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*

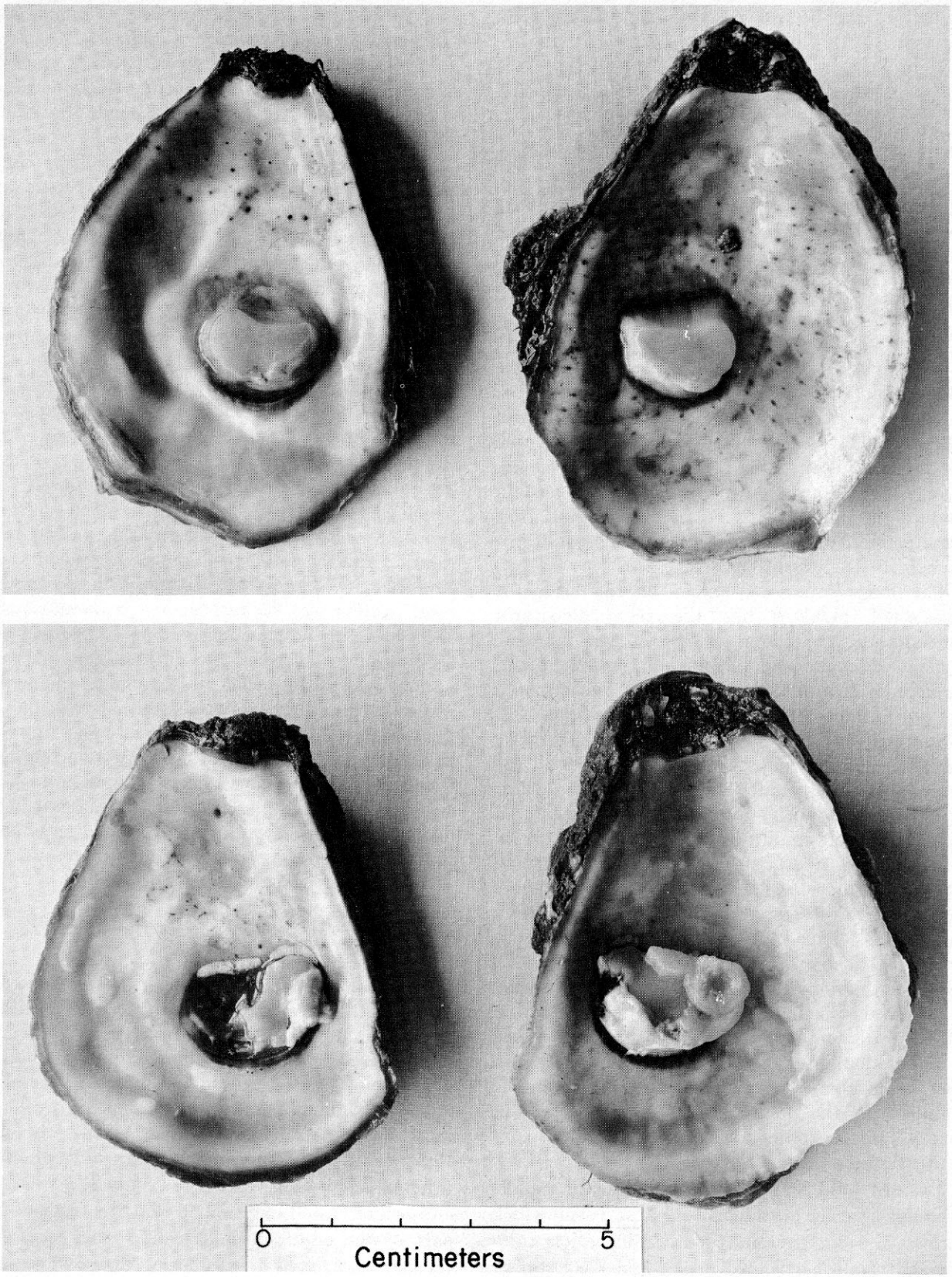
[Average percentages of fresh substance, 1934, 1935]

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.


0
5
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, adenosinetriphosphate (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 (diphosphopyridine-nucleotide) 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.

