CHAPTER X

ORGANS OF DIGESTION AND FOOD OF THE OYSTER

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The system of organs concerned with the ingestion and digestion of food and elimination of feces consists of the mouth, short esophagus, stomach, crystalline style sac, digestive diverticula, midgut, rectum, and anus (fig. 197). With the exception of a short section of the rectum, the entire alimentary canal lies within the visceral mass and is completely immobilized by the surrounding connective tissue. In the absence of peristaltic movements the food is moved from the mouth toward the anus exclusively by the strong ciliary motion of the epithelial lining of the system. It is difficult to reveal all anatomical details of the alimentary tract by dissecting the tissues. Better preparations can be obtained by making casts of latex or other suitable material injected through the esophagus or via the rectum and left until it sets. Yonge (1926a) used for this purpose a warm, concentrated solution of gelatin. Awati and Rai (1931) employed a mixture of paraffin and resin colored with carmine. Satisfactory results were obtained in the Bureau's shellfish laboratory by using red or yellow latex injected into the esophagus through a wide mouth pipette with a rubber balloon. The preparation was immediately placed in 5 percent formalin in which the latex sets and remains tough and flexible for a long time. Casts of the entire alimentary canal or of its parts can be obtained in this way.

MOUTH

The mouth is a compressed U-shaped slit between the two lips (fig. 104) and is lined with columnar ciliated epithelium set on a narrow basal membrane. The epithelial cells of the mouth are taller than those of the labial palps and contain only a few mucous glands. In the surrounding connective tissue are large vesicular cells, numerous muscle fibers, and blood spaces which are occasionally filled with leucocytes. Leucocytes are also found in narrow spaces between the tissue cells and on the surface of the mantle lining from which they are discarded.

ESOPHAGUS AND STOMACH

The esophagus, a short, funnel-shaped, and dorso-ventrally compressed tube, is lined with epithelium similar to that of the mouth. It leads to the stomach, which occupies a central position within the visceral mass (fig. 197). The stomach is an irregularly shaped, large sac (figs. 198 and 199) with several outgrowths or pouches. At the entrance of the esophagus the wall of the stomach forms an anterior chamber, a, which leads into a broader posterior chamber, b (figs. 198 and 199). An oblique outgrowth or pouch called the caecum, c, is the most conspicuous structure which arises from the ventral side of the anterior chamber. Both the anterior and posterior ends of the caecum are curved and form the anterior and posterior appendices (a.ap., p.ap.). The larger posterior appendix is a strip curved ventrally and toward the right of the stomach. The configuration and relative sizes of the appendices vary but the structures are recognizable in all the casts. A groove along the wall of the caecum leads to the opening of the midgut (m.g.) and serves for sorting of food (Yonge, 1926a). On the left side below the caecum the wall of the stomach bulges out to form a broad pyloric caecum (p.c.), which leads to a long outgrowth alongside the midgut, the crystalline sac (cr.s.).

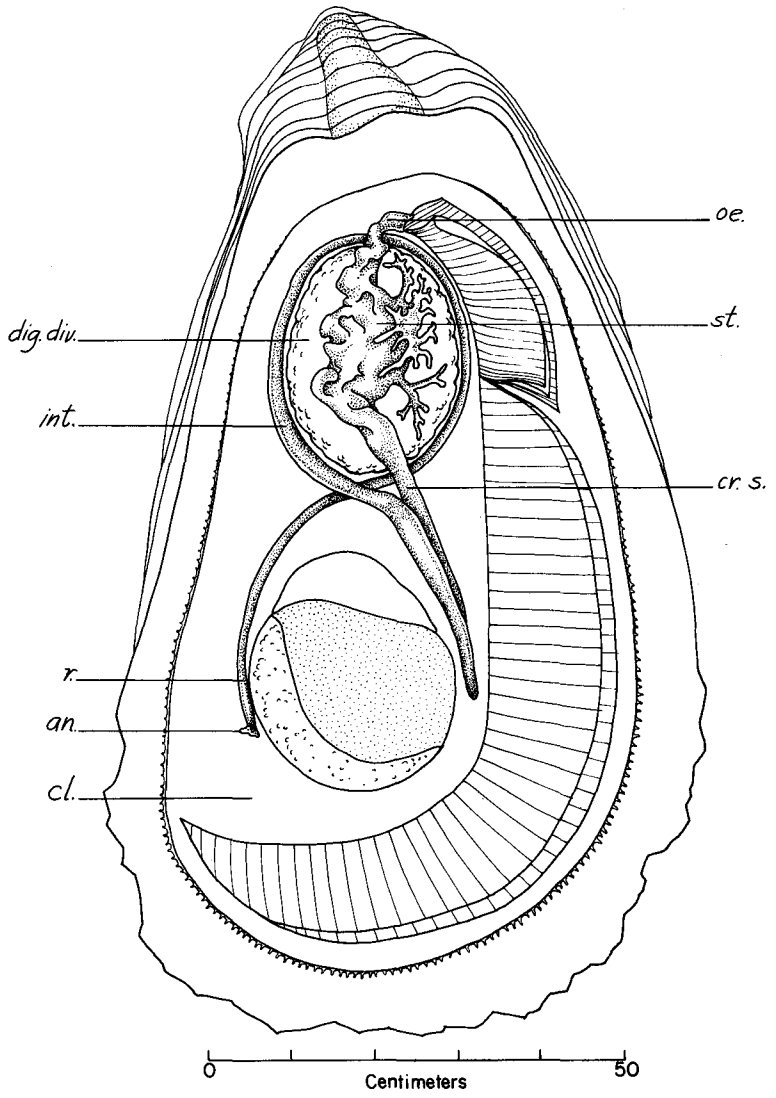

FIGURE 197.—Digestive system of the oyster, *C. virginica*, drawn from the dissected preparation after the injection of latex. The right outer labial palp was cut off to expose the esophagus. The parenchymal tissues over the stomach and intestine were removed. an.—anus; cl.—cloaca; cr.s.—crystalline style sac; dig. div.—digestive diverticula; int.—intestine (mid-gut); oe.—esophagus; r.—rectum; st.—stomach.

Three groups of wide ducts emerging from the wall of the stomach lead to the digestive diverticula. Two of them (fig. 199. d_1, d_2) originate at the anterior chamber and one (d_3) from the posterior chamber.

The internal lining of the anterior chamber forms a number of irregular ridges and furrows covered with ciliated epithelium. A broad ridge separates the anterior from the posterior chamber and apparently directs the food particles. The left ventral wall of the posterior chamber is covered by a translucent membrane, the gastric shield (fig. 200), which lies directly opposite the opening of the long sac occupied by the crystalline style (cr.s.).

Ciliary tracts of the stomach lining are very complex. Detailed observations on the course followed by food particles after they enter the stomach were made for *O. edulis* and *Mya arenaria* by Yonge (1923, 1926a), who studied them by carefully cutting off the wall and adding fine powdered carborundum or aquedag to the exposed surface. In general the pattern of ciliary movements in the stomach of the American oyster is similar to that of *O. edulis*. The direction of ciliary beat along different ridges and channels

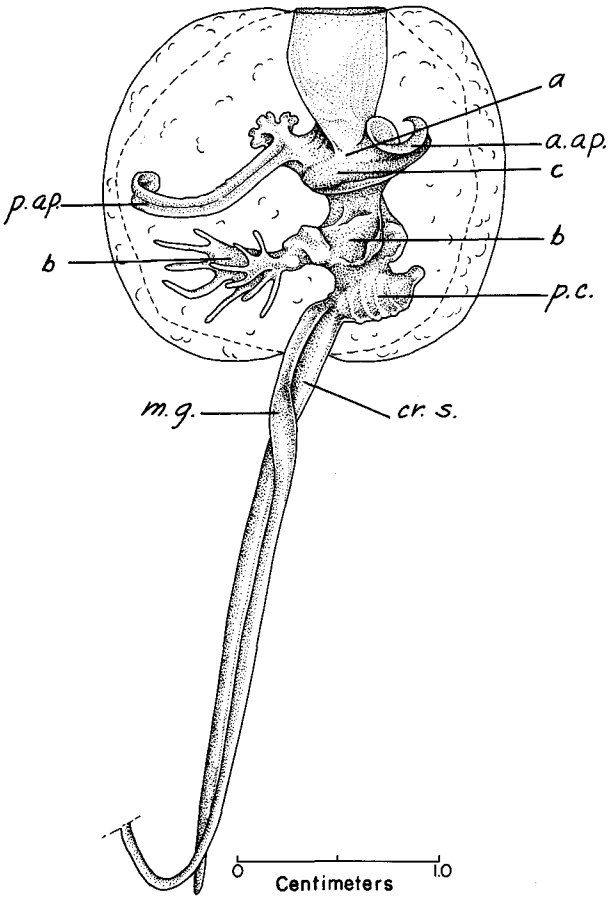

FIGURE 198.—Latex cast of the stomach, crystalline style sac, and esophagus of a large *C. virginica*, viewed from the left side. Upper part of the esophagus is slightly distended by injection. The visceral mass was dissected and the injected parts left in their natural position. Drawn from a preparation preserved in 5 percent formalin. a.—anterior chamber; a.ap.—anterior appendix of the caecum; b.—posterior chamber; c.—caecum; cr.s.—crystalline style sac; d.p.—dorsal pouch; d.—group of ducts of the digestive gland; m.g.—midgut; p.ap.—posterior appendix of the caecum; p.c.—pyloric caecum.

