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

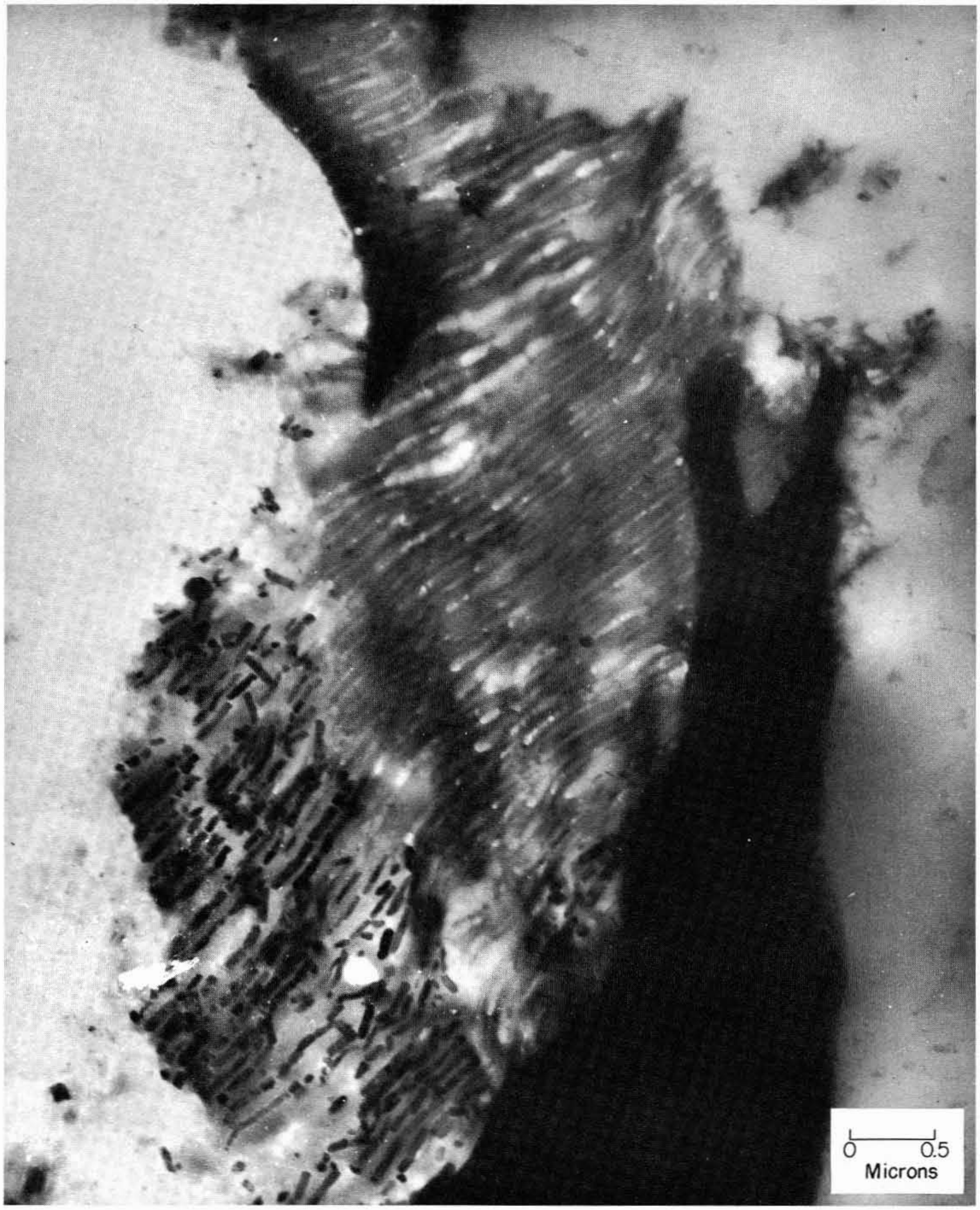

FIGURE 61.—Electron micrograph of the ligament of *C. virginica* sectioned parallel to the fibrils.

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

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[5] Translation by Paul S. Galtsoff.

