STUDIES ON THE COMPARATIVE PHYSIOLOGY OF DIGESTION.

I.—The Mechanism of Feeding, Digestion, and Assimilation in the Lamellibranch Mya.

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1. Introduction.

There is no aspect of invertebrate physiology which has been more neglected in recent years than that of digestion. The attempt has been made in this paper, the first, it is hoped, of

a series on the subject of digestion, to give as complete an account as possible of the course and disposal of food particles taken in through the inhalant siphon of *Mya arenaria*, and of the organs with which they come in contact. Previous workers on the Lamellibranchia have, without exception, confined their attention to particular aspects of the problem. Thus we have the work of Stenta, Wallengren, Orton, Kellogg, on the ciliary currents; of Coupin, Mitra, Nelson, Edmondson and others on the crystalline style; of Fredericq and Roaf on the other digestive enzymes; of Gutheil, Matthias, Thiele, and Siebert on the histology of the food collecting organs and alimentary canal; of Peck, Ridewood, and others on the gills; and finally, of particular interest in this research, the careful monograph of Vlès on the anatomy of *Mya arenaria* itself. The literature bearing on each of these points will be reviewed in detail at the appropriate place.

Mya arenaria has been chosen for this research for a variety of reasons. It is of common occurrence along our coasts and is one of the largest of British Lamellibranchia. It is extremely hardy and can, if kept in a cool place, be maintained alive and healthy out of water for a fortnight or longer. As already stated it has been ably monographed by Vlès,[44] while Edmondson,[18] working in the United States, employed it in his experiments on the regeneration of the crystalline style. It has been found exceptionally suitable for the application of experimental methods.

The investigation has been carried out under the supervision of Professor J. H. Ashworth. The greater part of the work on the enzymes was carried out in Dr Crew's laboratory at the Animal Breeding Research Department, under the direction of Dr L. T. Hogben, Lecturer in Experimental Zoology, to whom the author is also indebted for many suggestions on other sections of the work. The expenses of the research were defrayed by a grant from the Earl of Moray Fund, and the micrographic illustrations are due to Mr J. M. A. Chisholm, Artist to the Animal Breeding Research Department.

2. Description and Habits.

In *Mya arenaria*, the Clam, the two shell valves are practically equal in size, the right valve being slightly the larger of the two. In shape it is irregularly ellipsoidal, the anterior end being blunter and more smoothly rounded than the posterior end which is drawn out a little more. A good average specimen is 6 in. in length from end to end and $3\frac{1}{4}$ in. wide at the umbal region. The umbo is only slightly prominent and points anteriorly. When the valves are adducted to the utmost extent there is still a large gape at the posterior end in which lie the retracted siphons. When fully extended the siphons, over which the periostracum of the shell is continued, may reach a length of 50 cm. (Vlès). The mantle edges are fused except for a short extent on the antero-ventral surface where the pedal opening is found. The small wedge-shaped foot, which can be protruded through this opening, is situated, as in all burrowing forms, on the anterior edge of the visceral mass.

Mya arenaria has a wide distribution throughout the Northern Hemisphere. It occurs usually at the mouths of rivers and is not to be found on open sea beaches. Weymouth[49] states in explanation that, as it increases in size, Mya loses the ability to move quickly and hence to care for itself when exposed. Storms are thus fatal to the larger specimens. Moreover, it is incapable of renewing communication with the surface of the ground when its siphons have been choked by shifting sand; it needs protection and a firm substratum in which a hole is semi-permanent. At the same time the water from which it strains its food must not be stagnant but moving, and contain a supply of micro-organisms sufficient for the needs of the animal. Provided the above conditions are fulfilled, Mya will thrive in water that is brackish even although the temperature falls below 0° C.

It is obvious that these ideal conditions will be found in the tidal mud of a river estuary, and it is here that Mya occurs as one of the characteristic members of the brackish water fauna. The specimens on which this work has been performed were obtained from the estuary of the River Esk at Musselburgh, near Edinburgh.

During life the animal lies entirely buried, anterior end downward, in the mud, with only the tip of its siphons flush with the surface (fig. 1).

Alder and Hancock[1] have described it as it occurs at the mouth of the River Tyne. "Mya . . . buries itself to a depth of 6 or 8 in. in a stiffish clay mixed with shingle; and, in shallow pools left by the tide, the siphonal tubes may be seen just level with the surface of the muddy bottom in full action. The mud lies closely packed against the walls of the tubes, so that nothing is to be seen but the internal surface of the expanded lips of the siphonal orifices fringed with numerous tentacles. When it happens that the surface of the water is only a little above the orifices, a strong current can be distinctly seen to boil up from the anal siphon, and another, with a constant steady flow, to set into the branchial one. These currents were quite visible to the naked eye without the aid of a glass, so long as the mollusc remained undisturbed."

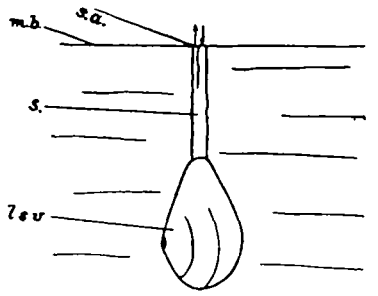

FIG. 1.—*Mya arenaria*, in natural habitat. l.s.v., left shell valve; m.b., muddy bottom; s., siphon; s.a., siphonal apertures with arrows showing direction of water currents.

3. Feeding.

a. **Literature.**—The literature dealing with the ciliary food currents of the Lamellibranchia is extensive, but comparatively little of it is of any great importance. The presence of inhalant and exhalant currents and of food currents on the gills and palps in Mya was demonstrated by Alder and Hancock[1] in 1851. M'Alpine,[27] in 1888, published a paper on "the movements of the entire detached animal, and of detached ciliated parts of bivalve molluscs," in which he described numerous experiments which revealed the presence of cilia on all free surfaces in the mantle chamber. He was erroneous in his views that "the palps act as guards and not guides to the mouth," and that food is not passed on from the gills to the mouth. Coupin,[9] in 1893, gave an account of the

removal of foreign matter from the mantle chamber of certain Lamellibranchs. In 1886 Thiele[42] described the labial palps of certain Lamellibranchs and gave his views on their physiology, but he regarded them not only as food conducting organs, but also as "Nebenkiemen," *i.e.* partly respiratory in function. List,[26] in his classic work on the Mytilidæ, showed that the palps in these forms are food conducting organs. Stenta[40, 41] has described the backwardly directed currents present in the mantle chamber in certain species.

The most important contributions on the subject are, however, those of Kellogg,[23, 24] Wallengren,[47, 48] and Orton.[31] The first, in a series of papers, has described with great detail the ciliary mechanisms present in a large number of species. It has been found impossible to agree with his views as to the direction of food currents on the palps. Wallengren's papers on "Die Wasserströmungen" and "Die Nahrungsaufnahme" in certain Lamellibranchs (including Mya) are of the utmost importance. It is a great misfortune that his work is so little known; both Kellogg and Orton, who worked to a large extent after the publication of his papers (1905), appear to have been unaware of them. He has described and figured the ciliary currents, and in particular those of the palps, with the greatest care. Orton has worked upon the ciliary currents in a number of species, and has also given an account of the functions of the different groups of cilia borne upon the gill filaments. Siebert,[39] in a paper on the histology of the body epithelium of *Anodonta cellensis*, records his observations on the physiology of the gills and palps. Allen [2] has given an account of the food and feeding habits of freshwater mussels. With regard to the gills, the only observer (apart from Vlès) who has figured the gill of Mya is Posner,[35] whose paper is of little value owing to his belief that the gill filaments arise as a result of the splitting up of flat lamellæ—the exact converse of the true explanation. The work of Peck[32] and Ridewood[34] is of great importance, furnishing as it does the basis of our present understanding of the structure and origin of the gills among the Lamellibranchia.

b. **Methods.**—The structure of the gills and palps has been studied in the organs *in situ*, while pinned out for more careful observation, and in microscopic sections. They have been examined carefully in the living condition under the binocular, and under medium powers of the compound microscope, in order to discover the nature and direction of beat of the cilia. Carmine, and in certain cases sand, grains have been employed whenever necessary in order to demonstrate the course of the currents. It is of great importance when using carmine to allow the particles to settle and, best of all, get entangled in the mucus, which is freely secreted on irritation by all free surfaces, before beginning to take note of the direction in which they are carried. Sections fixed in Bouin's fluid and stained with Delafield's hæmatoxylin and erythrosin, or with picro-indigo-carmine, have been employed for the histological examination of these tissues. The latter stain is the better for demonstrating the presence of cilia and of the chitinous rods in the gill filaments. The ciliary currents on the mantle folds and visceral mass are easily observed by the naked eye after a little carmine has been added to the water.

c. **Circulation of Water through the Mantle Chamber.**—The mantle chamber communicates with the exterior by three openings: the inhalant (branchial) and exhalant (anal) siphonal apertures, and the pedal opening (see fig. 2). When the animal is living under natural conditions the pedal opening is of no apparent use, but if a specimen be placed in water a slight current is observed to pass in through this aperture, while on removal from the water, the contraction of the shell adductors and of the siphons causes a powerful stream of water to be expelled through it.

The presence of currents passing in and out of the siphonal openings has already been commented upon. Water passes in through the inhalant or ventral siphon. The orifice is edged with a ring of short inwardly directed tentacles. The tentacles of freshwater mussels have been referred to by Allen[2] as possessing tactile and gustatory functions. "Upon being disturbed mechanically they are withdrawn into the shell, while a continued teasing or a strong chemical stimulus results in the closing of the shell, or perhaps only the siphons." That

they do possess a tactile sense, causing the aperture to become closed on the application of mechanical stimulus, or whenever the water becomes too heavily laden with particles, would appear to be the case, but the presence of anything in the nature of a sense of taste is far from proved.

The water, laden with food particles, passes into the infrabranchial cavity where it is strained through the gills, leaving all foreign particles behind. It then passes dorsally into the suprabranchial cavity, which lies between the gills and the dorsal region of the mantle, and finally makes its

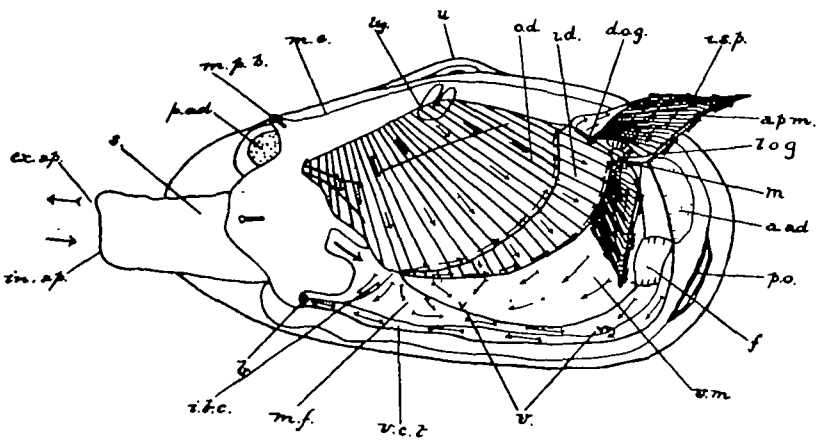

FIG. 2.—*Mya arenaria*, right shell valve and mantle fold removed. × ½. a.ad., anterior adductor; a.p.m., anterior palp margin; b., bay; d.o.g., distal oral groove; ex.ap., exhalant aperture; f., foot; i.b.c., infrabranchial cavity; i.d., inner demibranch; in.ap., inhalant aperture; i.s.p., inner surface of palp; lig., ligament; l.o.g., lateral oral groove; m., mouth; m.e., mantle edge; m.f., mantle fold; m.p.b., mantle pinned back; o.d., outer demibranch; p.ad., post. adductor; p.o., pedal opening; s., siphon; u., umbo; v., vortex; v.c.t., ventral ciliated tract; v.m., visceral mass. Plain arrows denote direction of ingoing currents; feathered arrows denote direction of outgoing currents.

way out through the exhalant siphon. The mechanism which induces this water current is to be found in the ciliation of the gill filaments and will be dealt with later.

When a specimen of Mya is strongly stimulated, or removed from the water in which it has been lying with expanded and freely functioning siphons, violent contraction takes place. The shell valves are forced together and the siphonal tubes withdrawn within the pallial sinus. At the same time the exhalant aperture is closed so that water is expelled through the inhalant siphon—a reversal of the normal procedure.

There is a communication between the infrabranchial and suprabranchial cavities, the upper surface of the inner demibranchs on either side being unattached to the visceral mass for a short distance about midway in their course, thus leaving a slit-like opening. Normally the free upper surface is closely applied to the visceral mass, so that no water passes through the opening except under the exceptional circumstances just related.

d. **Food.**—The stomach contents consist of very finely divided particles of organic debris, sand, and micro-organisms, *e.g.* diatoms, singly and in chains; Foraminifera; minute, probably larval, bivalves; ostracods and other microscopic Crustacea, with parts of larger specimens; spores and eggs of various kinds; sponge spicules, and spines of all sizes. The great mass of material consists of small sand grains. The largest particles present are thin filamentous strips of alga up to 1 mm. in length, the largest solid particles being not more than $\frac{1}{8}$ mm. in diameter, while the majority do not exceed $\frac{1}{50}$ mm. in diameter. This fine division is the result of the very beautiful sorting mechanism present on the palps.

e. **Gills**—(1) *Structure.*—The gills of *Mya arenaria* have been figured by Posner,[38] but neither his figure nor his observations are of any value. Ridewood[54] figures the gills of Lutraria, a closely allied genus, and makes a few comments on those of Mya. Vlès[44] gives an account of the morphology of the gills, but makes no reference to the ciliary currents. Their structure is that of a typical eulamellibranch gill. Four demibranchs are present, two on either side of the visceral mass, and are attached at the junction of the visceral mass and mantle. Posteriorly they arise immediately ventral to the exhalant siphon (fig. 2), and for some distance the gills of either side are united by the upper surfaces of their inner demibranchs. They are parted by the visceral mass for the rest of their extent. Ventrally they stretch over two-thirds of the visceral surface. The outer demibranchs (o.d.) extend anteriorly to the posterior edge of the labial palps, but the inner demibranchs (i.d.) are continued forward between the palps for half the distance of their united edges.

