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ON THE ORIGIN, NATURE, AND FUNCTION OF THE CRYSTALLINE STYLE OF LAMELLIBRANCHS¹

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SEVENTEEN FIGURES

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1. INTRODUCTION

"What has not been written concerning the crystalline style, and in how many ways has not the mind of man sought to understand its true meaning, and to get to the bottom of its nature?"²

With these words a recent investigator (Matthias, '14) sums up well the history of the crystalline style of the lamellibranchs. Few molluscan structures have excited more interest among

¹ Submitted as thesis for the degree of Doctor of Philosophy, June, 1917.
² Translation is mine.

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scientists than this singular organ, and yet, where described in many of the modern text-books, its function is ignored or held more or less doubtful. It seems that we really know little more regarding the biological rôle of the crystalline style than we did two centuries ago. A survey of the literature shows that the conclusions of the many investigators are decidedly contradictory. Much of the evidence advanced is not well supported and the conclusions in many instances are mere guesses.

It is to be noted, furthermore, that some recent authors (von Fürth, '03; Guthel, '11; Matthias, '14; Allen, '14, and others) have accepted the work of Mitra ('01) as conclusive. The investigations of this scientist were comprehensive and carefully conceived as far as they were carried out. However, as we shall see later, much of the credit given to Mitra rightfully belongs to earlier workers. He gives no survey of the literature beyond the theories presented in four text-books, and some of his conclusions are untenable in the light of more recent investigations.

The present contribution is an attempt to bring all known data under one head and to attack the problem from every possible angle.

My own investigations, together with such results of other workers as are applicable, will be considered under the main topics of morphology, histology, physiology, embryology, ecology, and evolution.

The crystalline style

In figure 1 is shown a fresh-water mussel, *Anodonta grandis*, dissected to show the crystalline style and the part of the alimentary canal in which it is lodged. As these structures as found in this species are fairly typical of the more common lamelli-branches, *Anodonta* will be used as the type form.

The intestine leaves the posterior end of the stomach and runs in a posteroventral direction along the base of the foot to within a short distance of the posterior margin. Here it turns dorsad and, making a wide bend, continues dorso-anteriorly through the hepato-pancreas to a point below the anterior end of the heart. Here the intestine makes a backward turn upon itself, and after

and yet, where described in function is ignored or held we really know little more in crystalline style than we did literature shows that the con- structure is decidedly contradictory. well supported and the guesses.

Some recent authors (von Allen, '14, and others) are conclusive. The investigations are extensive and carefully conducted. However, as we shall see, the literature beyond the work of Allen and others is not conclusive. The investigations are extensive and carefully conducted.

It is attempted to bring all known facts of the problem from every possible source.

With such results of other workers considered under the main heading of embryology, ecology, and evolution.

In the case of *Anodonta grandis*, the part of the alimentary canal consisting of these structures as found in the more common lamellibranch type form.

The stomach and runs from the base of the foot to within the mantle. Here it turns dorsad and runs anteriorly through the anterior end of the heart. It then turns upon itself, and after

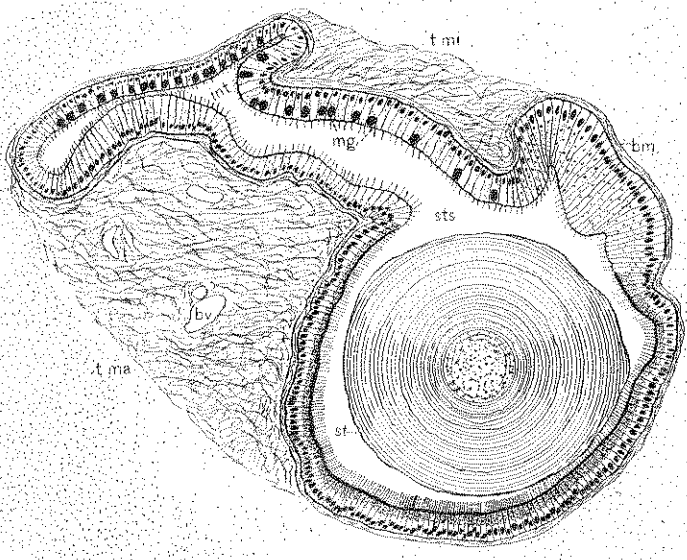
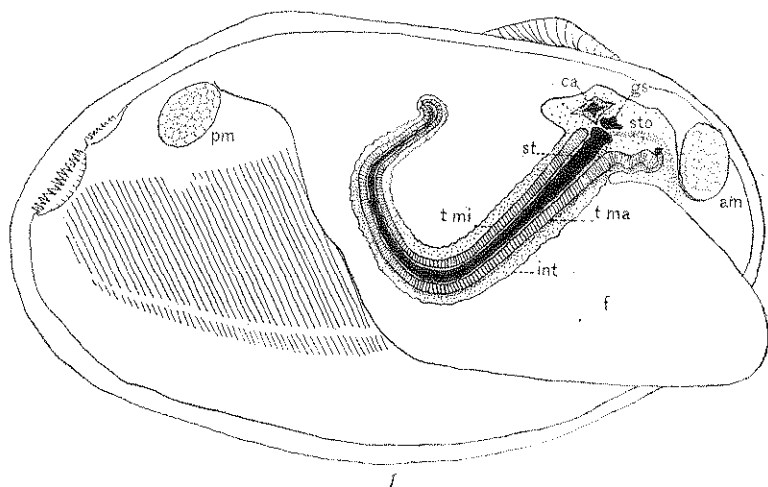


Fig. 1 *Anodonta grandis*: dissection from right side. *am*, anterior adductor muscle; *ca*, opening into dorsal caecum of stomach; *f*, foot; *gs*, gastric shield; *int*, intestine; *pm*, posterior adductor muscle; *st*, crystalline style; *sto*, stomach; *t ma*, major typhlosole; *t mi*, minor typhlosole.
 Fig. 2 *Lampsilis anodontoides*: transverse section through style sac and intestine near anterior end. *bm*, basal membrane; *bv*, blood-vessel; *int*, intestine; *mg*, mucous gland; *st*, crystalline style; *sts*, style sac; *t ma*, major typhlosole; *t mi*, minor typhlosole.

three more loops in the visceral mass passes to the rectum and anus.

The first portion of the intestine (the 'Magendarm' of the Germans) which lies between the stomach and the sharp bend beneath the heart, consists essentially of two regions. On the right side is the intestine proper and on the left the groove which lodges the style. Since this groove or tube is open at both ends, in the type under consideration, I shall refer to it as the style sac, rather than as the style caecum, as it is commonly called.

Incompletely separating the intestine and style sac are a dorsal and a ventral ridge, forming two 'typhlosoles' which extend throughout this portion of the intestine. The broad surfaces of these ridges are in apposition, leaving a narrow slit through which the style sac and intestine are in communication throughout their extent. The relation between these structures is better understood from figure 2, a transverse section through this portion of the alimentary tract.

The style itself, when fully formed, completely fills the lumen in which it lies. It tapers gradually from before backwards, becoming a more tenuous thread at the posterior end of the style sac. The anterior end projects from the pylorus across the cavity of the stomach to the opposite wall.

At the point of contact between the head of the style and the gastric mucosa there is developed a plate-like structure of cartilaginous consistency. It conforms to the outline of the stomach wall at that point, and may bear an apical projection near its center. In some forms (*Pholas*, *Donax*, *Martesia*) this cartilaginous sheet lines the whole of the stomach cavity. As this structure has not, to my knowledge, been described by English or American investigators, I propose the name 'gastric shield.'