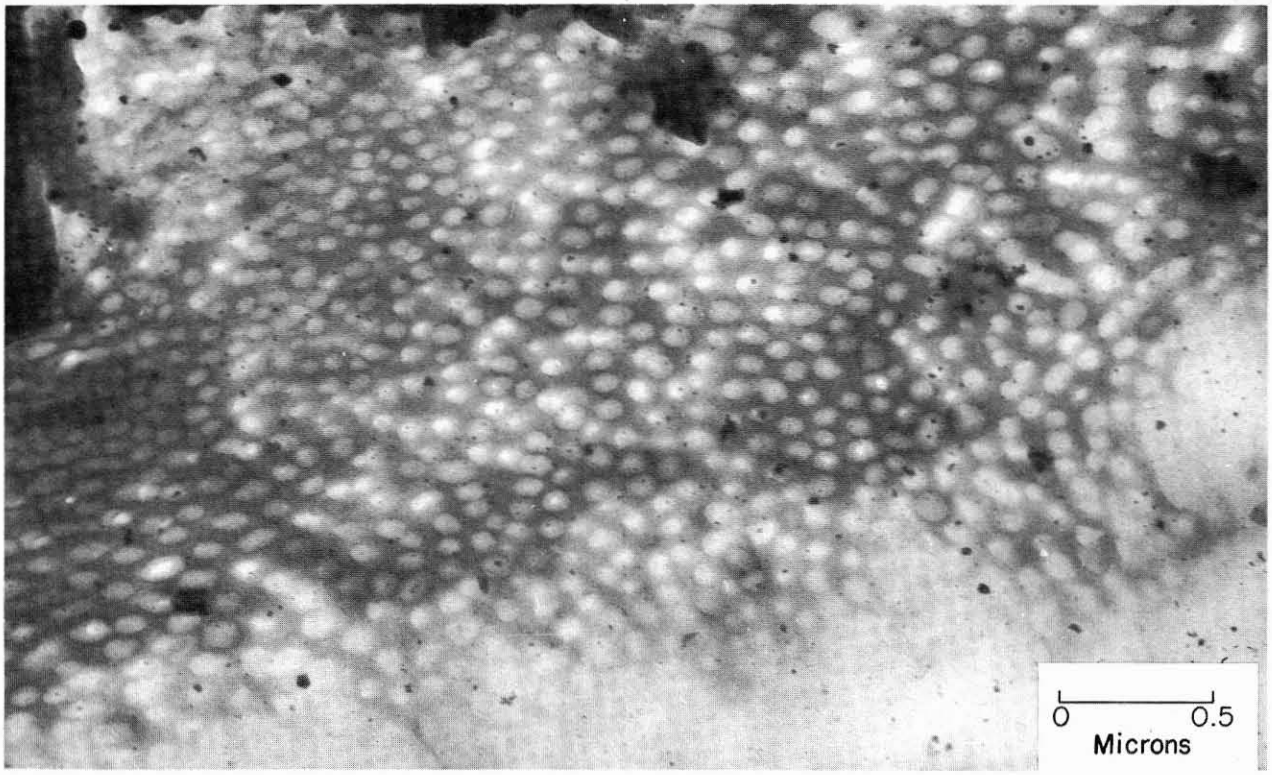

FIGURE 62.—Electron micrograph of a section of the ligament of *C. virginica* made across the fibrils.