A cross section of the gill lamellæ (fig. 6) exhibits the

following structure. The individual filaments are extremely small and are frequently fused by lateral interfilamentary junctions (i.f.j.), thus forming a firm lamella. The plication is well marked, the ascending and descending lamellæ of each demibranch being united by interlamellar junctions about every thirty-three filaments.

The individual filaments (fig. 3) when highly magnified exhibit an interesting structure. Frontal and large latero-frontal cilia are present and can easily be seen in well-fixed sections. The lateral cilia are smaller and more difficult to distinguish. There are no abfrontal cilia developed on the interlamellar end of the filament. A striking feature of the gill structure is the occurrence of two stout curved rods lying one on either side of the frontal ends of the filament. They stain a bright green with picro-indigo-carmine and show up much more vividly than the internal chitinous skeleton. They would appear to be formed of fused cilia, but no reference to similar structures in any previously described gill has been found. The interlamellar end of the filament may be greatly enlarged and extend for some distance into the cavity (see fig. 6).

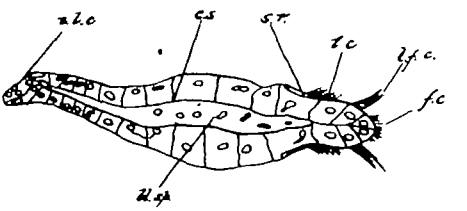

FIG. 3.—Mya. Transverse section of single filament. × 330. bl.sp., blood space; c.s., chitinous skeleton; f.c., frontal cilia; l.l.e., interlamellar end; l.c., lateral cilia; l.f.c., latero-frontal cilia; s.r., strengthening rods.

The free ventral edges of the demibranchs are characterised by the presence of food grooves. If the gill be examined in the living condition the groove is found to be wide open, but the sides are invariably drawn together during fixation. The transverse section of the edge of the demibranch (fig. 4) demonstrates the interesting fact that the groove is not situated precisely at the junction of the ascending and descending lamellæ, but a little to one side. In the outer demibranch the groove is a little to the outer side, and in the inner demibranch a little to the inner side. The large cilia characteristic of this region are not shown in the section.

(2) *Physiology.*—The lateral cilia of the gill filaments are responsible for the water currents which pass through the mantle chamber. Beating inwardly, they produce a streaming of the water into the interlamellar cavity, whence it passes up between the lamellæ into the suprabranchial cavity. Particles of any kind present in the water are caught by the large latero-frontal cilia of adjacent filaments, which interlock and form a sieve. The presence of the current can be demonstrated by pinning out a strip of gill under water and extending the lamellæ so that the latero-frontal cilia no longer meet. If carmine grains be added to the water they will be seen to be carried past the latero-frontal cilia into the groove between the two filaments, and to accumulate there in masses. Moreover, in many cases when examining animals that had been kept in the laboratory for some time, it was found that the gills were black in parts. This was due to the presence of particles of mud in the interlamellar cavities owing to the atrophy of the latero-frontal cilia, which apparently are more easily affected by adverse conditions than the lateral cilia.

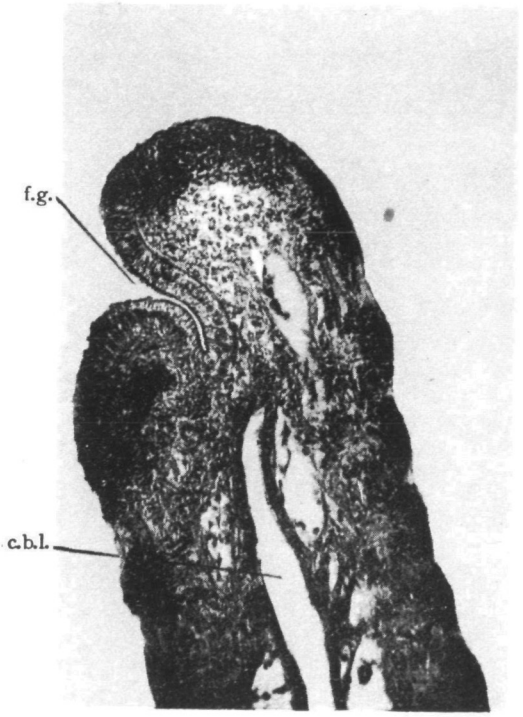

FIG. 4.—Mya. Cross section through food groove. × 80. c.b.l., cavity between ascending and descending lamellæ; f.g., food groove.

The movement of the latero-frontal cilia (l.f.c., fig. 5) tends to throw the particles caught among them towards the middle of the frontal edge of the filament where they are caught in the current created by the frontal cilia (f.c.). In Mya this current is everywhere directed towards the free ventral edges of the demibranchs. The food particles, entangled

in mucus, are carried down until they reach the margin, where they come under the influence of the powerful anteriorly directed current created by special large cilia (l.c., fig. 5) present on the crests and in the trough of the marginal groove.

A third anteriorly directed current is found on either side along the gill axis at the base of the two demibranchs. Fig. 7 shows very clearly the large cilia (cirri of Wallengren) which appear to be characteristically present on all powerful anteriorly directed ciliary tracts.

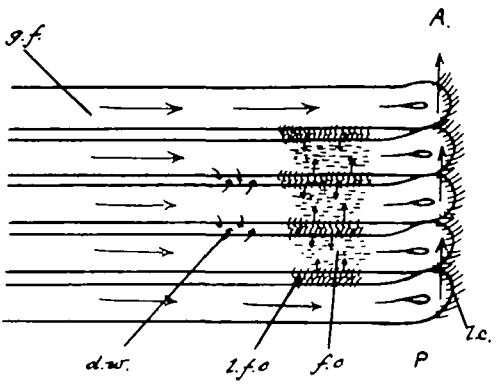

FIG. 5.—Mya. Food currents on gills, diagrammatic. A., anterior; d.w., direction of water current (shown by curved arrows); f.c., frontal cilia; g.f., gill filament; l.c., large cilia along ventral edge of demibranch; l.f.c., latero-frontal cilia. Food currents denoted by straight arrows.

The currents on the margin of the outer demibranch and the gill axis deposit their contents at the posterior end of a groove formed by the union of the edges of the inner demibranch and the outer labial palp. This has been named by Kellogg the distal oral groove (d.o.g., fig. 2). A forwardly directed current carries the particles along this groove to the anterior edge of the inner demibranch, where they are joined by the material collected by that organ. The groove, now bounded by the inner and outer palps, continues to the mouth, and this stretch has been named by Kellogg the lateral oral groove (l.o.g., fig. 2).

f. **The Labial Palps**—(1) *Structure.*—The most important and most highly developed organs concerned in the selection of food material and its transmission to the mouth are the labial palps. These consist of triangular flaps (fig. 2) attached one pair on either side of the mouth, the bases of each pair being united, while the anterior basal corners of the outer and inner palps of either side are united respectively above and below the mouth opening. Normally, the two palps composing a pair lie facing one another, but in fig. 2 the

outer palp of the right side has been turned back so as to expose the inner face.

The outer faces appear smooth on naked eye examination; in transverse sections, however, the epithelium (o.ep., fig. 8) is seen to be puckered into numerous minute folds. The inner or apposed faces are much more complicated (i.s.p., fig. 2). The posterior basal corner and thin strips lying along the anterior and posterior margin possess a smooth surface, but the remaining surface is crossed by a series of folds having a characteristic structure. They arise near the posterior margin as low ridges, coming off at an acute angle (see fig. 10) and pass diagonally across the palp face, increasing in size as they go, until they reach the anterior margin in the form of high folds which overlap one another in the direction of the mouth. They lie practically at right angles to the anterior margin. In section the epithelium of the margins and the posterior basal corner is seen to be smooth and thickly ciliated. A cross section through the folds (*i.e.* longitudinal section through the palp as a whole) near the anterior margin, where they are best developed, shows the structure as in fig. 8.

The epithelium consists of thickly ciliated columnar cells, which are distinctly taller on the distal than on the proximal slope, though the structure is the same. The shortest epithelial cells are to be found at the bottom of the furrows (f.). The nuclei, oval in the longer cells and almost circular in the more cubical ones, contain granules of chromatin and a distinct nucleolus. Mucus glands (m.g.) are numerous on the distal, but extremely rare on the proximal, slopes. Siebert[89] found a similar distribution of gland cells in *Anodonta cellensis*, and he also states that they become fewer in number as the mouth is approached. Cilia are present on the free surfaces of all the epithelial cells (except where mucus is being secreted); but although there is seen to be a distinct difference in the size and inclination of the cilia on the various regions when the palp folds are examined in the living condition, it is extremely difficult to make out these differences in sections. It is only possible to distinguish the large cilia present on the summit of the folds (l.c.s.). There is a well-marked basement membrane (b.m.) between the epithelium and the underlying tissue. This

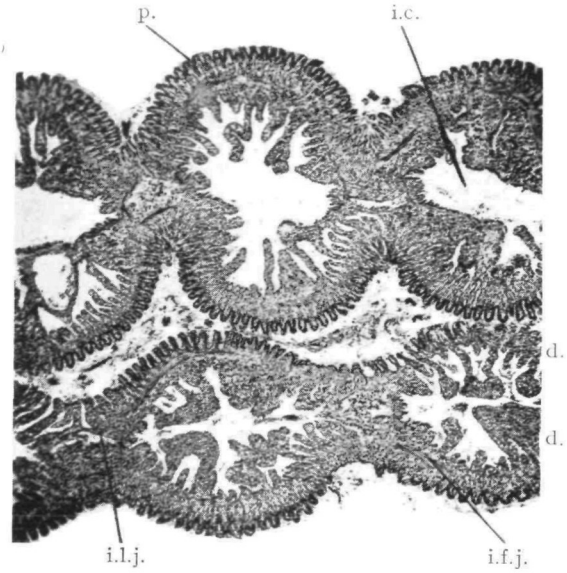

Fig. 6.—Mya Cross section through two demibranchs. × 55. d., demibranch; i c., interlamellar cavity; i.f.j., interfilamentary junction; i.l.j., interlamellar junction; p , plica of 33 filaments.

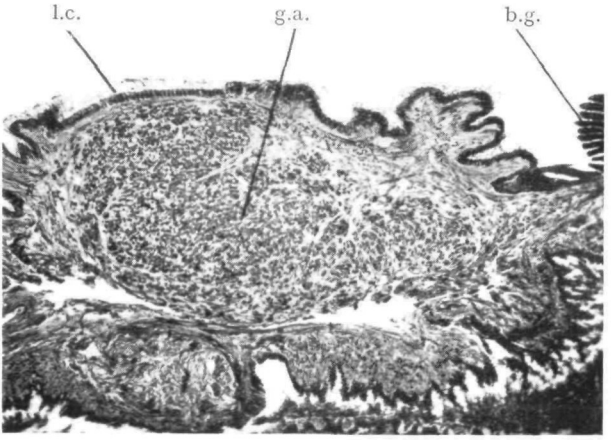

Fig. 7.—Mya. Transverse section through gill axis. × 65. b.g., beginning of gill filaments; g.a., gill axis; l.c., large cilia on axis epithelium.

latter consists of connective tissue fibres (c.t.s.) running in all directions, together with round, deeply staining nuclei (c.t.n.). Muscle fibres (m.f.) are present beneath the level of the furrows. The epithelium of the outer face (o.ep.) is puckered into minute folds and papillæ, and consists of more or less cubical cells containing round, deeply staining nuclei. Cilia are present but they are much smaller than those of the inner face, and are seen with extreme difficulty in sections. Immediately beneath this outer epithelium is a layer of clear tissue (c.l.), and below that again a thin layer of longitudinal fibres (l.c.t.).

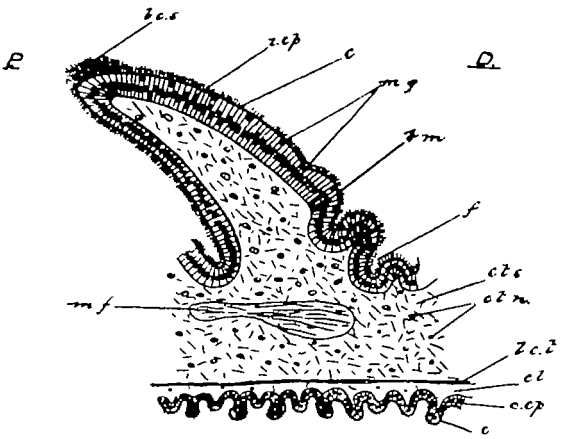

FIG. 8.—Mya. Transverse section through single palp fold. × 90. b.m., basement membrane; c., cilia; c.l., clear layer of tissue; c.t.n., connective tissue nuclei; c.t.s., connective tissue strands; P., proximal side; D., distal side (in relation to palp base); f., furrow between two folds; i.ep., epithelium of inner face; l.c.s., large cilia of summit of fold; l.c.t., longitudinal connective tissue strands; m.f., muscle fibres; m.g., mucus glands; o.ep., epithelium of outer face.

The folds do not extend to the basal edge of the palp (see i.s.p., fig. 2), the area on either side of the oral groove possessing an irregularly wrinkled, though, as elsewhere, thickly ciliated surface.