To avoid any misunderstanding, there is introduced here a synonymy of terms used by the earlier investigators. The crystalline style of English-speaking scientists is the 'stylet cristallin,' of Barrois ('89), 'tige hyalin,' of Siebold ('48), 'tige cristalline,' of Coupin ('00), the 'Dünndarmkörper' of Hazay ('81), and the 'stylus cristallinus' of its discoverer, Anton de Heide (1686).

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The gastric shield is represented by the 'Cristallgriffin,' of Bojanus ('19), 'Knorpelstiel,' of Hazay ('81), 'dreizackige Pfeil' of Jordan ('13), 'fleeche tricuspid,' or 'sagitta tricuspis,' of Poli ('1791), and the 'stomachal cuticle' in Pelseneer's volume on the Mollusca in Lankester's Treatise.

Historical

A complete chronological review of the literature dealing with the crystalline style would be beyond the limits of the present paper. A comprehensive digest of all former investigations and theories is, however, necessary. I shall, therefore, sum up most of these data, not chronologically, but group the various authors according as their conclusions favor one theory or another.

The following account is based chiefly upon the historical summaries found in the works of Barrois ('89, '90) and List ('02). Wherever discrepancies occur between the accounts as given here and for all of the important works, reference has been made to the original articles.

Anton de Heide (1686), in his monograph of the common mussel, describes the 'stylus crystallinus.' He refers to the work of Willis on the oyster, six years previous, in which he described a structure analogous with the 'spinali medullae' of the vertebrates. From the text it is probable that Willis referred not to the style, but to the typhlosole separating the style sac and true intestine. We are therefore justified in recognizing de Heide as the discoverer of the crystalline style. Its function was ignored by this investigator who, however, hazarded the guess that it might be an alimentary ferment or perform some rôle in the act of generation.

Lister (1696), in his anatomy of bivalve molluscs, pictures the stylus of *Pectunculus vulgaris* and *Mytilus marinus* (*edulis*), but gives no appreciation of the structure or usage of them.

Swammerdam (1737) refers to the presence of the style in *Mytilus belgicus*, evidently a fresh-water species.

Lesser (1744) reviews the literature to date, but adds no contributions of his own.

The first comprehensive investigation of the nature and function of the crystalline style was made by Poli (1791-95). He describes accurately the appearance of the style and its relations to the rest of the alimentary system. He was the first to note the presence of the gastric shield, which he likened to a cartilaginous three-pointed arrow, 'sagitta tricuspis,' borne on the head of the style.

Investigating the physical and chemical properties of the style, he found it to be soluble in water and coagulated by boiling water and alcohol. He believed that it served in some way to regulate the flow of 'bile' from the biliary crypts. Cuvier (1805) adds nothing new, but admits the conclusions of Poli.

From this point on investigators are so numerous that it is best to classify them according to the theory which they supported.

A. *An organ of support.* From its position in the alimentary tract, Carus, (1818), believed it to be a vestige of a splanchnic skeleton, homologous with the teeth of the sea-urchin. Garner ('41) believed its function was to give rigidity to the foot. DeBlainville ('48) claimed the simultaneous existence of several styles arising in the sinuses of the biliary canals. He had nothing to say regarding their use.

B. *Mechanical functions.* Meckel ('29) considered it homologous with the radula of the Cephalophores, and consequently an organ of mastication. Garner ('41) agreed with this interpretation, and thought that the points of the gastric shield served to modify the flow of 'bile' as first held by Poli. Deshayes ('48) gives good figures of the gastric shield which he believed to be important in mastication. Clark ('50) thought that the style acted as a pestle to grind up the alimentary matter.

Huxley ('53) was the first to consider the style an epithelial secretion. He says, regarding the style of *Pteroceras*,

The end of the style is opposed by one or two cartilaginous plates (the gastric shield) upon the principal elevation. It seems probable that the style is secreted by the walls of the pyloric caecum, and that it plays the rôle of a gastric plate to aid in grinding up the alimentary matter, though its transparent and delicate structure seems ill fitted to this purpose.

Milne-Edwards ('59) believed that its function was to stir the contents of the stomach during digestion. Hessling in the same year agreed with him that the style was an epithelial secretion. Both evidently were ignorant of the earlier work of Huxley.

Vanstone ('93) thought that the style was homologous with the stomach plates of snails and served to grind up the food.

Sabatier ('77), in his monograph of the common mussel, gives the first exhaustive histological treatise on the style region. He recognized the two ridges, or 'typhlosoles,' incompletely separating the style sac and intestine, and their relation to the style. His study of the heavy ciliation of the wall of the style sac led him to believe that the food of the mussel was caught between the cilia and the style, and mixed and rolled around, the style playing the part of an organ of mastication. He considered that the epithelium of the style sac served for the absorption of dissolved matter. He did not suspect the real relation between the style and the secretory epithelium which he describes.

C. *An aid to absorption.* Krunkenberg ('86) considered the style to have a function similar to that of the typhlosole of *Lumbricus*, pressing the alimentary matter against the absorbing epithelium. Von Fürth ('03) agrees with this interpretation of the anatomical function of the style. Grave ('03) believed that the style of the oyster acts as a plug to prevent the too rapid movement of alimentary materials and to exclude foreign particles of large size from the intestine.

D. *Reserve of nutriment.* Hazay ('81) made detailed observations of the occurrence of the style in the Unionidae, and distinguished the 'Knorpelstiel,' the gastric shield, and a hyaline string, the 'Dünndarmkörper,' or style proper. According to his observations, the gastric shield is very rudimentary in the spring and summer, attaining its greatest size in the autumn. The style is, in his opinion, formed from a gelatinous mass ('Magengallerte') in the stomach, and is pushed back into the intestine where it remains as a reserve of nutriment. This is kept from flowing into the stomach by the presence of the gastric

shield, which forms a sort of valve over the orifice. During the winter this reserve serves to nourish the animal. His conclusions are based on a study of two closely related fresh-water forms.

The presence of such a reserve of nutriment in the intestine was held by Krunkenberg ('86) to be untenable.

The inaugural thesis of Haseloff ('88) is the most extensive single work on the crystalline style. He studied seven different forms, but based his conclusions largely upon *Mytilus*. After describing the general anatomical relations of the style, the author cites experiments in which *Mytili* were kept in filtered and in normal sea-water. Finding that the style grew thinner and finally disappeared in those animals which were starved, he concluded that it must be a reserve of nutriment.

Haseloff was not aware of the work of Hazay until after he had finished his investigations. From his own results and those of Hazay, he concluded that the style was a structure built up in time of excess nutrition and absorbed in times of want. As for its origin, he believed it to be a chemical transformation of surplus nutriment, and not an epithelial secretion. Chemical tests applied by him showed the style to be albuminous in nature. He concluded that the presence of the style is almost universal in the lamellibranchs.

Dubois ('92) thought that the style serves as nutriment for parasites useful to their host.

Stempell ('98) believed the style to be lacking in the *Nuculidae*. The large gastric shield which he found in these forms was held to be a reserve material, absorbed by the gastric epithelium and used in the development of the sexual organs.

List ('02) in his beautiful monograph of *Mytilus*, gives a most comprehensive treatment of the subject. The historical summary is fairly complete and accurate, save that he does not give proper credit to some former workers. His investigations cover the anatomical, histological, and certain physiological aspects of the problem. He was the first to study the formation of the style in the living animal, and found that color particles taken up by the mussels in feeding were built up into the body of the style.

The nutriment taken into the stomach he believed to be mixed with the style substance, carried to the hepatopancreas and there digested. The course of the color granules was traced into the hepatopancreas, back to the stomach, and thence to the intestine. He believed that the style might serve as a reserve of nutriment in the Unionidae during hibernation, but that in marine forms this nutriment would be superfluous and therefore thrown away. Matthias ('14) in discussing List's idea of superfluous nutriment, considers that the mussels with all their ascribed stupidity are not so utterly 'stumpfsinnig' that they would willingly throw out the nutriment which they had so painstakingly gathered.