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

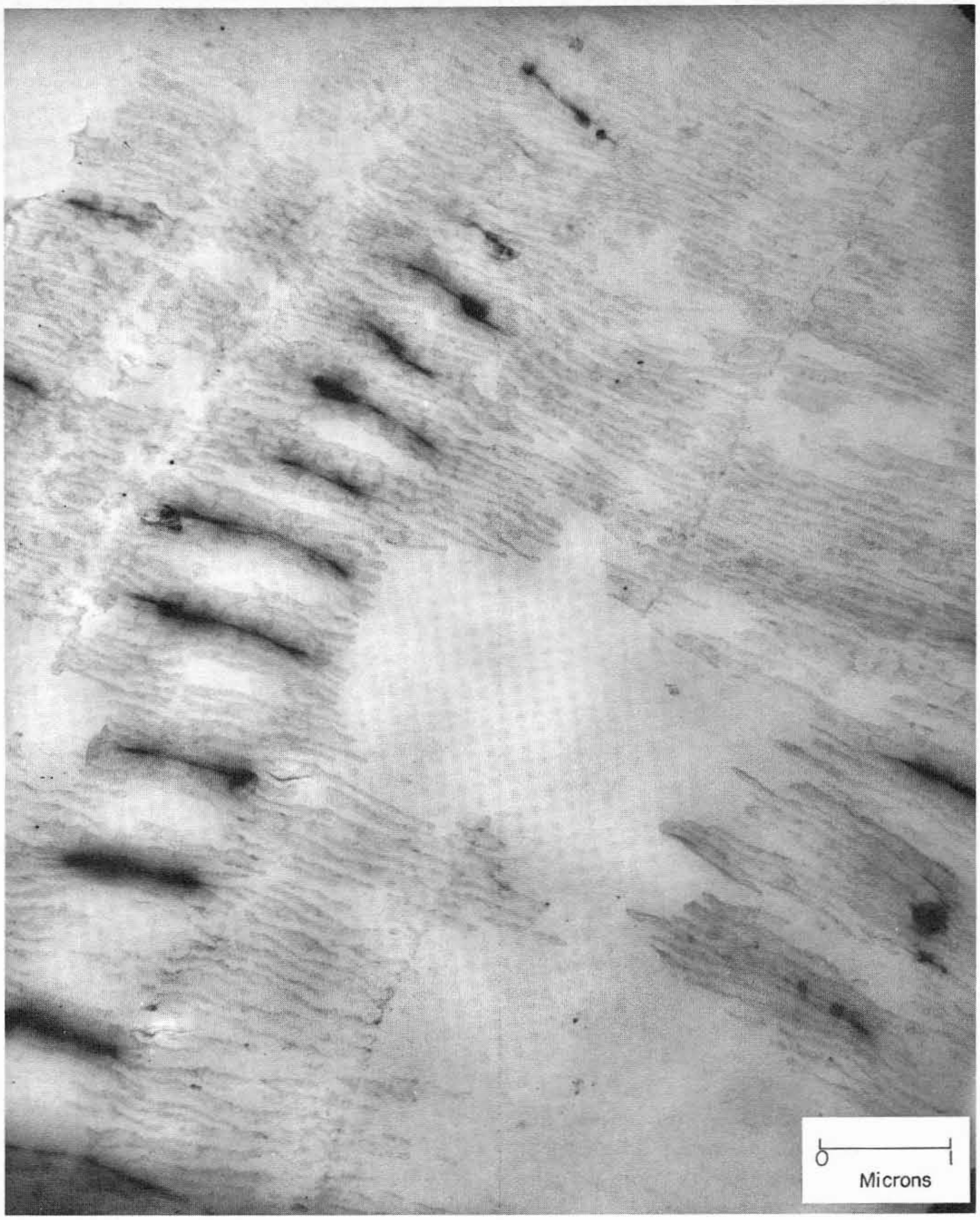

FIGURE 63.—Electron micrograph of the ligament made near one of the arches parallel to the fibrils of *C. virginica.* Dark bodies probably correspond to the smallest globules seen in the light microscope.

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

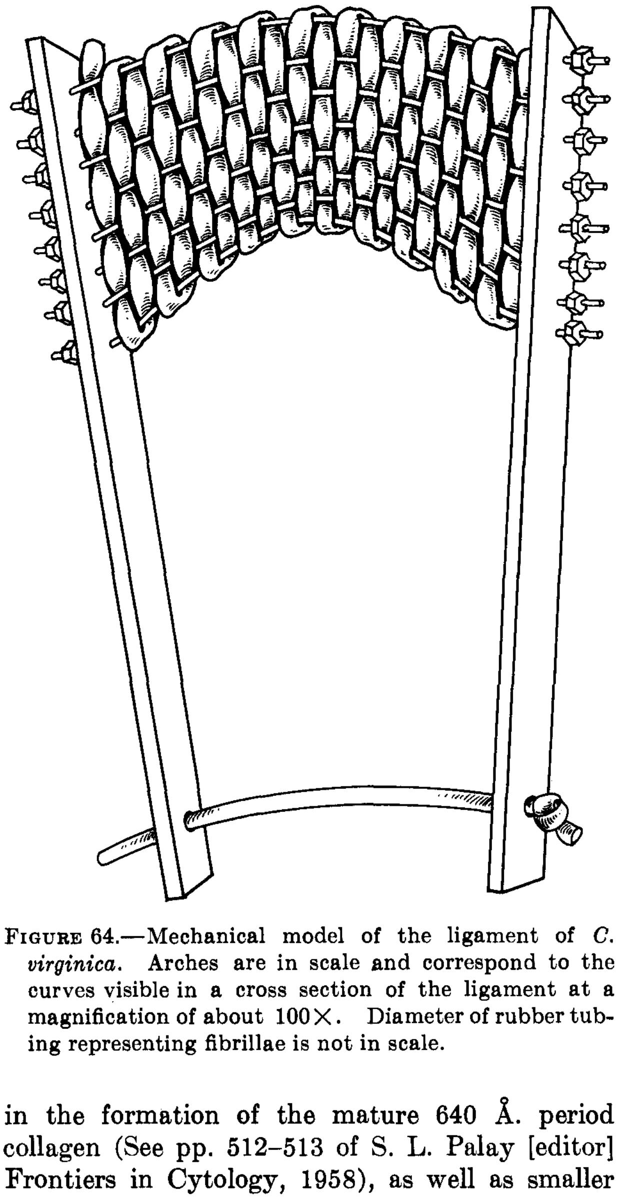

FIGURE 64.—Mechanical model of the ligament of *C. virginica*. Arches are in scale and correspond to the curves visible in a cross section of the ligament at a magnification of about 100×. Diameter of rubber tubing representing fibrillae is not in scale.

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 dimethylparaphenylenediamine 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 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.

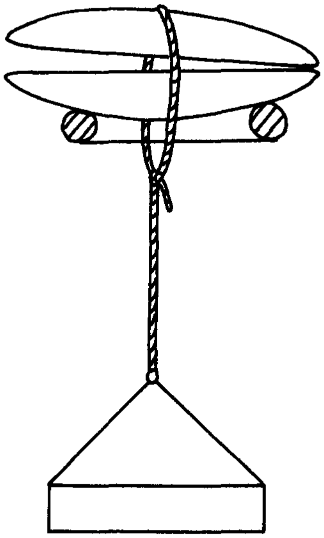

FIGURE 65.—Plateau's method of measuring elasticity of the ligament.

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