(2) *Physiology.*—Food particles collected by the inner demibranchs are transferred from the marginal grooves directly on to the inner palp faces. Material from the outer demibranchs and gill axis passes into the distal oral groove, but little of it proceeds directly to the mouth, the greater portion being caught by outwardly directed ciliary currents present on the posterior basal corner of the palps and carried on to the

inner faces. Particles present in the water surrounding the palps are collected on both inner and outer faces.

The direction of the ciliary currents present on the outer palp face is shown in fig. 9. They pass diagonally across from the anterior to the posterior margin, except at the extreme distal region where they are directed towards the tip. On reaching the posterior margin, particles are carried over the edge into the distally directed current that runs along that margin on the inner face. A strongly marked distally directed current flows along the anterior margin ; but this does not affect

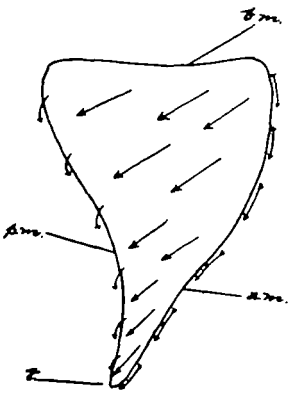

FIG. 9.—Mya. Direction of ciliary currents on outer palp face. a.m., anterior margin ; b.m., basal (attached) margin ; p.m., posterior margin ; t., tip of palp.

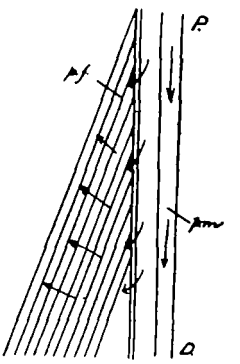

FIG. 10. — Mya. Posterior margin of inner palp face, diagrammatic. D., distal ; P., proximal ; p.f., palp folds ; p.m., posterior margin.

particles collected by the outer face, practically all of which being carried on to the inner face, there to be selected or rejected as the case may be.

A general examination of the inner face results in the detection of the following well-marked currents. There are distally directed streams running along the two margins (fig. 2) and on the surface generally a basally directed current passing at right angles over the palp folds. But, whereas the current in the anterior margin carries particles directly to the tip of the palp, from whence they are deposited on the mantle or visceral surfaces in the case of the outer and inner palps respectively, particles caught in the posterior marginal current have a different fate. As they pass backward sooner or later they are

caught in the forward currents of the palp folds which, as already noted, come off at an acute angle to this margin, and, following the direction of the curved arrows in fig. 10, they pass over on to the folded surface and are carried towards the base of the palp. This fate befalls all particles brought over from the outer face.

The course of the currents on the folded surface is extremely complicated. It has been worked out in full only by Wallengren.[47, 48] Kellogg's observations are unsatisfactory, and

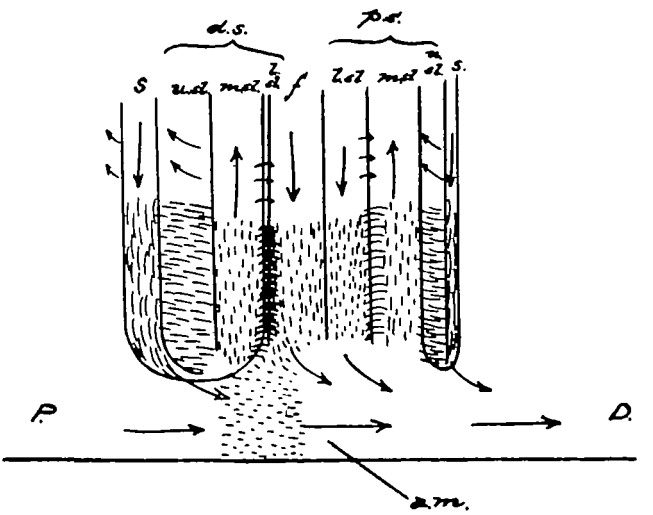

FIG. 11.—Mya. Anterior margin of inner palp face, palp folds pulled apart, diagrammatic. a.m., anterior margin; D., distal; d.s., distal side of palp fold; f., furrow; l.sl., lower region of palp slopes; m.sl., middle region of palp slopes; P., proximal; p.s., proximal side of palp fold; s., summit of fold; u.sl., upper region of palp slopes.

his conclusions were disproved by the present writer before he became acquainted with the work of Wallengren.

Fig. 11 gives a diagrammatic representation of the anterior margin with two folds arranged so that the more proximal of the two is inclined forward, exposing its distal slope, while the other is inclined backward, showing its proximal slope. There is a strong distally directed current created by small cilia present along the marginal tract (a.m.). The folds may conveniently be divided into the following regions for descriptive purposes—the summit, upper, middle, and lower slopes, and the furrow between adjacent slopes. The summit (s.) is occupied by a tract of conspicuously large anteriorly directed cilia, while

a similarly directed tract composed of much smaller cilia is present in the furrows (f.). Siebert,[89] working on Anodonta, states that this latter current flows in the opposite direction, but Wallengren, working on the same species, obtains contrary results. The upper slope on the distal side is covered by a broad band of large cilia, the beat of which is directed towards the base of the palp, though with a slight tendency posteriorly. The middle slope bears a tract of small posteriorly directed cilia. The lower slope ciliation on this side consists of a very narrow band of large cilia which beat downwards and carry particles into the furrow currents. On the proximal side the upper slope tract is narrower but contains similar large cilia, which beat in the same direction as those on the corresponding distal area. The middle slope on this side also is occupied by a tract of small posteriorly directed cilia. The lower slope is broad and possesses small anteriorly directed cilia which serve as an extension of the furrow tract.

These diverse currents work together in a strikingly efficient manner. Under normal conditions there is always an overlap, the folds being directed forward as shown in fig. 8, and covering all but the summit and distal upper slope of the preceding folds. Accordingly, the proximally directed cilia on the distal upper slopes lie on the functional summit of the folds, and it is they that give rise to the powerful current so well marked on the inner face of the palps. They are assisted to some extent by the narrow tract of similarly directed cilia on the proximal upper slopes, while the slight posterior tendency of the currents helps to counteract the influence of the anteriorly directed streams on the true summit. If a palp be removed and pinned out under sea-water and sand grains be placed upon its inner face, these are immediately conveyed by the action of the summit tracts to the anterior surface and so to the tip. According to Wallengren, under these conditions the folds contract down, so that they no longer overlap one another but stand upright with only their true summits exposed, the anteriorly directed current of which being brought into play to the exclusion of all others. Allen[3] states that the removal of objectionable matter is accomplished by the folds turning back and overlapping in the opposite

direction, thus bringing into play distally directed cilia which he considers to be present on the proximal upper slopes (he quotes the authority of Wallengren for this, though the views of that author are totally different). Kellogg,[24] speaking of Schizotherus, says that a similar contraction of the palp folds occurs but, in his opinion, so as to expose the furrow tracts and allow their ciliary currents to come into operation. (Kellogg appears to be unaware of the anteriorly directed summit tracts.)

As a matter of fact, however, if both sand and carmine grains are placed on the palp surface at the same time, the former are carried off to the anterior margin, while the latter are borne to the mouth. Obviously, none of the preceding explanations can account for this, all of them postulating the action of one current to the exclusion of all others. No contraction of the palp folds has been observed in Mya under any conditions, and the explanation of the selection and rejection of particles would appear to be as follows. All heavy particles which make their way on to the palp face will naturally tend to settle down, and, if they are more than a certain weight, they will, in spite of the proximal currents, come to rest in the grooves formed between the upper distal slope of the one fold and the summit of the preceding one. In this position they will come under the influence of the powerful summit current and be rolled along to the anterior margin. On the other hand, the lighter particles, such as carmine grains, will be, as it were, thrown over these grooves from upper distal slope to upper distal slope until they reach the oral groove. This current is decidedly swifter than that directed towards the margin.

In the same way, if particles are carried down between the folds lighter particles will be caught in the posteriorly directed currents on the middle slopes, and so finally make their way via the posterior margin, if not sooner, on to the palp face again; whereas heavier particles will fall on to the lower slopes or furrow and be carried to the anterior margin. The whole palp mechanism tends to separate small from large particles, the criterion of size being weight. Thus, as we have seen, the only particles of any size found in the stomach are filamentous

shreds of algae which are extremely light. The largest solid particles are $\frac{1}{8}$ mm. in diameter and the majority only $\frac{1}{10}$ mm., while an average sand grain is at least $\frac{1}{2}$ mm. across.

The reaction to chemically poisonous or irritable substances is different, and they might quite conceivably cause abnormal contraction of the palps and folds.

Kellogg states that, in Schizotherus, the anterior edge of the palp may curl over and remove, by means of its current, any excess of material present on the surface; this has not been observed in Mya.

From the palps the selected food particles make their way into the lateral oral groove and so round to the mouth. Wallengren considers that they may be accepted or rejected here, the mouth opening or closing as the case may be. His evidence is questionable. The mouth region is covered by dense but very short cilia on which particles move extremely slowly. So long as the amount of material passed on from the palps and gills is not too great, it proceeds eventually into the œsophagus; but if it is in excess a large deposit forms round the mouth, and the outlying portions of this mass become caught in the anterior marginal currents of the four palps and so removed.

g. **Removal of Material from the Mantle Chamber.**—It is of the utmost importance that the mantle chamber should be maintained free from any accumulation of foreign matter which might impede and finally destroy the free flow of water. Larger particles brought in by the inhalant current will speedily find their way, owing to their greater weight, on to the surface of the mantle or visceral mass. Moreover, larger particles present in the marginal food currents of the outer and inner demibranchs will be transferred to the mantle and visceral surfaces respectively, an operation made easier by the morphological position of these grooves. Matter rejected by the palps is, as we have seen, carried to the tip and from there deposited on the visceral surface, in the case of the outer palp, or the mantle surface, in the case of the inner palp.

Strong ciliary currents (denoted by feathered arrows in fig. 2) lead away the rejected matter. The visceral epithelium is covered with cilia which give rise to currents that converge

at a point on the postero-ventral border, where a vortex (Kellogg) is formed (v., fig. 2). Particles entangled in mucus are here rolled into balls which, after reaching a certain size, fall over by their own weight on to the mantle surface. This is also ciliated throughout, the currents being directed towards the mid ventral region where they merge into two anteriorly directed streams, one on either side of the fused mantle edges. Particles are carried forward in these currents until they reach a position just posterior to the pedal opening. Here a vortex (v.) is formed into which lead other currents from the anterior regions of the mantle chamber.

The mass formed at the vortex is caught by cilia, which carry it over into the trough formed by the fused mantle edges in which is situated the ventral ciliated tract (v.c.t., fig. 2). This is a powerful backwardly directed current which has been carefully studied by Stenta[40, 41] and named by him the "untere Rückenström." It terminates in a bay (b.) beneath the internal opening of the inhalant siphon. When a considerable quantity of material has accumulated here the animal makes a movement of sudden contraction and, closing the exhalant siphon, forces a current of water out through the inhalant opening, carrying with it the deposit of rejected matter.

The anus opens at the end of a short papilla immediately behind the posterior adductor. The fæcal matter is thus deposited in the suprabranchial cavity, caught in the water currents, and carried out through the exhalant siphon.

4. Anatomy, Histology and Ciliary Currents of the Alimentary Canal.

a. **General Description.**—A semi-diagrammatic sketch of the alimentary system viewed from the left side is given on page 34. The mouth opening is continued by an œsophagus which leads into a large flask-shaped stomach lying at the base of the visceral mass. The brown mass of the hepatopancreas surrounds the œsophagus and all but the postero-dorsal aspect of the stomach. This gland has a racemose structure, being composed of an immense number of tubules uniting into ducts which open into the stomach by three asymmetrically placed

apertures. The remainder of the visceral mass is occupied by the gonad. The openings of the style-sac and intestine lie side by side on the postero-ventral region of the stomach. During life the crystalline style occupies the lumen of the style-sac, while its head bears against a gelatinous structure—the gastric shield—secreted by the left antero-dorsal wall of the stomach. The style-sac passes directly downward through the substance of the gonad and then bends anteriorly, its distal end being externally visible behind the hinder margin of the foot. The intestine passes antero-ventrally into the visceral mass and, after describing a series of four spiral curves, turns abruptly backward and, proceeding to the right of the style-sac, passes into the rectum. This proceeds directly upward until it attains the same height as the roof of the stomach, when it turns posteriorly, passes through the ventricles of the heart and, after running dorsally to the posterior adductor muscle, bends ventrally to open at the anus, which is situated on a small papilla just posterior to the muscle.

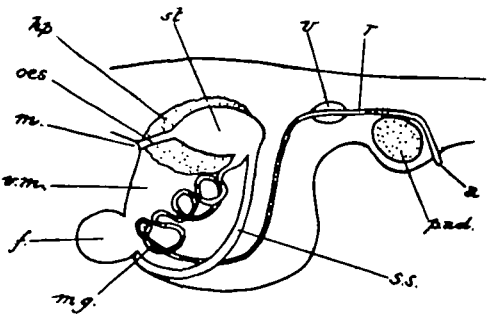

FIG. 12.—Mya. Alimentary system from left side. a., anus; f., foot; hp., hepatopancreas; m., mouth; m.g., mid-gut; œs., œsophagus; p.ad., post. adductor; r., rectum; s.s., style-sac; st., stomach; v., ventricle; v.m., visceral mass.

Material for histological examination was fixed in Bouin's fluid which gave much better results than Flemming's solution. Sections were stained with Delafield's hæmatoxylin and erythrosin for general examination, with picro-indigo-carmine for a closer study of the connective tissue and cilia (incidentally this is an excellent stain for hepatopancreatic tissue), with Heidenhain's iron hæmatoxylin for the nuclei, and with mucicarmine for the detection of mucus.