E. Relation to the organs of generation and the process of reproduction. De Heide (1686) thought the style might serve some purpose in the act of generation, but gave no reason for his belief. Poli (1791) advanced the same theory, also without any evidence in its support. In later years both of these investigators abandoned this interpretation of the function of the style.

After nearly two hundred years this rejected theory was again advocated by Cailliaud ('56). He held the Pholadidae to be hermaphroditic (since disproved) and that the style served some function during fecundation. Carus ('18) believed that the style might have some connection with the sex function.

Hoffmann ('14) following Stempel ('98), claimed to have ascertained a reciprocal relation existing between the gastric shield and the sexual organs. Comparing the mass of the gonad, the gastric shield, and the style, he concluded that the shield was not as well developed in males and females with mature gonads as in a male specimen where the testes were immature.

F. Serves as a means of transport for the nutriment; lubricating function of the style. Although the conclusions of Barrois ('89-'90) place his work under this head, it must be said in all fairness that his treatise on the crystalline style of the lamellibranchs is the most comprehensive and accurate contribution to the subject which thus far has appeared. Many subsequent workers have evidently paid but little heed to it, his results

either being ignored or else appropriated without giving proper credit. Much of the value of this work of Barrois lies in the thorough critical analysis of all former work which it contains. The historical summary is quite complete to date of publication. Most of his results will be considered in their proper connection on the following pages, but it may be said in passing that the investigations covered an exhaustive study of many forms, dealing with the anatomy, histology, chemical composition, and the physiological significance of the style and its attendant structures. The weakness of this contribution to the subject lies in Barrois' failure rightly to interpret the facts which were before him. He observed that the style was pushed forward into the stomach, there to be dissolved under the action of the gastric juice. He believed that the resulting viscous mass formed a sort of cement, which surrounding the sand grains and other foreign materials present, encrusted them, and thus prevented injury to the delicate lining of the alimentary canal. The gastric shield was believed by him to have a protective function similar to that of the 'Trichter' of insects.

Pelseneer, in Lankester's *Treatise on Zoology* ('06) and in his own earlier paper ('91) follows Barrois, and believes that the function of the style is to lubricate the alimentary mass. Schultze ('90) accepts the results of Barrois in their entirety, while Kellogg ('92) disagrees with both Barrois and Pelseneer, since where large amounts of sand are ingested the style could not form a sufficient protective covering. Coupin ('00) thought that the mucus of the style might serve to surround sharp particles and thus protect the epithelium.

G. *A mass of enzyme, or of enzyme and mucus.* Anton de Heide (1686), the discover of the style, hazarded the guess that it might be a mass of digestive fluid, but made no attempt to determine the fact. Wilson ('77) believed the style to be a mass of oesophageal mucus and gastric juice, but did not demonstrate any action of the style substance on food material.

The first attempt to determine the presence of enzymes in the style was made by Coupin ('00). He believed that the style was a mass of mucous substance, saturated with digestive fluid,

and he demonstrated the action of the amylolytic ferments present:

Mitra ('01), with no review of the literature save the theories given in the text-books of Gegenbaur, Balfour, and Claus and Sedgwick, repeated the work done by Barrios, Coupin, and some others, and concluded that the style was a mass of enzyme, in the nature of a globulin. Apparently he did not know of the existence of the gastric shield. His conclusions will be considered in some detail in the discussion of this paper.

Von Fürth ('03) followed Mitra, but believed it more reasonable to suppose as did Coupin ('00), that the style was a mass of mucus saturated with diastases, rather than a solid mass of enzyme. Matthias ('14), whose work has already been considered, admitted the conclusions of Mitra. Allen ('14), accepting the conclusions of Mitra in the main, showed that the formation and dissolution of the style are dependent wholly or in part on the feeding activities.

In addition to the above authors may be listed the following, whose work is of less importance:

Selenka ('68), in the description of the anatomy of *Trigonia*, notes the presence of the style, lying in a blind sac opening into the stomach. Egger ('87) describes the appearance of the style with its co-axial layers in *Jouannetia cumingii*. Grobben ('92) gives an account of the nature of the cilia of the intestine and of the style sac. Mention of the style is made by Moquin-Tandon ('85) in the Unionidae, and by Blanchard ('61) in the Pholadidae.

Other investigators and their conclusions, not listed here, will be considered in their appropriate connection in the main body of the present paper.

EXPERIMENTAL

Materials and methods

The investigations reported in this paper were carried on in the Zoological Laboratory of the University of Wisconsin, and at the floating laboratory of the New Jersey Agricultural Station for research in oyster culture, on the coast of New Jersey. The

work with fresh-water forms was done at Madison, using chiefly *Anodonta grandis*, *Lampsilis luteolus*, and *L. anodontoides*. These were collected from the near-by lakes and kept supplied with lake water. The marine species were studied at the summer station at Tuckerton, N. J., which is situated on the natural beds of the oyster, *Ostrea virginica*. Within a few feet of the laboratory also occurred the hard clam, *Venus mercenaria*, and the ribbed mussel, *Modiolus modiolus*. The bulk of the work with salt water forms was confined to these three species.

In studying the formation and physiological significance of the style, dissections were made of the living animals. Care was exercised to open the molluscs immediately after removal from the water, and to dissect open the stomach and intestinal walls in such a manner as to interfere as little as possible with the main nerve commissures. Wherever ciliary action was investigated the animal was kept in a small trough under the binocular, supplied with a constant stream of water at the same temperature as that in which it had been living.

Ciliary currents were traced by the movement of food material, and also by the introduction of carmine grains or fine sand.

Some difficulty was experienced in sectioning the style sac since with ordinary methods of fixation the style becomes so brittle that it invariably crumbles. The best results were obtained by fixing the material in Bouin's fluid, running up through the alcohols as rapidly as possible, and clearing in wintergreen oil, after which the tissue was imbedded in paraffin.

The sections were stained in Heidenhain's iron-hematoxylin and counterstained either with eosin, acid fuchsin, or mucicarmine. Fixation with osmic-acid solutions followed by safranin and gentian violet was very unsatisfactory, due chiefly to the slow action of the fixative on the rather large pieces of tissue necessarily used.

With Bouin's fluid the fixation of the cilia is almost instantaneous and, as a result, the separate cilia stand up clearly and distinctly, instead of being in a confused mass as usually happens during fixation.

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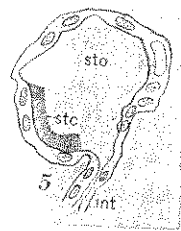
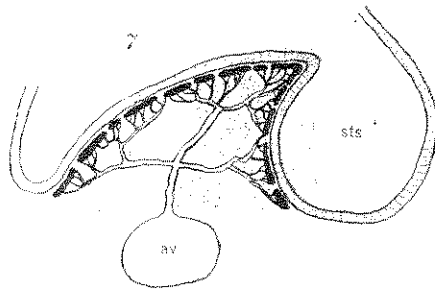
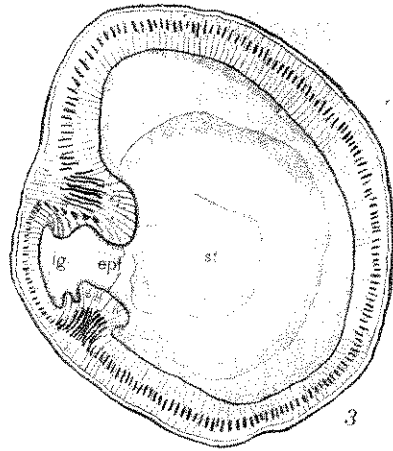
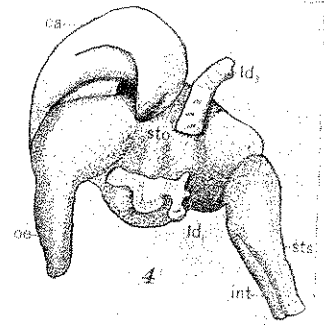
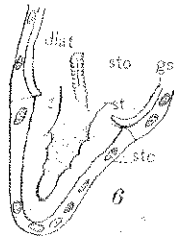
Gross structure of the style-bearing organs

The crystalline style and its attendant structures in Anodonta have been described, p. 56. The condition found in the other groups of the lamellibranchs follows:

The style occurs in its simplest form in the more primitive groups, the Protobranchs and Filibranchs. In *Nucula*, as an example, the style lies in the intestine itself, the alimentary canal showing no indication of a separation into two tubes. In the Filibranchs are certain transitional forms. *Arca*, for instance, contains species which, according to Matthias, show the simple condition, as in *Nucula*, and others in which there is the beginning of the formation of a separate style sac.

