muscles of *Pecten*, *Lima*, *Teredo*, *Spondylus*, and other bivalves leads to a widely accepted belief that the dark portion of the adductor muscle, also described by some authors as yellow, grey, or tinted (Kawaguti and Ikemoto, 1959), consists of cross striated fibers and that quick movements of these animals are brought about by their contraction.

From their study of the translucent fibers of the adductor of *C. angulata*, Hanson and Lowy (1961)

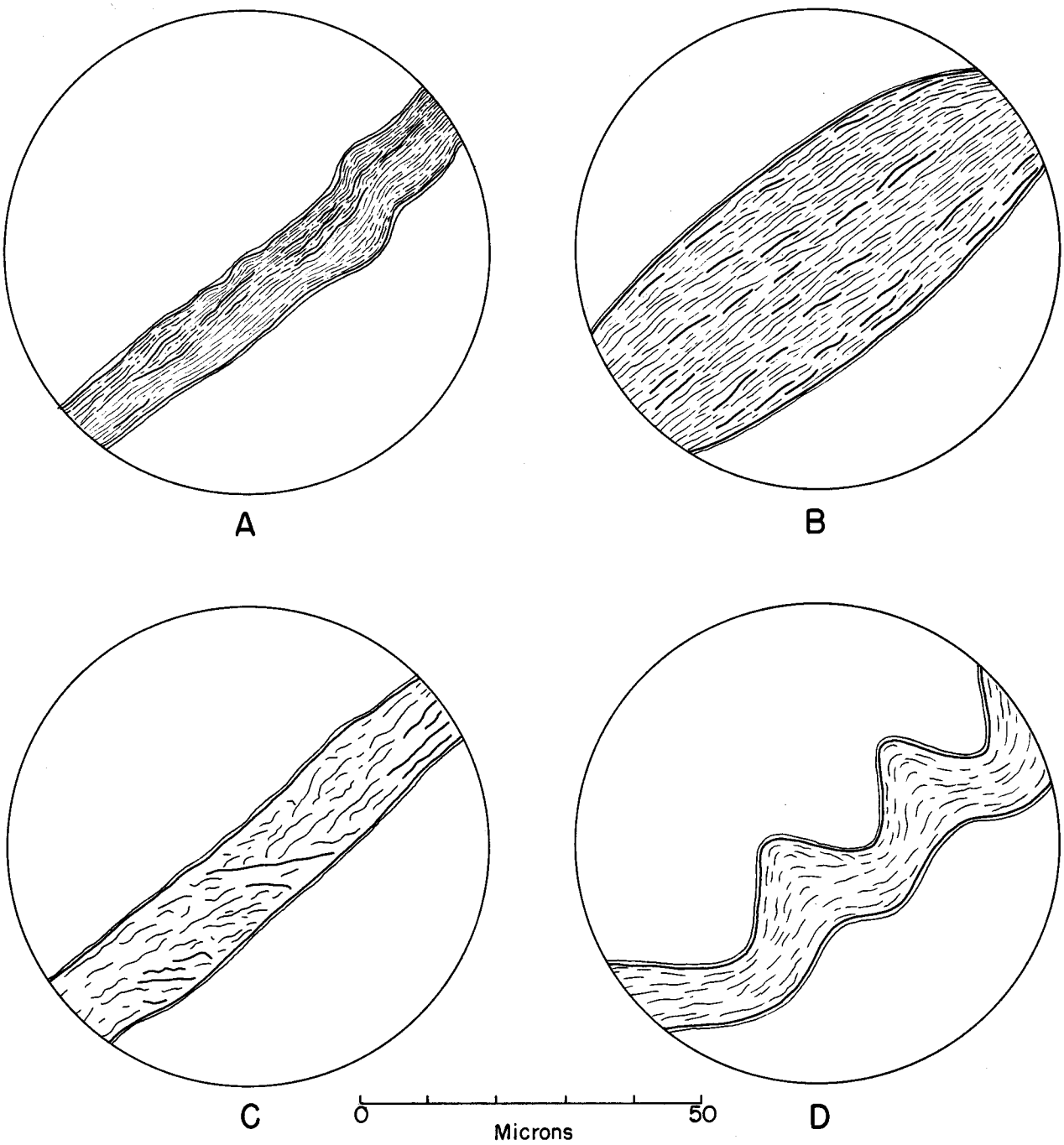

FIGURE 142.—Small pieces of four white fibers of the adductor of *C. virginica* seen under phase contrast lens. Whole mounts of a preparation teased after treatment with nitric acid. Glycerol. A—completely relaxed fiber from narcotized oyster; B—strongly contracted fiber; C—slightly contracted fiber; D—folded but not contracted fiber. Figures B and D are from one preparation of a highly contracted adductor.

concluded that the fibers of that part of the muscle differ from true cross striated muscles in that the bands (A and I) lie at about a 10-degree angle to the fiber axis and are arranged helically around the outer part of the fiber; this produces the double oblique striation visible in the light microscope. Hanson and Lowy's observations were based on electron microscopy, and the bands they refer to as A and I are not visible under the light microscope.

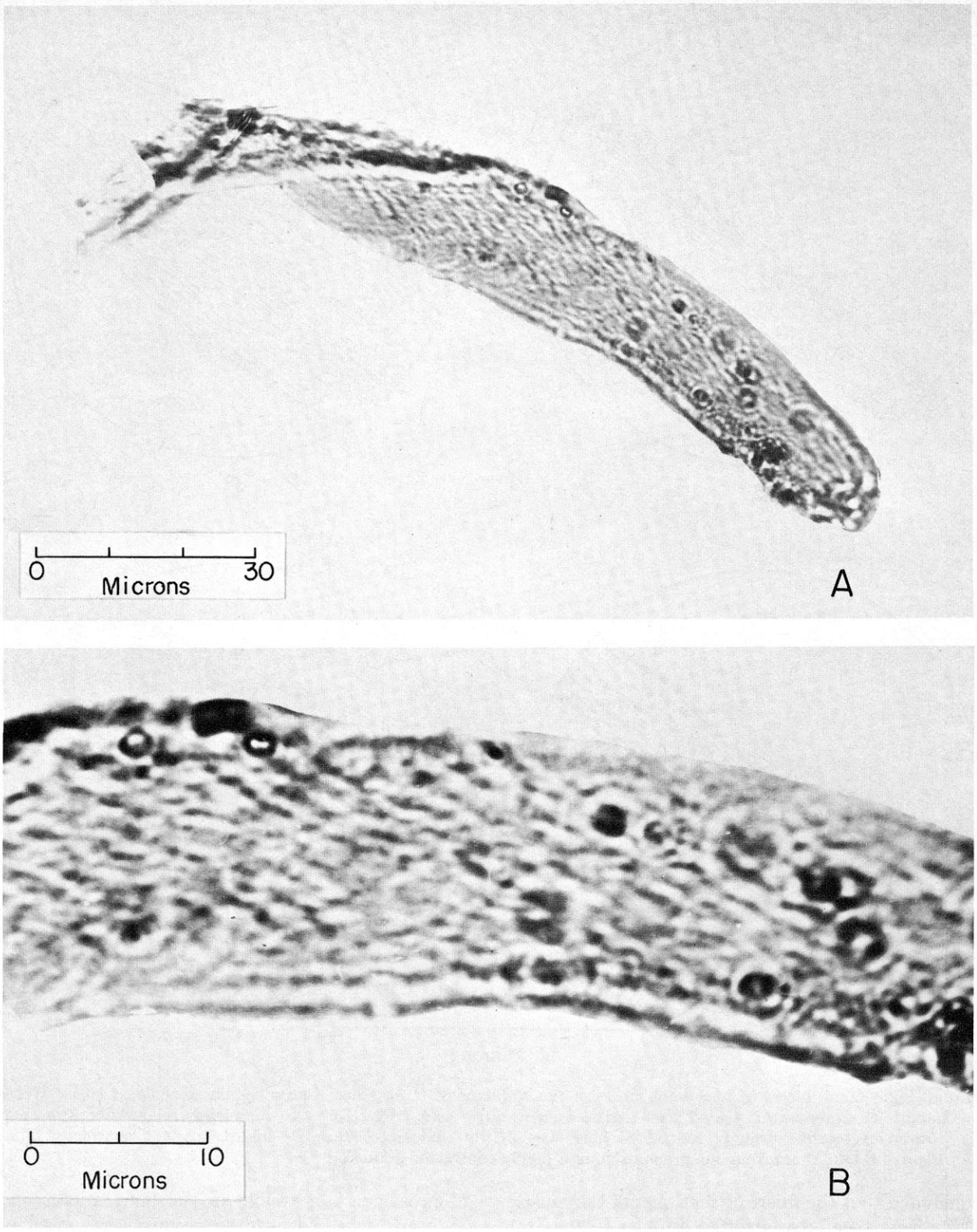

FIGURE 143.—Small piece of dark muscle fiber from the contracted adductor muscle of *C. virginica*. A—Whole mount in glycerol after nitric acid treatment. B—Small portion of the same negative magnified. Round globules are artifacts. Phase contrast lens.

Examination of relaxed fibers of *C. virginica* with phase contrast lenses shows the existence of a distinct diamond lattice pattern shown in figure 143. In the relaxed dark fibers this double oblique striation is absent and the fibrils are oriented parallel to the axis of the cell. My observations confirm the description made by Hanson and Lowy (1957), who found that in helical configuration of myofibrils of the "yellow" part of the adductors of oysters and *Ensis ensis* the angles between the helix and the axis of the fiber increased as the muscle relaxed. The so-called diamond lattice pattern of striation is not a permanent feature of the translucent fiber. It becomes visible in a contracted muscle and is usually confined to the cut ends of the fiber. This observation made by Bowden (1958) for *Ostrea edulis* and *C. angulata* is in accordance with my observations on *C. virginica*.

Considerable advance in the understanding of fine structure of bivalve muscle cells was made by Philpott, Kahlbrock, and Szent-Györgyi (1960), in the work on *C. virginica, Mya arenaria, Mercenaria mercenaria,* and *Spisula solidissima*. Similar studies of *C. angulata* were made by Hanson and Lowy (1961).

With respect to the ultrastructure of the fibers of the adductor muscles of these species, the results of the two investigations are in agreement although they present different theories of the so-called catch mechanism of the adductor, which is discussed later. In both parts of the muscle the fibrils consist of two types of filamentous structures that can be clearly seen on the electron micrograph of the transverse section of the fibril (fig. 144). The thick filaments form the largest part of the fibril; their diameter varies from 250 to 1,500 Å. The thin filaments which occupy the space around the thick ones are about 50 Å. in diameter. The thick filaments have the 145 Å. periodicity associated with paramyosin. The authors surmise that actomyosin is localized in the thin filaments. Hanson and Lowy (1961), in confirming the presence of two kinds of filaments in the fibrils of *C. angulata,* assume that the thinner filaments contain mainly actin. Accord-

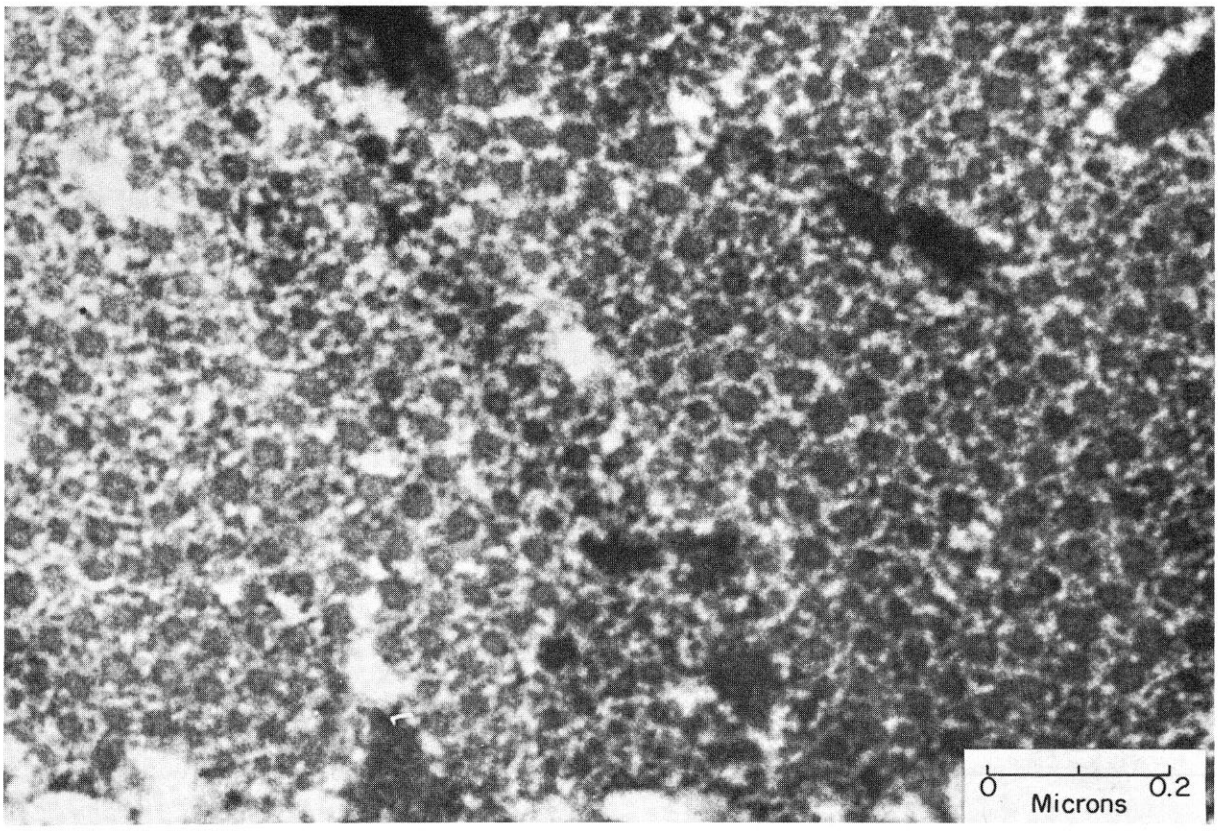

FIGURE 144.—Electron micrograph of a small portion of a muscle fiber of the translucent part of the adductor of *C. virginica*. Courtesy of Philpott and Szent-Györgyi.

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ing to their interpretation of the electron micrographs which accompany their paper, both types of filaments are relatively short in comparison with the length of the fiber; they lie parallel to the fiber axis and are grouped with separate arrays which alternate with each other and appear to be cross-linked by means of transverse projections which belong to the thick filaments. The existence of the projections does not seem to be firmly established, and their connections with the two types of filaments require corroboration. It is obvious from the electron micrographs published by Philpott, Kahlbrock, and Szent-Györgyi, (1960) that filaments are randomly distributed throughout the cross-sectioned area of the fibril.

In the relaxed state the muscle cells are stretched and on longitudinal sections of either part of the adductor appear to be arranged in parallel lines separated in places by connective tissue (fig. 145).