The histological examination of the alimentary tract has brought out three features of importance. (*a*) The universal presence of cilia throughout the alimentary tract, with the exception of that region of the stomach which secretes the

gastric shield. This would seem to be correlated with an almost complete absence of muscular tissue in the gut wall. Gutheil[17] has already pointed out that in Lamellibranchs ciliary action has replaced peristalsis as the means of the propulsion of food along the gut, and an examination of the ciliary currents present in the alimentary canal confirms the histological evidence. It is possible that a slight peristaltic contraction may occur in the rectum and may aid defæcation, but this is the only part of the gut where it is possible. (*b*) Mucus glands are everywhere numerous, particularly in the œsophagus and rectum. (*c*) There is a great abundance of phagocytes. These occur throughout the alimentary tract and hepatopancreatic ducts, both in the epithelium and the connective tissue. They are small round cells with a central darkly staining nucleus in which individual chromatin granules are difficult to distinguish. A vacuole is present but is not easily seen except when occupied by foreign matter. This typically takes the form of brownish masses several times the size of the cells which are ingesting them. These masses usually stain darkly with osmic acid and appear to be of fatty nature. This supports the evidence of Gutheil who studied the masses in Anodonta and regarded them as nutrient material. Dakin[11] considers the phagocytes to be excretory in function owing to their resemblance to the excretory cells found in the pericardial gland. In Mya although common in the connective tissue they rarely contain ingested matter in that area and, moreover, there is a certain area in the stomach in which phagocytic action is intense, the material ingested consisting of small sand grains, diatom tests and other indigestible matter. It is thus extremely difficult to say whether the phagocytes are primarily nutritive or excretory, and the probability is that they perform both functions.

b. **Œsophagus.**—In cross section (fig. 16) the œsophagus is seen to be dorso-ventrally compressed and to possess well-marked longitudinal grooves, the two lateral ones being more pronounced than the others. If the œsophagus be cut open along the mid-dorsal line, pinned out under water and the ciliary currents examined, particles, entangled in mucus, are seen to pass slowly towards the stomach. Owing to the

small size of the cilia only minute particles can be carried in the currents. A transverse fold (fig. 14), interrupted opposite the two lateral grooves, separates the œsophagus from the stomach, and particles may be observed to pass rapidly over this on to the gastric epithelium.

The epithelium of the œsophagus is composed of tall narrow cells, possessing abundant short cilia on their free borders, and bounded internally by a basement membrane. Connective tissue lies external to this, and beyond that again are circular and longitudinal muscle fibres (m.st.).

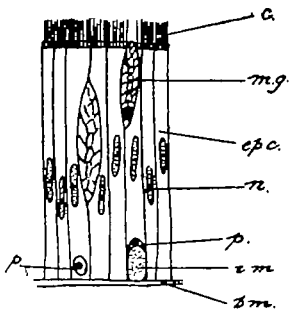

FIG. 13. — Mya. Portion of the epithelium of the œsophagus. × 315. b.m., basement membrane; c., cilia; ep.c., epithelial cell; i.m., ingested matter in phagocyte; m.g., mucus gland; n., nucleus of epithelial cell; p., phagocytes.

The epithelial cells (fig. 13) possess oval nuclei (n.) which contain numerous chromatin granules and a large nucleolus, and are situated usually near the middle of the cell. Mucus glands (m.g.) are especially abundant occurring even under the basement membrane, their nuclei being distinctly rounder than those of the epithelial cells. Phagocytes are present in large numbers, both in the epithelium and in the connective tissue, and are frequently to be seen in the epithelial region ingesting large masses of brownish matter.

c. **Stomach**—(1) *Anatomy.*—The anatomy of the stomach is somewhat complicated and may best be explained by reference to fig. 14. The œsophagus is separated from the stomach, as we have seen, by a transverse fold of epithelium (t.f.). Posterior to this the epithelium is raised into a series of ridges running antero-posteriorly and possessing a wavy outline; beyond this it is comparatively smooth for a short distance. The most conspicuous anatomical feature consists of a broad fold which lies along the floor of the stomach, and which does not maintain itself erect but has a permanent inclination to the right. In its origin it is connected with the crenated folds which line the opening of the principal hepatopancreatic duct (h^1), while it is greatly enlarged in the posterior region of the

stomach over the apertures of the mid-gut (m.g.) and style-sac (s.s.), which lie respectively to the right and left of it. It finally passes down the mid-gut as a broad typhlosole. A second series of ridges and folds is present in the left anterior region of the stomach and bounds the aperture of the second of the hepato-pancreatic ducts (h^2). To the left of this, on the wall of the stomach, is situated the third of the ducts (h^3), the dorsal lip of

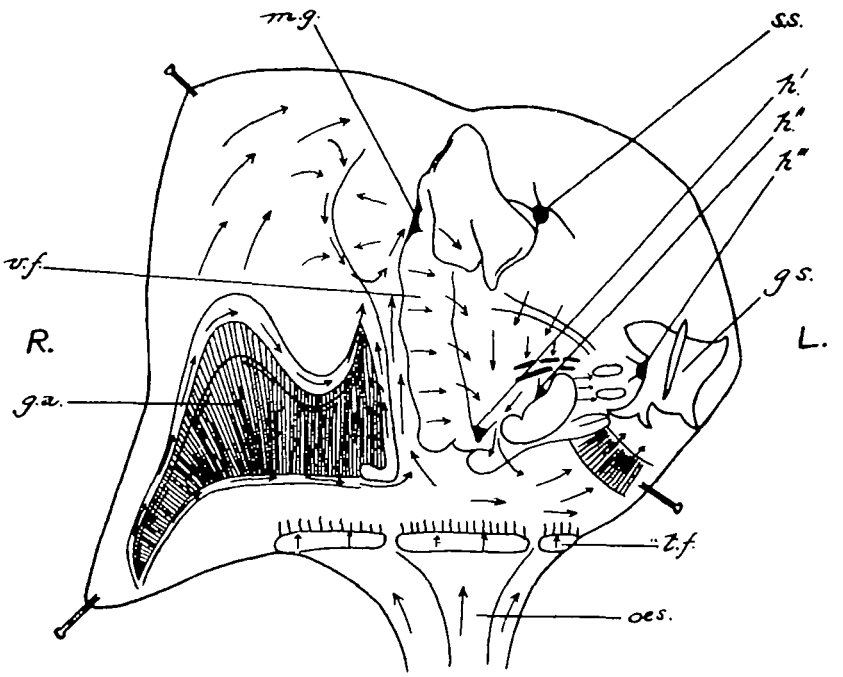

FIG. 14.—Mya. Stomach cut open along the left dorsal border and pinned out. × 2½. g.a., grooved area; g.s., gastric shield; h.', h.'', h.''', apertures of hepatopancreatic ducts; L., left; m.g., mid-gut; œs., œsophagus; R., right; s.s., style-sac; t.f., transverse fold; v.f., ventral fold.

which is bordered by the gastric shield (g.s.). This shield is spread over the left wall and roof of the anterior portion of the stomach. Edmondson[18] describes the gastric shield of Mya as consisting of "a cartilage-like layer applied to the epithelium of the stomach wall growing thicker from the posterior region forward, where it develops into an irregular structure with three curved processes which clasp prominent folds of the dorso-lateral wall of the stomach." It is secreted by an epithelium having a characteristic structure and consists of stratified

layers; it is extremely resistant and Nelson,[80] who examined it, considers it to be in the nature of chondrin.

The most interesting structure in the stomach, however, consists of a grooved area (g.a.) which, commencing as a broad strip to the right of the anterior end of the ventral fold, passes up the right anterior wall, becoming narrower and turning upon itself as it does so, crosses over the œsophageal opening on the upper surface of a ledge of tissue which divides the stomach into two in this region, and ends at a point in the middle of the left wall, immediately anterior to the gastric shield. It consists of a series of narrow ridges arranged at right angles to its long axis. It is not flat but V-shaped in cross section, one arm of the V being much longer than the other owing to the presence of a longitudinal furrow near the posterior border. The remainder of the stomach is bounded by smooth ciliated epithelium.

(2) *Ciliary Currents.* — Food particles pass down the œsophagus, over the transverse fold, and so on to the floor of the stomach. Here they may be carried in one of two directions. If they are deposited on the left side they are caught in currents which lead towards the gastric shield. If, on the other hand, they are deposited on the right side a strong current leads them directly along the right border of the ventral fold to the opening of the mid-gut. It is only the heavier particles, however, which are carried directly to the gut. Smaller particles are caught in side currents present on the grooved area and on the exposed left side of the ventral fold. Particles caught by the latter currents are carried into the middle of the stomach and thence in an anterior direction, between the openings of the first and second hepatopancreatic ducts, on to the left anterior surface of the floor of the stomach, and finally to the base of the gastric shield and the opening of the third hepatopancreatic duct.

The ciliation of the grooved area is more complicated. Light particles are carried along the longitudinal groove and deposited with the other particles at the edge of the gastric shield. This current is due to the presence of cilia beating in this direction on the summit of the ridges. Heavier particles are caught in strings of mucus freely secreted in this region

and are carried by other cilia, present in the furrows between the grooves, to the anterior margin of the grooved area. Currents running along this margin carry particles back to the anterior end of the ventral fold and so to the opening of the mid-gut. There are similar currents along the posterior border.

Ciliary currents present in the posterior region of the stomach tend to carry particles round to the gastric shield; but here again heavier particles which drop down are caught in an anteriorly directed current which runs above the opening of the mid-gut, and which carries them forward and then round and into the intestinal aperture.

It will be observed that the ultimate fate of the great majority of particles is to be deposited in the region of the gastric shield. Only the larger particles are carried directly into the mid-gut, while there is present in the grooved area a beautiful mechanism, having many resemblances to that of the palps, for the separation of large and small particles. Nelson[80] has described a food-sorting mechanism of a different pattern in the stomach of Modiolus. The crystalline style normally projects from its sac and bears against the gastric shield, which serves at the same time as a protection to the stomach epithelium and as a grinding surface for the head of the style. It has been shown by Nelson[80] that the style revolves, and as it does so it entangles the food particles which congregate round the gastric shield in the soft, sticky mucus of its head. The whole mass is thus set in motion, and the food is brought thoroughly into contact with the enzyme contained in the style while, at the same time, it is bathed in the hepato-pancreatic secretion which is discharged from the three ducts. Digestion having been effected in this manner, the food is ready to pass down the mid-gut.

(3) *Histology.*—The general epithelium of the stomach is ciliated except in the area of the gastric shield, mucus glands are rare, but phagocytes are everywhere abundant. There is a complete absence of muscle, the epithelium being separated from the underlying gonad or hepato-pancreas by a layer of connective tissue through which run blood vessels.

The gastric shield is secreted by an epithelium (fig. 15) composed of exceptionally long and narrow cells containing small oval nuclei which are situated about one-third of the way up the cells. The free border of the cells is bounded, not by cilia, but by a thin hyaline layer which stains deeply with erythrosin; it is this secretion which gives rise to the gastric shield. A distinct basement membrane borders the inner edge of the cells, and beneath it is a broad layer of connective tissue. Fig. 15 shows the edge of the gastric shield area, and the great reduction in depth, together with the presence of cilia, will be noted as characteristic of the ordinary gastric epithelium.

The epithelium running along the summit of the ridges in the grooved area is seen to be grey in colour when examined

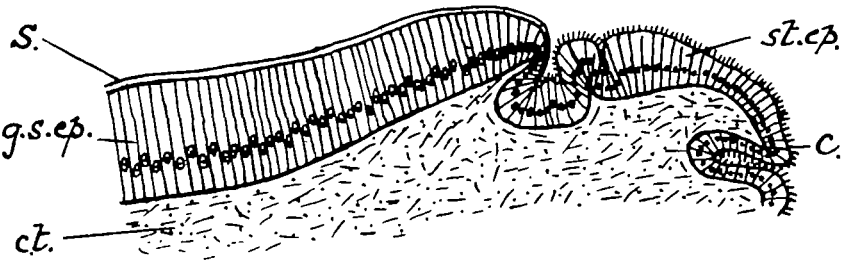

FIG. 15.—Mya. Section through edge of the gastric shield epithelium. × 55. c., cilia; c.t., connective tissue; g.s.ep., gastric shield epithelium; s., hyaline secretion; st.ep., stomach epithelium.

under the binocular microscope. On sectioning this is found to be the result of intense phagocytic action. Fig. 17 shows the typical condition of the epithelium in this region. The ingested matter consists, for the greater part, of small sand grains, empty diatom tests, and other inorganic material which is obviously quite indigestible. The particles are enclosed, as usual, within the limits of a cell vacuole at one side of which, on careful focussing, can be distinguished the small deeply-staining nucleus of the phagocyte. The ultimate fate of the particles is doubtful. They cannot be carried away through the tissue, and, if discharged again from the epithelium, nothing will have been gained; for the careful selection of indigestible particles for ingestion would seem to imply that this is but another aspect of the sorting mechanism known to be present in the grooved area, since in no other part of the alimentary tract

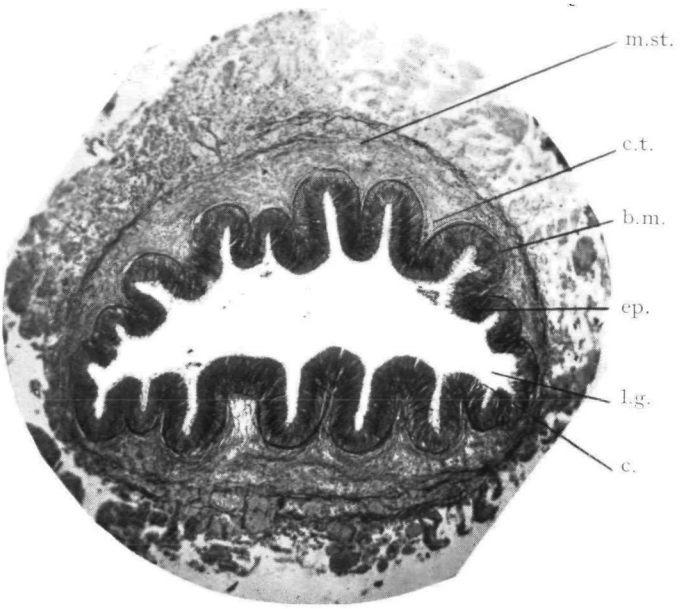

Fig. 16.—Mya. Transverse section through œsophagus. × 40. b.m., basement membrane; c., cilia; c.t., connective tissue; ep., epithelium; l.g., lateral groove; m.st., circular and longitudinal muscle strands.

