

# Structure and Physiology of the Organs of Feeding and Digestion in *Ostrea edulis*.

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With 42 Figures in the Text.

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## I. INTRODUCTION.

THIS research is the outcome of a recommendation made by Dr. J. H. Orton in his report (1923) on the cause of the mortality of oysters during 1920 and 1921, in which he pointed out the necessity of our obtaining more precise information regarding the physiology of the oyster, both for its own sake and for its possible economic applications. I have endeavoured, therefore, to give as complete an account as possible of the structure and function of the food collecting and digestive organs in the oyster—larval, "spat," and adult—in the hope of so determining the optimum conditions for feeding and digestion, and, consequently, for growth and "fattening." In view of the fact that no complete account of the anatomy and histology of these organs exists, it has been necessary to devote considerable time to this aspect of the work, since a sound knowledge of the structure of any organ is essential if the function is to be determined. The research covers a great deal of ground, so that it has been impossible in the time available to investigate in detail every problem that has been encountered or to perform all the experiments that have suggested themselves, but no problem of the first importance has been neglected, while it is hoped in the near future to carry out further investigations into those aspects of the work which have been found the most important.

The work on the adult oysters was carried out at the Plymouth Laboratory, the oysters being obtained from the River Yealm, and the work on the larval and "spat" oysters at the Fisheries Experimental Station at Conway during July and August, 1925. I wish to express my gratitude for their kindness and help to the Director and members of the Staff, especially Dr. J. H. Orton, of the Plymouth Laboratory, and to Dr. Dodgson, Mr. H. P. Sherwood, and the other members of the Staff at Conway.

## 2. ANATOMY AND HISTOLOGY OF THE ALIMENTARY SYSTEM.

### A. ADULT OYSTERS.

#### I. ANATOMY.

The arrangement of the food collecting and digestive organs in the oyster can best be described by reference to Fig. 1 in which an oyster is shown lying on the lower (left) shell valve with the right fold of the mantle cut away. The surface of the mantle (L.M.F.) is transversely ridged, and is bounded by a thickened margin bearing two rows of small tentacles. The mantle cavity is divided into inhalent (I.C.) and exhalent chambers (E.C.), the former being some four times the larger, and containing the gills (G.), which consist of four demibranchs, the inner ones being broader than the outer ones, the inner one on the left (under) side being the broadest of all. The outer demibranchs are attached directly to the mantle, the inner ones being attached to the mantle on the outer side and to one another on the inside. In the oyster the two pairs of demibranchs are not separated by a protruding foot or visceral mass. The gills extend in a semicircle from the junction between the right and left mantle folds, which forms the division between the inhalent and exhalent chambers (D.B.C.), to the labial palps (L.P.). The latter consist of triangular flaps attached by a broad base and arranged in two pairs, one on each side of the mouth (M.). The inner, opposing surfaces are ridged (see Fig. 2), the outer surfaces being smooth. The palps enclose the gills for a short distance, the outer and inner demibranchs of each side lying between the corresponding pairs of palps, the inner demibranchs arising slightly nearer the mouth than the outer, and immediately behind the most distal fold on the palp surface. Unlike the majority of Lamelli-branchs, the inner and outer palps of the two sides are united to one another in the region of the mouth (M.), which lies in the middle line in the groove formed by the continuation of the grooves between the two

sets of palps. The outer palps are united for about a quarter of their length, so that the mouth is entirely enclosed.

The mouth is a narrow horizontal slit and leads into a short œsophagus (O.) which has the the same shape in cross section and passes backwards and downwards into the stomach (S.). This is an irregular sac-shaped organ which is surrounded on all sides by the brown mass of the digestive

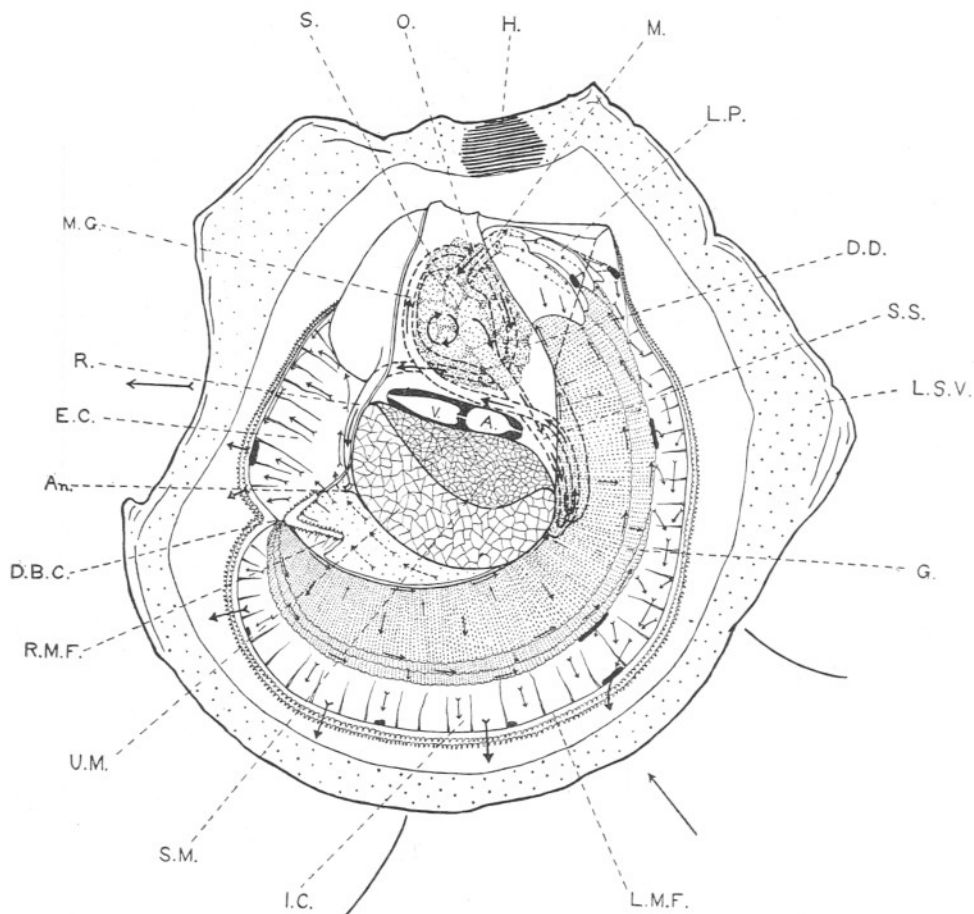


FIG. 1.—*Ostrea edulis*, right shell valve and mantle fold removed.  $\times 1$ . A., auricle; An., anus; D.B.C., division between inhalent and exhalent chambers; D.D., digestive diverticula; E.C., exhalent chamber; G., gills; H., hinge; I.C., inhalent chamber; L.M.F., left mantle fold; L.P., labial palps; L.S.V., left shell valve; M., mouth; M.G., mid-gut; O., œsophagus; R., rectum; R.M.F., right mantle fold; S., stomach; S.M., adductor muscle, portion with striated fibres; S.S., style-sac; U.M., adductor muscle, portion with smooth fibres; V., ventricle. Large arrows external to shell denote direction of ingoing and outgoing currents, within shell plain arrows denote direction of ingoing currents and feathered arrows direction of outgoing currents, broken arrows (except in gut) denote currents on under surfaces.

diverticula (D.D.), while internally the walls are thrown into a series of ridges and folds so that the exact shape of the stomach in the living animal is difficult to determine when it is opened for inspection. In order to obtain a clear idea of the anatomy, casts of the stomach were made by injecting, by way of the œsophagus, a warm, concentrated solution of gelatin. This was allowed to cool and solidify, the tissues were then dissected away, and the cast hardened in formalin and stained lightly with hæmatoxylin. Gutheil (1912) used plaster for making casts of the stomach

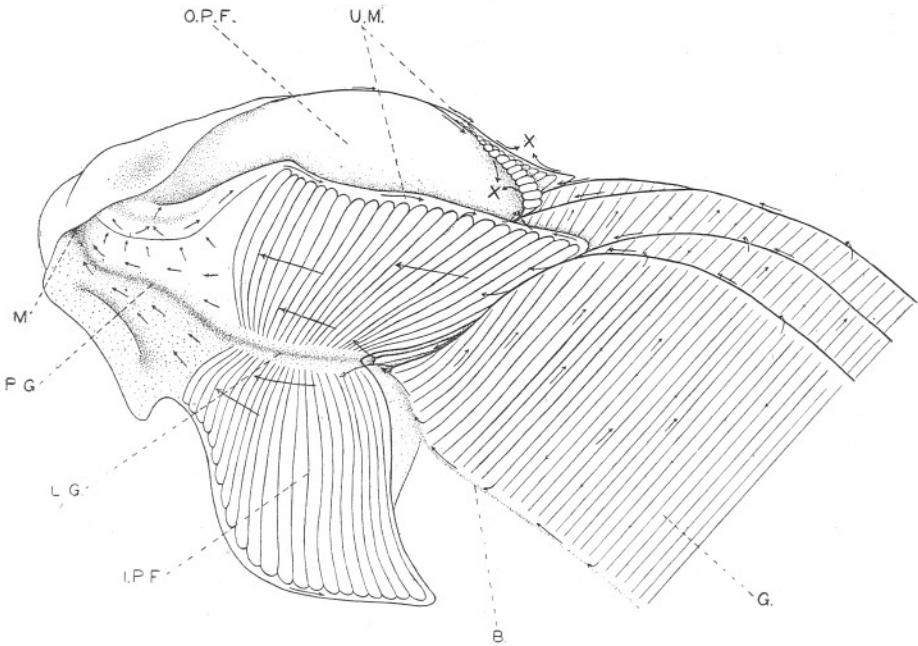


FIG. 2.—Junction of palps and gills, right palps opened out so as to expose inner, ridged surfaces.  $\times 8$ . B., base of gill demibranch; G., gill; I.P.F., inner palp face; L.G., lateral oral groove; M., mouth; O.P.F., outer palp face; P.G., proximal oral groove; U.M., upper margin of palps; X., point where material is rejected from palps.

of Anodonta, but I have found the gelatin method much more satisfactory, and by its use have been able to demonstrate in detail the anatomy of the stomach—a much more complex and important organ in the Lamelli-branchs than it has usually been considered—and its associated organs. Figs. 3 and 4 are drawings of a cast, the former from the ventral aspect and the latter from the dorsal aspect. The most conspicuous structure in the stomach is the long, grooved food sorting cæcum (F.C.), which extends backwards beneath the floor of the stomach, and is connected

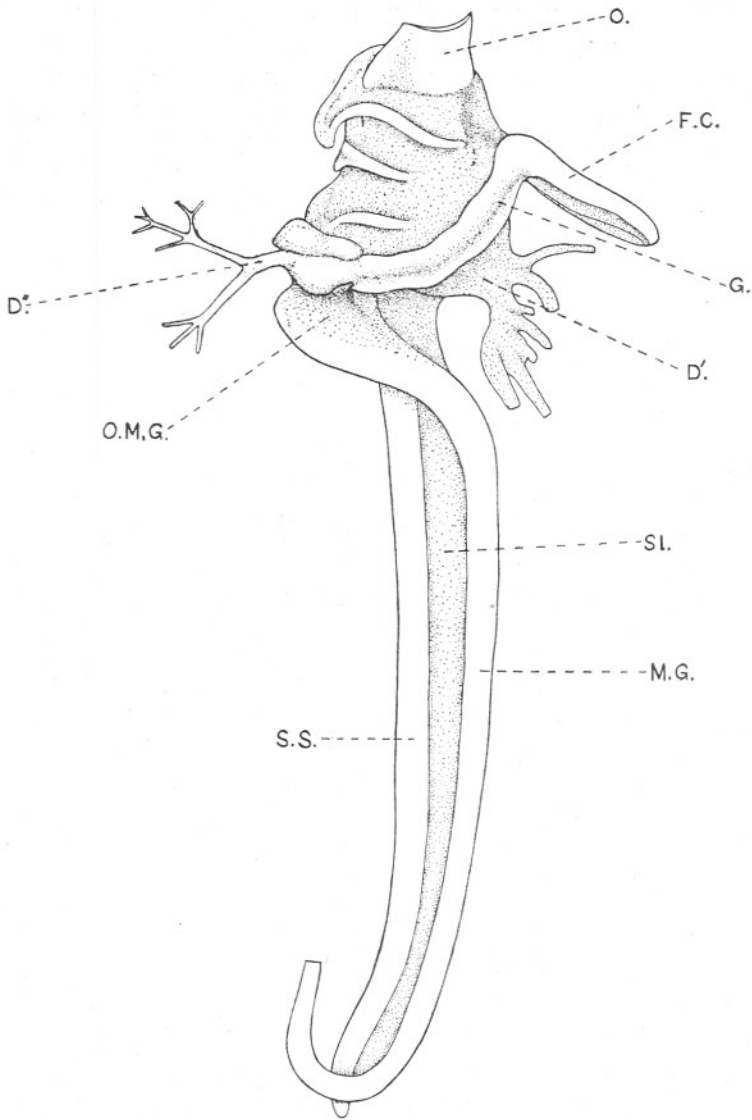


FIG. 3.—Gelatin cast of stomach with style-sac and first part of mid-gut and portion of oesophagus, from ventral aspect.  $\times 4$ . D', larger, left duct of digestive diverticula; D'', smaller, right duct of same; F.C., food sorting caecum; G., ventral groove; M.G., mid-gut; O., oesophagus; O.M.G., opening of mid-gut; S.S., style-sac; Sl., slit connecting mid-gut and style-sac.

with the opening of the mid-gut (O.M.G.) by means of a deep groove (G.), which runs across the floor of the stomach. On opposite sides open the two ducts which lead into the digestive diverticula, that on the left side (D'.) being the larger and dividing into a greater number of smaller

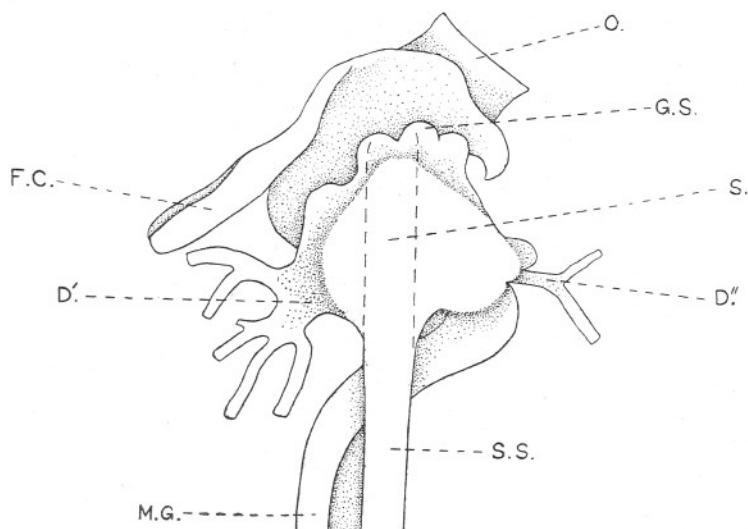


FIG. 4.—Stomach cast from dorsal aspect.  $\times 4$ . G.S., gastric shield S., style. Other lettering as in Fig. 3.

ducts than the one on the right (D''). On the dorsal wall of the stomach is borne the gastric shield (G.S.), a cuticular structure of somewhat irregular shape (see Fig. 5) consisting of two broad lobes united by a narrow neck, the larger of the lobes being thin and smooth, while the smaller is thicker and bears a number of teeth, which are also shown in Fig. 4. It is against this shield that the crystalline style bears, and the dotted line in Fig. 4 shows the position of the style as it projects into the stomach from the style-sac (S.S.), and bears against the gastric shield on the opposite wall. The cavities of the style-sac and mid-gut are united by a narrow slit (Sl.), and pass downwards and slightly forwards from the stomach, as shown in Fig. 1.

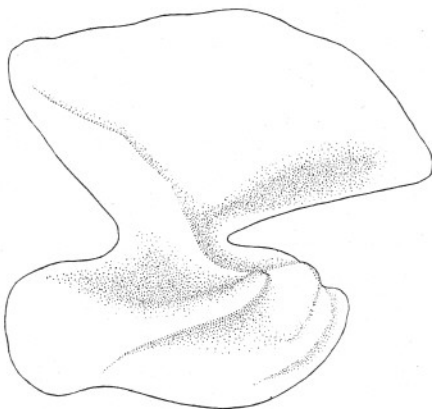


FIG. 5.—Gastric shield.  $\times 20$ .

The style-sac is practically straight but the mid-gut, which opens into the stomach on the right side of the style-sac (Figs. 3 and 4), twists round to the left side immediately behind. At the distal end of the style-sac, the gut turns anteriorly and then completely back on its course, subsequently passing dorsal to the heart (Fig. 1, A. and V.) and encircling the stomach on the left side. Finally, it passes into the rectum (R.), which runs round the posterior margin of the adductor muscle (U.M.), and ends at the anus (A.), which lies at the tip of a small papilla on the posterior ventral surface of the muscle, and opens into the exhalent chamber.

## II. HISTOLOGY.

Material for histological examination was fixed in Bouin's fluid or in Flemming's strong fluid, sections were cut  $6\mu$  thick, unless otherwise stated, and were stained with Delafield's hæmatoxylin and erythrosin; or with iron hæmatoxylin either with acid fuchsin as counterstain or with mucicarmine for the demonstration of mucus glands; or with alum carmine and picro-nigrosin.

### (a) Gills.

An excellent account of the gills of *Ostrea edulis* has been given by Ridewood (1903), the substance of whose statements is as follows: "There are 9-12 filaments to the plica. The front of the principal filament has the form of a broad ridge. The filaments adjacent to the principal filament are slightly larger than usual, and have been called *transitional filaments* by Kellogg (1892). . . . The interlamellar junctions have the form of septa. At a short distance up, the interlamellar septa occur only in relation with alternate principal filaments, but the order is not absolutely regular. Higher up still each fourth septum only persists. The bars which run across the floor of the suprabranchial cavity from descending to ascending lamella are the thickened upper edges of alternate high septa. They recur at intervals of about eight plicæ. Most of the interfilamentar junctions are bands of tissue running horizontally round the inner surface of the plica, but each third or fourth in a vertical series extends across the plica as a horizontal septum. . . . The frontal and lateral cilia are normal. There are short cilia on the interlamellar edges of the principal filaments. No intrafilamentar septum is present. There is a fair amount of muscle in the interfilamentar junctions and in the inner edge of the horizontal septa. . . ."

Many of the characteristics of the gill of *Ostrea* given by Ridewood, and also other points which he does not emphasize but which are of importance functionally, are shown in Fig. 6, which represents a trans-



verse section through a single lamella (i.e. one side only of a complete demibranch). A principal filament (P.F.) is figured with four filaments on either side, all being united by an interfilamentar junction (I.). (For the structure of the interlamellar junctions and the horizontal interfilamentar septa, reference must be made to Ridewood's figure.) The large size of the principal filament and the thickness of the chitinous supporting rods (C.R.) within it and the two transitional filaments (T.F.) one on either side of it are well marked. There are many strands of horizontal muscle (H.M.) in the interfilamentar junction and also a slight development (not mentioned by Ridewood) of vertical muscle (V.M.)

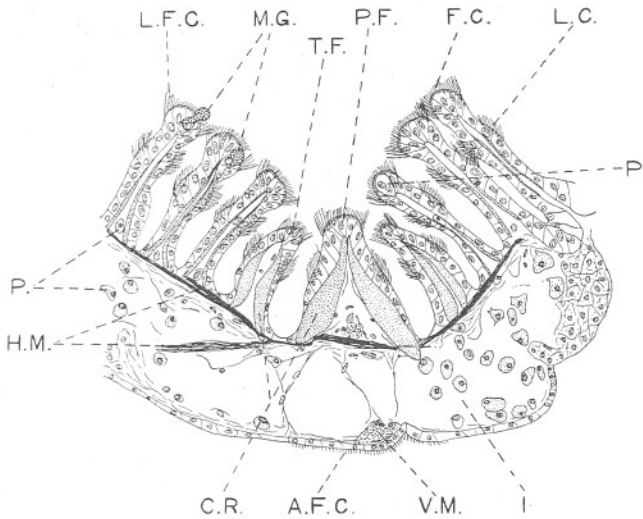


FIG. 6.—Transverse section single gill lamella through groove between plicæ. Delafield's hæmatoxylin and erythrosin.  $\times 220$ . A.F.C., abfrontal cilia; C.R., chitinous supporting rod; F.C., frontal cilia; H.M., horizontal muscle; I., interfilamentar junction; L.C., lateral cilia; L.F.C., laterofrontal cilia; M.G., mucus glands; P., phagocytes; P.F., principal filament; T.F., transitional filament; V.M., vertical muscle.

in the principal filament. The abfrontal cilia (A.F.C.) noted by Ridewood are shown in the figure, and also the lateral (L.C.), frontal (F.C.), and laterofrontal (L.F.C.) cilia. The latter are not well developed in *Ostrea* and are difficult to distinguish in sections, but are easily seen in fresh material (see Fig. 21, p. 323). Mucus glands (M.G.) occur in the epithelium of the filaments, particularly in the frontal region. Wandering blood cells (P.) are present in large numbers within the filaments and the interfilamentar junctions, and are also frequently to be found actually between the cells of the epithelium. These cells, as will be shown later, are phagocytic, and will be referred to as phagocytes in the remainder of this account.

Along the free, lower margins of the demibranchs and along their axes there are ciliated grooves.

(b) *Palps.*

The folds on the inner palp surfaces arise near the attached base of the palps, and run across the face to the upper free margin, gradually increasing in height and breadth. In cross section (Fig. 7) they are seen to bend forward slightly on the proximal side (i.e. in the direction of the mouth), a tendency which is most pronounced near the free margin.

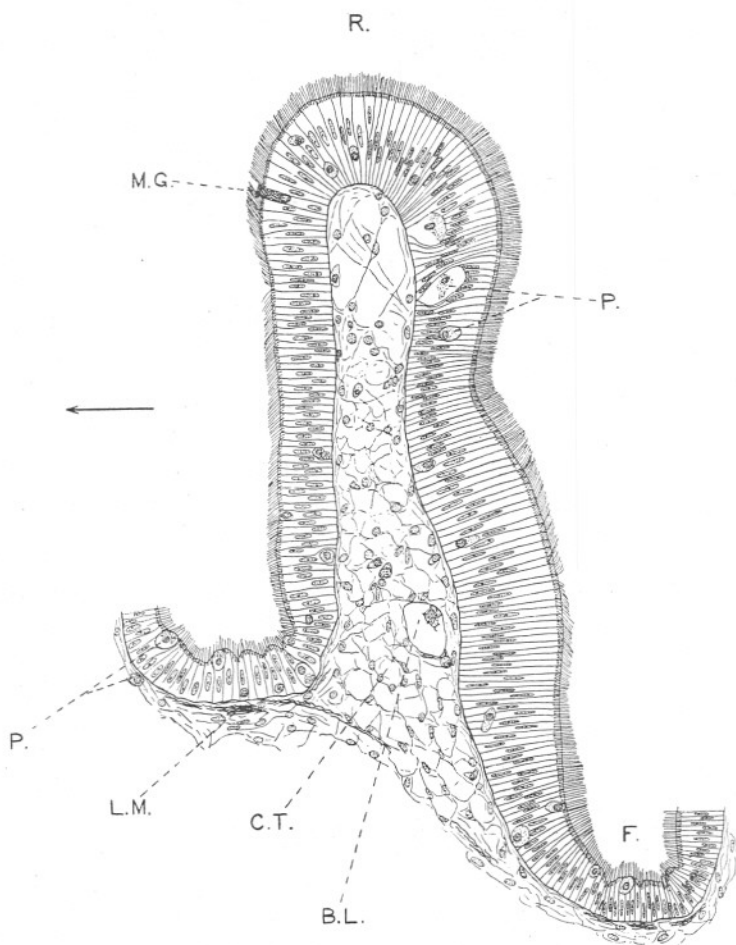


FIG. 7.—Transverse section through fold on inner palp face. Delafield's hæmatoxylin and erythrosin.  $\times 330$ . B.L., blood lacuna; C.T., connective tissue; F., furrow between ridges; L.M., longitudinal muscle; M.G., mucus gland; P., phagocytes; R. summit of ridge. Arrow indicates direction of mouth.

The proximal wall of the folds is comparatively straight, but on the distal wall there is a well-developed longitudinal groove about one-third of the distance between the summit of the fold (R.) and the furrow (F.), which lies between adjacent folds. The epithelium of the folds is composed of long, regular cells with oval nuclei and bearing a thick covering of long cilia. These cilia, as can be seen by the directions in which they lie in the sections, do not all beat in the same direction. It is, however, impossible to distinguish all the different tracts except in the living tissue, an account of which is given in a later section. Unicellular mucus glands (M.G.) of the goblet type are present, almost exclusively near the summits of the folds. Siebert (1913) has also found sense cells in the epithelium of the palps in Anodonta. Between the epithelial cells are many phagocytes (P.), which are also present in the connective tissue and blood lacunæ (B.L.), some of them containing yellow or brown granules. The connective tissue is very open in character, consisting of a network of fine strands. There are longitudinal muscle fibres (L.M.) under the epithelium of the furrows, and running through the connective tissue at the base of the ridges. There are also occasional fibres running across to the smooth surface of the palp, and a feeble development of circular muscle immediately beneath the epithelium of the furrows. It is important to note that there are *no muscles within the folds* such as could cause it to contract downwards.

The epithelium of the smooth surface of the palps (Fig. 8) is very different. The cells are lower and more irregular, cilia are present but often difficult to distinguish in sections, so that some workers have denied their presence. I have often seen them in my sections of *Ostrea*, while experiments on the living palp demonstrate immediately their presence. Mucus glands of the usual type are extremely numerous. The contents of these cells may be granular, and stain darkly with iron hæmatoxylin or contain a

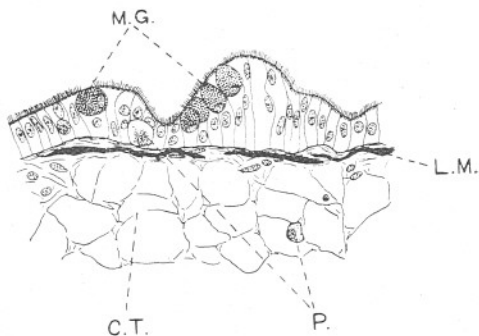


FIG. 8.—Transverse section epithelium of smooth, outer palp face. Delafield's hæmatoxylin and erythrosin.  $\times 330$ . Lettering as in Fig. 7.

mass of swollen granules or spheres which stain lightly with mucicarmine. Since intermediate stages between these two types are frequently found (this applies wherever mucus cells are found, from the mantle to the rectum), it seems probable that the granules represent an early

stage in the elaboration of the secretion. The glands invariably stain deeply with erythrosin, and have a great affinity for many stains. Beneath the basement membrane there is a well-developed layer of longitudinal muscle (L.M.). There is the usual network of connective tissue (C.T.) with darkly staining nuclei among which are many phagocytes (P.), which may also penetrate into the epithelium.

(c) *Mouth and Œsophagus.*

The epithelium of the mouth is a continuation of that of the grooves between the palps and consists of extremely long, thin cells, about four times the height of those on the folded surface of the palps. Long cilia are borne by the cells, mucus glands occur but not in large numbers, while phagocytes are present in great abundance.