A contracted adductor muscle is strikingly different in appearance from one which is relaxed. Most of the muscle fibers are folded and the entire organ has a herringbone appearance (fig. 146). The uniform thickness of the folded fibers indicates that their actual length is not shortened by the contraction; the fibers are compressed to occupy a shorter distance between the valves. Folding implies the existence of a force that acts parallel to the longitudinal axis of the fibers. The question arises as to the nature of the force that produces this effect. In an attempt to answer

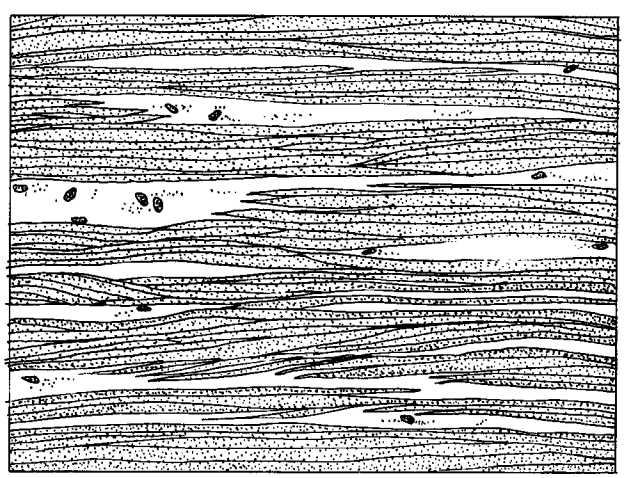


FIGURE 145.—Longitudinal section through a completely relaxed translucent part of the adductor muscle. Bouin, hematoxylin-eosin.

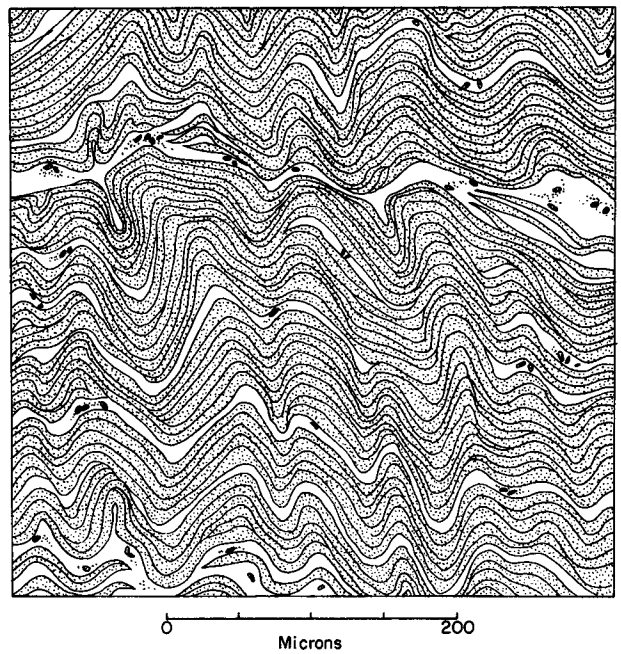


FIGURE 146.—Longitudinal section of the white part of the adductor muscle of *C. virginica* preserved in Bouin with formalin solution. Muscle is in a highly contracted state. Hematoxylin-eosin. Camera lucida drawing.

this question I examined a series of sections of muscles preserved at various degrees of contraction. Oysters were stimulated to close their valves and were preserved in that state by using a strong and rapidly acting fixative applied through an opening cut in a portion of the shell. In such preparations contracted muscle fibers were found only in the area near the attachment to the valves. In the two photomicrographs (fig. 147) the contracted fibers, nearest to the valve (left side), are short, thick, and deeply stained with eosin. The fibers to the right in the same preparation are narrow and folded.

In a partially closed oyster the contracted fibers may be scattered between the folded fibers throughout the entire cross-sectional area. This, condition, shown in figure 148, is drawn from preparations preserved in osmic acid and stained with iron hematoxylin. The contracted fibers appear as isolated dark bodies scattered throughout the moderately folded fibers. It may be deduced from the histological picture that only a small number of muscle fibers are in a contracted state. In order to explain the folding of the noncontracted portion of the adductor it is necessary to assume that a rigidity develops in the contracted fibers in two places—near their contact

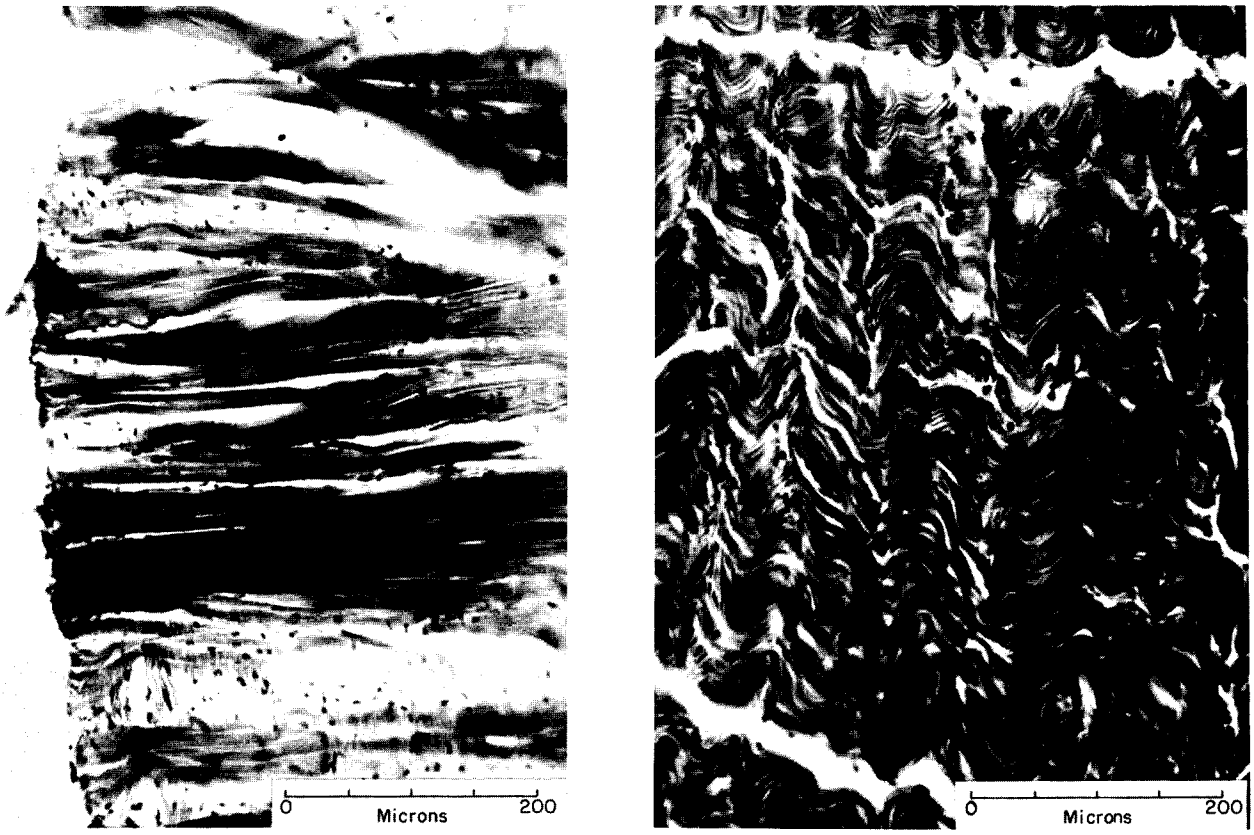


FIGURE 147.—Two photomicrographs of a longitudinal section of the translucent part of the adductor muscle near the valve (the left side of the photograph). The muscle was preserved in a contracted state in Bouin with formalin solution. Note the thick, short contracted fibers on the left and the beginning of folding at the right edge of it. Contracted fibers are deeply stained with eosin. The photomicrograph on the right shows folded fibers a short distance away from the area of the same section shown at left.

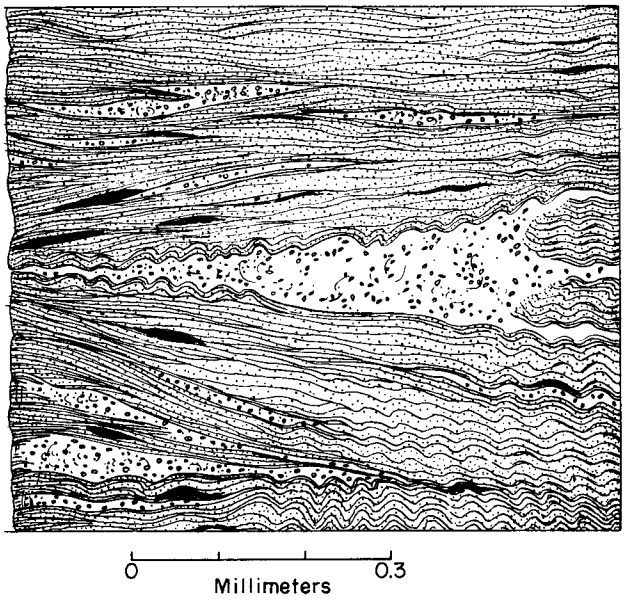


FIGURE 148.—Longitudinal section of partially contracted translucent portion of the adductor. Contracted fibers appear as black spindles. Osmium fixation. Iron hematoxylin.

with the folded fibers and at their anchorage in the connective tissue. Under this condition the contracted portions will bring the valves together and compress the noncontracted fibers into folds. This gives the oyster a considerable degree of flexibility in controlling the degree of opening of the valves.

Observations by Bandmann and Reichel (1955) on *Pinna nobilis* deal with similar conditions. In the smooth muscle of this mollusk plastic lengthening is combined with an orientation of the fiber structure without any changes of its elastic properties. The reverse process (disorientation) takes place during contraction, which is accompanied by an increase in dynamic stiffness. The authors attribute plastic and contractile length alterations to two different mechanisms: change in orientation and change in molecular shape within the contractile elements.

No observations have been made in the living oyster of the contractions of small bundles of fibers that run parallel to the surface of the valves.

These fibers, which are at right angles to the main fibers extending from one valve to the other, are found near the attachment of the adductor to the valves (fig. 149). Their position suggests that they act as braces by bringing together and tightening the principal bundles.

FIGURE 149.—Longitudinal section of a piece of partially relaxed muscle near the attachment to the valve (right side). Note band of muscles at right angle to the main fibers. Kahle, hematoxylin-eosin.

ATTACHMENT TO SHELL

The adductor muscle of *C. virginica* is fastened so strongly to the shell that when the valves are forced apart the muscle breaks in the middle instead of tearing from the shell. The adhesion sometimes withstands a pulling force of 10 kg. (22 pounds). On the other hand, the connection between the muscle and the shell can be weakened or completely destroyed by applying heat to the shell over the area of the muscle scar. This connection is smooth and glossy.

Brück (1914) found that in the shells of *Anodonta* and *Cyclas* the muscles are fastened by means of a specialized layer of cells which he called holding or adhesive epithelium ("haft epithelium").

Hubendick (1958) used both electron and light microscopy to demonstrate the presence of adhesive epithelium in the areas of attachment of the muscles of the fresh-water snail *Acroloxus lacustris* (Maxwell). The surface of the cells has a dense brush border of minute microvilli which are transversed by very thin cytoplasmic fibrils originating in the base of the cell. The epithelial cells are fastened to the underlying connective tissue by the evaginations which extend into the base of the cells. Since the muscles used by Hubendick were fixed in osmic acid, which resulted in their detachment from the shell, the electron micrographs published in his paper do not show the actual connection between the microvilli and shell material. The shell surface over the area of the attachment has, however, small depressions into which fit the tops of the microvilli. It is, therefore, likely that in *Acroloxus* the adhesion of the muscle is accomplished in this manner.

The holding epithelium of *C. virginica* can be seen on transverse sections of decalcified shell and muscle preparations. Individual cell boundaries are indistinct, but the position of each cell is clearly marked by a large round nucleus (fig. 150). Fine strands resembling those described by Hubendick originate in the base of the cells and terminate at their surfaces. They are not visible at low power but can be seen under oil immersion. The holding epithelium of the oyster is a modification of the surface epithelium of the mantle; the transition from one type to another can be seen in the areas adjacent to the muscle attachment (fig. 151). The holding epithelium of *C. virginica* secretes an organic film of about 2 μ in thickness that consists of adhesive material by which the muscle fibers are attached to the shell. The chemical nature of this film was not determined, but staining properties suggested the presence of collagen. Since it is known that under proper conditions collagen is digested by collagenase, I made a series of experiments at Woods Hole to determine the effect of this enzyme on the attachment of muscles. Small amounts of phosphate buffer solution (pH 8.4) containing 1 mg. of collagenase per ml. were injected into adductor muscles through holes

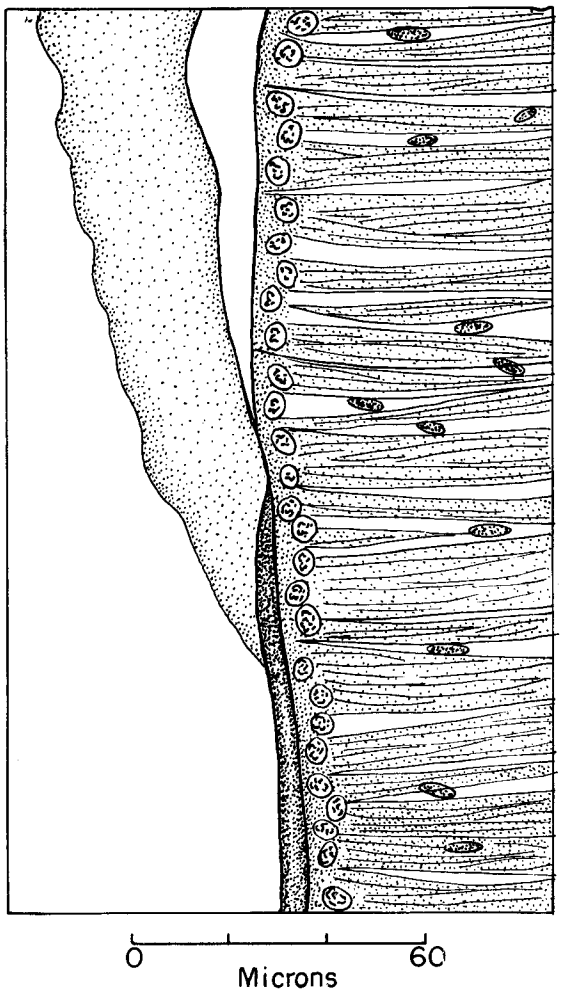


FIGURE 150.—Longitudinal section of the adductor muscle of *C. virginica* where the muscle fibers are attached to the shell. Note the holding epithelium and the cement layer which in the upper part of the illustration is separated from the epithelium and turned over. The decalcified shell is out of the field of view. The cement film is partially detached at the upper half of the preparation and twisted exposing its surface facing the shell; the width of the upper part of the film corresponds to the thickness of the section. Kahle. Hematoxylin-eosin.