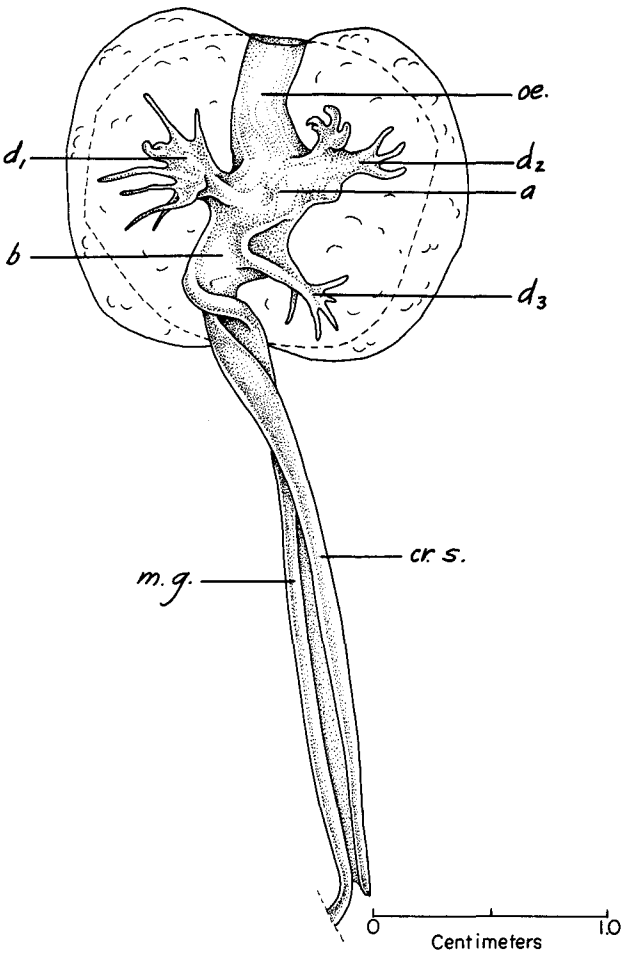

FIGURE 199.—Latex cast of the stomach, crystalline style sac, and esophagus of a large *C. virginica* viewed from the right side. The visceral mass was dissected, and the injected parts were left in their natural position. Drawn from a preparation preserved in 5 percent formalin. a.—anterior chamber; b.—posterior chamber; cr.s.—crystalline style sac; d_1, d_2, d_3—ducts leading to the digestive diverticula; m.g.—midgut; oe.—esophagus.

brings the food from the esophagus to the caecum where the food materials are separated according to size. Some of the larger particles entering the midgut may be voided without being digested while the smaller particles are pushed toward the gastric shield. Other groups of cilia conduct the particles toward the ducts leading to the digestive diverticula. The ducts branch out into a large number of smaller passages that ramify and extend deep into the mass of diverticula.

Nearly the entire inner surface of the stomach is covered by ciliated epithelium; only the areas under the gastric shield and near the posterior end of the stomach are nonciliated. The epithelium is of columnar type with very long cilia, which are particularly prominent on the ridges (fig. 201). The height of the cells gradually decreases toward the caecum. Mucous cells are abundant, particularly near the junction with the midgut, and phagocytes are numerous between the epithelial cells and in the underlying connective tissue. There is no well-developed muscular layer under the epithelial lining, but a few smooth

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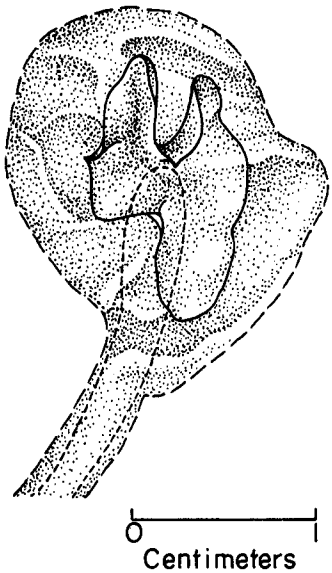

FIGURE 200.—Gastric shield viewed in its natural position in a dissected stomach. The position of the crystalline style is indicated by the dotted line. Drawn from an unpreserved preparation.

muscle fibers may be found under the basement membrane. In general, the histological picture of the stomach of an adult *C. virginica* is similar to that described for the spat of this species by Shaw and Battle (1957), *C. angulata* by Leenhardt (1926), and *O. chilensis* and *O. edulis* by Dahmen (1923) and Yonge (1926a) respectively.

GASTRIC SHIELD

The stomach wall in front of the openings to the midgut and style sac is covered by a thin but tough, irregularly shaped membrane (fig. 200) made of translucent and slightly striated material. The structure, named the gastric shield by Nelson (1918), rests on a prominent epithelial ridge of narrow columnar cells with oval nuclei, rich in chromatin (fig. 202). The cells are devoid of cilia. The shield is made of two portions of different size, joined together by a narrow middle piece (fig. 200). The thicker portion of the shield lies over the peak of the ridge and is underlined by the tallest cells in the area. On both sides of the peak the epithelium flattens and at the edges changes into the typical ciliated lining of the stomach. The surface of the shield is roughened by the remnants of food particles embedded in it.

The origin of the shield has not been fully explained. Obviously, it is the product of the underlying cells, but the process of its formation has not been studied sufficiently. One view, advanced by Gutheil (1912) and shared by some investigators, assumes that the shield is formed by the droplets secreted by the epithelial cells. No evidence in support of this view can be found in the histological preparations of the stomachs of *O. edulis* and *C. virginica*. No droplets could be seen in the sections of stomach, and no other indication of the secretory activities of these cells could be found. Yonge (1926a) thinks that the shield is very likely formed by the fusion of the cilia and in support of this view points out that the structure is attached to the epithelium by fine threads which transverse the substance of the shield and resemble the cilia. Indistinct transverse striation can be seen in the sections of the stomachs of *C. virginica* fixed in osmic acid and stained with iron hematoxylin (fig. 202). The question could be settled by electron microscopy, which would reveal the structure of the cilia if the latter are present within the shield substance. So far no such studies have been made.

The shield is not destroyed by boiling in a 40 percent solution of potassium hydroxide. Treatment with iodine followed by a strong solution of zinc oxide gives the deep violet coloration that is characteristic of the color reaction for chitin (Zander reaction). These facts support Berkeley's (1935) findings that the material of the shield of the common Pacific coast clam, the Pacific gaper (*Schizothaerus nutalli nutalli* Conrad), is made of chitin and contains no chondrinlike constituent.

In *C. angulata* Leenhardt (1926) described the torch bearing cells near the edges of the area occupied by the gastric shield. The function of the cells is not known. They are not found in my preparations of *C. virginica* and are not mentioned

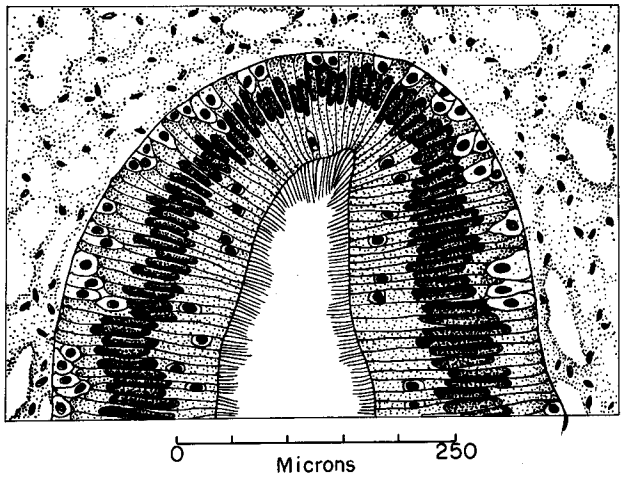

FIGURE 201.—Cross section of the wall of the stomach. Kahle, Hematoxylin-eosin stain.