The œsophagus (Fig. 9) is exceptionally large in the oyster and much compressed dorso-ventrally. The epithelium consists of narrow cells of

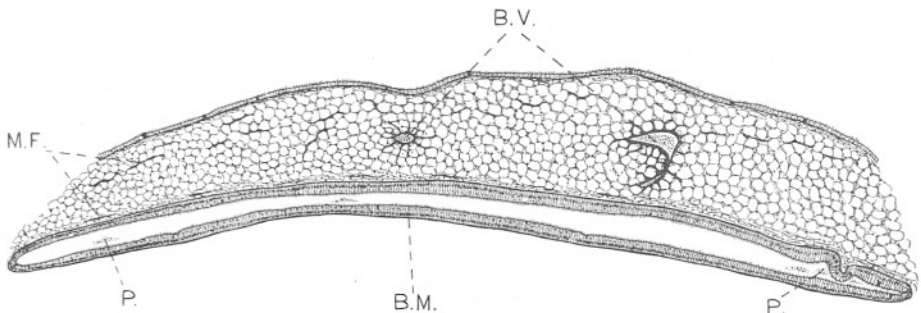


FIG. 9.—Transverse section œsophagus. Iron hæmatoxylin and acid fuchsin.  $\times 18$ . B.M., basement membrane; B.V., blood vessels; M.F., muscle fibres; P., phagocytes and food matter in lumen.

much the same height as those of the mouth region, but with cilia of only about a third the length. Phagocytes are very numerous between the cells of the epithelium and also *free in the lumen* (P.), sometimes with ingested matter. Mucus glands are here very rare, but Gutheil (1912) has described and figured what he considers to be secretory cells in the œsophagus of *Anodonta cellensis* and similar cells can be distinguished in *Ostrea*. They stain more lightly and have rather more vacuolated protoplasm than the neighbouring cells, and have, in addition, no cilia. On the other hand, their nuclei are identical with those of the ciliated cells, and there is really very little evidence that they are secretory cells; it seems more probable that they are damaged or degenerating epithelial cells. Particles are continually passing over the epithelium, which must suffer in the process, and it is quite common, moreover, to

find dividing nuclei near the free surface of the epithelium. An exceptionally thick basement membrane (B.M.), through which phagocytes pass, surrounds the epithelium, and outside this there are thin strands of circular and longitudinal muscle fibres (M.F.). Muscle strands also occur in the vesicular connective tissue in which the oesophagus is embedded and which contains large blood-vessels (B.V.), in and out of which the phagocytes pass.

(d) *Stomach.*

The epithelium of the stomach is of two kinds, that composed of typical ciliated cells, which covers the greater part of the surface, and that which lies beneath the gastric shield. Fig. 10 represents a section through the junction between the two. The ciliated epithelium consists of narrow cells, a little higher than those of the oesophagus and possessing longer cilia. The border cuticle (B.C.) is particularly well developed here,

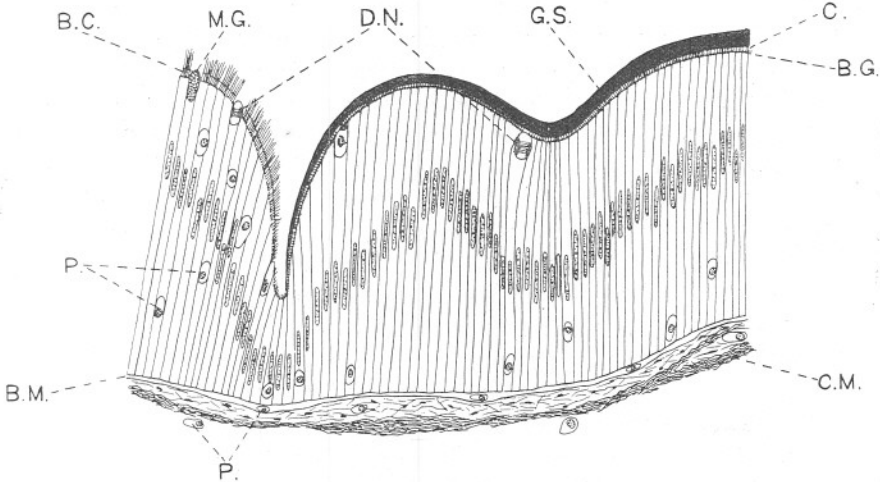


FIG. 10.—Transverse section stomach epithelium at junction between gastric shield area and ciliated epithelium.  $\times 425$ . B.C., border cuticle; B.G., basal granules; B.M., basement membrane; C., cilia-like strands between edge of cells and gastric shield; C.M., circular muscle; D.N., dividing nuclei of epithelial cells; G.S., gastric shield; M.G., mucus gland; P. phagocytes.

consisting (as in all the ciliated cells) of a clear cuticular layer distal to the line of basal granules, from which the cilia arise. The distinct nature of this layer is not shown by the usual staining methods, but after staining with Prenant's three-colour process (iron hæmatoxylin, erythrosin, and light green) the border cuticle is stained by the light green and the cytoplasm by the erythrosin. Mucus glands (M.G.) are occasionally found; phagocytes (P.) are very abundant in the connective tissue, basement membrane, between the cells of the epithelium and free in the lumen.

Dividing nuclei (D.N.) of epithelial cells are frequently seen, the nuclei passing to the surface of the cells in the manner characteristic of the dividing nuclei of ciliated cells (see Ehrhard (1910), and particularly Gutheil (1911), who has described and figured in detail the process of mitosis). There is a fairly thick basement membrane (B.M.), and, beneath that, strands of muscle (C.M.) the whole being surrounded by vesicular connective tissue in which lie embedded the tubules of the digestive diverticula.

The epithelium which lies beneath the gastric shield resembles closely that of the rest of the stomach. Mucus glands are never present, and phagocytes, though invariably present, occasionally even in the substance of the gastric shield, are not so numerous. Dividing nuclei are frequent. The gastric shield (G.S.) in cross section appears as a homogeneous substance composed of indistinct horizontal strata. It stains vividly with light green, moderately deeply with erythrosin (except in Prenant's stain) and very lightly with mucicarmine. Gutheil considers that it is formed by droplets of secretion from the cells beneath, and this has been the general view with regard to its formation. In my sections, however, I have failed to find any evidence of secretion from the cells, while the substance of the shield is united to the epithelium by fine strands having the appearance of cilia (C.) and arising from basal granules (B.G.) at the edge of the cells, as shown in Fig. 10. It is possible in certain places to observe the continuation of the strands transversely through the whole substance of the shield, while, as we have seen, the shield takes up light green in the same way as the border cuticle. In view of these facts and also that the cells of the gastric shield area are in no way different from those of the rest of the epithelium with regard to either nucleus or cytoplasm, it seems very probable that the gastric shield is *not* a secretion, but is formed by the fusion of cilia, originally in response to the irritation caused by the head of the style. Nelson (1918) thinks the shield is probably in the nature of chondrin, which would appear to support this view.

#### (e) *Digestive Diverticula.*

These consist of a brownish mass of blind tubules which surround the stomach. They have been called "liver" and "hepatopancreas," but, as I have emphasized in a recent paper (1926) to which reference should be made for a detailed account of the structure and function of these organs, they are organs of assimilation and of intracellular digestion with none of the functions of a true liver or pancreas, and I suggested, therefore, that they are more suitably termed digestive diverticula.

They communicate with the stomach by way of two large ducts (Figs. 3 and 4, D'. D''). These ducts are quite distinct in structure from the

tubules with which they communicate. They are usually circular in cross section, though the lumen is irregular owing to the variation in height of the epithelium, which is similar to that of the stomach of which it is a direct continuation. Cilia are always present, the protoplasm is not vacuolated and stains darkly with erythrosin. Mucus glands are present and also phagocytes in large numbers, both in the epithelium and in the lumen. There is a layer of circular muscle beneath the basement membrane.

The tubules (Fig. 11) are quite different. Cilia are never present in sections nor can a border cuticle be distinguished as in the case of some

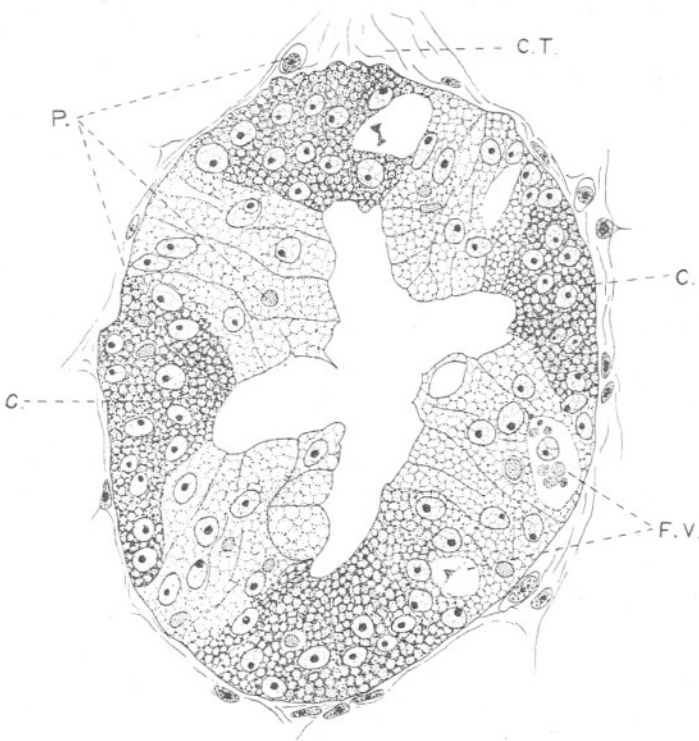


FIG. 11.—Transverse section through tubule of digestive diverticula. Iron hæmatoxylin and acid fuchsin.  $\times 350$ . C., crypts of darkly staining young cells; C.T., connective tissue; F.V., food vacuoles; P., phagocytes.

Lamellibranchs, and the outline of the cells is frequently very irregular. The tubules are surrounded by a few strands of connective tissue (C.T.), but muscle is *never* present. The nuclei are very characteristic, being circular and possessing a large nucleolus. In cross section the lumen is usually tripartite or in the form of a cross, and in the crypts (C.) at the

end of the arms are low areas of darkly staining protoplasm with many nuclei, the outline of the cells being very indistinct. The remaining cells are larger, very vacuolated, and consequently more lightly staining. As I have pointed out (1926) there is every reason, "From the histological character, the distribution, and the behaviour of these small dark cells . . . for considering them young cells which, by dividing, are able to make good the loss resulting from the casting off of the old cells." Large vacuoles (F.V.), sometimes with ingested food material which stains with erythrosin, are frequently found in the older cells, while phagocytes occur everywhere. There is never any indication of secretion.

As already noted, cilia are never to be seen in sections (similar observations have been made on *Ostrea* by Carazzi (1896, 1897), MacMunn (1900), and Vonk (1924)), nor have I observed them in fresh material; but in many Lamellibranchs (though never possible to see more than a border cuticle in sections), it is possible to see long cilia beating in the tubules when fresh material is examined, as Potts (1923) and I (1926) have shown. As will be shown later, there is a constant stream of food particles passing into the diverticula and of rejected particles passing out, and as there is no system of circular and longitudinal muscles such as ensures a similar circulation in the Crustacea (in which the diverticula are organs of both assimilation and secretion), there is strong presumptive evidence that cilia are present in the tubules of *all* Lamellibranchs. In many cases, including *Ostrea*, these cilia appear to be retracted very readily, and so cannot be seen when the tissue is pressed out under a coverslip for examination.

The tubules are embedded in vesicular connective tissue, in which lie many phagocytes often containing included granules, which frequently, as MacMunn (1900) has described and figured, take the form of brown or yellow spheres, which often are blackened by osmic acid after fixation with Flemming. The nature of the pigment will be discussed in the section on Assimilation (p. 340).

(f) *Style-Sac.*

Except for a short diverticulum where it arises (see Fig. 3), the style-sac is united for its entire length with the mid-gut. The two cavities (Fig. 12) are separated by two typhlosoles which, however, are not so well marked as in such genera as *Anodonta* (Nelson, 1918) or *Crepidula* (Mackintosh, 1925). The epithelium of the gut is quite distinct from that of the style-sac, and will be described later. The epithelium of the style-sac (Fig. 13) is very characteristic, consisting of cells of medium height, very regularly arranged, with large oval nuclei and long, stout cilia all of the same length. The structure of the style-sac in *Crepidula fornicata* (which,



although a Gastropod, has a style and style-bearing organs of exactly the same nature as those of the Lamellibranchs) has been investigated in detail by Mackintosh (1925). He has shown that the cilia are continued into the cell where they form an "internal fibrillar apparatus," the fibres of which are greatly thickened below the nucleus, so as to form "a bundle of thick rod-like bodies." I have observed the same arrangement in *Ostrea* (Fig. 13, I.F.), the fibres showing very clearly after staining with iron hæmatoxylin (though whether or no they really represent fibres in the living tissue cannot be stated). Mackintosh has also demonstrated the presence of a series of "intra-epithelial" canals,

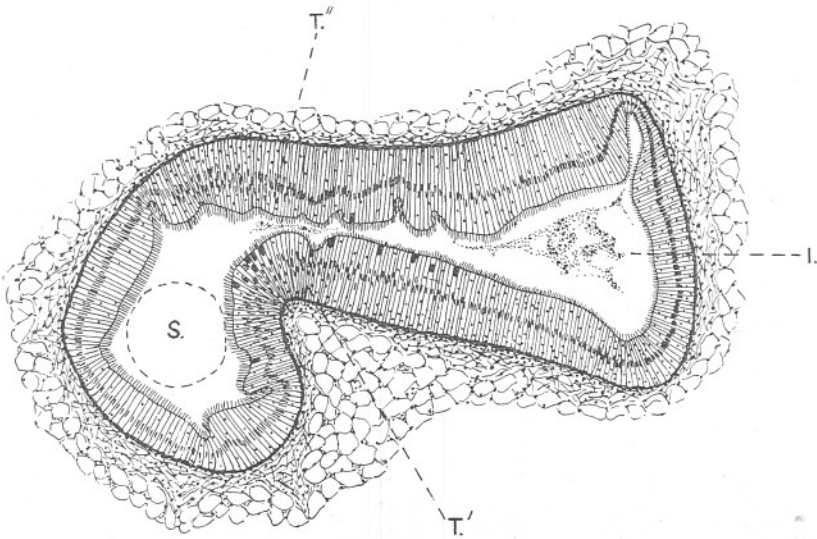


FIG. 12.—Transverse section style-sac and mid-gut. Iron hæmatoxylin and mucicarmine.  $\times 56$ . L., lumen of gut; T'', larger typhlosole; T''', smaller typhlosole; S., position of style in sac.

which appear in transverse sections near the base of the cells, and are filled with a very lightly staining, stringy substance. A similar state of affairs exists in *Ostrea*, the canals (I.E.C.) appearing to pass through and not between the cells, though it is difficult to be certain. Mackintosh has further shown that the larger canals extend longitudinally down the style-sac, and are connected with one another by smaller canals, and this appears also to be the case in *Ostrea*. Judging by their staining reactions, he is of the opinion that the contents are of the nature of connective tissue fibres, and considers that the function of the whole apparatus is to lend extra strength to the epithelium, which bears a considerable strain in revolving and pushing forward the style. The epithelium of the larger typhlosole (T', Fig. 12), which corresponds to the *minor* typhlosole

of Anodonta and Crepidula, or the *right* typhlosole of *Mya* as described by Edmondson (1920), consists of long, very narrow cells with cilia a

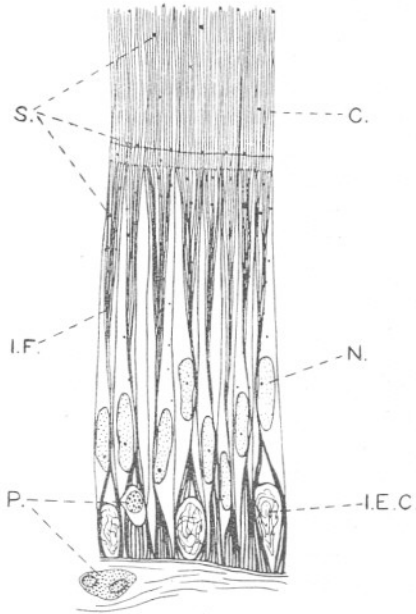


FIG. 13.—Transverse section epithelium of style-sac. Iron hæmatoxylin and acid fuchsin, secretion granules demonstrated by iron technique.  $\times 900$ . C., long cilia of epithelium; I.E.C., intra-epithelial canals; I.F., internal fibrillar apparatus; N., nuclei of epithelial cells; P., phagocytes; S., droplets of secretion containing iron in solution.

little shorter than those of the groove. Mucus glands are very numerous in this region and also occur in the other typhlosole (T'')—which is covered with long cells which gradually merge into the epithelium of the mid-gut—but never in the epithelium of the style-sac. The intra-epithelial canals also occur in the typhlosoles, but in decreasing numbers as these merge into the epithelium of the gut. Phagocytes occur everywhere, though they are not so numerous in the epithelium of the style-sac as they are in that of the typhlosoles, in which they are exceptionally numerous. The whole is surrounded by a few strands of muscle, the typhlosoles being filled in with vesicular connective tissue of the usual type.

It is very difficult to determine where and how the substance of the style is secreted.

List (1902), Nelson (1918),

Edmondson, and Mackintosh all think that it is secreted by the narrow cells of the minor typhlosole, but they have been unable to produce definite evidence. Gutheil describes and figures clear vesicular granules above the nuclei in the cells of the style-sac. In *Ostrea*, sections prepared for histological examination showed no sign of any secretion. It has been shown (1926) that the presence of minute droplets of secretion containing iron in solution can be demonstrated in the style-sac epithelium of *Mytilus edulis* four hours after a 0.5% solution of iron saccharate in sea-water has been injected by way of the foot. This method of demonstrating the presence of secretory cells has been employed with success for Crustacea, Insecta, and Gastropoda (for full details and literature see my papers (1924, 1926)). I have employed the same methods with *Ostrea*, injecting the same

solution by way of the adductor muscle, afterwards washing the animals so as to prevent any of the fluid entering the mouth. The style-sac was fixed (by the methods described in the section on assimilation) two, four, and six hours later, and sections prepared which were treated so as to demonstrate the presence of iron by the Prussian blue method, the sections being stained with alum carmine. In the style-sac of the animal which had been fixed four hours after injection, it was easily possible to distinguish fine blue granules in the cytoplasm above the nuclei and in the process of being passed into the lumen. The position of the granules is indicated in Fig. 13 (the internal fibrillæ do not appear after staining with alum carmine, but were drawn from sections stained with iron hæmatoxylin). There was *no trace of similar granules in the epithelium of the gut*, nor could I determine their presence with certainty in the narrow cells of the larger typhlosole though they are present in the cells of the other typhlosole so long as they retain the character of the style-sac epithelium. In view of the presence of these granules, it seems probable that Gutheil is correct, and that the substance of the style is secreted by the cells of the groove and not of the larger typhlosole, and that it is then revolved by the cilia of the style-sac, so that, as Edmondson has shown in his experiments on the regeneration of the style in *Mya*, it comes to lie against the larger typhlosole the cilia of which have a different function, as will be described in the section on ciliary currents.

The style during life lies in the groove of the style-sac, as indicated by the broken circle in Fig. 12. It is a gelatinous rod, whose structure has been described too often for further detailed description to be necessary. In the oyster the central core is very fluid and flows freely to and fro, the outer portion being firmer and consisting of co-axial layers. The style is seldom white, usually yellowish or brown, but the colour depends on the nature of the food, as in all cases where the style-sac is in communication with the mid-gut. Spirochætes of the genus *Cristispira* are very numerous, particularly in the outer layers, and are able to move about freely in the substance of the style.

#### (g) *Mid-Gut.*

This region (Fig. 14) is characterised in cross section by the possession of a large typhlosole with a groove down the centre. The cells of the epithelium are invariably ciliated, mucus glands are present, but not in large numbers, while there is a complete absence of muscle around the epithelium, which is bounded by a broad basement membrane. Phagocytes are very plentiful both in and around the epithelium, and in the lumen, where they are to be seen lying among the food

particles and mucus therein contained, particularly in the groove of the typhlosole.

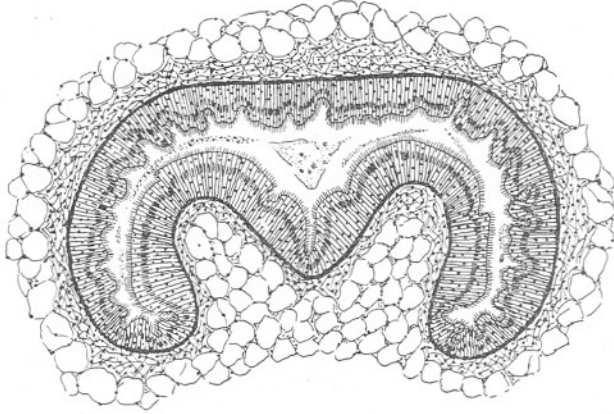


FIG. 14.—Transverse section mid-gut. Iron hæmatoxylin and acid fuchsin. Round dots in epithelium indicate phagocytes, dark masses indicate mucus glands.  $\times 56$ .

(h) *Rectum.*

The rectum (Fig. 15) is practically circular in cross section, the lumen being larger than that of the mid-gut. The typhlosole is here thrown into more prominent folds, and the central groove is practically obliterated, owing to the coming together of the two halves of the typhlo-

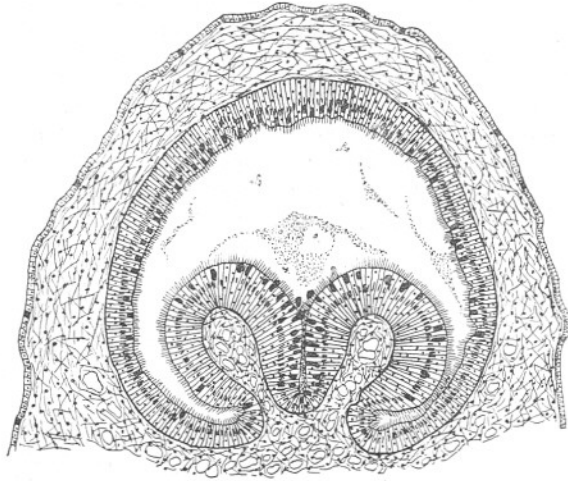


FIG. 15.—Transverse section rectum. Delafield's hæmatoxylin and erythrosin. Great numbers of mucus glands in epithelium.  $\times 56$ .

sole. Mucus cells are extremely numerous, more so than in any other region of the alimentary canal (the same is true for *Mya arenaria* (Yonge (1923))). All the other cells of the epithelium are ciliated, phagocytes are very plentiful everywhere; there is no surrounding muscle, while the basement membrane is thinner than that of the mid-gut. The surrounding connective tissue is more compact than in any other region of the gut. In the lumen are found food particles, or excreta, mucus, and phagocytes.

(i) *The Phagocytes.*

As will have been noted from the foregoing account, one of the most striking features about the gills, palps, and entire alimentary tract is the universal presence of wandering phagocytic cells. They are always easy to distinguish because their nuclei, unlike those of the epithelial cells which are oval and lightly staining, are small, spherical and contain a great number of fine granules of chromatin, which stain darkly with hæmatoxylin. The cytoplasm of the phagocytes stains lightly with erythrosin. No less than seven different types of blood cells in the Lamellibranchs have been distinguished by de Bruyne (1896), but it is doubtful how many of these represent different stages in the same type. In this paper no attempt is made to divide the phagocytes into different types, although further work on the subject is contemplated.

The presence of these phagocytes is characteristic of the Lamellibranchs (with the possible exception of the Septibranchs), and attention has been drawn to their presence by many workers, although their great importance in the physiology of digestion in these animals has not always been recognised. Lankester (1886, 1893) seems to have been the first to note the presence of the phagocytes in the gills of green oysters; de Bruyne (1893, 1896) gave a long account of the wandering of phagocytic cells into the epithelium of the gills and mantle in a number of Lamellibranchs; Herdman and Boyce (1899) gave a full account of their activities, especially in connection with green leucocytosis in the American oyster; List (1902) noted their presence in and around the gut in the Mytilidæ; Gutheil (1912) gives a full account of their occurrence throughout the alimentary tract of Anodonta and in the connective tissue and blood-vessels, and he also observed them dividing amitotically in the region of the gut; Matthias (1914) observed the presence of great numbers of phagocytes in the ventral portion of the stomach of *Arca barbata*; Orton (1923) has noted their great numbers and widespread distribution throughout the tissues, and particularly round the gut, of *Ostrea edulis*; I have myself (1923, 1926) observed and figured them in the gut of *Mya*, and in connection with the digestive diverticula in the same animal and in *Nucula*, *Cardium*, and *Teredo*.

In the oyster they are abundant everywhere, and appear to pass freely through the tissues. Fig. 16 represents a blood-vessel from the region of the œsophagus (it is an enlarged drawing of the smaller blood-vessel shown in Fig. 9). In the lumen can be seen a mass of blood cells,

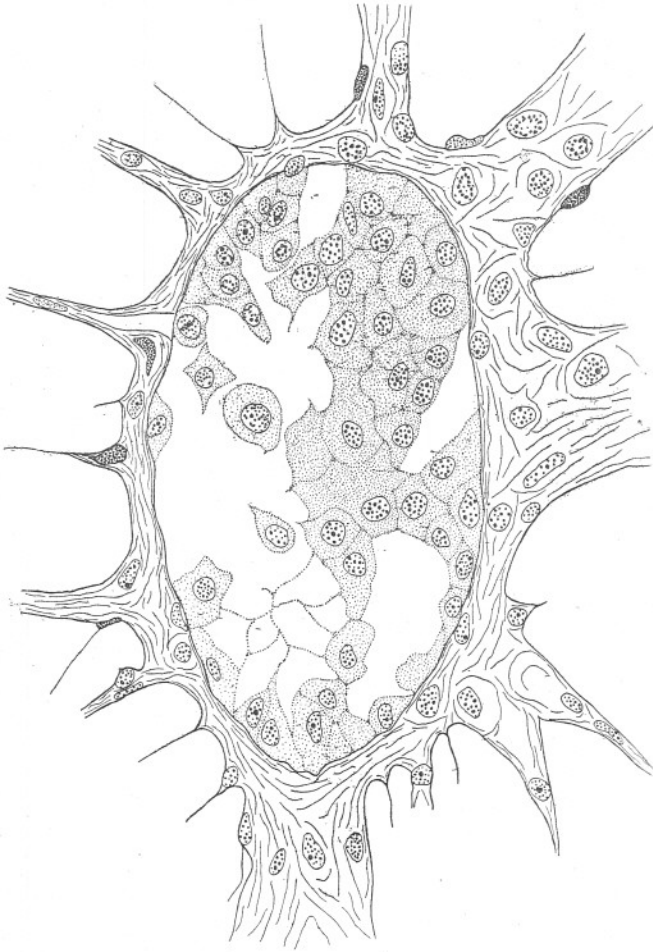


FIG. 16.—Transverse section through blood vessel near œsophagus.  
Many amœboid blood cells in lumen and passing through walls.  
Iron hæmatoxylin and acid fuchsin.  $\times 1200$ .

which have probably come together as a result of fixation. Similar cells can be seen passing through the wall of the vessel, though it is impossible in this region to distinguish more than the characteristic nuclei; the nuclei of the connective tissue which forms the wall of the blood-vessel are usually smaller, spindle-shaped, and stain intensely black. There can

be no doubt that the cells are amœboid, and have the power of wandering at will through the tissues and in and out of the lumens of the gut and of the blood-vessels. An account of the very important part they play in the assimilation of food will be given in the appropriate section.