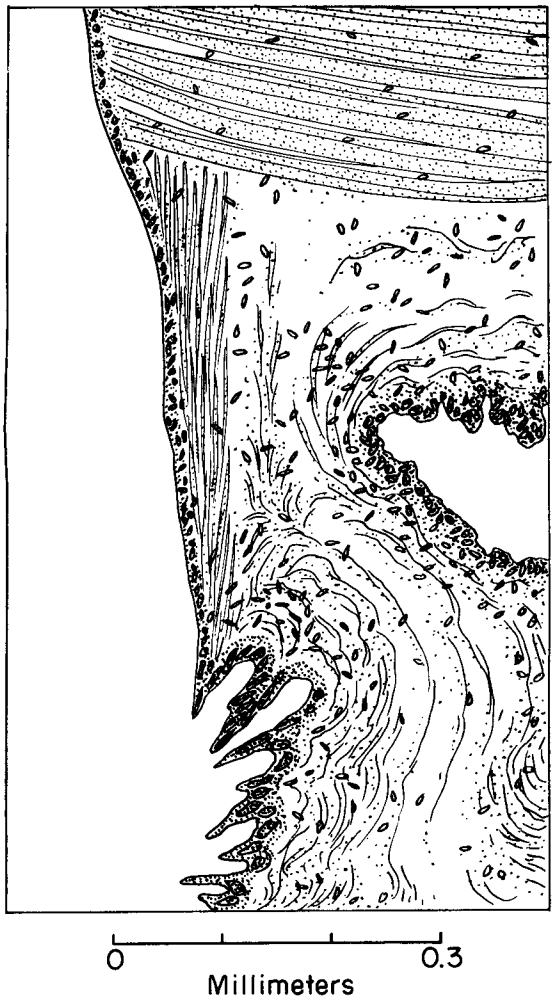


FIGURE 151.—Cross section of the visceral mass of *C. virginica* near the adductor muscle. Notice the gradual change of typical mantle epithelium (left side) into holding epithelium covering the adductor muscle. The shell is not shown. Kahle, hematoxylin-eosin.

drilled in the valves. In another set of experiments the muscles of oysters with the shells attached to them were immersed in the solution of collagenase and were kept at a temperature of 24° to 25° C. for 24 to 48 hours. Solutions of trypsin and phosphate buffer alone, without collagenase, were used for control experiments. In all cases the muscles treated with collagenase became detached within 36 hours. In the controls they remained attached to the shells (fig. 152).

CHEMICAL COMPOSITION OF THE ADDUCTOR MUSCLE

The chemistry of the adductor muscle of oysters has received less attention than that of the muscles of clams, scallops, and sea mussels. Probably the differences in the chemical composition of the muscles of various marine lamellibranchs are not of fundamental nature, although the proportion of various components may vary greatly between the species and even within mollusks of the same species living in different environments. Older reviews dealing with the comparative physiology of the adductor muscle make no distinction be-

tween the various groups of mollusks and combine the data under the general and nonscientific designation of "shellfish" (Katz, 1896; Riesser, 1936). Gross analysis of the adductor muscle of the oyster (*O. imbricata*) (Grimpe and Hoffmann in: Tabulae Biologicae, 1926) shows the following composition: water 66.58 percent; protein 11.38 percent; fat 4.8 percent; and ash 1.1 percent.

INORGANIC SALTS

Studies of the content of the metallic salts in the body of oysters and other bivalves were made by many investigators interested in the problem of osmotic regulation in marine invertebrates. Observations on European oysters, presumably *O. edulis*, made by Krogh (1938) are of particular significance. He found that in the oysters living in waters of high salinity (35°/oo) in France the concentrations of chlorine, sodium, and potassium expressed on the basis of tissue water, were as follows: chlorine 256 mM/kg.[6]; sodium 265 mM/kg.; potassium 46 mM/kg. The next day the oysters were placed in water of lowered salinity (25°/oo) in Limfjord, Denmark, and individual samples were taken at intervals of 1 to 2 days. The results, though somewhat irregular owing to individual variations, showed a decrease in chlorine (221 to 138 mM/kg.) and in sodium (258 to 139 mM/kg.). The potassium increased from 46 to 98 mM/kg.

The mean values for the concentrations of some elements in the adductor muscle of the Australian oyster, *Crassostrea* (*Saxostrea*) *commercialis*, were found to be as follows (Humphrey, 1946):

	Percent Mg.
Potassium	381.7 ±18.9
Sodium	327.9 ±13.0
Calcium	45.76± 3.28
Magnesium	79.93± 3.03
Chlorine	733.4 ±17.3

[6] Values given in millimoles per kilogram of water.

In this case sodium and potassium were present in almost equal amounts (Na:K=0.98) while in *O. edulis* potassium was present in much smaller concentrations and the Na:K ratio varied from 1.6 to 5.8. The concentrations of calcium and magnesium in the whole adductor muscle of *C. commercialis* were found to be 1.1 and 1.5 x 10^2 M, respectively. Both elements are uniformly distributed between the two parts of the muscle (Humphrey, 1949).

ORGANIC COMPONENTS

Glycogen

Bivalve mollusks accumulate considerable quantities of glycogen in their tissues, including the muscles. This reserve material is deposited primarily in the connective tissue of the body parenchym and in the mantle and in smaller quantities is found in the gills and adductor muscles. Analyses made in the Bureau's shellfish laboratory show that on a percentage basis the adductor muscle stores smaller quantities of glycogen than do the gills or visceral mass (table 19).

TABLE 19.—*Solids, water, and glycogen content of the adductor muscles, gills, and remainder of the bodies of* 15 C. virginica *of good quality from the vicinity of Charles Island, Long Island Sound*

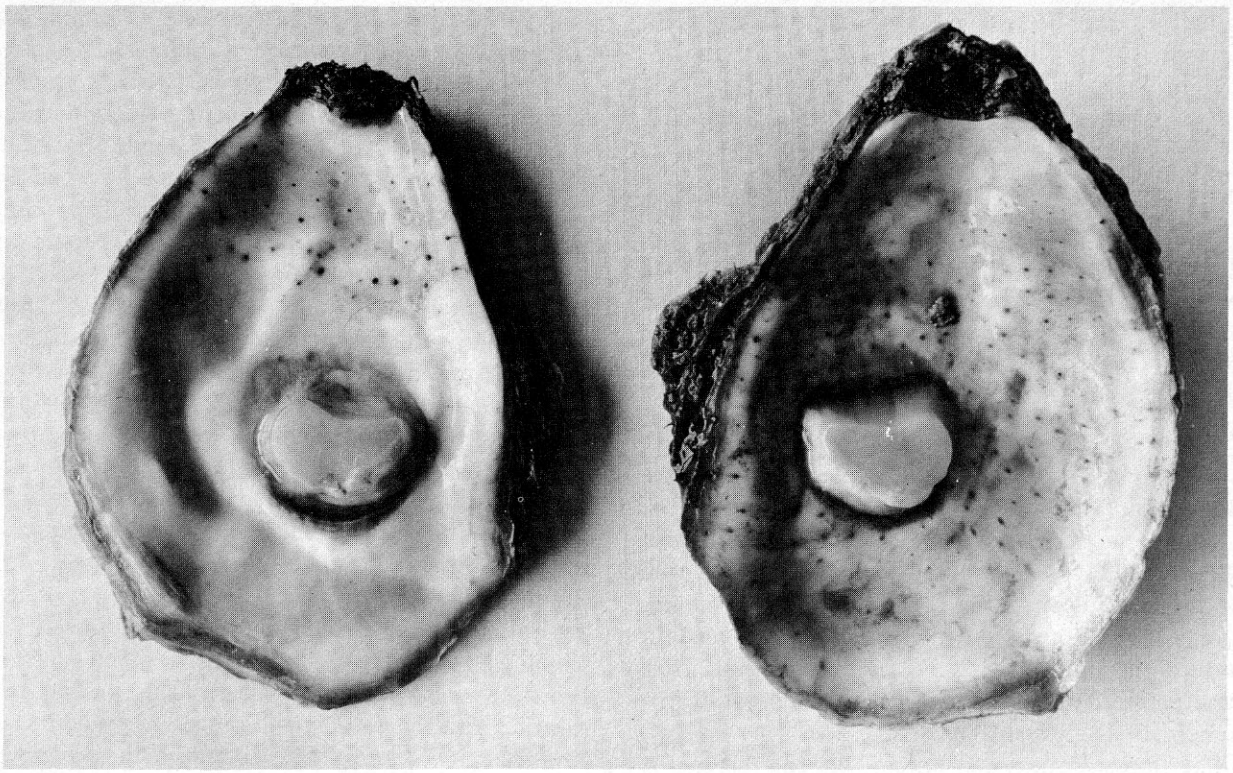
[Average percentages of fresh substance, 1934, 1935]

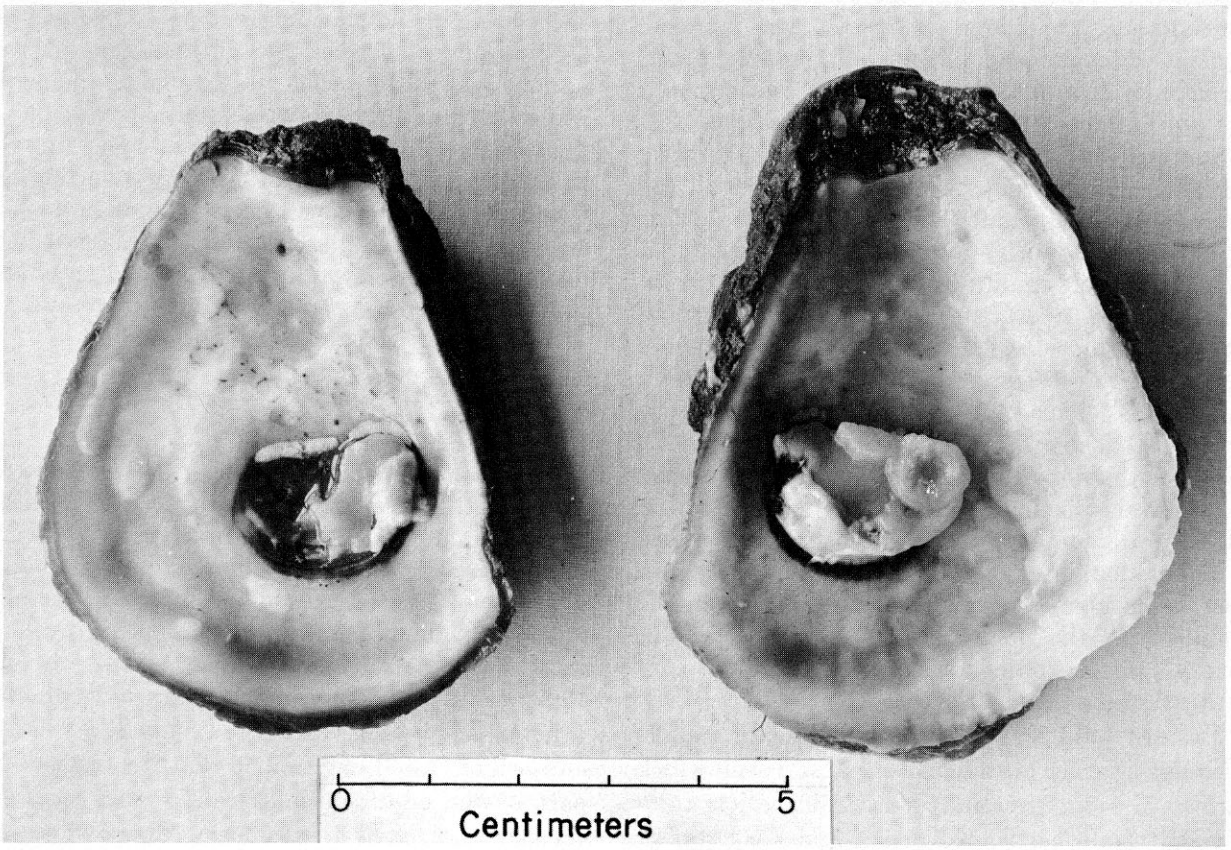
Item	Mantle	Body	Gills	Adductor muscle
November 30:				
Water	----------	73.24	80.20	78.57
Total solids	----------	26.76	19.80	21.43
Glycogen	----------	7.96	4.68	1.69
January 3:				
Water	78.60	74.0	88.52	79.04
Total solids	21.40	26.0	11.48	20.96
Glycogen	3.37	3.96	1.53	1.40

Samples consisted exclusively of adult Long Island Sound oysters of good commercial quality and high content of solids; they were analysed within a few hours after removal from the bottom.

In the Japanese species, *O. circumpicta*, the percentage of glycogen in the two parts of the

FIGURE 152.—Effect of collagenase on the attachment of the muscle of *C. virginica*. Upper row: control—trypsin injected through the hole in the right valve (on left) has no effect on the attachment of the muscle. Lower row: part of the adductor is detached from the right valve after an injection of collagenase. The detached part is seen on the left valve (right side). Twenty-four hours after injection, 24° to 25° C. Left valves of each oyster are on right.

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Centimeters

adductor has been calculated as follows (Kobayashi, 1929):

Translucent portion—	
October	1.12 percent.
November	1.0 percent.
White portion—	
October	1.43 percent.
November	1.29 percent.

The figures are not essentially different from those for *C. virginica*. The questions of how much of the glycogen in the adductor muscle is part of the muscular mechanism and how much of it is stored have not been answered with certainty.

Proteins

According to the data quoted from Tabulae Biologicae (1926), the fresh adductor muscle of *O. imbricata* contains 11.38 percent protein and 4.8 percent fat. No published data are available for the protein content of the muscle of *C. virginica*. It may be assumed that in this species the protein content is not essentially different from that usually found in plain muscles in which it forms from 14 to 18 percent (Evans, 1926).

The contractile mechanism of the adductor muscle of bivalves has the same structural elements as are found in vertebrate muscles: myosin (Florkin and Duchâteau, 1942), actin, and adenosinetriphosphate (ATP). The actin and myosin extracted from muscles of *O. edulis*, *Mytilus edulis*, and *Pinna nobilis* (Lajtha, 1948) have solubility relationships similar to those of the corresponding substances of rabbit muscle (Szent-Györgyi, 1951). The myosin is soluble in distilled water, insoluble in dilute potassium chloride solution (0.002–0.08 M), and again soluble in 0.1 M potassium chloride and higher. It is also soluble in the 0.1 M and stronger solutions of chloride and magnesium chloride. Myosin and actin can be precipitated at isoelectric points of 5.2 and 4.7. They both show double refraction which disappears in dilution or at higher concentration (0.4 M potassium chloride for myosin). Actin has a higher double refraction than myosin. It also has the peculiar property of undergoing reversible change from the globular to the fibrous state and vice versa, depending on the pH and ionic concentration of the medium.

