CHAPTER XVII

CHEMICAL COMPOSITION

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Regardless of the zoological group to which an animal belongs the greatest mass of materials which form the tissues and organs, exclusive of skeleton or shells, consists of three major groups of organic compounds: proteins, carbohydrates, and lipids (fats). Many analyses reported in the literature show that, in spite of great variability in the composition of meat of several species of *Ostrea* and *Crassostrea*, the order of magnitude of the three components is common to all the species studied. The proteins make up 50 percent or more of the solids, carbohydrates are less than 25 percent, lipids constitute less than 20 percent.

PROXIMATE COMPOSITION OF OYSTER MEAT

A general idea of the proximate composition of the meat of *C. virginica* can be deduced from tables published by the U.S. Department of Agriculture for dietitians, nutritionists, physicians, and others engaged in planning diets or in calculating the nutritive value of foods (Watt and Merrill, 1950, p. 36). The material used for such analyses represents the average sample available for purchase at the market or delicatessen store. The figures do not refer, therefore, to oysters of any particular locality or to time of the year. For convenience in making a comparison all the values originally given for 1 cup (240 g.) of raw oysters were recomputed for 100 g., which corresponds to five to eight medium-size oysters. The sample contained 9.8 g. of protein, 5.6 g. of carbohydrates, 2.1 g. of fat, 94.1 mg. of calcium, 143 mg. of phosphorus, and 5.6 mg. of iron, and 80.5 g. of water.

When oysters are prepared for the market the meats are shucked and washed, either in fresh water or sea water. During this process the water is stirred and air is blown through it to remove grit, pieces of broken shell, and mud. The procedure affects the chemical composition because some of the soluble salts present in the body are lost, and the less soluble constituents, the proteins and fats, then make up the greater proportion of solids. Consequently the values for these two components quoted above are somewhat higher than for unwashed oysters. Correspondingly the values of mineral salts in Watt and Merrill's data are lower.

SEASONAL AND LOCAL VARIATIONS

Variations in the chemical composition of oysters follow distinct patterns related to environment and season of the year. The major environmental factor affecting chemical composition is the salinity of water. *C. virginica* is an estuarine species which may be found in waters ranging from almost 40‰, as in the sheltered bayous of the Gulf Coast, to less than 3‰ at the upper reaches of bays after heavy rainfall (upper Chesapeake Bay, Mobile Bay, Apalachicola Bay, and others). A change from wet to dry spells produces a pattern of fluctuations in the contents of mineral salts in oysters growing in waters of fluctuating salinity. Such conditions prevail in the waters of the south Atlantic and Gulf states where the annual range of changes from maximum to minimum ash content was reported to be 5.3 to 31.1 percent on a moisture-free basis. The solids, for the same period of time, varied between 7.5 and 18.4 percent of the wet weight of oysters (Lee, Kurtzman, and Pepper, 1960). Fluctuations in the moisture content due to absorption of water and loss of solids are the most significant features of changes in the chemical composition of oyster meat which affect their commercial quality. Good oysters contain two and one-half times more solids

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per unit of volume or weight, and obviously have higher nutritive value than the poor ones containing over 92.5 percent water.

Oysters living under marginal conditions (see chapter XVIII) are usually low in solids throughout the year. A comparison of the mean annual composition of meat based on a series of regular observations discloses these differences. Table 38 summarizes the chemical studies made for 2 consecutive years on oysters from six southern states (Lee and Pepper, 1956; Lee, Kurtzman, and Pepper, 1960). The lowest values of total solids, and of proteins, carbohydrates, and fat were found in Georgia and the highest in Louisiana and Alabama oysters. Data on seasonal variation in the composition of meat for the southern oysters were analyzed by Lee and Pepper (1956). Solids increase steadily from 9.5 percent in October to about 13.5 percent in March; in the middle of May they begin to decline and reach the lowest value of 9.2 percent in September. The fat content followed the trend approximately. The changes are associated with the gonad development and spawning, which in the southern oysters begins earlier and continues longer than in oysters of the northern waters.

TABLE 38.—*Proximate mean composition of meat of* C. virginica *from southern waters for 2 consecutive years, from October 1954 to October 1956 inclusive, in percent of their net weight*

[Recalculated from the data published by C. F. Lee and L. Pepper (1956) and C. F. Lee, C. H. Kurtzman, and L. Pepper (1960)]

State	Solids		Protein		Carbohydrate		Fat		Ash	
	1st year	2d year	1st year	2d year	1st year	2d year	1st year	2d year	1st year	2d year
Louisiana	11.5	12.2	5.8	6.3	2.7	3.0	1.1	1.3	1.9	1.5
Mississippi	11.2	12.5	5.1	6.1	3.0	3.3	1.1	1.6	2.0	1.5
Alabama	11.8	13.8	5.8	6.1	3.3	4.6	1.3	1.6	1.4	1.4
Florida	10.8	11.7	5.0	5.6	2.5	2.9	0.8	1.1	2.5	2.1
Georgia	10.7	10.0	5.0	4.4	2.3	2.1	0.7	1.0	2.7	2.5
South Carolina	13.5	13.9	6.2	6.6	3.7	4.0	1.0	1.1	2.6	2.9

YIELD AND QUALITY OF MEAT

Some idea of geographical differences in the productiveness of oyster bottoms may be gained by comparing the yield of oysters in pounds of meat per bushel. It can be seen from table 39 copied from Power (1962) that the recorded yield of market oysters in the waters of Delaware and further north varies from 6.6 to 7.5 pounds per standard bushel and is significantly higher than in the southern states, from Maryland to Texas, in which the yield is from 3.15 to 5.07.

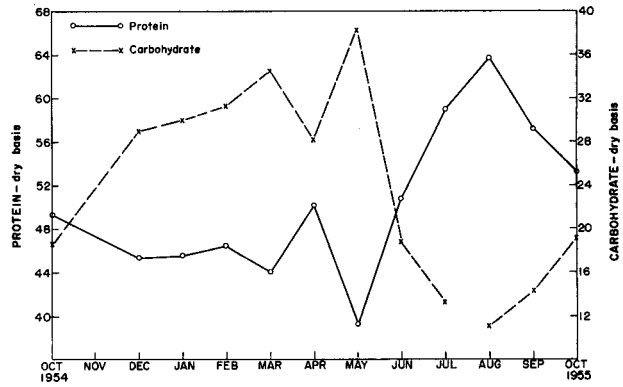

FIGURE 355.—Average protein and carbohydrate content in monthly samples of southern oysters in percent of dry weight. Oysters for analysis were collected at the shucking plant but were not subjected to the routine washing and air bubbling procedure which causes large salt and fluid losses. From Lee and Pepper, 1956.

The quality of oyster meat is related primarily to the amounts of protein and carbohydrates. The ratio between the two components changes with the season and reproductive cycle. The percentage of protein sharply decreases in May to less than 40 percent of the dry weight while at the same time the carbohydrates reach their maximum of about 60 percent (fig. 355). The actual changes in the protein content are less pronounced because of the increase in solids due to storage of glycogen.

Decline in the ash (mineral matter) content of oyster meat from the highest value of almost 25 percent (dry weight basis) in October to about 5 percent in May (fig. 356) and a gradual increase during May to September are probably related to changes in the salinity of water from which the

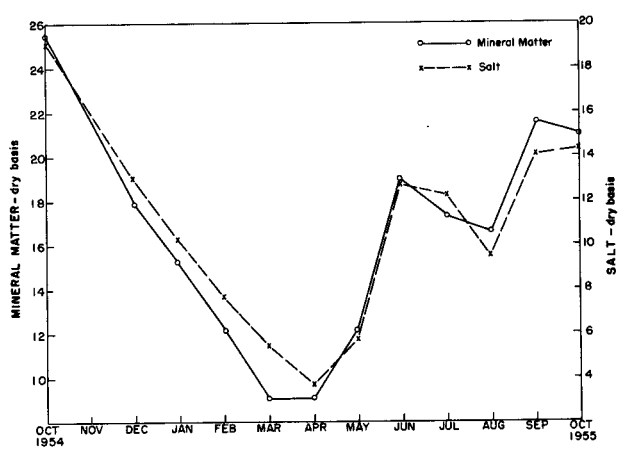

FIGURE 356.—Average mineral matter and salt content in the monthly samples of southern oysters (unwashed) in percent of dry weight. From Lee and Pepper, 1956.

TABLE 39.—*Yield of market oysters 1960, pounds of meat in U.S. standard bushel*

[From Power, 1962]

Maine	7.50	North Carolina	4.21
Massachusetts	6.50	South Carolina	2.93
Rhode Island	7.00	Georgia	3.15
Connecticut	7.70	Florida, east coast	4.20
New York	7.50	Florida, west coast	4.22
New Jersey	7.01	Alabama	4.17
Delaware	6.60	Mississippi	3.86
Maryland	4.58	Louisiana	4.54
Virginia	4.19	Texas	5.07

oysters were taken, but the problem requires further study.