In *Arca platei* the intestine is roughly ovoid in cross section and is nearly filled by the style. At one side the intestinal wall forms a slight groove down which pass the waste materials. In *Arca barbata* this groove is somewhat deepened and, by the infolding of the intestinal wall, forming two ridges, this furrow is partially separated from the part bearing the style. These infoldings, which represent the typhlosoles of Anodonta in their most primitive condition, are not in apposition in this species, but leave a wide space through which the intestinal groove and the style-bearing portion are in communication (fig. 3).

A condition intermediate between that found in these two species occurs in *Modiolaria marmorata* where, according to List ('02) only a very slight infolding of the intestinal wall occurs. The epithelium of the alimentary tract is covered with large and powerful cilia, by means of which, on the one hand, the style is pushed forward into the stomach, and on the other, the waste matter is passed outward. By continued growth and differentiation, the epithelial infoldings of the intestine develop broad flat surfaces which come into apposition and separate the intestine from the style sac save for a narrow slit, as found in Anodonta (fig. 2). Coincident with these changes to form the typhlosoles, the epithelium of the style-bearing region becomes thrown up into a series of transverse folds, extending throughout



the length of the style sac, forming a series of 'bearings' upon which the style rests (figs. 9, 10).

The typhlosoles vary in size and position according to the species. In the forms examined by me they are dorsal and ventral, and thus separate the alimentary canal into right and left halves as far as the end of the style sac. In all these species I have examined, the ventral typhlosole is the larger. As a result of the bending of the alimentary canal the typhlosole, which is dorsal at the anterior end, becomes ventral throughout the posterior half of the style sac. I shall, therefore, speak of the typhlosoles as major and minor, rather than as dorsal and ventral.

These structures gradually diminish in size from before backward. In an adult *Anodonta*, 14.5 cm. long, the major typhlosole measures about 3 mm. across at the anterior end, narrowing down to 2.5 mm. at the posterior end of the sac. The minor typhlosole measures 2 mm. and 1 mm., respectively.

In the species which have just been considered, where the style caecum and intestine are completely separated, the two tubes merge into one at the end of the style-bearing region. Where the separation is more nearly complete, as in some of the Unionidae a small diverticulum occurs at the end of the style sac at the point where the intestine makes the sharp backward

Fig. 3 *Arca barbata*; transverse section through alimentary canal posterior to the stomach (Matthias, fig. 17). *cpf*, epithelial folds; *ig*, intestinal groove; *st*, crystalline style.

Fig. 4 *Anodonta cellensis*; plaster-of-Paris mold of stomach, oesophagus, and anterior portion of intestine and style sac (Guthrie, fig. 3). *ca*, dorsal caecum; *int*, intestine; *ld*, *ld*, left and dorsal ducts of hepato-pancreas; *sto*, stomach; *sts*, style sac.

Fig. 5 *Dreissensia polymorpha*; sagittal section through stomach, intestine, and anlage of the style caecum (Meisenheimer, fig. 132). *int*, intestine, *stc*, wall of stomach which evaginates to form the caecum of the crystalline style; *sto*, stomach.

Fig. 6 *Dreissensia polymorpha*; longitudinal section through the style caecum (Meisenheimer, fig. 133). *diat*, diatoms; *gs*, gastric shield; *st*, crystalline style; *stc*, caecum of the crystalline style; *sto*, stomach.

Fig. 7 *Anodonta cellensis*; transverse section through the major typhlosole and style sac showing distribution of blood-vessels (Schwaneeke, fig. 24 a, somewhat diagrammatic). *av*, visceral artery; *sts*, style sac.

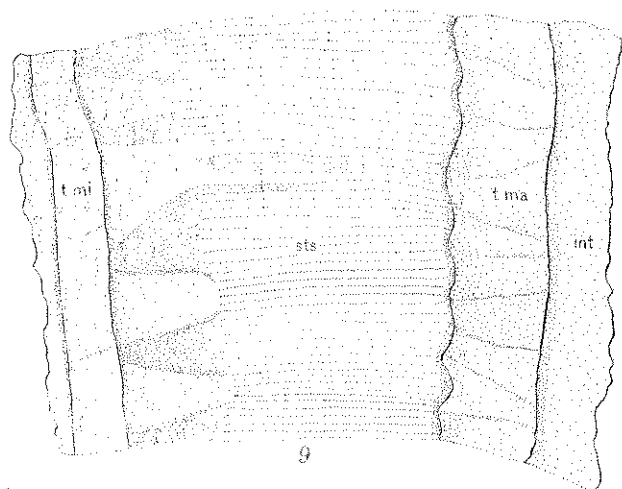
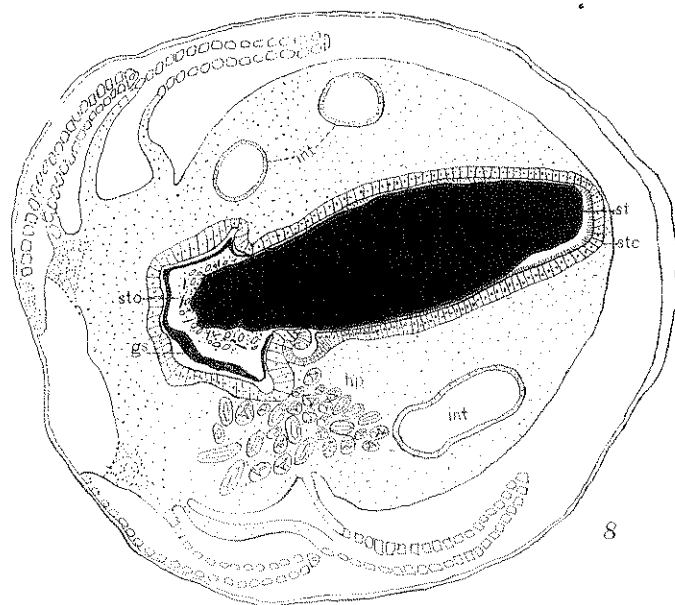


Fig. 8. *Martesia*; median transverse section of entire body. *gs*, gastric shield; *hp*, hepatopancreas; *int*, intestine; *st*, crystalline style; *stc*, caecum of the crystalline style; *sto*, stomach.

Fig. 9. *Anodonta grandis*; enlarged view of the surface of the typhlosoles, intestine, and style sac, opened from right side. *int*, intestine; *sts*, style sac; *t ma*, major typhlosole; *t mi*, minor typhlosole.

bend beneath the heart. In some forms (e.g., *Mytilus galloprovincialis*, according to List, '01) this diverticulum is prolonged a short distance posteriorly as a blind sac distinct from the intestine.