### B. LARVAL OYSTERS.

The development and structure of the larvæ of *Ostrea edulis* have been described in detail by Horst (1886), while Stafford (1913) has given an

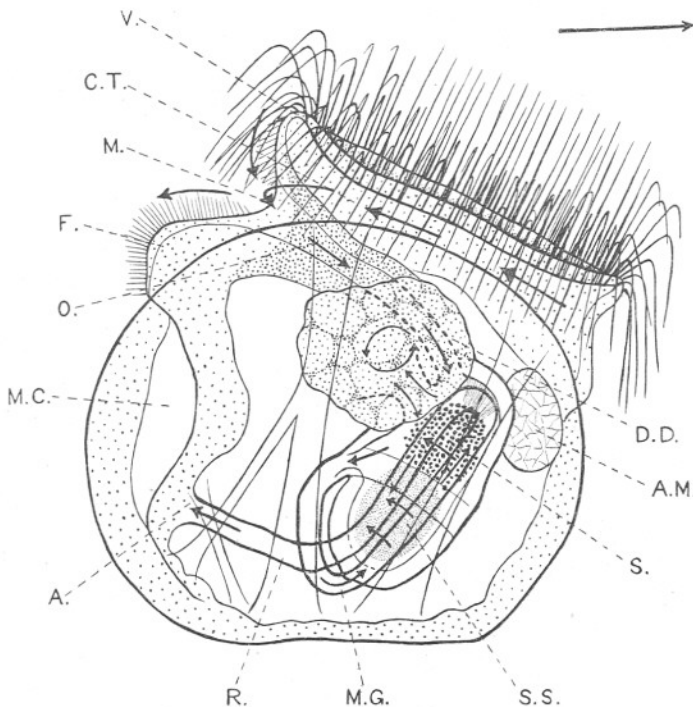


FIG. 17.—*Ostrea edulis*, veliger larva showing alimentary organs, drawn from life.  $\times 330$ . A., anus; A.M., adductor muscle; C.T., ciliated tract at base of velum; D.D., digestive diverticula; F., foot; M., mouth; M.C., mantle cavity; M.G., mid-gut; O., oesophagus; R., rectum; S., stomach; S.S., style-sac with contained style; V., velum. Large arrow above figure shows direction of movement, smaller arrows in figure show direction of food eurrents caused by cilia.

account of the developmental stages in the American oyster, *Ostrea virginica*, with a summary of the previous work on both species. Here it is necessary only to describe the alimentary organs of the veliger larvæ of *Ostrea edulis*. Fig. 17 represents such a larva, the dimensions of whose shell were  $0.2 \times 0.165$  mm., drawn from life so as to show the alimentary organs. The velum (V.), which is protruded—when retracted the organs

are tightly packed together and difficult to distinguish—is crowned with extremely long cilia, while there are smaller cilia round the base. The mouth (M.) lies behind the velum, between it and the rudiments of the foot (F.). It is a wide, funnel-shaped orifice which leads into an œsophagus (O.), whose thick walls are pigmented. This passes forwards and downwards in the middle line and opens into the head of the stomach (S.), an oval-shaped organ divided by an annular thickening of the wall from the style-sac (S.S.). On the posterior wall of the stomach open the two simple lobes of the digestive diverticula (D.D.), which are arranged symmetrically one on either side, their more ventral portions overlapping the œsophagus. They are darkly pigmented and even at this stage have the structure of the adult diverticula (see Fig. 42, p. 353). The style-sac contains the style which, though difficult to distinguish normally, can easily be seen if the larvæ are placed for several hours in a very dilute solution of brom thymol blue in sea-water when the substance of the style stains a light yellow, and can be seen revolving rapidly in the stomach. It may be a single oval mass (as represented in Fig. 17), or be composed of from two to four rounded masses. The mid-gut (M.G.) begins on the posterior side of the stomach at the line of its junction with the style-sac, and passes dorsally and then ventrally, describing a loop on the right side of the stomach before turning backwards as the rectum (R.), which ends in the anus (A.) on the dorsal side of the mantle cavity (M.C.). The whole of the gut is lined with large and very active cilia (cilia cannot be seen in sections of the digestive diverticula, but there is evidence that they are present in the living tissue), though in the figure the only cilia shown are the group of extremely large ones on the antero-ventral wall of the stomach. The external dimensions of the various parts of the gut are: œsophagus, 0.02 mm.; stomach, 0.046 mm.; mid-gut, 0.012 mm. Sections of the larvæ do not demonstrate any points in the structure of the alimentary system, which cannot be seen in an examination of the living larvæ.

### C. "SPAT" OYSTERS.

The structure of the food collecting and digestive organs in recently settled or "spat" oysters, though they quickly come to resemble those of the adult, show many interesting features. Unfortunately, 1925 proved a bad year for spat at Conway, and I was unable to obtain specimens in the act of settling, and so get stages in the metamorphosis from the larval to the adult structure, a process which takes place with great rapidity. The larvæ come to lie on the left valve cementing themselves firmly to the surface by means of a secretion from the byssus gland in the temporarily developed foot. A full account of the metamorphosis of the American oyster has been given by Stafford. Fig. 18 is a



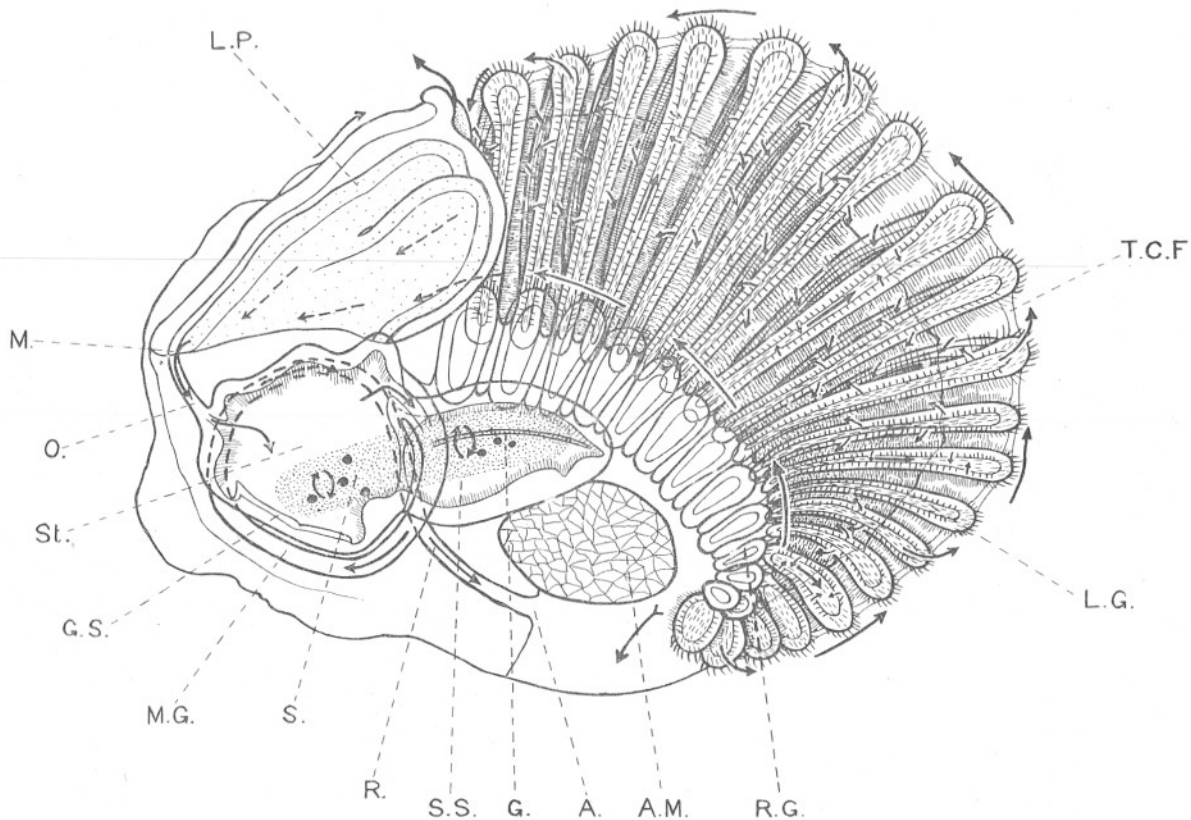


FIG. 18.—*Ostrea edulis*, "spat" shortly after settling, drawn from life after removal of shell (1.2 mm. deep), digestive diverticula not shown.  $\times 155$ . A., anus; A.M., adductor muscle; G., groove down style-sac; G.S., gastric shield; L.G., left gill; L.P.; labial palps; M., mouth; M.G., mid-gut; O., oesophagus; R., rectum; R.G., right gill; S., style; S.S., style-sac; St., stomach; T.C.F., transparent connections between free ends of filaments. Arrows indicate direction of ciliary currents and movement of style.

drawing of the smallest settled oyster which I obtained, the shell (not figured) measured 1.2 mm. from the umbo to the margin and the body, after removal from the shell and consequent contraction of the mantle folds (i.e. as shown in the figure),  $0.59 \times 0.66$  mm.

At this stage there is one simple gill on each side which represents the inner demibranch of the adult. There is a marked difference in the degree of development, the lower or left gill (L.G.) being much larger than the upper, right one (R.G.). Moreover, there are twenty filaments present on the left and only thirteen on the right. No firm lamella is formed, the filaments being united solely by thin strands of transparent tissue (T.C.F.) at their free extremities. The ascending and descending portions of the filaments are also unconnected. Lateral, frontal, and laterofrontal cilia are all to be distinguished on the filaments and also large cilia on the free extremities. The labial palps (L.P.) are much larger in proportion to the rest of the body than in the adult. The outer palps are completely united to form a hood which encloses the inner palps, which are united for about half their length. The mouth (M.) leads into a short œsophagus (O.), which opens into the large stomach (St.). Seen from the side this is a somewhat squarish organ with a well-developed gastric shield (G.S.) on the dorsal wall, against which bears the style (S.), which can readily be distinguished as a stout rod in which lie embedded diatoms and other particles. The wall of the stomach is covered with large cilia and so is that of the style-sac (S.S.), which forms a wide tubular diverticulum posterior to the stomach. Along one side of the sac is a narrow groove (G.). The stomach is surrounded by tubules of the digestive diverticula, though these have not been shown in Fig. 18 as they would have obscured the other organs; they are best studied in sections. On the postero-ventral side of the stomach is the opening of the mid-gut (M.G.), still quite distinct from the style-sac. As in the adult, the gut describes a circle round the left side of the stomach before passing dorsally and backwards as the rectum (R.); the anus (A.) opens into the exhalent chamber on the dorsal side of the adductor muscle (A.M.).

Transverse sections through a slightly larger specimen—the shell was 2 mm. across—are shown in Figs. 19 and 20. The former represents a section through the middle of the stomach. Owing to the direction of the cut, the section has passed transversely through a number of the gill filaments and the disparity in numbers between the filaments of the two sides is again demonstrated. The stomach lumen is practically filled with the style, the dorsal walls—with the exception of the extreme dorsal end—being covered with the gastric shield, the remainder of the wall being thickly ciliated. On the ventral side is the opening of the mid-gut (O.M.G.); the gut has been cut twice (M.G.) in its course round

the left side of the stomach. On the left wall of the stomach opens one of the ducts (O.D.) of the digestive diverticula, tubules of which (D.D.) are present on all sides of the stomach. Unlike the adult condition, however, the ducts have the same structure as the tubules—i.e. there are no

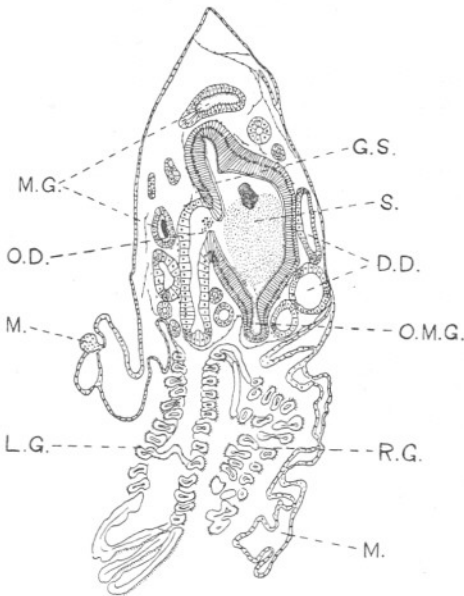


FIG. 19.—Transverse section through 2 mm. spat in region of stomach. Alum carmine.  $\times 80$ . D.D., digestive diverticula; G.S., gastric shield; L.G., left gill; M., mantle; M.G., mid-gut; O.D., opening of digestive diverticula into stomach; O.M.G., opening of mid-gut into stomach; R.G., right gill; S., style.

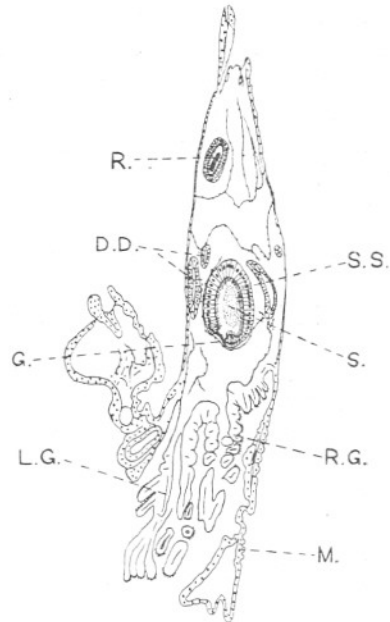


FIG. 20.—Section from same series as Fig. 19, more posterior.  $\times 80$ . G., groove in style-sac; R., rectum; S.S., style-sac. Other lettering as in Fig. 19.

ducts strictly speaking. The tubules are identical in structure with those of the adult, nests of darkly staining young cells lying between the more lightly staining, vacuolated and older cells. The dark masses in the stomach, the opening of the digestive diverticula, and in the lumen of the mid-gut are iron saccharate on which the animal had been fed one day before fixation.

Fig. 20 represents a section from the same series as Fig. 19, but more posteriorly, the section passing transversely through the style-sac (S.S.), the structure of which is shown clearly. The epithelium consists of large cells, very clearly demarcated, containing large oval nuclei and covered with thick, long cilia. On the ventral side lies the groove (G.), which is formed of extremely low ciliated cells bounded on each side by a group of tall, narrow cells. It is along the line of this groove that the union of

style-sac and mid-gut must later take place, and the areas of tall cells are, no doubt, identical with the cells of the future typhlosoles. The lumen of the sac is incompletely filled by the substance of the style (S.). In view of the fact that in *Ostrea* the style-sac and mid-gut are separate in the larvæ and early stages of the adult, it would be interesting to know whether in species such as *Mya arenaria* in which these structures are also separated in the adult, the separation represents a persistent embryonic condition or is secondary. The structure of the style-sac in the adult *Mya* would suggest that there has been union between the two and secondary separation. The other points of interest in Fig. 20 are the backward prolongations of the digestive diverticula (D.D.) on either side of the style-sac, and the rectum (R.) which here contains a mass of iron in the lumen.

### 3. FEEDING. THE COURSE OF THE CILIARY CURRENTS.

The course of the ciliary currents was followed in the intact tissues under the binocular microscope, and in small pieces of excised tissue under the high powers of the monocular microscope.

Carmines and carborundum powder of varying grades were employed to demonstrate the direction of the currents. The literature on this branch of the subject is extensive, and reference has been made to only the most important papers.

#### A. ADULT.

##### I. IN THE MANTLE CAVITY.

###### (a) *The Gills.*

Although in the oyster the mantle folds are not united except at the point of division between the inhalent and exhalent chambers, the food current is not drawn in along the whole of the inhalent chamber since, as described and figured by Orton (1912), the mantle folds are normally opposed except for the short distance on the ventral surface between the thick lines in Fig. 1. The ingoing current is caused by the action of the lateral cilia on the gills, a fact fully established by the work of Wallengren (1905) and Orton (1912). These cilia cause a strong current of water to pass between the gill filaments from the infrabranchial chamber into the suprabranchial chamber, which is in free communication with the exhalent chamber, as shown by the dotted arrows ventral to the adductor muscle in Fig. 1.

As a result of this current, any particles in suspension in the water will be carried into the inhalent chamber. As soon as the ingoing current has passed through the comparatively narrow inhalent aperture, its

speed will be reduced and the largest particles in suspension will drop on to the mantle folds. This may be called the first selection of particles. Smaller particles remaining in suspension will be deposited on the surface of the gill which serves as a very efficient filter, the water passing between the filaments while the particles are stopped by the action of the latero-

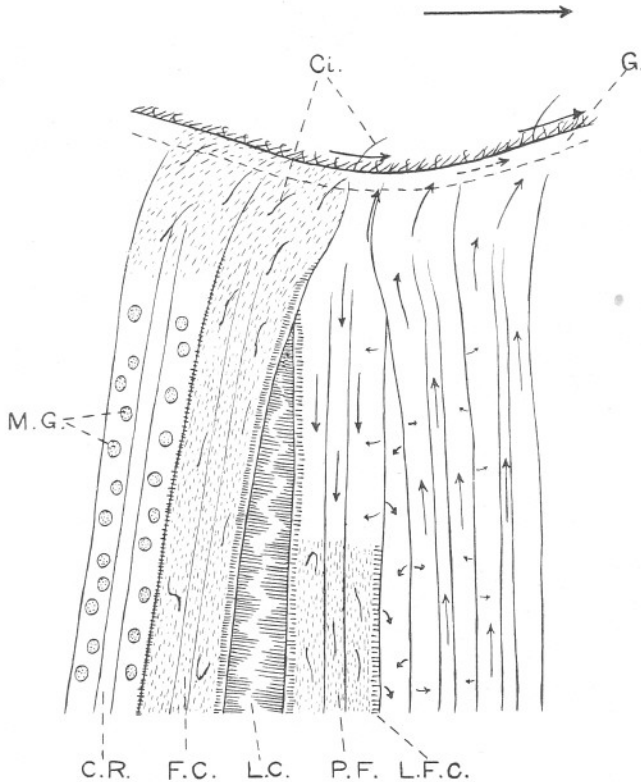


FIG. 21.—Semi-diagrammatic representation of five gill filaments and free margin of demibranch, several of the filaments being drawn apart to show cilia between.  $\times 375$ . C.R., chitinous supporting rod in filament; Ci., cirri; G., ciliated groove at free margin; F.C., frontal cilia; L.C., lateral cilia; L.F.C., laterofrontal cilia; M.G., mucus glands; P.F., principal filament. Arrow above figure indicates direction of mouth, smaller arrows in figure show direction of beat of cilia.

frontal cilia (Fig. 21, L.F.C.), which lie at the edges of the filaments, so that those of adjacent filaments interlock, while at the same time they beat across the surface of the filament, and so throw particles on to the frontal surface. Fig. 21, which represents several filaments pulled apart, shows these cilia very clearly and also the lateral cilia (L.C.) beneath. On account of their consecutive beat, these cilia appear to beat up the

side of one filament and down the side of the one opposite; but in reality the effective beat is inward into the interlamellar space which communicates with the suprabranchial chamber. The abfrontal cilia (see Fig. 6) no doubt assist in the formation of the current into the suprabranchial

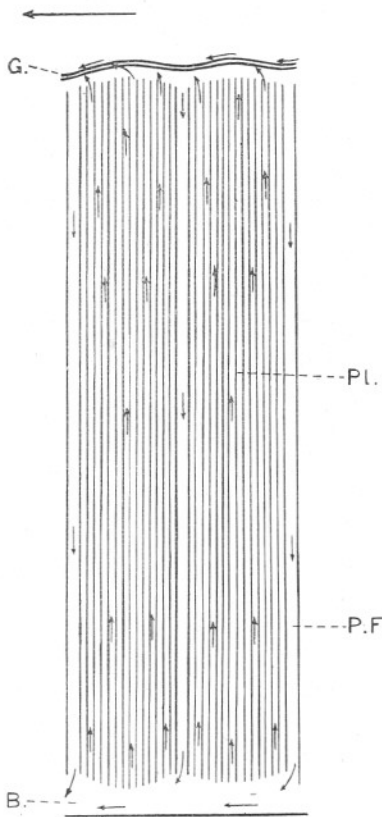


FIG. 22.—Diagrammatic figure of two plicae and three principal filaments of gill lamella.  $\times 10$ . B., base of gill, arrows show direction of current along gill axis; G., groove at free margin; P.F., principal filament; Pl., plica. Arrow above figure shows direction of mouth, smaller arrows direction of currents on face of gill.

chamber. The frontal cilia (F.C.) are smaller than the laterofrontals, but here and there are especially large cilia, or cirri (Ci.) as they have been called by Wallengren who regards them as characteristic of the ciliated tracts along which food is carried. The frontal cilia are concerned solely with the transport of the particles which drop upon them or are thrown upon them by the laterofrontals, the contact of solid particles immediately causing the mucus glands (M.G.), with which the surface of the filaments is covered, to secrete and so entangle the particles with mucus. The beat of the frontal cilia on the principal filaments in the bottom of the grooves between the plicae is the reverse of that on the other filaments. The former beat towards the base of the demibranch, the latter towards the free margin, as shown diagrammatically in Fig. 22. Conditions are the same on all four demibranchs.

Kellogg (1915) has described a similar state of affairs in *Pecten*, but this difference in the beat of the cilia on two kinds of filaments is not usual in Lamellibranchs. Particles carried to the free margin are passed into the ciliated groove (G., Figs. 21 and 22), which runs along it and in

which they are carried towards the palps and mouth, while particles taken to the base of the gills by the cilia of the principal filaments are also carried forward by the ciliated tracts present at the gill axes (see Figs. 1, 2, and 22).

It is the opinion of Kellogg that the arrangement of the ciliary currents

on the gills is an adaptation which ensures that feeding shall take place only when very limited numbers of particles are present in the ingoing currents. These will tend to fall into the grooves and be carried by the cilia on the principal filaments to the base of the gill, and so direct to the palps. But when the water is heavily laden with silt, particles will fall on all parts of the gill, become embedded in a common mass of mucus, and *all* be dragged to the free margin of the gill under the action of the frontal cilia on the summits of the plicæ. Individual particles or thin strings of mucus in the ciliated groove are carried to the palps, as we have seen, but large particles or heavily laden mucus strings tend to fall out of the groove on to the mantle surface, from which they are expelled. Kellogg's observations have been confirmed; small particles alone are passed to the base of the gills, and from thence direct to the palps (except when they occur in such numbers that they accumulate in masses, are caught by the cilia on the plicæ and carried across to the free margin of the gill), while material in the grooves may or may not reach the palp according to its size. His deductions from these observations will be discussed in the section on the palps. There are thus *two selective mechanisms on the gills*, one on the surface of the filaments and one on the free margin, both of which act by selecting the smaller particles or masses for passage to the palps and mouth and reject the larger.

There is also a certain degree of muscular activity in the gills which has often been overlooked. Kellogg, however, with his accustomed keenness of observation, has noted (p. 674) that in *Pecten* "much material causes the gill grooves . . . to open wide, and then to close with so sudden a contraction that material is thrown out of them. Often this violent bending of filaments, which results in spreading open and then constricting the grooves, occurs in about a second of time. The whole demibranch, also, may present a wavy surface, and sway, fanwise, towards the mantle and inwards." Similar movements have been observed in the gills of *Ostrea*, the opening and sudden contraction of the grooves being of frequent occurrence. It has already been shown that horizontal muscles are present in the interfilamentar connections and also vertical muscles in the principal filaments, and it is the contraction of these muscles, presumably, which causes the movements of the gills. These types of movement, sudden contractions and bending of the filaments, result in excess of material being transferred from the grooves to the crests of the plicæ or from the surface of the gills to that of the mantle, and form yet another sorting mechanism, though of a less exact nature than the ciliary ones.

*(b) The Palps.*

The junction between the gills and palps is shown in Fig. 2 (p. 299), and on examination of this figure it will be seen that particles from the free margin of the gills are transferred to the middle of the inner palp face, whereas those from the gill axes (i.e. the smaller particles) pass into the groove between the palps, although, on account of the slight development of cilia in the grooves particles are passed on to the lower folds, as shown in the figure. In accordance with the terminology suggested by Kellogg, this groove will be called the lateral oral groove (L.G.), while the groove which leads from it to the mouth between the non-folded region of the palps will be known as the proximal oral groove (P.G.). There is a third, distal oral groove in Lamellibranchs in which the outer demibranchs do not extend so far forward as the inner, but this is practically absent in the oyster. Material passed on to the folded surface of the palps comes under the action of the long and very active cilia with which it is covered which, as will be described in detail shortly, conduct it either to the upper margin (U.M.) or across the palp folds in the direction of the mouth. There is a powerful backwardly directed current along the upper margin of the palps in which particles are carried back to a point marked X in Fig. 2 within a short distance of the tip, where it meets a forwardly directed current from the tip. A vortex is created in which particles are rolled into masses which, on attaining a certain size, fall off on to the mantle, directly from the left palps and by way of them in the case of the right palps.

The direction of the ciliary currents on the outer smooth surfaces of the palps is shown in Fig. 23. The cilia being shorter the currents are much weaker than those of the inner surfaces, and particles are carried diagonally backwards, those near the upper edge being transported into the upper marginal current on the inner face—and so finally rejected at X—while those in the central or lower areas are passed to the distal edge round which they are carried on to the folded surface, there to be either taken to the mouth or rejected as the case may be.

Passing now to a detailed examination of the inner, folded surface; Fig. 24 represents such a surface, the arrows showing the direction of the currents. The number of folds varies according to the age of the animal but this in no way affects their action. Each fold (as shown in the cross section in Fig. 7, p. 304) bends towards the mouth, overlapping to some extent the fold immediately proximal to it. In the middle of the exposed distal surface of each fold runs the longitudinal groove. There are no less than five distinct ciliated tracts on the exposed surface of the folds, details of which are shown in Fig. 25. Beginning with the most distal; there is a tract of cilia (*a*) which beats downward into the furrow between



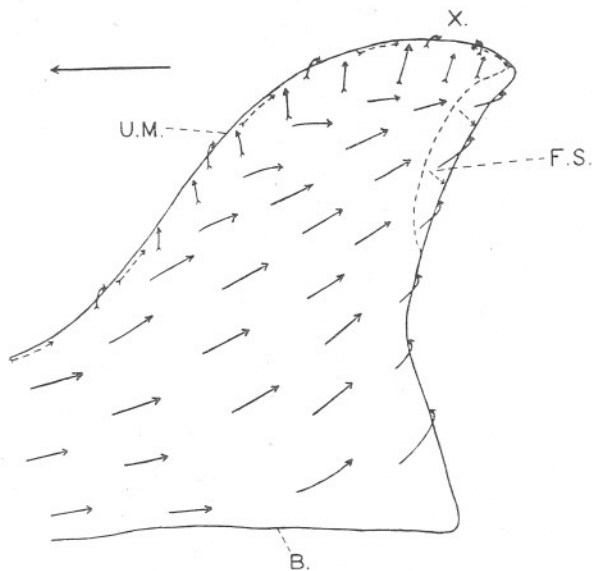


FIG. 23.—Smooth, outer surface of palp showing direction of ciliary currents.  $\times 10$ . B., base of palp; F.S., folded, inner surface frequently exposed along dotted line; U.M., upper margin of palp; X., point where material rejected from palp. Large arrow shows direction of mouth.