Variations in the chemical composition of the meat of *O. edulis* are similar to those which take place in *C. virginica*. Gaarder and Alvasker (1941) give a detailed account of these changes in the oysters of Norwegian waters. The extent of annual fluctuations that took place in 1936 are given in table 40.

TABLE 40.—*Extent of changes in the chemical composition of* Ostrea edulis *in Norway in percentage of wet weight*

[According to Gaarder and Alvasker, 1941]

	Weight of meat	Moisture	Protein	Carbohydrate	Glycogen	Fat	Ash
	g.						
Maximum	14.6	81.9	11.2	9.6	7.9	2.5	1.5
Minimum	7.6	76.0	8.8	6.3	5.1	1.6	1.2
Average for 1936	11.3	78.7	10.0	7.8	6.8	2.2	1.3

In a comparison with the analysis of *C. virginica*, the *O. edulis* has a relatively higher carbohydrate-protein ratio and higher fat content. This may be due primarily to the fact that European oysters are grown on oyster farms while the sample of southern American oysters was taken from wild populations. Likewise, the higher yields of *C. virginica* in the waters of northern latitudes is primarily the result of skill in cultivation by private oyster growers of New England and the North Atlantic states rather than geography.

INORGANIC CONSTITUENTS

The mineral content of the edible portion of the oyster consists primarily of sodium chloride (fig. 356), but it also contains almost every chemical element present in sea water. Spectrographic analysis of 22 samples of oyster ash (exclusive of shell) made by the U.S. Bureau of Mines in 1940 at the request of the Bureau of Fisheries (data on file in the library of the Bureau of Commercial Fisheries Biological Laboratory, Woods Hole, Massachusetts) showed that the samples consisted mainly of sodium, potassium, calcium, magnesium, and phosphorus; and low concentrations of the following elements: copper, iron, silicone, aluminum, strontium, lithium, rubidium, nickel, silver, titanium, zinc, vanadium, platinum, manganese, gold, and zirconium. The results are predictable since sea water which contains these elements enters the composition of the oyster's body fluids.

IODINE

The presence of iodine in various sea food animals has been generally known for a long time and has been studied primarily from the point of view of dietitians and nutritionists. Coulson (1934) found that one average serving of *C. virginica* (110 g.) would furnish to the diet 54 μg. of iodine, an amount higher than that found in one serving of red salmon, milk, various vegetables, and beef. The iodine content of fresh oysters handled in the usual commercial manner varied from 1,000 to 11,530 parts per billion on the dry basis, or from 194 to 1,652 parts per billion on the original wet weight basis. When the means of individual variation are considered statistically, there appears to be no significant variation in the iodine content of oysters from different Atlantic and Gulf states nor any significant variation with season. There is, however, a significant difference between the Atlantic and Pacific Coast oysters, (*C. virginica* and *O. lurida*): the Pacific species have a lower iodine content than the Atlantic species. The mode of accumulation of iodine in the oyster tissue and the role it plays in the physiology of the oyster are not known.

The iodine content in oyster meat can be artificially increased by placing live oysters in sea water to which free iodine has been added. In experiments with *C. angulata* at Arcachon, France, Loubatié (1931) showed that the concentration of iodine in the tissues of oysters increased 700 times over its normal value after live oysters were kept for 4 days in water containing up to 3 mg./l. of free iodine. In 1932 a commercial concern at Bordeaux, France, artificially produced such "super-iodized" oysters and advertised their beneficial effect in cases of anemia and other maladies attributed to iodine deficiency. When I visited Arcachon in 1932 there was apparently a good demand for these oysters, which had a strong iodine flavor.

HEAVY METALS

The ability to accumulate various elements present in sea water at very low concentrations

is common to many marine invertebrates. Of particular interest is the ability of many bivalves to accumulate various heavy metals, such as zinc, copper, iron, manganese, lead, and arsenic. The problem is of importance because in polluted coastal waters shellfish may store substances that may be dangerous to human health. Hunter and Harrison (1928) showed that oysters affected by industrial pollution in certain coastal areas in Connecticut, New York, and New Jersey contained traces of lead (determined as Pb) and arsenic (As_2O_3), the concentration of the arsenic varying, depending on locality, from 0.6 mg./kg. to 3.0 mg./kg. of dry weight.

The accumulation of copper causes green discoloration of the mantle and gills of oysters and gives them an unpleasant coppery flavor. The problem of greening has attracted many investigators, especially since Lankester (1886) demonstrated that green color in some oysters may be due to an excess of copper, while in the green-gilled European oysters of the west coast of France the bluish-green coloration was caused by absorption of a pigment from a diatom, *Nitzschia ostrearia*, called marenin (Ranson, 1927). Green oysters similar to those of Marennes, France, occur occasionally along the Atlantic coast in Virginia (Mitchell and Barney, 1917) and in North Carolina (personal observation). Accumulation of iron, zinc, and manganese does not change the color of oyster meat.

The degree of concentration of heavy metals in the oyster body is related to the environment. Oysters from the North Atlantic States are poorer in iron and richer in copper than oysters of the South Atlantic and Gulf States in which the relation is reversed. This has been shown by Coulson, Levine, and Remington (1932), who analyzed a number of samples collected from various states in April and again in November-December, 1931. Their observations are summarized in table 41. The data show that the iron content of oyster meat significantly increases from north to south while the copper content decreases. The samples show no significant variations in the manganese content. The increase in iron content is associated with a greater percentage of iron (as Fe_2O_3) in the river water of the South Atlantic States discharged into the estuaries than is present in the runoff waters of the North Atlantic States. High copper content in the oysters of New Jersey, New York, Connecticut, and Rhode Island is possibly associated with the discharge of chemical wastes from shore installations of these highly industrialized states.

OBSERVATIONS ON NEW ENGLAND OYSTERS

Seasonal changes in the composition of oysters can best be studied by regularly taking samples from a single bed containing a population of oysters of known age. Such an investigation was made by taking samples of oysters from a commercial bed in Long Island Sound, off Charles Island, and simultaneously recording the temperature, salinity, and pH of the water. The work was conducted from the Bureau of Commercial Fisheries Biological Laboratories at Woods Hole and Milford. For experimental purposes and for checking analytical methods a large number of 4- to 5-year-old oysters were kept in the outdoor tanks near the laboratories. Samples of 25 oysters were taken once or twice a month for a period of 22 months from July 1933 to August 1935. Ten of the oysters were used for a chemical analysis of ash, 10 for the extraction of glycogen, and 5 for biological studies.

ANALYTICAL PROCEDURES

Oyster meats being prepared for chemical analyses are easily contaminated with iron while they are being removed from the shell. We found that the following analytical procedure was most satisfactory. The surface of the shells was cleaned with a stiff nonmetal brush, and the whole oysters

TABLE 41.—*Iron, copper, and manganese content of oysters from the Atlantic and Gulf coasts*

[Results are expressed in mg./kg. (wet basis). From Coulson, Levine, and Remington, 1932]

Locality		Spring samples			Winter samples		
		Iron	Copper	Manganese	Iron	Copper	Manganese
North Atlantic States	Range	24.9– 32.1	41.2–122.9	1.05–3.00	31.5– 47.1	34.4–137.2	1.56–2.82
	Average	28.9± 1.1	71.8± 6.6	2.09±0.20	40.7± 1.1	85.2± 9.1	2.47±0.1
South Atlantic States	Range	50.0–104.5	4.6– 38.0	1.26–4.16	45.1–135.3	3.4– 36.9	2.0–2.87
	Average	66.0± 8.0	16.1± 3.8	2.49±0.25	70.3± 8.1	17.0± 3.0	2.57+0.08
Gulf States	Range	37.5– 74.8	5.9– 26.8	2.93–4.09	65.2–113.8	19.1– 48.2	3.07–4.40
	Average	59.8± 3.4	16.0± 1.9	3.50±0.15	82.5± 5.0	26.7± 3.0	3.77+0.13

were put in glass containers and placed in an oven at 50° C. for about 1 hour. The meats were then removed with a glass spatula from the gaping valves. The shell liquor remaining in the containers was added to the meats, and a sample of 10 oysters was weighed and placed in a porcelain dish for drying at 90° C. to a constant weight.

The dried samples were pulverized in a glass mortar. Then several grams of the powdered and well-mixed sample were weighed into a silica dish, charred over a low flame, and ashed in an electric muffle at a temperature of 500° C. for 3 to 4 hours. After cooling the sample was moistened with water and 1 ml. of concentrated nitric acid was added. The sample was evaporated to dryness on a hot plate and returned to the muffle, this time at a temperature of 400° C. One application of nitric acid was usually sufficient to complete ashing. Ash was dissolved by heating in a 1:1 solution of hydrochloric acid, 10 drops of hydrogen peroxide were added, and heat was applied until the liberation of oxygen ceased. Finally the sample was weighed, transferred to a 100-ml. volumetric flask and diluted to the 100 ml. mark.