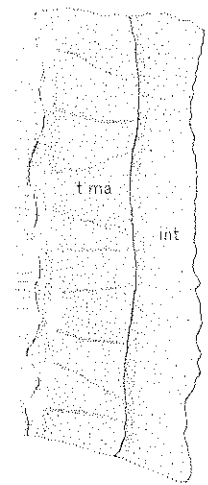
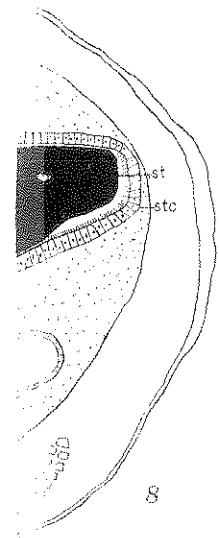
The style sac in certain of the higher lamellibranchs has become completely cut off from the intestine, forming thus a distinct caecum. This is entirely independent of the intestine, and opens into the stomach by a separate orifice. Such a condition is found in *Martesia* (fig. 5).

As has been pointed out by Matthias ('14), there exist, therefore, three distinct types of lamellibranchs as regards the position of the style, with transitional forms making a graded series from one to the other. First, those species in which the intestine itself bears the style; second, those in which the style sac and intestine form two tubes, incompletely separated by the typhlosoles, and finally the forms in which the style sac exists as a diverticulum distinct from the intestine proper.

The epithelium around the opening of the style sac into the stomach is raised into a ring which forms a sort of 'bushing' about the style. When the latter is fully formed it fits this orifice so snugly that no particles from the stomach could possibly pass between the style and the wall of the sheath, even if the effective stroke of the cilia were in such a direction as to permit it.

In the forms where the intestine and style sac are incompletely separated by the typhlosoles, the major typhlosole extends anteriorly into the stomach, forming a sort of tubercle as in *Anodonta* or a long ridge on the ventral wall of the stomach as in *Mourolus* (fig. 12). Other lamellibranchs examined have shown a somewhat similar prolongation of the major typhlosole.

The stomach presents the most diverse modifications in the different species. In general it is an elongated, oval sac, the lateral walls of which are more or less compressed. Various out-pocketings of the epithelium are present, usually in connection with the orifices of the hepatic ducts. The number of these varies, according to Pelseneer, from three in certain of the *Nuculidae*, to as many as twelve in *Mytilus*. Three are present in *Anodonta* (fig. 4).



re body. *gs*, gastric shield;
stc, caecum of the crystal-

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t, intestine; *stc*, style sac;

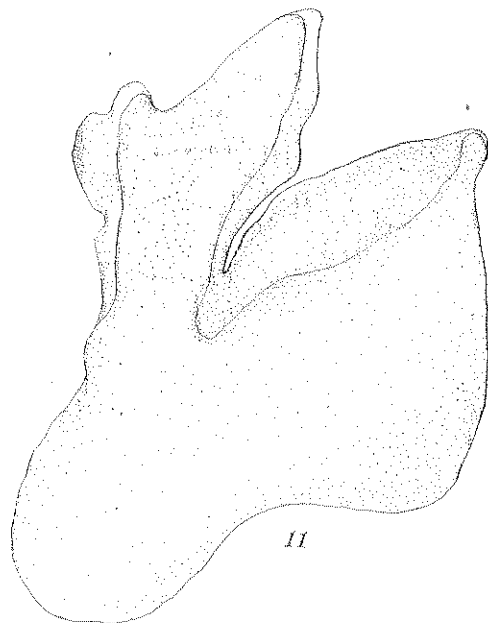
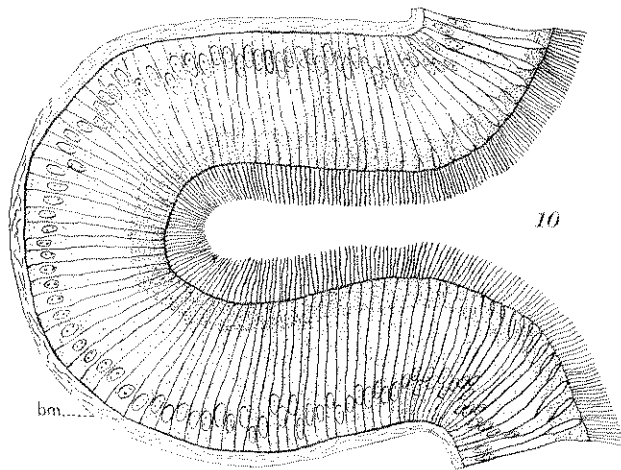


Fig. 10 *Lampsilis anodontoides*; longitudinal section of the wall of the style sac, showing one of the transverse furrows. *bm*, basal membrane.

Fig. 11 *Modiolus modiolus*; the gastric shield, concave aspect.

The epithelium of the stomach is thrown into many folds and ridges, with deep grooves passing between them. Figure 16 gives the appearance of the stomach of *Anodonta* when opened along the right margin of the dorsal wall, and spread out flat. It also shows the relation of the two orifices, opening side by side and separated by the typhlosole, and the large deep groove leading across the wall of the stomach and entering the intestine proper.

In some forms a portion of the stomach is more or less separated by a constriction, forming, in *Anodonta*, a pouch-like diverticulum on the dorsal side (fig. 1) or a distinct caecum extending the length of the stomach, beneath its ventral wall, as in *Modiolus*. This blind sac performs in the latter genus, a very important function, as will be shown later.

The gastric shield

On the anterior wall of the stomach, at the point where the style comes in contact with the mucosa is a singular structure which I have called the gastric shield. This is a thin, plate-like sheet, as clear as glass, and of the consistency of cartilage. In an adult *Anodonta* it measures approximately 8 x 5.5 mm. in its greatest extent. It assumes many diverse forms in different species, but in general I find three main lobes, and a blunt apical projection (fig. 11). The edges of these lobes are firmly held between the folds of the epithelium, and, in some species at least, the border of the shield dips down into the crypts of the hepatic ducts. In some instances a sharp spur of the shield may run for some distance into the orifice.

The cells beneath the shield are, according to Gutheil ('11) the only columnar cells of the entire alimentary tract which are devoid of cilia. Their function is to secrete droplets of a colorless matter which harden to form the shield.

This structure is closely applied to the epithelium lying beneath it, and, as it rests between folds of the stomach wall, it presents an outer surface concave in outline. In *Venus* an outward projection of the epithelium forms a sort of tubercle which