Besides actin and myosin the adductor muscle contains another protein called paramyosin, which differs in solubility and X-ray diffraction from myosin (DeRobertis, Nowinski, and Saez, 1954). Paramyosin was first detected in the adductor muscle of the clam (*Mercenaria* (*Venus*) *mercenaria*) by using electron stains (Hall, Jakus, and Schmitt, 1945). Preparations of muscle fibrillae treated with phosphotungstic acid reveal a periodic structure of alternate bands that show affinity for the stain. The distance between the bands averages 145 Å. At the same time there is a larger period of 720 Å. which is repeated every five spaces of the smaller period (145 × 5). It was concluded by Hall, Jakus, and Schmitt (1945) that the fibrillae of this type consist of paramyosin. Its content in various bivalves varies but is quite high in *Mytilus edulis* in which, according to Lajtha (1948), it exceeds the content of myosin. Paramyosin of the adductor muscle of *C. virginica* was separated from actomyosin by precipitation with three volumes of ethanol at room temperature (Philpott, Kahlbrock, and Szent-Györgyi, 1960) and resuspension of the precipitate in 0.6 M potassium chloride at pH 7.4, which was then dialyzed against the same solution. By such treatment the paramyosin passed into solution and the actomyosin remained precipitated. The yield of paramyosin extracted in percent of total protein was 22 percent in the opaque part, 16 percent in the translucent part. On the basis of biochemical studies the authors suggest that paramyosin is localized in the thick filaments, while the thin filaments consist of actomyosin.

Paramyosin is not found in vertebrate muscles but is the principal protein in many invertebrates (Engström and Finean, 1958). Although its particular role in muscular contraction has not been determined with certainty, it appears probable that this protein is responsible for the maintenance of the tonus of the adductor muscles.

PHYSIOLOGY OF THE ADDUCTOR MUSCLE

The zoologists of the middle 19th century were aware of the difference in the function of the two parts of the adductors of bivalves. They regarded the white part as a bunch of elastic bands which counteracted the pulling force of the valve ligament and the translucent part as an ordinary muscle which brought the valves together (Bronn, 1862, p. 359). Coutance (1878) and Jhering (quoted from Marceau, 1904a) and later Jolyet and Sellier (1899) maintained that the translucent part of the adductor muscle of *Pecten maximus* consists of striated anastomosing fibers whose exclusive function is to close the valves; they

observed that the white part of the adductor contracts very slowly and can remain in a contracted state for a long time. Marceau (1904a, 1904b) confirmed these results by a series of experiments. He cut off either white or translucent portions and found that in *O. edulis* the rapid closing of the valves is accomplished by the contraction of the translucent part of the muscle while the elasticity and tonus of the white part counteract the pulling force of the ligament. Useful reviews of many investigations dealing with the muscle physiology of bivalves and other invertebrates are found in the papers of Ritchie (1928), Jordan (1938), Evans (1926), and others.

It is a well-established fact that the two parts of the adductor muscle contract at different speeds. In scallops the isolated striated (translucent) portion contracts in about 100 microseconds (μ sec.); its relaxation time is about 0.1 second (sec.) (Bayliss, Boyland, and Ritchie, 1930). In the slow part of the adductor the contraction time varies from 500 μ sec. to 2.5 sec. and the relaxation time is from 10 to 45 sec. The contraction of the adductor muscle of oysters is always several times faster than its relaxation, the ratio varying according to the type of muscular reaction. Marceau (1909) published a number of tracings of the spontaneous movements of the valves of *O. edulis* in which only the white (slow) part of the muscle was left. The time of relaxation was from 15 minutes to 1 hour.

In many bivalves the adductor muscle can remain contracted, keeping the valves closed tightly, for a long time. This behavior varies, however, in different species. For instance, common scallops of the American and European coastal waters, *Astropecten irradians* and *Chlamys opercularis*, close their valves for only a short time. Soon after being taken out of water they gape, lose shell liquor, and perish. My observations on pearl oysters of the Hawaiian Islands and Panama (*Pinctada galtsoffi*, *P. mazatlanica*), show that shortly after being taken out of water their shells gape and the muscle fails to contract. These species cannot be transported over long distances unless they are kept in frequently renewed water all the time. On the other hand, the bivalves in which the adductor muscle remains contracted for a long time can survive long exposure and can be shipped alive over great distances.

Oysters living within the tidal range on flats thrive in this situation because they can keep their valves closed during the time of exposure. It is obvious that this ability provides a great survival value for those sedentary animals that can withdraw within their heavy shells to avoid desiccation and remain protected against unfavorable conditions or attacks of predators.

The ability of bivalve muscles to keep the shells closed is frequently called a "catch" or locking mechanism. The idea originated from observations made by Uexküll (1912) on the scallop; if a piece of wood is pushed between the valves the adductor contracts with such force that the edges of the shells may be splintered. The wooden wedge is held as firmly as if it were in a vise and can be removed only by twisting and pulling. The valves, however, remain motionless, and the muscle that holds them in their position shows no elasticity. The muscular fibers seem to be frozen solid. The shell cannot be opened, but if the valves are pressed on both sides they may be brought nearer together and remain fixed in their new position. This ability Uexküll called "Sperrung", which in English means "locking." Bayliss (1924) interpreted Uexküll's expression using the word "catch," probably influenced by Grützner's (1904) suggestion that the muscle fibers of the bivalve adductor must somehow be "hooked up" by a mechanical arrangement similar to a ratchet consisting of two pieces with teeth facing each other. In his proposal the upper piece could be pushed only in one direction, shortening the total length of the model, and the upper teeth could not move back unless the two pieces were separated from each other by the depth of the teeth. There is nothing in the structure of the muscle fiber which even remotely suggests the existence of such a mechanism. The expression "catch mechanism" implies some mechanical device and is, therefore, misleading. It has been used, however, for such a long time that the literary meaning of the words has been lost and the term simply refers to the continuous state of contraction of the closing muscle of bivalves.

Several theories have been proposed to explain the locking or catch mechanism of the adductor muscles. Some investigators assumed that the muscle twitch (i.e., the contraction in response to single brief stimulus) is common to all muscles and the difference between the behavior of the adductors of bivalves and of the muscles of other

types is due to the differences in time scale and the condition of stimulation. It was claimed, (Ritchie, 1928, p. 86), although not proved, that tonus of the adductor muscle is maintained by tetanic contraction. Another view (Winton, 1930), which is more in harmony with the biochemical data, explained the locking mechanism as a result of physical changes during contraction, particularly the alteration in viscosity of muscle proteins. Experiments with byssus retractor of *Mytilus* showed that after stimulation by direct current the viscosity of the muscle was raised and remained high for about 2 hours. No such effect was obtained if alternating current was used. These observations suggest that viscosity changes are involved in the contractions of the adductor of bivalves.

The difference between the white and the translucent parts of the adductor muscle may be primarily of a quantitative character. This suggestion was made by Shukow (1936), who found that in *Anodonta* and *Unio* the two parts of locking muscles actively participate in single, spontaneous contractions and in the maintenance of tonus. Shukow's observations indicate the inadequacy of the theory that makes the maintenance of the tonus the exclusive function of the white fibers.

Studies of the electric phenomena in the smooth adductor muscles of lamellibranchs (*Mytilus*, *Modiolus*, and smooth part of *Chlamys*) lead Lowy 1953, 1955) to conclude that the hypothesis of "catch mechanism" is unnecessary because, according to his observations, the tonus in the intact muscles of these mollusks is due to a shifting pattern of tetanic stimuli controlled by the nervous system, bringing it in line with the tonus in other muscles. Since action potentials were observed in muscles which were isolated from the ganglia, Lowy suggested that they may be of myogenic nature. The question of whether the tonic activity of lamellibranch muscles is neurogenic or myogenic remains open. Lowy makes an interesting statement that "lamellibranch muscles maintain a certain level of tension all the time due to the activity of a peripheral automatic system, which works by successive activation of limited areas."[7] This conforms with the histological observations described above which show that in an intact adductor muscle of the oyster preserved in a contracted state only certain muscle bands are in a true contracted state while others are folded. Lowy concludes that further studies are needed before it is decided whether lamellibranch muscles are directly innervated by excitatory and inhibitory nerves or are acted on indirectly via a peripheral ganglionic plexus. The existence of inhibitory axons in *Pecten* was demonstrated by Benson, Hays, and Lewis, (1942), who found that the relaxation of the adductor of the scallop was considerably accelerated by stimulating certain nerve bands going to the msucle. This is in accord with the evidence presented by Barnes (1955) for the adductor muscles of *Anodonta*. His work implies that the adductor of *Anodonta* is innervated by three types of nerves: one group of motor fibers supplies the striated muscles and produces phasic contractions which may summate and produce tetanus; another group of activating fibers supplies the unstriated muscles and produces increased tonus; the third group consists of inhibitory fibers which decrease the tonus. Barnes points out that the nervous mechanism controlling the adductor activity in *Mytilus* may be the same as in *Anodonta*. *Mytilus* is capable of both phasic and tonic contractions, but there is no obvious differentiation of the muscle into two parts. It must be accepted, therefore, either that all muscle fibers are capable of exhibiting both types of contraction or that the two types of fibers are present but completely interspersed.

Electrical activities associated with the contraction of the adductor muscle of the oyster have not been studied enough to warrant an evaluation of their role in the locking mechanism of these mollusks. An attempt to solve the paradox of the catch muscle mechanism was made recently by Johnson, Kahn, and Szent-Györgyi (1959) and is based on the study of the property of paramyosin. The solubility of this protein was found to be critically dependent upon the pH and ionic strength of the medium. Similar dependence was shown in the glycerinated fibers of the anterior byssus retractor of *M. edulis*. The fibers were stretched, and the tension thus developed was measured. To reduce the effect of actomyosin, 10^{-4} M Salygran and 10^{-2} M pyrophosphate were added to the medium. Stiffness of the fibers was measured at various values of pH. Below pH 6.5 and at low ionic strength of 0.07 M potassium chloride the fibers were relatively stiff. This is a range in which paramyosin crystallizes out of

[7]—Underscoring is mine. P. S. G.

solution. At higher pH values the fibers were relatively plastic. The authors think that parallel with the actomyosin system which produces initial tension of the adductor there is a second, or paramyosin system, capable of maintaining the tension developed by the first one by crystallization of the paramyosin component caused by pH shift within the muscle. The theory was tested by Hayashi, Rosenbluth, and Lamont, (1959) on the muscle extracts of *Mercenaria* (*Venus*) *mercenaria* and *Spisula solidissima*. The results of these experiments tend to support the hypothesis that crystallization of paramyosin effectively freezes the adductor muscle at any state of contraction.

In two papers dealing with the fine structure of the small fibers of the oyster (*C. angulata*) and other bivalves, Hanson and Lowy (1959, 1961) have proposed two possible explanations of the mechanism by which the closing muscles of mollusks maintain tension "very economically," i.e., without using much energy. According to their view, based on examination of electron micrographs of muscle, the thick filaments of the fibril (see p. 157 and fig. 144) are discontinuous and do not contract; they slide as the muscle shortens the relative portions of the thick and thin filaments. The tension is maintained by cross links between the two types of filaments. According to their view the alternative hypothesis, which supposes that tension is maintained by change in the physical state of the protein within a paramyosin system, is difficult to reconcile with their observations. The sliding or so-called interdigitatory model of the contractile structure is based primarily on the studies of striated muscle (Huxley, 1960), and the extension of the theory to nonstriated muscles of bivalves is very attractive. It is impossible, however, to state at present which of the two theories interprets correctly the catch mechanism. Further experimental studies are needed to solve the puzzle which for a century has baffled the biologist.

In spite of the substantial advance of biochemical investigations, the problem of the locking mechanism requires further study. So far no evidence has been presented to show that the shift in the pH needed for the crystallization of paramyosin actually takes place in the whole living muscle of a bivalve. It seems that the solution to the locking paradox should consider the problem in its entirety, by taking into account all the biochemical and biophysical processes which accompany the prolonged tonus of the adductor muscle.

Chemical changes during muscular activity

Chemical changes occurring during the contraction and relaxation of the muscle are extraordinarily complex. The reader interested in this problem should consult the textbooks of general physiology (Scheer, 1948), biochemistry (Needham, 1932; Baldwin, 1957), and particularly the comprehensive reviews of more recent works given by Szent-Györgyi (1951) and Weber (1958). Most of the work on the chemistry of muscular contraction has been performed on vertebrate muscles. In general the results were found to apply to the muscles of scallops (*Pecten, Astropecten, Chlamys*), sea mussels (*Mytilus*), edible oysters (*Ostrea, Crassostrea*), and *Anodonta*.

A complex chain of events is involved in muscular contractions. I will consider only the high points. Glycogen appears to be the principal, if not the only source of energy in this process. Its content in the adductor muscles of bivalves varies from less than 1 to about 3 percent. The immediate source of energy for muscular contraction is not derived, however, from the breakdown of glycogen. Considerable quantities of phosphate are released by the organic compounds called phosphagens. These substances contain (Weber, 1958, p. 5) an energy-rich phosphate bond and, therefore, are the "stores of immediately available energy." Creatine phosphate, identified as a phosphagen of vertebrate muscle, does not occur in mollusks; its place is taken by arginine phosphate. Phosphagen decreases during contraction and is formed again during rest. After prolonged contractions the tissues of the fatigued muscle become acidic due to the accumulation of lactic acid. Glaister and Kerly (1936) found that iodoacetate, which inhibits the formation of lactic acid in *Mytilus* muscle does not materially interfere with muscular contraction.

The key substance involved in the energy transformation in the muscles is, however, adenosine-triphosphate (ATP); the presence of ATP is a prerequisite to contraction. According to Szent-Györgyi's theory ATP has a great affinity to myosin and is strongly linked to it. Excitation of the muscle implies the formation of actomyosin (from actin + myosin), a process which does not take place in the absence of ATP (Szent-Györgyi,

1951). Dephosphorylization of ATP to adenosinediphosphate (ADP) is believed to be the most important reaction closely connected to the liberation of energy in the contracting muscle. The function of ATP, according to Weber (1958) is twofold: "it acts as a contracting substance if it is split and as a relaxing and plasticizing substance if it is present without being split." The ATP used in contraction is restored "almost as rapidly as it is broken down by transphosphorylation of phosphagens."

Since the phosphorylization of ATP is the main stage in the energy-providing reaction in the muscle, it is of interest to know the splitting capacity of this compound in the adductor muscles. Investigation of this problem by Lajtha (1948) showed that the phosphatase activity is much lower in bivalve muscles (*Mytilus* and *Pinna*) than in rabbit muscle. Lajtha suggests that this is correlated with the slow working of the adductor muscle, which does not require the quick energy changes needed in the more rapidly functioning muscles of vertebrates and insects.

Chemical changes in the adductor muscle of the oyster (*C. commercialis*) were studied by Humphrey (1944, 1946, 1949, 1950), who demonstrated the presence of arginine phosphate and of several phosphorylated breakdown compounds of glycogen. The glycogen can be synthesized in both parts of the muscle from glucose-1-phosphate, but synthesis is more readily effected in the translucent portion.

In the glycolysis of the oyster muscle the glycogen breaks down in the presence of added potassium, magnesium, and DPN (diphosphopyridinenucleotide) and yields a mixture of pyruvic and lactic acids (Humphrey, 1949). The glycolytic ability of the adductor muscle of the oyster is several hundred times less powerful than that of rabbit muscle.

