CHAPTER III

THE LIGAMENT

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APPEARANCE AND STRUCTURE

The significance of the ligament in the phylogeny and classification of bivalves was a favored subject in malacological studies of the past century. Lengthy theoretical speculations about this structure are found in the papers of Bowerbank (1844), Jackson (1890, 1891), Tullberg (1881), Dall (1889, 1895), Reis (1902), Biedermann (1902), Stempell (1900), and others. A review of the literature from the earlier years to 1929 is adequately presented by Haas (1935). These investigations give little information, however, concerning the microscopic structure, origin, chemical composition, and function of the ligament. The latter subjects receive attention in the more recent works of Mitchell (1935) on the ligament of *Cardium corbis*, in a series of detailed studies by Trueman (1942, 1949, 1950a, 1950b, 1951, 1952, 1953a, 1953b) on the ligaments of *Mytilus*, *Pecten*, *Nucula*, *Ostrea edulis*, *Tellina tenuis*, and the Semelidae, and in the paper of Owen, Trueman, and Yonge (1953) on the ligament in the bivalves.

The ligament of the Atlantic oyster is a narrow band of dark, elastic material situated along the edge of the hinge between the two valves. The ligament does not extend deep into the shell, is not visible from the outside, and is called internal or ligamentum internum by Haas (1935) and "alvincular" by Dall (1889). The latter term is no longer used in malacological literature.

The ligament performs a purely mechanical function. Its elastic material, compressed when the contraction of the adductor muscle closes the valves, expands and pushes the valves apart when the tension of the adductor is released. The extent to which the valves may gape depends largely on the shape and size of the beaks. In the specimen shown in figure 17 the large, triangular space beyond the hinge permits wide excursions of the valves and their gaping may consequently be very broad.

On the other hand, the narrow and crooked beaks shown in figure 53 greatly restrict the movement of the valves along the pivotal axis regardless of the degree of relaxation of the muscle. Small pebbles, pieces of broken shell, and other foreign particles often found lodged between the beaks may further limit the opening of the valves. The possibility that such purely mechanical obstructions can impede the movement of the valves should be kept in mind in evaluating the results of physiological tests in which the degree of shell opening is recorded.

The youngest part of the ligament is that which touches the inside of the valves; the oldest portion, which is usually dried, cracked, and nonfunctional, faces the outside. When the

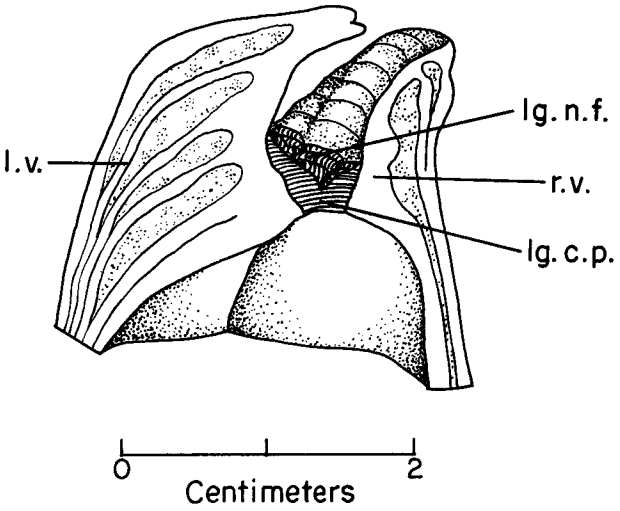

FIGURE 53.—Longitudinal section through the beak and ligament of *C. virginica*. l.v.—left valve; r.v.—right valve; lg.c.p.—functional, compressible part of the ligament; lg.n.f.—nonfunctional, old part of the ligament.

valves are forcibly separated, the ligament breaks approximately along the pivotal axis of the shell (fig. 54, piv. ax.) and the two parts remain attached to the respective valves.

The three parts of the ligament at the edge of the valves differ in color, size, and shape. The usually brownish central (inner) part called resilium forms a bulging ridge marked by fine striations visible to the naked eye or under a low magnification. The resilium is attached to a groove called resilifer or chondrophore (figs. 54, 2, 16). The dark olive anterior and posterior portions of the ligament called by Olsson (1961) tensilia are attached to the edges of the valves (nymphae).

The resilium consists of tightly packed lamellae arranged at about right angles to the longitudinal axis of the ligament; they can be seen on the exposed surface of the central part. These lamellae are intersected by fine striations visible on the side of the resilium after the removal of the adjacent lateral part (fig. 55).

When the valves are closed the resilium is compressed because of its considerable thickness while both lateral parts (tensilia) are slightly stretched. It can be seen in a series of cross sections of the hinge made at right angles to its pivotal axis (fig. 56) that the curved lines of the compressed resilium (2) are deeply arched, while those in the lateral parts are almost straight. This observation agrees with the description of the operation of the ligament of *O. edulis* by Trueman (1951). Since the beaks of the oyster illustrated are asymmetrical, the distance between the two valves is greater at the anterior than at the posterior end (fig. 56, 3, and 1) and, consequently, the anterior portion

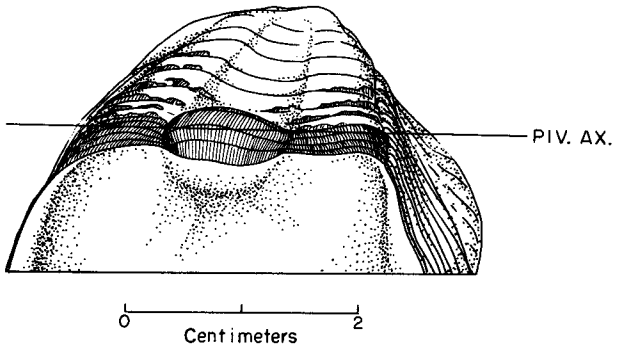

FIGURE 54.—Ligament of large *C. virginica* attached to the right valve. View from the inside. piv. ax.—pivotal axis. The resilium occupies the central position and on both sides is flanked by tensilium. Slightly magnified.

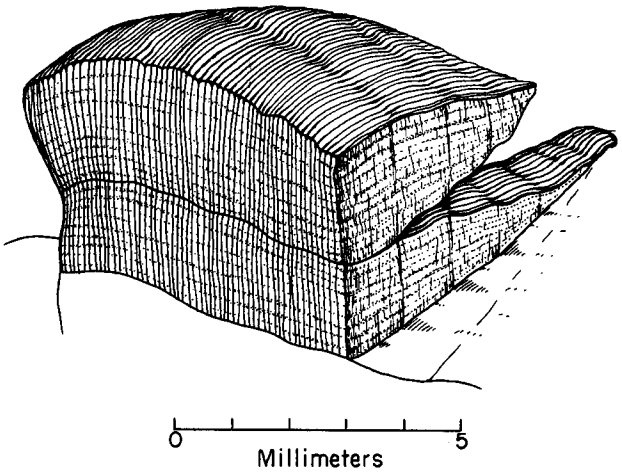

FIGURE 55.—Central portion of the ligament (resilium) attached to the groove (bottom) of a valve. Note the lamellar structure and fine striations visible on the right side of the figure. *C. virginica.*

of the ligament stretches more than its posterior part.

The ligament effectively seals the space between the dorsal edges of the valves and forms an elastic, watertight joint that prevents the entry of water and organisms which otherwise could easily invade the mantle cavity.