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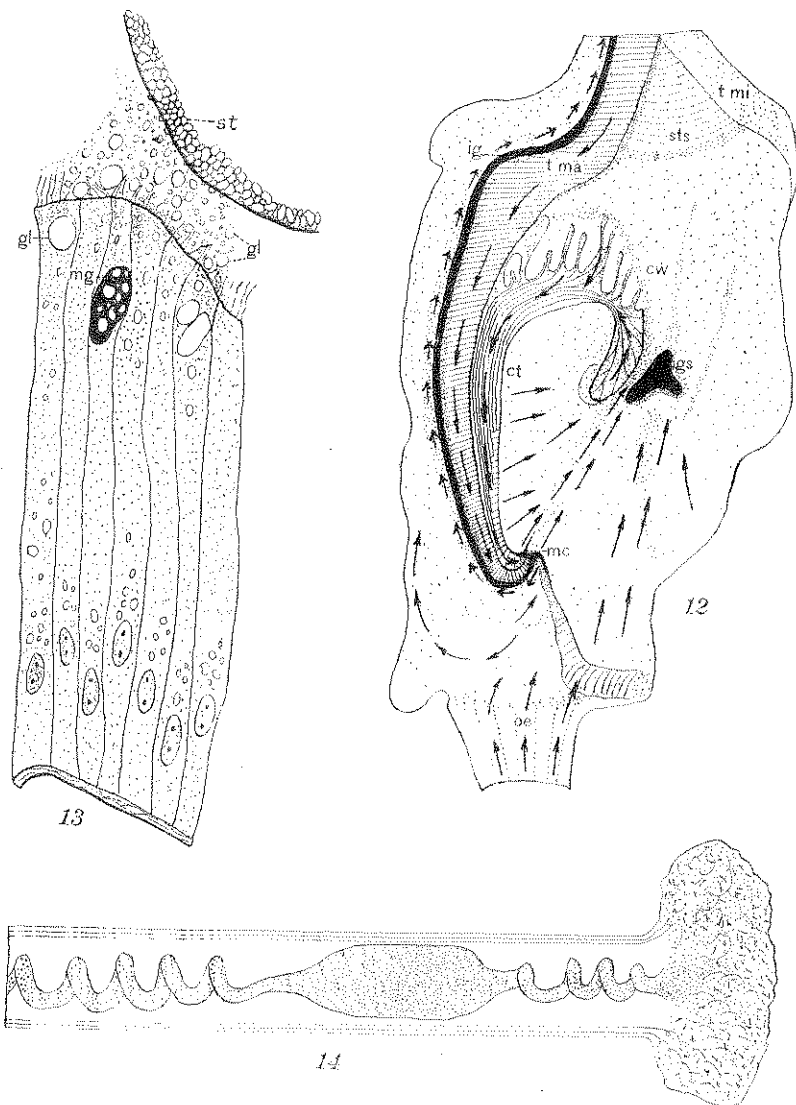


Fig. 12. *Mediolus modiolus*; surface of inner wall of the stomach opened from the right side; long arrows indicate direction of ciliary currents on the ridges and exposed surfaces of the mucosa, short arrows show direction in the furrows and grooves. *ct*, ciliated tract leading to mouth of caecum; *ctw*, ciliated whorl from which this tract originates; *gs*, gastric shield; *ig*, intestinal groove; *mc*, mouth of caecum; *oc*, oesophagus; *sts*, style sac; *t ma*, major typhlosole; *t mi*, minor typhlosole.

Fig. 13. *Lampsis anodontaoides*; enlarged view of the secreting cells of the dorsal wall of the style sac. *gl*, globules of secreted material; *mg*, mucous gland; *st*, style.

Fig. 14. *Anodonta grandis*; optical section of freshly removed style showing inner spiral core of food materials, and the solution of the head of the style.

acts as a brace for the shield. In *Ostrea* the smallest of the three lobes is concave on its upper surface, forming a bowl-like depression.

In *Donax*, according to Barrois, the gastric shield covers nearly the entire wall of the stomach. At the crypts of the hepatopancreas it dips down into the orifices and lines them. *Martesia* (fig. 7) exhibits the same type of shield.

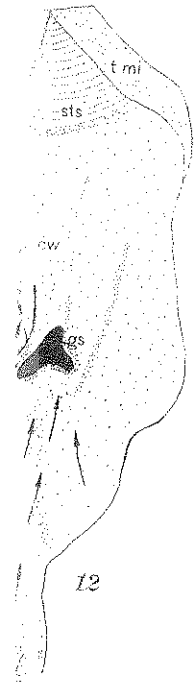
In all cases which I have examined the shield bears at its apex a blunt projection of much heavier nature than the lobes themselves. Under natural conditions the head of the style is directed against this projection, while the food mass around the style is in contact with the lateral lobes of the shield. The significance of this will appear later.

Nervous and vascular connections of the style sac

According to Schwanecke ('13), blood is supplied to the style sac and other parts of the alimentary canal through the visceral artery, a large branch which leaves the anterior aorta just ventrad the stomach, and courses posteriorly to the upper part of the intestine and style sac. Here it divides to form two large trunks, which in turn supply the greater part of the alimentary canal. Most of the blood going to the intestine is carried to the typhlosoles. Here there are no true capillaries but the smaller arteries form large lacunae beneath the epithelium. As a result, an almost continuous haemal cavity is formed in close conjunction with the secretory and ciliated cells (fig. 7).

The venous system is less well defined than the arterial. The blood from the lacunae is collected by several large veins and carried to the sinus venosus.

The lamellibranchs, according to Pelseneer (Lankester's Treatise), have no differentiated stomatogastric nervous system. Nerve strands to the alimentary canal are given off from the median faces of the two branches of the cerebrovisceral commissure. More recently, Splittstoesz (13), reviewing the work of Keber and Duvernoy on the nervous system of molluscs, has presented a careful and extensive investigation of the nervous



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system of *Anodonta cellensis*. According to him, three gastric nerves arise from the cerebrovisceral commissures of each side, just posterior to the cerebral ganglia. These send branches to the oesophagus, but principally supply the wall of the stomach. As a result of the intercrossing of many nerve fibers there is formed here a 'solar plexus' from which fibers extend downwards to the wall of the intestine and style sac. This latter region is, therefore, in close nervous connection with the anterior centers of control.

Histology of the style sac and accompanying organs

By far the most extensive and careful part of all former work on the crystalline style has been concerned with the structure of the cells of this region. When one sees the long ciliated cells, with their large distinct nuclei, besides the several types of secreting cells, it does not seem strange that so many investigators, setting out to study the style in all its relations, should have been sidetracked by the beautiful histological material before them. Inasmuch as the present problem lies rather in the realm of physiology than that of histology, I shall consider here only such details of cell structure as are necessary to understand the mechanism for the secretion and movement of the style. For a more complete account of the histology of the alimentary canal of lamellibranchs the reader is referred to the works of Sabatier ('77), Barrois ('89), List ('02), and Gutheil ('11). An extensive treatise on the structure of ciliated cells, and the mechanism of ciliary action with special reference to the typhlosole of *Anodonta*, will be found in the work of Erhard ('10).

Figure 2 is a transverse section of the style sac and intestine of *Lampsilis anodontoides*, showing the typhlosoles incompletely separating the two tubes. The typhlosoles are highly vascular, and are composed of loose connective tissue lying between the lacunae or haemal spaces. Very few nuclei occur here. The epithelium of this region is composed of a single layer of long ciliated cells resting on a basal membrane. The cytoplasm is usually filled with many refractive granules and stains rather

lightly. The nucleus is very large, is situated proximally and may contain one or two nucleoli. Vacuoles or granules of absorbed food material often occur in the cytoplasm. From the distal end of each cell project a number of large cilia, the basal fibers of which extend down some distance into the cytoplasm.

Beneath the basal membrane are bands of musculature in the carnivorous *Septibranchs*, but in the other lamellibranchs, as pointed out by Gutheil ('11), ciliary action has entirely replaced peristalsis, hence muscular elements in this region are lacking.

Lying between the ciliated cells are two other types, namely, secreting cells and mucous glands. The secretory cells are of long goblet-cell type, and when filled with secretion are greatly distended. They occur most numerous in the minor typhlosole.

The mucous glands are large unicellular structures which take a deep red color with mucicarmine, and are sharply differentiated from all surrounding cells. The nucleus lies near the base, and when the upper portion of the cell becomes filled with mucus it is so distended as to appear ovoid or nearly spherical in shape (fig. 13). These glands in *Lampsilis* are confined to the minor typhlosole and the intestine. I have never found them in this species in the major typhlosole or the style sac (fig. 2).

Where the minor typhlosole joins the style sac there is a striking modification of the epithelium. The surface of the cells dips down to form a furrow which extends parallel to the typhlosole. The cells bordering this groove are twice as long as the other cells of the alimentary canal, are very narrow, and have large, densely staining nuclei. Their distal ends bear few and small cilia (fig. 13). In favorable material great numbers of globules of a substance staining like the style are found in and between the cells. In figure 14 many of these globules are shown beneath the epithelium. At the surface some of these drops have coalesced to form a mass which cements the style to the epithelium at this point.