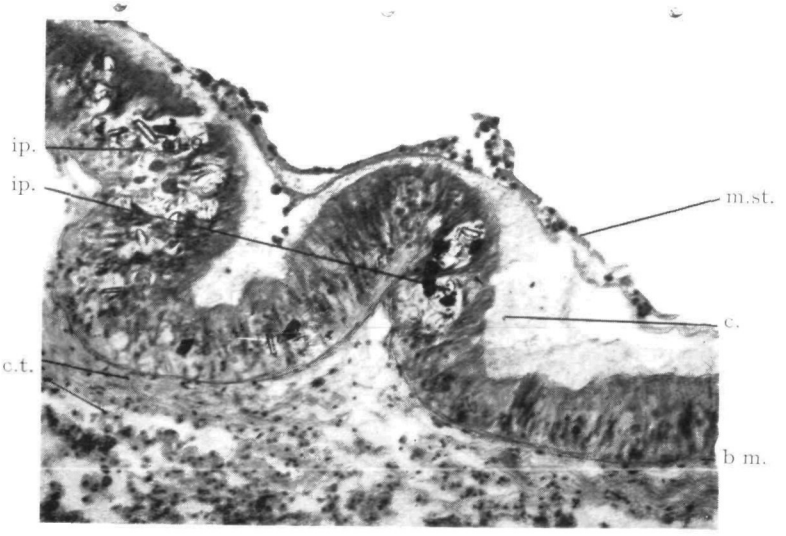

Fig. 17.—Mya. Section through the grooved area showing the nature of the ingested matter. × 200. b.m., basement membrane; c., cilia; c.t., connective tissue; i.p., ingested particles; m.st., mucus string.

are indigestible particles ingested by the phagocytes. It is just possible that the phagocytes have no selective action one way or the other, but that they are especially numerous in this region and ingest both digestible and indigestible particles indiscriminately, in which case the grooved area may serve the double purpose of a sorting mechanism and a special absorptive area. Matthias[28] has described a channel in the "ventral fundus" of the stomach of *Arca barbata* into the epithelium of which phagocytes enter, probably for the purpose of absorbing food material.

The cells have the typical structure, possessing well-developed cilia throughout and abundant mucus glands in the furrows. The presence of blood vessels in the connective tissue is additional evidence in favour of the view that the grooved area is an absorptive region.

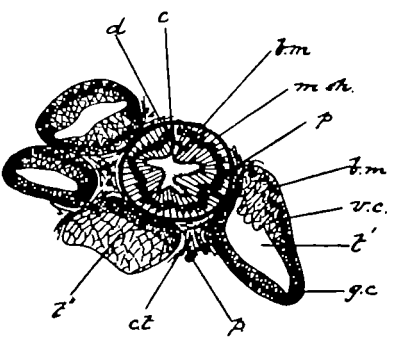

FIG. 18.—Mya. Cross section through small part of the hepatopancreas. × 100. b.m., basement membrane; c., cilia; c.t., connective tissue; d., duct; g.c., granular cells; m.sh., muscular sheath round duct; p., phagocytes; t.', tubule; t.'', tubule cut at blind end; v.c., vacuolated cells.

d. **Hepatopancreas.**—In transverse section (fig. 18) the gland is seen to be composed of a great number of tubules and ducts bound together by strands of connective tissue. The tubules are oval in cross section and are enclosed by a darkly staining basement membrane. The cells are distinguished into two categories: those opposite the narrow ends of the lumen (g.c.) which are short and narrow and contain a mass of darkly staining granules, and those bordering the rest of lumen (v.c.) which are larger in every way and contain no granules but only vacuolated cytoplasm. The nuclei, which lie near the base, are large and round and possess a darkly staining chromatin mass in the middle. There are no cilia on the cells, which are often to be observed in the process of being shed into the lumen. The probability is that the smaller darkly staining cells are gradually transformed into the larger ones which are the functional secretory cells.

Gutheil[17] has observed mitosis and the formation of new cells in the crypts of the hepatopancreatic tubules in Anodonta. List[26] has shown that the secretory cells of the digestive gland of Mytilus possess the power of absorbing particles of colouring matter such as indian ink, and this has been confirmed by the work of Dastre and Floresco,[12] on Anodonta, and Carazzi,[7] on Ostrea, but has not been observed in Mya. Phagocytes, though plentiful elsewhere, are never to be found in the cells of the tubules.

The ducts have a different structure. A basement membrane and strands of circular muscle (m.sh.) surround the epithelium, which is composed of narrow cells of varying length. Thus, though the ducts are usually circular in cross section, the lumen is irregular in outline. Short cilia cover the free surface of the epithelium, while oval nuclei occur at varying heights in the cells, the cytoplasm of which (unlike that of the tubules) stains deeply with erythrosin. Phagocytes, often laden with granules, are present in large numbers in the epithelium, muscular sheath, and connective tissue. The latter occurs everywhere between the ducts and tubules, and Sabatier,[28] working on Mytilus, has described the presence of blood lacunæ in its substance.

e. **Style-Sac with Contained Crystalline Style**—(1) *Histology of the Style-Sac.*—Edmondson has described the anatomy and histology of the style-sac in *Mya arenaria*, and his observations have been entirely confirmed. The sac is united with the mid-gut for a short distance below the floor of the stomach (fig. 19), the latter structure being anterior, and the two cavities being all but separated by two prominent folds which have been named the right and left typhlosoles. This union resembles in every way the condition which prevails throughout the entire length of the style-sac in these species in which it is not separated from the intestine. After the separation of the two tubes the original area of union remains in the form of a groove (G), which runs down between the two typhlosoles on the antero-lateral border of the sac. It is lined by cells having the same histological character as those of the mid-gut, and everything points to its being a remnant of

the mid-gut carried away by the style-sac when the two structures separated. The two typhlosoles maintain their identity throughout, the right one remaining the more prominent.

The histological character of the epithelium of the style-sac is important. The cells of the right typhlosole (Rt.) are exceptionally long and narrow, their nuclei being compressed and irregularly arranged. Thick cilia, a little shorter than those on the remainder of the surface, cover the free edges. The left typhlosole (Lt.) may possess a small group of similar cells, or they may be absent. The epithelium of the greater part of the lining consists of strikingly regular cells possessing oval nuclei with a distinct nucleolus, which are situated in every case near the middle of the cells. Dense cilia, half the length of the cells, clothe the free surface. There is a basement membrane and, beneath that, connective tissue. Phagocytes are absent, being confined to that part of the alimentary tract through which food passes.

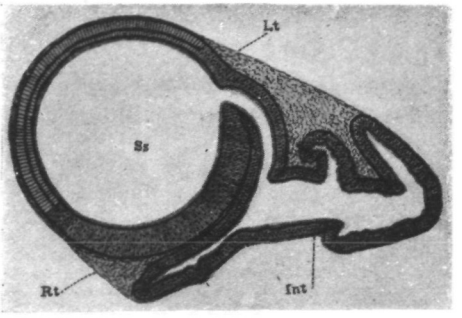

FIG. 19.

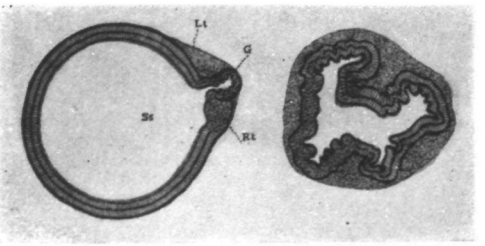

FIG. 20. FIG. 21.

FIGS. 19, 20, 21 (Edmondson).—Mya. Transverse sections through proximal, middle, and distal regions of the style-sac. × 19, 12½, 12½. G., groove; Int., intestine; Lt., left typhlosole; Rt., right typhlosole; Ss., style-sac.

The characteristic cells of the right typhlosole become fewer in number as they pass away from the stomach (compare figs. 19 and 20). Finally, both groove and typhlosoles are lost among the complex folding of the distal region (fig. 21). Longitudinal sections through the style-sac reveal the presence of transverse folds, which begin as low indulations proximally but become pronounced irregular ridges in the distal region.

These folds do not extend on to the groove or typhlosoles, which remain throughout perfectly smooth.

(2) *The Crystalline Style.*—Throughout life the style-sac is invariably occupied by the firm gelatinous crystalline style, (fig. 22) which in Mya is extremely resistant, varying in size only in accordance with the size of the animal possessing it. Edmondson, by comparing the lengths of the styles in a number of individuals with the shells, found that the length of the style was equal on the average to 72 per cent. of the length of the shell. A medium-sized style is about 3 mm. in diameter at the proximal end and a little less at the distal end. In cross section it is seen to be made up of 80 to 100 concentric layers of varying thickness.

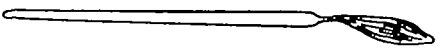

FIG. 22.—Mya. Crystalline style showing softened substance with entangled particles at proximal end. × ½.

(3) *Composition of the Style.*—The chemical analyses of Barrois,[8] List,[26] and Mitra[29] agree in showing the fresh style to have roughly the following composition :—

	Water	about 87 per cent.
Solid matter	Organic	„ 12 „
	Inorganic (salts, etc.) . .	„ 1 „

The organic matter exists largely in the form of a globulin.

(4) *Function of the Style.*—There is an extensive literature dealing with the crystalline style in the Lamellibranchs and this which has been fully reviewed by Barrois,[8] List, and more recently (918) by Nelson,[30] who has summarised the whole under the seven chief theories regarding its functions which have been held by various authors. It may now be regarded as fully established that the style is an albuminoid mass saturated with an amylolytic enzyme. It is interesting to note that Anton de Heide,[20] the discoverer of the style in 1686, also made the suggestion that it might be of the nature of a digestive fluid, though this was not based on any definite evidence.

Coupin,[10] in 1900, first demonstrated the presence of an amylolytic ferment in the style of *Cardium edule*, though the credit for the discovery is usually given to Mitra,[29] who

worked on the style of Anodonta in 1901 and gave an account of its physical, chemical, and physiological properties. Since that date their work has been completely confirmed by List, Van Rynberk,[48] Gutheil, Matthias,[28] Nelson, and others.

Barrois,[8] in his exhaustive work on the style (in which he did everything except discover its true function), states as his opinion that the style is pushed forward into the stomach, at the same time revolving on its long axis. List, judging from the histological character of the style-sac, expressed the view that the style must be turned round by the cilia present. Nelson quotes Hoffman[21] as stating that the style of Tagelus is rotated by the cilia of the style-sac. None of these authors actually observed the movement, and it was left to Nelson to describe the revolutions of the style in Anodonta and Modiolus, the movement being in a clockwise direction when observed from the anterior aspect.

The style-sac of Mya was carefully dissected out and the ciliary currents examined. A strong current in the direction of the stomach, accompanied by a copious secretion of mucus, was observed along the groove and typhlosoles. On the rest of the surface the ciliary currents were slow and difficult to detect, but a movement of particles transversely from right to left was finally observed. The style presumably is pushed forward into the stomach by the first current and revolved by the second.

As we have already seen, the head of the style is worn down against the gastric shield.

(5) *Origin of the Style.*—Mitra[29] was of the opinion that the style substance was secreted by the hepatopancreas and only stored in the style-sac. This view has since been disproved and it has been definitely established, as a result of the work of Edmondson, that the style is secreted by the style-sac itself. By means of a neat cut near the distal end of the style-sac, where it lies exposed on the ventral edge of the visceral mass, Edmondson removed the style from a series of animals and then replaced them in their natural habitat. About 50 per cent. of them survived the operation. At the end of about four days a new style began to appear, lying on one of the typhlosoles, usually the right one. More-

over, the short distal portion of the style-sac, which closed up after the operation and had thus no connection with the rest of the alimentary system, also secreted a short style when conditions were favourable, and thus afforded conclusive proof of the site of formation of the style. In sections of the style-sac stained with muci-carmine a certain amount of mucus can be detected in the cells lining the groove, and as a dense mass of secretion in the long cells of the typhlosoles, while no sign of mucus is to be seen in the remaining cells. There is every reason to believe that it is the cells of the typhlosoles which secrete the substance of the style-sac.

Mitra also believed the style to be a mass of pure enzyme, but the probability is that the style consists of globules of a colloid substance upon the surface of which the enzyme is borne (an adsorption phenomenon). This substance is secreted and pushed forward by the cells of the typhlosoles, while the cilia on the remainder of the epithelium beat it round and determine the concentric structure of the mature style.

(6) *Permanence of the Style.*—It is a striking fact that, whereas in certain species of Lamellibranchs (e.g. *Ostrea virginica*) the style is dissolved and reformed twice daily, in Mya it is a permanent structure, never absent in the living animal, while, after an artificial extraction, Edmondson found that it took seventy-four days under the most favourable conditions before the style was completely regenerated.

It was observed so long ago as 1829 by Meckel (quoted by Nelson) that the style disappeared in a number of species after they had been kept out of water for a short time. The view of Hazay[19] and Haseloff[18] that the style was of the nature of reserve food material was based upon the observations that the style disappeared from specimens of Mytilus after they had been kept in filtered sea-water, and was reformed when the same animals were fed. Allen,[2] who worked on Anodonta, states that the style is invariably present when the animals have plenty of food, and is only absent when they are starved. Dakin[11] states that the style of Pecten reappears and disappears under similar conditions.