The spring-like action of the ligament is a function of the elasticity of its component parts. Examination of transverse and longitudinal sections of fresh ligament under low power discloses its amazingly complex structure. A cross section made with a razor blade at a right angle to the pivotal axis of the valves shows a series of well-defined curved lines extending from the right to the left valve, and a complex system of lamellae arranged perpendicularly to the curves. Both systems are clearly seen in unstained preparations mounted in glycerin jelly or in balsam (fig. 57). The pivotal axis of the ligament lies in the center of the drawing, perpendicular to the plane of the paper; the valves (not shown in the figure) are on the right and left sides, and the newly deposited portion of the ligament lies at the bottom of the drawing. The most conspicuous arches extend almost without interruption from one side to another; the lighter ones can be traced only for short distances over the cross-sectional area. The structure of the resilium resembles a leaf plate of an old-fashioned automobile spring, suggesting that the arches are the lines of stresses corresponding to the deformation of the ligament under compression. Within the mass of the ligamental ma-

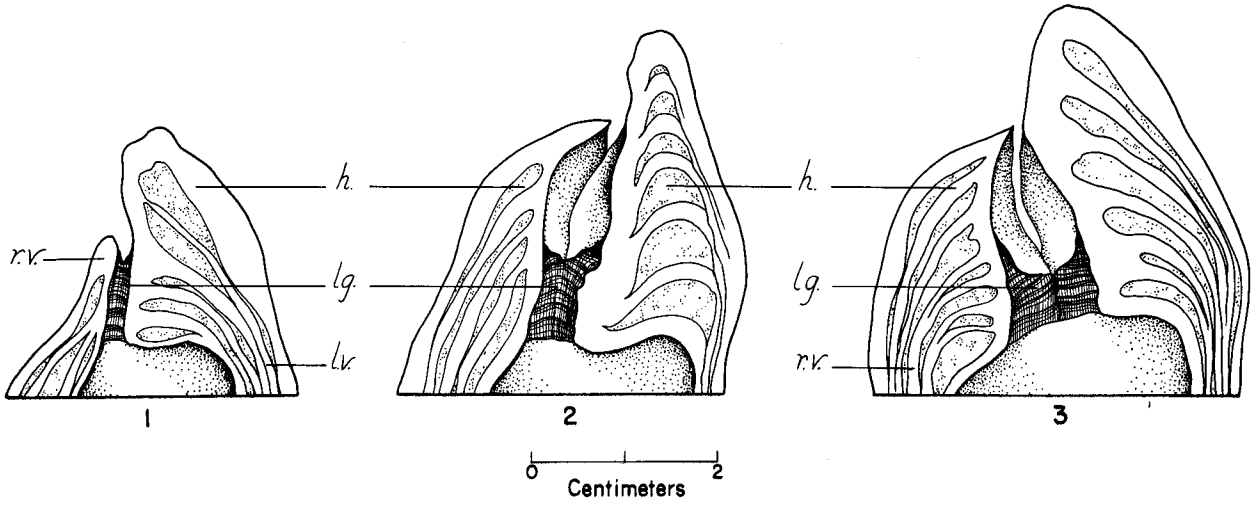

FIGURE 56.—Three longitudinal sections through hinge and ligament of the shell of *C. virginica*. (1) posterior portion; (2) central portion or resilium; (3) anterior portion, h.—beaks, lg.—ligament, l.v.—left valve, r.v.—right valve. Note the arched lines of the resilium (lg.) in the central drawing (2). Slightly magnified.

terial they are the visual evidence of these stresses. Since the "springs" of the resilium do not consist of separate structure parts joined together into a complex unit, the comparison is only superficial.

The ligament is a nonliving structure secreted at a varying rate by the highly specialized epithelium of the subligamental ridge of the mantle (see p. 89). Structurally, the arches, visible at low magnification, represent stages of growth; functionally and in accentuated form, they reflect compressional deformation in the operating structure of the ligament.

Under a binocular microscope the lamellae of the resilium, when separated with fine needles, appear slightly bent and zigzagged. A small piece of the resilium cut in the dorsoventral plane and magnified about 250 times (fig. 58) can be seen to consist of fibrillar material and of dark bands of variable width composed of tightly packed, oval, birefringent globules. Pressure over the cover slip does not change the shape of the globules, which appear to be firmly embedded in the ground substance. The globules contain no acid-soluble material since they are not affected by strong hydrochloric acid, nor are they soluble in alcohol or xylol. Preparations mounted in balsam present the same appearance as nondehydrated sections mounted in glycerin jelly. Besides the globules concentrated in the dark bands within a delicately fibrillar ground substance, some of them are arranged in longitudinal lines at right angles to the dark bands. Some of the horizontal bands (upper part of figure 58) are of much greater complexity than the others; they consist of oval-shaped light areas surrounded by globules. The two structural elements, namely, the bands of fibrils and the rows of globules, repeat themselves with regularity, the successive layers varying only in width and in the concentration and size of globules. The fibrils intersect the arches either perpendicularly or at about 45° (lower part of figure 58) and probably exert additional elastic force under compression.

The anterior and posterior parts of the ligament, the tensilium of Olsson (1961) or outer layer of Trueman (1951), are made of tenacious material which withstands considerable stretching without breaking. This can be easily ascertained by trying to tease or to pull apart the dissected parts of the tensilium. In this respect the material of the tensilium differs from that of the resilium, which is weak under tension but strong under compression. The color of the tensilium differs from that of the resilium. In New England oysters it is usually dark green on the surface, while the resilium is light brown. The tensilium is made of tough lamellae which in a transverse section appear as slender, transparent cylinders of slightly yellowish substance (fig. 59). Both resilium and tensilia are secreted by highly specialized epithelial cells which underly the ligament. The thickness of each lamella corresponds to the width of a ruffle at the edge of the secreting epithelium (See chapter V, p. 89). At low magnification the material of the tensilium appears to be non-