For iron determination Kennedy's colorimetric method of potassium thiocyanate was employed (Kennedy, 1927), using ferrous ammonium sulfate (dried to constant weight) as standard. Copper was determined by Biazzo's method as described by Elvehjem and Lindow (1929) and Elvehjem and Hart (1931). Zinc was determined by Birckner's method, using a nephelometer for comparison of the turbidity of samples (Birckner, 1919) and zinc oxide solution in hydrochloric acid as the standard. Manganese was found by Richards' method (Richards, 1930). To determine the reliability of analytical procedures, several analyses were made in duplicate and occasionally known quantities of metal salts were added to the samples and recovered. In this way error due to analytical procedures was found to vary between 0.5 and 2.5 percent.

Differences in the results of analyses of oyster meat often are due to the method of obtaining samples. The percentage of solids in a sample and the corresponding figure of moisture content depend on the method of drying. Sometimes the sample is dried on a steam bath at a temperature of 90° C.; in other cases the oyster meat is kept in an electric oven at 95° or 97° C. The results will also differ if the sample is first homogenized or if the whole oyster is used for drying.

The main source of inconsistency in the analyses results from methods of discarding the fluid retained in the mantle cavity and in the water tubes and chambers of the gills. This fluid consists primarily of sea water with some blood cells and excretion from the kidney. Oysters removed from the shell with no injury to the mantle and pericardium nevertheless continue to lose blood from the severed ends of the muscle and from blood sinuses in the body proper. The loss of body fluid is very rapid during the first half hour after removal from the shell. For as long as 2 hours after shucking the oyster may lose a quantity of fluids equivalent to 26 percent of the original body weight (Fingerman and Fairbanks, 1956a, 1956b). Puncturing the mantle and pericardium results in up to 50-percent loss of body weight.

To minimize losses of weight caused by prolonged bleeding, oyster meats may be placed on a screen and drained for 5 minutes. More consistent results are obtained if the water captured between the organs is discarded. If the valves are forced apart slightly and jammed open by a small wooden wedge, shaking the oyster with 10 sharp jerks is sufficient to dislodge the water from the gills. This method gives a lower percent of solid content than those obtained with other procedures, because bleeding is minimized.

The percent of moisture in the meat is usually determined by the difference between the total and dry weight of the sample. Direct determination of water content can be made by distillation in xylene in a flask with a reflux condenser. The sample is boiled continuously for 1 hour at a rate of approximately 5 ml. of reflux per minute and for 3 hours at double that rate. Without interrupting the boiling, two drops of 95 percent ethanol are added through the top of the condenser. After the violent ebullations have ceased, boiling is continued for 5 minutes (Calderwood and Piechowski, 1937), then the volume of water accumulated in the side arm of the condenser is measured.

The glycogen content of oyster tissues is determined by digesting them in 30 percent sodium hydroxide for 1 hour at 80° C. Glycogen is precipitated by 95 percent ethanol, washed, dissolved in hot water, hydrolyzed with hydrochloric acid for at least 4 hours at 92° C. and the dextrose present determined in aliquot sample by use of the Hagedorn-Jensen procedure. Details modifying the method to make it suitable for obtaining glycogen in a high state of purity from oyster

tissues are given by Calderwood and Armstrong (1941).

VARIATIONS IN GLYCOGEN CONTENT

Glycogen is the reserve material of the oyster. It is stored primarily in the connective tissue of the mantle and labial palps. During the rapid proliferation of sex cells the reserve supply is used, and by the end of the reproductive cycle the amount of glycogen is at a minimum and the mantle is reduced from a thick heavy layer to a thin transparent membrane. Soon after spawning the oysters begin to form and store glycogen and, in the parlance of oyster growers, become fat. The expression fatness as it is used in trade is a misnomer because it does not refer to an increase in lipids. In New England waters the accumulation of glycogen reaches its maximum during late autumn but sometimes continues even in winter. As a rule the glycogen remains at a high level until the beginning of rapid proliferation of sex cells in May. Seasonal fluctuations in glycogen content are common to all the species of oysters that have been studied. The pattern of changes varies in different localities and in different species depending on local conditions—temperature and abnormal salinity of water, abundance and type of food available, and intensity of feeding.

Seasonal changes in the glycogen content of New England oysters show a definite cycle related to gonad development and spawning. The rapid increase in the number of sex cells in the gonad exhausts the reserve materials and brings the glycogen content to its minimum, which usually occurs immediately after spawning. After a short period of relative inactivity during which the unspawned sex cells are reabsorbed the oysters begin to accumulate and store glycogen in their tissues. The process may be rapid, as for instance in September to December 1933 (fig. 357) or gradual as in the same period in 1934. The glycogen count of oysters of the same population varies from year to year. It can be seen in fig. 357 that in 1934 the content of glycogen after spawning was significantly higher than in the preceding year. Microscopic examination of these and Cape Cod oysters showed that sometimes the glycogen reserve is not depleted during the growth of the gonad and remains at a relatively high level throughout the spawning season. Another interesting fact noticeable in the annual glycogen curve is the continuing increase in glycogen during the cold months of winter when feeding ceases.

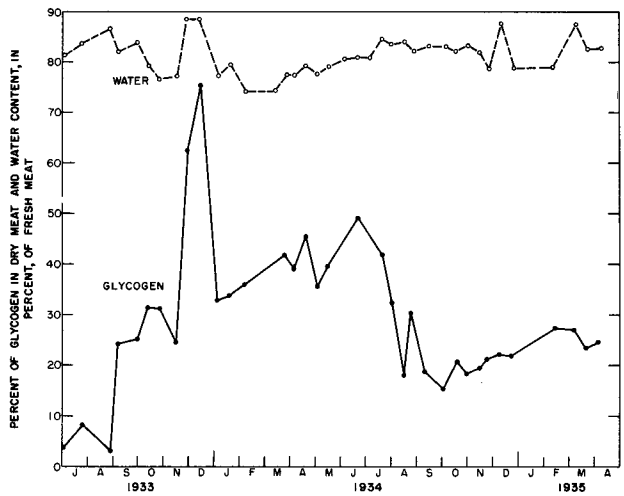

FIGURE 357.—Glycogen and water content of adult oysters (5-years-old in 1933) from commercial oyster bed off Charles Island, Long Island Sound.

The amount of glycogen stored in the tissues at a given moment is the balance resulting from the glycogen formed (glycogenesis) and that broken down (glycolysis). Biochemistry of both processes known in great detail in mammals, has not been adequately investigated in bivalves. It appears, however, reasonable to postulate that the tissue glycogen continues to be synthesized by the oyster from the carbohydrates accumulated with food during the period of active feeding or from indigenous sources of intermediary metabolism.

Increase in glycogen content is usually associated with an increase in solids and a corresponding decrease in water. There are, however, unusual instances as in the oysters found in November and December 1933 (fig. 357) which had a high glycogen content in spite of an increase of water to 88 percent and corresponding loss of solids.

The annual glycogen cycle in oysters of the York and Piankatank Rivers, Va. (Galtsoff, Chipman, Engle, and Calderwood, 1947) follows the general pattern similar to that of Long Island with the only difference that the lowest concentrations were observed in July to September and the highest in November to February. In Louisiana the period of low glycogen was found by Hopkins, Mackin, and Menzel (1954) to extend from April to the end of November. All the differences mentioned above are associated with the longer reproductive periods in warmer climates.

The cyclic change in glycogen content has been described for *O. edulis* and *C. angulata* by Bierry, Gouzon, and Magnan (1937); Bargeton (1945);

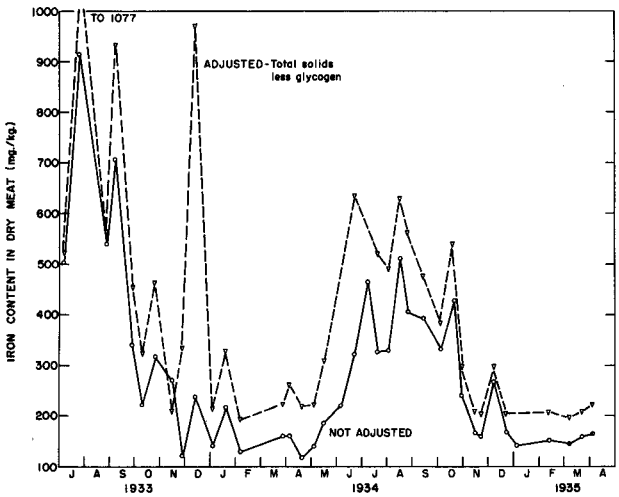

FIGURE 358.—Seasonal changes in iron content in adult oysters from Long Island Sound in mg./kg. of dry weight adjusted to weight of total solids less glycogen (broken line), July 1933 to March 1935.