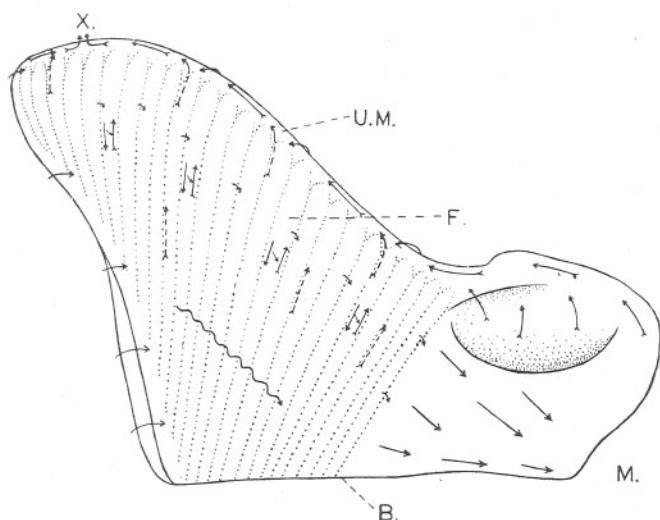


FIG. 24.—Folded, inner surface of palp showing direction of ciliary currents.  $\times 10$ . B., base of palp; F., flds; M., position of mouth; U.M., upper margin; X., point where material rejected.

adjoining folds, but this region is largely covered by the adjacent overlapping fold; next there is a narrow tract (*b*) within the longitudinal groove whose cilia beat in the direction of the base of the palp; then a narrow tract (*c*) which directs particles diagonally across the palp towards the mouth; then a tract (*d*) in which particles are carried to the upper margin of the palp; and, finally, a tract of cilia (*e*) whose beat is directed at right angles to the line of the folds and towards the mouth. Moreover, in the furrows between the folds are tracts of cilia which

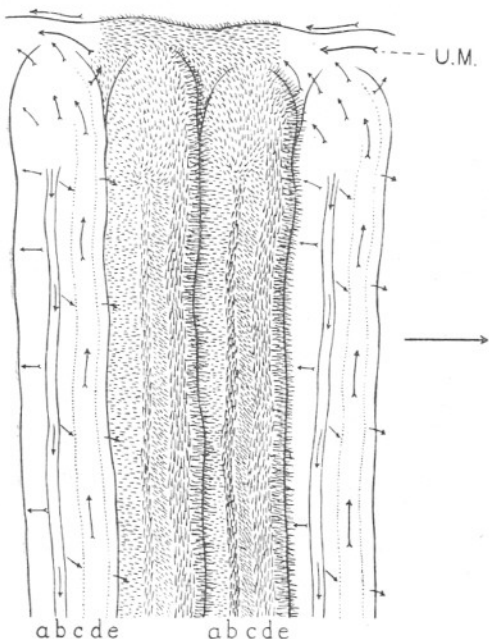


FIG. 25.—Enlarged semidiagrammatic figure of palp folds, showing direction of ciliary currents.  $\times 60$ . a, b, c, d, e, tracts of cilia on exposed surface of folds; U.M., upper margin. Large arrow shows direction of mouth.

lead, invariably, towards the upper margin (Siebert (1913) states that the cilia in the furrows in *Anodonta* beat in the opposite direction, but his findings have not been confirmed by other workers). The cilia on the proximal surface of the folds which are not exposed are difficult to observe, but appear in the main to beat into the furrows, and so are concerned with the rejection of particles. The path taken by particles carried on to the folded surface is the resultant of the action upon them of these different tracts of cilia, the interaction of which is very difficult to investigate. There can be no doubt, however, that the whole forms an extraordinarily

efficient sorting mechanism. As the folds lie normally the effect of the five exposed ciliated tracts will be that light particles, such as carmine grains, are carried diagonally across the palp face, the individual particles being thrown lightly from fold to fold, largely by the action of the large cilia of tract (*e*), and following the somewhat serpentine course indicated by the long undulating arrow in Fig. 24. Large particles, such as carborundum, or smaller particles imbedded together in long mucus strings (which amounts to rather the same thing since the larger the particles the more mucus is secreted) tend to be drawn down within the furrows under the action of the cilia

in tract (*a*), and so, finally, expelled. So strong are the cilia in the furrows that if any portion of a mucus string comes under their influence, the whole string is carried to the upper margin. It is difficult to be certain as to the true state of affairs when the opposing palps, as always happens in life, are working in conjunction; experimentally it is only possible to examine the working of one exposed surface. Though gravity may have some influence on the selection of particles or strings of mucus lying on the under surface, that, of course, cannot influence particles attached to the upper surface, though the larger particles will tend to fall on to the under surface. Undoubtedly the mucus is of great importance; the larger the strings or masses, the more they will come into direct contact with the cilia, and the more chance that some portion will be drawn into the furrows and removed. Kellogg thinks there is a muscular retraction of the proximal edge of the folds concerned which causes particles to fall into the furrows; but I have never seen any such action in the oyster.

There is, however, an immediate muscular reaction when large particles are placed on the palp surface, the entire palp curling back in the manner shown in the left palps in Fig. 2. This is caused, no doubt, by the thick layer of longitudinal muscle which lies beneath the epithelium of the smooth surface (see Fig. 8, p. 305). As a result, the inner surface becomes convex and the folds are drawn apart, thus exposing the furrows into which the majority of the material on the palp will fall and be removed. The palps may occasionally curl inwards—by a contraction of the muscles under the epithelium of the folded surface—so that the folds are puckered and spaces left through which particles can drop into the furrows. These muscular responses are of the first importance in the functioning of the palps as was first noted by Wallengren, who originally described the different tracts of cilia on the palp folds, but who considered that, as a result of their *individual* contraction, different tracts were brought into play, and in this way the direction taken by the particles was controlled. Kellogg has described a curling over of the ventral (upper) margin of the palp in *Schizotherus*, whereby material is drawn off directly from the palp surface on to the outgoing marginal tract. Such a movement has not been observed in the palps of *Ostrea*, which are not free from one another, as in *Schizotherus*, but are attached for a quarter of their length. Allen (1914, 1921) follows Wallengren's account, and ascribes selection to the action of the different tracts of cilia brought into play during different states of contraction and relaxation in the folds; he claims further (apparently owing to a faulty reading of Wallengren's paper) that by this means cilia are brought into action which led particles in the *opposite* direction to that of the mouth. Grave (1916) considers that there is a reversal of the beat of certain cilia (pointing to the similar conditions

described by Parker for the sea anemone *Metridium*). I have never observed any sign of a reversal of cilia in the palps of any Lamellibranch, nor have Grave's opinions been supported by any more recent worker. Cobb (1918) has shown that the palps of *Anodonta* respond by muscular contractions to a variety of stimuli; mechanical, electrical, chemical, photic, and thermal. He also found that the detached palp reacts as effectively as one attached to the body (a fact also noted during these experiments on the palps of *Ostrea*), showing that "the palp contains within itself the neuromuscular organization necessary for all the responses described . . . and . . . possesses an autonomy even more complete than that of the vertebrate heart and comparable with what is shown by the tentacle of an actinian." Churchill (1924) has observed the muscular curling of the palps under normal feeding conditions in young, transparent fresh-water mussels. Nelson (1924) watched the feeding of spat oysters under similar conditions, and states that the rejection of particles is due to "reflex erection of the ridges of the palps which brings into play groups of cilia which beat away from the mouth." He does not state whether this erection is due to a general curling back of the palp surface, but describes the palps at this stage as consisting of "isolated filaments which are capable of independent movement." (This is certainly not the case in the spat of *Ostrea edulis*, where, as we have seen, the palps are more united than in the adult.) Nelson placed spat in 1/20 sat. magnesium sulphate, and states that the filaments of the palps lost the power to erect, with the result that masses of material passed over to the mouth and eventually blocked it. He concludes that feeding in the oyster is accomplished "through the delicate co-ordination of nervous, muscular, ciliary, and mucus secreting elements in which mechanical sorting of the materials plays the most important part"; an admirable summary of the state of affairs.

Herdman and Boyce (1899) have described in the oyster the presence of thin bands of muscle arising one on each side at the surface of the mantle near the anterior edge of the visceral mass and being inserted at the junction of the gills and palps, and have identified them with the protractor pedis muscle of other Lamellibranchs. They suggest that in the oyster they may function by pulling apart the inner and outer palps and gill demibranchs of each side, and so allowing food particles to reach the mouth more easily. It is difficult experimentally to prove this or to see its necessity since other Lamellibranchs function perfectly well without it, but, as they state, the opening of the shell will, by separating the points of attachment of the two muscles, cause "the opening up of the food avenues."

A considerable controversy has arisen around the question whether in the Lamellibranchs the selection of particles for swallowing is qualita-

tive or quantitative. The view that there is a definite selection of particles, having food value has been upheld chiefly by Lotsy (1893), Allen (l.c.) and Grave (1916), but the majority of workers, including List (1902), Kellogg (l.c.), Yonge (1923), Nelson (1924), and Churchill (1924) have failed to find anything other than a purely mechanical selection having as its object the reduction of the quantity of matter passed to the mouth, large particles or many small particles embedded in mucus being rejected and smaller particles or mucus masses passed on to the mouth quite irrespective of their food value. This appears to be confirmed by examinations of stomach contents by Savage (1925), and the majority of previous investigators whose work he summarises. Churchill found that when fresh-water mussels were kept in suspensions of mixed organic and inorganic matter they took in a sample of everything small enough to enter the mouth. In some cases where the inorganic particles are the larger there may be—incidentally—a selection of particles having food value (as Nelson thinks is the case in spat oysters). Nothing but a purely mechanical or quantitative selection has been found in the oyster, and this has, I think, been made clear in the preceding account, but attention may again be drawn to the series of selective mechanisms which exist.\*

1. The heaviest particles in the ingoing current drop on to the mantle and never reach the gills.

2. The smaller particles on the gills are carried by the cilia on the principal filaments to the base, the larger ones passing to the free margin.

3. The largest particles or mucus masses fall out of the groove on the free margin on to the mantle.

4. Muscular contractions in the gills cause material to be transferred from the grooves on to the crests of the plicæ, and from the surface of the gills to that of the mantle.

5. Material passed on to the inner face of the palps from the free margin of the gills is there most rigorously sorted, larger particles or masses being rejected and only the smallest crossing towards the mouth.

6. The smaller particles from the gill axes which pass into the lateral oral groove are not so rigorously sorted, since the folds at the base of the palps are lower and closer together and the effect of the curling back of the palp surface is much slighter.

Experiments with four grades of carborundum powder demonstrated the efficiency of these sorting mechanisms very clearly. The particles

\* Lamellibranchs, such as *Syndosmya*, *Tellina* or *Gari*, which have long, free siphons and are classified by Hunt (1925) as deposit feeders, may exercise a certain qualitative selection by means of the inhalent siphon which is fringed with sensory tentacles. Possibly the Protobranchs may do the same, though to a less extent, by means of the extrusible appendages of the outer pair of labial palps. In both cases, however, qualitative selection, if it occurs, takes place *outside* the mantle cavity.

were in all cases dropped lightly on the middle and posterior regions of the gills, and with the following results (the coarser grades being taken first):—

Grade 120. Particles passed to the free margin of gill; fall on to mantle before they can reach the palps.

Grade 220. All carried to free margin; majority drop off, a few of the thinner mucus strings reach palps, there *all* rejected.

Grade F. All carried to free margin; comparatively little falls off, *all* rejected by palps.

Grade FF. All carried to free margin; very little falls off, great majority rejected by palps, a very little carried to mouth.

As already stated, Kellogg is of the opinion that Lamellibranchs can only feed in waters that are comparatively clear. This has been denied by Grave (1916), Nelson (1921)—who supplies the *definite evidence* that oysters can feed in waters bearing as high as 0.4 grams dry weight of suspended matter per litre—and Churchill (1924), and, I think, with reason. Certainly the more particles carried into the mantle cavity, the more wholesale is the rejection, but, as Churchill has shown for fresh-water mussels, although the main surface of the palp is concerned with the rejection of the large masses passed on from the marginal grooves on the demibranchs, the finer matter which enters by way of the lateral oral groove will find its way to the mouth. I place a similar interpretation on my experiments, although in the oyster the selective mechanisms, both on palps and gills, are more efficient than in the majority of Lamellibranchs—a correlation, no doubt, with the sessile mode of life and consequent danger of silting up—in which the frontal cilia of all gill filaments usually beat in the same direction, and food can pass to the mouth by way of the distal and lateral oral grooves without ever coming into contact with the folds on the palps; *Mya arenaria* is a good example of this type of Lamellibranch. Even in the oyster, however, I have observed the passage of a certain amount of material from the gills to the palps under all conditions approaching the normal (if the gills are absolutely covered with a mass of particles these are all removed, whatever their individual size, but this would never occur in nature); carmine grains are carried to the mouth along the lateral oral groove while the rest of the palp surface is ridding itself of carborundum. It is not impossible, however, that Lamellibranchs, and especially such highly specialised species as the oyster, feed with the *maximum of efficiency* in waters that are comparatively clear; they can, moreover, by frequent closing of the shell valves clear the water to some extent and prevent any too great accumulation of sediment within the inhalent chamber.

It remains to describe the passage of particles from proximal folds to the mouth, the course of which is shown in Fig. 2 (p. 299). The cilia in this region are short, and matter accumulates along a line parallel to the last fold and then passes slowly in the direction of the mouth. There is never an accumulation of material about the mouth, particles which do not pass deep in the proximal oral groove being caught by ciliated tracts which lead them upwards and then either distally on to the upper margin of the inner palp face, or over on to the outer face of the inner palps. In either case they are rejected finally. Material which reaches the mouth passes slowly into the œsophagus.

(c) *Removal of Material from the Mantle Surface.*

Material dropped on to the surface of the mantle is carried away by the ciliary tracts shown in Fig. 1 (p. 298). There are ciliary tracts in the anterior region of the mantle cavity which carry particles back to a point about the middle of the inhalent aperture where they accumulate, since the thickened ridge which bounds the mantle is not ciliated. The masses thus formed are expelled from time to time by sudden contractions of the valves. Nelson (1921) by very ingenious experiments has shown that ejections of this nature are most numerous when the water in which oysters are living is at its maximum turbidity. Along the posterior region of the inhalent chamber, and also in the exhalent chamber, matter passes directly to the edge of the mantle, there to accumulate and be expelled in the manner described.

## II. IN THE GUT.

Mucus laden with particles passes slowly along the œsophagus, particularly in the grooves at the extremities of the lumen, as described by Vonk (1924), into the stomach. Fig. 26 represents the stomach and œsophagus opened out for examination of the ciliary currents. As a result of the position of the cut, the ventral wall of the stomach lies on the left side of the figure. The relative positions of the various parts is seen in Figs. 3 and 4, and by reference to these and to Fig. 26 some idea of the physiology of the stomach will be gained.

Particles entering the stomach will tend to pass into the food sorting cæcum (F.C.) either directly or by way of the ciliated tract (C.T.), which leads into it from the floor of the stomach. The cæcum, as shown in Figs. 3 and 4, is a long grooved diverticulum, which extends backwards under the floor of the stomach. Fig. 27 represents it opened out, the broken lines indicating the line of the cut. The ingoing ciliated tract (C.T.) is situated on a ridge, which passes down the right side of the

cæcum to the extremity and then along the left side, terminating abruptly at a point level with the opening of the cæcum. Particles are carried very rapidly along this tract until they reach the point X, when they may be carried in either of two ways. If they are heavy (e.g. carborundum) they are rolled round and fall over into the groove on the right of the ridge, as shown by the arrows and dark mass in Fig. 27. The cilia in this groove conduct particles slowly round in the reverse direction to the cilia on the ridge and out of the cæcum into the deep ventral groove (G., Figs. 3, 4, 26, and 27), which runs across the floor of the stomach

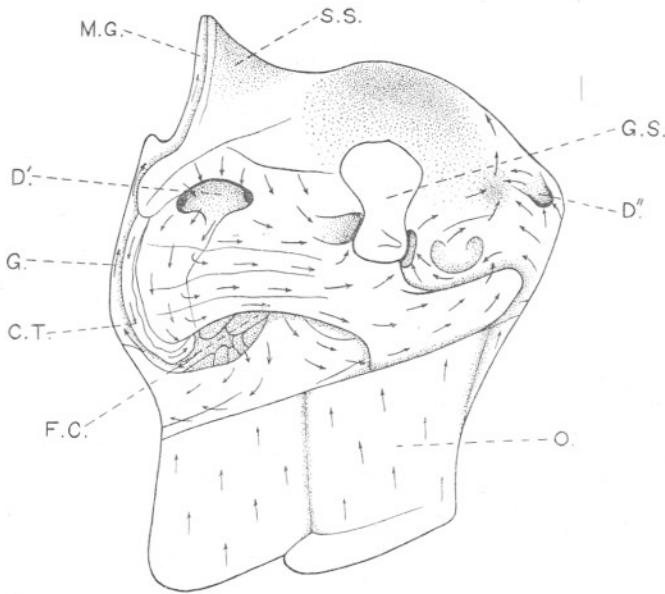


FIG. 26.—Stomach opened out to show direction of ciliary currents, cut made along right ventral surface.  $\times 4$ . C.T., ciliated tract leading into food sorting cæcum; D', larger, left duct of digestive diverticula; D'', smaller, right duct; F.C., food sorting cæcum; G., ventral groove leading from food sorting cæcum to mid-gut; G.S., gastric shield; M.G., mid-gut; O., oesophagus; S.S., style-sac.

and is continued into the mid-gut, as shown in Fig. 26. On the other hand, light particles, such as carmine grains, not embedded in great masses of mucus, pass along the ciliated tract past the point X, and are wafted out of the left side of the cæcum in the direction indicated by the dotted arrows in Fig. 27, being ultimately carried to the region of the gastric shield, as shown in Fig. 26. The cæcum constitutes yet another sorting mechanism wherein larger particles are separated from smaller ones without any apparent regard to their food value, the larger ones being removed from the stomach by way of the mid-gut, and the smaller being retained in the stomach and passed towards the head of the style.



Nelson (1918) has described a similar cæcum in *Modiolus*, and I have given an account of a food sorting area in the stomach of *Mya* (1923). In both of these cases the mechanism is more complicated than in *Ostrea*, presumably because in the latter the selective powers of the gills and palps are better developed, a fact testified to by the smaller size of the particles in the stomach of the oyster. The remaining cilia on the wall of the stomach either conduct particles towards the gastric shield, where they come under the action of the style, or else towards the ducts of the digestive diverticula (D' and D'', Figs. 3, 4, and 26). These ducts are bounded on the one side by an overhanging wall over which the cilia beat *into* the opening, but on the other the opening lies flush with the epithelium of the stomach, and the cilia on this side lead particles *away* from the opening. There is thus a mechanism whereby particles enter

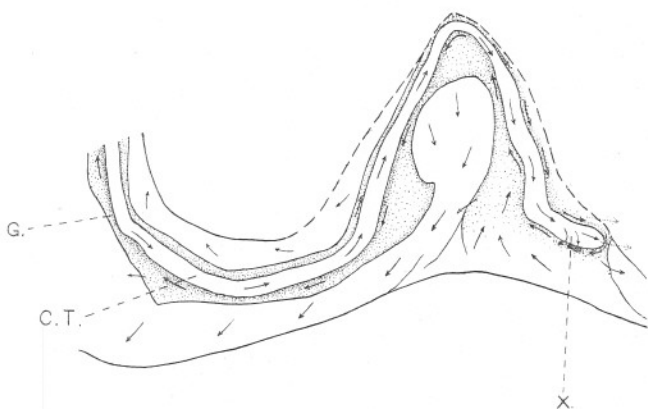


FIG. 27.—Food sorting cæcum opened up along dorsal surface, line of cut shown by broken lines. X S. C.T., ciliated tract leading into cæcum; G., ventral groove leading out; X., point where large and small particles separated, larger passing into groove and smaller following line of dotted arrows to gastric shield region.

the ducts on the one side and leave them on the other, which is essential if a circulation is to be maintained within the diverticula.

The main agent concerned with the movement of material within the stomach, however, is the style. It was originally suggested by List that the style was probably revolved by the action of the cilia in the style-sac, but it was left to Nelson (1918), as the result of careful opening of the stomach, to observe the actual revolution of the style in the stomach of *Anodonta* and *Modiolus*. He found that the maximum number of revolutions per minute in *Anodonta* at 11.5° C. was 11, and in *Modiolus* at 25° C. was 13. In both cases the direction of the movement was clockwise when viewed from the anterior end of the animal. It has never been possible to observe the movement in the adult oyster, in which

the stomach is less exposed than in the majority of Lamellibranchs, but I have seen it in the larvæ and spat (as will be described later), while Churchill (1924) has observed it in young, transparent fresh-water mussels, and there can be no doubt, if only from the nature of the ciliation of the style-sac, that the style revolves in all Lamellibranchs. The head of the style is continually being dissolved away, and in the sticky mass become embedded particles and strings of mucus, and it may well be, as Orton (1923) has suggested, that material is in some cases drawn into the stomach as a result of the mucus strings being wound round the "shredded revolving head of the style like a capstan." As we have seen, muscle is practically absent from the gut of the oyster (in common with all Lamellibranchs except the Septibranchs), the place of peristalsis being taken by ciliary activity. One of the principal functions of the style, as Nelson (1918, 1925) has pointed out, is that of stirring and mixing particles in the stomach, an operation performed in many other animals by peristaltic contractions. Although, of necessity, the different activities of the stomach have been described separately, in life, of course, they are all proceeding simultaneously, food entering from the œsophagus, being sorted in the cæcum, being revolved in the head of the style, passing in and out of the ducts of the digestive diverticula, and being removed by way of the mid-gut all at the same time.

The disposition of the ciliary currents in the style-sac and first part of the mid-gut is shown in Fig. 28. Particles enter the mid-gut from the stomach by way of the ventral groove (G.) and pass quickly down it along the channels at the base of the typhlosoles (T' and T''). The cilia on the typhlosoles beat diagonally away from the stomach and into the gut. The groove of the style-sac is ridged transversely, and the beat

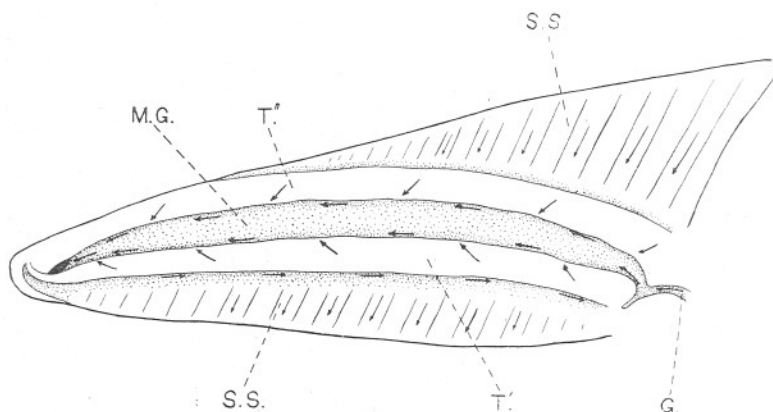


FIG. 28.—Style-sac opened along middle of surface so as to expose ciliary currents in style-sac and mid-gut.  $\times 4$ . G., ventral groove in stomach; M.G., mid-gut; S.S., style-sac; T', larger typhlosole; T'', smaller typhlosole.

of its large cilia is very difficult to determine ; particles placed upon them appear to get caught between the cilia, as they can be seen trembling with the movement of the cilia but are moved extremely slowly. Such movement as there is, however, is from right to left (looking at the style-sac from the stomach), i.e. in the direction which would revolve the style in a clockwise direction when seen from the same standpoint. There is a tract of cilia beating in the direction of the stomach on the side of the larger typhlosole (T'), which is somewhat easier to demonstrate and is accompanied by the production of great quantities of mucus. The same disposition of cilia is found in the style-sac of *Mya* (Yonge (1923), and there is the same difficulty in demonstrating the direction of their beat. The style, presumably, is revolved by the first set of cilia and pushed forward by the other, and the difficulty of demonstrating the direction of beat may be due to the cilia not being adapted for the movement of small particles which rest lightly on them, but for the movement of a firm body which is pressed firmly against them. In spite of the presence of the ciliary currents leading from the style-sac into the gut over the face of the typhlosoles, a certain amount of material which has been carried down the gut gets caught up in the substance of the style, wrapped round it spirally as it revolves, and carried back to the stomach. This "retrieving function" of the style has been commented on by Nelson (1918, 1925), Allen (1921), and Orton (1923)—the latter having figured the spiral bands of mucus laden food strings wrapped round the style of the oyster—and is probably of some importance in Lamellibranchs, such as *Ostrea*, in which the style-sac and mid-gut are in communication.

Throughout the remainder of the gut material is passed slowly backwards under the influence of the cilia, and is finally ejected by way of the anus into the exhalent chamber (see Fig. 1); where it comes under the influence of the exhalent currents and of the cilia on the mantle surface and is removed from between the shell valves.

## B. THE LARVÆ.

The arrows in Fig. 17 (p. 317) indicate the direction of the ciliary currents in the larvæ. If larvæ are placed in suspensions of carmine, indian ink or other fine particles these are thrown by the large cilia of the velum on to the ciliated tract (C.T.), which runs round the base of the velum, where they are embedded in mucus and carried back to the mouth. The velum, therefore, acts both as a swimming and as a food collecting organ. Not all the material passes into the mouth, any surplus being carried off by the cilia on the lobe which represents the rudiments of the foot (F.), so that a larva swimming through a thick suspension leaves behind it a trail of particles embedded in a long string of mucus. If

the suspension is excessively thick the larvæ become embarrassed in their movements and turn repeatedly over and over in their efforts to free the cilia from the mass of particles which they automatically collect.

Material is passed into the stomach by way of the cesophagus and is there rotated, as indicated by the arrows in the figure, by the cluster of large cilia at the anterior end and also by the action of the smaller cilia which line the wall of the stomach. At the same time, the style, in which particles become embedded, is rotating in the style-sac. The direction of rotation appears to be constant for the individual, but to vary in the different larvæ, in some clockwise, in others anti-clockwise. Nelson, on the other hand, states that in the larvæ of *Ostrea virginica* the movement is always clockwise. The speed varies greatly, as few as 36 and as many as 90 revolutions per minute having been counted. Partly as a result of this movement, particles are thrown into the cavity of the digestive diverticula in which they can be seen in active movement, as shown by the curved arrows in Fig. 17. We may therefore assume that cilia are present in the cavity of the diverticula. Particles leave the stomach by the mid-gut, and are carried rapidly through the remainder of the alimentary tract and ejected at the anus. When larvæ were kept in heavy suspensions of carmine the gut became packed with a continuous red stream, and under these conditions the action of the cilia was practically inhibited, owing to the pressure of the enclosed mass; movement became exceedingly slow and a certain amount of peristaltic activity was observed. The gut in the larvæ is, it may be noted, unlike that of the adult, free from the surrounding tissue. The fæces are rolled into a ball by the action of the cilia in the mantle cavity, and are then expelled.

### C. THE SPAT.

All mechanisms concerned with the rejection of surplus matter are well developed in the spat. Thus the cilia of the mantle are larger and more active than those in the adult, while the palps are relatively of immense size and reject the great majority of particles passed on to them from the gills, which collect them in the usual manner as indicated by the arrows in Fig. 18 (p. 319). Fig. 29 shows the palps seen from the free end after they had been dissected out of a 1 mm. spat, the two inner palps (I.P.) being enclosed by the hood formed by the outer palps (O.P.). It is extremely difficult at this early stage in their development to distinguish the direction of all the ciliary currents on these small organs. No folds are present, although there is a groove on each of the palps along which the cilia beat in the direction of the free extremities of the palps, as in the furrows of the fully developed palp, and there are ciliated tracts leading in the same direction along the outer edges of the outer palps

Other tracts lead across the palps, but, after careful examination, only one tract—on the right inner palp—was distinguished in which the cilia beat towards the base. When particles are placed upon the palps only a minute proportion succeed in passing between the inner and outer palps of each side and so reaching the mouth, the majority are passed

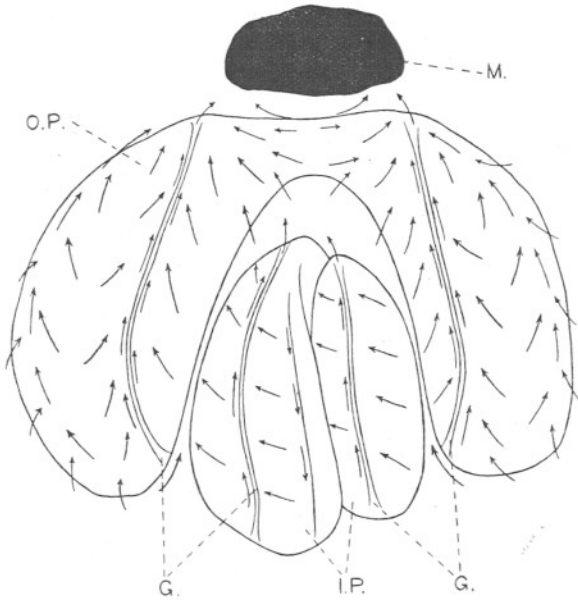


FIG. 29.—Palps from 1 mm. spat dissected out and drawn from free end, i.e. looking in direction of mouth.  $\times 275$ . G., grooves on inner faces of palps; I.P., inner palps; M., mass of rejected material accumulated in depression at junction of outer palps; O.P., outer palp.

rapidly to the depression at the junction of the outer palps (see Figs. 18 and 29), where they are rolled into a large ball (M.), which finally falls on to the mantle surface. In experiments on whole spat the palps were found to be very active and to respond readily to stimulation by drawing back and upwards, probably by so doing exposing to their maximum the outgoing tracts.