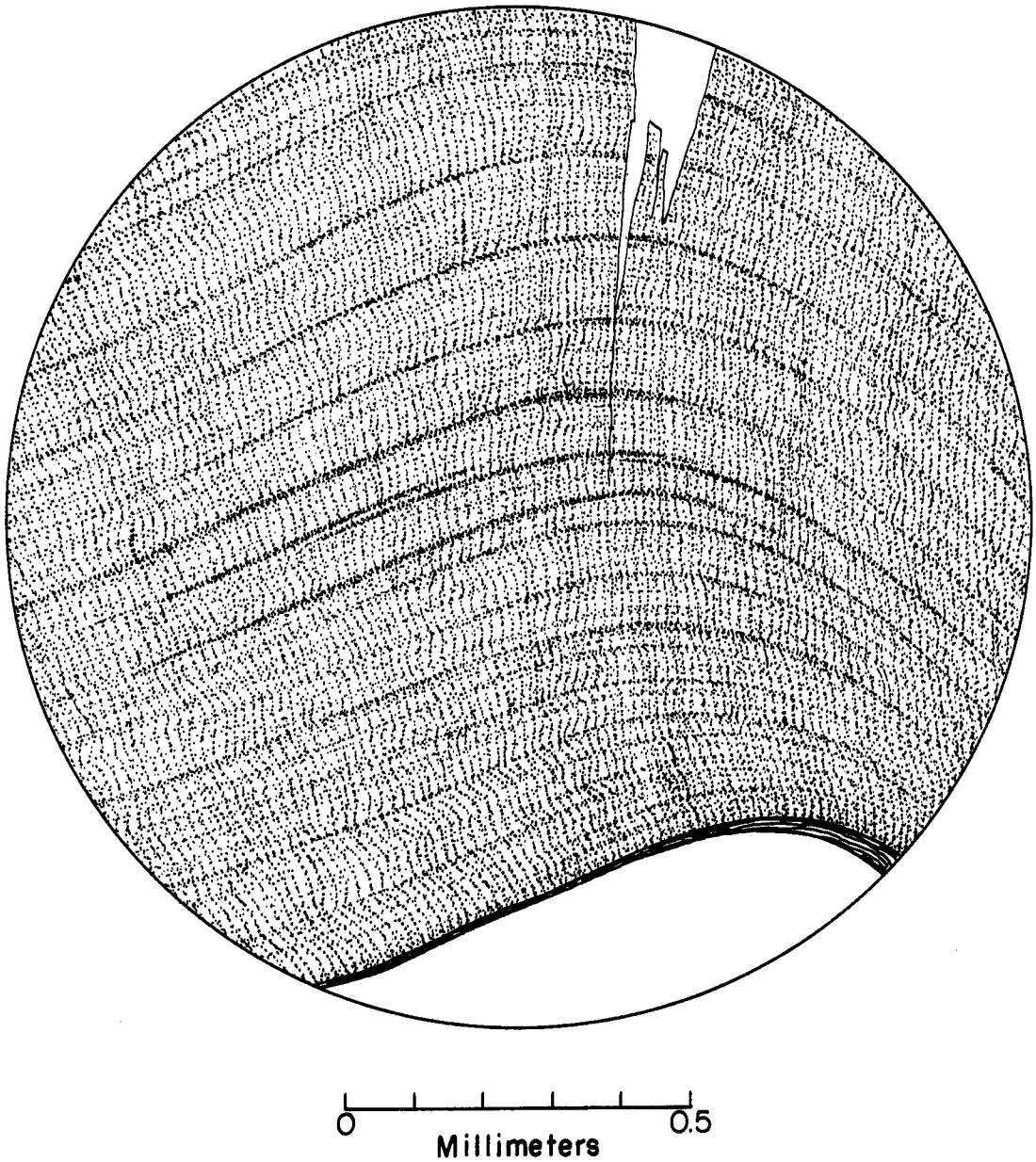

FIGURE 57.—Cross section of the central portion of the ligament of *C. virginica* made perpendicular to the pivotal axis of the valve. Arches (curved lines of dense material) extend from left to right; the valves are not shown in the drawing.

fibrillar, but at higher magnification the fibrillar structure becomes clearly visible. Two types of fibrils can be distinguished on the photomicrograph of tensilium shown in figure 60. Heavy and well-defined bundles of fibers originated along the vertical plane of the lamellae (up and down bundles in fig. 60) and short and slender fibrils in places at right angles to the large bundles (the lower half of fig. 60). Large oval-shaped bodies on the upper right and lower left part of the figure are the accumulation of calcium carbonate crystals. Single minute crystals are scattered over the body of the lamella. The outer dark layer is very thin, its color is due to densely packed narrow fibrils. Large and small globules which are conspicuous in the architecture of the resilium are absent in the tensilium, and the structure of the latter lacks the complex arrangement of globules and fibrils found in the former.

The complexity of the microscopic structure

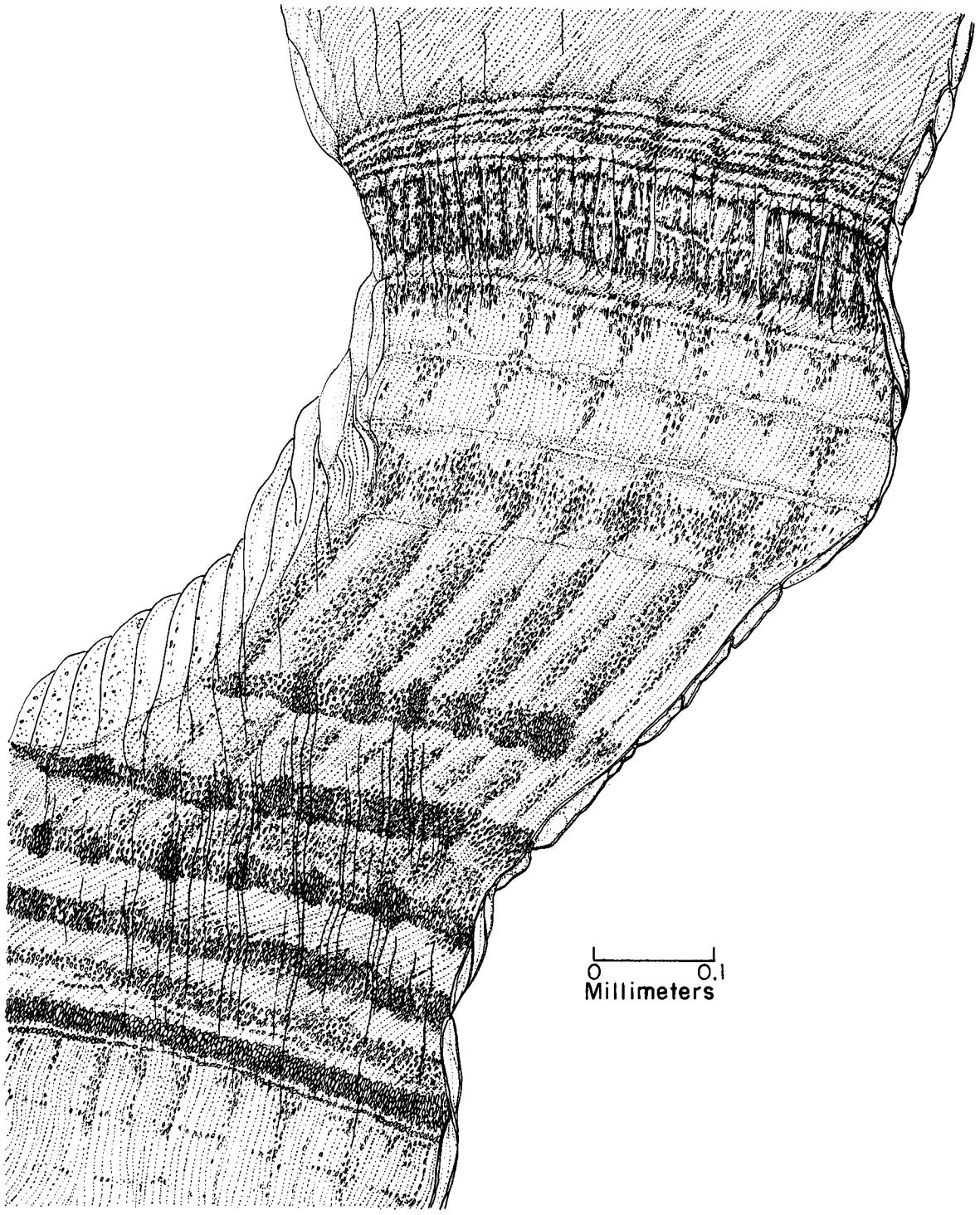

FIGURE 58.—Longitudinal (dorsoventral) section of the resilium of *C. virginica*. Two structural elements are seen: Band of fibrillae extending in vertical direction in the plane of the picture, and horizontal bands of various thicknesses consisting of numerous globules.