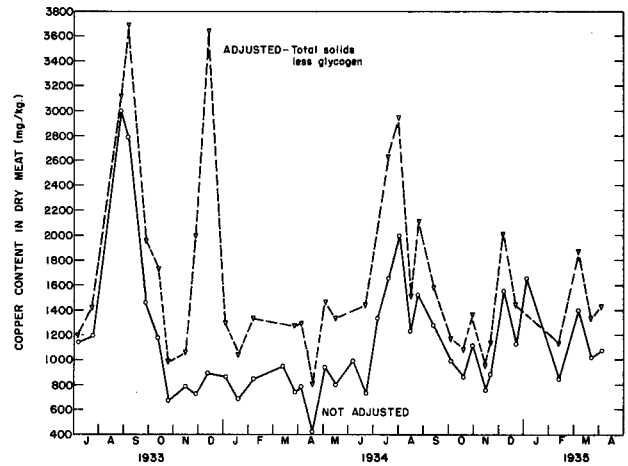

FIGURE 359.—Seasonal changes in copper content in adult oysters from Long Island Sound in mg./kg. of dry weight adjusted to weight of total solids less glycogen (broken line), July 1933 to March 1935.

Gaarder and Alvsaker (1941), and many others. In general the changes are similar to those observed in *C. virginica*, the lowest content occurring during the summer.

The cycle of fat has not been studied for *C. virginica*. According to Watt and Merrill (1950) the average content of fat of raw oyster meat sold in U.S. markets is equal to 2.1 percent. Gaarder and Alvsaker (1941) found that the fat content of *O. edulis* in Norwegian ponds varied from 2.52 to 1.56 percent with the annual average of 2.17 percent. The observed fluctuations were not seasonal.

IRON, COPPER, ZINC, AND MANGANESE

The four metals present in the meat of Long Island Sound oysters were found primarily in the gills and mantle; lesser quantities were in the muscle and gonads. Only the ovaries had manganese in quantities greatly exceeding the content of this metal in other organs. These findings are based on the series of chemical analyses of different organs and on histochemical reactions used for the localization of various metals. The curves in figures 358 to 361 showing the seasonal changes in the contents of metals expressed in mg./kg. of dry weight have a common pattern despite large differences in the levels of concentration. The amounts of metals increase during summer and decline in the following fall and winter. The increase in metals during the warm feeding season cannot be associated with the possible presence of food particles in the intestinal tract, since the total weight of food and fecal masses inside the intestines constitutes only a minute fraction of the body weight, and because the mantle and gills are the principal storage places for iron, copper, and zinc. Likewise the increase in metal content is not caused by the loss of glycogen since the general trend of the curves is not affected by adjusting the values of concentrations to the weight of solids less glycogen (dotted lines in figures 358 to 361).

With minor exceptions the two types of curves (adjusted and nonadjusted) run parallel. It appears, therefore, a firmly established fact that the content of the four metals increases during the

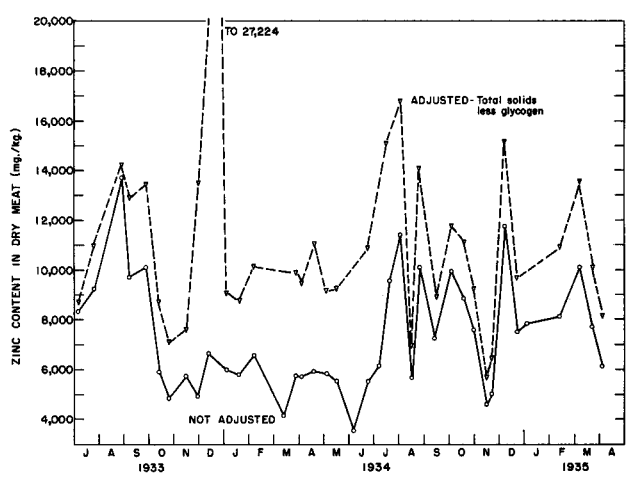

FIGURE 360.—Seasonal changes in zinc content in adult oysters from Long Island Sound in mg./kg. of dry weight adjusted to weight of total solids less glycogen (broken line), July 1933 to March 1935.

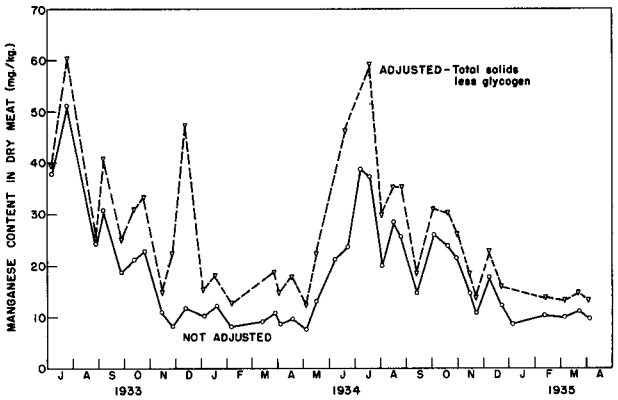

FIGURE 361.—Seasonal changes in manganese content in adult oysters from Long Island Sound in mg./kg. of dry weight adjusted to weight of total solids less glycogen (broken line), July 1933 to March 1935.

summer and decreases in winter. Heavy metals are accumulated in the oyster tissues by direct absorption from sea water, ingestion in the intestinal tract with food, and dispersal by blood cells throughout the visceral mass.

Individual variations in iron, copper, and zinc contents are large, and oysters living side by side frequently were found to vary in the contents of these metals. This is particularly easy to observe in green oysters, for the color varies in intensity in direct relation to the copper content. In the case of pronounced green discoloration the presence of metallic copper may be demonstrated by inserting in the tissues a well-polished steel knife; the surface becomes copper plated in a short time. This simple method can be used profitably for a qualitative demonstration of the presence of copper. The green pigment of the oyster can be isolated by grinding the meats with pure sand previously treated with strong hydrochloric acid and carefully washed. The proteins in the extract are precipitated with ammonium sulfate $(NH_4)_2SO_4$, but the pigment remains in solution. It was shown by S. Lepkofsky (quoted in Galtsoff and Whipple, 1931) that the green compound is not even remotely related to hemocyanin and that it exists in the oyster as a readily diffusable material. The green extract is readily soluble in methyl alcohol, less so in ethyl alcohol, and quite insoluble in butyl or amyl alcohol. It is insoluble in chloroform, ether, acetone, or benzene, but is soluble in pyridine.

When the extract is left standing for 4 months or longer in sealed glass tubes it turns to a reddish-chocolate color, but the green color returns if it is shaken with methyl alcohol, ethyl alcohol, or pyridine. Bubbling air or oxygen fails to bring back the green color.

The content of copper in the tissues can be artificially increased by placing the mollusks in sea water containing an excess of this metal. Green discoloration develops in the oysters kept in sea water which is in contact with copper pipes or valves. Within about 6 summer weeks the copper content in oysters kept under such conditions increased up to 20 times and the meats became deep green. Analyses of samples of Woods Hole water taken in the harbor and from the laboratory supply pipe showed that the copper content in the laboratory water sometimes exceeded 20 to 40 times its concentration in the harbor near the intake pipe.

The iron content of oyster meat may be artificially augmented by adding ferric salts to the water in which the oysters are kept. The iron in sea water was enriched by suspending several pounds of iron nails in the large outdoor tank with the oysters or by adding ferrous iron sulphate (copperas). Although large quantities of iron oxide particles were formed and remained in suspension, the concentration of iron dissolved in sea water did not change significantly in 28 days but the content of iron in suspension increased about five times. Particles of iron oxide were noticeable in the feces, which contained as high as 13,000 mg. of iron per kg. (dry basis). Oysters being prepared for chemical analysis were placed for several days in running sea water containing no iron particles in suspension so that all loose sediment in the mantle cavity and the gills would be discarded. The removed meats were thoroughly inspected and rinsed in sea water. Microscopic examination of sections of the gills and other organs was made at intervals varying from 20 minutes to several days following the initial feeding with iron oxide suspension. The oysters treated with potassium ferrocyanide and hydrochloric acid (Prussian blue reaction) show that leucocytes on the surface of the gills actively ingest iron particles, migrate throughout the body, and aggregate near the wall of the intestines and in blood vessels (fig. 362). No iron was detected in the digestive diverticula, sex cells, or in the adductor muscle. Some iron is eliminated through the epithelial cells of the mantle (fig. 363).