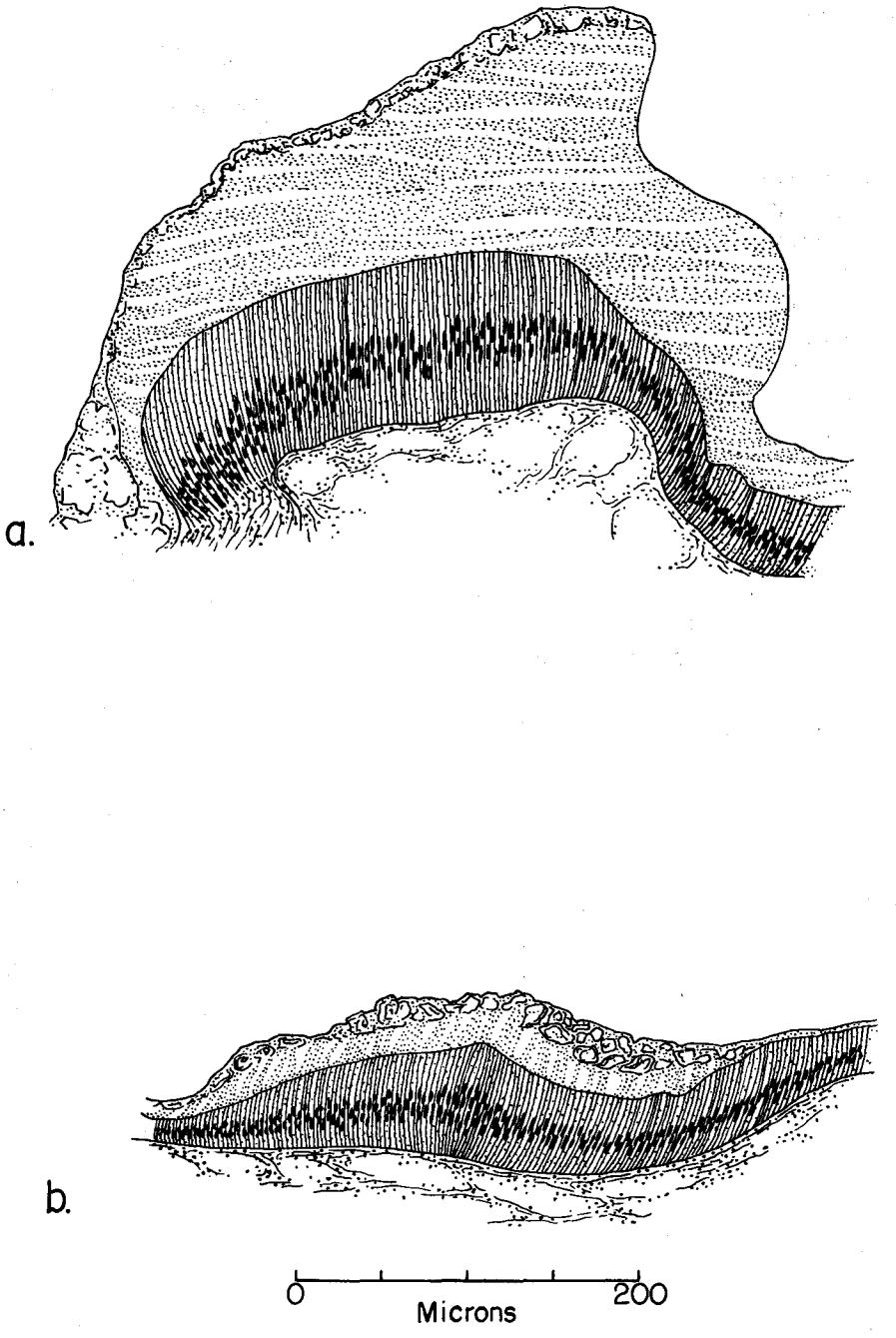

FIGURE 202.—Two cross sections of the wall of the stomach of *C. virginica* under the gastric shield. A—the thickest portion of the shield. Bouin, hematoxylin-eosin stain. B—cross section near the periphery of the shield. Osmic acid, iron hematoxylin. The surface of the shield is rough due to embedded and partially ground food particles. Note cross striation of the shield visible in B.

by Shaw and Battle (1957) in their work on the microscopic anatomy of the digestive tract of this species.

The function of the shield is to provide a base for grinding of food by the rotating head of the crystalline style.

CRYSTALLINE STYLE

The posterior wall of the stomach leads to an elongated outgrowth or sac which extends a considerable distance along the ventral arm of the visceral mass (fig. 197, cr.s., and fig. 203) on the antero-ventral side of the adductor muscle. A

narrow slit joins the sac over nearly its entire length to the midgut; near the entrance to the stomach the two structures are separated. The sac is slightly twisted around the midgut and occupies a somewhat dorsal position, while the midgut forms the ventral portion of the common structure (figs. 198, 199). A cross section of the sac and midgut shows (fig. 204) that the two channels are separated in the middle by a narrow slit compressed by the two protruding lobes or typhlosoles. In figure 204 the style sac is at the top; its lumen is usually larger than that of the midgut (lower part of the figure). This relationship between the style sac and midgut is similar to the topography of this organ in *O. chilensis* (Dahmen, 1923), *O. edulis* (Yonge, 1926a), *Mya* (Edmondson, 1920), *Ensis* (Graham, 1931a), *Mytilus edulis* (Sabatier, 1877), *M. latus* and *M. magellanicus* (Purdie, 1887), and *Anodonta* (Nelson, 1918). In the old literature the structure was called a "tubular stomach" by Sabatier (1877), and "pyloric appendix" by Purdie (1887), names which have not been accepted in malacological literature.

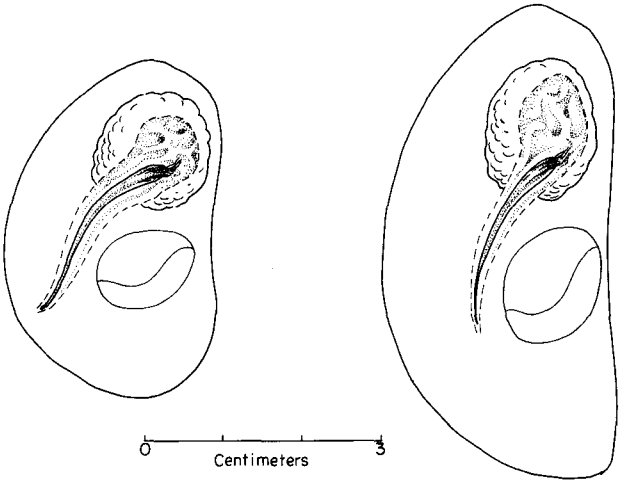

FIGURE 203.—Crystalline styles of *C. virginica* (left) and *C. gigas* (right) in their natural position. Drawn from live preparations. The wall of the stomach and of the crystalline style sac has been dissected.

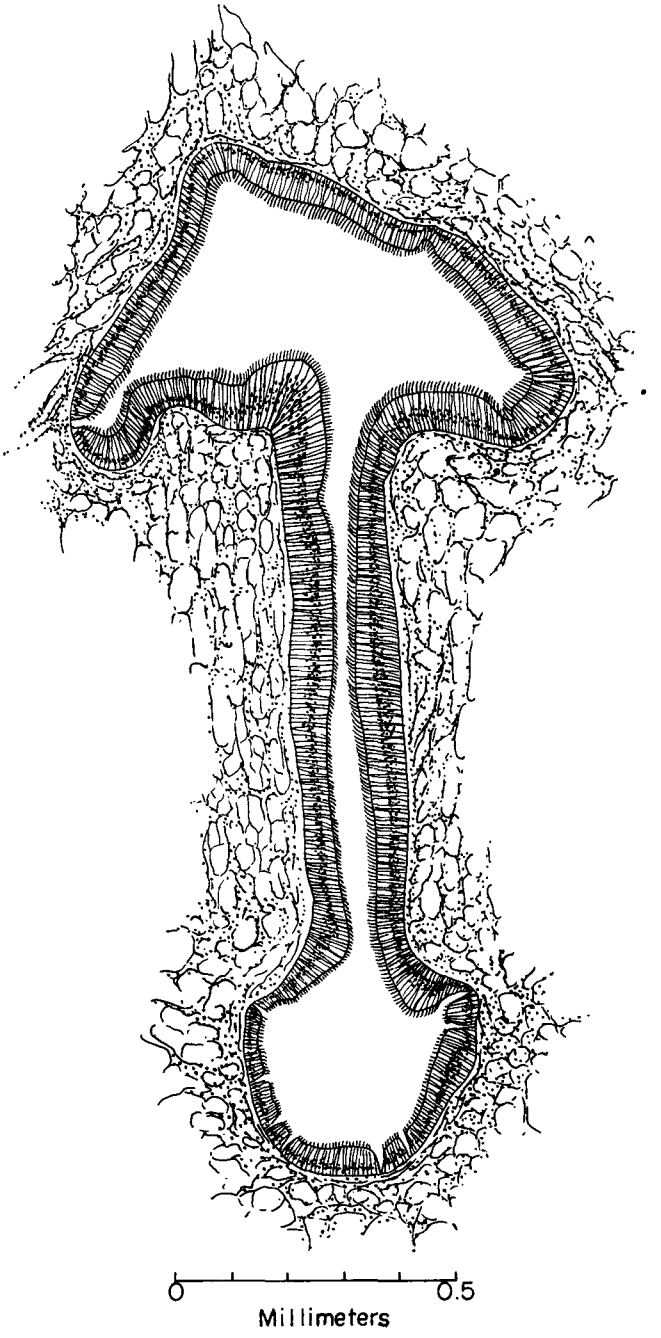

FIGURE 204.—Transverse section of the style sac (upper part) and midgut (lower part). The crystalline style is absent. Kahle, hematoxylin-eosin.