FIGURE 59.—Transverse section of tensilium showing lamellar structure and darkly pigmented surface, *C. virginica*. Photomicrograph of unstained and nondecalcified preparation.

suggested that electron microscopy might reveal some interesting details. Small pieces of the resilium fixed in 1 percent osmic acid were embedded in plastic and sectioned. Although the material is very hard, it was possible to obtain sections from 0.3 to 0.5μ in thickness. The electron micrograph (fig. 61) shows bands of fibrils varying in diameter from 370 to 500 Å.

A section made across the plane of the arches (fig. 62) shows a membrane honeycombed by holes about 500 Å in diameter. Two interpretations seem possible: (1) that the fibrils are tubular, the light areas corresponding to the centers of the tubes, or (2) that the empty circles represent spaces between the fibrils. The first interpretation is more plausible because of the gradation

FIGURE 60.—Large and small bundles of fibers of the tensilium of *C. virginica* seen in unstained and nondecalcified preparation of the material teased in glycerin. Photomicrograph.

from circular to elliptical light areas as the plane of section of the fibrils becomes tangential (see fig. 62).

Sections made at right angles to the fibrils (fig. 62) demonstrate a certain similarity to those of the organic membranes of the aragonitic part of the shells of mollusks and pearls. According to Grégoire, Duchâteau, and Florkin (1950, 1955), such organic membranes have a lace-like structure consisting of meshes and holes of different size and pattern. In these investigations by Belgian biologists the material was first decalcified, and the layers of organic substance then separated by ultrasonic oscillation to obtain the ultrathin membranes suitable for electron microscopy. The films of the calcite-ostracum layer of the shells of

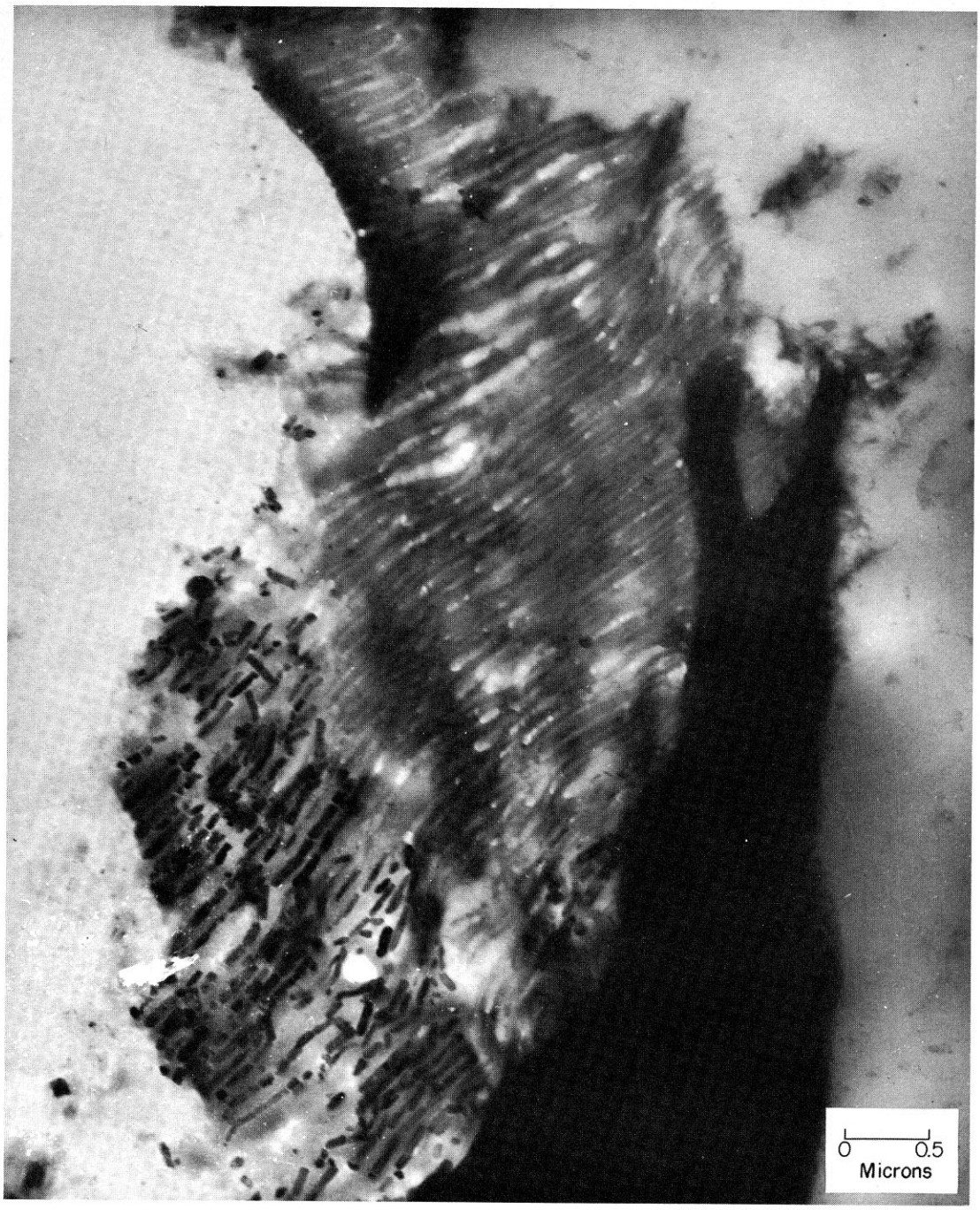

FIGURE 61.—Electron micrograph of the ligament of *C. virginica* sectioned parallel to the fibrils.

pelecypods which have no true nacre (*O. edulis*, *O. tulipa*, *Yoldia*, *Acra*, and others) were found to consist "of heterogenous material, the more representative elements of which are amorphous, vitreous plates, sometimes granular and devoid of visible (or unquestionable) pores." (1950, p. 30).[5] In the absence of ultrasonic equipment in my laboratory this method could not be used at Woods Hole, Mass. Comparison of figures published by Grégoire and his associates with the photograph reproduced in figure 62 suggests that

[5] Translation by Paul S. Galtsoff.