The epithelium of the rest of the style sac differs markedly from that of the above region in that it is composed of shorter and thicker cells. The nuclei are large, rich in chromatin, and

lie close to the basal membrane. Most striking of all are the great bristle-like cilia on these cells. Of such size are they that one gains the impression that the very ends of the cells themselves have been drawn out into the fiber. The distal portion of the cells is very rich in protoplasmic material which stains deeply, and forms a distinct region extending from the groove on the dorsal side, entirely around the style sac to the edge of the major typhlosole (fig. 2). A better conception of the cilia on these cells may be gained from figure 10, which shows a section through the style sac at right angles to the transverse folds.

The only cells of the epithelium of the stomach which need concern us are, first, those on the folds of the walls and, second, the cells which lie beneath the gastric shield. The former, according to Sabatier ('77), are much more heavily ciliated than the surrounding furrows. The latter, following Gutheil ('11), are the only columnar cells of the entire alimentary tract which are devoid of cilia. Their function is to secrete globules of a clear fluid which hardens to form the gastric shield.

The ciliary mechanism

Nowhere in the animal kingdom is ciliary activity brought to the high degree of development and complexity that it attains in some of the molluses. Not only has it replaced muscular peristalsis in many of these forms, but, as is shown in the excellent works of Allen ('14) and Kellogg ('15), the lamellibranchs have developed very efficient and delicately balanced modes of feeding, entirely by the use of cilia.

Allen found that the quality of the food permitted to enter the mouth is controlled by bringing into action one or the other of two opposed sets of cilia. These are situated on opposite faces of the transverse folds of the palps. According as these folds lie in their normal position or are erected by reflex muscular action, the food matter, entangled in mucus, is directed into or away from the mouth. By means of this mechanism, streams of food material which contain too large a percentage of foreign particles are directed backwards, and enter the re-

urrent ciliary channels leading to the base of the incurrent siphon, whence they are expelled. The mouth is a slit-like aperture situated between the palps. It is provided with muscles by means of which it may be closed in case any objectionable matter gets by the palps. So far as I know, no one has followed the ciliary mechanism beyond this point.

The oesophagus is a thin-walled tube, dorsoventrally compressed. Its inner surface is furrowed by a number of distinct longitudinal grooves, one of which is very much larger than the others. Where the oesophagus joins the stomach the epithelium is raised to form a distinct ridge encircling the orifice; in *Lampisilis* this ridge forms a sort of shelf on the ventral side, which projects into the stomach, nearly to the gastric shield.

All of the longitudinal grooves of the oesophagus end at the stomach except the large one, which extends across the left side of the ventral wall of the stomach to the base of the gastric shield. The cilia in the oesophagus carry the streams of food material up to the shelf-like projection in its ventral wall and also up the large furrow to the base of the gastric shield.

The lateral walls of the stomach in proximity to the gastric shield bear tufts of very powerful cilia. When the walls are in their normal position the effective stroke of these is such as to set into rotary motion any material in contact with them. This rotation is in a clockwise direction when viewing the animal from the anterior end.

All ridges and furrows of the stomach wall, except the region beneath the gastric shield, bear strong cilia which keep up constant currents within the stomach. In general these may be resolved into two main groups: first, those currents on the ridges and raised parts of the gastric epithelium, together with all streams from the oesophagus, which lead to the region around the gastric shield; second, those of the deeper grooves and furrows of the stomach wall which, save for exceptions to be noted later, lead into the large furrow which passes to the right of the major typhlosole and forms the intestine proper.

A detailed description of the comparative anatomy of the stomach and the ciliary mechanisms in each of the lamelli-

branches studied would lead too far afield. With minor modifications the figure and description of the stomach of *Anodonta* serves equally well for *Lampsilis*, *Ostrea*, and *Venus*.

In *Modiolus*, however, there is an important accessory structure of the stomach which is closely connected with one of the functions of the style in this form. That part of the ventral wall of the stomach which lies between the orifice of the style sac and the base of the gastric shield forms a spirally shaped structure suggesting a whorl of a small shell. Close examination proves this to be a series of conical orifices, each connected with a deep groove (fig. 12). These furrows, at first separate, reunite to form a tract which crosses the ventral wall of the stomach to the left of the extended major typhlosole, and enters the ventral diverticulum or blind sac mentioned above (p. 71). The surface of the conical orifices and the furrows leading from them bear large and powerful cilia, which beat in the direction indicated by the arrows in figure 12. The tract formed by the union of these separate channels bears regular transverse furrows which lead to a deep groove on the left side of the major typhlosole. The same structures are continued down into the caecum, where the transverse furrows become somewhat longer.

Figure 15 illustrates the appearance of the caecum when cut along the dorsal wall and opened out flat. The major typhlosole, T , passes to the further end of the caecum, where it ends blindly. In apposition to it under normal conditions is a similar ridge, T' , while between them the epithelium is thrown up into regular ridges and furrows.

The cilia on the surface of the typhlosole, as well as those on the crests of the transverse ridges, beat inward toward the end of the caecum. This holds true for the region $R'-T-R$ of figure 15, while in the region $R-T'-R'$ the cilia on these same structures beat in the opposite direction.

In the transverse furrows the cilia are very large and powerful and beat toward the deep groove which follows the base of the major typhlosole and its opposing ridge as shown by the arrows in figure 15. As a result of this mechanism, a particle of debris falling into the transverse furrows at any point would be car-

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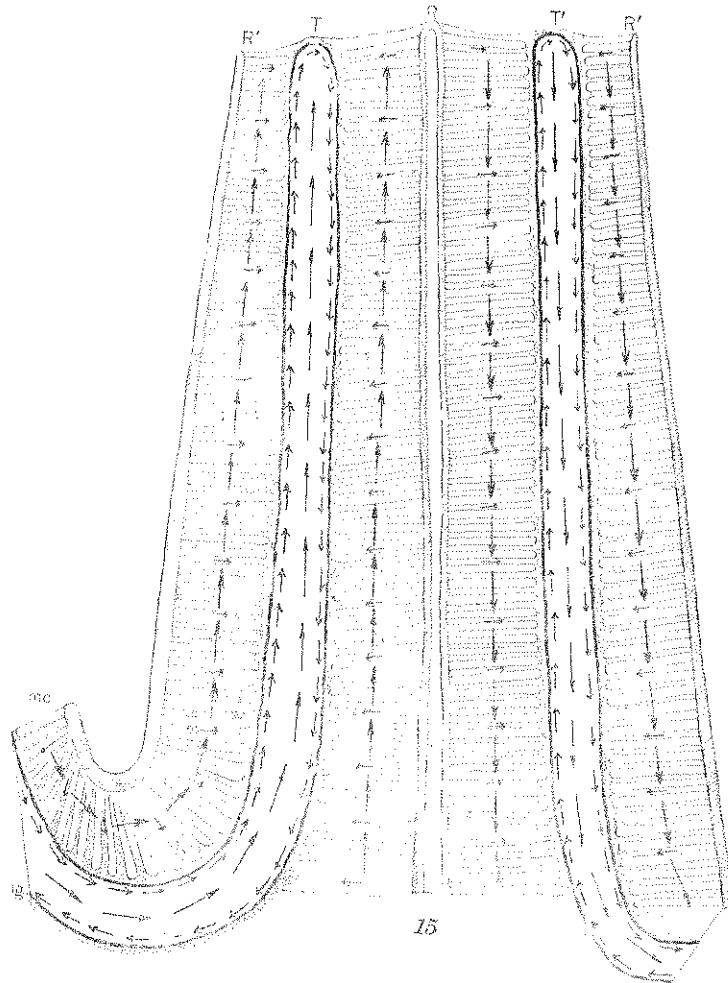


Fig. 15 *Modiolus modiolus*; food sorting caecum opened from the dorsal side, along the ridge *R'*; long arrows indicate course of currents on ridges, short arrows show direction in furrows; *R, R'*, narrow ridges; *T*, extension of major typhlosole; *T'*, broad ridge apposing typhlosole in life; *mc*, mouth of caecum; *ig*, intestinal groove.

ried into the deep furrow and thence to the intestinal groove. In this groove, or the intestine proper, the effective stroke of the cilia is toward the posterior end of the body, and thus the waste matter is carried to the outside.