The style sac is lined with densely packed cylindrical cells that have large oval nuclei and long cilia measuring about 20 μ. The intracellular fibrillar apparatus is well developed. Phagocytes and mucous cells are scarce. The basal membrane rests on a thin layer of collagenous fibers; circular muscles are sparsely arranged, as in the stomach, and there is no distinct muscular layer. The epithelial cells of the two lobes (typhlosoles) of the sac and midgut gradually change from robust, long cells to shorter cells with smaller cilia, typical for the lining of the midgut. The mucous cells are more abundant in the midgut than in the sac.

The connective tissue around the sac and under the typhlosoles consists of typical vesicular cells.

In actively feeding oysters the lumen of the sac is occupied by a gelatinous rod with a bulging head protruding inside the stomach (fig. 203) and the pointed tail extending to the distal part of the sac. The color of the style varies from greyish white to deep yellow and brown, depending on the type of food consumed by the oyster. The head is usually darker than the rest of the style because of the food particles wrapped around it.

Inside the sac the style is rotated by the ciliary action of the epithelium. The rotary motion was originally suggested by List (1902) in his work on mussels, but the demonstration that the rotation actually takes place in *Anodonta* and *Modiolus* was made by Nelson (1918). According to Yonge (1926a), the large cilia of the groove of the sac of *O. edulis* move in such a way as to produce a slow clockwise rotation of the style when seen from the stomach. There is, however, a tract of cilia on the side of the larger typholsole which beats in the direction of the stomach and presumably pushes the style forward. Food particles that enter the sac are carried by the currents down the gut but some of them tangle in the substance of the style, are wrapped around it, and carried back to the stomach. This process, observed by Nelson (1918, 1925), Allen (1921), and Orton (1924), may be significant for the bivalves in which, like in *Ostrea*, the style sac is in direct communication with the midgut.

As the style rotates and rubs against the gastric shield, aiding in mixing and grinding food particles it slowly dissolves in the gastric juice and yields digestive enzymes.

FORMATION

The crystalline style is not a permanent structure. In oysters removed from water and left in the air the style dissolves in a short time. This observation, reported for *O. edulis* by Orton (1924), has been confirmed for *C. virginica* and *C. gigas*. At room temperature of 21° to 22° C. the crystalline styles of the American species removed from the sac and left exposed to air completely dissolve within 45 to 60 minutes. In the body of the oysters (*C. virginica*) taken out of water the style disappears in 2 to 3 hours. The absence of the style is frequently observed in nonfeeding oysters. The symptom is useful, but not entirely reliable because under certain conditions the style may be present in oysters which do not take food. Observations made in winter in the Woods Hole laboratory showed that in late December, at temperatures varying from 5.4° to 5.7° C. about 4 out of 10 oysters had crystalline styles. No trace of food was found in these oysters, which were examined within a few minutes after they had been taken out of water.

Yonge (1926a) states that in *O. edulis* the style is always present in healthy oysters, even when they are starved, and is absent only under abnormal conditions and in diseased specimens.

The style must be the product of secretion but investigators do not agree on the manner and site of its formation. List (1902), Nelson (1918), Edmondson (1920), and Mackintosh (1925) think that the style is secreted by the narrow cells of the smaller typhlosoles but do not present conclusive evidence in support of this view. For freshwater *Anodonta*, Gutheil (1912) demonstrated the presence of vesicular granules around the nuclei of the epithelial cells of the sac and probably interpreted them correctly as a sign of active secretion. No such granules were found, however, in the histological preparations of *O. edulis* (Yonge, 1926a) and in my slides of the sac of *C. virginica*. Evidence of the secretory activity of the style sac was produced by Yonge (1926a) by injecting 0.5 percent solution of iron saccharate into the adductor muscle, washing the animals, and then dissecting and fixing the sac at 2-hour intervals. The sections were treated by potassium ferricyanide in acid solution to demonstrate the presence of iron by Prussian blue reaction. Fine blue granules indicative of the presence of iron salt were found in the cytoplasm above the nuclei and between the cilia of the epithelial cells. No iron was detected in the epithelium of the midgut or of the larger typhlosoles, although some of the metal was present in the epithelial cells of the minor typhlosole. The experiments may indicate the secretory function of the epithelial cells, but they cannot be considered as evidence of the formation of the style from the secreted granules.

CHEMICAL COMPOSITION

Analysis of the crystalline style of *Cardium* made by Barrois (1889, 1890) showed the following composition: water 87.11 percent; solid organic matter 12.03 percent; solid inorganic matter 0.86 percent. The organic component of the style was considered to be a globulin with traces of mucus or chondrinlike substance. Berkeley (1935) dem-

onstrated that the styles of four species of bivalves (*Crassostrea gigas*, *Mya arenaria*, *Schizothaerus nuttalii*, and *Saxidomus giganteus*) in addition to protein, yield, on acid hydrolysis, glucionic and sulphuric acids and a hexamine, the essential constituents of both mucin and chondrin. The ease of the hydrolysis and the solubility of the style materials indicate that mucin rather than chondrin is involved. The variations in the solubility and the quantitative differences in the chemical composition of the styles suggest, according to Berkeley, that the less readily soluble styles contain larger quantities of mucin.

All examined styles are carriers of certain enzymes which they yield upon dissolution. The role the styles play in digestion is discussed later (see p. 230 of this chapter).

MIDGUT AND RECTUM

The portion of the intestine between the stomach and the rectum is called the midgut. It begins at the ventral wall of the stomach next to the opening of the crystalline sac and runs parallel to the sac as far as its distal end, then turns sharply backward parallel to its previous course (fig. 197, int.). The ascending branch of the intestine makes a loop that completely encircles the stomach and continues as a descending branch which ends with the rectum and anus (r., a.).

Throughout its entire length the midgut is characterized by a well-developed typhlosole which extends along its inner wall (fig. 205). The gut is lined with columnar ciliated epithelium; there is an abundance of mucous cells and of wandering leucocytes. The muscular layer is absent.

The rectum (fig. 197, r.) runs along the dorsal side of the heart. In this respect the oyster differs from many other bivalves (sea mussels, clams, fresh-water mussels) in which the rectum runs through the heart. The structure of the rectum is similar to that of the midgut; the main difference is the disappearance of the well-developed typhlosole near the anal region where the lining is thrown into numerous small folds (fig. 206). A distinct feature of the rectum is a circular layer of smooth muscles which, however, do not form a sphincter at the anus (fig. 207). According to Leenhardt (1926), the anal sphincter is present in the Portuguese oyster. The surface epithelium of the anal region is well developed and abounds in mucous cells.

DIGESTIVE DIVERTICULA

The stomach is surrounded by an irregularly shaped mass of dark tissue which has been called the "liver" or "hepatopancreas." Its color varies from light yellow to dirty green and dark brown. In most cases the color is not visible through a white or creamy layer of connective tissues rich in glycogen.

Yonge (1926b) has shown that assimilation and intracellular digestion takes place in this mass of darkly colored tissues; it has none of the known functions of the liver or pancreas. He named it "digestive diverticula", a term that correctly describes its role.

The digestive diverticula are made of a large number of blind tubules emptying into several large ducts which lead to the interior of the stomach. The structure of the tubules is simple. In cross section (fig. 208) they are usually round with a lumen in the form of a cross. The tubules are surrounded by connective tissue in which muscle fibers are absent. The digestive cells which form the interior of a tubule are large and well vacuolated, with large nuclei at their base. Food vacuoles can be seen in them during feeding. At the corners of the "cross" of the lumen one usually finds crypts of young cells with dark staining protoplasm, large and compact nuclei, and indistinct cell boundaries. Cells from these crypts replace the old cells that are cast off. The digestive cells of the American oyster are non-ciliated, but the cells in the diverticula of other bivalves (*Teredo*) have been reported to have cilia (Potts, 1925). Yonge (1926a, 1926b) believes the cilia are present in the tubules of *edulis* but probably retract so rapidly that they cannot be seen in the fragments of tissues pressed by a cover slip. I was not able to detect them in *C. virginica*. Phagocytes are very abundant between the cells and in the surrounding connective tissue.

The ducts that connect the tubules with the stomach are circular in cross section and are lined with ciliated epithelium (fig. 209). Their lumen is, however, irregular due to the variations in the height of the epithelial cells. The epithelium is similar to that of the stomach and contains many mucous glands and phagocytes.