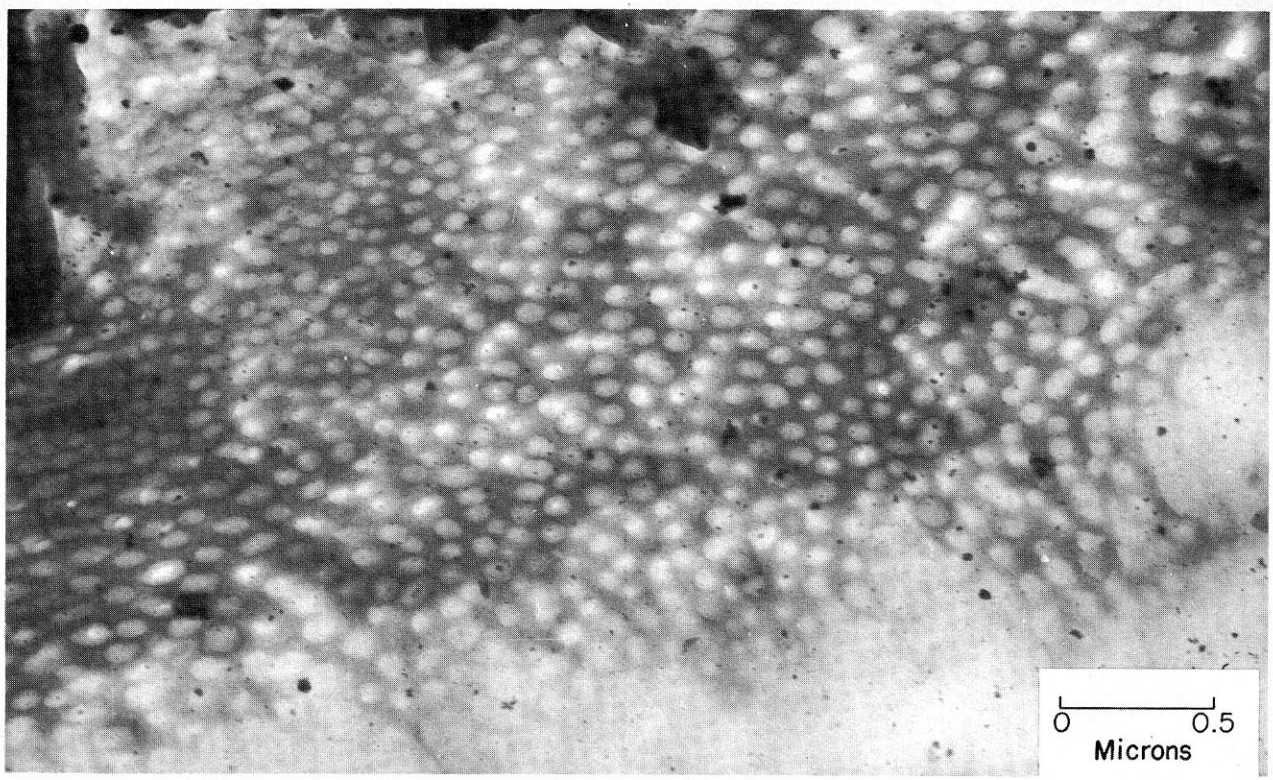

FIGURE 62.—Electron micrograph of a section of the ligament of *C. virginica* made across the fibrils.

the structure of the ligament of *C. virginica* has some similarity to that of the organic membranes of the aragonite shells. Recently Stenzel (1962) has found that the resilium of the Ostreidae contains aragonite.

One of the sections of the ligament of *C. virginica* studied with the electron microscope shows a series of black, oval-shaped bodies arranged along curved lines and separated from one another by fibrils (fig. 63). The black bodies probably correspond to the small globules visible under the light microscope. Their nature has not been determined.

The action of the ligament can be demonstrated by a rather crude model consisting of two slightly curved pieces of wood, representing the valves, joined by a series of brass rods. The rods are bent and arranged to correspond to the course of the arches as the latter are seen in an enlarged photograph of a transverse section of the ligament (fig. 57). Thin rubber tubing interwoven between the arches corresponds to the bundles of fibrils. Since the diameter of rubber tubing used in the construction of the model greatly exceeds the comparable diameter of the fibrils, this portion of the model is not in scale. Another departure from actual conditions is the interweaving of the rubber tubing between the arches, a method used to simplify construction although no such arrangement of fibrils was disclosed by microscopy. The model is shown in fig. 64. If the sides of the structure are pressed together, the arches curve up and exert lateral pressure at the same time that the increased rigidity of the rubber tubing adds to the elastic force. One can easily feel this pressure by touching the rubber tubing with the finger tips while bringing the "valves" together.

CHEMICAL COMPOSITION

The chemical composition of the ligament is essentially the same as that of the organic matrix of the shell (Mitchell, 1935: Trueman, 1949, 1951). The proteins forming the lateral (tensilium) and the central (resilium) portions of the ligament are not, however, identical. The difference can be demonstrated by staining reactions and by various chemical tests. For instance, in *Tellina tenuis* the lateral parts of the ligament are stained red or yellow by Mallory triple stain, while the inner part turns blue, a difference comparable to that between the staining reaction

FIGURE 63.—Electron micrograph of the ligament made near one of the arches parallel to the fibrils of *C. virginica*. Dark bodies probably correspond to the smallest globules seen in the light microscope.

of the conchiolin of the prismatic layer and that of the calcite-ostracum discussed on p. 42. Trueman (1949) concludes that the two types of conchiolin seem to correspond respectively to the two components of the ligament. The tensilium gives a positive reaction with the xanthoproteic, Millon's, and Merker's reagents, whereas the reaction of the resilium to these reagents is negative. Brown (1949) points out that most of the epithelial skeletal proteins of invertebrates that have been examined seem to be collagens and that their physical properties depend upon degree of hydration. The electron micrographs of the ligament (figs. 61 and 63) do not, however, show the axial periodicity of about 640 angstrom (Å.) which is the most common characteristic of collagen fibrils (Gross, 1956). Other authors describe fibrils of 270 Å. period which participate

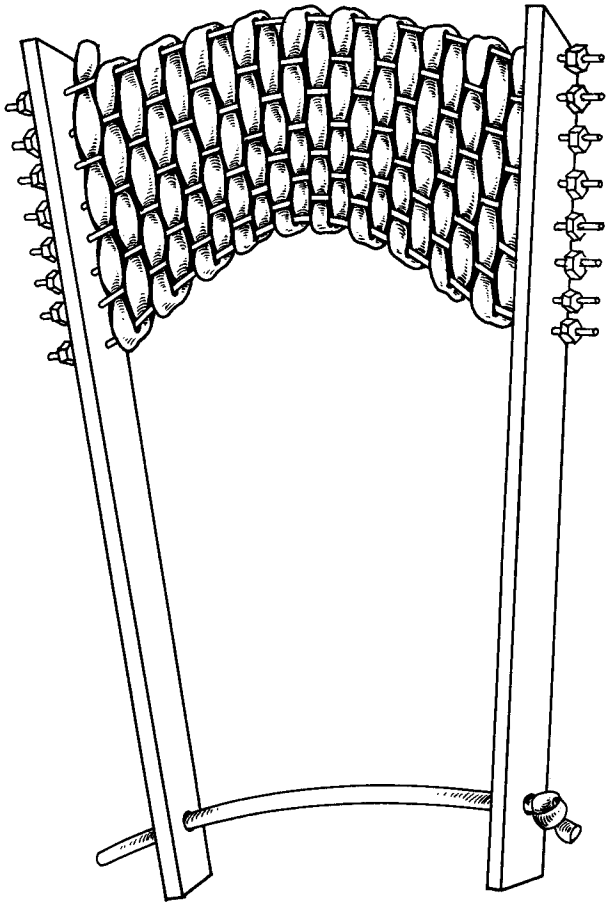

FIGURE 64.—Mechanical model of the ligament of *C. virginica*. Arches are in scale and correspond to the curves visible in a cross section of the ligament at a magnification of about 100×. Diameter of rubber tubing representing fibrillae is not in scale.

in the formation of the mature 640 Å. period collagen (See pp. 512–513 of S. L. Palay [editor] Frontiers in Cytology, 1958), as well as smaller fibrils in the embryonic tissues. The latter probably represent a very early stage in the formation of collagen.

Collagen fibers can be tanned in vitro, that is, they can be converted by various agents to a form in which they swell less and develop greater chemical resistance. The tanning of protein structures by an orthoquinone occurs naturally among many invertebrates and has been demonstrated for the cuticles of a number of arthropods (Dennell, 1947; Pryor, 1940; Pryor, Russell, and Todd, 1946) and for the chaetae of earthworms (Dennell, 1949). There is also evidence that a similar phenomenon takes place in the ligaments of bivalves (Friza, 1932). In *Anodonta*, for instance, the amber coloration of the lateral layer of the ligament is considered to be the result of tanning by an orthoquinone. This conclusion is based on the fact that even after boiling this layer induces rapid oxidation of the mixture of dimethyl-paraphenylenediamine and α-naphthol (Nadi reagent), which is frequently employed to indicate the presence of orthoquinones in the cuticles of insects and crustaceans (Dennell, 1947). In the ligament of *O. edulis* the differentiation between the two layers may be made visible by Mallory triple stain. The lateral layer (tensilium) consists of quinone tanned protein whereas the central layer (resilium) is built of calcified proteins (Trueman, 1951).