Particles which succeed in reaching the mouth are passed rapidly through the oesophagus into the stomach (see Fig. 18). There they are whirled round by the cilia and in the head of the style, which in spat of this size can be seen in rapid movement through the wall of the stomach. It consists of a somewhat irregular rod which bears against the gastric shield and revolves in a clockwise direction when viewed from the anterior, the speed observed varying between 60 and 70 revolutions per minute.

Embedded in its substance, especially near the head, are particles of all sizes, the largest observed being specimens of the spherical diatom *Coscinodiscus* having a diameter of about  $23\mu$ . These are shown in Fig. 18. It is difficult to observe the disposition and interior of the digestive diverticula in the spat, but movements were seen within them, while peristaltic movements were well marked in the mid-gut, especially in the region nearest the stomach, particles being moved by this means and by ciliary activity through the mid-gut and rectum and expelled at the anus, which opens in the exhalent chamber.

The great development of the organs concerned with the removal of surplus matter in the spat can readily be understood, since a small sessile organism of this nature is in constant danger of being smothered by falling silt unless this can speedily be removed from within the mantle cavity. The presence of peristaltic activity in the gut of both larvæ and spat seems to indicate that the absence of peristalsis in the adult is not primitive. Peristalsis is usually well developed in the Gastropods, which may represent, in this respect at any rate, the more primitive condition.

#### 4. ASSIMILATION.

##### I. LITERATURE AND METHODS.

Although in animals such as the Vertebrates, digestion precedes assimilation, this is the case only to a very limited extent in the Lamellibranchs, since food is ingested directly both by the phagocytes and the tubules of the digestive diverticula and digestion then takes place *intracellularly*. The only extracellular enzymes in the gut of Lamellibranchs are those set free by the dissolution of the head of the style. In this paper, therefore, an account of the process of assimilation is given before passing to a consideration of the digestive enzymes.

I have recently had occasion (1926) to review the literature dealing with assimilation in the digestive diverticula, so that it is unnecessary to discuss the matter in detail. As a result of a study of previous work and as the outcome of my own experiments, the conclusion was reached that the digestive diverticula are organs of absorption and of intracellular digestion, since they absorb soluble matter such as iron sulphate (Carazzi (1897) on *Ostrea*) or iron saccharate (Yonge (1926) on *Nucula*, *Mya*, and *Teredo*), and ingest solid matter such as Indian ink (List (1902) on *Mytilus*, Potts (1923) on *Teredo*, Vonk (1924) on *Ostrea*), carmine (List on *Mytilus*) or blood corpuscles from dogfish (Yonge (1926) on *Teredo*). Sigerfoos (1908) and Potts have further shown that the *Teredinidæ* ingest wood fragments in digestive diverticula specialised for that purpose. Matter which may be of use to the animal such as iron (Carazzi (1897),

Yonge (1926)) or blood corpuscles (Yonge) is carried away in amoebocytes, but useless material such as Indian ink is rejected into the lumen of the diverticula shortly after ingestion and carried out of the body (List, Vonk).

Carazzi (1896, 1897) claims that iron is absorbed by the epithelial cells of the gills, palps, and oesophagus, and then carried to the digestive diverticula by way of the amoebocytes. Since, however, he kept his oysters for *four months* in a solution of iron sulphate in sea water so that they had time to become thoroughly permeated with iron, an entirely contrary interpretation may be placed on his results, namely, that the phagocytes become loaded with iron either from the digestive diverticula or by direct ingestion and then carry it to all the tissues. In the same way the Marenin from *Navicula* is taken in by the phagocytes and carried to all free surfaces of the oyster, so that the gills, palps, and gut are coloured green (for full details on the subject of green oysters see Lankester (1886), Herdman and Boyce (1899), Mitchell and Barney (1916), and other papers quoted by them). Guthel (1912) found fat globules in the ciliated epithelium of the gut in *Anodonta*, except in the style-sac and the region of the gastric shield. He therefore concluded that the epithelium could absorb, although he carried out no controlled experiments by first starving and then feeding animals, but argued from the presence of fat in the epithelium of fresh animals. Churchill (1915, 1916) states that after keeping fresh-water mussels in very dilute solutions of soap, egg albumen, or starch stained with iodine, these substances are taken by the outer epithelial cells of the body, mantle, foot, gills, and palps, some being carried away by blood cells, which he observed on occasion between the epithelial cells. His experiments were in most cases kept up for a considerable number of days, and, though the same objection cannot be made to them as to those of Carazzi, since he plugged the mouth of many of his animals thus preventing the passage of food to the digestive diverticula, yet the presence of these substances in the epithelial cells did not necessarily mean that they had been absorbed directly by them. Canegallo (1924) kept *Unio* in soap solution and found that this was absorbed to a far greater extent by the epithelium of the intestine than of the gills, the fat being carried away by leucocytes. Ranson (1926) considers that molluscs can absorb organic matter in solution through any free surface as well as by the intestine. In my own work on *Nucula*, *Cardium*, *Mya*, and *Teredo* (1926) the absorption of iron saccharate was never observed except in the tubules of the digestive diverticula.

Large particles are taken in directly by phagocytes, to the universal presence of which attention has already been drawn. De Bruyne (1893, 1896) considered that they ingested damaged or degenerating epithelial

cells particularly in the gills. Gutheil (1912) has described and figured in Anodonta the passage of phagocytes laden with material from between the epithelial cells through the basement membrane and into the connective tissue and blood vessels. Cuénot (1914) has observed phagocytosis in the blood cells of Lamellibranchs, as a result of the injection of Chinese ink. Canegallo (1924), by injecting olive oil stained with Sudan III into *Unio*, found that this was quickly taken in by leucocytes. I have described (1923) the presence of great numbers of these phagocytes in the gut of *Mya*, and shown that they may contain large, hard particles such as sand grains or the tests of diatoms, often in such numbers that the gut is coloured grey. After feeding *Cardium* and *Mya* with blood corpuscles of dogfish it was found (1926) that the corpuscles were ingested by phagocytes lying between the epithelial cells in the stomach and ducts of the digestive diverticula. They were carried into the connective tissue and there digested. Reference has already been made in this paper to the presence of phagocytes in all parts, and to the fact that they often contain green or brown granules, the colour being due to a pigment investigated by MacMunn (1900) and named by him *Enterochlorophyll*, on account of its close relationship to chlorophyll.

Oysters after appropriate periods of starvation in water which had been passed through filter cloth were fed with suspensions in sea-water of iron saccharate, of blood corpuscles from dogfish, of a pure culture of the diatom *Nitzschia*, and with an emulsion of olive oil stained with Nile blue sulphate. No experiments were carried out with Indian ink, the recent and conclusive work of Vonk (1924) on *Ostrea* having rendered them unnecessary. Animals were removed, and the various regions of the alimentary system fixed, at varying intervals after the commencement of feeding. After feeding with iron saccharate tissues were fixed in equal parts of 5% of ammonium sulphide in 95% alcohol and Bouin's fluid, sections being later treated for ten minutes with a 10% solution in water of potassium ferrocyanide, and then for a few minutes in a very dilute solution of HCl in order to demonstrate the presence of iron by the Prussian blue reaction, the sections being stained with alum carmine. After feeding with the other substances tissues were fixed either in Flemming's strong fluid or in Bouin. If by the former method, sections were stained with a saturated solution of safranin in 70% alcohol and later differentiated in clove oil saturated with orange G, the osmicated fat by this method standing out very clearly against the red nuclei and yellow cytoplasm. After fixation in Bouin sections were stained with Delafield's hæmatoxylin and erythrosin.

Larvæ and spat were fed on carmine and iron saccharate, and fixed respectively in Bouin and in the ammonium sulphide-alcohol Bouin mixture.



## II. FEEDING EXPERIMENTS ON ADULTS.

(a) *With Iron Saccharate.*

This substance was taken in readily, a thick brown suspension in sea-water being rapidly cleared. Oysters opened within a few hours of feeding were found to have the stomach full of a brown mass of iron saccharate, a great deal of which was embedded in the head of the style. After sectioning, iron was found in the lumen of all parts of the gut up to two days after feeding (it was present in very great quantity in the rectum six hours after feeding), sometimes it was seen ingested in phagocytes lying free in the lumen and—very rarely—being carried by them in between the cells of the epithelium. *But in the epithelium of neither the gills nor the palps nor any part of the gut except the tubules of the digestive diverticula was it absorbed.* In the cells of the latter it is ingested freely, slight traces being present six hours after feeding, a maximum being reached from one to two days after feeding, very slight traces being found after three days and none after any longer period.

The typical conditions of absorption are shown in Fig. 30, which represents two cells from a digestive tubule two days after feeding with iron saccharate. The free surface of the cells is very irregular, and iron is taken into large vacuoles and accumulates in the form of irregularly round or oval masses. It is *never* absorbed in the form of fine granules or in a diffuse condition. Exactly the same conditions were found in *Nucula*, *Mya*, and *Teredo*, while List and Vonk found that Indian ink was taken into vacuoles in the diverticula of *Mytilus* and *Ostrea* in a similar manner. It is impossible, as Vonk has also noted, to distinguish a bounding membrane around the masses which appear to lie

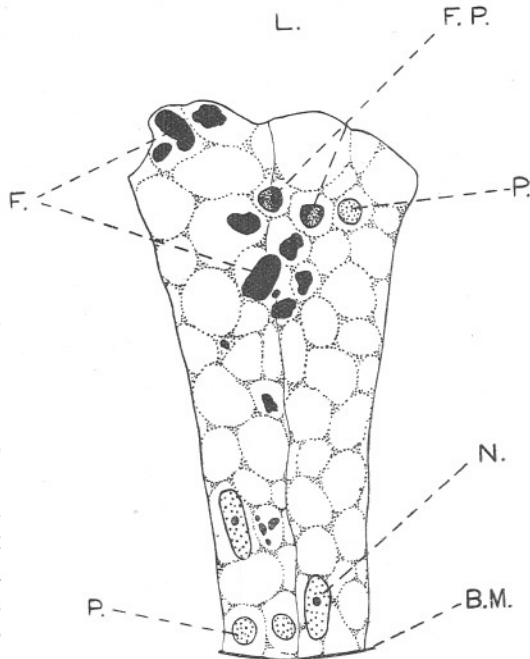


FIG. 30.—Two cells of digestive diverticula two days after feeding with iron saccharate. Iron technique.  $\times 1350$ . B.M., basement membrane; F., masses of ingested iron; F.P., iron partly ingested; L., lumen; N., nucleus of cell; P., phagocytes.

free in cavities in the protoplasm, but the manner in which the iron first forms a ring (Fig. 30, F.P.) the interior of which is later filled up, seems to point to the presence of such a membrane. This manner of absorption is unlike that found in animals, such as Arthropods and Annelids, in which digestion is extracellular and only the soluble products of digestion are absorbed, and would appear to be an indication of the presence of intracellular digestion, as would also the irregular outline of the free surface of the cells.



FIG. 31.—Phagocyte from connective tissue between digestive diverticula and gonad, four days after feeding with iron saccharate. Nucleus at one end, fine pseudopodium at the other, body of phagocyte full of iron. Iron technique.  $\times 2400$ .

Phagocytes are almost invariably present in the cells in which iron is being ingested, they may or may not be present in the others. Usually only their nuclei (as in Fig. 30) can be distinguished, although occasionally the outline of the cells can be seen, particularly when they are full of iron which they have collected from the cells. Four days after feeding, though no trace of iron was found in the tubules, many phagocytes full of minute granules of iron were to be seen immediately round the tubules, in the connective tissue (e.g. the phagocyte in Fig. 31), and occasionally in the blood vessels and in the gonads (Carazzi considered that the final destination of the iron was the gonad). There was never any indication of rejection of iron into the lumen, in the

manner described by List and Vonk after feeding with Indian ink.

#### (b) *With Blood Corpuscles.*

A quantity of fresh blood from a dogfish was added to the filtered sea-water in a large bell-jar in which a number of oysters had been starved. The corpuscles were taken in rapidly by the oysters. The stomach contents of an oyster opened three hours after the blood had been added consisted exclusively of mucus, blood corpuscles of dogfish, phagocytes of the oyster and a few ciliates and spirochætes. The style was intact. The great majority of the corpuscles were in perfect condition, the outline being smooth and the nucleus quite clear, some were lying free in the stomach, some entangled in mucus or in the substance of the head of the style, while others were in *process of being ingested by phagocytes*. This process is shown in Figs. 32 and 33; in the former a

phagocyte is beginning to engulf a corpuscle, while in the latter one has already been ingested, and is lying in a vacuole within the phagocyte which is beginning to engulf a second corpuscle. The stomach contents of an animal opened six hours after feeding were a diffuse red, probably owing to the presence of free hæmoglobin, very few corpuscles could be distinguished, and there were many fewer phagocytes free in the lumen, those present being often large, and containing the remnants of many corpuscles in an advanced state of digestion. Many other phagocytes contained no ingested

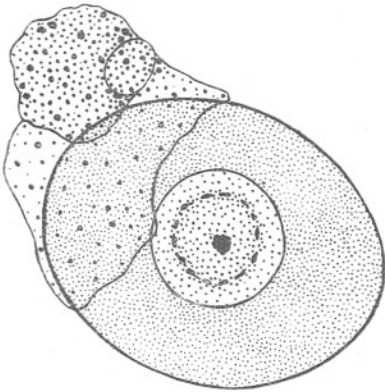


FIG. 32.—Phagocyte in stomach ingesting blood corpuscle three hours after feeding with blood from dogfish. Drawn from life.  $\times 2400$ .

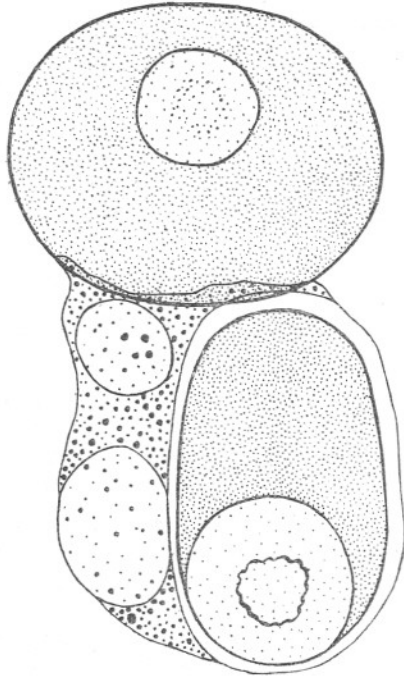


FIG. 33.—As above, phagocyte with one corpuscle ingested and beginning to ingest a second. Drawn from life.  $\times 2400$ .

matter. Phagocytes with ingested corpuscles could be distinguished passing into the epithelium of the stomach. Eighteen hours after feeding there was only a slight redness in the stomach, which contained very few corpuscles, the outline of which was often serrated.

As a result of sectioning it was found that corpuscles are taken in between the cells of the epithelium in all regions by the phagocytes. This was most rare in the rectum, few corpuscles passing so far in the lumen, and in the digestive diverticula where, although corpuscles were occasionally found ingested in the cells, they only appeared to be digested with consequent formation of fat globules in the presence of phagocytes. Ingestion by phagocytes took place to a small extent in the gills, palps and cesophagus, to a greater extent in the mid-gut, but the principal centre of phagocytic activity was found to be the stomach and ducts of

the digestive diverticula, immense numbers of phagocytes making their appearance in the lumen and epithelium in these regions. The course of phagocytic ingestion of corpuscles was followed in detail in the stomach epithelium.

It is very difficult to remove all traces of fat from oysters, even after prolonged starvation. Feeding experiments were carried out on animals which had been starved four and eleven weeks, and in both cases, though the quantity of fat was substantially less than in fresh animals, there

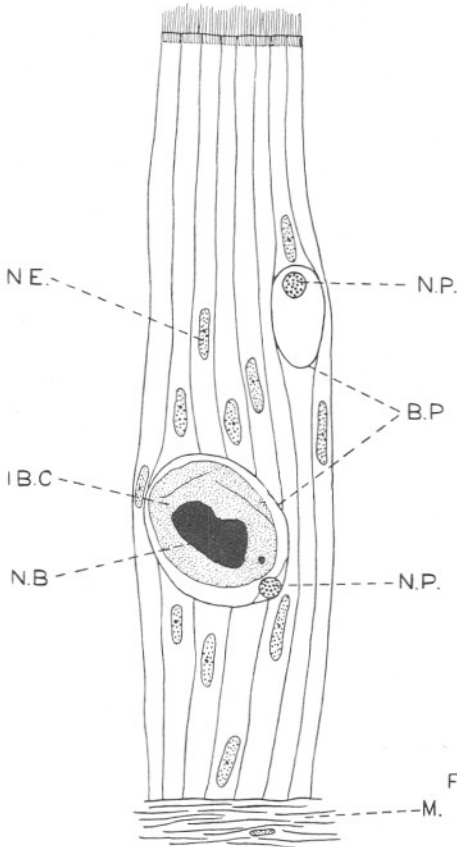


FIG. 34.—Stomach epithelium, showing ingestion of a blood corpuscle in a phagocyte three hours after feeding. Oyster starved for eleven weeks previously. Fixed strong Flemming, stained safranin and orange G.  $\times 900$ . B.P., boundary of phagocyte; I.B.C., ingested blood corpuscle; M., muscle; N.B., nucleus of blood corpuscle; N.E., nucleus of epithelial cell; N.P., nucleus of phagocyte.

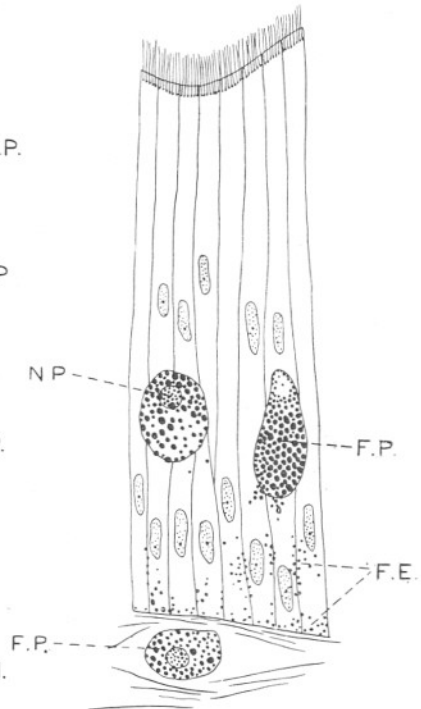


FIG. 35.—Stomach epithelium, ingestion and digestion of blood corpuscles in phagocytes six hours after feeding. Oyster starved for eleven weeks previously. Fixed Flemming, stained safranin and orange G.  $\times 900$ . F.E., fat in epithelium; F.P., fat in phagocytes; N.P., nucleus of phagocyte.

was still a certain amount present, especially in the connective tissue, in the phagocytes and very occasionally at the base of the epithelial cells. Nevertheless, the difference in the fat content after feeding with blood corpuscles was quite unmistakable. Fig. 34 shows the ingestion

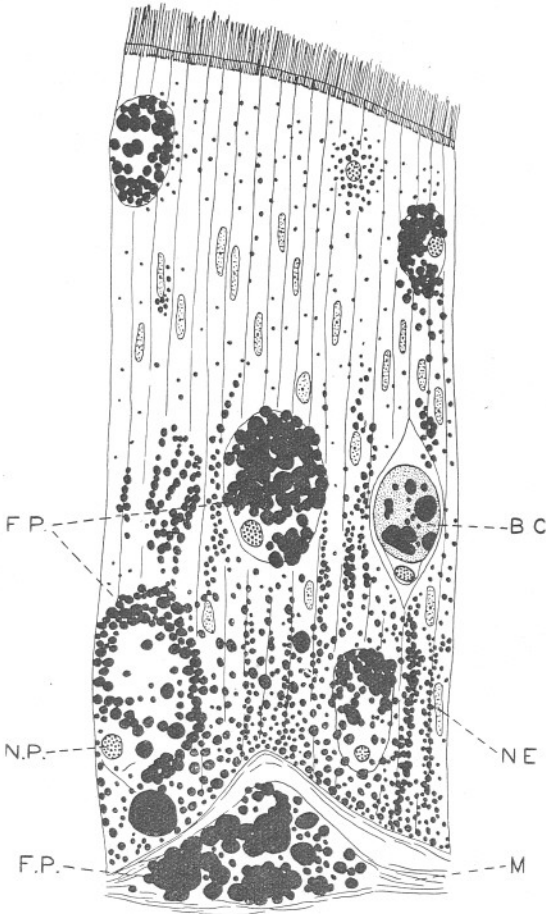


FIG. 36.—As above, twelve hours after feeding. Oyster starved for four weeks previously. Technique as before.  $\times 900$ . B.C., corpuscle in early stages of digestion; F.P., fat in phagocytes; M., muscle; N.E., nucleus of epithelial cell; N.P., nucleus of phagocyte.

of a corpuscle three hours after the commencement of feeding. The corpuscle lies in a phagocyte in the middle of the epithelium, the outline is uneven and the nucleus is degenerating. At this period there are few phagocytes in the epithelium, and there is practically no sign of fat in either the ingested corpuscles or in the epithelial cells. The condition

six hours after feeding is shown in Fig. 35. There are now many times more phagocytes in the epithelium than at the preceding period; the ingested corpuscles are difficult to distinguish, consisting now, as a result of the digestive action of the phagocytes, of a mass of fat globules. Two phagocytes laden with fat are shown in the epithelium in the figure and a third is seen passing through the circular muscle into the connective tissue. Fat is being passed from the phagocytes to the cells, in which it is accumulating near the base. Fig. 36 represents a portion of the epithelium twelve hours after feeding. Owing to the mass of fat globules it is difficult to distinguish the outline of the epithelial cells, of the phagocytes, and of the nuclei. Phagocytes are present in the epithelium in vast numbers; in the figure one phagocyte contains a corpuscle (B.C.) in the early stages of digestion, the other phagocytes containing great numbers of fat globules, which in some cases probably represent the products of digestion of two or more corpuscles. Fat has passed from the phagocytes to the cells, the whole epithelium appearing black with osmicated fat. Fat is also being transported into the connective tissue, though the nuclei and outline of the phagocyte or phagocytes which carry it are obscured by fat. There is a sharp distinction between the ciliated epithelium of the stomach and the epithelium of the gastric shield area, the latter containing no fat and few phagocytes, which never contain ingested corpuscles.

One day after feeding conditions were much the same as twelve hours after, but there was a still greater accumulation of fat in the phagocytes, in the epithelium, and in the vesicular connective tissue cells in which it is deposited by the phagocytes. It is never carried by them into the blood vessels. Conditions remain substantially the same two and three days after feeding. At the end of the latter period great quantities of fat were observed at the base of the cells of the gastric shield area, phagocytes were rare, and only at the base of the cells, and it is they, presumably, which carry the fat here from the ciliated epithelium, since there is no indication that phagocytes can pass through the substance of the gastric shield. Five days after feeding, though the connective tissue contains great quantities of fat, there are only slight traces in the epithelium, while the few phagocytes which contain fat are in most cases either at the base of the epithelium or in the basement membrane. Very similar conditions prevail six days after feeding, while after eight days there is a complete absence of fat in both epithelium and phagocytes in the ciliated areas of the stomach, but in the region of the gastric shield there is still abundance of fat near the base of the cells, though phagocytes are very rarely seen. There is a reduction in the amount of fat in the vesicular connective tissue. In an oyster fixed eleven days after feeding, however, there was no trace of fat in the epithelium of the gastric shield

area, but a little, especially in the phagocytes, in the ciliated epithelium; there was a considerable reduction in the quantity of fat in the vesicular connective tissue. Fourteen days after feeding there was a considerable quantity of fat in the ciliated epithelium, phagocytes, and connective tissue. These individual variations are due probably to variations in the number of corpuscles taken in, not all the oysters having opened their shell valves for the same period, and to the degree to which they had been deprived of fat by starvation, which depends on the amount present previous to starvation. The same degree of phagocytic activity was observed in the ducts of the digestive diverticula; similar activity in the ducts has been described and figured (1926) for *Mya arenaria*.

(c) *With Olive Oil.*

An emulsion of olive oil stained red with Nile blue sulphate was injected by means of a pipette either into the mouth or mantle cavity of oysters, parts of whose shells had been drilled away so as to permit of the operation. The shell valves were then clamped, and the oysters placed in water with the drilled valve undermost, so as to prevent the light oil from floating out. The animals were examined after one day.