Studies of the glycolysis in extracts of the adductor muscle of *C. commercialis* (Humphrey, 1944) disclosed three essential facts: (1) phosphate, potassium, magnesium or manganese, and DPN are the essential parts of the system resulting in the production of acid; (2) lactic and pyruvic acids are produced simultaneously; and (3) acid production is inhibited by fluoride and iodoacetate. The glycolysis in oysters and other invertebrates still is not well understood, particularly with respect to the metabolism of pyruvate by oyster muscles.

The ATP present in the adductor muscle has a definite relationship to glycolysis. The amount of ATP in the muscle decreases when oysters are left out of water. From this observation Humphrey advances the hypothesis that the breakdown of glycogen provides the energy for the muscle to resist the pull of the ligament. He thinks that the regeneration of ATP proceeds through glycolysis, which continues under both aerobic and anaerobic conditions. Both conclusions require further corroboration.

NORMAL SHELL MOVEMENTS

Studies of shell movements can give valuable information regarding the physiological state of the oyster and its reactions to the changes of environment. The only type of motion that can be performed by an adult oyster consists of two distinct components: the contractions of the adductor muscle that bring the opposing valves together and may completely seal off the soft parts of the oyster, and the springlike action of the ligament that pushes the valves apart during the periods of relaxation. The purely mechanical action of the ligament is counteracted by the tonus of the muscle, which retains a certain degree of elasticity even in the state of maximum stretching. If the muscle is cut off at the maximum gaping, the valves are pushed farther apart by the elastic force of the ligament.

METHOD OF RECORDING

Oysters selected for long-term observation (several weeks or months) should be free of boring algae and animals. The surface of the shell is scrubbed with a metal brush, washed, and dried. The left valve is embedded in a rapidly setting mixture of cement, sand, and unslacked lime in proportion 1:2:1. Care should be exercised to keep the edges of the valves free of cement mixture and to wipe out and wash with sea water all excess material. Mounted oysters are left in the air at room temperature for 12 to 24 hours.

A small metal loop cut from a paper clip may be used to attach strings which lead to a recording lever. The two arms of the U-clip are bent horizontally, and the loop is placed on the clean, dry surface of the right valve and sealed in a vertical position by a few drops of hot colophonium cement. For recording the up and down movements of a valve heart and muscle levers available at scientific supply houses can be used. Adequate levers can be made of strips of appropriate length cut from a sheet of plastic and mounted on pivots

of a small glass rod inserted in a hole drilled in the supporting arm. It is convenient to have at hand levers of various lengths so that records of shell movements of several oysters can be made simultaneously on one kymograph drum. Unless there are some special reasons for not changing the sea water during the observations, the oysters are placed in running sea water, and the temperature of the water is recorded on a thermograph and its salinity checked at regular intervals.

The records reproduced in this book were obtained by using a slow-motion kymograph. The uppermost position of the writing pen always corresponds in these tracings to the position of a completely closed right valve; the lower position of the line marks the various degrees of opening of the shell. The magnitude of the up and down excursions of the writing pen depends on the ratio between the two arms of the lever, the distance between the hinge ligament and the place of the attachment of the string, and the height of the oyster. The magnification of shell movements recorded in the Bureau's shellfish laboratory at Woods Hole varied from three to seven times the actual excursions of the valves. A baseline representing the position of the writing pen when the shell is completely closed (not shown in the records reproduced here) may be obtained by rotating the drum rapidly before beginning observations.

Under ordinary circumstances the opening and closing movements of the shell are so small that the corresponding up and down tracings on kymograph paper are relatively short and are not distorted by the actual movement of the lever, which on wider tracings describes an arc on the side of the rotating cylinder. In case of wide gaping produced by experimental stretching of the muscle the distortion becomes serious since the writing point at the bottom moves ahead of the time marker and draws a gentle slope instead of a steep curve. To avoid possible misinterpretation the true position of the writing lever at the time of maximum stretching and its return to the top as the muscle contracts are shown on the records by dotted lines.

For long-term observations the speed of the kymograph drum is adjusted to slow movement of about 1 inch per hour. When studies are made of the reactions of oysters to various stimuli the speed of the rotation should be increased to about three-eighths of an inch (1 cm.) per minute. With the fast- and slow-motion kymograph used in the Bureau's shellfish laboratory, the latter speed corresponded to one complete revolution of the drum per hour. With this technique several thousands of records of shell movements of oysters were obtained under a great variety of conditions using both normal and diseased oysters. Specimens used in the tests were taken from New England waters, Chesapeake Bay, South Carolina, the west coast of Florida, Mississippi, Louisiana, and Texas. A relatively small number of records were made of shell movements of *C. gigas* and *O. lurida* of the Pacific Coast. Many records were obtained while oysters were subjected to various types of poisons (chlorine, phenol, black liquor and red liquor of pulp mill wastes, crude oil, thiocyanates, etc.) or while they were given various concentrations of carbohydrates and suspensions of pure culture of *Escherichia coli*.

For a study of shell movements under normal conditions the oysters were kept in running sea water delivered at 10 times, at least, faster than the rate at which it was transported through the gills. Under this condition one can be certain that the products of metabolism were removed and the oysters were not deprived of food.

Shell movements play an essential part in the respiration, feeding, and rejection of silt, mucus, and excreta that otherwise may accumulate in the pallial cavity of the oyster. Material settled on the gills and mantle is rejected by rapid and powerful snapping of the valves. In addition to this rejection reaction there are smaller and slower changes in the tonus level of the adductor which may be interpreted as adjustments to a steady flow of water through the gills. It is not surprising that shell movements of oysters show great variations both in the rate and type of contraction. Analysis of the records made under known conditions in the laboratory indicates that in spite of this variability the movements of individual oysters can be grouped into five major types characterized by their responses to various conditions.

FIVE MAJOR TYPES OF SHELL MOVEMENTS

In comparing the records of shell movements it is necessary to know the following essential points: the highest and lowest level reached by the writing pen during the periods of closing and opening of the valves, the frequency at which

the contractions occur, and the speed of rotation of the drum. Published reports frequently fail to mention these significant details. Another feature of importance is the general level corresponding to the tonus of the muscle to which the valve returns after each brief closing. Under normal conditions the adductor muscle is never completely relaxed. The distance to which the valves are pushed apart by the hinge ligament is, therefore, indicative of the degree of relaxation.

During my years of study, more than 2,000 tracings of shell movements of oysters were obtained under a great variety of conditions. It was possible to group them into five principal types which for the sake of brevity are designated by the first five letters of the alphabet.

Type A

The three curves shown in figure 153 (A–1, A–2, and A–3) indicate normal behavior of the oyster. The differences in the appearance of the curves are due primarily to differing speeds of drum rotation. Curve A–2 is a continuation of curves A–1 with the drum movement reduced from 15.3 cm. to 3.6 cm. per hour. The extreme right portion of curve A–2 indicates the summation of several stimuli that caused brief closing of the valves. The curve A–3 is a variation of type A–1 and is essentially similar to curves A–1 and A–2. The writing lever in curve A–3 was set in such a way that the magnification of the vertical excursions was only one-third of that used in curves A–1 and A–2. The contractions were, however, more frequent. Several downward excursions of the pen indicate brief attempts to widen the opening of the valves, but the general tonus level of the adductor remained fairly constant.

Type A shell movement, shown in figure 153, represents movements of an undisturbed oyster that maintains a steady current of water for the ventilation of the gills and for the collection of food. The general level of opening of the valves is fairly constant (curves A–1 and A–2). Relaxation of the muscle immediately after rapid contraction is slow, and the resulting curve slopes down gently (see right parts of curves A–1 and A–2). Sudden snapping of the valves is associated with the discard of rejected food, mucus, detritus, and other particles that accumulate on the inner surface of the pallium. This rejection reaction is an important feature of oyster behavior for it is the principal method of keeping the pallial cavity free from the accumulation of foreign matter.

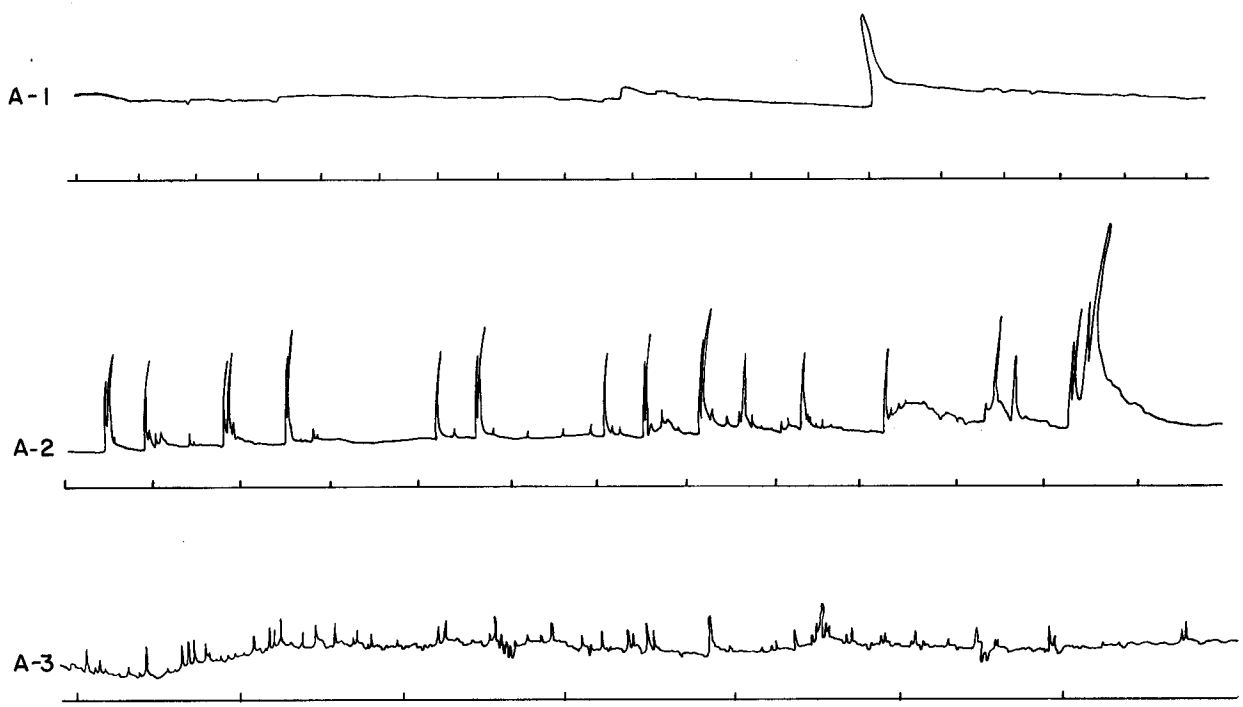

FIGURE 153.—Shell movements of normally feeding oyster. Type A. Vertical magnification of curve A–3 is about one-third of that in A–1 and A–2. In each curve the uppermost point corresponds to the position of the lever when the shells are completely closed. Time interval: A–1, 5 min.; A–2, 30 min.; A–3, 1 hour.

Numerous minor contractions (fig. 153, A–2) that occur between the rejection reactions only slightly reduce the opening between the valves and are more difficult to interpret because they are not accompanied by the discharge of any material. Possibly they represent the fine adjustments made by the oyster in maintaining a steady flow of water through the gills. On the other hand, it is also possible that they are responses to minor physical disturbances such as vibrations of laboratory floors and slight changes in illumination. None of the existing laboratories in the United States have the shockproof floors and walls that would assure complete elimination of the outside disturbances caused by street traffic and footsteps within the building.

Type B

Type B shell movement is characterized by the increased frequency and well-pronounced periodicity of contractions and corresponds to the state of increased excitability (fig. 154). Curve B–1 was observed in oysters which were exposed to a rapid rise of temperature from 13° to 25.6° C. B–2 represents the behavior of oysters affected by the metabolites accumulated in stagnant and unaerated water. The uniform and rapid contraction shown in B–2 stopped immediately when the water was changed.

Curve B–3 represents a similar activity recorded on a rapidly moving drum. The relaxation periods are much shorter, but the level of the muscle tonus remains constant. Shell movements of this type were frequently observed in oysters which were left after spawning in water containing large quantities of oyster eggs and sperm. Normal movements of the type A–1 were resumed as soon as the water was changed.

Type C

The curve of type C shell movements (fig. 155) illustrates periods preceding or following changes in the degree of opening and closing of the valves. Both periods are characterized by a series of minor contractions and relaxations until the final tonus level is reached. The type shown in C–2 (left part of the curve) is a typical "staircase" or "Treppe" reaction of the adductor muscle, which contracts in several distinct stages. This reaction is the response to an irritating substance added

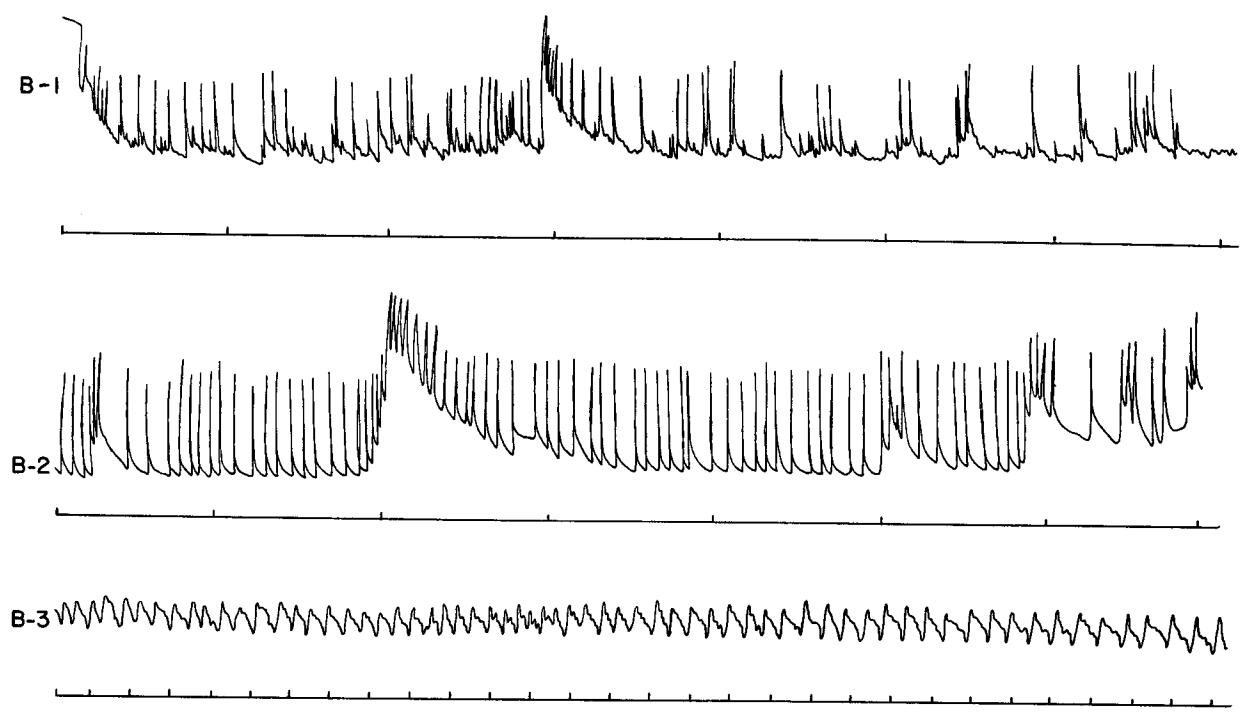

FIGURE 154.—Shell movements of type B are typical for the state of increased excitability frequently caused by the accumulation of metabolites in sea water or rapid rise of temperature. Vertical magnification in B–3 is about one-fourth of that in B–1 and B–2; uppermost points correspond to closed shells. B–1 temperature increased from 13° C. at the start to 25.6° C. at the end. B–2, B–3 increased muscular activity due to the accumulation of metabolites. Time interval: B–1, 1 hour; B–2, 1 hour; B–3, 1 minute.