Few chemical studies have been made on the ligaments of oysters, but chemical analysis of the two portions of the ligament of the related pelecypod *Tellina* made by Trueman (1949) shows the following differences summarized in table 10.

It is rather surprising to find that an elastic, nonliving structure functioning through a considerable period of time (according to Trueman, several years in *Tellina*) is heavily calcified. The resilium of *C. virginica* contains a much larger amount of calcium carbonate than the outer parts: determinations made in my laboratory on the ligaments of 5- and 6-year-old oysters dried at 55° C. show that the calcium carbonate content of the resilium varied from 30 to 67 percent of the total weight of the sample, while in the tensilium the content of calcium carbonate was only from 5.3 to 8.5 percent.

It is apparent that knowledge of the chemistry of conchiolins and other substances found in molluscan shells and ligaments is incomplete and that much remains to be discovered about the composition and structure of these proteins which play such an important role in the life of all bivalves.

TABLE 10.—*Results of chemical tests of the ligament of* Tellina tenuis, *according to Trueman*

Test	Outer layer	Inner layer
Five percent HCl	No effect	
Saturated KOH (hot)	All dissolves	
Xanthoproteic reaction	+	−
Millon's reagent	+	−
Biuret reaction	−	−
Ninhydrin	+	+
Morner's reagent	+	−
Chitosan test (Campbell)	−	−
Chitin test (Schulze)	−	−
Argentaffine	Faint	−

ELASTIC PROPERTIES

It has long been known that the ligament performs a mechanical function by automatically pushing the valves apart when the tension of the adductor muscle relaxes. In a live oyster, however, the gaping of the valves never attains the potential maximum limited by the angle and length of the beaks. This can be demonstrated by a simple test: if the entire adductor muscle is severed, the valves open to a much greater angle than that maintained by a fully narcotized oyster with a completely relaxed muscle attached to the shell. It follows from this observation that during the entire life of the oyster the adductor muscle, even at the periods of its greatest relaxation, exerts a certain pulling force against the elastic tension of the ligament.

In view of the voluminous literature dealing with the structure and function of bivalve muscles it is surprising to find how little attention has been given to the study of the physical properties of the ligament. The first attempt to determine the pulling force of the muscle sufficient to counteract the elasticity of the ligament was made in a rather crude manner in 1865 by Vaillant who tried to measure the elastic force of the ligament of *Tridacna* shells. Trueman (1949) erroneously gives credit for this pioneer work to Marceau (1909), who only repeated the method used by earlier investigators (Plateau, 1884).

After removing the soft body of *Tridacna*, Vaillant set the empty shell on a table with the flat valve uppermost and placed a glass graduate on top of it. Water was poured into the graduate until the valves closed. Then the volume of water was read and its weight computed. The weight of the water plus the weight of the glass container and of the valve gave Vaillant a value which he called the resistance of the ligament. For a shell of *Tridacna*, apparently one of small size, he gives the following figures: weight of water required to close the valves—250 g.; weight of the vessel—700 g.; weight of the valve—632 g. The total force needed to overcome "the resistance" of the ligament is, therefore, 1,582 g.

A similar method was used by Plateau (1884), the only differences being that weights were added to a metal pan suspended from a loop encircling the valves, as shown in figure 65, and that the shell was placed on a metal ring. The elastic force exerted by the ligaments of several common bivalves, as determined by Plateau, was found to

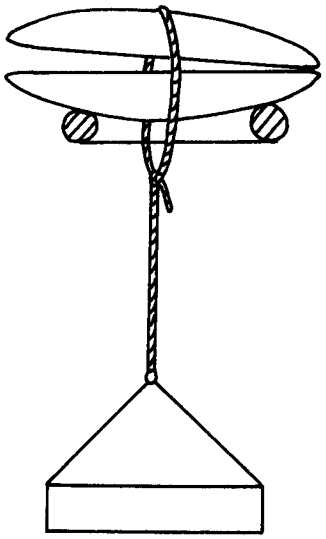

FIGURE 65.—Plateau's method of measuring elasticity of the ligament.

be as follows: *Ostrea edulis*—333.8 g.; *Venus verrucosa*—500.0 g.; *Mya arenaria*—620.0 g.; and *Mytilus edulis*—1,051.8 g. In Marceau's paper of 1909 the data taken from Plateau's work are repeated without change or verification.

Trueman's investigation of the ligament of *Tellina* (1942) marks a renewal of interest in the study of the physical properties of the ligament. In a later paper (1951) he finds that in very young *O. edulis* the outer layer of the ligament (tensilium according to our terminology) forms a continuous band along the entire dorsal margin of the hinge, but that in adults this outer layer separates into the anterior and posterior portions, leaving the inner layer (resilium) exposed at the dorsal edge. The axis about which the valves of the adult *O. edulis* open (pivotal axis) is the same in *C. virginica* (figure 54, piv. ax.). In the closed shell of *Ostrea* and *Crassostrea* the central part of the ligament (the resilium) is under compression and the two flanking portions (tensilium or outer layer of Trueman) are under tension.

To measure the opening moment of thrust of a hinge ligament, Trueman (1951), uses the following method, shown diagrammatically in figure 66: soft parts of the body are removed and the lower valve embedded in plasticine; a counterbalanced beam is erected above the valve in such a way that the weight placed on the pan at the left end is applied at the center of the upper valve. The distance from the left end of the beam to the arm touching the centroid of the

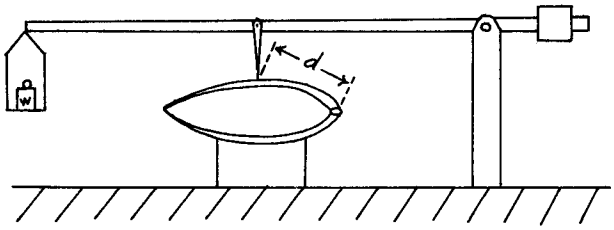

FIGURE 66.—Trueman's method for measuring the moment of thrust of bivalve ligament. Redrawn from Quarterly Journal of Microscopical Science, 1951, series 3, vol. 92, part 2, p. 137.

valve is so adjusted that the weight at the point of application to the centroid is twice that placed on the pan. Weights are gradually added until the valves just close so that the opening moment M is exactly counterbalanced. The ratio M′ between the opening moment M and the surface area of the valve A is determined by the formula: $M' = d\frac{(2W+V)}{A}$, where d is the straight line distance from the point of weight application on the shell to its pivotal axis; W is the weight applied; and V is the weight of the upper valve.

There are two objections to this method. The central point of the valve can be accurately determined only for round, symmetrical shells; for the irregularly curved shells of *C. virginica*, *C. angulata*, or *C. gigas*, its position can only be guessed. Another more serious objection refers to the determination of the weight under which the valves "just closed." Experimenting with *C. virginica*, I found that visual observation, even with a magnifying glass, is not sufficient to determine when the valves are completely closed. Frequently a tiny slit between the valves cannot be seen but becomes apparent on a magnified kymograph record of shell movement. Trueman's method with modifications was used by Hunter and Grant (1962) to study the mechanical characteristics of the ligament of the surf clam, *Spisula solidissima*. They found that the ligament of the clam is about 3.5 times stronger (in terms of opening moments) than that of *Mya arenaria*. The mechanical differences, according to their opinion, reflect the modes of life of the two clams.