It will be noted that all these experiments have been performed on animals possessing a style which lies free in the

intestine. In Mya the style is removed from the influence of the intestinal contents, and this explains its greater persistence, for, after fourteen days' starvation, there is little sign of any dissolution, except for a slight softening at the gastral end.

Experiments were made with a view to testing the influence of the hepatopancreatic secretion upon the style.

An extract of six hepatopancreases was made in 90 c.c. of toluol water, and the following experiments performed :—

A. 15 c.c. extract + 1 c.c. H_2O + 1 style (0.30 gm.).
B. ditto boiled + 1 style (0.28 gm.).
C. 15 c.c. extract + 1 c.c. 0.1N HCl + 1 style (0.35 gm.).
D. ditto boiled + 1 style (0.40 gm.).
E. 15 c.c. extract + 1 c.c. 0.1N Na_2CO_3 + 1 style (0.355 gm.).
F. ditto boiled + 1 style (0.34 gm.).

These were incubated at a temperature of 32° C. for two days and then examined, with the following result :—

A. Weight of style . . . . . 0.0 gm.
B. „ . . . . . 0.28 „
C. „ . . . . . 0.15 „
D. „ . . . . . 0.40 „
E. „ . . . . . 0.0 „
F. „ . . . . . 0.12 „

The style is dissolved by the unboiled hepatopancreatic secretion, particularly in neutral and alkaline media. To attempt to account for the partial dissolution of the style in the sixth (control) experiment, the following additional experiment was performed :—

A. 1 style (0.43 gm.) + 10 c.c. sea-water + 1 c.c. H_2O
B. „ (0.33 gm.) + „ „ + 1 c.c. 0.1N HCl
C. „ (0.37 gm.) + „ „ + 1 c.c. 0.1N Na_2CO_3

These were maintained at 32° C. and the time required for complete dissolution noted. Thus :—

A. Style dissolved in 20 hours.
B. Style only partially dissolved after 18 days.
C. Style dissolved in 6 hours.

i.e. the style dissolves in sea-water with an alkaline reaction (ordinary sea-water was slightly alkaline to litmus), but exists for lengthy periods in an acid medium. Since the "liver"

secretion has a decided acid reaction this will tend to preserve the style from the influence of the sea-water, and only under the exceptional conditions of natural or artificial starvation, where there are no food particles to the stomach for the secretion to act upon, will the style substance be severely attacked. It is probably always attacked to a slight degree as an aid to the normal wearing down of its tip. In such animals as Mya, however, the style is completely protected against the destructive powers of the stomach juices, and is naturally a much more substantial and permanent structure than in those in which it is liable to be dissolved whenever conditions are unfavourable.

(7) *General Remarks.*—The crystalline style is invariably present in Lamellibranchs, and from the small semi-permanent rod, flying free in the intestine of the Prosobranchs, has evolved the firm permanent style, enclosed in its own cæcum, characteristic of the higher Eulamellibranchs. It should be noted that the graduation in the complexity and separation of the style-cæcum in the various genera does not follow the modern classification of the Lamellibranchs based on the gill structure. Matthias[28] has pointed this out, and he has distinguished three groups in which are found a progressive separation of the intestinal and style-secreting tracts. It would seem as though either the taxonomy of the Lamellibranchs is defective, or else that independent evolution has occurred within the class, giving rise to many striking cases of convergence. This aspect of the matter has been dwelt upon by Robson,[87] who has also summarised the extensive literature dealing with the presence of a style and style-cæcum in the Prosobranch Gastropoda. Here again there is a graduated series from species possessing a style which lies free in the pyloric region of the stomach to those in which it lies encased in a cæcum. This parallel development in the two classes of the Mollusca is striking and, as Robson remarks, is another instance of the fundamental unity that characterises that phylum.

The greater development of the style in the Lamellibranchs is doubtless due to the absence of salivary glands, and the need for some accessory supply of a starch-splitting ferment.

That it should have taken the form of the crystalline style, strange though it may appear at first sight, is readily understood in a class which is characterised by a universal presence of cilia and of mucus-secreting glands.

f. **Mid-Gut.**—The mid-gut (fig. 23) possesses a large typhlosole (t.) which almost fills the lumen, and thus an ample absorptive surface is provided. Two main ciliary currents (f.st.) pass along the grooves on either side of the base of the typhlosole. The epithelium is thrown into a series of longitudinal folds and is everywhere ciliated. Comparing it with that of the œsophagus, the cells are only two-thirds as high, the nuclei are rounder, while the cilia are practically twice the length. Mucus glands occur, but in smaller numbers, and phagocytes, either empty or with ingested brownish particles, are present everywhere in the epithelium. The basement membrane (b.m.) is unusually thick and is surrounded by a few fine strands of circular muscle tissue (m.f.), and outside that by a little connective tissue. Similar connective tissue fills the interior of the typhlosole. A blood vessel (not shown in the figure) is present in the connective tissue just beneath the base of the typhlosole.

g. **Rectum.**—The rectum (fig. 24), unlike the rest of the alimentary canal, is surrounded by a thick muscular sheath composed of intermingled circular and longitudinal strands, the former predominating. A layer of connective tissue, in which lie many phagocytes, and a basement membrane separate the muscular sheath from the epithelium. This resembles the epithelium of the mid-gut in histological character but varies considerably in height. It possesses abundant cilia of about the same length as those of the mid-gut. Phagocytes are exceptionally numerous and can be distinguished as small black spots (p.) in the epithelium shown in the figure. There is no typhlosole but the epithelium is thrown into longitudinal grooves, one of the grooves (m.l.g.) being much more pronounced than the rest, and being bounded by especially long epithelial cells whose contents give a dense red colour with mucicarmine. There are no mucus glands in the remainder of the epithelium and the assumption, from the histological evidence, is that the greater part of the food passes down the

channel provided by this groove. The cilia beat in the direction of the anus, and food particles are carried slowly in that direction.

5. The Digestive Processes.

a. **Literature and General Remarks.**—Little satisfactory work has been done on the digestive enzymes of Lamellibranchs apart from the style ferment. C. Bernard[5] demonstrated the presence of a lipase and an amylase in the acid stomach juices of Ostrea. Fredericq[14] described a protease in Mya and Mytilus which acted in acid media. Mitra showed the presence of an amylase and invertase in Anodonta. Van Rynberk[18] found a reduction of starch but no action upon cellulose or egg albumen by the hepatopancreatic extract of Mytilus. Krukenberg[25] observed the presence of a pepsin-like enzyme in Ostrea which he called "Conchopepsin," and declared to be characterised by its being destroyed by 2 per cent. oxalic acid, a statement which Biedermann[6] has denied. Dakin[11] states that an amylase, a protease, and a lipase are present in Pecten. In view of the paucity of evidence, Jordan[22] considered that the hepatopancreas was an absorptive, and not a secreting, organ, basing his views on the work of List and Carazzi.

The hepatopancreatic secretion is a brown fluid with a decided acid reaction to litmus. The stomach contents, however, do not always give an acid reaction in Mya (as Krukenberg also observed in Ostrea). Feeding would appear to be a steady process in the Lamellibranchs but, as Allen[2] has pointed out, digestion fluctuates; he states that "at times nearly all the intestinal contents are found to be at least partially digested; while again much material is found, even in the rectum, in perfect preservation, and often the fæces themselves contain forms which are apparently unaffected." Nelson has shown that the motion of the style in Modiolus and Anodonta is not continuous but is "interrupted by periods of inactivity, preceded by a gradual slowing down. . . . The presence or absence of food in the stomach itself seems to be of no consequence so far as this activity is concerned." It is an interesting speculation whether or no there is any connection

between the periodic outpourings of acid digestive secretion and the irregular movements of the style. The work of Gray[15] on the effect of ions upon ciliary activity suggests the possibility that the acidity of the hepatopancreatic secretion may serve as a stimulus to the cilia of the style sac, the stimulus being effected at the proximal end and then extending throughout the length of the sac.

Vogt and Yung[45] state that "il est probable que c'est surtout dans l'estomac que la digestion s'effectue." Allen fed a number of starved mussels on a culture of Paramæcium, but found few living specimens beyond the stomach. Extracts of the mid-gut of Mya fail to show any enzymatic properties, and the probability is that digestion takes place in the stomach and absorption in the stomach, mid-gut, and rectum.

b. **The Hepatopancreatic Secretion.**—Extracts of the hepatopancreas were made by grinding up the tissue and then extracting it with distilled water. Toluol was employed as an antiseptic in the case of carbohydrate, and HCN in the case of fat and protein, digests. Incubation was carried out at a temperature of 32° C. The methods of Roaf[85] were largely employed. Benedict's solution was used for the estimation of reducing sugars and Barfoed's solution for detecting the reduction of disaccharides. The biuret test was employed for the detection of albumenoses, bromine water for typhophane, and Millon's reagent for tyrosine. Experiments with olive oil emulsion for the detection of lipase were unsuccessful, so recourse had to be made to the less satisfactory methods of milk and methyl acetate. Rigorous control experiments were set up.

The table on page 52 provides a summary of the more important experiments.

The hepatopancreatic enzymes can reduce starch (optimum medium acid), glycogen, sucrose, maltose, and lactose among carbohydrates. It is improbable that a separate enzyme is responsible for each of these reactions for, as Roaf[86] has pointed out, the enzymes of the invertebrates would not appear to have evolved to the same degree of specificity as those of the vertebrates. The most prolonged digests failed to reveal the presence of a cytase such as Biedermann found in Helix.

No. of Glands.	Experiment.	Time.	Results.
			Titrated into 10 c.c. Benedict.
3	A. 10 c.c. + 5 c.c. 1 per cent. starch .	3 hours	A. 5·5 c.c.
	B. ditto, boiled .	,,	B. 10·35 c.c.
3	A. 10 c.c. + 10 c.c. starch + 1 c.c. H_2O	4 hours	A. 5·1 c.c.
	B. ditto + 1 c.c. 0·1N HCl . .	,,	B. 4·2 c.c.
	C. ditto + 1 c.c. 0·1N Na_2CO_3 . .	,,	C. 6·5 c.c.
4	A. 10 c.c. + 10 c.c. 1 per cent. sucrose	3 hours	A. 4·8 c.c.
	B. ditto, boiled .	,,	B. 20·2 c.c.
	C. 10 c.c. + 10 c.c. 1 per cent. glycogen	,,	C. 13·7 c.c.
	D. ditto, boiled .	,,	D. 20·0 c.c.
2	A. 10 c.c. + 10 c.c. 1 per cent. inulin .	3 hours	A. 11·5 c.c.
	B. ditto, boiled .	,,	B. 11·8 c.c.
5	A. 10 c.c. + 10 c.c. 1 per cent. cellulose	8 days	A. 11·8 c.c.
	B. ditto, boiled .	,,	B. 12 c.c.
			1 c.c. + 5 c.c. Barfoed for 10 min.
4	A. 10 c.c. + 10 c.c. 2 per cent. maltose	2 days	A. Reduction.
	B. ditto, boiled .	,,	B. No reduction.
	C. 10 c.c. + 10 c.c. 2 per cent. lactose .	,,	C. Reduction.
	D. ditto, boiled .	,,	D. No reduction.
6	A. 15 c.c + 5 c.c. boiled milk + phenol red + 1 c.c. 0·1N Na_2CO_3	24 hrs.	A. Bright yellow.
	B. ditto, boiled .	,,	B. Remained pink.
			0·1N Na_2CO_3 titrated in.
4	A. 10 c.c. + 5 c.c. 5 per cent. M. acetate	2 days	A. 16·7 c.c. to neutralise.
	B. ditto, boiled .	,,	B. 3·8 c.c. ,,
4	A. 10 c.c. + 5 c.c. calcified milk . .	1 day	A. Coagulated.
	B. ditto, boiled .	,,	B. Not coagulated.
			Fibrin. Biuret. Bromine. Millon.
10	A. 10 c.c. + 0·4 gm. fibrin + 5 c.c. H_2O	3 days	A. 0·235 gm. fair mod. no
	B. ditto + 5 c.c. 0·1N HCl . .	,,	B. 0 . good good good
	C. ditto + 5 c.c. 0·1N Na_2CO_3 . .	,,	C. 0·4 gm. . poor mod. no
			Biuret. Bromine. Millon.
10	A. 10 c.c. + 5 c.c. 2 per cent. peptone .	3 days	A. yes no yes
	B. ditto + 5 c.c. 0·1N HCl . .	,,	B. yes yes yes
	C. ditto + 5 c.c. 0·1N Na_2CO_3 . .	,,	C. yes no yes
	D. 10 c.c. boiled + 5 c.c. H_2O + peptone	,,	D. yes no yes
			Fibrin. Bromine. Millon.
6	A. 10 c.c. + 5 c.c. 0·1N Na_2CO_3 . .	3 days	A. 0·2 gm. no no
	B. ditto + 0·2 gm. fibrin . . .	,,	B. 0·2 gm. no no
	C. 10 c.c. alone	,,	C. 0 gm. yes yes
	All left for 18 hours. A and B then neutralised and 5 c.c. 0·1N HCl added to each and 0·2 gm. fibrin.		