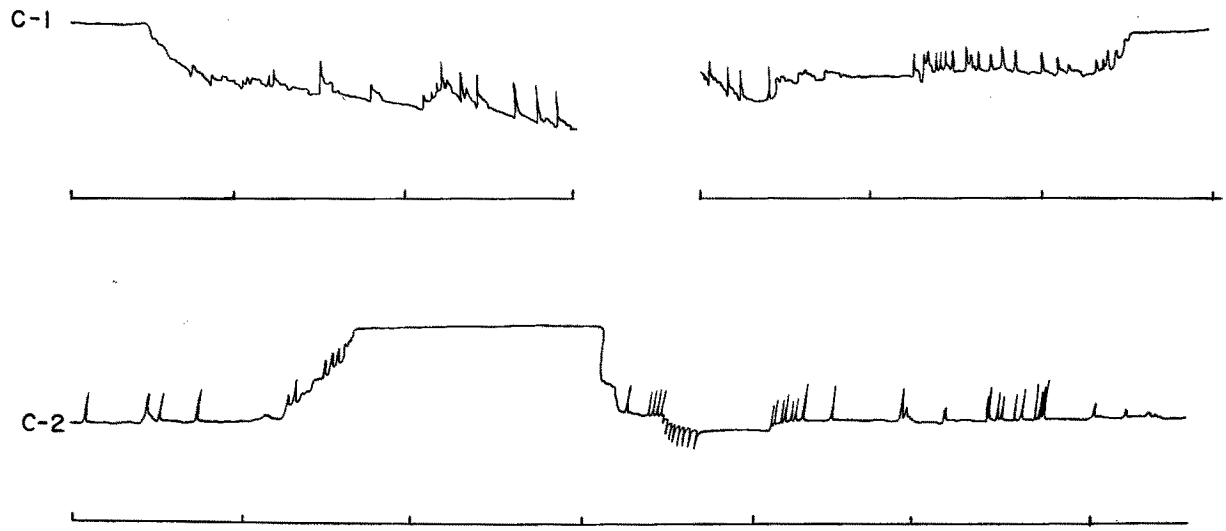

FIGURE 155.—Shell movements of type C, preceding the closing of valves (left side) and following their opening (right side). Note staircase movement in C–2. Time interval: C–1 and C–2, 1 hour each.

to the water. A "staircase" in reverse direction sometimes takes place during the opening of the valves (C–2, right half). This behavior was provoked by small doses of oyster sperm, vitamins, and sugars injected between the valves into the pallial cavity. The "reversed staircase" may be interpreted as a testing reaction of the oyster, which adjusts the opening of the valves to a needed rate of ventilation.

Type D

Shell movement of type D (fig. 156) was observed in oysters affected by various poisons which caused increased excitability of the adductor muscle (D–1). In case of a prolonged action of poison the periods of greater excitability (D–2 and D–3) are interrupted by gradually increasing durations of periodical closure (D–4 and D–5). This type of shell movement is a symptom of a highly advanced pathological condition resulting from poisoning, disease, or exposure to adverse physical conditions. It is typical for dying mollusks (D–5).

Type E

The E type of shell movement associated with the spawning of the female oyster is characterized by great regularity, rapidity, and rhythmic up and down strokes (fig. 157). At the beginning of the reaction the time needed to reach the maximum relaxation level is very brief, almost equal to the time of the contraction (see: E–2). During the relaxation phase (downward stroke) there is a brief period of slowing down in the decrease of muscular tension. On the curve this period is represented by a small plateau. This moment coincides with the passage of eggs through the gills into the pallial cavity. The eggs in the pallial cavity are dispersed into the surrounding water by rapid contractions of the adductor.

Shell movements that take place during the spawning of a female do not occur at any other time and cannot be induced by drugs. They cease with the cessation of spawning. The factor that induces female spawning (temperature or chemical stimulation by sperm) has no effect on the type of shell movement of a male and is ineffective on nonspawning females. It is probable that this type of muscular activity is associated with the discharge of eggs from the gonads.

DURATION OF PERIODS OF OPENING AND CLOSING

The length of time the shells remain open or closed and the conditions that affect this behavior are of importance to oyster biology. Obviously the normal functions of the organism, such as respiration, feeding, and elimination of waste products, can be performed only when the valves of the mollusk are open. It does not follow, however, that the opening of the shell indicates that the mollusk is feeding or is ventilating its gills. Under certain conditions water may be shut off from the pallial cavity by the pallial curtain or by the cessation of ciliary motion while the valves remain open. However, in the majority of laboratory observations of the behavior of oysters in unadulterated sea water the opening of the valves coin-

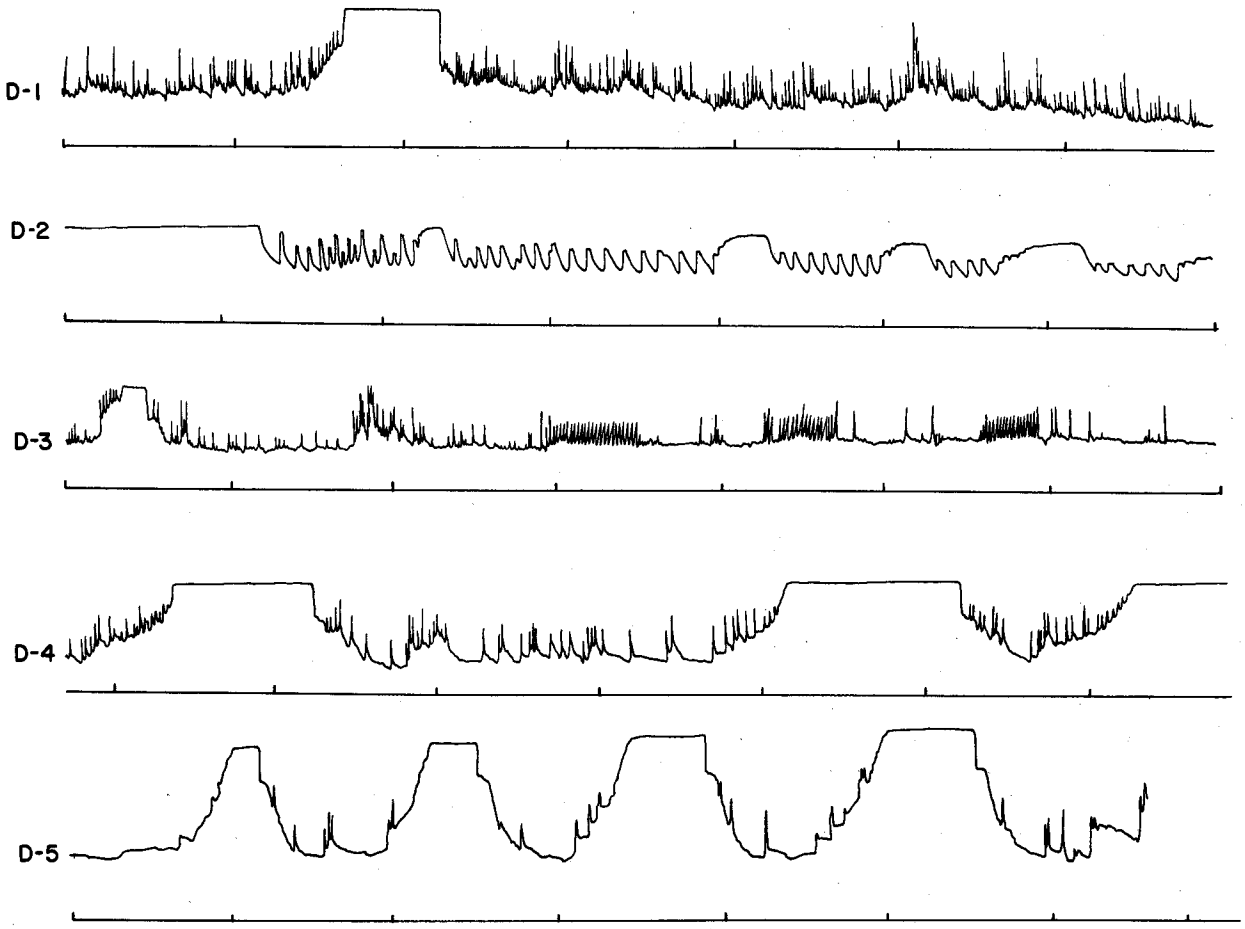

FIGURE 156.—Shell movements of type D are observed in the oysters poisoned by toxic substances or weakened by adverse environment. D–5 shell movements of a dying oyster. Vertical excursions of the writing pen are magnified three times in all tracings. Uppermost level of the curves corresponds to closed shells. Time interval: D–1, D–2, D–3, D–4, D–5, 1 hour each.

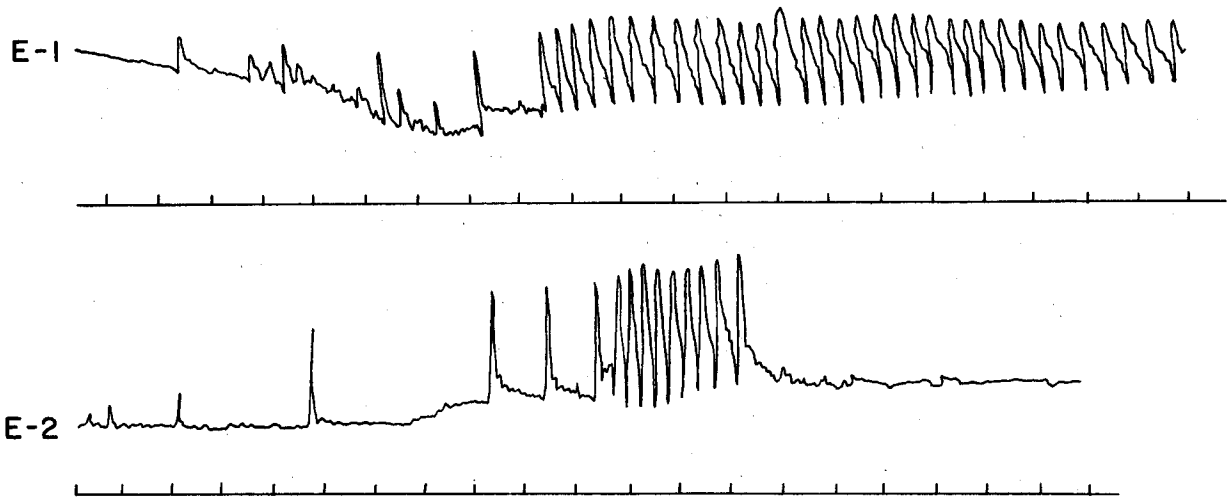

FIGURE 157.—Shell movements of a spawning female. Note the frequencies of up and down movements, brevity of the relaxation periods and slowing down at the middle of the downward strokes; this brief period coincides with the penetration of eggs through the gills. Time interval: E–1 and E–2, 1 minute each.

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cided with the maintenance of a steady cloacal current.

The determination of the number of hours the oyster remains open under average normal conditions is of significance in studies of reactions of the mollusk to changes in its environment. Certain industrial wastes discharged in sufficient concentrations into natural waters reduce the time the oysters stay open. It was found, for instance, that the red liquor which is the waste product of pulp mills using acid digestion of wood and the black liquor of pulp mills which employ a sulfate process exert this effect on the Olympia oyster (Hopkins, 1931) and on *C. virginica* (Galtsoff, Chipman, Engle, and Calderwood, 1947). Any condition that forces oysters to remain closed for an abnormally long time deprives them from taking in food and eventually may harm them.

The percentage of time during each 24-hour period that the oysters are open can be used as an index of normal behavior, provided the shell movements of the mollusk do not indicate pathological conditions of the type shown in curves D–1 to D–5. Failure to recognize the significance of this type of shell movement while recording the time the oyster remains open may lead to serious misunderstandings and errors. Unfortunately there are many published data in which the "time open" was recorded without observing the character of shell movements.

The length of time *C. virginica* remains open is also influenced by temperature and by the state of the oyster itself. Since the shell movement is influenced by several external and internal factors, it is not surprising that there is a great discrepancy in the estimates of the average duration of "open shells" reported by various investigators.

In the Bureau's shellfish laboratory at Woods Hole from June 15 to October 15, 1926, 132 daily records of 34 oysters observed gave an average of 17 hours 7 minutes for open shells. The temperature of the water during this period ranged from 13° to 22° C., but daily fluctuations of temperature were insignificant, never exceeding 1.5° C. (Galtsoff, 1928). Records of the three oysters kept by Nelson under observation for 21 days in New Jersey water indicated that the shells remained open on the average of 20 hours per day at temperatures varying between 22° and 25° C. (Nelson, 1921). For oysters kept in running sea water at a Beaufort, N.C., laboratory, average time open in October to November varied between 10 and 14 hours (Hopkins, 1931). The temperature of water was not recorded. Two hundred and one daily records of 49 York River (Virginia) oysters kept under observation in the laboratory at Yorktown showed that the periods of opening varied from 19.2 to 24.0 hours a day (Galtsoff, Chipman, Engle, and Calderwood, 1947). Within the temperature range of 17.0° to 28.0° C. Long Island Sound oysters were found to remain open for an average period of 22.5 hours. The latter data are based on 64 records of 18 oysters (Loosanoff and Nomejko, 1946). *O. lurida* of the Pacific Coast remained open for an average of 20 hours a day at the temperature range of 5° to 17° C. (Hopkins, 1931).

A sample of oysters always includes several individuals that may remain closed for 24 hours or longer. One or two of them will reduce unduly the average figure based on a small number of observations. Furthermore, under identical conditions of the normal environment (i.e., not affected by pollution, dredging, or other disturbances) an oyster may keep its shell open or closed for varying periods of time depending on the requirements of the organism for food and oxygen. I found that immediately after spawning the female oysters have a tendency to keep their shells closed for several days. On the other hand, oysters left overnight out of water open almost immediately upon being returned to sea water. It is reasonable to assume that they accumulated an oxygen debt during the period of closure. In view of these observations the differences in the duration of periods of opening or closing described for oysters of different localities have no particular significance. The average value may be useful, however, in determining the adverse effects of the changes in the population of oysters in a given locality and in making a comparison between the behavior of these individuals in clean and polluted waters.