ALIMENTARY TRACT AND FORMATION OF FECES

Food ingested by the oyster is moved through the alimentary canal by the ciliary action of the

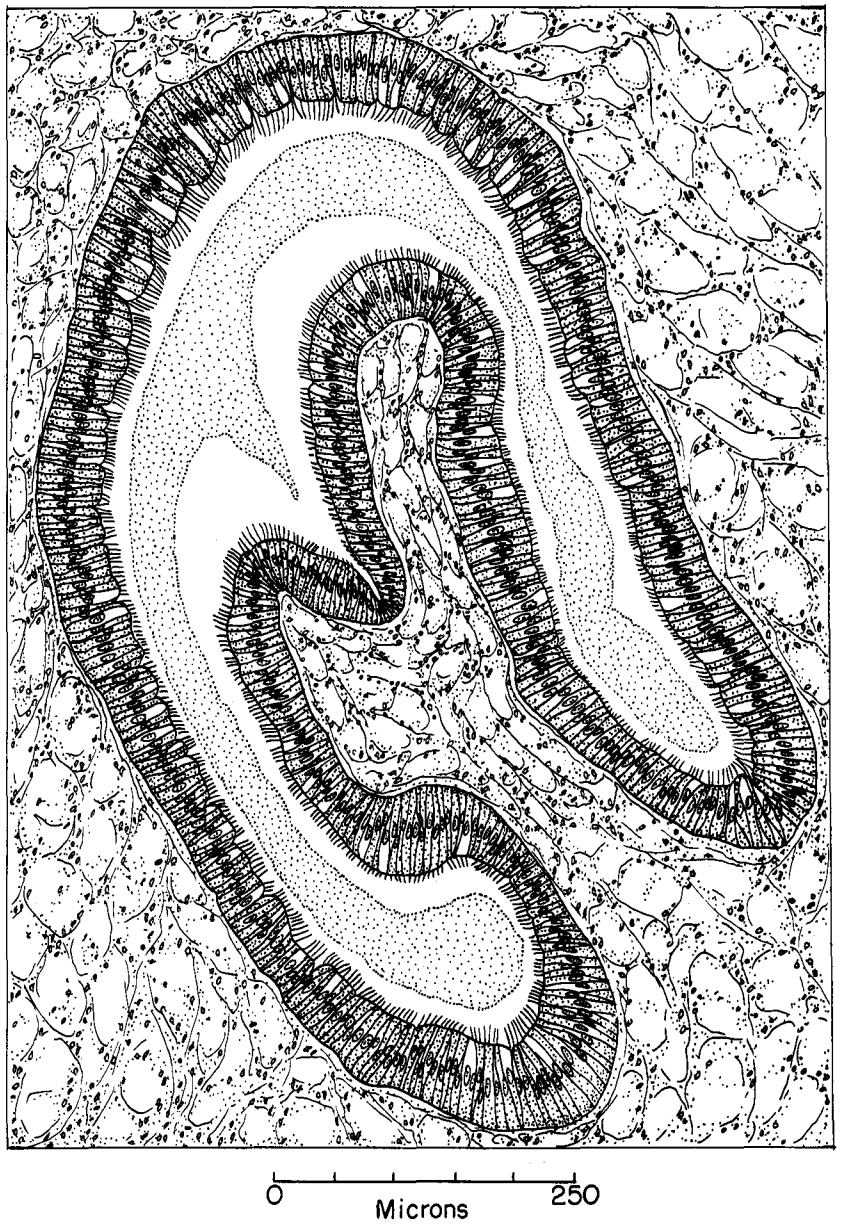

FIGURE 205.—Cross section of the midgut. Bouin, hematoxylin-eosin.

epithelium. There is no peristaltic motion since the muscular layer of the intestines is either absent or poorly developed, and the feces are discharged in a continuous ribbon which is carried away by the cloacal current and eventually settles. The time required for food to pass through the entire intestinal tract can be measured by recording the time between addition of a suspension of carmine or yeast to the gills and the appearance of the red or white particles in the feces. The rate of passage naturally depends on the length of the intestinal tract and the rate of feeding. In large oysters (about 10 by 6 cm.) kept in running sea water of about 15° to 16° C. the time required for food to pass through the entire intestinal tract varied from 90 to 150 minutes. The length of the intestinal tracts of the oysters used in these tests was measured on latex casts which were left in situ and exposed by dissecting the tissues above them. The lengths of the alimentary tracts were as follows:

In an oyster measuring 11 by 6 cm	14.5 cm.
In an oyster measuring 10.0 by 7.5 cm	11.1 cm.
In an oyster measuring 11 by 6 cm	12.9 cm.
In an oyster measuring 11.5 by 5.5 cm	12.6 cm.

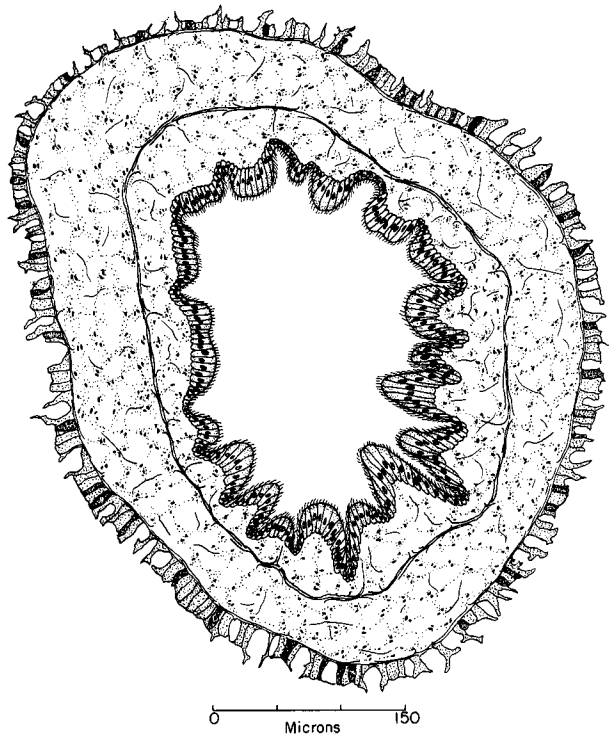

FIGURE 206.—Cross section of the rectum near the anal region. Bouin, hematoxylin-eosin.

The rates of discharge of fecal ribbons observed on actively feeding oysters in laboratory sea water of 15.0° to 15.7° C. are given in table 30. The average of the observed rate was 8.1 cm. per hour. Assuming that the average length of the intestines was 12.8 cm., the estimated time of passage of food through the entire alimentary tract was 95 minutes.

The feces of the oyster are voided from the rectum as a compact and slightly flattened ribbon of sufficient consistency to withstand the velocity of the cloacal current. In an actively feeding oyster the ribbon is maintained in a horizontal position along the axis of the current, but being heavier than the sea water it sinks down to the bottom as soon as the cloacal current slows down or ceases. Large masses of fecal ribbons accumulate on the bottom a short distance from the opening of the cloaca. The ribbon remains intact for 2 to 3 days until it is disintegrated through decomposition and mechanical disturbance. The appearance of the fecal masses of the oyster is typical and can be recognized by their shape. It was shown by Moore (1931) that specific identification of fecal pellets can be made for a number of marine invertebrates.

Fecal ribbons of oysters contain many live cells—diatoms, dinoflagellates, yeast, and others which are not killed by the gastric and intestinal juices and can be recultured.

TABLE 30.—*Rate of formation of fecal ribbons (in cm.) in* C. virginica *during feeding in laboratory sea water, Woods Hole*

Date	Time	Temperature	Length of ribbon	Rate of formation
	Minutes	*°C*	*Cm.*	*Cm./hr.*
May 18	108	15.6	23	7.7
18	17	15.0	3.2	11.3
18	102	15.0	9.8	5.8
May 19	70	15.2	14.2	12.2
19	123	15.0	9.3	4.5
May 23	60	15.7	7.2	7.2

DIGESTION

The digestion and absorption of food in the oyster are primarily an intracellular process which takes place in the digestive diverticula. This was demonstrated by Yonge (1926a, 1926b) in a series of carefully executed feeding experiments in which the solutions of iron saccharate, suspension of carmine powder, oil emulsion, and dogfish blood corpuscles were fed to European oysters. He produced convincing evidence that very small food particles are absorbed by the cells of the digestive diverticula, while the diatoms and other

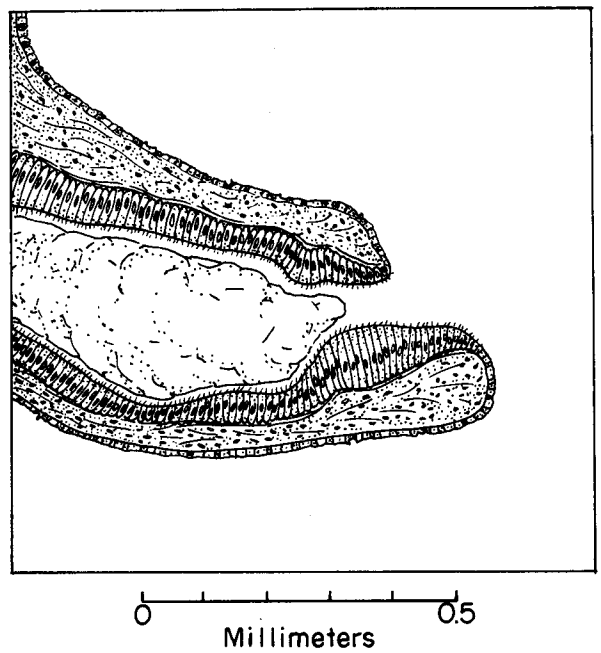

FIGURE 207.—Longitudinal section of the anus and adjacent portion of the rectum. Note the fecal mass inside and the absence of a sphincter. Bouin, hematoxylin-eosin.