Histological localization of copper is not entirely reliable. According to Mallory (Lillie, 1948; Glick, 1949) copper compounds produce a

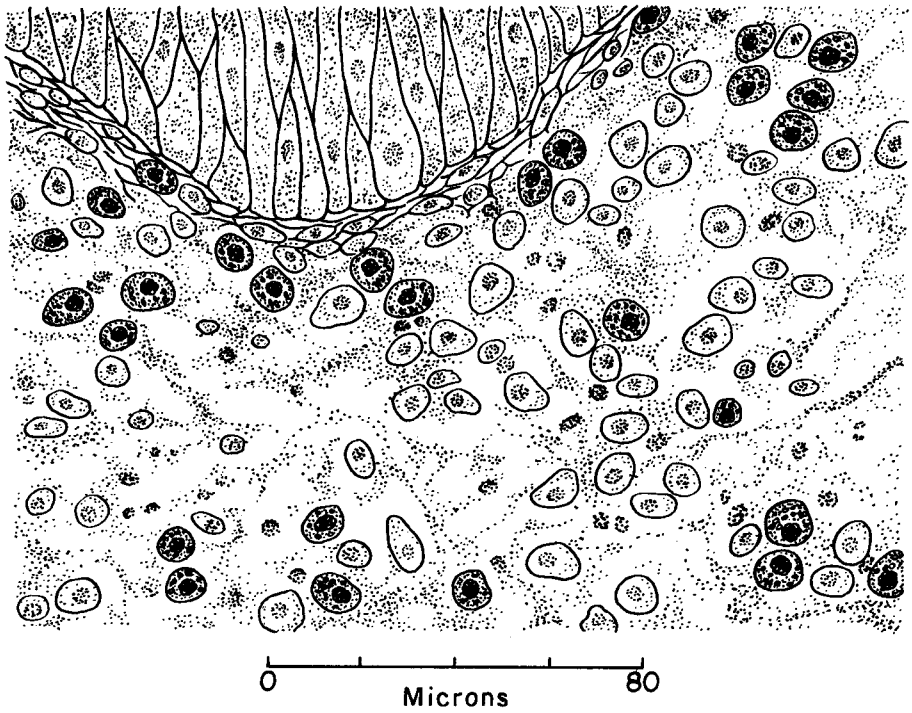

FIGURE 362.—Blood cells of *C. virginica* containing iron in the connective tissue under the digestive tract. Drawing of a section of oyster fed iron particles and treated with potassium ferrocyanide and hydrochloric acid.

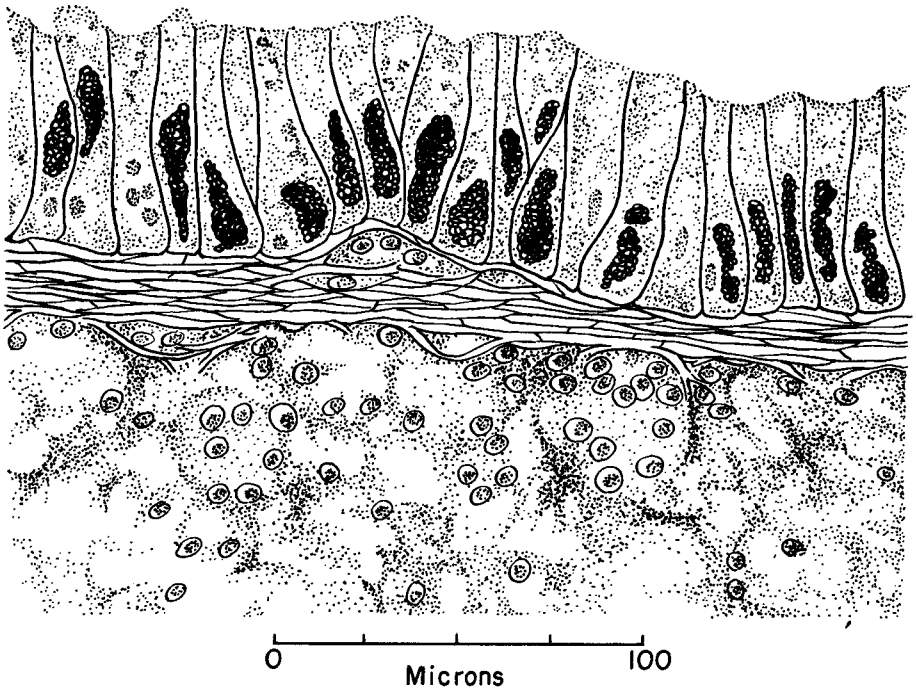

FIGURE 363.—Iron particles in the mantle epithelium of *C. virginica* fed iron oxide. Treated with ferrocyanide and hydrochloric acid.

light to dark blue color with an unoxidized fresh aqueous solution of hematoxylin made by dissolving from 5 to 10 mg. of pure hematoxylin in 0.5 to 1 ml. of 100 percent ethyl alcohol and 10 ml. of distilled water boiled 5 minutes to drive off carbon dioxide. Sections of celloidin-embedded tissues were stained for 1 hour or longer. Copper compounds appeared as a light to clear blue color. The reaction is to a certain extent obscured by a mass of yellow to brown colors produced by the iron in the tissues. The surface of the mantle and gills of green oysters usually contains large masses of blood cells loaded with dark granules which react strongly with Mallory reagent. It is obvious that a large proportion of the copper in the oyster is found in the blood cells.

For histological localization of zinc, the nitroprusside reaction proposed by Mendel and Bradley (1905) can be used. The reaction is considered by Lison as specific (Lillie, 1948). The method involves treatment of the paraffin section of tissues for 15 minutes at 50° C. in 10 percent sodium nitroprusside solution. The section is washed for 15 minutes in gently running water. Then a drop of sodium or potassium sulfide solution is introduced under one side of the cover glass. The reagent elicits an intense purple color in the zinc precipitated by the nitroprusside. In many preparations of green oysters treated by this method a diffuse purple coloration of varying degrees of intensity was produced in different organs, the mantle and gills staining conspicuously deeper than the rest of the body. The concentration of zinc within the blood cells could not be demonstrated by this method. It appears probable that zinc is present in a soluble state and is more universally distributed through the tissues than iron, copper, and manganese. Observations on the uptake and accumulation of radioactive zinc Zn^{65} confirm this view. Chipman, Rice, and Price (1958) demonstrated that zinc in surrounding water is rapidly taken up in great amounts by the bodies of oysters, clams, and scallops. The gills of oysters were found to accumulate almost twice the concentration of radioactive zinc, as did the organs and tissues. The digestive diverticula and body mass contained a considerable amount of Zn^{65}, The zinc content of sea water along the Atlantic and Gulf of Mexico inshore waters averages 10.6 μg./1.

Several histochemical reactions for the localization of manganese in the oyster tissue have been tried without success. So far as I know there is no satisfactory method for demonstrating this element in the cells and tissues.

The distribution of manganese in the oyster body is related to the female reproductive cycle, because the concentration of this element in fully developed ovaries (see fourth column of table 42), is 15 times that of the spermary (Galtsoff, 1943) and its total concentration materially decreases after the discharge of eggs. No such relationship is apparent for the other three metals.

The role of heavy metals in the physiology of the oyster is not clear. It is reasonable to assume that manganese performs some function during the rapid propagation of ovocytes, possibly as a catalyst.

Iron, copper, and zinc may be stored in the tissues and in some blood cells as excess materials which are slowly eliminated. Observations on excretion of iron by the mantle epithelium (fig. 363) and accumulation of iron, copper, and zinc in the mantle and gills support this view. The distribution of the four metals in different organs of Woods Hole oysters was studied analytically. The organs were excised by fine scissors, weighed, and analyzed separately. The results of the analyses are shown in table 42 as means of 10 samples taken from natural environment. The lower part of the table summarizes the results obtained after keeping the oysters in a tank with an excess of copperas. It appears significant that both mantle and gills have absorbed relatively large quantities of the metals.

TABLE 42.—*Distribution of metals in the body of adult* C. virginica *in Cape Cod waters (mg./kg., dry weight)*

[Mean of 10 samples. Early August and October, 1936]

Body portion*	Iron	Copper	Zinc	Manganese	Remarks
Gills	382	178	4,480	39	Summer samples from natural environment.
Muscle	136	65	1,420	4	
Ovary	151	63	1,710	60	
Spermary	136	65	1,420	4	
Residue	252	153	4,630	9	
Mantle	184	1,840	22,000	14	Autumn samples from tanks with excess of copperas after 26 days of exposure.
Gills	194	1,920	19,400	25	
Muscle	75	172	1,590	4	
Residue	401	1,490	14,400	9	

*In summer the mantle could not be separated without contaminating the sample with underlying gonads; in the autumn, after spawning, the gonads contain only few undifferentiated cells of germinal epithelium.

VARIATIONS IN THE CONTENT OF PROTEINS, AMINO ACIDS, AND VITAMINS

The protein content in oyster meat of *C. virginica*, determined by the Kjeldahl method as N $\times$ 6.25, fluctuates between 5.1 and 9.8 percent

of a fresh wet sample and between 42 and 57 percent of a dry sample. The figures quoted above from the paper by Wentworth and Lewis (1958) refer to the oysters of Apalachicola Bay, Fla., the extent of fluctuations is probably common to all oysters of the Atlantic and Gulf states since occasional observations on oysters from various states fall within this range (Jones, 1926). According to monthly observations by Gaarder and Alvsaker (1941), the protein content in the meat of *O. edulis* from Norwegian ponds ranks somewhat higher, varying from 8.8 to 11.2 percent (fresh, wet basis) with an annual average of 10.5 percent.

Interesting biological observations were made by Duchâteau, Sarlet, Camien, and Florkin (1952) on free amino acids in the muscles of marine bivalves, *O. edulis* and *Mytilus edulis*, and the fresh-water mussel, *Anodonta cygnea*. The muscles of these mollusks were isolated after bleeding, boiled for 5 minutes to inactivate proteolytic enzymes, homogenized, and treated with tungstic acid. Protein-free samples were hydrolyzed and analyzed. The results (table 43) show that the amino acid contents differ greatly between the marine and fresh-water species. Generally higher concentrations of amino acids in the muscles of marine forms is related to the osmotic equilibrium with the blood, which in these animals has nearly the same concentration as that of sea water. Because the concentration of inorganic ions in the tissues is lower than in the blood, a relatively high concentration of free amino acids in the tissues is necessary for maintaining osmotic equilibrium.