EFFECT OF TEMPERATURE

Temperature as such has no direct influence on the duration of shell opening. There was no significant difference in the length of time the Woods Hole oysters remained open when kept at temperatures varying from 15° to 30° C. (Galtsoff, 1928). It is rapid change in temperature, often occurring in those laboratories where sea water is subject to wide diurnal fluctuations, that has a pronounced effect on shell movements. *O. lurida*,

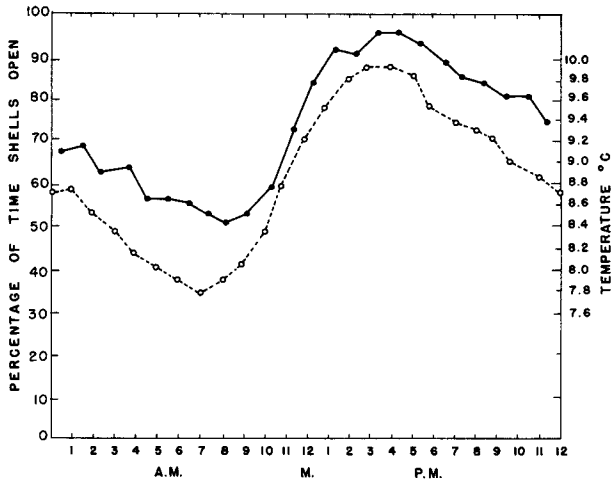

FIGURE 158.—The average percentage of time open of two specimens of *O. lurida* at each hour of the day observed over the 29-day period (solid circles). Average temperature readings for each hour during the same period (open circles). From Hopkins (1931), fig. 4, p. 6.

for instance, has a tendency to close with the falling of temperature and open with a rise of temperature (Hopkins, 1931). The sensitivity of this oyster to temperature changes was reported to be greater at the lower range. At 4° to 6° C. the oysters remained closed a relatively high percentage of the time; at 6° to 8° C. they were open only about 6 hours, while at the maximum of about 15° C. they remained open over 23 hours per day. In both cases the diurnal curve of shell activity was parallel to the curve of temperature fluctuation observed by Hopkins (1931) although the percentage of time open in warmer water was consistently higher (fig. 158). It would be of interest to repeat these observations and compare them with controls kept at a constant temperature since Hopkins' temporary laboratory near Olympia, Wash. lacked adequate equipment for regulation of temperature. He concluded that "change of temperature is more important in affecting the length of time Olympia oysters remain open than the degree of temperature itself." The results of his observations on *C. virginica* at Beaufort, N.C., bear close resemblance to those described above but, unfortunately, they were not accompanied by thermograph records and so are not entirely convincing. His conclusions need clarification.

EFFECT OF LIGHT AND DARKNESS

Periods of light and darkness have no apparent effect on the closing or opening of the valves. Analysis of 103 daily records of shell movements of oysters kept in the Bureau's Woods Hole laboratory in running sea water at nearly constant temperature (daily fluctuations ±0.5° C.) and constant salinity shows that of the total number of 831 hours of inactivity (shell closures), 266 hours or 32 percent occurred during the 8-hour period of darkness and the balance of 565 hours, or two-thirds of the total, took place during the remaining two-thirds of daylight (Galtsoff, 1928). During the summer, from June to August inclusive, the Long Island Sound oysters kept their shells open for 94.4 percent of the total time during daylight and 93.8 percent during the hours of darkness (Loosanoff and Nomejko, 1946). These observations repudiate Nelson's conclusion that the periods of inactivity (or closings) occur during darkness (Nelson, 1921, 1923c).

EFFECT OF TIDE

There is no evidence that the opening and closing of oyster valves is related to the stages of tide. The idea that oysters living below the low-water mark are relatively inactive during the outgoing tide and that the times of cessation and commencement of feeding are correlated to stages of the tide, was several times expressed by Nelson (1922, 1923a, 1923c, 1938) and without verification was accepted by Orton in his article in Encyclopedia Britannica (Orton, 1929). Loosanoff and Nomejko (1946) analyzed the kymograph tracings of shell movements of oysters kept under virtually natural conditions on a platform installed on a small oyster bed on the bottom of Milford Harbor in Long Island Sound. They found that the shells remained open on an average of 93.4 percent of the time during the flood periods and 95.2 percent of the time during the ebb periods. The tidal changes in Long Island Sound are not accompanied by the excessive changes in the temperature, salinity, pH, and turbidity of water which frequently take place in the tidal streams of the southern Atlantic states and may influence the shell movements of oysters.

POWER OF THE ADDUCTOR MUSCLE

Anyone who attempts to open a live oyster by inserting and twisting a knife between the two valves becomes aware of the considerable resistance exerted by the mollusk. As a rule the valves of healthy oysters just taken out of sea water are difficult to pry apart. The power of the adductor muscle, which is solely responsible for keeping

the valves tightly closed, varies greatly in oysters of the same size and environment. Prolonged exposure to air so weakens the adductor that oysters left out of water for several days can be easily opened.

In attempts to measure the power of the adductor of various bivalves Plateau (1884), Marceau (1905a, 1905b), and Tamura (1929, 1931) drilled holes near the edge of the shells and inserted rods or hooks to which they attached weights. The opposite valve was immobilized. Assuming that the adductor muscle is an elastic body, the amount of work (W) done by the adductor against the loaded weight (G) was calculated by using a simple formula $W=\frac{ac}{ad}G$ where ac is a distance in centimeters from the ligament to the attachment of the weights; ad is the distance in centimeters from the ligament to the center of the adductor muscle; and G is the weight in grams applied to the valve. Under a known pulling force the shell movements were traced on a kymograph and a record was made of the time and load under which the muscle fibers were torn off. Continuous irritation of the adductor by the foreign body (hook or rod) inside the shell near the mantle makes this technique objectionable. Furthermore, the end point of the experiment, the tearing off of the muscle, is of no biological significance compared to a determination of the tensile force of the muscle fibers.

The method used in the Bureau's shellfish laboratory eliminates these objections. The left valve of the oyster is mounted on a heavy cement block, using a very strong mixture of portland cement and sand to which a small amount of plaster of paris is added (fig. 159). The base is bolted to the frame D which may be placed in the aquarium tank B supplied with running sea water. A galvanized iron screw (a) about 1 inch in length is inserted into the valve at the center of the attachment of the adductor muscle. Its tip should not penetrate the valve. Enough portland cement or other highly adhesive mixture is applied to the shell surface around the screw to make a cone of about 1 inch in diameter; the top of the screw (a) should protrude above the cement. A metal stirrup (E) consisting of a pair of iron bars (b) with pronged arms at the lower end and a hook (d) mounted at the upper end connect the valve and the pan (e) of the laboratory balance

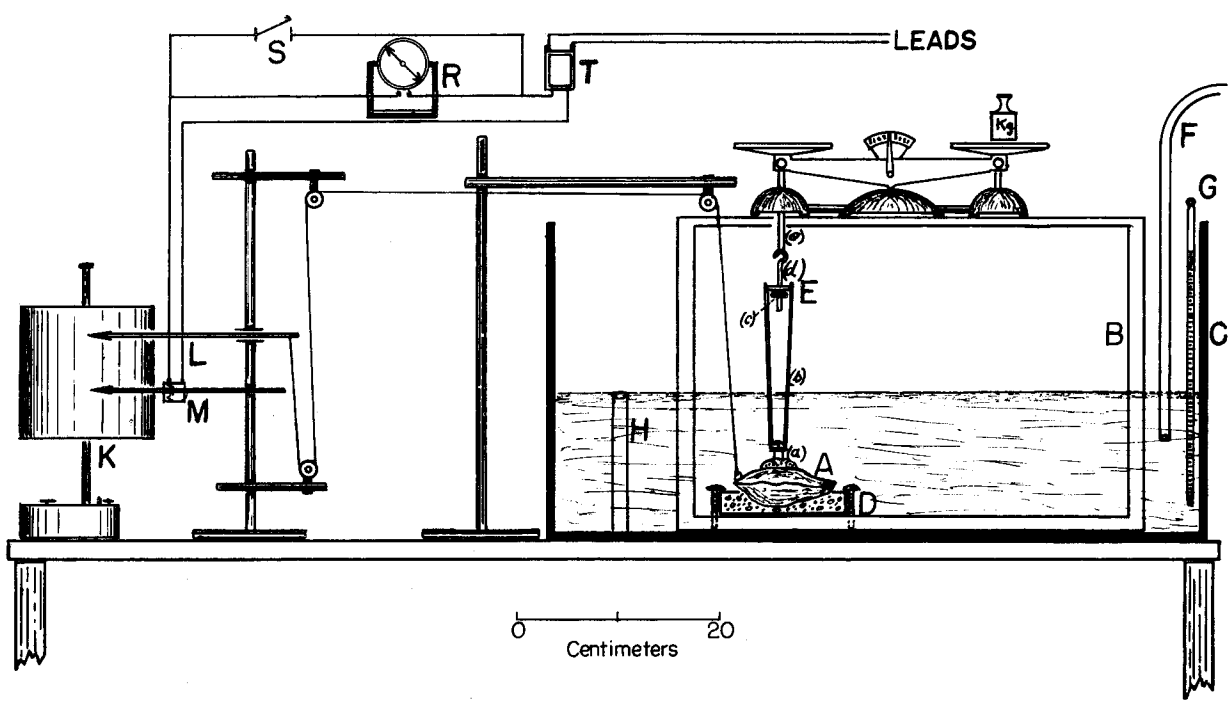

FIGURE 159.—Method of determining the resistance of the adductor muscle of *C. virginica* to a pulling force. A—cement base, bolted to wooden frame D and placed in tank B; a—galvanized iron screw; b—bars of the stirrup E; c—adjusting nut; d—hook for connecting the stirrup to the balance; e—left pan of the balance; F—seawater intake; H—overflow; K—kymograph; L—writing lever; M—signal magnet and pen; R—Telechron timer; T—transformer.

placed on frame D. The length of the hook is adjusted by turning the nut (c). The two pans of the balance are placed in a zero position, and the desired weight is put on the right pan. The right valve of the oyster is connected to the writing lever (L) of the kymograph (K). The writing pen (M) is attached to a signal magnet which is activated by an electric timer (R) and transformer (T). The timer is made by mounting a plastic disc on the axis of a Telechron motor making one revolution every hour. A short piece of copper wire at the periphery of the disc, indicated in figure 159 by the arrows, completes the circuit every 30 minutes (at the vertical position of the arrow). The weight of the balance is sufficient to keep the platform from floating when it is placed under water. Sea water is supplied through the intake (F); the overflow (H) controls the water level. This setup was successfully used in a large number of tests made both in the air and under water.

Occasionally the bond between the cement cup and the surface of the shell was insufficient for a pull of 8 to 10 kg. and had to be adjusted by using a stronger mixture and slightly roughening the surface of the valve. In the majority of cases the connection between the valve and the cement cap remained intact even when the pulling force of about 10 kg. was applied and occasionally the muscle itself was torn in the middle.

The purpose of the test was twofold: to study the behavior of the adductor under variable pulling force and to determine the time required to cause the loss of tonus by the muscles that were being stretched by weights varying from 2 to 10 kg. directly over the muscle scar.

New England oysters kept in the harbor near the laboratory were used in all the tests. The oysters were about 5 inches in height and appeared to be in good condition with the shells undamaged by boring sponge.

TESTS MADE IN AIR AND IN WATER

Adult oysters exposed to air at room temperature are able to withstand the pulling force of several kilograms for several days. Under the weight of 7 to 8 kg. the adductor muscle opened immediately (fig. 160). A force of 8 kg. (2,185.8 g./cm.^{2} of cross-sectional area of the adductor) caused immediate stretching of the adductor to about one-third of the maximum gaping distance, which was attained within 6.5 hours. During the 5 hours following the initial stretching there was no shell movement but the adductor retained its tonus level; the response to pricking (several small upward strokes on the record) was very slight. Final stretching to 1.5 cm. gaping distance of the valves was relatively rapid. At this stage the adductor lost the tonus and failed to respond to stimulation. Upon removal of the weights the muscle regained its elasticity and contracted (right side of fig. 160). For several hours an oyster weighing only 18.2 g., exclusive of shell, was capable of maintaining a constant tonus level against the pull of 8 kg.

In all tests in which the pulling force of 10 kg. per oyster was applied (from 2.5 to 3.0 kg./cm.^{2} of the muscle area) the muscle stretched immediately and the gape of the valves reached the maximum width of 15 to 18 mm. The muscle failed to respond to pricking or to the application of 0.1 *N* hydrocholoric acid but retained a certain degree of elasticity and was able to counteract the pulling force of the ligament. As soon as the muscle was cut off the valves opened several millimeters beyond their former position.

Individual variations in the time required for a muscle to reach maximum stretching are considerable. The time needed to produce tonus loss is inversely related to the weight applied to the valves. The pulling force of 0.5 kg. (131 to 136 g./cm.^{2}) applied for 15 days had no effect on the opening of the oyster shell in the air (at room temperature of 15° to 18° C.). At the pulling force of about 500 g./cm.^{2} the loss of tonus and failure to respond to stimulus developed in 300

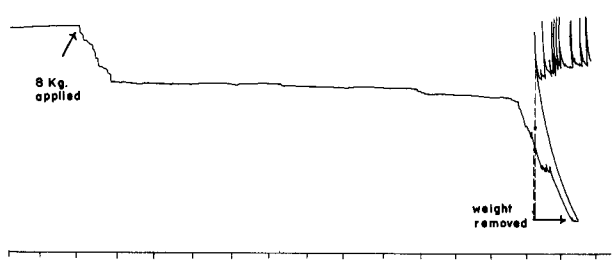

FIGURE 160.—Record of shell movement of *C. virginica* kept in air under the pulling force of 8 kg. (2185.8 g./cm.^{2} of cross-sectional area of the adductor muscle). Arrows indicate time when the weight was applied (upper left) and removed (lower right). Temperature 18° to 23° C. Total weight of oyster meat 18.2 g.; of shell 166 g. Maximum gap (right end of the curve) 1.5 cm. The distortion of the lowermost position of the lever with reference to the horizontal axis is marked by the heavy arrow. Time interval: 0.5 hour.

hours. To avoid desiccation the oyster in this experiment was surrounded by a small moist chamber. With the increase in weight the time of complete loss of tonus rapidly decreases (fig. 161).

Muscles which were kept for several hours under a pulling force of about 1.7 kg./cm.^{2} of cross-sectional area suffered a temporary injury which resulted in abnormal shell movements after the return of the oysters to sea water (fig. 162). The two tracings reproduced in this figure are almost identical, although in the case of oyster A a pulling force of 6 kg. was used while 8 kg. were applied to oyster B. In both instances the pulling force per unit of muscle scar area was the same, 1,676 in A and 1,675 g./cm.^{2} in B. After a few days in running sea water both oysters completely recovered and their shell movements became normal.