When opened the epithelium of the mantle, free surface of the visceral mass, gills, palps, and stomach was in many cases found to be coloured *blue* in patches, while under the microscope the gill mucus was seen to be full of phagocytes, most of them ingesting oil. Fig. 37 represents such a phagocyte. The large vacuoles (shown empty in the figure) are filled with unchanged oil, but the smaller vacuoles (black in the figure) are vividly blue, owing to the transformation of the neutral fat into fatty acids by the lipase of the phagocyte, with a consequent change in the colour of the stain. The use of Nile blue sulphate provides a very graphic demonstration of the digestion of fats. The blue colour of the epithelia was found to be due, when the tissues were cleared in glycerine, to the presence of great numbers of phagocytes, all laden with fat and fatty acids, both on the surface, in the epithelium, and in the deeper layers. The condition in the gill is shown in Fig. 38, in which

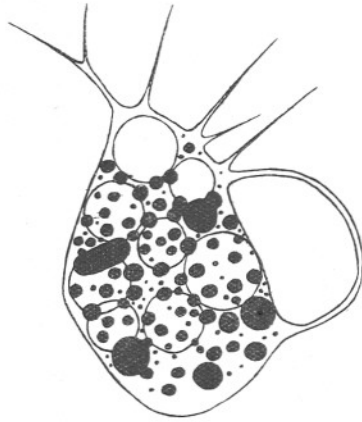


FIG. 37.—Phagocyte from gill mucus after ingestion of olive oil stained with Nile blue sulphate. Large vacuoles full of red oil, small vacuoles (shown black) containing blue stained fatty acid. Drawn from life.  $\times 2700$ .

phagocytes are seen lying free on the surface of the gill, while others are passing into the tissue, and there are a line of them down the centre

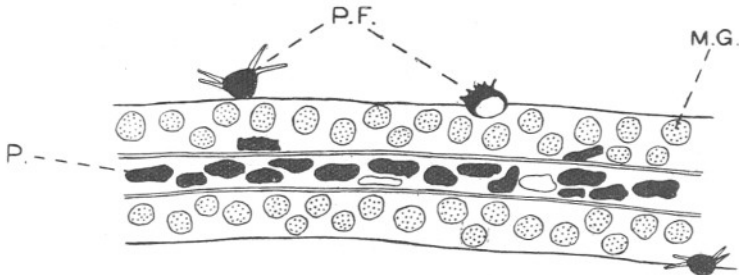


FIG. 38.—Portion of gill one day after feeding with olive oil stained Nile blue sulphate, cleared with glycerine.  $\times 480$ . M.G., mucus glands; P., phagocytes with fat in centre of gill filament, others passing in; P.F., phagocytes free on surface of gill, full of fat and fatty acids.

of the filament in the blood channel, most of them containing fat. Similar conditions prevail in the mantle, as represented in Fig. 39, the centre of the mantle tentacle being deep blue with darker spots denoting

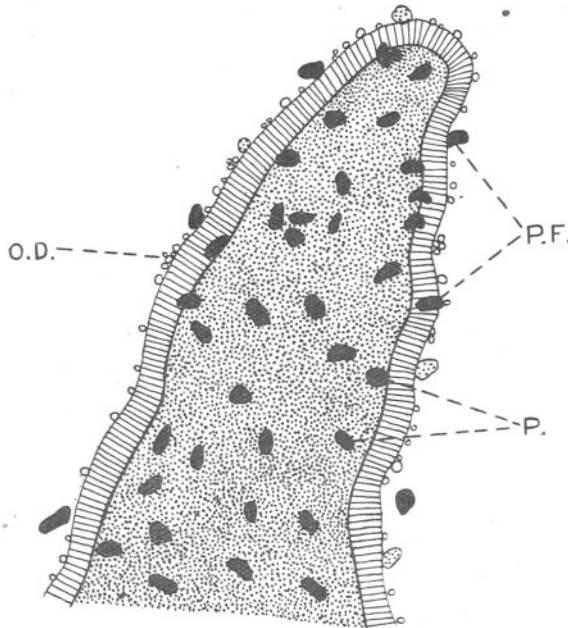


FIG. 39.—Tentacle from edge of mantle one day after feeding with olive oil stained Nile blue sulphate, cleared in glycerine.  $\times 240$ . O.D., droplets of oil on surface of epithelium; P., phagocytes coloured blue owing to fatty acids, lying deep in tissues; P.F., phagocytes containing fat and fatty acids free on surface and passing through epithelium.



the presence of phagocytes near the surface. Other phagocytes are passing through the epithelium on the surface of which are more phagocytes and droplets of oil.

In the lumen of the stomach there were immense numbers of phagocytes, most of them with ingested oil. In certain cases they collected in great numbers round large droplets of oil, which had turned blue under the influence of their enzymes. *All oil droplets lying free in the stomach and not surrounded by phagocytes retained the red colour*—evidence of the absence of lipase in the stomach. Nelson (1918) also noted the absence of extracellular lipase in the stomach of other Lamellibranchs. Portions of the epithelium cleared in glycerine showed that phagocytes laden with oil were passing through it in large numbers.

(d) *With Nitzschia*.

Oysters which had been starved for three months were fed with a pure culture of *Nitzschia*, a quantity of which was added daily to the filtered sea-water. The oysters were observed to open their valves more widely than usual. One oyster was opened after seven days. In the stomach were many phagocytes ingesting *Nitzschia*, such as the one shown in Fig. 40, which has ingested three diatoms, and there were also very many free diatoms, while at the head of the style was a brown mass consisting exclusively of entangled diatoms. There were fewer phagocytes in the stomach than after feeding with blood corpuscles or oil. Many of the phagocytes contained green or yellow globules, the result probably of the ingestion of the brown chromatophores of the diatoms. Digestive diverticula pressed out and examined under a coverslip were largely colourless, except for the presence in some tubules of light green or brown vacuoles, which were not seen in the diverticula of starved animals. Substantially the same conditions were found after two weeks of feeding with *Nitzschia*.

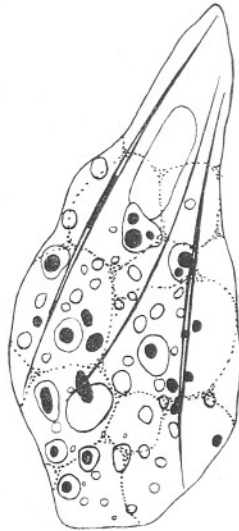


FIG. 40.—Phagocyte from stomach ingesting three *Nitzschia*. Drawn from life.  $\times 2400$ .

Sections of the stomach and mid-gut, fixed in Flemming, showed many fat globules in the epithelium and great numbers of phagocytes. It was difficult to see ingested diatoms in the phagocytes, but in Fig. 41 is shown a portion of the edge of the stomach epithelium, in which lie two phagocytes, each containing an ingested diatom. As a result of the digestion

of the diatoms, there is a quantity of fat in the phagocytes, and some has been passed into the cells of the epithelium. Conditions are thus essentially the same as after feeding with blood corpuscles. It was never possible to detect ingested diatoms in the digestive diverticula. Vonk

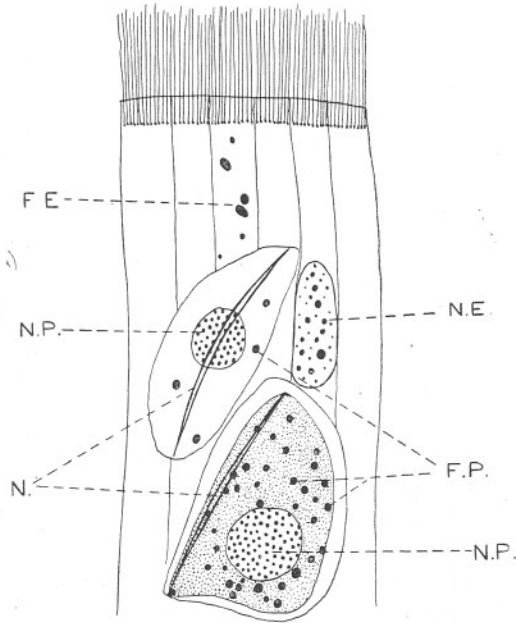


FIG. 41.—Edge of stomach epithelium, two phagocytes ingesting *Nitzschia*.  $\times 2700$ . F.E., fat in epithelial cells; F.P., fat in phagocytes; N., *Nitzschia*; N.E., nucleus of epithelial cell; N.P., nucleus of phagocyte.

after feeding starved oysters with plankton, never observed the presence of whole diatoms in the cells of the "liver," only numerous green inclusions of very irregular form, though occasionally green algæ appeared to be taken in entire.

### III. FEEDING EXPERIMENTS ON LARVÆ AND SPAT.

Larvæ placed in a suspension of iron saccharate in sea-water took it in in large amounts. A study of sections shows that it was assimilated *exclusively* in the cells of the digestive diverticula. Fig. 42 represents a transverse section through one of the two simple diverticula twenty-one hours after feeding with iron saccharate. This has been absorbed in large quantities and lies in discrete round masses in vacuoles in most of the

cells. It is never in the form of fine granules or diffuse. It is being passed from the cells to phagocytes, two of which are seen in the connective tissue around the diverticula, both of them so packed with iron that only the nucleus, and that with difficulty, can be distinguished.

In the spat, iron was taken in exclusively by the cells of the digestive diverticula in the same manner as in the larvæ and adult, although iron was found in the lumen of all parts of the gut (see Figs. 19 and 20).

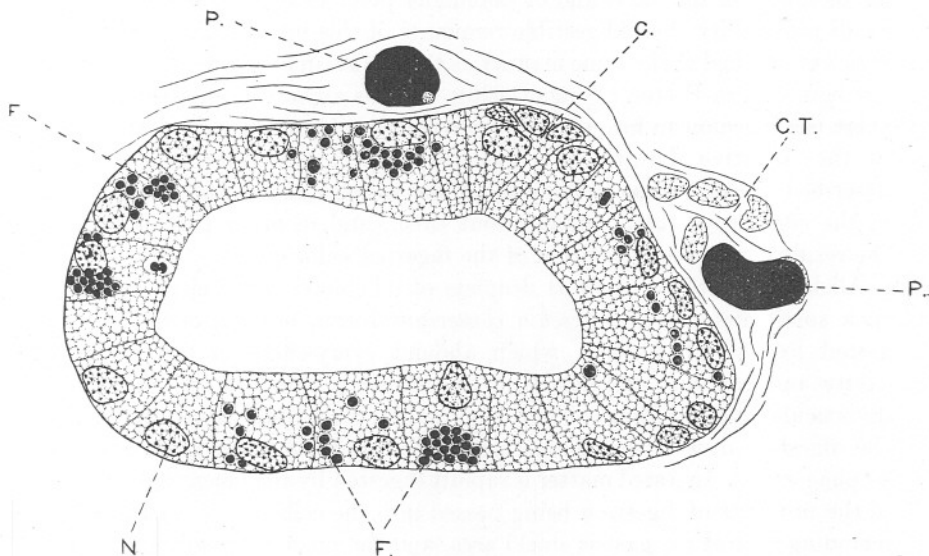


FIG. 42.—Transverse section through digestive diverticulum of one side in larval oyster. Fed on iron saccharate twenty-one hours before fixing. Iron technique.  $\times 1800$ . C., crypt of young cells; C.T., connective tissue; F., iron lying in round vacuoles in cells; N., nucleus of cell of diverticulum; P., phagocytes laden with iron.

Carmine was also taken in by the spat in such quantity that in sections stained with Delafield's hæmatoxylin the lumen of the gut appeared as a uniform red. Carmine is ingested by the cells of the digestive diverticula in precisely the same manner as the iron saccharate, and in no other region of the gut.

#### IV. DISCUSSION OF RESULTS.

Soluble matter, such as iron saccharate, is absorbed exclusively in the cells of the digestive diverticula in larva, spat, and adult, being invariably taken into large vacuoles and carried away by leucocytes. Presumably, therefore, the products of extracellular digestion in the stomach due to the action of the digestive enzymes from the style are here absorbed. Fine particles, such as carmine grains or Indian ink (List and Vonk), are ingested by the cells of the digestive diverticula, being also taken into

large vacuoles and being expelled later if of no food value. It may be assumed that the contents of the green or brown vacuoles, seen in the digestive diverticula of freshly fed animals (see Fig. 11), consist of finely divided vegetable matter which has been ingested by the cells and is in process of being digested intracellularly within the vacuoles. The presence of bright yellow or brown concretions, which alone remain in the diverticula after prolonged starvation, and which are also found in the lumen of the ducts and of the hinder portions of the gut represents in all probability the indigestible remnants of this intracellular digestion which is expelled in the same manner as the Indian ink. These concretions are best seen in *Pecten* (Yonge (1926)). It has already been shown that there is a mechanism for ensuring a circulation of particles in the tubules of the digestive diverticula. The presence of the enterochlorophyll described by MacMunn in the cells of the tubules and in the leucocytes in the connective tissue round about them, and in other parts will be the result of the decomposition of the ingested chlorophyll.

All larger particles, such as droplets of oil, blood corpuscles, or even such small diatoms as *Nitzschia closterium* forma *minutissima*, are ingested by the phagocytes, which abound everywhere in the mantle cavity and gut, but particularly in the stomach, ducts of the digestive diverticula and mid-gut. They very rarely pass into the tubules of the digestive diverticula, those that enter the ducts being there seized by phagocytes. Ingested matter is rapidly digested by the phagocytes, part of the products of digestion being passed into the cells of the epithelium, including that of the gastric shield area, and the remainder carried to the vesicular connective tissue cells, or Langer's vesicles, and there stored. *No evidence of any absorption in the epithelium of the gut or of any free surface in the mantle cavity, other than by the agency of phagocytes, was found*, and previous accounts of direct absorption by the ciliated epithelium on further investigation will probably be found to be the result of the action of phagocytes and the transference of material from them to the cells.

## 5. THE DIGESTIVE ENZYMES.

Digestive enzymes are present in the style and in the tissue of the digestive diverticula, the former are released into the stomach when the style dissolves and the latter remain in the tissues, where they act intracellularly. It is clear from the results of the experiments described in the last section that the phagocytes must also possess powerful digestive enzymes of various kinds. The enzymes were obtained by grinding up the styles or the excised tissue of the digestive diverticula with sand, and then extracting for two or three days with distilled water (the extracts being as efficacious as those prepared in sea-water and being easier to

deal with), toluol being used as antiseptic. Except when otherwise stated, extracts of the style were always of a strength of 1% and extracts of the digestive diverticula 10%, and incubation took place at about 30° C. Rigorous controls consisting of boiled extracts were invariably set up. All experiments were confined to adult oysters.

#### I. THE STYLE.

The presence of digestive enzymes in the style was first discovered by Coupin (1900), who found an amylase and a weak invertase in the style of *Cardium*; this has been confirmed, amongst others, by Mitra (1901), who found amylase and glycogenase in *Anodonta*, Van Rynberk (1908), who found amylase and invertase in *Mytilus*, Nelson (1918), who found similar enzymes in *Anodonta*, and Yonge (1923), who found amylase and glycogenase in the style of *Mya*, and showed that the amylase had all the properties of a typical enzyme. More recently Berkeley (1923) has found an oxidase in the styles of several Lamelli-branches. Barrois (1889) gives a detailed chemical analysis of the styles of *Cardium* made for him by Lambling, who found that they consisted of 87.11% water, 12.03% solid organic matter, and 0.86% solid inorganic matter. The mass of the organic matter consisted of a globulin, though traces of a mucin or chondrin like substance were found. List also found the latter in the styles of *Mytilus*. Mitra (1901), in ignorance of the work of Barrois, made a thorough examination of the styles of *Anodonta*, with almost identical results. Mackintosh (1925) finds that the style of *Crepidula* consists largely of globulin with some mucus. Mitra thought that the style represented a mass of enzyme, but Nelson (1918) advances the more probable view that the enzymes are *adsorbed* on the surface of globules of albuminoid substance.

In view of the complete agreement of previous workers, I have not carried out a chemical examination of the style of *Ostrea*, there being no reason to doubt that it differs in any important degree from those which have been analysed.

##### (a) *Specificity.*

In common with previous workers, I have failed to find any trace of proteoclastic or lipoclastic enzymes in the style, experiments with calcified milk, methyl acetate, and phenol red milk, all giving negative results. No action was found on the glucosides, amygdalin and salicin, on pectin or on lactose, maltose, raffinose, cellulose, or sucrose (in spite of the contrary assertions of Coupin and Mitra with regard to the latter). Starch and glycogen, as in *Mya*, were the only substances acted on by the enzymes of the style, both being rapidly converted into reducing sugars, as shown

by their action on Fehling's and Benedict's reagents. The properties of the amylase have been studied in detail.

(b) *Influence of Temperature on Amylase.*

The experiments shown in Table I were carried out to determine the optimum temperature for the working of the style amylase. The enzyme was destroyed after incubation, the contents of each tube filtered and made up to the original volume.

TABLE I.  
OPTIMUM TEMPERATURE OF STYLE AMYLASE.

10 c.c. of extract with 10 c.c. of 1% starch solution in each experiment.

| 1. Extract of 1.14 gms. of style made in 70 c.c. toluol water. Experiments incubated for 4 hours. |                               | 2. Extract of 0.93 gms. of style made in 50 c.c. toluol water. Incubated for 3 hours. |                               |
|---|-------------------------------|---|-------------------------------|
| Temperature.  | Titred with 10 c.c. Benedict. | Temperature.  | Titred with 10 c.c. Benedict. |
| 16° C.  | 9.95 c.c. needed.             | 40° C.  | 15.05 c.c. needed.            |
| 25° C.  | 9.7            ,,             | 43° C.  | 14.35            ,,           |
| 30° C.  | 9.2            ,,             | 46° C.  | 14.7             ,,           |
| 35° C.  | 9.05          ,,              | 49° C.  | 15.45           ,,            |
| 40° C.  | 8.9           ,,              | 52° C.  | 16.25           ,,            |
| 48° C.  | 8.9           ,,              |   |                               |
| 55° C.  | 10.0          ,,              |   |                               |

These two experiments demonstrate that the optimum temperature lies at, or a little below, 43° C. The pH was 5.9 in both experiments.

Experiments were carried out to determine the temperature of destruction. The heating of 5 c.c. of enzyme extract for fifteen minutes at eight temperatures between 100° C. and 56° C. resulted in complete destruction of the enzyme, as shown by subsequent incubation for twenty hours at 30° C. with 1% starch solution. Heating at 55° C. and all lower temperatures resulted in some of the enzyme remaining active.

It appears that the enzyme is destroyed at 56° C. There was practically no action on starch at 0° C., the enzyme being inactivated, not destroyed. It is interesting to compare the above optimum temperature and temperature of destruction with those found for the style enzyme of *Mya* (1923), which were 32° C. and 51° C. respectively. The difference between the optima is very striking, and may reflect differences in the habitat of the two animals; it is known that the oyster will breed and flourish in the Norwegian pools, where the temperature may rise as high as 90° F. (32° C.), but in neither animal does the optimum temperature represent

anything approaching the temperature at which digestion must normally proceed.

(c) *Influence of pH.*

Table II shows the results of an experiment to determine the optimum pH for the working of the amylase.

TABLE II.

OPTIMUM pH OF STYLE AMYLASE.

Extract of 1.36 gms. of style made in 90 c.c. toluol water. 10 c.c. of extract and 10 c.c. of 1% starch solution in each experiment with acid or alkali, volume made up to 25 c.c. with water. All incubated for 6 hours at 32° C.; pH determined by Clark and Lubs' indicators.

|    | ·01N HCl. | ·01N NaOH. | Initial pH. | Titrated with<br>10 c.c. Benedict. |
|----|-----------|------------|-------------|------------------------------------|
| A. | 3.0 c.c.  | —          | 3.4         | No reduction.                      |
| B. | 0.5 c.c.  | —          | 4.9         | 55.4 c.c. needed.                  |
| C. | 0.3 c.c.  | —          | 5.2         | 13.5 „                             |
| D. | —         | —          | 5.9         | 9.1 „                              |
| E. | —         | 0.1 c.c.   | 6.2         | 9.7 „                              |
| F. | —         | 0.2 c.c.   | 6.6         | 10.15 „                            |
| G. | —         | 0.5 c.c.   | 7.3         | 11.6 „                             |
| H. | —         | 0.7 c.c.   | 7.6         | 13.05 „                            |
| I. | —         | 1.0 c.c.   | 9.3         | 21.0 „                             |

The optimum is very sharply defined, and lies at about 5.9, i.e. at the pH produced by the dissolution of the style in water, on either side of this point, and particularly on the acid side, the efficiency of the enzyme being rapidly reduced.

(d) *Influence of Salts.*

In view of the fact that if the pancreatic amylase is dialysed it loses its power to act upon starch, as shown by Bierry, Giaja, and Henri (1906), and that the amylase from the liver is inactivated in the same manner (Starkenstein (1910, 1910a)), action being restored in the former case by the addition of the electro-negative chloride or bromide ions, and in the latter by the addition of sodium chloride, experiments were carried out to determine whether the amylase of the style is similarly dependent for its efficacy on the presence of electrolytes.

An extract of 0.75 gms. of style was made in 40 c.c. of toluol water. After three days the enzyme was precipitated by the addition of 200 c.c. of absolute alcohol, the precipitate being filtered off, thoroughly washed

with absolute alcohol, dried, and then dissolved in 40 c.c. of glass-distilled water. The experiments in Table III were then carried out, the sea-water used in experiments B and C being made acid until the pH approximated to the optimum.

TABLE III.

## ACTION OF PURIFIED ENZYME WITH AND WITHOUT SALTS FROM SEA-WATER.

10 c.c. extract with 10 c.c. 1% starch solution in each experiment. Incubated for 5 hours at 32° C., enzyme destroyed and titrated.

|    | Added.                                     | pH. | Titred with 10 c.c. Benedict.* |
|----|--|-----|--------------------------------|
| A. | 20 c.c. distilled water                    | 5.8 | 175 c.c. needed                |
| B. | 20 c.c. 100% sea-water                     | 5.7 | 28.05 "                        |
| C. | 20 c.c. 200% sea-water                     | 5.5 | 25.6 "                         |
| D. | 20 c.c. dis. water and<br>1 drop sea-water | 5.8 | 72.75 "                        |

In the absence of the salts present in sea-water the enzyme is almost inactivated (A), action is restored to some extent by the addition of a trace of sea-water (D), and fully restored in a medium of 50% (B) or 100% (C) sea-water, action being slightly less in the former.

A series of dialysis experiments were then carried out, details of which are given in Tables IV, V, and VI.

TABLE IV.

## ACTION OF DIALYSED EXTRACT.

0.75 gms. style extracted in 80 c.c. toluol water for 3 days, divided into two parts, A and B, each of 40 c.c. These dialysed in separate parchments for 3 days, surrounding fluid being changed daily, contents of each finally made up to 40 c.c. 10 c.c. extract with 10 c.c. 1% starch solution in each experiment. Incubated for 5 hours at 32° C., enzyme destroyed and titrated.

|    | Added.  | pH. | Titred with 10 c.c. Benedict.* |
|----|---|-----|--------------------------------|
| A. | 1. 10 c.c. distilled water                    | 5.8 | 275 c.c. needed.               |
|    | 2. 10 c.c. acidified sea-water                | 5.8 | 24.05 "                        |
|    | 3. 10 c.c. surrounding fluid                  | 5.8 | 230 "                          |
|    | 4. 10 c.c. 1% NaCl                            | 5.8 | 28.75 "                        |
| B. | 1. 10 c.c. distilled water                    | 5.8 | 290 "                          |
|    | 2. 10 c.c. 1% Na <sub>2</sub> SO <sub>4</sub> | 5.8 | 290 "                          |
|    | 3. 10 c.c. 1% NaBr                            | 5.8 | 50 "                           |
|    | 4. 10 c.c. 1% KCl                             | 5.8 | 40.1 "                         |

\* Or suitable aliquot part.



TABLE V.

## ACTION OF DIALYSED EXTRACT.

0.45 gms. style extracted in 40 c.c. toluol water, dialysed 3 days, water changed 4 times.  
Experiments conducted as in Table IV.

|    | Added.                                       | pH. | Titrated with 10 c.c. Benedict.* |
|----|--|-----|----------------------------------|
| C. | 1. 10 c.c. distilled water                   | 6.4 | 241.5 c.c. needed.               |
|    | 2. 10 c.c. 1%Na <sub>2</sub> CO <sub>3</sub> | 9.8 | 186.5     ,,                     |
|    | 3. 10 c.c. 1%KI                              | 6.6 | 96.5     ,,                      |
|    | 4. 10 c.c. 1%CaCl <sub>2</sub>               | 5.7 | 23.0     ,,                      |

TABLE VI.

## ACTION OF DIALYSED EXTRACT.

0.64 gms. style extracted in 60 c.c. toluol water, dialysed 4 days, water changed 4 times.  
Experiments conducted as in Table IV.

|    | Added.                                      | pH. | Titrated with 10 c.c. Benedict.* |
|----|---|-----|----------------------------------|
| D. | 1. 10 c.c. distilled water                  | 6.6 | 270 c.c. needed.                 |
|    | 2. 10 c.c. 1%MgCl                           | 5.8 | 46.5     ,,                      |
|    | 3. 10 c.c. 1%NaF                            | 6.0 | 270     ,,                       |
|    | 4. 10 c.c. 1%NaNO <sub>3</sub>              | 6.2 | 123.5     ,,                     |
|    | 5. 10 c.c. 1%K <sub>2</sub> SO <sub>4</sub> | 6.1 | 300     ,,                       |
|    | 6. 10 c.c. 1%BaCl                           | 5.8 | 40     ,,                        |

An examination of the results of these four sets of experiments shows that the dialysed extract is almost without action on starch, but that action is restored to a slight degree on the addition of 10 c.c. of the water into which the salts had passed from out of the parchment (A3), while action was completely restored as before on the addition of sea-water (A2). Action was also restored in the presence of the chlorides of sodium (A4), potassium (B4), calcium (C4), magnesium (D2), and barium (D6), and in that of sodium bromide (B3). To a less extent it was restored in the presence of the iodide of potassium (C3), the nitrate of sodium (D4) and the carbonate of sodium (C2), in spite of the high pH in the last case. There was no increase in activity in the presence of the sulphates of sodium (B2) or potassium (D5), or in that of sodium fluoride (D3). The amylase of the style appears, therefore, to need for its action the presence of electro-negative ions—preferably those of chlorine or bromine—the identity of the electro-positive ion being immaterial. Conditions are the same as in the case of the amylase of the pancreas or of the liver in Vertebrates. Since the extracts of the style made up in distilled water have the same efficacy as those prepared in sea-water it appears that these ions are present in sufficient quantity in the substance of the style.

\* Or suitable aliquot part.

## II. THE DIGESTIVE DIVERTICULA.

The presence of digestive enzymes in extracts of the digestive diverticula has been shown by Fredericq (1878), who found protease in *Mya* and *Mytilus*; Mitra (1901), who found amylase and invertase in *Anodonta*; Van Rynberk (1908), who found amylase in *Mytilus*; Dakin (1909), who found amylase, protease and lipase in *Pecten*; Heymann (1914), who found protease, lipase and a variety of sucroclastic enzymes in *Ostrea*; and Yonge (1923), who found in *Mya* sucroclastic enzymes which acted on starch, glycogen, sucrose, maltose, and lactose, also a protease acting in acid media and a lipase. Most of these workers considered that the

TABLE VII.

## ACTION OF 10% EXTRACT OF DIGESTIVE DIVERTICULA ON CARBOHYDRATES, ETC.

10 c.c. extract with 10 c.c. of substrate, controls of boiled extract, incubated at 30° C.

| No.  | Substrate.    | Time.  | Experiment.<br>Titrated with 10 c.c. | Control.<br>Benedict. |
|--|---------------|--------|--------------------------------------|-----------------------|
| 1.   | 1% starch     | 2 hrs. | 4.3 c.c.                             | 9.0 c.c.              |
| 2.   | 0.5% glycogen | 1 day  | 5.9 c.c.                             | 9.0 c.c.              |
| 3.   | 5% sucrose    | „      | 4.8 c.c.                             | 9.0 c.c.              |
| 4.   | 1% raffinose  | 3 days | 6.8 c.c.                             | 9.0 c.c.              |
| 5.   | 1% inulin     | „      | 8.2 c.c.                             | 8.2 c.c.              |
| 6.   | 1% salicin    | „      | 4.2 c.c.                             | 8.3 c.c.              |
| 7.   | 1% amygdalin  | „      | 4.2 c.c.*                            | 8.1 c.c.              |
| 1 c.c. boiled with 5 c.c. Barfoed's sol. 10 min. |               |        |                                      |                       |
| 8.   | 2% maltose    | 1 day  | Reduction                            | No reduction.         |
| 9.   | 2% lactose    | „      | Reduction                            | No reduction.         |

\* Smell of CN.

digestive diverticula were secretory, and that these enzymes were discharged into the stomach. As shown in detail in a previous paper (1926) there is no evidence, histological or physiological, of any secretion in the diverticula which are organs of absorption and of intracellular digestion, the digestive enzymes acting on material ingested.

(a) *Sucroclastic Enzymes.*

*Specificity.*—Owing to the presence of reducing sugars in the extract of the digestive diverticula, it is necessary to estimate the sugar in both experiments and controls. In Table VII are shown the results of experiments on the simpler carbohydrates and glucosides. Starch, glycogen,

sucrose, raffinose (to a slight degree), maltose, and lactose were all digested by the enzymes in the extract and also the two glucosides, salicin and amygdalin. Inulin was not digested. A series of longer experiments was set up to determine whether cellulose or pentosans are digested. Both of these are of great importance since cellulose must bulk large in the food

TABLE VIII.