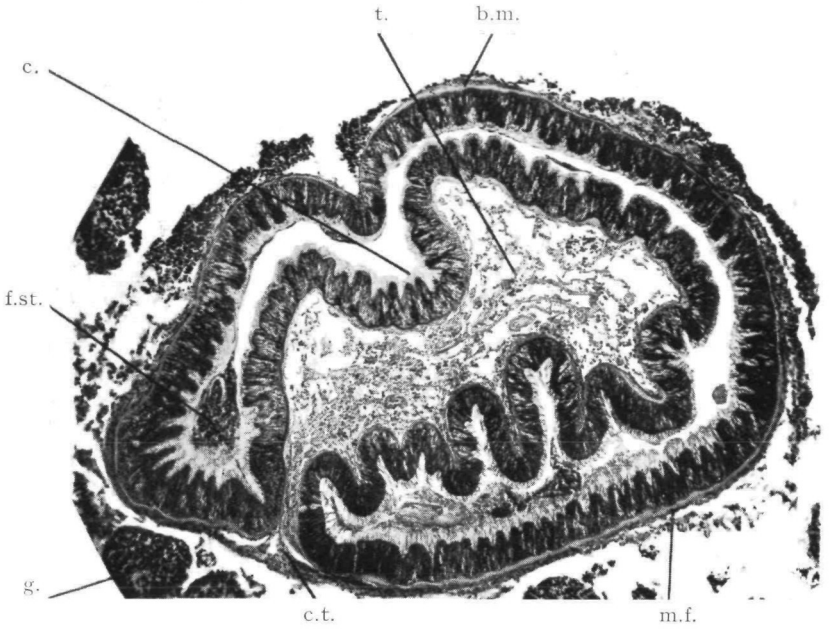

FIG. 23.—Mya. Cross section through mid-gut. × 36. b.m., basement membrane; c., cilia on epithelium; c.t., connective tissue; f.st., food stream; g., gonad, m.f., muscular fibres; t., typhlosole.

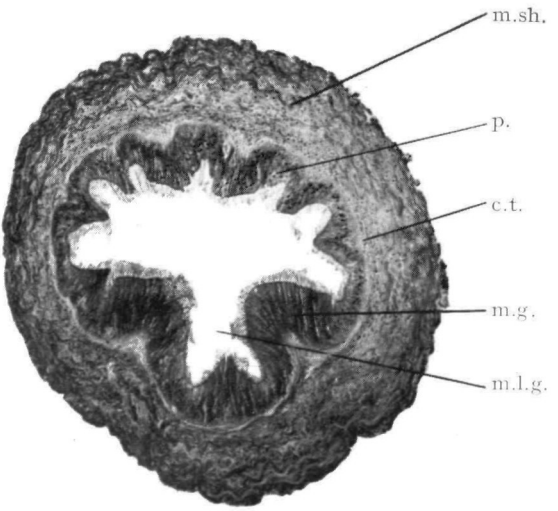

FIG. 24.—Mya. Transverse section through rectum near anus. × 70. c.t., connective tissue; m.g., mucus glands; m.l.g., main longitudinal groove; m.sh., muscular sheath; p., phagocytes in epithelium.

A strong protease is present which acts in an acid medium almost exclusively, being destroyed by alkali even in the presence of fibrin. It might be described as a pepsin were it not for the fact that it reduces globulins to amino acids instead of merely to peptones as is the case with vertebrate pepsin. It also coagulates milk in the presence of calcium chloride. A lipase is present.

A portion of carefully separated, minced gland was extracted several times with absolute alcohol. The extract had a rich brown colour and turned green on the addition of concentrated H_2SO_4 or HCl, showing that the pigment is in the nature of a lipochrome. The alcohol was then evaporated and the solid residue extracted with water. The fluid obtained had a light brown colour. The following tests were applied :—Oliver's and Gmelin's tests, negative; Jaffe's test, negative; Biuret, Millon's and bromine water tests, all negative; Fehling's test, positive. There would appear, therefore, to be no bile salts or pigments present, no creatinine, and no evidence of the products of protein digestion although traces of reducing sugars were found. The brown residue left after the watery extract had been removed was immediately taken up by absolute alcohol and is probably pure lipochrome pigment.

c. **The Style Enzyme.**—Experiments were made with the style extract in order to determine the properties of the enzyme it contains. It is seldom that a supply of enzyme of so pure a quality can be obtained and, though the amount of enzyme present in each style is small, very satisfactory results can be obtained if the experiments and final estimations are carefully carried out.

(1) *Specificity.*—Careful experiments failed to reveal the presence of a lipase. When it is remembered that the style substance is rapidly dissolved by the protease of the hepatopancreas, and that the style will remain intact in distilled water for a practically indefinite period, the presence of a protease in its substance is obviously impossible.

The following experiments were performed in order to test for the presence of carbohydrate-splitting enzymes. Toluol was employed as antiseptic and the digests were maintained at

a temperature of 32° C. Reducing sugars were tested for with Fehling's solution :—

Styles.	Experiment.	Time.	Results.
8	A. 10 c.c. + 10 c.c. 1 per cent. starch B. ditto, boiled	3 hours „	A. Strong reduction. B. No reduction.
6	A. 10 c.c. + 10 c.c. 1 per cent. inulin B. ditto, boiled C. 10 c.c. + 10 c.c. 1 per cent. glycogen D. ditto, boiled	2½ hrs. „ „ „	A. No reduction. B. „ „ C. Strong reduction. D. No reduction.
8	A. 10 c.c. + 10 c.c. 1 per cent. cellulose suspension B. ditto, boiled	8 days „	A. No reduction. B. „ „
4	A. 10 c.c. + 10 c.c. 1 per cent. sucrose B. ditto, boiled	4 hours „	A. No reduction. B. „ „ + Barfoed sol. for 15 min.
	A. 10 c.c. + 10 c.c. 2 per cent. maltose B. ditto, boiled C. 10 c.c. + 10 c.c. 2 per cent. lactose D. ditto, boiled	2 days „ „ „	Very slight reduction in all.

The style enzyme reduces starch and glycogen but, despite the contrary evidence of Coupin and Mitra who worked on Cardium and Anodonta, not sucrose. Both these animals, however, possess styles which lie free in the intestine, and which become contaminated by the powerful invertase discharged from the hepatopancreas.

Starch is rapidly reduced by the enzyme and does not give the characteristic blue colour with iodine within half an hour of the beginning of an experiment. An intermediate compound of the nature of dextrin is formed but this speedily disappears, the final product of reaction being, on the evidence of the osazone crystals, glucose. At the same time the enzyme will not reduce commercial maltose to glucose.

(2) *Optimum Medium.*—An extract of six styles in 30 c.c. of water was prepared, and the following experiment performed :—

A. 10 c.c. + 10 c.c. 1 per cent. starch + 1 c.c. H_2O
B. „ + „ „ + 1 c.c. 0.1N HCl
C. „ + „ „ + 1 c.c. 0.1N Na_2CO_3

Incubated for three hours at 32° C., then titrated into 5 c.c. Benedict solution.

A. 7.95 c.c. reduced Benedict.
B. about 20 c.c. „
C. about 20 c.c. „

i.e. the enzyme acts very much better in neutral than in acid or alkaline media. This is interesting in view of the fact that the amylase and protease of the hepatopancreas both have their optimum in an acid medium.

(3) *Influence of Temperature.*—A series of preliminary experiments demonstrated the fact that the optimum tempera-

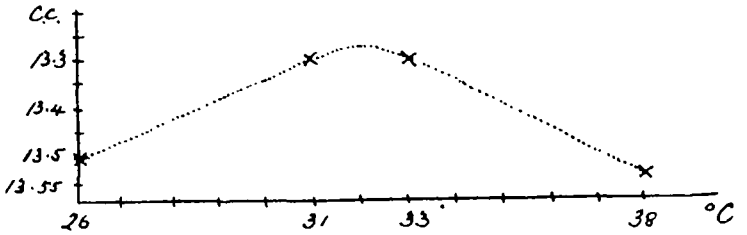

FIG. 25.—Temperature curve.

ture for the action of the style enzyme lay somewhere between 31° and 33° C. The following final experiment was made.

An extract of sixteen styles made in 40 c.c. of toluol water.

A. 10 c.c. + 10 c.c. 1 per cent. starch solution at 26° C.
B. „ + „ „ „ 31° C.
C. „ + „ „ „ 33° C.
D. „ + „ „ „ 38° C.

Left for three hours and then boiled. Each made up to 25 c.c. and titrated into 20 c.c. Benedict solution.

A. 13.5 c.c. needed
B. 13.3 „ „
C. 13.3 „ „
D. 13.55 „ „

The optimum temperature is seen to lie at about 32° C.

Further experiments were carried out to determine the temperature of destruction.

An extract of twelve styles in 60 c.c. of toluol water prepared.

A.	10 cc. + 10 cc.	1 per cent. starch	at	48° C.	for 15 mins.
B.	,, + ,,	,,	,,	49° C.	,,
C.	,, + ,,	,,	,,	50° C.	,,
D.	,, + ,,	,,	,,	51° C.	,,
E.	,, + ,,	,,	,,	52° C.	,,
F.	,, + ,,	,,	,,	53° C.	,,

All six digests were incubated for four hours at 32° C. and then tested with Fehling's solution, with the following results :—

A. Big reduction.
B. Reduction.
C. Very slight reduction.
D., E., F. No reduction.

i.e. the enzyme is entirely destroyed at a temperature of 51° C.

(4) *Variation of Concentration.*—The following experiment was carried out in order to ascertain the influence of varying concentrations of enzyme upon the rate of activity.

An extract of eighteen styles made in 72 c.c. of toluol water.

A.	16 c.c. + 10 c.c.	1 per cent. starch	—*i.e.* conc. of	1	
B.	14 c.c. + 2 c.c.	H_2O + 10 c.c.	1 per cent. starch	—*i.e.* conc. of	$\frac{7}{8}$
C.	12 c.c. + 4 c.c.	,, +	,,	,,	$\frac{3}{4}$
D.	10 c.c. + 6 c.c.	,, +	,,	,,	$\frac{5}{8}$
E.	8 c.c. + 8 c.c.	,, +	,,	,,	$\frac{1}{2}$
F.	6 c.c. + 10 c.c.	,, +	,,	,,	$\frac{3}{8}$
G.	4 c.c. + 12 c.c.	,, +	,,	,,	$\frac{1}{4}$

All maintained at 32° C. for two and a half hours, then boiled, made up to 30 c.c. and titrated into 5 c.c. Benedict.

A.	6.0 c.c.	E.	15.3 c.c.
B.	6.3 ,,	F.	32.6 ,,
C.	6.6 ,,	G.	62.5 ,,
D.	8.8 ,,		

The velocity of the reaction is not in direct linear proportion to the quantity of enzyme present, *i.e.* high concentrations are relatively less active than lower. This agrees with the known laws of enzyme action. As Bayliss[4] says, "Where the enzyme

is in considerably smaller concentration than the substrate, the velocity of the reaction is in direct linear proportion to the quantity of enzyme present, owing to the whole of it being able to enter into effectual combination with the substrate" (see results concs. $5/8 - \frac{1}{4}$). "As the concentration of the substrate diminishes, another law begins to make its appearance, so that greater quantities of enzyme have relatively less effect" (results concs. $1 - \frac{3}{4}$).

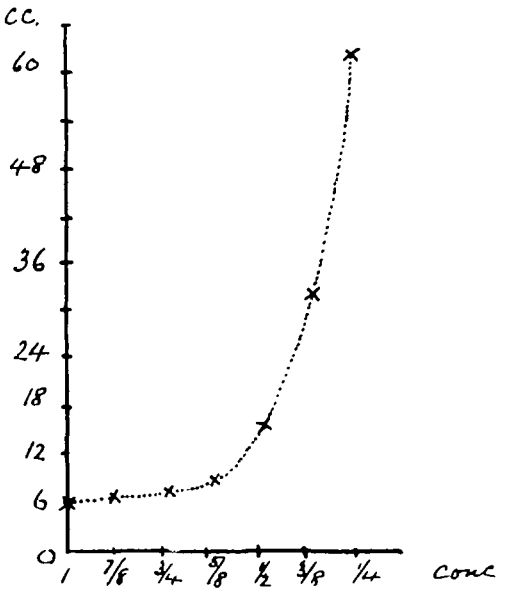

FIG. 26.—Concentration of enzyme curve.

A second experiment was performed in order to determine what influence the variation of the concentration of the substrate had upon the action of the enzyme.

An extract of fourteen styles was made in 70 c.c. toluol water :—

A.	10 c.c.	+	10 c.c.	1 per cent. starch	—*i.e.* conc. of	1
B.	„	+	„	7·5	„	„ $\frac{3}{4}$
C.	„	+	„	5.0	„	„ $\frac{1}{2}$
D.	„	+	„	2.5	„	„ $\frac{1}{4}$
E.	„	+	„	1.25	„	„ $\frac{1}{8}$
F.	„	+	„	0.625	„	„ $\frac{1}{16}$
G.	„	+	„	0.3125	„	„ $\frac{1}{32}$

All incubated at 32° C. for two and a half hours, boiled,

made up to 25 c.c., and titrated into 10 c.c. of Benedict's solution with the following results :—

A.	1.66 c.c.	E.	11.2 c.c.
B.	2.35 „	F.	19.0 „
C.	3.2 „	G.	33.8 „
D.	6.8 „		

The curve approximates very nearly to what would have been obtained had the velocity of reaction increased exactly

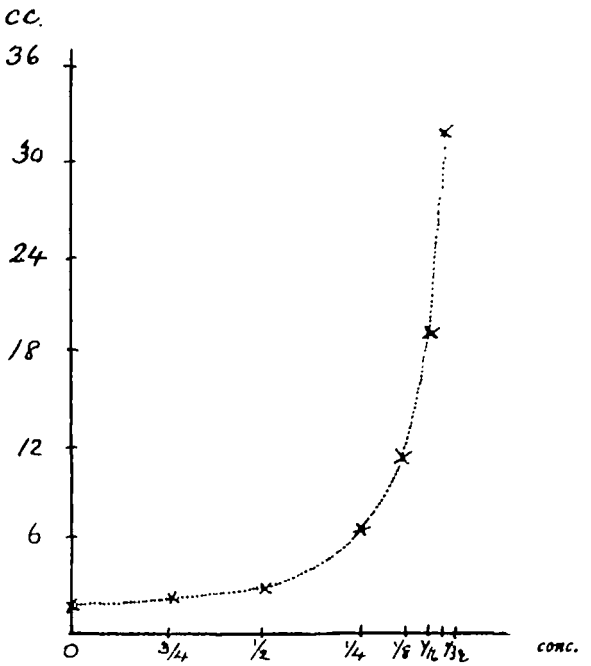

FIG. 27.—Concentration of substrate curve.

with the increase of concentration of the substrate. This is exceptional, the only cases in which it occurs being due to the concentration of the substrate being very low as compared with that of the enzyme (Bayliss, p. 105).