In oysters kept in sea water the relationship between the weight applied to the valves and the time needed to attain tonus loss is less regular and individual differences are much greater than for oysters left in the air. With a pulling force of about 1.5 kg./cm.^{2} of muscle area some of the oysters showed tonus loss in less than half an hour while others remained closed for many hours. The relationship between the increasing pulling force and the time required to develop loss of tonus is shown in figure 163.

Changes in the character of shell movements of

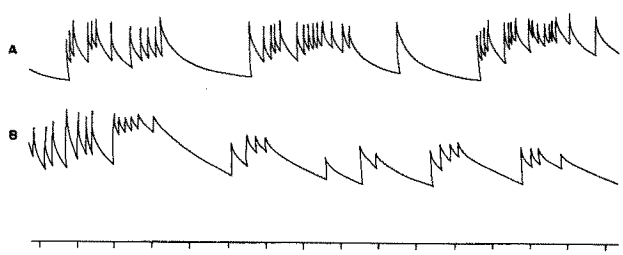

FIGURE 162.—Shell movements of *C. virginica* in sea water after the removal of weight of 6 kg. or 1,676 g./cm.^{2} of muscle area (upper line) and 8 kg. or 1,675 g./cm.^{2} of muscle area (lower line) applied to the valves. Weights were removed after complete loss of tonus was attained in 43.52 and 52 hours exposure in air at temperature 23° to 24° C. Water temperature 13.5° C. Time interval: A and B, 0.5 hour each.

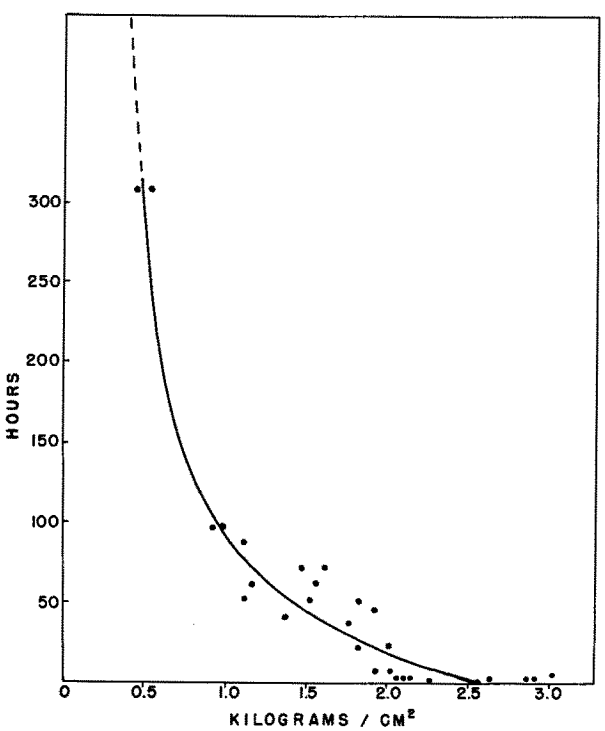

FIGURE 161.—Time in hours required to obtain loss of tonus of the intact adductor muscle kept under constant pull in kg./cm.^{2} of the cross-sectional area of the adductor. Experiments with *C. virginica* kept in air at temperatures between 18° and 24° C.

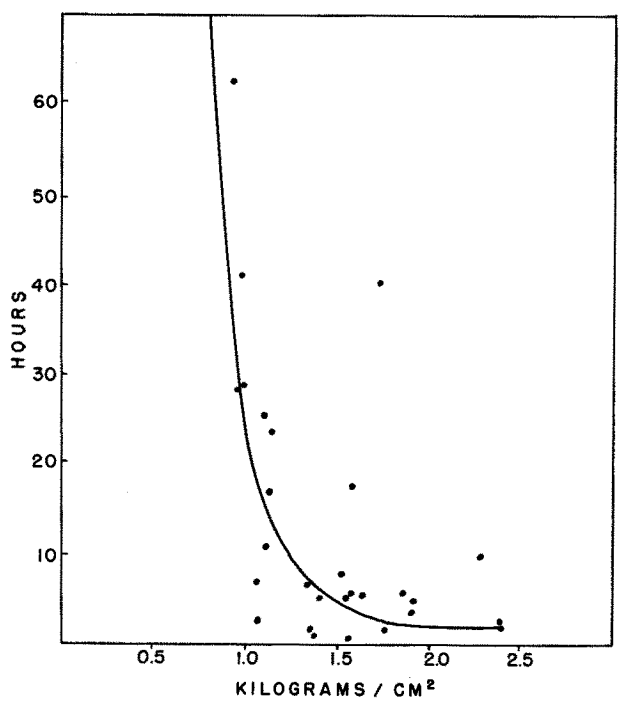

FIGURE 163.—Time in hours required to obtain complete loss of tonus in the adductor muscle of *C. virginica* kept in water under a constant pulling force expressed in kg./cm.^{2} of the cross-sectional area of the adductor muscle. Temperature 13.9° to 18.0° C. At the pulling force of 0.59 kg. complete tonus loss was obtained in 274 hours (11.5 days).

an oyster kept under the continuous pull of the relatively light weight of 2 kg. (606 g./cm.^{2} of cross section of muscle area) are shown in figure 164. The five lines represent excerpts of about 7.5 hours duration from a continuous recording made at a temperature of 13.9° to 14.1° C. and salinity of 31.3 °/oo. In line A the movements are normal. Their amplitude is increased after the application of a pulling force of 2 kg.; at the same time the frequency of contraction decreases (line B). This condition continues until the 67th hour (line D, middle part) when the muscle begins to stretch and the number of contractions greatly increases. At the 71st hour (end of line D) the muscle does not respond to stimulation. After removal of the weight (line E) shell movements are restored. The frequency of contractions during the recovery period is greater than under normal conditions. Within the next 48 hours normal shell movements of the type shown in line A are resumed.

Similar experiments in the air at higher temperatures varying from 18.5° to 24.0° C. gave slightly different results shown in figure 165. The pulling

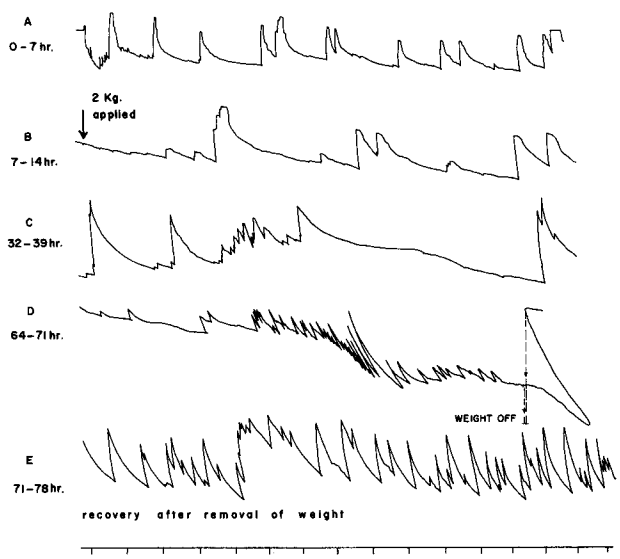

FIGURE 164.—Shell movements of *C. virginica* in sea water under continuous pull of 2 kg. (606 g./cm.^{2} of cross-sectional area of the adductor muscle). Temperature 13.9° to 14.1° C. Salinity 31.3 °/oo. A—normal shell movements before the application of weight. B—immediately after the application of weight. C—after 32 hours; note increased gaping. D—after 64 hours; wide gaping, complete loss of tonus and lack of response to stimulation. Maximum valve opening 1.5 cm. E—increased muscular activity during the recovery period following the removal of the weight. Time interval: A, B, C, D, and E, 0.5 hour each.

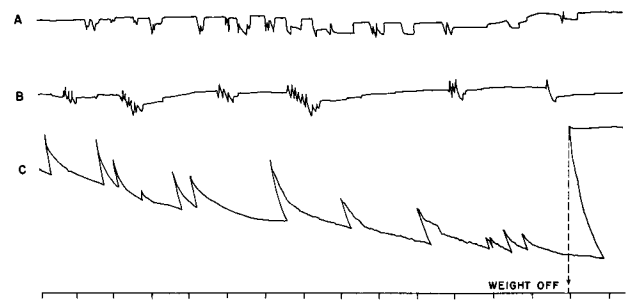

FIGURE 165.—Excerpts of the continuous records of shell movements of *C. virginica* in air under the pulling force of 2 kg. (590 of the cross-sectional area of the adductor). A—line ends at the 96th hour after the application of force; B—at 190th hour; C—at 274th hour when the muscle failed to respond to stimulation. Widest gap of valves 1.5 cm. Temperature 18.5° to 24° C. Time interval: A, B, C, 0.4 hour each.

force of 2 kg. per oyster applied in this case was equivalent to 590 g./cm.^{2} of the cross-sectional area of the muscle. Loss of tonus was attained in this case after 274 hours (line C) when the gap between the valves reached the maximum of 1.5 cm. Pathological condition of the muscle was apparent after 96 hours (line A) and became pronounced at 190 hours (line B). After removal of the weights the oyster was left in running sea water but failed to recover and died in 2 days.

A lighter weight (315.5 g./cm.^{2} of muscle area) applied to an adult oyster kept in running sea water at temperatures ranging from 13.9° to 18.0° C. produced very slow changes in the normal shell movements (fig. 166). The upper line of figure 166 represents normal movements recorded immediately after the application of the weight. A noticeable increase in the amplitude of contractions began on the 3rd day and continued through the 11th and 12th days. During the 13th and 14th days the amplitude of up and down strokes was greatly reduced; loss of tonus and failure to respond to stimulation developed by the 18th day. The last line shows the typical staircase contraction following the removal of the weight, indicating that the muscle retained some of its elasticity. At the maximum amplitude of the contractions (9th and 11th days) the oyster periodically lifted the weight of 1 kg. to the height of about 1 cm. Ten days after the end of the test the oyster recovered completely and its shell movements became normal.

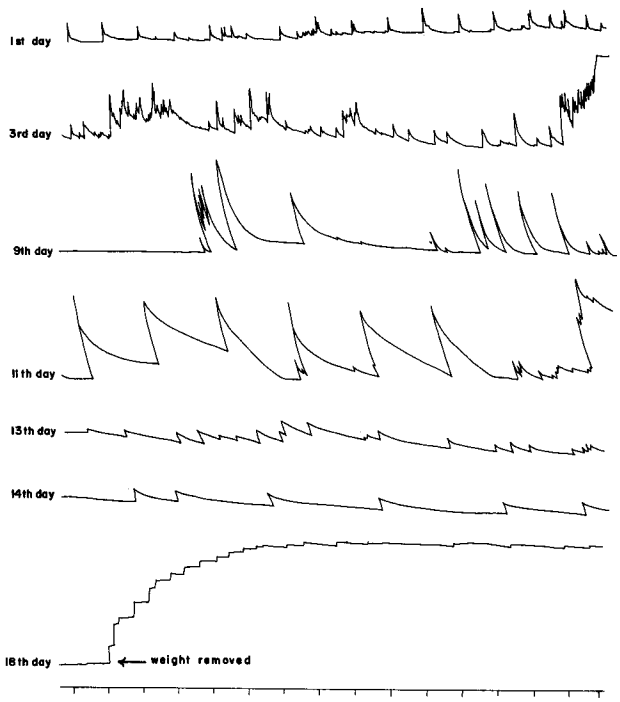

FIGURE 166.—Shell movements of *C. virginica* in running sea water under a continuous pull of 1 kg. (312.5 g./cm.^{2} of the cross-sectional area of the adductor muscle). Temperature 13.9° to 18.0° C. Time interval: 0.5 hour.

If much greater weight (4 kg. per oyster or 1,150 g./cm.^{2} of muscle area) is applied shell movements become abnormal at the very beginning of the test. This is demonstrated in the records of two Cotuit (Mass.) oysters (*C. virginica*) and one *C. gigas* shown in figure 167.

The stretching of the adductor muscle by a pulling force not exceeding 4 kg. per oyster did not interfere with their feeding; a strong current was maintained by the gills, and the feces were formed and discharged in a normal way. However, the secretion of mucus by the mantle and gills was greatly increased. Vast quantities of slimy material accumulated at the mantle edge and were discarded as pseudofeces.

The resistance of the adductor muscle to a pulling force exceeds by many times the force required to overcome the elasticity of the ligament and close the shell. This additional force is apparently needed to keep the valves hermetically sealed. The ability to keep the valves tightly closed has definite survival value. Mollusks possessing it are able to protect themselves against desiccation when exposed to air, or against adverse conditions caused by the presence of toxic substances in the water. Powerful muscular mechanism also helps

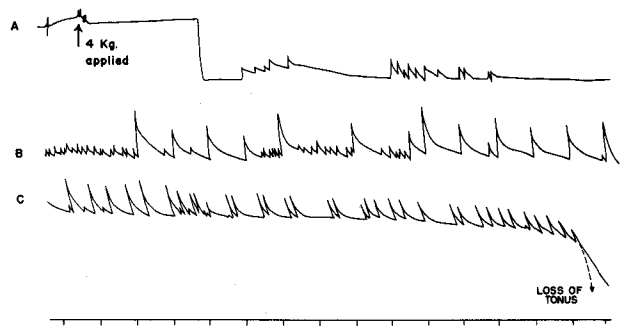

FIGURE 167.—Shell movements of two Cotuit oysters, *C. virginica* (lines A and B), and *C. gigas* (line C) in sea water under a continuous pull of 4 kg. or about 1,150 g./cm.^{2} of cross-sectional area of the adductor muscle. Temperature 14.5° to 16.5° C. Salinity 32.0 to 32.3 °/oo. The exact time of tonus loss is shown by the broken line and arrow. Time interval: A, B, and C, 0.5 hour each.

them to resist attacks of starfishes, crabs, and other enemies that attempt to pry open their valves.

CYCLES OF SHELL MOVEMENTS

There is no indication of any periodicity in muscular activity in the kymograph records of shell movements of oysters that were kept in running sea water in the laboratory or kept outside on a suitable platform submerged from a pier (Loosanoff and Nomejko, 1946). Brown and his associates (Brown, 1954; Brown, Bennett, Webb, and Ralph, 1956) claim, however, that *C. virginica* possesses a persistent lunar cycle of activity with the maxima occuring at about 12.5 hour intervals. Oysters used for obtaining tracings of shell movements were kept for a fortnight or longer in about 4 or 5 l. of sea water which was not changed but was adjusted by occasional addition of distilled water to compensate for evaporation. The mean daily cycles were calculated for 15-day periods by obtaining the average value of opening for each hour of the day and applying to the data a very complicated method of adjustment. The main conclusions reached by the authors were that: (1) oysters and quahogs display "statistical rhythms of opening of shell while the overt rhythms are not apparent from kymograph records", (2) short periods of opening tend to occur about 6:00 a.m. and more or less prolonged periods of openings happen through much of the remainder of the day. The observations and their mathematical treatment are of interest from a theoretical point of view, but the ecological

significance of the times of maxima and minima of activities in the daily cycle of the oyster are difficult to imagine at the present time.

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