The concentration of protein in blood plasma in *O. edulis*, *Pecten maximus*, *Mya arenaria*, and *Mytilus edulis* is about 0.1 percent (Florkin and Blum, 1934). Samples of blood were collected after tearing off the gills, and the cells were removed by centrifugation.

TABLE 43.—*Free amino acids (mg./100 g. of water) in the muscles of marine and fresh-water bivalves**

	Ostrea edulis	*Mytilus edulis*	*Anodonta cygnea*
Alanine	646.0	340	8.8
Arginine	66.6	415.5	36.5
Aspartic acid	26.1	200.4	4.4
Glutamic acid	264.0	317.0	29.4
Glycine	248.0	399.0	13.2
Histidine	22.9	12.1	2.5
Isoleucine	19.2	24.8	6.3
Leucine	12.9	15.4	3.6
Lysine	22.0	39.4	8.2
Methionine	8.4	9.8	0.4
Phenylalanine	8.5	9.6	1.6
Proline	166.0	29.0	1.0
Threonine	9.7	30.5	3.6
Tyrosine	10.3	12.7	2.2
Valine	10.8	14.4	3.3

*From: Duchâteau, Sarlet, Camien, and Florkin, 1952. The French investigators express the concentration in a rather unique manner as mg./100 g. of "d'eau de fibre."

Nutritional studies have been made by feeding raw and frozen oysters to albino rats suffering from artificially induced vitamin deficiency (Randoin and Portier, 1923; Jones, Murphy, and Nelson, 1928; Whipple, 1935). Experimental results showed that oysters are a good source of vitamins A, B, and D. Daily feeding of 2 g. of fresh Chesapeake Bay oysters (0.32 g. on a dry basis) furnished sufficient vitamin A to cure rats of xerophthalmia (chronic inflammation and thickening of the conjunctiva of the eye) in 18 to 20 days. According to Whipple's data the vitamin content of oysters taken in October from Great South Bay, Long Island, N.Y., was approximately three U.S.P. units/g. The vitamin D content of oysters harvested from the same bay in the fall was approximately 0.05 U.S.P. units/g. and the vitamin B (B^1) content was found by Whipple to be approximately 1.5 Sherman units/g. Oysters are a very modest source of vitamin D and their antiricketic value is low.

In more recent work Wentworth and Lewis (1958) determined by chemical analyses the contents of niacin, riboflavin, and thiamine (table 44). None of these vitamins was found to have a distinct pattern of seasonal fluctuation.

TABLE 44.—*Range of vitamin contents in Apalachicola, Fla., oysters in mg./100 g. wet weight*

[From February 1955 to August 1956 From Wentworth and Lewis, 1958]

	February	August
Niacin	1.41	2.52
Riboflavin	0.06	0.28
Thiamine	0.08	0.13

Thiamine content of raw shucked oysters studied by Goldbeck (1947) varied by region. Oysters collected in the waters of Connecticut and New York contained more thiamine per unit of fresh weight than those from Louisiana, Georgia, Virginia, and Maryland (table 45). The determination of thiamine was made by chemical method (using thiochrome) and by rat growth method, which gave values about 9 percent smaller than the chemical tests.

The sterol mixtures of bivalves are of particular interest, because in certain species they are the richest natural sources of provitamin D. In *Modiolus demissus* of the Atlantic coast of America the content of provitamin D was found to be suffi-

ciently rich to warrant commercial exploitation for the manufacture of vitamin D preparations (Bergmann, 1962).

The cholesterol in bivalves constitutes but a small portion of the sterol mixtures in comparison with those obtainable from gastropods. In *C. virginica* and *C. gigas* Bergmann (1934) found a new sterol which he named ostreasterol. Similar compounds found in the sponge *Chalina* and in Japanese oyster (*C. gigas*) which were named chalinasterol and conchasterol. Reinvestigation of bivalve sterols proved the identity of ostreasterol, chalinasterol, and conchasterol with 24-methylenecholasterol (Bergmann, 1962).

The conditions and type of food which favor the enrichment of the bivalve body with sterols and vitamins are not known.

TABLE 45.—*Thiamine contents per 100 g. of raw oysters from different states*

[From Goldbeck, 1947]

State	Thiamine in μg.	State	Thiamine in μg.
Connecticut	170	Virginia	100–110
New York	170–180	Georgia	98–106
Maryland	100–103	Louisiana	110–130

CONDITION INDEX

Oysters of good quality have relatively large amounts of meat in relation to their total volume. Their glycogen content is high, and the meat has a creamy color and pleasant flavor. Determination of glycogen and of the total solids is a time-consuming procedure which cannot be regularly used in the oyster trade. To Caswell Grave (1912) belongs the credit of expressing the quality or fatness of oysters as the percentage of the volume of space enclosed between the two valves occupied by the oyster body. Hopkins (quoted from Higgins, 1938, p. 49–50) developed this idea further and suggested that the ratio

$$\frac{\text{dry weight of meat in g.} \times 100}{\text{volume of cavity in ml.}}$$

is a useful index of quality. Since then the ratio between the dry or wet weight of meat to the volume of the cavity has been used by many investigators in determining the condition index of oysters. The volume of the cavity can be measured by displacement. The oyster shells are thoroughly scrubbed with a wire brush, and each oyster is placed in a glass container provided

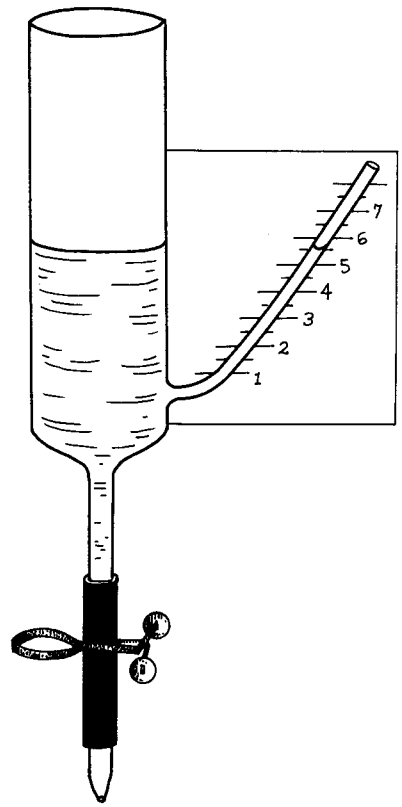

FIGURE 364.—Glass container used for determining the volumes of whole oysters and oyster shells by displacement.

with a side arm set at an angle to the side wall of the container (fig. 364). First the zero level of water is marked; then the oyster is introduced, and the level is brought back to zero position by draining the water through a drain pipe at the bottom. The water is collected, and its volume measured. The oysters are then taken from the container, opened carefully, and the meats removed. The volume of shells without meat is measured, and the volume of shell cavity is found by the difference between the volume of the whole oyster and the volume of its shell.

The methods used in determining condition index have not been standardized and, therefore, the values given by different investigators vary. Baird (1958) applied statistical analysis in evaluating the significance of variation of the index. He also demonstrated that little accuracy is gained by using dry weights as an index measurement and that even with fairly large samples the fluctuations may be considerable. He concludes that 50 oysters per sample is the largest practicable number.

The condition index has a practical use for oyster growers. It gives an objective method of comparing the quality of oysters taken from different commercially exploited oyster beds, but this comparison is valid only if oysters of the same species and of approximately the same age are used. Oysters from overcrowded natural reefs and young oysters are usually flat, with very little inner space between the valves; consequently their condition factor will be relatively high because the bodies occupy almost the entire shell cavity.

Westley (1961) found that the condition index of samples of *C. gigas* in Oakland Bay, Wash., in 1956 varied between 6.2 and 8.1. The condition index of the oysters in North Bay, Wash., in August 1957 was 12.3 to 15.0.

Observations were made on *O. lurida* in Oyster Bay on July 11 and repeated on August 8, 1957. On the first date the range of condition index varied from 6.6 to 7.2, while a month later it had increased to 16.0 to 17.3. The improvement was probably associated with an accumulation of glycogen after discharge of the larvae. In this case the volume of the oysters was measured by weighing them first in air and then in water, and computing the volume from the difference between the weights. The removed meats were oven-dried to constant weight (at 100° C.).

Although the condition index may be useful to oyster growers as a measure of quality of oyster meats, it provides no advantages for physiological studies, and cannot be used for the study of growth.