## ACTION OF 10% EXTRACT OF DIVERTICULA ON CELLULOSE, PENTOSANS, AND INULIN.

| No. | Experiment.                            | Temp.  | Time.    | Titrated with<br>10 c.c. Benedict. |
|-----|--|--------|----------|------------------------------------|
| 1.  | A. 20 c.c. with 0.5 gm. sawdust.       | 32° C. | 3 wks.   | A. 3.2 c.c.                        |
|     | B. Ditto boiled.                       | "      | "        | B. 5.2 c.c.                        |
|     | C. 20 c.c. extract alone.              | "      | "        | C. 3.2 c.c.                        |
| 2.  | A. 20 c.c. with 0.5 gm. sawdust.       | "      | 2 wks.   | A. 2.55 c.c.                       |
|     | B. Ditto boiled.                       | "      | "        | B. 3.2 c.c.                        |
|     | C. 20 c.c. extract alone.              | "      | "        | C. 2.55 c.c.                       |
| 3.  | A. 10 c.c. with 10 c.c. 1% pectin.     | "      | 15 days. | A. 5.6 c.c.                        |
|     | B. Ditto boiled.                       | "      | "        | B. 7.0 c.c.                        |
|     | C. 10 c.c. with 10 c.c. water.         | "      | "        | C. 5.0 c.c.                        |
| 4.  | A. 20 c.c. with 10 c.c. 1% pectin.     | "      | 16 days. | A. 4.45 c.c.                       |
|     | B. Ditto boiled.                       | "      | "        | B. 7.30 c.c.                       |
|     | C. 20 c.c. with 10 c.c. water.         | "      | "        | C. 4.5 c.c.                        |
| 5.  | A. 20 c.c. with 10 c.c. 5% gum arabic. | "      | 2 wks.   | A. 2.4 c.c.                        |
|     | B. Ditto boiled.                       | "      | "        | B. 2.92 c.c.                       |
|     | C. 20 c.c. with 10 c.c. water.         | "      | "        | C. 2.4 c.c.                        |
| 6.  | A. 20 c.c. with 10 c.c. 5% gum arabic. | "      | 3 wks.   | A. 2.2 c.c.                        |
|     | B. Ditto boiled.                       | "      | "        | B. 4.5 c.c.                        |
|     | C. 20 c.c. with 10 c.c. water.         | "      | "        | C. 2.6 c.c.                        |
| 7.  | A. 10 c.c. with 10 c.c. 2% inulin      | "      | 2 wks.   | A. 5.85 c.c.                       |
|     | B. Ditto boiled.                       | "      | "        | B. 9.8 c.c.                        |
|     | C. 10 c.c. with 10 c.c. water.         | "      | "        | C. 6.0 c.c.                        |
| 8.  | A. 20 c.c. with <i>Ulva</i> 3" × 1"    | "      | 3 wks.   | A. <i>Ulva</i><br>unchanged        |
|     | B. Ditto boiled.                       | "      | "        | B. <i>Ulva</i><br>unchanged.       |

of the oyster, while Petersen (1911), in his work on the food of oysters in the Limfjord, maintained that detritus was the principal source of food, and Boysen Jensen (1914) has shown that the main constituent of this detritus consists of pentosans. No experiments were made by him on the digestion of pentosans, although Heymann (1914), by somewhat questionable methods, found that pectin was digested by extracts of the

"liver" of the oyster. The same author also maintaining that inulin is digested, a longer experiment was set up in order to confirm the results of the experiment in Table VII. In view of the autolysis which proceeds if tissue extracts are left for any long period, resulting in the formation of additional quantities of reducing sugars, two controls were necessary for these experiments, one with boiled extract and substrate and the other with unboiled extract without substrate. Sawdust was used for the experiments on cellulose, owing to the presence in it of hemicelluloses, which do not occur in filter paper. Table VIII gives the result of these experiments.

None of these substances were digested, the only increase in reducing sugar being due to autolysis. Cellulose in the form of sawdust or as the green alga, *Ulva*, was not digested even after three weeks incubation. It would have been surprising if it had since the power of digesting cellulose by means of an enzyme is rare, being confined, in the Mollusca, to certain of the herbivorous Pulmonates and Tectibranchs, which secrete an extracellular cellulase and, in the Lamellibranchs, to the specialised Teredinidæ, which digest wood intracellularly (for résumé of work on this subject see Yonge (1925a)). After incubations of two and three weeks there was no indication of the digestion of the pentosans, pectin and gum arabic. It is impossible to confirm the findings of Heymann, nor is confidence in his work strengthened by the negative results of the experiments here performed on inulin. The implications of these results on the theories of Petersen and Boysen Jensen will be discussed later.

*Influence of Temperature on Amylase.*—Similar experiments to those carried out on the style amylase were done on the amylase from the digestive diverticula. The experiments in Table IX were performed to determine the optimum temperature.

TABLE IX.

## OPTIMUM TEMPERATURE OF AMYLASE.

10 c.c. 10% extract with 10 c.c. 1% starch solution, digests in left column incubated 2 hours, in right column 3 hours.

| Temperature. | 10 c.c. Benedict. | Temperature. | 10 c.c. Benedict. |
|--------------|-------------------|--------------|-------------------|
| 18.5° C.     | 7.75 c.c. needed. | 40° C.       | 5.2 c.c. needed.  |
| 28° C.       | 6.8               | 43° C.       | 5.0               |
| 35° C.       | 6.4               | 46° C.       | 5.0               |
| 40° C.       | 6.3               | 49° C.       | 5.2               |
| 45° C.       | 6.25              | 52° C.       | 5.4               |
| 50° C.       | 6.8               |              |                   |
| 55° C.       | 8.0               |              |                   |
| 60° C.       | 9.45              |              |                   |

These experiments show that, at pH 5.5, the optimum temperature is 44.5° C., i.e. slightly higher than that of the style amylase, where the pH, however, was 5.9.

Experiments to determine the temperature of destruction showed that the heating of 5 c.c. of enzyme extract for fifteen minutes at four temperatures between 100° C. and 67° C. resulted in complete destruction of the enzyme as shown by subsequent incubation for twenty hours at 30° C. with 1% starch. The enzyme remained active after heating at 64° C. and all lower temperatures. The temperature of destruction therefore, at pH 5.5, lies between 64° C. and 67° C. This is considerably higher than that of the style amylase (56° C.); there, however, the pH was 5.9, and as Compton (1921 and previous papers therein quoted) has shown that temperature optima of enzyme actions are dependent, amongst other things, on pH, there is not rigorous proof that the two enzymes are distinct in their properties. The action of the amylase from the diverticula is practically inhibited at 0° C.

*Influence of pH.*—The experiment in Table X was carried out to determine the optimum pH for the action of the amylase.

TABLE X.

## OPTIMUM PH OF AMYLASE FROM DIVERTICULA.

10 c.c. 10% extract and 10 c.c. 1% starch solution with acid or alkali, volume made up to 25 c.c. with water. Incubated for 2 hours at 30° C.

|    | HCl.        | NaOH.        | Initial pH. | Titrated with 10 c.c. Benedict. |
|----|-------------|--------------|-------------|---------------------------------|
| A. | 5 c.c. .1N  | —            | 2.4         | 10.8 c.c. needed.               |
| B. | 3 c.c. .1N  | —            | 3.2         | 9.6        "                    |
| C. | 1 c.c. .1N  | —            | 3.6         | 8.4        "                    |
| D. | 3 c.c. .01N | —            | 4.0         | 7.45       "                    |
| E. | 2 c.c. .01N | —            | 4.6         | 6.65       "                    |
| F. | 1 c.c. .01N | —            | 5.0         | 6.1        "                    |
| G. | —           | —            | 5.5         | 5.8        "                    |
| H. | —           | 1 c.c. .01N  | 5.8         | 6.0        "                    |
| I. | —           | 2 c.c. .01N  | 6.2         | 6.03       "                    |
| J. | —           | 3 c.c. .01N  | 6.6         | 6.1        "                    |
| K. | —           | 5 c.c. .01N  | 7.0         | 6.45       "                    |
| L. | —           | 0.8 c.c. .1N | 7.8         | 7.3        "                    |
| M. | —           | 1 c.c. .1N   | 8.6         | 8.0        "                    |
| N. | —           | 2 c.c. .1N   | 9.6         | 9.4        "                    |

The optimum lies at about pH 5.5, i.e. somewhat lower than that of the style (5.9). The optimum is not so sharply defined as in the case of

the style enzyme, the efficacy of the enzyme not decreasing so rapidly on either side of that point.

*Influence of Salts.*—A number of experiments were conducted to determine the action of salts on the activity of the amylase, the results being given in Tables XI and XII.

TABLE XI.

ACTION OF PURIFIED ENZYME WITH AND WITHOUT SALTS  
FROM SEA-WATER.

15 gms. diverticula extracted in 60 c.c. toluol water, enzyme precipitated with alcohol and purified as in expts. on style. Ppt. dissolved in 40 c.c. glass distilled water. 10 c.c. extract with 10 c.c. 1% starch in each experiment, incubated for 5 hours at 32° C.

|    | Added.                                      | pH. | Titrated with 10 c.c. Benedict. |
|----|---|-----|---------------------------------|
| A. | 20 c.c. distilled water.                    | 6.4 | 29.0 c.c. needed.               |
| B. | 20 c.c. 100% sea-water.*                    | 5.8 | 17.4     ,,                     |
| C. | 20 c.c. 200% sea-water.*                    | 5.6 | 17.3     ,,                     |
| D. | 20 c.c. dis. water and<br>1 drop sea-water. | 6.4 | 24.4     ,,                     |

TABLE XII.

ACTION OF DIALYSED EXTRACT.

50 c.c. 10% extract dialysed for 3 days, water changed 4 times. Experiments conducted as in Table XI.

|    | Added.                         | pH. | Titrated with 10 c.c. Benedict. |
|----|--------------------------------|-----|---------------------------------|
| A. | 10 c.c. distilled water.       | 6.6 | 38.8 c.c. needed.               |
| B. | 10 c.c. 1% NaCl.               | 5.8 | 23.25     ,,                    |
| C. | 10 c.c. 1% NaBr.               | 9.6 | 28.2     ,,                     |
| D. | 10 c.c. 1% CaCl <sub>2</sub> . | 5.8 | 22.65     ,,                    |
| E. | 10 c.c. 1% KI.                 | 6.0 | 31.75     ,,                    |

Here again the enzyme requires for its working the presence of the salts in sea-water, only a trace of which has a considerable effect (Table XI, D). The action of the enzyme is definitely inhibited after dialysis. It is more difficult to purify the enzyme from the tissue extract than from the style and hence the greater activity of the dialysed enzyme in this case. Action was restored in the presence of the chlorides of sodium and calcium (Table XII, B and D) and of the bromide of sodium (C)—the reduced action in the last case being due to the high pH, owing to the presence

\* Acidified.

of impurities in the salt—and to a slighter extent in the presence of potassium iodide (E).

(b) *Lipoclastic Enzymes.*

The results of a series of experiments on the lipase in the digestive diverticula are shown in Table XIII.

TABLE XIII.

ACTION OF 10% EXTRACT OF DIGESTIVE DIVERTICULA ON FATS AND ESTERS.

| No. | Extract. | Substrate.  | Time.   | Acidity in terms of .1N NaOH. |                 |
|-----|----------|---|---------|-------------------------------|-----------------|
|     |          |   |         | Experiment.                   | Control.        |
| 1.  | 15 c.c.  | 30 drops olive oil emulsion   | 7 days  | 3.2 c.c.                      | 2.5 c.c.        |
| 2.  | 25 c.c.  | 30 drops „ „  | 14 „    | 2.5 c.c.                      | 2.1 c.c.        |
| 3.  | 20 c.c.  | 30 drops „ „  | 21 „    | 1.05 c.c.                     | 0.85 c.c.       |
| 4.  | 10 c.c.  | 10 c.c. 5% methyl acetate   | 7 „     | 9.0 c.c.                      | 2.5 c.c.        |
| 5.  | 10 c.c.  | 10 c.c. 10% „ „   | 14 „    | 17.35 c.c.                    | 5.15 c.c.       |
| 6.  | 5 c.c.   | 5 c.c. boiled milk with<br>3 c.c. 2% Na <sub>2</sub> CO <sub>3</sub> and<br>phenol red. | 17 hrs. | Yellow.                       | Remains<br>Red. |
| 7.  | 5 c.c.   | ditto with 1 c.c. Na <sub>2</sub> CO <sub>3</sub>                                       | 19 „    | Yellow.                       | Remains<br>Red. |

The action on olive oil is very slight even after three weeks incubation; but there is considerable action on methyl acetate, and experiments with phenol red milk were all positive. Since fat is taken in freely by the phagocytes and there digested, very little appearing in the digestive diverticula, there is probably no necessity for the presence of a powerful lipase in the latter. Indeed, the slight lipolytic action of the extract may be due, in part at any rate, to the phagocytes in the tissue extracted and *not* to enzymes from the actual absorptive tubules.

(c) *Proteoclastic Enzymes.*

It is very difficult to test for the presence of proteoclastic enzymes in extracts of the diverticula on account of the weakness of their action. No digestive activity on coagulated egg albumen or congo red fibrin could be demonstrated. Satisfactory results were obtained by the method of Dernby (quoted by Bodansky and Rose). A 10% solution of gelatin was prepared and a series of experiments performed with extracts of the diverticula at different hydrogen ion concentrations. At stated intervals

5 c.c. of the digests were removed and the process of digestion determined by placing them in ice for fifteen minutes, and then observing the degree of liquefaction. The gelatin is liquefied as digestion proceeds, and fails to solidify to a greater or less extent dependent upon the degree to which digestion has proceeded. Dernby used the following scale of numbers to denote the approximate degree of digestion :—

- 0 = Completely solid.
- 1 = Solid, but small pieces may be torn off by strong shaking.
- 2 = Solid, but the surface moves somewhat when tubes are shaken.
- 3 = Soft.
- 4 = Half liquid.
- 5 = Almost liquid.
- 6 = Entirely liquid.

Table XIV shows the result of an experiment of this nature which confirmed the results of a previous experiment. There appear to be two optima, one at a pH of about 3.7 and the other at and above pH 9.0. There is *no* action at the normal pH of the tissue extract, while optimum conditions prevail at a degree of alkalinity which cannot be present

TABLE XIV.

## DIGESTION OF GELATIN BY EXTRACT OF THE DIVERTICULA.

10 c.c. 15% extract with HCl or NaOH with 15 c.c. 10% gelatin with water = 30 c.c.

| No. | HCl.          | NaOH.        | Initial pH. | Degree of liquefaction after incubation at 32° C. for :— |         |         |         |
|-----|---------------|--------------|-------------|--|---------|---------|---------|
|     |               |              |             | 2 days.  | 3 days. | 4 days. | 5 days. |
| 1   | 2.5 c.c. N    | —            | 2.2         | 1-2  | 1-2     | 2       | 3       |
| 2   | 1.0 c.c. N    | —            | 3.7         | 5  | 6       | 6       | 6       |
| 3   | 5.0 c.c. .1N  | —            | 4.2         | 2  | 3       | 4       | 6       |
| 4   | 2.5 c.c. .1N  | —            | 4.8         | 1-2  | 2       | 2       | 2       |
| 5   | 5.0 c.c. .01N | —            | 5.4         | 1  | 1       | 1       | 1       |
| 6   | —             | —            | 5.8         | 0  | 0       | 0       | 0       |
| 7   | —             | 0.7 c.c. .1N | 6.0         | 0  | 0       | 0       | 0       |
| 8   | —             | 1.0 c.c. .1N | 6.4         | 0  | 0       | 0       | 0       |
| 9   | —             | 2.0 c.c. .1N | 7.3         | 0-1  | 1       | 1-2     | 3       |
| 10  | —             | 3.0 c.c. .1N | 8.6         | 2  | 2       | 3       | 6       |
| 11  | —             | 4.0 c.c. .1N | 9.2         | 2-3  | 3       | 6       | 6       |
| 12  | —             | 5.0 c.c. .1N | 9.8         | 3  | 3       | 6       | 6       |
| 13  | —             | 1.0 c.c. N   | 10.2        | 3  | 3-4     | 6       | 6       |
| 14  | —             | 2.0 c.c. N   | 10.8        | 6  | 6       | 6       | 6       |
| 15  | —             | 3.0 c.c. N   | 11.3        | 6  | 6       | 6       | 6       |



normally in the tissues. It may be that we are dealing here with two enzymes, as Bodansky and Rose (1922) believe to be the explanation of similar double optima obtained in experiments of the same nature on certain Coelenterates. In the case of the oyster, one enzyme may come from the digestive diverticula and the other from the phagocytes; Heymann, indeed, states that there are three proteases in the oyster: a trypsin, a "liver" pepsin, and a blood pepsin. He knew nothing, however, of the digestive powers of the phagocytes. It is useless to call one pepsin and the other trypsin, as it is possible at the optimum of either for the extract to act on peptone with the formation of amino acids, as proved by positive results of tests for tryptophane eighteen hours after the commencement of experiments. On the other hand, *after three weeks digestion with gelatin there is no indication of the presence of amino acids* in any medium. The extract coagulates calcified milk in four or five hours.

The important point in connection with this work is not whether there are two enzymes or one, but the *weakness* of the proteoclastic enzyme. Although albumenoses are split up with formation of amino acids, gelatin is not, while albumen and fibrin are not attacked. It takes two days for the digestion of gelatin to proceed to the stage it attained in one or two hours in the experiments of Bodansky and Rose on the Coelenterates, Physalia and Stomolophus. These animals are by nature carnivorous, whereas the oyster is not, and it is an interesting fact that in the Ascidian, *Ciona intestinalis*, which also feeds by ciliary currents and has a similar diet to the oyster, it was found (1925) that there is a similar weakness of proteoclastic enzymes, though in this case digestion is exclusively extracellular. Apparently there is a correlation between the nature of the food and the variety and strength of the digestive enzymes in animals. In *Ciona*, as in the Lamellibranchs, the sucroclastic enzymes are very powerful. In the omnivorous Crustacea both sucroclastic and proteoclastic enzymes are highly developed (Yonge (1924)).

### III. STOMACH CONTENTS.

Digests with the stomach fluid show the presence of enzymes, presumably derived from the style, capable of quickly digesting starch and glycogen. Slight traces of reducing sugars were found after three days incubation with sucrose, maltose and amygdalin, but none after five days incubation with lactose. This action is far slighter than that of the extract of the digestive diverticula and can safely be attributed to the phagocytes, great numbers of which are always present in the stomach. To the same origin, no doubt, can be attributed the traces of lipase and protease. Phenol red milk made alkaline with 2 c.c. of 2%  $\text{Na}_2\text{CO}_3$  is

turned yellow in two hours by the action of stomach fluids and calcified milk is coagulated in the same time. These experiments were repeated with filtered and unfiltered fluid, since in the former the phagocytes would be absent. The experiments were otherwise identical in all respects and controls were set up. In the test for lipases the phenol red milk was turned yellow in two hours by the unfiltered fluid and in twelve hours by the filtered fluid; in the test for protease the calcified milk was coagulated in twelve hours by the unfiltered and in forty hours by the filtered fluid, the control also coagulating after forty hours in the latter case. As we have seen there is *no action on olive oil* by enzymes free in the stomach, only by phagocytes. There is thus no evidence that the digestive diverticula secrete enzymes into the stomach, since the only enzymes of any power proceed from the style, the traces of other enzymes having their origin in the phagocytes. The lack of powerful digestive enzymes in the digestive tract is confirmed by the presence—noted by many workers—of living and apparently unprotected organisms, both plant and animal, in the mid-gut, rectum and fæces of the oyster and other Lamellibranchs. No naked organism, unless protected chemically like intestinal parasites, could survive the action of powerful enzymes.

#### IV. GILL MUCUS.

Gorka (1916), in a paper which I have been unable to see, but which is quoted by Vonk, states that he found enzymes in the gill mucus of *Anodonta* and *Unio* capable of digesting polysaccharides, glucosides and fat, and in the mucus of the palps he also found a protease. A series of experiments on the gill mucus of *Ostrea* were carried out, the mucus being obtained by covering the gills with fine carborundum and collecting the mucus laden strings and extracting them in toluol water, later filtering off the carborundum. After four days incubation no trace of action on any carbohydrate or glucoside was found, nor did digests with phenol red milk or olive oil give positive results, although there was a slight increase in acidity after two weeks incubation with methyl acetate. Traces of tryptophane were found after two weeks incubation with peptone, while calcified milk was coagulated after three days. In both cases controls gave negative results. There appear therefore to be traces of lipase and protease in the mucus, but if that is examined under the microscope many phagocytes are seen which wander freely on the surface of the gill, as already described in the section on feeding with olive oil. There seems no doubt that the slight development of enzymatic action in these experiments, and probably in those of Gorka, is due to enzymes from these phagocytes.

## V. OXIDASES.

It is most convenient here to refer to the presence of oxidases in the tissues. Berkeley (1923) has shown that extracts of the styles of *Saxidomus giganteus*, *Paphia staminea*, and *Mya arenaria* have a marked oxidising action on guaiacum, paraphenyldiamine, and pyrogallol in the absence of  $H_2O_2$ , and in its presence after boiling, and further that the oxidation of guaiacum takes place as rapidly in the absence of air. He suggests, in connection with his theory that the style is concerned with anærobic respiration (to which reference will be made later), that the substance is a complex of an oxidising agent and an enzyme which can convey oxygen to the tissues. He also found a slight action on guaiacum by extracts of the palps and traces by those of the mantle and "digestive gland," but none by those of the gills, gonad, or siphons.

In the oyster, extracts were made of the mantle, gills, palps, digestive diverticula, style, gonad, and muscle. Catalase was tested for by adding 2 c.c. of  $H_2O_2$  to 5 c.c. of the extracts. There was a great evolution of oxygen, showing the presence of catalase, with the gonad and digestive diverticula, a medium evolution with the palps, gills, and muscle, but none with the style and mantle. Peroxidases were tested for with tincture of guaiacum, hydroquinone, and pyrogallol, in every case 5 c.c. of extract being used, and to it added 2 c.c. of  $H_2O_2$  and twelve drops of freshly prepared guaiacum, 2% hydroquinone or 1% pyrogallol. With guaiacum oxidation was very slow, and after a day slight traces of activity were found only with the style, palps, and gills. After five hours hydroquinone was turned a decided green-brown colour with the style extract, a light yellowish brown with the gills, palps, mantle, and digestive diverticula, and pale yellow with the muscle and gonad. Pyrogallol after five minutes was turned dark red-brown with the style extract, a medium brown with the digestive diverticula and muscle and a light yellow with the other tissues. The action of minced tissue was also tested with the indophenol reagent in the absence of  $H_2O_2$ , the results, which are striking, being given below :—

1. Style—deep purple almost immediately.
2. Gill—deep purple in a few minutes.
3. Mantle—purple in four to five minutes.
4. Palps—light purple in ten minutes.
5. Dig. diverticula—light purple in fifteen minutes.
6. Gonad—light purple in fifteen minutes.
7. Muscle—light purple in twenty minutes.

The different reagents all give different results with the various tissue extracts, except in the case of the style which in *all cases* gives the most

decided reaction, and clearly contains, since it can act in the absence of  $H_2O_2$ , a complete oxidase system. It is strange that action on guaiacum should be so much less with the styles of *Ostrea* than with those of *Saxidomus* in Berkeley's experiments. Time has not permitted further work on this subject, but the presence of this enzyme in the style may be of great importance in the metabolism of the Lamellibranchs.

## 6. HYDROGEN ION CONCENTRATION IN THE GUT AND PERMANENCE OF THE STYLE.

### I. HYDROGEN ION CONCENTRATION.

Table XV shows the pH of the fluid in the mantle cavity and in all regions of the gut, and of the substance of the digestive diverticula and

TABLE XV  
pH IN FRESH OYSTERS.

| No.                   | Mantle cavity. | Eso-phagus. | Stomach. | Style. | Dig. div. | Mid-gut. | Rectum. |
|-----------------------|----------------|-------------|----------|--------|-----------|----------|---------|
| 1.                    | 7.0            | 6.0         | 5.5      | 5.2    | 5.8       | 5.6      | 6.0     |
| 2.                    | 7.1            | 5.9         | 5.4      | 5.2    | 5.8       | 5.5      | 6.0     |
| 3.                    | 6.8            | 5.8         | 5.6      | 5.2    | 5.7       | 5.6      | 5.9     |
| 4.                    | 7.2            | 5.9         | 5.5      | 5.2    | 5.9       | 5.8      | 5.9     |
| 5.                    | 6.8            | 5.8         | 5.6      | 5.2    | 5.8       | 5.8      | 5.8     |
| 6.                    | 6.8            | 5.6         | 5.6      | 5.2    | 5.8       | 5.8      | 5.8     |
| 7.                    | 6.8            | 5.9         | 5.5      | 5.2    | 5.7       | 5.8      | 6.0     |
| 8.                    | 7.0            | 6.0         | 5.4      | 5.2    | 5.8       | 5.8      | 6.3     |
| 9.                    | 6.8            | 5.8         | 5.4      | 5.2    | 5.6       | 5.9      | 5.8     |
| 10.                   | 7.1            | 5.6         | 5.4      | 5.2    | 5.7       | 6.0      | 5.8     |
| 11.                   | 7.0            | 5.8         | 5.6      | 5.2    | 5.8       | 5.7      | 5.9     |
| 12.                   | 6.9            | 5.8         | 5.4      | 5.2    | 5.8       | 5.8      | 6.0     |
| Range                 | 6.8-7.2        | 5.6-6.0     | 5.4-5.6  | 5.2    | 5.6-5.9   | 5.5-6.0  | 5.8-6.3 |
| Mid-point<br>of range | 7.0            | 5.8         | 5.5      | 5.2    | 5.75      | 5.75     | 6.05    |
| Average               | 6.94           | 5.83        | 5.5      | 5.2    | 5.77      | 5.76     | 5.93    |

style. Clarke and Lubs' indicators were used for the estimations, drops of fluid, or fragments of tissue being mixed with the indicators on a white plate and the colours compared with those of the same indicators added to drops of standard buffer solutions; the usual corrections for salt error were made. The pH in twelve healthy animals all with firm, well-developed styles was determined, the range, mid-point of range and

average of each set of values being given. The results agree with those recorded (Yonge (1925b)) for *Pecten*, *Mya*, and *Ensis*, the style, which had in all cases a pH of 5.2, being the most acid substance in the gut, while of the fluids that of the stomach with an average pH of 5.5 is the most acid, followed by that of the mid-gut, oesophagus, rectum, and mantle cavity in the order named. The tissue of the digestive diverticula had an average pH of 5.77. Similar results were obtained with oysters starved for twelve weeks, as shown in Table XVI, the figures

TABLE XVI.

## pH IN STARVED OYSTERS.

| No.     | Mantle cavity. | Oeso-phagus. | Stomach. | Style. | Dig. div. | Mid-gut. | Rectum. |
|---------|----------------|--------------|----------|--------|-----------|----------|---------|
| 1.      | 7.0            | 5.7          | 5.5      | 5.4    | 5.8       | 5.8      | 6.0     |
| 2.      | 7.0            | 5.8          | 5.6      | 5.4    | 5.7       | 5.9      | 6.1     |
| 3.      | 6.8            | 5.8          | 5.65     | 5.4    | 5.8       | 5.8      | 5.9     |
| 4.      | 7.0            | 5.7          | 5.7      | 5.4    | 5.7       | 5.8      | 5.8     |
| 5.      | 7.0            | 5.8          | 5.6      | 5.4    | 5.9       | 5.8      | 6.0     |
| 6.      | 7.0            | 6.0          | 5.9      | —      | 5.9       | 5.9      | 6.0     |
| Average | 6.97           | 5.8          | 5.66     | 5.4    | 5.8       | 5.83     | 5.95    |

being slightly higher, i.e. conditions less acid, in all cases. The style was present in five out of the six oysters. The digestive diverticula were in all cases very pale, but the pH remained practically the same as in the fresh oysters.