The results of these two concentration experiments prove that the style ferment, when employed in the quantities used in these digests, is a powerful enzyme having the characteristic properties of such a substance.

d. **Mid-Gut.**—Repeated experiments have failed to show the presence of lipolytic or proteolytic enzymes in the mid-gut,

digests with peptone failing to reveal the presence of even an erepsin-like ferment. It is difficult to test for the presence of an amylolytic ferment owing to the invariable presence of a certain quantity of gonad tissue which gives a strong reduction after standing, presumably owing to the autolysis of glycogen. If there is any carbohydrate-splitting ferment in the mid-gut it is in minute quantities.

6. Reserve Food Materials.

Food material is stored in Mya in the form of glycogen and of fat. It has already been noted that the gonad extract (male or female) after a short incubation gives a strong reduction with Fehling's solution. This does not occur if the extract has been previously boiled, but the style enzyme will act upon the boiled extract which will then give a well-marked reduction. There is thus reason for thinking that glycogen is present in quantity in the gonad and with it an enzyme capable of converting it into sugar. No appreciable increase in the amount of reducing sugar normally present in the hepatopancreas was obtained after an extract had been incubated for several hours at 32° C., and the assumption is that little or no glycogen is present in that gland (which might safely be described as a simple digestive gland). This is supported by the evidence of the majority of workers on the subject, Griffiths[16] failing to find glycogen in the hepato-pancreas of *Mya arenaria* itself. Creighton (quoted by List) found glycogen among Lamellibranchs in the plasma cells of the submucosa of the alimentary tract and in other places. Collip[8] states that the glycogen content of perfectly fresh *Mya arenaria*, calculated as percentage of wet drained tissue, may be as high as 11 per cent. This falls to 2 per cent. after the animals have been kept for two weeks, and again to 0.25 per cent. after they have been sealed up for six days (*i.e.* living as anaerobic organisms).

Sections stained with osmic acid show the almost universal presence of fat, but especially in the gonad. Voit,[46] working on the pearl oyster, found that the fat content of the hepato-pancreas is always higher than that of other organs. His

figures are : hepatopancreas, 9.7 per cent. ; ovary, 7.9 per cent. ; foot muscle, 4.3 per cent. ; mantle, 3.8 per cent. ; and gills, 1.3 per cent.

7. Summary.

The observations recorded in this paper on the feeding, the alimentary organs, and the digestive processes in *Mya arenaria*, may be epitomised as follows :—

1. The food consists of organic debris, sand particles, and micro-organisms suspended in food currents, which are created by the ciliary action of the gills, and conveyed to the mouth by ciliary currents on the surface of the gills and labial palps.

2. Particles are carried towards the ventral margin of the demibranchs, where they are caught in the currents created by the large cilia present in the region of the marginal food groove, and carried towards the mouth. A third anterior current, also created by large cilia, runs along the gill axis.

3. The direction of the ciliary currents on the labial palps has been described in detail, and the view expressed that the ciliary mechanism present on the inner face of the palps is devoted entirely to the separation of the food into large and small particles, the former being despatched to the tip of the palp, and the latter carried forward to the mouth.

4. Coarser particles which do not reach the gills, and other particles rejected by the gills and palps, are carried away by ciliary currents present on the visceral mass and mantle, and are finally expelled from the mantle chamber.

5. The anatomy and histology of the alimentary canal and the hepatopancreas have been described.

6. The presence of muscle fibres in the wall of the gut is practically restricted to the œsophagus and rectum, but the entire alimentary tract, with the exception of the area of the gastric shield, is ciliated and abundantly provided with mucus-secreting glands.

7. The presence of ciliary currents in all parts of the gut has been demonstrated.

8. The ciliary currents present in the stomach have been described, and the definite separation of the food into larger and smaller particles, particularly by the mechanism of the

grooved area, has been shown. The larger particles are carried straight into the intestine and the smaller particles to the base of the gastric shield, where they are caught in the substance of the tip of the style.

9. The universal presence of phagocytes or wandering cells throughout the gut has been noted, and their special activity in the grooved area of the stomach described. The balance of evidence has been shown to be in favour of the view that they are nutritive in function, although they may also have an excretory function, as they have been shown to be capable of ingesting matter of absolutely no food value.

10. The histology of the style-sac, the origin of the style, and the ciliary currents present in the sac have been described.

11. The mature style has been described and evidence brought forward to prove that it is an albuminoid mass saturated with an amylolytic enzyme, which is revolved, and, at the same time, pushed forward into the stomach by the action of the various cilia present on the epithelium of the style-sac. The tip bears against the gastric shield and is gradually worn down against this surface and through the action of the hepatopancreatic secretion.

12. The permanence of the style in Mya has been shown to be due to its protection from the corroding effects of the protease present in the hepatopancreatic secretion owing to the possession of a separate style-cæcum.

13. The importance of the style as a factor in the evolution of the higher Lamellibranchs, the presence of an homologous structure in certain of the Gastropods, and the question of its taxonomic importance have been touched upon.

14. The periodicity of digestion, as contrasted with the mechanical regularity of feeding, has been discussed.

15. The absence of digestive enzymes in the intestine, together with the evidence of previous workers, has been advanced in favour of the assertion that digestion takes place in the stomach only, and absorption in the stomach, mid-gut, and rectum.

16. The hepatopancreatic secretion has been shown to possess amylolytic, proteolytic, and lipolytic enzymes, and the action of these has been examined.

17. The style enzyme has been examined and found to reduce starch and glycogen but not sucrose. The optimum medium has been shown to be neutral, the optimum temperature to lie near 32° C., and the temperature of destruction at 51° C. Experiments in which the concentration of the enzyme and substrate were varied gave results which prove that the style enzyme has the typical properties of such a substance.

18. The presence of reserve supplies of glycogen and fat has been shown.

8. References.

[1] Alder, J., and Hancock, A. (1851), "On the Branchial Currents in Pholas and Mya," *Ann. Mag. Nat. Hist.*, **8**, 2nd series.

[2] Allen, W. R. (1914), "The Food and Feeding Habits of Freshwater Mussels," *Biol. Bull.*, **27**.

[3] Barrois, T. (1889-90), "Le Stylet cristallin des Lamellibranches," *Revue biol. N. France*, **3**, **4**, **5**.

[4] Bayliss, W. M. (1919), *The Nature of Enzyme Action*, 4th edition, London.

[5] Bernard, C. (1855-66), *Leçons de Physiologie Expérimentale Appliquée à la Medicine*, Paris.

[6] Biedermann, W. (1910-11), "Die Aufname, Verarbeitung, und Assimilation der Nahrung," *Handb. d. vergl. Physiol.*, H. Winterstein, **2**, pp. 1929-1940, Jena.

[7] Carazzi, D. (1897), *Internat. Monatshr. Anat. Physiol.*, **14**.

[8] Collip, J. B. (1921), "A Further Study of the Respiratory Processes in *Mya arenaria* . . ." *Journ. of Biol. Chem.*, **49**.

[9] Coupin, H. (1893), "Sur l'élimination des Matières étrangères chez les Acéphales . . ." *C. R. Acad. Sci.*, Paris, **117**.

[10] Coupin, H. (1900), "Sur les Fonctions de la Tige cristalline des Acéphales," *Ibid.*, **130**.

[11] Dakin, W. J. (1909), "Pecten," *L.M.B.C.*, Memoir No. xvii.

[12] Dastre, A., and Floresco, N. (1899), "Contrib. Chlorophylles anim," *C. R. Acad. Sci.*, Paris, **128**.

[13] Edmondson, C. H. (1920), "The Reformation of the Crystalline Style in *Mya arenaria*," *Journ. Exp. Zool.*, **30**.

[14] Fredericq, L. (1878), "La Digestion des Matières albuminoides chez quelques Invertébrés," *Arch. Zool. Exp.*, **7**.

[15] Gray, J. (1920), "The Effects of Ions upon Ciliary Movement," *Q.J.M.S.*, **64**.

[16] Griffiths, A. B. (1888), "Researches on the Problematic Organs of the Invertebrata," *Proc. Roy. Soc.*, Edin., **14**.

[17] Gutheil, F. (1911), "Über den Darmkanal und die Mittledarmdrüse von *Anodonta cellensis*," *Zeit. wiss. Zool.*, **99**.

[18] Haseloff, B. (1888), "Über den Krystallstiel der Muscheln nach Untersuchung verschiedener Arten der Kieler Bucht," *Biol. Centr.*, **7**.

[19] Hazay, J. (1881), "Die Molluskenfauna von Budapest," *Malak. Bl. L. Pfeiffer. N. F.*, Bd. **4**.

[20] Heide, A. de (1686), *Experimenta circa sanguinis missionem . . . Mytuli*, pp. 35-36, Ed. Nua., Amsterdam.

[21] Hoffman, F. (1914), "Beiträge zur Anatomie und Histologie von *Tagelus dombergi*," *Jen. Zeit. f. Natur.*, **52**.

[22] Jordan, H. (1913), *Vergleichende Physiologie wirbelloser Tiere*, Jena.
[23] Kellogg, J. L. (1900), "The Ciliary Mechanisms in the Branchial Chamber of the Pelecypoda," *Science*, **11**.
[24] Kellogg, J. L. (1915), "Ciliary Mechanisms of Lamellibranchs, with Descriptions of Anatomy," *Journ. of Morph.*, **26**.
[25] Krukenberg, F. W. (1886), *Vergleichend-physiologische Vorträge*, **1**, Heidelberg.
[26] List, T. (1902), "Die Mytiliden," *Fauna und Flora des Golfes von Neapel*, **27**.
[27] M'Alpine, D. (1887), "Observations on the Movements of the Entire Detached Animal, and of Detached Ciliated Parts of Bivalve Molluscs . . ." *Proc. Roy. Soc.*, Edin., **15**.
[28] Matthias, M. (1914), "Untersuchungen über den Darmkanal und das Herz einiger Arcaceen," *Jen. Zeitschr. f. Natur.*, **52**.
[29] Mitra, S. B. (1901), "The Crystalline Style of Lamellibranchs," *Q.J.M.S.*, **44**.
[30] Nelson, T. C. (1918), "On the Origin, Nature, and Function of the Crystalline Style of Lamellibranchs," *Journ. of Morph.*, **31**.
[31] Orton, J. H. (1912), "The Mode of Feeding of Crepidula . . ." *Journ. Mar. Biol. Ass.*, **9**, N.S.
[32] Peck, R. H. (1877), "The Minute Structure of the Gills of Lamellibranch Mollusca," *Q.J.M.S.*, **17**.
[33] Posner, C. (1875), "Ueber den Bau der Najadenkieme," *Arch. f. Mikr. Anat.*, **11**.
[34] Ridewood, W. G. (1903), "On the Structure of the Gills of the Lamellibranchia," *Phil. Trans. R. Soc.*, London, **195**.
[35] Roaf, H. E. (1906), "A Contribution to the Study of the Digestive Gland in Mollusca and Decapod Crustacea," *Bioch. Journ.*, **1**.
[36] Roaf, H. E. (1908), "The Hydrolytic Enzymes of Invertebrates," *Bioch. Journ.*, **3**.
[37] Robson, G. C. (1922), "On the Style-Sac and Intestine in Gastropoda and Lamellibranchia," *Proc. Malacal. Soc.*, **15**.
[38] Sabatier, A. (1877), "Anatomie de la Moule Commune," *Ann. Sci. Nat.*, **5**, 6th series.
[39] Siebert, W. (1913), "Das Körperepithel von *Anodonta cellensis*," *Zeit. f. Wiss. Zool.*, **106**.
[40] Stenta, M. (1901), "Über eine bei Lamellibranchiaten beobachtete untere Rückströmung sowie über die Wimperrinne des Mantels von Pinna," *Zool. Anz.*, **11**.
[41] Stenta, M. (1903), "Zur Kenntnis der Strömungen im Mantelraume der Lamellibranchiaten," *Arbeit. Zool. Inst.*, Wien., **14**.
[42] Thiele, J. (1886), "Die Mundlappen der Lamellibranchiaten," *Zeit. f. Wiss. Zool.*, **44**.
[43] Van Rynberk, G. (1908), "Sur le rôle fonctionnel du 'Stylet crystallin' des Mollusques," *Arch. Ital. Biol.*, **49**.
[44] Vlès, F. (1908), "Monographie Sommaire de la Mye," *Mém. Soc. Zool. France*, **21-22**.
[45] Vogt, C., et Yung, E. (1894), "Acéphales," *Traité d'Anatomie Comparée*, **1**, Paris.
[46] Voit, C. (1860), "Anhaltspunkte für die Physiologie der Perlmuschel," *Zeit. Wiss. Zool.*, **10**.
[47, 48] Wallengren, H. (1905), "Zur Biologie der Muscheln: I.—Die Wasserströmungen; II.—Die Nahrungsaufnahme," *Acta. Univ. Lunds.*, ser. 2, **1**, Nos. 2 and 3.
[49] Weymouth, F. W., "The Edible Clams, Mussels, and Scallops of California," *State of California Fish Bull.*, No. 4.