ANTIBACTERIAL AGENTS

Antibacterial and antiviral agents were found in the meat of the oyster (*C. virginica*), in abalone (*Haliotis rufescens*), and in a number of other mollusks. These substances have been isolated in the laboratory of the U.S. Public Health Service and their activity tested in vitro on a number of pathogenic bacteria (Li, Prescott, Jahnes, Martino, 1962), and on various strains of influenza and polio viruses. In vitro tests were made by using cultures of monkey kidney tissue; tests in vivo were conducted by feeding white mice with the extracts and recording the death rate after infection. There are two different extracts which the authors call Paolin 1 and Paolin 2 (according to Li, Paolin is a Chinese word which means "abalone extract"). Paolin 1 was found to inhibit the growth of *Staphylococcus aureus*, *Streptococcus pyogenes*, *Salmonella typhosa*, *Shizella dysenteriae*, and others. Paolin 2 fed to white mice decreased the death rate of animals experimentally infected by virus. The decrease was from 36 percent in the controls, which received no extracts, to 10 percent in the animals fed with oyster or abalone extracts before infection (Li and Prescott, 1963). The discovery by Li and his coworkers is of great practical significance and opens a new chapter of research into the role of antibacterial and antiviral agents in the tissues of mollusks.

BIBLIOGRAPHY

BAIRD, R. H.
1958. Measurement of condition in mussels and oysters. Journal du Conseil, vol. 23, No. 2, pp. 249–257.

BARGETON, MARIE.
1945. Sur la teneur en glycogène des diverticules digestifs de l'huître. Comptes Rendus Hebdomadaire des Séances de l'Académie des Sciences, tome 220, pp. 623–625.

BEARD, HARRY R. (Compiler).
1926. Nutritive value of fish and shellfish. [U.S.] Bureau of Fisheries, Report of the Commissioner of Fisheries for the fiscal year 1925, appendix 10 (Document 1000), pp. 501–552.

BERGMANN, WERNER.
1934. Contributions to the study of marine products. III. The chemistry of ostreasterol. Journal of Biological Chemistry, vol. 104, No. 3, pp. 553–557.
1962. Sterols: their structure and distribution. *In* Marcel Florkin and Howard S. Mason (editors), Comparative biochemistry, a comprehensive treatise, vol. 3, part A, Constituents of life, ch. 2, pp. 103–162. Academic Press, New York.

BIERRY, HENRI, BERNARD, GOUZON, AND COLETTE MAGNAN.
1937. Les variations de la teneur en glycogene des Huitres de consommation. Comptes Rendus Hebdomadaires des Séances de l'Academie des Sciences, tome 204, pp. 1895–1897.

BIRCKNER, VICTOR.
1919. The zinc content of some food products. Journal of Biological Chemistry, vol. 38, No. 2, pp. 191–203.

CALDERWOOD, H. N., and ALFRED R. ARMSTRONG.
1941. Determination of glycogen in oysters. Journal of the Association of Official Agricultural Chemists, vol. 24, No. 1, pp. 154–165.

CALDERWOOD, H. N., and R. J. PIECHOWSKI.
1937. Modified distillation method for moisture. Industrial and Engineering Chemistry, analytical edition, vol. 9, pp. 520–521.

CHIPMAN, WALTER A., JR.
1947. Seasonal changes in the fattening of oysters. Addresses Delivered at the Convention of National Shellfisheries Association, June 3–5, 1947, pp. 28–32.

CHIPMAN, WALTER A., THEODORE R. RICE, and THOMAS J. PRICE.
1958. Uptake and accumulation of radioactive zinc by marine plankton, fish, and shellfish. U.S. Fish and Wildlife Service, Fishery Bulletin 135, vol. 58, pp. 279–292.

COULSON, E. J.
1933. Studies on the nutritive value of oysters. [U.S.] Bureau of Fisheries, Investigational Report No. 17, vol. 1 (1936), 30 pp.
1934. The iodine content of oysters. [U.S.] Bureau of Fisheries Investigational Report No. 18, vol. 1 (1936), 10 pp.

COULSON, E. J., HAROLD LEVINE, and ROE E. REMINGTON.
1932. Oysters and anemia. American Journal of Public Health, vol. 22, No. 11, pp. 1141–1146.

COULSON, E. J., ROE E. REMINGTON, and KENNETH M. LYNCH.
1934. Studies on the metabolism of copper. [U.S.] Bureau of Fisheries, Investigational Report No. 23, vol. 1 (1936), 12 pp.

DICK, JAMES, and L. I. PUGSLEY.
1950. The arsenic, lead, tin, copper, and iron content of canned clams, oysters, crabs, lobsters, and shrimps. Canadian Journal of Research, Section F, vol. 28, pp. 199–201.

DUCHÂTEAU, GHISLAINE, HENRI SARLET, MERRILL N. CAMIEN, and MARCEL FLORKIN.
1952. Acides aminés non protéiniques des tissus chez les Mollusques Lamellibranches et chez les Vers. Comparaison des formes marines et des formes dulcicoles. Archives Internationales de Physiologie, vol. 60, fascicule 1, pp. 124–124.

ELVEHJEM, C. A., and E. B. HART,
1931. Synthetic rations and hemoglobin building. A note on the Drabkin-Waggoner modification of the Biazzo method for determining copper. Journal of Biological Chemistry, vol. 91, No. 1, pp. 37–42.

ELVEHJEM, C. A., and C. W. LINDOW.
1929. The determination of copper in biological materials. Journal of Biological Chemistry, vol. 81, No. 2, pp. 435–443.

ENGLE, JAMES B.
1958. The seasonal significance of total solids of oysters in commercial exploitation. Proceedings of the National Shellfisheries Association, vol. 48, August 1957, pp. 72–78.

FINGERMAN, MILTON, and LAURENCE D. FAIRBANKS.
1956a. Osmotic behavior and bleeding of the oyster *Crassostrea virginica*. Tulane Studies in Zoology, vol. 3, No. 9, pp. 149–168.
1956b. Bleeding and heat death in the southern oyster. U.S. Fish and Wildlife Service, Commercial Fisheries Review, vol. 18, No. 10, pp. 4–5.
1956c. Some factors affecting fluid loss in southern oysters. U.S. Fish and Wildlife Service, Commercial Fisheries Review, vol. 18, No. 1, pp. 10–11.

FLORKIN, MARCEL.
1949. Biochemical evolution. Edited, translated, and augmented by Sergius Margulis. Academic Press, New York, 157 pp.

FLORKIN, MARCEL, and HAROLD F. BLUM.
1934. Sur la teneur en protéines du sang et du liquide coelomique des invertébrés. Archives Internationale de Physiologie, vol. 38, fascicule 4, pp. 353–364.

FOX, H. M., and H. RAMAGE.
1931. A spectrographic analysis of animal tissues. Proceedings of the Royal Society of London, series B, vol. 108, pp. 157–173.

GAARDER, TORBJØRN, and E. ALVSAKER.
1941. Biologie und Chemie der Auster in den norwegischen Pollen. Bergens Museums Årbok, 1941, Naturvitenskapelig Rekke, Nr. 6, pp. 1–236.

GALTSOFF, PAUL S.
1932. The life in the ocean from a biochemical point of view. Journal of the Washington Academy of Sciences, vol. 22, No. 9, pp. 246–257.
1942. Accumulation of manganese and the sexual cycle in *Ostrea virginica*. Physiological Zoology, vol. 15, No. 2, pp. 210–215.
1943. Copper content of sea water. Ecology, vol. 24, No. 2, pp. 263–265.
1951. The oyster industry of the world. *In* Donald K. Tressler and James McW. Lemon (editors), Marine Products of Commerce, ch. 26, pp. 550–575. 2d ed. Reinhold Publishing Corporation, New York.
1953a. Accumulation and excretion of manganese, iron, copper, and zinc in *Ostrea virginica*. XIX International Physiological Congress, Abstracts of Communications, Montreal, August 31 to September 4, 1953, pp. 376–377. Thérien Frères Limitée, Montreal.
1953b. Accumulation of manganese, iron, copper, and zinc in the body of American oyster, *Crassostrea (Ostrea) virginica*. [Abstract.] Anatomical Record, vol. 117, No. 3, pp. 601–602.

GALTSOFF, PAUL S., WALTER A. CHIPMAN, JR., JAMES B. ENGLE, and HOWARD N. CALDERWOOD.
1947. Ecological and physiological studies of the effect of sulfate pulp mill wastes on oysters in the York River, Virginia. [U.S.] Fish and Wildlife Service, Fishery Bulletin 43, vol. 51, pp. 59–186.

GALTSOFF, PAUL S., and DOROTHY V. WHIPPLE.
1931. Oxygen consumption of normal and green oysters. Bulletin of the U.S. Bureau of Fisheries, vol. 46, for 1930, pp. 489–508. (Document 1094.)

GLICK, DAVID.
1949. Techniques of histo- and cytochemistry. Interscience Publishers, Inc., New York, 531 pp.

GOLDBECK, CLARA GALE.
1947. Some studies on the content of thiamine and anti-thiamine factor in fishery products. [U.S.] Fish and Wildlife Service, Commercial Fisheries Review, vol. 9, No. 8, pp. 13–21.