It was shown in the paper cited above that the origin of the acidity of the gut lay in the style and *not*, as previously thought, in a secretion from the "liver." This provides, incidently, yet further evidence that the digestive diverticula do not secrete. By removing the style from *Mya* or inducing it to disappear from *Mytilus* by keeping animals out of water for four days or by placing them in boiled or deoxygenated water for six days, it was found that the pH of the stomach rose even though that of the mantle cavity fell on account of the accumulation of CO<sub>2</sub>. Similar results were obtained with *Crepidula* kept out of water for two days, and with *Tapes* whose shell valves had been clamped together for seven days.

The last method has been found the most satisfactory, and in Table XVII are shown the results of a series of experiments with oysters which had been clamped for one, two, three, four, five, and six days. Twelve animals were used for each experiment, each, after clamping, being replaced in the tanks and so kept at normal temperature. In all case

*the style was absent*, and, as a result of the accumulation of CO<sub>2</sub>, the average pH in the mantle cavity fell from the normal average value of 6.94 to 6.7, 6.56, 6.53, 6.51, 6.44, and, finally, 6.41 respectively after from one to six days clamping, while during the same periods the pH of the stomach rose from a normal of 5.5 to 5.67, 5.7, 5.84, 5.9, 6.02, and 6.14. Thus while the pH in the mantle cavity dropped by 0.53, the pH in the stomach

TABLE XVII.

## PH IN CLAMPED OYSTERS.

|                       | No. | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  | 11  | 12  | Average |
|-----------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|---------|
| Clamped 1 day.        |     |     |     |     |     |     |     |     |     |     |     |     |     |         |
| pH in mantle cavity . |     | 6.7 | 6.6 | 6.6 | 6.8 | 6.8 | 6.8 | 6.7 | 6.6 | 6.6 | 6.7 | 6.7 | 6.8 | 6.7     |
| pH in stomach .       |     | 5.7 | 5.7 | 5.6 | 5.7 | 5.6 | 5.7 | 5.6 | 5.7 | 5.8 | 5.6 | 5.7 | 5.7 | 5.67    |
| Clamped 2 days.       |     |     |     |     |     |     |     |     |     |     |     |     |     |         |
| pH in mantle cavity . |     | 6.6 | 6.7 | 6.6 | 6.6 | 6.5 | 6.6 | 6.6 | 6.4 | 6.4 | 6.6 | 6.6 | 6.5 | 6.56    |
| pH in stomach .       |     | 5.6 | 5.7 | 5.8 | 5.7 | 5.6 | 5.7 | 5.8 | 5.6 | 5.8 | 5.7 | 5.7 | 5.6 | 5.7     |
| Clamped 3 days.       |     |     |     |     |     |     |     |     |     |     |     |     |     |         |
| pH in mantle cavity . |     | 6.6 | 6.4 | 6.5 | 6.6 | 6.5 | 6.4 | 6.6 | 6.5 | 6.6 | 6.7 | 6.5 | 6.5 | 6.53    |
| pH in stomach .       |     | 6.0 | 5.8 | 5.9 | 5.8 | 5.8 | 5.8 | 6.0 | 5.8 | 5.6 | 5.9 | 5.9 | 5.8 | 5.84    |
| Clamped 4 days.       |     |     |     |     |     |     |     |     |     |     |     |     |     |         |
| pH in mantle cavity . |     | 6.6 | 6.4 | 6.6 | 6.5 | 6.4 | 6.6 | 6.5 | 6.6 | 6.6 | 6.5 | 6.4 | 6.4 | 6.51    |
| pH in stomach .       |     | 6.1 | 5.8 | 5.9 | 5.8 | 5.7 | 6.0 | 5.8 | 6.0 | 5.9 | 6.0 | 5.9 | 5.9 | 5.9     |
| Clamped 5 days.       |     |     |     |     |     |     |     |     |     |     |     |     |     |         |
| pH in mantle cavity . |     | 6.5 | 6.3 | 6.6 | 6.5 | 6.3 | 6.6 | 6.3 | 6.4 | 6.4 | 6.5 | 6.4 | 6.5 | 6.44    |
| pH in stomach .       |     | 6.1 | 5.8 | 6.0 | 5.9 | 6.0 | 6.1 | 6.0 | 6.0 | 6.0 | 6.0 | 6.1 | 6.2 | 6.02    |
| Clamped 6 days.       |     |     |     |     |     |     |     |     |     |     |     |     |     |         |
| pH in mantle cavity . |     | 6.5 | 6.4 | 6.6 | 6.5 | 6.7 | 6.3 | 6.3 | 6.4 | 6.4 | 6.1 | 6.5 | 6.2 | 6.41    |
| pH in stomach .       |     | 6.3 | 6.2 | 6.4 | 6.1 | 6.2 | 6.2 | 6.1 | 6.1 | 6.0 | 6.0 | 6.1 | 6.0 | 6.14    |

rose, on account of the absence of the style, due to decrease in the rate of secretion, by 0.64, so that it came near to that of the mantle cavity. This experiment, together with those cited above, leaves no doubt that the acidity of the gut is due to the dissolution in it of the style. It is important to note that the pH thus produced in the stomach approximates to the optimum pH for the working of the amylase of the style (5.9).

## II. PERMANENCE OF THE STYLE.

The view was advanced (1925b) and has recently been reasserted (1926a) that the style is dissolved by the fluid in the stomach, and is only maintained as a result of a balance between the rate of secretion and the rate of dissolution. The view that its presence is correlated with the presence of food has been disproved by the work of Orton (1923), Martin (1923), Berkeley (1923), and Yonge (1925b), all of whom showed that Lamellibranchs retain the style after long periods of starvation, provided

they are kept perfectly healthy, whereas in the presence of abundant food the style may be absent in unhealthy animals. As shown in Table XVI, five of the six oysters starved for twelve weeks retained the style.

The style is dissolved and reformed at very different rates in different animals. After artificial extraction of the style of *Mya*, Edmondson (1920) found that it took seventy-four days completely to regenerate (though this may have been due in part to the injury caused by the operation), while in *Ostrea virginica* Nelson (1925) states that it is alternately formed and dissolved in a rhythmical fashion. The style is large and firm at flood tide when the animals are feeding actively, but at late ebb tide when "most of the sand has been sorted out and removed from the stomach and digestion is well under way the style may be reduced to a soft amorphous mass of jelly." A similar rhythm is shown in the production of other forms of digestive secretion, such as that of the salivary glands of Gastropods (Hirsch (1914), Krijgsman (1925)).

In *Ostrea edulis* the style is usually present as shown in Table XVIII. Out of fifteen healthy animals examined, all of which had been in the

TABLE XVIII.

## CONDITION OF STYLE AND DIGESTIVE DIVERTICULA IN FRESH OYSTERS.

| No. | Condition of Style. | Condition of Dig. Div. |
|-----|---------------------|------------------------|
| 1.  | Large, firm.        | Pale.                  |
| 2.  | " "                 | Dark.                  |
| 3.  | Absent.             | "                      |
| 4.  | Large, firm.        | "                      |
| 5.  | Medium, soft.       | "                      |
| 6.  | Large, firm.        | "                      |
| 7.  | " "                 | "                      |
| 8.  | " "                 | Pale.                  |
| 9.  | " "                 | Dark.                  |
| 10. | " "                 | "                      |
| 11. | Absent.             | "                      |
| 12. | Large, firm.        | "                      |
| 13. | " "                 | Pale.                  |
| 14. | " "                 | Dark.                  |
| 15. | " "                 | "                      |

tanks for a week so as to provide time for recovery from the effects of the journey from the beds, in only two cases was the style absent, and this was not correlated with the colour of the digestive diverticula, paleness of which Orton (1923) thought might be connected with absence of the

style. All the animals appeared in good condition; in those obviously in bad condition, with flabby watery tissues, the style is frequently absent or much reduced.

The style invariably disappears when oysters are kept out of water for any length of time. Table XIX shows the results of a series of experiments to test the speed at which the style was dissolved, thirty-six healthy oysters being kept out of water and opened six at a time at one-hour

TABLE XIX.

| CONDITIONS OF STYLE AFTER REMOVAL OF OYSTERS FROM WATER. |                           |                           |                           |
|--|---------------------------|---------------------------|---------------------------|
| Oysters out of water for:—                               |                           |                           |                           |
|  | 1 hour.                   | 2 hours.                  | 3 hours.                  |
| No.  | Condition of style.       | Condition of style.       | Condition of style.       |
| 1.   | Soft, $\frac{1}{2}$ size. | Firm, $\frac{3}{4}$ size. | Absent.                   |
| 2.   | Absent.                   | Soft, $\frac{1}{2}$ size. | „                         |
| 3.   | Practically intact.       | Absent.                   | Soft, $\frac{1}{4}$ size. |
| 4.   | Intact.                   | Soft, $\frac{1}{4}$ size. | Absent.                   |
| 5.   | „                         | Practically intact.       | „                         |
| 6.   | Firm, $\frac{3}{4}$ size. | „ „                       | Practically intact.       |
|  | 4 hours.                  | 5 hours.                  | 6 hours.                  |
| No.  | Condition of Style.       | Condition of Style.       | Condition of Style.       |
| 1.   | Absent.                   | Absent.                   | Absent.                   |
| 2.   | „                         | „                         | „                         |
| 3.   | „                         | „                         | „                         |
| 4.   | „                         | „                         | „                         |
| 5.   | „                         | „                         | „                         |
| 6.   | „                         | „                         | „                         |

intervals. After one hour in only one case was the style absent, and the same conditions were found after two hours, although the styles were much reduced. After three hours only two animals possessed styles, while after four, five, and six hours in no case was a style present.

It was found in the previous work on the subject (1925b) that styles were dissolved rapidly in alkaline or slightly acid media, but increasingly slowly as the pH was reduced until at a certain critical pH—probably corresponding to the isoelectric point of the globulin of the style—it ceased to be dissolved. This critical pH varied for the styles of different animals, being 4.4 for *Ensis*, 4.2 for *Mya*, and 3.6 for *Pecten*, *Mytilus*, and *Crepidula*. It was suggested that the differences might be due to the fact that in the former cases the style is lodged in a separate cæcum, and is a much firmer and more resistant body than in the other three in which it lies in free communication with the gut.



In Table XX are shown a similar series of experiments carried out on the styles of *Ostrea*, large, firm styles being placed in tubes containing 10 c.c. of standard buffer solutions, a little toluol being added to prevent decomposition. The styles were dissolved rapidly in pH between 10

TABLE XX.

## DISSOLUTION OF STYLE IN DIFFERENT pH.

| pH   | Length of style. | Time to dissolve. | pH.  | Length of style. | Time to dissolve. |
|------|------------------|-------------------|------|------------------|-------------------|
| 10.0 | 2.5 cm.          | 56 min.           | 2.6  | 2.6 cm.          | 90 min.           |
| 9.0  | 2.5 cm.          | 70 min.           | 2.3  | 3.0 cm.          | 22 hrs.           |
| 8.0  | 2.5 cm.          | 70 min.           | 1.9  | 2.5 cm.          | 15 days.          |
| 7.0  | 2.6 cm.          | 61 min.           | 1.65 | 2.4 cm.          | 13 days.          |
| 6.0  | 2.4 cm.          | 75 min.           | 1.42 | 2.8 cm.          | 13 days.          |
| 5.0  | 2.5 cm.          | 87 min.           | 1.25 | 2.4 cm.          | 9 days.           |
| 4.0  | 2.3 cm.          | 90 min.           | 1.14 | 3.1 cm.          | 7 days.           |
| 3.0  | 2.8 cm.          | 88 min.           | 1.04 | 2.6 cm.          | 7 days.           |

and 2.6, more slowly at pH 2.3 and extremely slowly—it took fifteen days for a style 2.5 cm. long to be dissolved—in pH 1.9. Below this point dissolution was also very slow, though gradually increasing in speed down to pH 1.04, in which a style of 2.6 cm. took seven days to dissolve.

Unlike the other styles that of *Ostrea* is dissolved in all media, although the difference between the fifteen days needed for the process at pH 1.9 and the fifty-six minutes needed at pH 10 is very striking. Repeated experiments have confirmed these figures. The isoelectric point, 1.9, is much lower than the lowest, 3.6, recorded for the other molluscs examined, and as the style in *Ostrea* is exceptionally unstable, this gives additional evidence of the connection between the isoelectric point and the site of formation, and consequent firmness, of the style.

It is clear from the above experiments that the style must speedily be dissolved by the fluid in the stomach. It has been shown definitely that the presence of food is *not* necessary for the formation of the style, while Berkeley's theory that the style is a reserve of oxygen which is used in anaerobic respiration cannot be substantiated in view of the fact that there is no correlation between the size of the style in different species and the nature of the habitat; a criticism which has also been made by Nelson (1925). Moreover, in such animals as *Siliqua*, *Schizothærus*, *Macoma* (Edmondson (1920)), and *Mya* (Edmondson, Yonge (1923)), in which the style lies in a *separate cæcum* and so is protected from the action of the fluid in the gut, the style never dissolves even after death from starvation or from lack of oxygen. The style is continually being

dissolved, and is only maintained by the continual secretion of new substance in the style-sac ; any lowering of the vital or metabolic activities of the animals is at once reflected in a reduction or stoppage in the rate of secretion, but the rate of dissolution remains constant and the style disappears. This is a purely physical reaction which cannot take place if the style is protected in a cæcum. The gradual reduction in secretion is indicated by the gradual rise in pH in the clamped animals. Absence of a style is, therefore, an indication of lowered metabolism, and it is noteworthy that Allen (1921) has found that the style is formed less readily in autumn and winter than in summer, and has proved by experiment that this is the direct result of the difference of temperature. Edmondson also states that the style of *Mya* is reformed more rapidly after excision in summer than in winter. Allen further notes that there is no rhythmical loss and renewal of the style in fresh-water Lamellibranchs as there is in marine, tidal species ; the result, clearly, of the equable conditions under which the former species live. Spärck (1925) considers " that absence of the crystalline style must be interpreted as an indication of something not quite normal, as regards the state of metabolism or nutrition." Adverse conditions of any kind will cause a lowering of metabolism, and this, in Lamellibranchs such as the oyster, will result in the partial or total dissolution of the style, the state of which presents a valuable index of the condition of the animal.

## 7. RESERVE FOOD MATERIALS.

It is fitting that some reference should be made to the nature and distribution of the reserve food materials. So much work has already been carried out on this subject (the most recent investigation is that by Russell (1923), which contains a summary and bibliography of previous work) that further research was considered unnecessary. As already noted, fat is stored in the oyster particularly in the vesicular connective tissue cells, or Langer's vesicles, and is also present in the epithelium of the gut and of the digestive diverticula. Traces were found in the connective tissue after three months starvation. Material fixed in Carnoy's fluid and treated with iodine shows the presence of masses of glycogen in the vesicular connective tissue, but never in the epithelium of the digestive diverticula or of the gut. These results are in complete accordance with those of previous workers both on the oyster and on other Lamellibranchs.

Quantitative estimations of the fat and glycogen in oysters made by the Government Chemist (Russell) and by previous workers show that the latter is much the more abundant (ranging between 21.34 and 40.04%, according to the analyses of the Government Chemist), while "fattening"

of oysters is to be attributed, as pointed out by Mitchell (1916a), "to the accumulation of glycogen, which must be regarded as the chief storage substance for oysters." The same author (1916) has further found that oysters kept in a weak solution of glucose show an increase in the amount of glycogen in the tissues. There is a seasonal variation in the quantity of glycogen, which in the oyster, according to the estimations of the Government Chemist, is constantly high from July to January, the total carbohydrate and glycogen approximating closely, showing that practically all the carbohydrate is in the form of glycogen. It is to be assumed, as Russell points out, that "during this period the oyster accumulates reserve food substance in the form of glycogen." From February to April there is a fall in the glycogen content, although the total amount of carbohydrate remains constant, the former being presumably "broken down into an assimilative form which is then, in May and June (when the total carbohydrates fall), utilised in the formation of the sexual products" (Russell). There can be no doubt as to the primary importance of glycogen in the physiology of the oyster and all Lamellibranchs in which it seems to play the same part as does fat in the vertebrates. This throwing of the balance of metabolism on to the carbohydrate side is in close accordance with the results recorded on the nature of the digestive processes.

#### 8. GENERAL DISCUSSION.

In the oyster the organs of feeding and digestion are specialised for dealing with small particles *exclusively*. The elaborate ciliary mechanisms in the mantle cavity with the accompanying secretion of mucus ensure the capture of fine particles in suspension, of which the selective mechanisms reject the larger particles or mucus laden masses and allow only the smaller ones to pass to the mouth. There is a reduction in the individual size and general bulk of the particles swallowed, but no indication of any selection of particles having definite food value. In the gut, cilia and mucus glands are also universally distributed, ciliary activity, either directly or by the agency of the style, having taken the place of the muscular peristalsis necessary for the passage of large particles through the gut. The style is clearly correlated, here as elsewhere, with the presence of cilia, mucus glands, and a finely divided, and principally vegetable food.

The purely mechanical process of feeding is confirmed by the results of investigations into the stomach contents of oysters and other Lamellibranchs. Thus Savage (1925) states that "the oyster appears to ingest anything suitable that it can capture, and no evidence was found to show that selection takes place." The work of Savage and previous

investigators (quoted in detail by him) shows that in the stomach are found samples of all matter in suspension in the water in which the oysters live, and it is not surprising, therefore, to find that in the Limfjord, where there is a great development of *Zostera* and of detritus formed by its decomposition, the stomach contents of oysters should consist largely of detritus. This has led the Danish workers, notably Petersen (1911), Boysen Jensen (1914), Blegvad (1914), and Spärck (1925), to maintain that oysters are by nature detritus eaters. Recent American workers such as Nelson (1921), Churchill (1920), and Martin (1923) all consider that animate matter, and particularly diatoms, is of primary importance in the food of the oyster. Savage found that at Orford inanimate material provided the bulk of the stomach contents, animate matter never exceeding 10%. Hunt (1925), in his account of the stomach contents of Lamellibranchs, states that they consist of a mixture of micro-organisms and detritus. He is at variance with Blegvad in the latter's classification of Lamellibranchs as detritus feeders, adding, very aptly, that "When sand-grains are numerous in a stomach the proportion of detritus is correspondingly great, and the organisms present are largely bottom-living forms, but there is no reason to suppose that this preponderance of detritus signifies its value as food any more than the abundance of sand suggests the nutritive value of silica." Reviewing these results it is seen that the majority of workers have accepted the presence of material in the stomach of oysters or other Lamellibranchs as proof that it has been *deliberately swallowed and can be digested*. The Danish workers, in particular, do not appear to have studied either the mechanism of feeding or of digestion in Lamellibranchs.

Digestion is largely intracellular either in the tubules of the digestive diverticula or in the phagocytes. This is clearly correlated with the finely divided nature of the food, which is again sorted in the food cæcum in the stomach only the most minute particles entering the ducts and tubules of the digestive diverticula, the ramifications of the latter providing the large ingesting surface typical of the gut of animals which digest intracellularly. The larger particles in the gut are taken in directly by phagocytes. The only extracellular enzymes are those of the style which act exclusively on carbohydrates. This feeble development of extracellular digestion and particularly the complete absence of extracellular protease and lipase accounts for the passage of living organisms undamaged through the gut, their presence in the rectum and fæces having been noted by many authors, including Blegvad (1914), Coker, etc. (1921), Allen (1921), and Churchill and Lewis (1924). The two first of these, however, have drawn from the presence in the fæces of living diatoms, green algæ and other plankton organisms the *quite erroneous conclusion* that these are either useless or of secondary importance as

food. Attention to this error has also been drawn by Nelson (1925). Sherwood (statement in Savage's paper) and Nelson (1921) have both noted the presence of living oyster larvæ in the fæces of the adults.

An examination of the enzymes shows that oysters are unable to digest everything which enters the stomach, whereas the contrary has been too often assumed. Thus there is no indication of digestive action on cellulose or pentosans by the enzymes of either the style or the digestive diverticula. Boysen Jensen (1914) found that pentosans were the only non-nitrogenous substances present in estimable quantities in the detritus of the Limfjord, but the only evidence he could produce as to digestion was that pentosans are digested by herbivorous mammals and that cellulose is digested by *Helix*, finally stating that, "We may then perhaps conclude that also bivalves are able to digest pentosan, and that the considerable amount of pentosan present in the sea bottom—besides other possible substances (hemicelluloses generally) plays an important part as non-nitrogenous nourishment for a great portion of the bottom fauna." The known facts of the comparative physiology of digestion indicate that conditions in Mammals and Gastropods have *no bearing whatever* on conditions in Lamellibranchs, in which the digestive processes are particularly characteristic, and of which the only members capable of digesting cellulose (and there is no evidence as yet that they can digest pentosans) are the highly specialised wood-boring Teredinidæ.

Like all Lamellibranchs, oysters are particularly adapted for the digestion of carbohydrates. The only extracellular enzymes are those which digest starch and glycogen, while extracts of the digestive diverticula reveal the presence of powerful sacroclastic enzymes capable of digesting a variety of carbohydrates. On the other hand, lipoclastic and proteoclastic enzymes are very weak, and fats and proteins are probably digested largely in the phagocytes. In close connection with this concentration on the digestion of carbohydrates is the storage of great quantities of glycogen which represent the principal reserve food material. There is a close parallel to these conditions in *Ciona* (Yonge (1925)), in which digestion is also concentrated on carbohydrates, and there are large reserves of glycogen particularly in large cells in the epithelium of the mid-gut. It is clearly this dependence on carbohydrates which has enabled the Teredinidæ to live on a diet consisting almost exclusively of the carbohydrates in wood.

It follows that the food of the oyster must consist of small organisms rich in carbohydrates, i.e. of microscopic plant life. The following table taken from the paper of Brandt (1900) shows the relative amounts of protein, chitin, fat, and carbohydrates in the ash-free dried substance of diatoms, peridinians, and copepods.

|              | Diatoms<br>% | Peridinians<br>% | Copepods<br>% |
|--------------|--------------|------------------|---------------|
| Protein      | 28.7         | 13.7             | 65.1          |
| Chitin       | —            | —                | 5.1           |
| Fat          | 8.0          | 1.37             | 7.7           |
| Carbohydrate | 63.2         | 84.9             | 22.1          |

There is a much greater proportion of carbohydrates in the two former. Russell considers that growth is due to an increase in protein and "fattening" to an accumulation of carbohydrates; and the connection between "fattening" and the presence of large numbers of diatoms in the food has been noted by many workers, including Nelson (1921) and Savage. No doubt, in the spring, the abundance of algal spores provides ideal food, with their high carbohydrate content and delicate structure which renders them easy to assimilate. Martin (1923) has drawn attention to the importance of nannoplankton, especially small flagellates and peridinians, in the food of the oyster, and the structure and physiology of the digestive system supports this, since it is only organisms of this size which are ingested entire in the digestive diverticula. Only fragments of diatoms seem to be so ingested—whole diatoms are digested by the phagocytes—and it is only "detritus" of *this* nature, i.e. fragments of vegetable matter containing assimilative carbohydrates, which can be of use to the oyster.

It is clear from the results of this research that ideal conditions for "fattening," and incidently reproduction, in the oyster are found in the presence of abundant supplies of diatoms, peridinians, algal spores, and other microscopic vegetable matter. It is the quantity of *carbohydrate* which is important, the protein matter necessary for growth is probably always present in excess of the demands and powers of digestion of the oysters. Such conditions are provided artificially in the "claires" at Marennes and other places along the French coast. Immense numbers of diatoms and other microscopic organisms accumulate in them, and the speed with which the oysters "fatten" is proof positive of the fitness of the environment.

## 9. SUMMARY.

1. The anatomy and histology of the food collecting and alimentary organs of the adult oyster are described.

2. The anatomy of the stomach is investigated with the aid of gelatin casts and attention drawn to the food cæcum, the ventral groove, and the two ducts of the digestive diverticula.

3. Cilia and mucus glands are universal throughout the food collecting and alimentary organs.
4. There is evidence that the gastric shield is composed of fused cilia.
5. There is no evidence of secretion in the digestive diverticula.
6. The histology of the style-sac resembles that described by Mackintosh for *Crepidula*. There is evidence that secretion of the style takes place in the groove.
7. Phagocytes are everywhere numerous in the blood vessels, connective tissue and epithelia, and free in the gut and mantle cavity.
8. The alimentary organs of the larva are described.
9. The anatomy and histology of these organs in the "spat" is described, the palps are relatively large and the gills asymmetrical. The style-sac is distinct from the mid-gut.
10. The course of the ciliary currents on the gills and palps is described and the importance of the various selective mechanisms emphasized. Selection appears to be purely quantitative, large particles or mucus masses being rejected and smaller ones accepted.
11. Muscular activity is of great importance in the functioning of both gills and palps. Reversal of cilia has never been seen.
12. Rejected matter is removed from the mantle cavity.
13. Material is sorted in the food cæcum in the stomach, larger particles passing into the mid-gut and smaller ones towards the gastric shield and ducts of the digestive diverticula, within the tubules of which there is a constant circulation.
14. The rotation of the style assists in the stirring of matter in the stomach.
15. In the style-sac are cilia, which rotate the style and others which push it into the stomach.
16. In the larva the velum acts as a food collecting organ; the style lies in an extension of the stomach and rotates rapidly. Material passes freely into the digestive diverticula.
17. In the spat rejective mechanisms are highly developed. The style revolves at a speed of between sixty and seventy revolutions per minute.
18. The tubules of the digestive diverticula are the only place where soluble matter is absorbed, in adult, larvæ, or spat.
19. Fine particles are ingested and digested intracellularly in the tubules of the digestive diverticula, the products of digestion carried away by amœbocytes, and useless matter rejected into the lumen.
20. Larger particles are ingested and digested by phagocytes in all parts, the products of digestion being carried to the vesicular connective tissue cells and there stored.
21. Enzymes in the style digest starch and glycogen. The amylase, at pH 5.9, has an optimum temperature of 43° C., and is destroyed at

56° C. The optimum medium is pH 5.9. It is inactivated by purification with absolute alcohol or by dialysis, but action is restored on the addition of chlorides or bromides and to a less extent iodides, nitrates, and carbonates, but not with sulphates or fluorides.

22. Sucroclastic enzymes in the digestive diverticula act on starch, glycogen, sucrose, raffinose, maltose, lactose, salicin, and amygdalin, but not on inulin, cellulose, or pentosans.

23. The amylase, at pH 5.5, has an optimum temperature of 44.5° C., and is destroyed at between 64 and 67° C. It has an optimum pH of 5.5, and is inactivated after purification or dialysis, action being restored in the presence of chlorides or bromides.

24. There is a weak lipase and protease, the latter has two optima at pH 3.7 and at or above 9.0; its action is very slow.

25. The only enzymes free in the stomach are those from the style.

26. There is no evidence of any enzymes free in the gill mucus.

27. There is a powerful complete oxidase system in the style, and a catalase in the digestive diverticula and gonad, and traces in the palps, gills, and muscle.

28. The style is the most acid substance in the gut and the cause of the acidity of the gut.

29. The style is dissolved rapidly in fluid of pH 2.3 and above, but very slowly below that point. It is readily dissolved and reformed in the oyster, its presence depending on the maintenance of the balance between the rate of secretion and the rate of dissolution. Its condition is a valuable indication of the state of metabolism.

30. Glycogen and fat are stored, particularly in the vesicular connective tissue cells, the former furnishing the principal reserve food material.

31. The presence of abundant supplies of microscopic plant life rich in carbohydrates provides ideal food for the oyster, and represents optimum conditions for "fattening" and reproduction.

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