

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 ovster 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 value is connected to writing lever N of kymograph K. A glass hypodermic syringe of 10 ml. capacity, mounted on wooden frame G_{1} is placed so that its plunger F touches the value 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 stop- $\operatorname{cock} L$ 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 Sis 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 $1.971 \times 13.95 \times \text{H}$ 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-



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°/... to 24°/...); Chesapeake Bay, Md. $(10^{\circ}/_{\circ\circ}$ to $16^{\circ}/_{\circ\circ})$, 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 $(31^{\circ}/_{\circ\circ} \text{ to } 32^{\circ}/_{\circ\circ})$ 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, FlaB, 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.

BIBLIOGRAPHY

BEVELANDER, GERRIT, and PAUL BENZER.

1948. Calcification in marine molluses. Biological Bulletin, vol. 94, No. 3, pp. 176-183.

BIEDERMANN, W.

- 1902. Untersuchungen über Bau und Entstehung der Molluskenschalen. Jenaische Zeitschrift für Naturwissenschaft, Neue Folge, Band 29, pp. 1–164.
- 1914. Physiologie der Stütz- und Skelettsubstanzen. Hans Winterstein's Handbuch der Vergleichenden Physiologie, Band 3, 1 hälfte, Teil 1, pp. 319-1,188. Gustav Fischer, Jena.

BOWERBANK, J. S.

1844. On the structure of the shells of molluscous and conchiferous animals. Transactions of the Microscopical Society of London, vol. 1, pp. 123-154.

BROWN, C. H.

1949. Protein skeletal materials in the invertebrates. Experimental Cell Research, Supplement 1, pp. 351-355.

- 1950a. A review of the methods available for the determination of the types of forces stabilizing structural proteins in animals. Quarterly Journal of Microscopical Science, series 3, vol. 91, part 3, pp. 331-339.
- 1950b. Quinone tanning in the animal kingdom. Nature, vol. 165, No. 4190, p. 275.

DALL, WILLIAM HEALEY.

- 1889. On the hinge of pelecypods and its development, with an attempt toward a better subdivision of the group. American Journal of Science, series 3, vol. 38, pp. 445-462.
- 1895. Contributions to the tertiary fauna of Florida, with especial reference to the Miocene silex-beds of Tampa and the Pliocene beds of the Caloosahatchie River. Part III. A new classification of the Pelecypoda. Transactions of the Wagner Free Institute of Science of Philadelphia, vol. 3, part 3, pp. 479-570.

DENNELL, R.

- 1947. The occurrence and significance of phenolic hardening in the newly formed cuticle of Crustacea Decapoda. Proceedings of the Royal Society of London, series B, vol. 134, pp. 485-503.
- 1949. Earthworm chaetae. Nature, vol. 164, No. 4165, p. 370.

FRIZA, FRANZ.

- 1932. Zur Kenntnis des Conchiolins der Muschelschalen. Biochemische Zeitschrift, Band 246, pp. 29-37.
- GALTSOFF, PAUL S.
 - 1955. Structure and function of the ligament of Pelecypoda. [Abstract.] Biological Bulletin, vol. 109, No. 3, pp. 340-341.
- GRÉGOIRE, CHARLES, GH. DUCHÂTEAU and M. FLORKIN. 1950. Structure, étudiée au microscope électronique, de nacres décalcifiées de Mollusques (Gastéropodes, Lamellibranches et Céphalopode). Archives Internationales de Physiologie, vol. 58, pp. 117-120.
 - 1955. La trame protidique des nacres et des perles. Annales de l'Institut Océanographique, nouvelle série, tome 31, pp. 1-36.
- GROSS, JEROME.
 - 1956. The behavoir of collagen units as a model in morphogenesis. Journal of Biophysical and Biochemical Cytology, vol. 2, No. 4, part 2, suppl., pp. 261-274.

HAAS, F.

1935. Bivalvia. Teil 1. Dr. H. G. Bronns Klassen und Ordnungen des Tierreichs. Band 3: Mollusca; Abteilung 3: Bivalvia. Akademische Verlagsgesellschaft, Leipzig, 984 pp.

HUNTER, W. RUSSELL, and DAVID C. GRANT.

1962. Mechanics of the ligament in the bivalve Spisula solidissima in relation to mode of life. Biological Bulletin, vol. 122, No. 3, pp. 369-379.

JACKSON, ROBERT TRACY.

1890. Phylogeny of the Pelecypoda. The Aviculidae and their allies. Memoirs of the Boston Society of Natural History, vol. 4, No. 8, pp. 277-400.