The moment of thrust measured by Trueman's method is of no particular significance to the physiology of the oyster because it does not represent the pulling force which the adductor muscle must exert to close the valves or to keep them partially open. This force differs from Trueman's moment of thrust because the site of the attachment of the adductor muscle is located not in the center but in the ventroposterior quadrant of the valves. The following method overcomes these difficulties: the body of the oyster is removed without injuring the ligament; the gaping shell is placed with the left valve resting on concave cement support (fig. 67) and immobilized by small lead wedges. The right valve is connected to writing lever *N* of kymograph *K*. A glass hypodermic syringe of 10 ml. capacity, mounted on wooden frame *G*, is placed so that its plunger *F* touches the valve over the center of the muscle attachment area. The flattened end of the plunger is cut off, and its stem is sharpened to a point. A three-way stopcock *L* is attached by hard rubber tubing to the upper end of the syringe; one of its arms is connected to a hand pump *D* (automobile or bicycle tire type); the other arm leads to an open mercury manometer *C*. Two dry cell batteries *E* activate the recording electro-magnet *M* which makes a mark on the drum only when the key switch *S* is pushed down. As the pump is worked the pressure created in the system forces the plunger down, gradually closing the shell. Each time the mercury column rises 2 mm. the operator pushes the signal key down. Pumping is continued after the valves are closed until the horizontal line on the drum record indicates that increase in pressure produces no further change in the position of the upper valve. The point corresponding to the complete closure of the valves is easily determined by placing a ruler against the horizontal portion of the kymograph curve and noting the point at which the line begins to curve down (fig. 68). The number of signal marks from the beginning of the recording to the end of the curved line multiplied by two gives the height of the mercury column in millimeters. The manometer must be calibrated to correct for the error resulting from slight irregularities in the diameter of the glass tubing in its two arms.

To minimize friction between the walls of the syringe and its piston, several lubricants were tried until it was finally discovered that a minute quantity of high-speed centrifuge oil permits free movement of the piston under its own weight. The weight of the piston in the operating position, determined by placing the balance pan under the point of the piston, was recorded at 17.0g.; weight of the same piston taken out of the syringe was

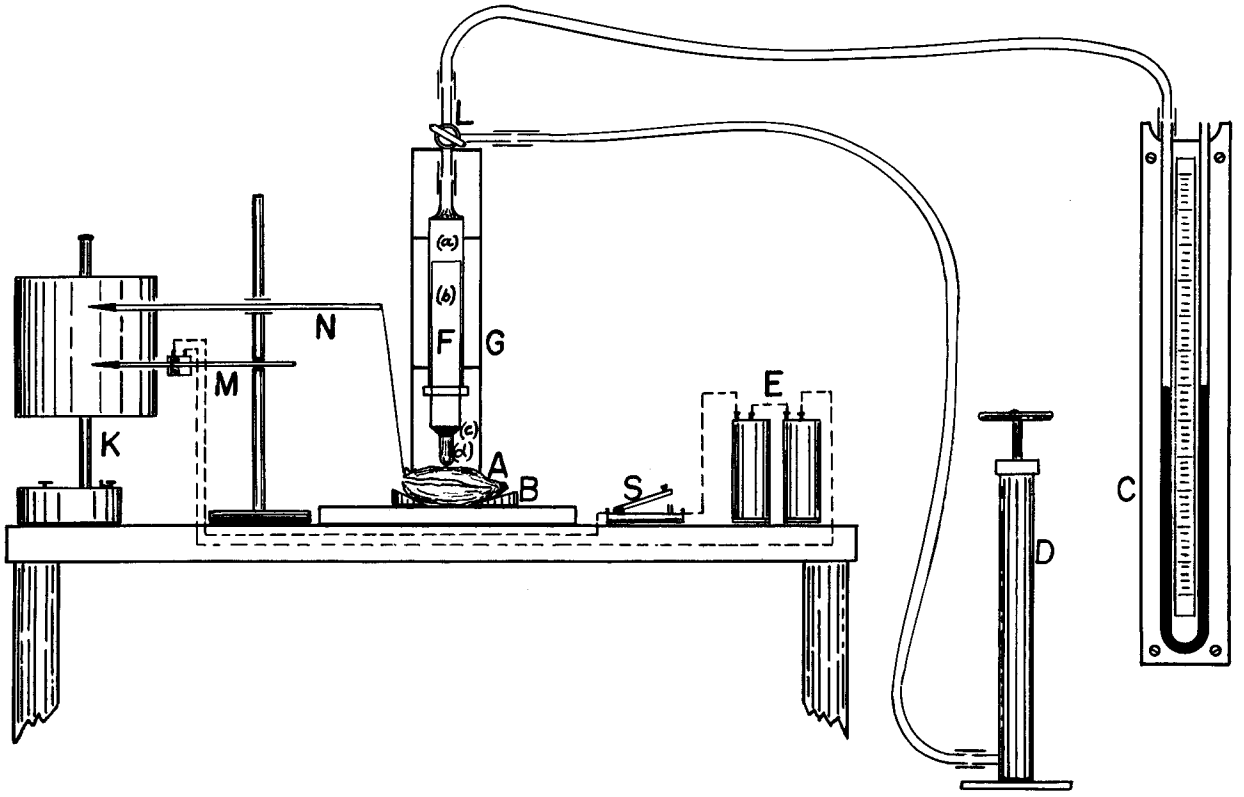

FIGURE 67.—Apparatus used for determining the elastic force of the ligament. A—oyster shell; B—support; C—mercury manometer mounted on wall; D—air pump; E—two dry cells to operate the signal magnet M; F—plunger of hypodermic syringe resting on right valve above muscle attachment area; G—stand upon which the syringe is mounted; K—kymograph; L—three-way stopcock; M—signal magnet with writing pen; N—lever connected to upper valve of the oyster A; S—key switch for signal magnet.

18.45g. Both syringe and piston were cleaned and lubricated at the beginning of each series of observations and the weight of the piston in the operating position checked frequently. Prior and during the determination, which required only a few minutes, the ligament was kept moist by frequent applications of a few drops of sea water.

To convert the manometer readings into force in grams, the following simple computation was made: since the cross-section area of the piston in the syringe is 1.971 cm.^{2} and the specific gravity of mercury is 13.95, the weight of the column of mercury is equal to 1.971 x 13.95 x H where H is the height of that column in centimeters. Determinations of elastic force made by this method are accurate within 5.3g. since readings were taken at 2 mm. intervals and the weight of a mercury column of 1 cm. height is 26.71g.