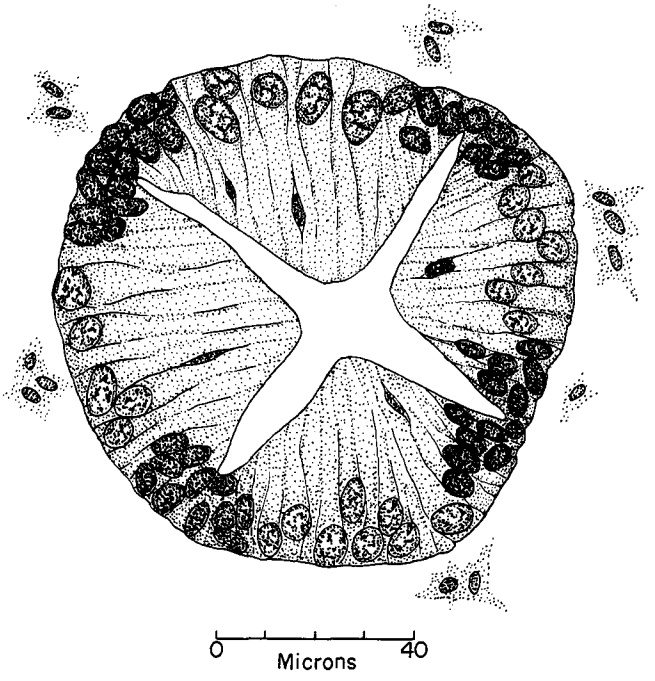

FIGURE 208.—Cross section of a single digestive diverticulum. Note the crypts of young cells at the corner of the cross of the lumen. Kahle, hematoxylin-eosin.

algae of larger size are ingested by phagocytes. Yonge's work fully confirmed the idea, first expressed by Saint-Hilaire (1893), that the digestive diverticula are the organs of absorption. He found no evidence of any secretion from the diverticula and demonstrated the importance of phagocytes in the digestive processes. Since the work of the earlier investigators is fully discussed by List (1902) and more recent investigations are summarized in several papers of Yonge (1926a, 1926b), the reader interested in the history of the problem is referred to these publications.

The digestion of food also takes place in the stomach where several digestive enzymes are present. On the basis of our knowledge, which admittedly is not complete, the process of digestion seems to take the following course. After being sorted several times by various mechanisms of the gills and labial palps, the food particles enter the stomach where the sorting continues and the larger particles are broken by the combined action of the crystalline style rotating against the gastric shield and the chemical action of enzymes which dissolve from the style. Very small particles are pushed by the cilia through the ducts into the digestive tubules where they are taken into the vacuoles of the digestive cells and are acted upon by the enzymes of these cells. Usable material is ingested by the phagocytes or is stored in the surrounding connective tissue. Indigestible substances like colloidal carbon of india ink are expelled. Some of the food particles, especially of larger size, are engulfed by the phagocytes which abound in the digestive tract. Circulation of food in the ducts is maintained by the ciliated cells.

The stomach contains free enzymes which are dissolved from the crystalline style. The most active among them are the amylase and glycogenase which digest starch and glycogen. Yonge's experiments (1926a, 1926b) showed that the optimum activity of oyster amylase is at approximately pH 5.9. Purification by dialysis or with absolute ethyl alcohol inactivates the enzyme, but its action can be restored by the addition of chlorides or bromides. Besides these two enzymes, the style contains a complete oxidase system. The presence of oxidases in the extract of styles was first demonstrated by Berkeley (1923) in the Pacific coast clam, *Saxidomus giganteus*, in rock cockle, *Paphia staminea*, and in soft-shell clam, *Mya arenaria*. This finding lead Berkeley to advance a theory that the crystalline style represents a reserve of oxygen and is a factor in the anaerobic respiration of mollusks. The theory is not supported by sufficient evidence and has not been accepted by the students of molluscan physiology.

The presence of oxidases in the styles of *Ostrea*

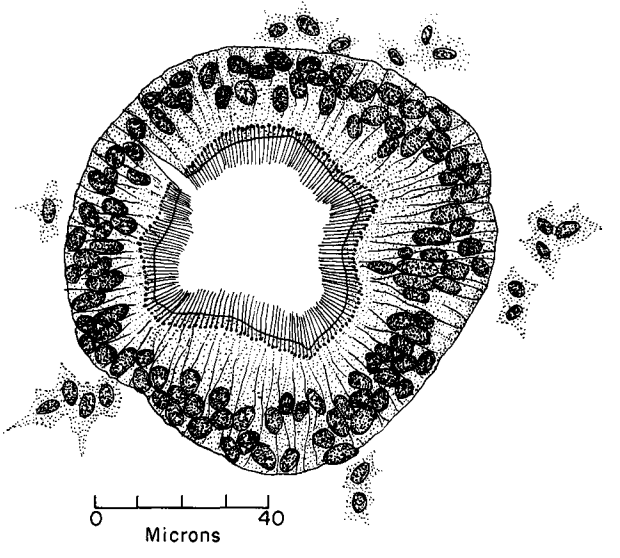

FIGURE 209.—Cross section of the duct leading to the digestive diverticula. Kahle, hematoxylin-eosin.

was confirmed by Yonge (1926a) and Graham (1931a, 1931b). The enzymes were obtained by grinding the styles with sand and extracting for 2 to 3 days in distilled water with a small amount of toluol as an antiseptic. For testing, the 5-ml. samples of 1 percent extract of style were treated with 2 ml. of hydrogen peroxide and 12 drops of 1 percent pyrogallol. After 5 minutes the sample turned dark red-brown. The extract produced color even in the absence of hydrogen peroxide, indicating the presence of a complete oxidase system. Reactions with guaiacum and 2 percent hydroquinine were less pronounced than with pyrogallol.

Sawano (1929) reported the presence of butyrase, an enzyme that clots milk, in the styles of *O. circumpicta* but his observation remains unconfirmed.

Extracts of digestive diverticula contain a large array of sucroclastic enzymes which act on starch, glycogen, sucrose, maltose, lactose, raffinose, and on some glucosides. The amylase, which converts starch into dextrin and dextrin into maltose, is present both in the style and in the digestive diverticula of the oyster. It has, however, different optima; the style amylase acts best at pH 6.0, whereas the enzyme from the diverticula has an optimum at pH 6.4 (Sawano. 1929).

The proteolytic enzyme of *O. edulis* is absent in the gut but can be found in the extract of the diverticula. It acts very slowly and has two pH optima at 3.7 and 8.5 when casein is used as a substrate. With gelatin the optima are 4.1 and 8.5.

Cellulase, the enzyme which hydrolizes cellulose, has not been found in the digestive extracts of the oyster. It must be assumed, therefore, that the oysters are unable to digest cellulose. The possibility is not excluded, however, that this enzyme may be present in the bacteria and fungi which happen to be in the gut. The presence of cellulase in mollusks has been established for the gastropods *Helix* and *Linnaea* and for the wood boring bivalve *Teredo*.

Fats are hydrolized to fatty acids and alcohols by the action of lipase. Yonge (1926a) demonstrated the presence of this enzyme by feeding the oysters an emulsion of olive oil stained red with Nile blue sulphate and watching the change of red color into blue as the digestion proceeded. Oil is ingested by phagocytes and is carried by them through the tissues, the gradual change of color serving as an index of the action of lipase. From the observation that the droplets of oil found free in the stomach retain the red color, Yonge deduced that free lipase is absent in the gastric juice. These findings are contradicted by the observations of George (1952) who showed that in *C. virginica* and in *Mytilus* the hydrolysis of neutral fats takes place extracellularly in the stomachs and that lipase can be extracted from the crystalline style. According to his observations, droplets of olive or peanut oil stained scarlet red with Sudan I or Sudan III are not deposited in the tissues. It is known that in mammals and birds the stained fat may be stored in the bodies (Gage and Fish, 1924). Several possibilities may be considered: (a) that the stained fat is rapidly metabolized; (b) that it may be deposited in connective tissue in minute quantities undetectable under the light microscope; and (c) that the mollusks are unable to utilize the peanut and olive oil because of the differences between the fatty acids of these oils and the unsaturated fatty acid of their natural food. So far no experimental evidence has been presented in support of any of these possibilities (George, 1952) and further studies of the problem of fat digestion in bivalves are needed.

pH CONTENT OF GUT AND STOMACH

The digestive fluids found in the alimentary tract are acid. The most acid conditions exist in the stomach (average pH 5.5) due to the dissolution of the crystalline style, which has a pH of 5.2 (5.4 in starved animals), and, according to Yonge (1926a), is the most acid substance in the gut. In the absence of the style the pH of the stomach fluids increases. This has been demonstrated on oysters with clamped shells, kept for 6 days out of water. Under these conditions the pH of the stomach rose from 5.67 to 6.14 while the pH of the liquid in the mantle cavity decreased due to the accumulation of carbon dioxide from 6.7 to 6.14. It is significant that the acidity in the stomach caused by the dissolution of the style approximates the optimum (pH 5.9) for the action of the style's amylase. The pattern of pH differences in various parts of the alimentary tracts as shown by Yonge is as follows: esophagus 5.6–6.0; stomach 5.4–5.6; style 5.2; midgut 5.5–6.0; rectum 5.8–6.3. The pH of the extracts of the styles of *C. virginica*, determined by placing the

styles across the one-drop electrode, was found by Dean (1958) to vary between 5.8 and 6.0.