GRAVE, CASWELL (editor).
1912. A manual of oyster culture in Maryland. Fourth Report of the Shell Fish Commission of Maryland, 1912, pp. 279–348. [Reprinted in 1944 by King Brothers, Inc., Baltimore, Md.]

GUNTER, GORDON.
1942. Seasonal condition of Texas oysters. Proceedings and Transactions of the Texas Academy of Science, vol. 25, pp. 89–93.

HIGGINS, ELMER.
1938. Progress in biological inquiries, 1937. [U.S.] Bureau of Fisheries, Report of the Commissioner of Fisheries for the fiscal year 1938, appendix 1 (Administrative Report No. 30), pp. 1–70.

HILTNER, R. S., and H. J. WICHMANN.
1919. Zinc in oysters. Journal of Biological Chemistry, vol. 38, No. 2, pp. 205–221.

HOPKINS, A. E.
1949. Determination of condition of oysters. Science, vol. 110, No. 2865, pp. 567–568.

HOPKINS, SEWELL H., J. G. MACKIN, and R. WINSTON MENZEL.
1954. The annual cycle of reproduction, growth, and fattening in Louisiana oysters. National Shellfisheries Association, 1953 Convention Papers, pp. 39–50.

HUNTER, ALBERT C., and CHANNING W. HARRISON.
1928. Bacteriology and chemistry of oysters, with special reference to regulatory control of production, handling, and shipment. U.S. Department of Agriculture, Technical Bulletin No. 64, 75 pp.

JONES, D. BREESE.
1926. The nutritional value of oysters and other sea food. American Journal of Public Health, vol. 16, No. 12, pp. 1177–1182.

JONES, D. BREESE, J. C. MURPHY, and E. M. NELSON.
1928. Biological values of certain types of sea food. II. Vitamins in oysters (*Ostrea virginica*). Industrial and Engineering Chemistry, vol. 20, No. 2, pp. 205–210.

KENNEDY, R. P.
1927. The quantitative determination of iron in tissues. Journal of Biological Chemistry, vol. 74, No. 2, pp. 385–391.

LANKESTER, E. RAY.
1886. On green oysters. Quarterly Journal of Microscopical Science, vol. 26, pp. 71–94.

LEE, CHARLES F., CAROLINE H. KURTZMAN, and LEONARD PEPPER.
1960. Proximate composition of southern oysters—factors affecting variability. U.S. Fish and Wildlife Service, Commercial Fisheries Review, vol. 22, No. 7, pp. 1–8.

LEE, CHARLES F., and LEONARD PEPPER.
1956. Composition of southern oysters. U.S. Fish and Wildlife Service, Commercial Fisheries Review, vol. 18, No. 7, pp. 1–6.

LI, C. P.
1960. Antimicrobial activity of certain marine fauna. Proceedings of the Society for Experimental Biology and Medicine, vol. 104, pp. 366–368.

LI, C. P., and B. PRESCOTT.
1963. Antimicrobial agents from shellfish. [Abstract.] Abstracts 16th annual Gulf and Caribbean Fisheries Institute, November 12–16, 1963, 2 pp. [Mimeographed.]

LI, C. P., B. PRESCOTT, W. G. JAHNES, and E. C. MARTINO.
1962. Antimicrobial agents from mollusks. Transactions of the New York Academy of Sciences, series 2, vol. 24, No. 5, pp. 504–509.

LILLIE, R. D.
1948. Histopathologic technic. The Blakiston Company, Philadelphia, Pa., 300 pp.

LOOSANOFF, V. L., and JAMES B. ENGLE.
1947. Effect of different concentrations of microorganisms on the feeding of oysters (*O. virginica*). U.S. Fish and Wildlife Service, Fishery Bulletin 42, vol. 51, pp. 31–57.

LOUBATIÉ, RENÉ.
1931. La fixation de l'iode à l'état organisé dans le corps de l'huître vivante. Journal de Médecine de Bordeaux et de la Région du Sud-Ouest, vol. 108, No. 4, pp. 99–110.

MARKS, GRAHAM WALLACE.
1938. The copper content and copper tolerance of some species of mollusks of the southern California coast. Biological Bulletin, vol. 75, No. 2, pp. 224–237.

MCCOLLUM, E. V.
1926. V. Vitamins in fish and shellfish. *In* Harry R. Beard (compiler), Nutritive value of fish and shellfish, pp. 546–548. [U.S.] Bureau of Fisheries, Report of the Commissioner of Fisheries for the fiscal year 1925, appendix 10 (Document 1000).

MCHUGH, J. L., and J. D. ANDREWS.
1957. Computation of oyster yields in Virginia. Virginia Fisheries Laboratory Contribution No. 55. Virginia Fisheries Laboratory of the College of William and Mary and the Commission of Fisheries, Contributions 1955 and 1956, Gloucester Point, Va., 6 pp.

MEDCOF, J. C., and A. W. H. NEEDLER.
1941. The influence of temperature and salinity on the condition of oysters (*Ostrea virginica*). Journal of the Fisheries Research Board of Canada, vol. 5, No. 3, pp. 253–257.

MENDEL, LAFAYETTE B., and HAROLD C. BRADLEY.
1905. Experimental studies on the physiology of the mollusks.—second paper. American Journal of Physiology, vol. 14, No. 4, pp. 313–327.

MITCHELL, PHILIP H., and RAYMOND L. BARNEY.
1917. The occurrence in Virginia of green-gilled oysters similar to those of Marennes. Bulletin of the U.S. Bureau of Fisheries, vol. 35, for 1915–1916, pp. 135–150. (Document 850.)

NILSON, HUGO W., and E. J. COULSON.
1939. The mineral content of the edible portions of some American fishery products. [U.S.] Bureau of Fisheries, Investigational Report No. 41, vol. 2 (1950), pp. 1–7.

POWER, E. A.
1962. Fishery statistics of the United States, 1960. U.S. Fish and Wildlife Service, Statistical Digest 53, 529 pp.

RANDOIN, L., and P. PORTIER.
1923. Étude des vitamines des mollusques. Présence du facteur antiscorbutique chez l'huître. Notes et Mémoires, Office Scientifique et Technique des Pêches Maritimes, No. 30, 15 pp.

RANSON, GILBERT.
1927. L'absorption de matières organiques dissoutes par la surface extérieure du corps chez les animaux

aquatiques. Annales de l'Institut Océanographique, nouvelle série, tome 4, fascicule 3, pp. 49–174.

RICHARDS, Marion B.
1930. The colorimetric determination of manganese in biological material. The Analyst, vol. 55, No. 654, pp. 554–560.

SEVERY, HAZEL W.
1923. The occurrence of copper and zinc in certain marine animals. Journal of Biological Chemistry, vol. 55, No. 1, pp. 79–92.

TRESSLER, DONALD K.
1926. II. Fish and shellfish as a source of protein. *In* Harry R. Beard (compiler), Nutritive value of fish and shellfish, pp. 527–532. [U.S.] Bureau of Fisheries, Report of the Commissioner of Fisheries for the fiscal year 1925, appendix 10 (Document 1000).

TRESSLER, DONALD K., and ARTHUR W. WELLS.
1924. Iodine content of sea foods. [U.S.] Bureau of Fisheries, Report of the Commissioner of Fisheries for the fiscal year 1924, appendix 1 (Document 967), pp. 1.–12.

U.S. DEPARTMENT OF AGRICULTURE.
1951. Food values in common portions. U.S. Department of Agriculture, Bureau of Human Nutrition and Home Economics, Government Printing Office, Washington, D.C., AIB–36, April 1951, 8 pp.

VINOGRADOV, A. P.
1953. The elementary chemical composition of marine organisms. Memoir, Sears Foundation for Marine Research, No. 2, 647 pp.

WATT, BERNICE K., and ANNABEL L. MERRILL.
1950. Composition of foods—raw, processed, prepared. U.S. Department of Agriculture, Agriculture Handbook No. 8, Bureau of Human Nutrition and Home Economics, Agricultural Research Administration, Government Printing Office, Washington, D.C., 147 pp.

WELLS, ARTHUR W.
1925. Iodine content of preserved sea foods. [U.S.] Bureau of Fisheries, Report of the Commissioner of Fisheries for the fiscal year 1924, appendix 6 (Document 979), pp. 441–444.

WENTWORTH, JANE, and HARVYE LEWIS.
1958. Riboflavin, niacin, and thiamine in Apalachicola Bay oysters. Food Research, vol. 23, No. 2, pp. 194–197.

WESTLEY, RONALD E.
1959. Olympia and Pacific oyster condition factor data. State of Washington, 1954–1958. State of Washington, Department of Fisheries, Shellfish Research Laboratory, Quilcene, Wash., 8 pp. [Mimeographed.]
1961. Selection and evaluation of a method for quantitative measurement of oyster condition. Proceedings of the National Shellfisheries Association, vol. 50, August 1959, pp. 145–149.

WHIPPLE, DOROTHY V.
1935. Vitamins A, D, and B in oysters—effect of cooking upon vitamins A and B. Journal of Nutrition, vol. 9, No. 2, pp. 163–173.