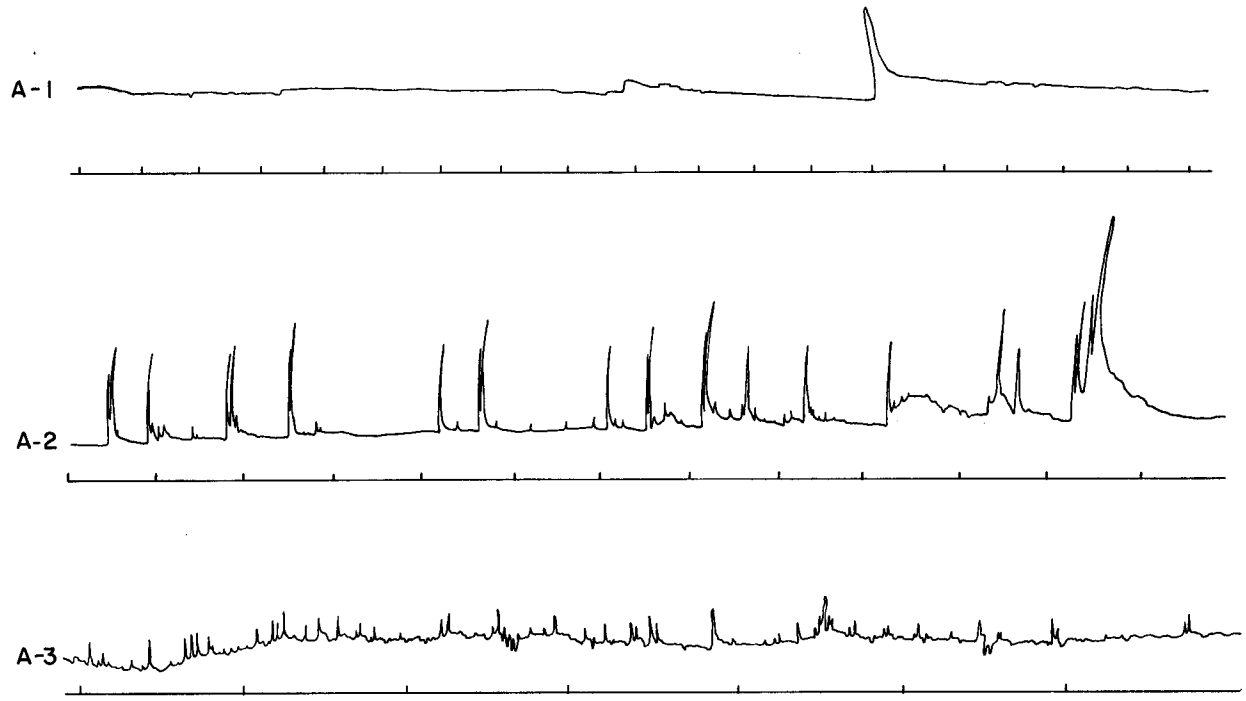


FIGURE 153.—Shell movements of normally feeding oyster. Type A. Vertical magnification of curve A–3 is about one-third of that in A–1 and A–2. In each curve the uppermost point corresponds to the position of the lever when the shells are completely closed. Time interval: A–1, 5 min.; A–2, 30 min.; A–3,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

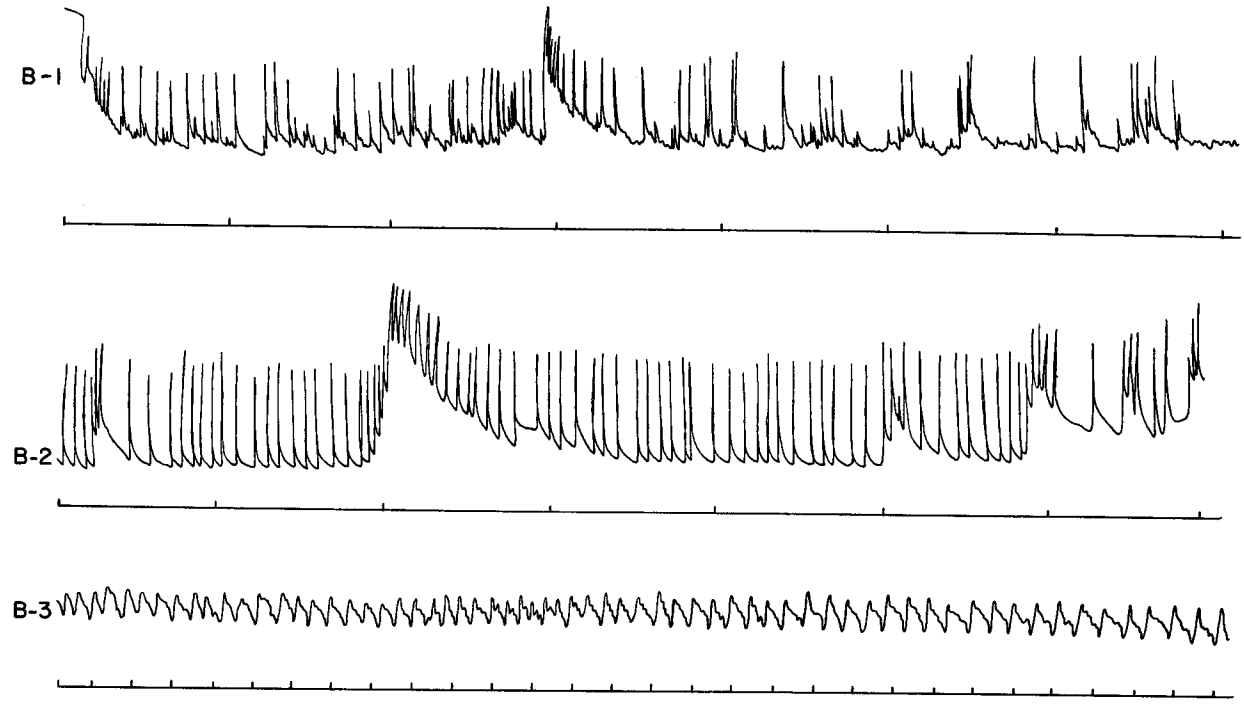


FIGURE 154.—Shell movements of type B are typical for the state of increased excitability frequently caused by the accumulation of metabolites in sea water or rapid rise of temperature. Vertical magnification in B–3 is about one-fourth of that in B–1 and B–2; uppermost points correspond to closed shells. 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.