Extracts of the digestive diverticula of *O. edulis* have pH values from 5.6 to 5.9; the variations are probably associated with the resting and active stages of digestion. The styles of *C. virginica* contain a heat-labile substance, probably an enzyme, which has the ability to attack certain algal cells only during the dissolution of the style or within a very short period after the dissolution. This has been reported by Dean (1958) who observed rapid disintegration of *Cryptomonas* cells in buffered sea water (pH 6.0) containing style extract. *Monochrysis* sp. were immobilized by the extract while *Isochrysis* sp. were not affected and were able to swim near or even touch the style. Dean thinks the "enzyme" may be a protease, lipase, or amylase. The observed results may be interpreted as the differences in the resistance to digestion by different species of algae used by the oyster as food.

It has been pointed out in support of the importance of extracellular digestion that fragments of partially disintegrated large diatoms (*Coscinodiscus, Melosira, Skeletonema*) are frequently found in the stomachs of *C. virginica* (Nelson, 1934), but the question of the significance of extracellular digestion in bivalves has not been settled. Weak proteolytic action was found in the stomach of the giant clam, *Tridacna*, the pearl oyster, *Pinctada* (Mansour-Bek, 1946, 1948), and in the crystalline style extract of *C. virginica* (Chestnut, 1949) and strong amylolytic activity in the stomach of the oyster was demonstrated by a number of investigators. Oysters apparently have a great capacity to utilize materials rich in carbohydrates.

ABSORPTION OF FOOD BY GILLS AND MANTLE

The idea that the exposed surfaces of bivalves, particularly the gills, palps, and mantle, absorb the organic matter dissolved in sea water (Ranson, 1926, 1927) is not substantiated by experimental evidence. In experiments with *O. edulis* Yonge (1928) has shown that the oyster absorbs glucose from the water but that this absorption takes place through the alimentary canal and digestive diverticula. No absorption was recorded in the animals in which the access of water to the esophagus was prevented by stuffing the mouth with wax plugs. Glucose may be absorbed, however, by the phagocytes which accumulate on the surface of the mantle. The results of Yonge's observations were confirmed by Koller (1930) in his experiments with *Mytilus edulis* and *Mya arenaria*.

Since phagocytes normally aggregate on the surface of the mantle and gills, it is possible that the oyster may absorb the substances present in the surrounding media by means of these wandering cells. Yonge admits this possibility in the case of oysters fed iron saccharate, and I observed that the particles of iron oxide added to the water in which I kept *C. virginica* were ingested by the phagocytes of the gills and transported to the deeper parts of the body.

FOOD AND FEEDING

The study of food of the oyster has attracted the attention of many investigators who examined the stomach contents and recorded the variety of organisms found in it. One of the earliest observations was made more than a century and a half ago by Reade (1844, 1846), who was "induced" to examine the contents of the stomach of British oysters and the "well known ciliary currents in the fringes of the oyster." His curiosity was well satisfied, for he found "myriads of living nomads, the *Vibrio* also in great abundance and activity, and swarms of a conglomerate and ciliated living organism, which may be named *Volvox ostrearius*, somewhat resembling the *Volvox globator*, but so extremely delicate a structure, that it must be slightly charred to be rendered permanently visible." He listed also a number of common diatoms, silicoflagellates, and desmids which he called "Infusoria." It is impossible to guess the true identities of the "*Vibrio*" and "*Volvox*."

Since the oyster is a filter feeder it is natural to expect that the contents of its alimentary canal would reflect the material suspended in water. Many of the investigators were unduly impressed by the occurrence of one or several species in the stomach and because of their abundance considered them to be of primary importance in the oyster diet. Opinions based on such examinations referred to the following forms found in the European oyster as important food materials: *Navicula fusiformis* v. *ostreari*, Grün. (Puységur, 1884); desmids, minute animals, and dead organic matter (Hoek, 1883); bottom diatoms *Nitzschia punctata, N. acuminata, N. sigma, Grammatophora oceanica*, and *Diploneis bombus* var. *densestriata*, the latter species being considered of special importance for

fattening of oysters (Hinard, 1923). American biologists made similar observations in *C. virginica*. McCrady (1874) concluded that "diatoms and spores of algae" constitute the food of Carolina oysters; Lotsy (1893) found that in the James River, Va., "oyster lives almost exclusively on diatoms"; according to Smeltz (1898), the natural food of Florida oysters "can be supplemented by . . . the pollen of our pine trees and the bloom of our palmetto", (p. 307) but no evidence was presented that pollens were found in the stomachs or that they can be digested by the oysters. The flourishing and fattening of oysters in Delaware Bay was attributed by Nelson (1947) to the abundance of the diatom *Skeletonema*, which he called "the most valuable of all diatoms in the food of oysters in New Jersey waters." In an earlier paper (1923b) and in the Report of the New Jersey Agricultural Experiment Station for the year 1924 (Nelson, 1925), he emphasized the significance of nannoplankton which "comprise by far the largest part of the food of the oyster" and at times is composed of small flagellates and other minute forms which may comprise 80 to 90 percent of the stomach's contents. Since no plankton analysis was made by Nelson of the Delaware Bay water at the time of the *Skeletonema* bloom, the conclusion that the species is "the most valuable" requires corroboration.

Moore (1910) found that eight species of diatoms constituted 98 percent of the total amount of food in the alimentary tract of Texas oysters and that organic detritus also might play an important part in nutrition. Experimental studies of the feeding of oysters made by Martin (1927b) showed no significant differences between the average increases in size of young oysters which were fed pure cultures of the diatoms—*Nitzschia palea, Amphora coffeaeformis, Nitzschia poleaceae, Amphora coffeaeformis* var. *lineata*, and one species of green alga, *Gloeocystis vesiculosa*. No check was made on the amount of food added to the water and the experiment lasted only 4 weeks. Water was changed only once during this period. Because of the obvious deficiencies in the experimental technique no definite conclusions could be made from these observations. Martin also suggested that zoospores of *Enteromorpha* and other algae (*Ulva, Monostroma, Ectocarpus*, and *Pylaiella*) form an important element in the food of plankton eaters (Martin, 1927a). A comprehensive investigation of the food of the European oyster

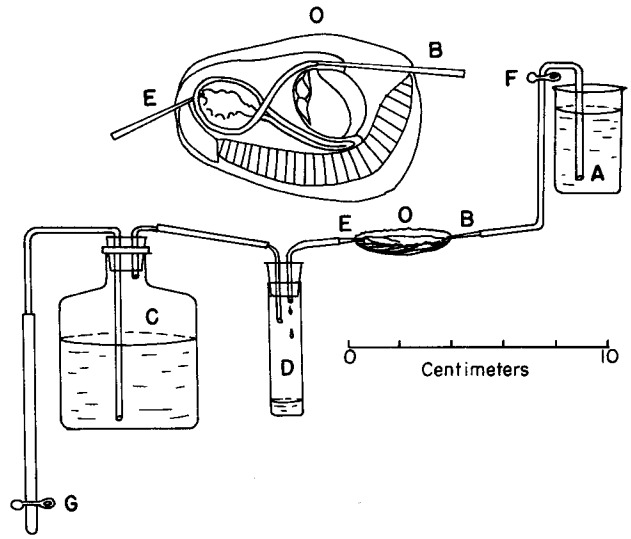

FIGURE 210.—Moore's method for washing out the stomach and intestinal content of the oyster. A—reservoir with sea water; B—canule inserted in the rectum; C—aspirator; D—collecting vessel; E—canule inserted into mouth of the oyster; F—siphon of the reservoir; G—siphon of the aspirator; O—oyster. Upper insert O—shows the details of the method of inserting canules B and E into the oyster.

was made by Savage (1925), whose work remains the most valuable contribution to the study of the problem. He used Moore's (1910) method of washing the entire alimentary canal; this technique is diagrammatically shown in figure 210. Two canules are introduced, one into the anus, B, and the wider one, E, into the mouth. Rubber tubing connects the anal canule with the siphon F inserted in glass container A filled with sea water. The oral canule leads to a small collecting vessel D, which is connected to the aspirator bottle C. By regulating the flow of water from the aspirator C the alimentary canal may be washed out without damaging the digestive tract. The volume of the collected material is measured and the collected microorganisms identified and counted. By this method Savage (1925) sampled at regular intervals the stomach contents of British oysters and analyzed throughout the year the seasonal fluctuations in the abundance of different species of algae. He considered that the following diatoms were the most important food items of the British (Oxford) oysters: *Nitzschiella parva, Pleurosigma* sp., *Coscinodiscus* sp., *Rhizosolenia* sp., and *Melosira* sp. The most significant conclusion made by Savage is that the greater part of the food found in the oysters examined by him consisted of organic

detritus and that "the animate food (i.e., living microorganisms) never exceeded 10 percent of the total" (by volume). He also advanced a hypothesis which, however, lacks experimental confirmation, that growth of Oxford oysters was due mainly to the inanimate food (detritus) and that fattening was caused by diatoms (*Nitzschiella longissima* f. *parva*). He found no evidence of selection of food by the oysters and commented that the actively feeding oyster appears to ingest anything that it can capture.