JACKSON, ROBERT TRACY.

1891. The mechanical origin of structure in pele-

THE LIGAMENT

cypods. American Naturalist, vol. 25, No. 289, pp. 11-21.

MARCEAU, F.

- 1909. Recherches sur la morphologie, l'histologie et la physiologie comparées des muscles adducteurs des mollusques acéphales. Archives de Zoologie Expérimentale et Générale, série 5, tome 2, fascicule 6 (tome 42), pp. 295-469.
- MITCHELL, HAROLD D.
- 1935. The microscopic structure of the shell and ligament of *Cardium (Cerastoderma) corbis* Martyn. Journal of Morphology, vol. 58, No. 1, pp. 211-220. OLSSON, AXEL A.
 - 1961. Mollusks of the tropical Eastern Pacific particularly from the southern half of the Panamic-Pacific faunal province (Panama to Peru). Panamic-Pacific Pelecypoda. Paleontological Research Institution, Ithaca, N.Y. Norton Printing Co., Ithaca, N.Y., 574 pp.

OWEN, G., E. R. TRUEMAN, and C. M. YONGE.

1953. The ligament in the Lamellibranchia. Nature, vol. 171, No. 4341, pp. 73-75.

PALAY, SANFORD L. (editor).

1958. Frontiers in cytology. Yale University Press, New Haven, Conn., 529 pp.

PLATEAU, FELIX.

1884. Recherches sur la force absolue des muscles des invertébrés. I. Force absolue des muscles adducteures des mollusques lamellibranches. Archives de Zoologie Expérimentale et Générale, série 2, tome 2 (tome 12), pp. 145-170.

PRYOR, M. G. M.

1940. On the hardening of the ootheca of *Blatta* orientalis. Proceedings of the Royal Society of London, series B, vol. 128, pp. 378-393.

PRYOR, M. G. M., P. B. RUSSELL, and A. R. TODD.

1946. Protocatechuic acid, the substance responsible for the hardening of the cockroach ootheca. Biochemical Journal, vol. 40, pp. 627-628.

REIS, OTTO M.

1902. Das Ligament der Bivalven. (Morphologie seines Ansatzfeldes, seine Wirkung, Abstammung und Beziehungen zum Schalenwachstum). Jahreshefte des Vereins für väterlandische Naturkunde, Württemberg, Band 58, pp. 179-291. Stuttgart. Carl Grüninger, K. Hofbuchdruckere: zu Gutenberg (Klett + Hartmann).

- STEMPELL, WALTER.
 - 1900. Ueber die Bildungsweise und das Wachstum der Muschel- und Schneckenschalen. Biologisches Centralblatt, Band 20, pp. 698-703; *ibid.* pp. 731-741.
- STENZEL, H. B.
 - 1962. Aragonite in the resilium of oysters. Science, vol. 136, No. 3518, pp. 1121-1122.
- TRUEMAN. E. R.
 - 1942. The structure and deposition of the shell of *Tellina tenuis*. Journal of the Royal Microscopical Society, series 3, vol. 62, pp. 69–92.
 - 1949. The ligament of *Tellina tenuis*. Proceedings of the Zoological Society of London, vol. 119, pp. 717-742.
 - 1950a. Observations on the ligament of *Mytilus* edulis. Quarterly Journal of Microscopical Science, series 3, vol. 91. part 3, pp. 225-235.
 - 1950b. Quinone-tanning in the Mollusca. Nature, vol. 165, No. 4193, pp. 397-398.
 - 1951. The structure, development, and operation of the hinge ligament of Ostrea edulis. Quarterly Journal of Microscopical Science, series 3, vol. 92, part 2, pp. 129-140.
 - 1952. Observations on the ligament of Nucula. Proceedings of the Malacological Society of London, vol. 29, part 5, pp. 201-205.
 - 1953a. The structure of the ligament of the Semelidae. Proceedings of the Malacological Society of London, vol. 30, pp. 30-36.
 - 1953b. Observations on certain mechanical properties of the ligament of *Pecten*. Journal of Experimental Biology, vol. 30, No. 4, pp. 453-467.

TULLBERG, TYCHO.

1881. Studien über den Bau und das Wachsthum des Hummerpanzers und der Molluskenschalen. Kongliga Svenska Vetenskaps-akademiens Handlingar, series 4, Band, 19, No. 3, pp. 3-57.

VAILLANT, LÉON.

1865. Recherches sur la famille des Tridacnidés. Annales des Sciences Naturelles: Zoology, série 5, tome 4, pp. 65-172.