With exposure to air the elasticity of the ligament changes, gradually losing its resilience. As drying progresses greater force must be applied to bring the valves together, and the ligament becomes harder and more brittle until it finally breaks along the pivotal axis. The rate of these changes was ascertained in two tests with large American oysters from Peconic Bay, N.Y. After the shell was placed in the apparatus (fig. 67) determinations were made at 15-minute intervals between which the ligament was not moistened. Room temperature varied slightly from 68° to 70° F., and relative humidity in the laboratory was 46 percent. The results of testing which continued for 5 hours and 5 minutes indicate that under the conditions of the experiment no significant change in the physical properties of the ligament is noticeable during the first 90 minutes. After that the hardness of the ligament increases steadily as can be seen from the shape of the curve in figure 69. The test repeated a second time yielded similar results. It can, therefore, be deduced that under the given experimental condi-

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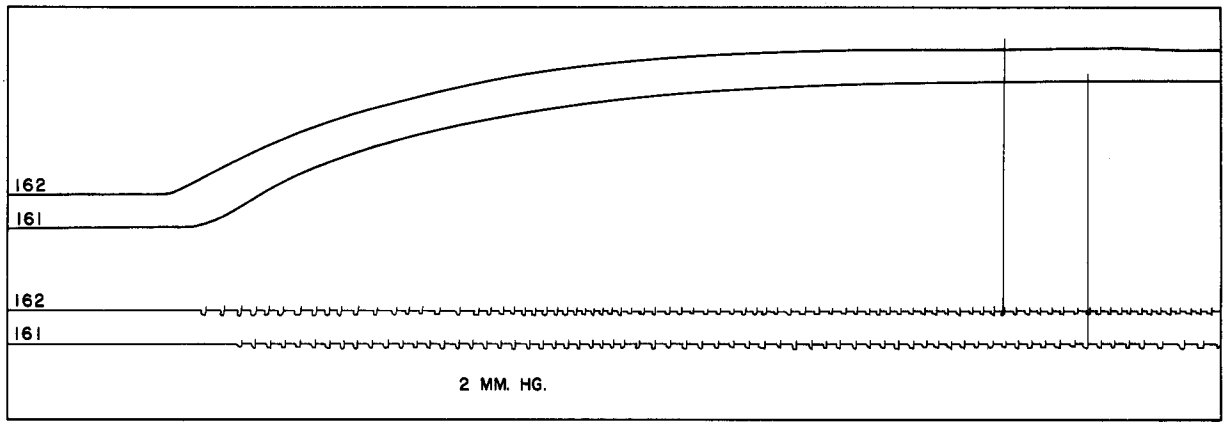

FIGURE 68.—Two kymograph records of the closing of oyster valves under pressure applied at the upper valve over the muscle attachment area. Marks on the bottom lines refer to each 2 mm. increase in the height of the mercury column in the manometer. Vertical lines indicate the point on the abscissa at which the final reading was made.

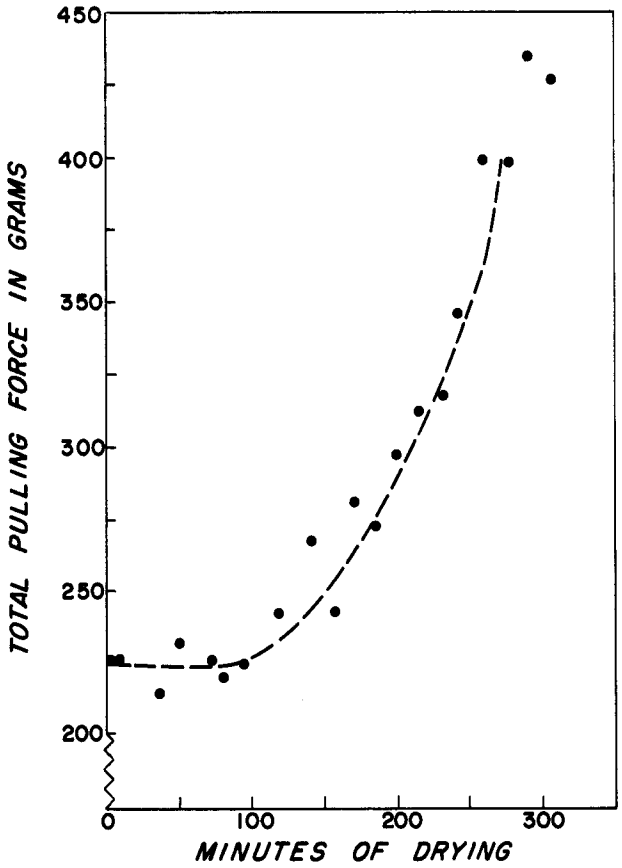

FIGURE 69.—Effect of drying on elasticity of the ligament of adult *C. virginica* from Peconic Bay, New York. (At temperature of 68° F.)

tions drying can not affect the values of readings obtained within a few minutes after the removal of the shells from water.

The question arises whether there are significant differences in the elastic properties of the ligaments of oysters living in different ecological environments. The problem was studied by obtaining samples of oysters from the following localities: Peconic Bay, N.Y. (nearly oceanic water of high and stable salinity); upper part of Narragansett Bay, R.I. (18°/oo to 24°/oo); Chesapeake Bay, Md. (10°/oo to 16°/oo), both localities characterized by considerable daily and seasonal fluctuations in salinity of water; Apalachicola Bay and East Bay, Fla., representing typical southern conditions of warm water and great fluctuations in salinity. Oysters from East Bay (near Pensacola, Fla.) were taken from three different zones: A—intertidal flat; B—bottom level; and C—below low water level in the area of exceptionally strong tidal currents. Each sample consisted of either 30 or 50 adult oysters of marketable size. After arrival at Woods Hole, Mass., they were kept at least 5 weeks in the harbor water (31°/oo to 32°/oo) before they were tested. All experiments were conducted during the winter when harbor water temperature was about 4° C. and laboratory air temperature about 21° C.

The results of the tests, expressed as the pulling force in g. per cm.^{2} of the muscle scar area necessary to counteract the elasticity of the ligament, are summarized in the series of histograms shown in figure 70. It is apparent that the elastic properties of the ligament vary greatly within each group but especially in the Peconic Bay and Apalachicola oysters. A comparison of the modes of the elastic forces in ligaments of oysters from different environments gives the following values

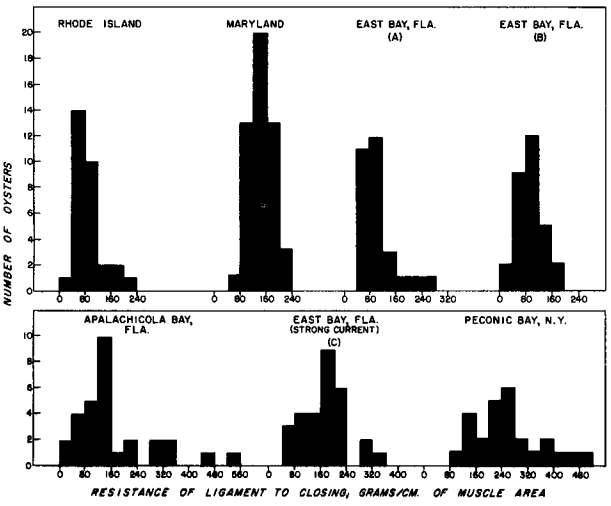

FIGURE 70.—Frequency distribution of the elastic property of the ligaments in seven groups of adult *C. virginica*. The elastic property is expressed in the pulling force of the adductor muscle (in g. per cm.^{2} of muscle area) needed to counteract the action of the ligament.

expressed in g. per cm.^{2} of transverse section of muscle area arranged in diminishing order:

Peconic Bay (Fireplace oysters)	252g.
East Bay, Fla.—C, fast tide	178g.
Apalachicola Bay	128g.
Chesapeake Bay, Md	99g.
East Bay, Fla.—B, bottom	93g.
East Bay, Fla.—A, intertidal zone	91g.
Narragansett Bay	79.g

Whether the values observed do actually depend on ecological conditions cannot be stated without further investigation.

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