The extreme view that phytoplankton is of no direct significance as food of *O. edulis* in Danish water was expressed by Blegvad (1914), who classified this mollusk as a "pure detritus eater." Phytoplankton, according to his view, contributes to the food only as part of the detritus after the death of the algae.

Petersen and Jensen (1911) attributed great importance to eel grass, *Zostera*, as a possible source of food for bottom organisms. On the basis of their observations Spärck (1926) experimented with *O. edulis*, which he kept in a tank with sea water to which he added a liberal supply of old brown *Zostera*. Examination of the stomach contents of these oysters showed many species of flagellates and some *Zostera* detritus, but the quantity of the latter was by no means greater than in the oysters from the natural bottoms in the fjord. Decaying *Zostera* probably fertilized the water and stimulated the growth of the plankton. Danish investigators emphasized the fact that pentosan released from the decaying *Zostera* is a principal source of organic food for bottom invertebrates. The substance is apparently useless to oysters because they are unable to digest it, as has been shown by Yonge's experiments (1926a). The question of the extent of utilization by the oyster of the organic detritus which is always present in its natural environment has not yet been settled.

Naked flagellates and infusoria are frequently found in the contents of the alimentary tract. Under the influence of gastric fluids these forms are rapidly destroyed and, therefore, cannot be enumerated with any degree of certainty. The same problem applies to the bacteria which reach the alimentary canal. That they may play a considerable role in the feeding of lamellibranchs is indicated by the experiments of ZoBell and Landon (1937), and ZoBell and Feltham (1938), with the California mussel, which was fed known amounts of red coccus and a spore-forming bacillus. Within 3 hours the mussel removed about 200 million bacteria per 1 ml. of water. The microorganisms were actually ingested and after 6 hours disappeared from the digestive tract. In 9 months the mussels which were fed red coccus gained an average of 12.4 percent, the bacillus fed animals gained 9.7 percent, and the fasting mussels, kept as controls, lost about 6.8 percent. These experiments suggest an explanation of the observations by Kincaid (1938) that oysters kept for several months in water with nothing to feed on except bacteria appeared to be normal and even increased their glycogen content. Kincaid's experiment should, of course, be repeated and the question of the role of bacteria should be adequately studied before a conclusion can be made of their significance in the feeding of oysters and other bivalves.

By feeding the oyster known concentrations of coliform bacteria, Galtsoff and Arcisz (1954) found that 15 minutes after the start of addition of the culture the two oysters retained from 21 to 49 percent of *Escherischia coli* available in sea water. The accumulation of bacteria soon reached the point at which no more microorganisms were retained and the effluent leaving the oysters contained more *E. coli* than the surrounding water. Retention and elimination of microorganisms are probably associated with the secretion and discharge of mucus by the gill epithelium. These results confirm the previous observations by Galtsoff (1928) that over 50 percent of the bacteria pass through the gills and that only a fraction of their total number is retained.

The organisms found in the stomach of the oyster reflect the composition of plankton and nannoplankton present in the surrounding water. Selection is made primarily by the size and shape of food particles, although the ability of the oyster to discriminate between two suspensions of microorganisms of different colors but of the same size was suggested by Loosanoff's experiments (1949). A more detailed study should be made, however, before the discriminating ability of the oyster is confirmed.

There are several weaknesses common to all the studies on the feeding of oysters. The conclusions are based on examinations of the contents of the stomach and composition of feces without giving proper consideration to the nutritive value of different forms and their digestibility. The simple

test of feeding the oysters inert materials such as carmine powder, carborundum, clay, pulverized williamite, and colloidal carbon would show that these undigestible materials, if fed gradually and not in excessive quantities, are swallowed and pass through the digestive tract. The fluorescent mineral williamite, which I used extensively in my studies, is particularly suitable for this purpose because it permits easy detection of the most minute granules of the mineral inside the intestinal tract or in the feces when illuminated by ultraviolet light. The fact that some of the microorganisms found in the stomach are not destroyed and can be recaptured alive in the feces has been known for a long time. The dinoflagellate *Prorocentrum micans* was seen by Blegvad (1914, p. 47) to pass unharmed. Living *Chlorella* and *Nitzschia closterium* given to *C. virginica* in large quantities can be recovered alive from the feces and recultured (Loosanoff and Engle, 1947). In studies of the effect of feeding oysters in the laboratory I frequently used a light suspension of Fleishmann's yeast, and observed that such a large number of yeast cells passed undigested that the feces acquired a milky color. Thus, the presence of an organism or its remnants in the alimentary tract in itself is not a proof that it is being used by the oyster as food and that it has nutritive value. Neither the enumeration of the organisms found in the stomach nor the determination of their volume gives satisfactory quantitative data. It is at present impossible to judge whether, for instance, one cell of *Coscinodiscus* equals or differs in nutritive value from a single cell of *Pleurosigma, Skeletonema, Nitzschia,* or other forms. Information is lacking about the caloric value and chemical composition of various forms and, therefore, it is impossible to determine the number that should satisfy the energy requirements of the oyster.

Through trial and error oyster growers know that certain grounds in their possession are particularly suitable either for the growth or for fattening and conditioning of oysters for market. Sometimes a great difference in the productive capacity of grounds may be found in the two areas located a short distance apart. In an ecological survey of the bottom it is relatively easy to detect conditions which are unsuitable for growth. It is, however, impossible at present to evaluate the potential productivity on the bottom because of the inadequacy of our knowledge of the nutrition of the oyster.

ARTIFICIAL FEEDING

So far only a few experiments on artificial feeding reported in the literature were successful in producing an increase in the weight of the oysters. As a rule oysters kept in the laboratory show lack of nutrition and die sooner or later. Better results may be obtained by keeping them in large outdoor tanks adequately supplied with sea water which has not been stored for any length of time. Experiments by Martin (1927a, 1927b) in feeding oysters with pure cultures of plankton forms resulted in very poor growth. Spärck (1926), experimenting with *Zostera* as a potential food for the European oyster, emphasized the fact that oysters "may thrive, increase in size and even spawn in very small limited water volumes without any renewal of water worth mentioning." Such conditions occur in the Norwegian oyster basins and in the French "parks" which, however, must contain "some source producing nourishment in sufficient quality and quantity." This material presumably may derive from the organic detritus. He also reports that in his experiments the "development of bacteria did not seem in any way to hurt the oyster, rather the opposite."

A unique experiment, unfortunately not well known to biologists, was made by Gavard (1927, quoted from Korringa, 1949) in Algiers. He fed the oysters an artificial detritus prepared from animal and plant material and obtained an increase of 15 kg. per 1,000 oysters per season. Korringa states that these results demonstrate the ability of the oysters to grow without using living organisms as food. Without access to Gavard's original paper it is impossible to judge if the detritus was directly consumed by the oysters as food or whether it stimulated the growth of bacteria and nannoplankton.

Artificial enrichment of sea water by adding commercial fertilizers at one time seemed to be a simple answer to the problem of providing increased food supply to the oyster. To test the idea a series of experiments was conducted in the Bureau of Commercial Fisheries Biological Laboratory at Milford, Conn., which resulted in the interesting discovery that an excessive concentration of microorganisms (*Chlorella* sp., *Nitzschia closterium, Prorocentrum triangulatum, Euglena viridis*) adversely affects the feeding of oysters

(Loosanoff and Engle, 1947). A large-scale "natural" experiment along the same line took place in Great South Bay where unbalanced fertilization of sea water by manure from the duck farms located along the banks of the bay boosted such reproduction of *Chlorella*-like organisms that the heretofore prosperous shellfish industry of the bay suffered a serious setback (Redfield, 1952).

Nelson (1934) made a series of tests of several substances as artificial foods for oysters. He used corn starch, ground alfalfa, soybean meal, and ground meat of the king crab. It is not clear in his report if the criterion of results was the weight of the oyster meat. Nelson states that only with corn starch "was any success obtained." The details of these experiments have not been disclosed.

In spite of doubtful results the artificial feeding of oysters appears to be a definite possibliity which should be carefully investigated. Since oysters are able to absorb glucose dissolved in sea water (Yonge, 1928), it seems desirable to explore more thoroughly this method of feeding. Furthermore, the diet of the oyster and the nutritive value of different diatoms and flagellates should be investigated together with the methods of their cultivation. It is reasonable to expect that certain forms richer in protein, may be more useful for obtaining better growth of oysters; others, richer in carbohydrates, may prove more valuable for their fattening. Research along these lines offers many interesting possibilities that may prove useful in the artificial culture of oysters.

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