On the broad surfaces of the typhlosoles the effective stroke of the cilia is inward toward the style sac, and somewhat in a posterior direction as well. The cilia on the faces of the two typhlosoles are not equally developed in all species. In *Lampsilis* I find that those on the major typhlosole are of about twice the length of those on the minor.

The regular transverse folds of the style sac, with their great bristle-like cilia, have been described (p. 65). The effective stroke of these is such as to put the style in rotation in a clockwise direction, and at the same time to push it anteriorly against the gastric shield.

Barrois ('89-'90, p. 356) believed that the style turned on its long axis while being pushed forward into the stomach. List ('02, p. 277), ignoring this statement of Barrois, expressed the opinion that the function of the large cilia of the style sac was to set the style in rotation, while pushing it forward into the stomach. Hoffmann ('14, p. 533) states that the style of *Tagelus* is rotated by the cilia of the style sac. In the text of none of these authors is there any statement indicating that the rotation of the style was actually observed. Their conclusions were based merely on the presence of the co-axial layers of which the style is built up.

The secretion and formation of the crystalline style

Regarding the point of origin of the crystalline style there is the same lack of unanimity of opinion among the various investigators that characterizes their speculations regarding its function. In general, three structures have been considered as being the seat of its origin: the stomach, the hepatopancreas, and the epithelium of the style sac.

The opinion of Huxley ('53) was mentioned in the historical summary (p. 58). Milne-Edwards ('59, p. 362) argued from the existence of the co-axial layers of the style that it must be the

product of epithelial secretion. Eighteen years later, Sabatier (pp. 28, 29), after an exhaustive study of the histological structure of the epithelium surrounding the style, found that the cells of this region were full of an unusually large number of protoplasmic granules. He considered that these cells therefore formed a secretory epithelium destined to furnish the stomach with a digestive fluid. Barrois ('89, p. 309) was of the same opinion regarding the origin of the style.

Haseloff ('88) and Hazay ('81), whose conclusions are given in the historical summary (p. 60) believed that the style was a mass of nutriment transformed in the stomach by the action of the gastric juice and stored in the style sac as a reserve. As their conclusions have been so ably disposed of by Barrois ('89), we need not consider them further than to say that they were based on false deductions, and have no valid evidence to support them.

Drew ('01, p. 352) says of *Nuclula*:

The posterior and part of the lateral walls of the stomach are formed by long and slender epithelial cells that stain but lightly. They secrete a mucus-like material that stains deeply, and probably corresponds to the crystalline style. In adults this structure seldom takes the form of a rod, but in embryos a rod is commonly present.

Mitra ('01, p. 601) says, regarding the secretion of the style,

There are grounds for believing it is secreted by the so-called liver. The chief ground is that there is in the liver an amylolytic ferment exactly like the ferment of the style. The ferment in the liver behaves exactly as the style ferment does. On the other hand, we could hardly detect any amylolytic ferment in the enteric epithelium. There is also another fact which must be allowed to have some force in this connection. It is that yellow pigment cells from the liver are occasionally seen to form the axial zone of freshly formed styles.

Biederman ('10-'11) argued from the almost universal presence of ciliated cells in the style-bearing region that, as these could not at the same time be secretory cells, the hepatopancreas was probably the organ of secretion.

By far the greatest weight of evidence, historically, however, places the seat of origin of the style in the typhlosoles. In forms where these do not occur the secretory activity is confined to a region of cells lying in the wall of the style sac itself.

List ('02, p. 274) fed finely ground color particles to *Mytilus*, and observed that these were built up into the structure of the style. He claims frequently to have seen the style in the process of formation, in which the typhlosoles furnished the secretion in large quantities. He concludes (p. 277), "dass der Krystallstiel im Darms entsteht, und dass hauptsächlich die Secrete der seitlichen Epithelwülste (typhlosoles) an seinem Aufbau theilhaftig sind."

Gutheil ('11), as a result of an extensive study of the alimentary tract of Anodonta, concluded that a ciliated epithelium might also be absorptive and secretory as well. He found cells in the 'Kristallstieldarm' which were loaded with clear vacuoles lying above the nucleus. These structures he is certain were neither artifacts nor products of degeneration, but material destined to become the style. His conclusions are, therefore, in entire accord with those of List.

My own studies of the secretion and formation of the crystalline style were made on the living material and with stained sections. It is evident from these observations that List was right in placing the origin of the style in the typhlosoles. We have already seen (p. 67) that in forms where the intestine and style sac are incompletely separated by the typhlosoles, a short diverticulum occurs at the end of the style sac. This communicates freely with the intestinal groove down which pass the waste materials from the stomach.

In animals taken during the height of feeding activity, and quickly dissected, a thin thread of large mucus globules may be found issuing from the diverticulum. At the same time there may be seen a string of whitish,ropy mucus, which is secreted by the walls of the intestine for a short distance beyond the diverticulum. The two streams unite at the mouth of the diverticulum and pass anteriorly into the style sac.

The cilia here, as we have already seen, beat so as to put into rotation any matter in contact with them. As a result, the round or flattened stream of mucus is twisted on itself as it starts on its way toward the stomach, thus giving rise to the spiral arrangement typical of the smaller, posterior end of the

style (fig. 14). This spiral structure was recognized by Barrois (1890) and illustrated in several figures.

In the stomach minute sand grains, tests of diatoms, and similar waste matter, entangled in mucus, are beaten by the cilia into compact masses. Passing into the intestine, this waste material is carried along in the deepest part of the intestinal groove. During periods of active feeding some food particles, diatoms, unicellular algae, etc., escape from the stomach and pass down the intestine with the sand and dirt. As this escaped food matter is less compact it comes to lie closer to the typhlosoles, and, if the particles be very small, they may be caught by the cilia on the edges of these structures and carried across to the style. Larger particles, such as the larger diatoms, pass downward until they reach the region of the diverticulum at the end of the style sac. Here they are caught by the cilia and carried into the stream of mucus at this point, while the waste materials pass on through the intestine. Thus it happens that this twisted mucous thread in the style sac so often contains food particles in a very fresh condition.

It has been seen (p. 75) that in the typhlosoles there are two types of secreting cells, long goblet cells, and mucous glands. As the spiral mucous thread is passed anteriorly from the diverticulum, in contact with the edges of the typhlosoles on the right side of the style sac, the secretions from these unicellular glands are carried by ciliary action across the faces of the typhlosoles and applied to it. By the action of the thousands of powerful cilia of the style sac these globules of secretion are beaten into a homogeneous mass around the mucous thread, which forms the core.

As this structure moves anteriorly, turning on its axis, successive layers of secretion are added to it, until as it nears the stomach, further additions cease and the fully formed crystalline style passes out of the style sac into the stomach.

The styles of different individuals of the same species exhibit the most diverse modifications, depending on the physiological state of the animal prior to examination. A specimen which has been starved for some time and then put into water contain-

ing abundant food material will ordinarily show a style of very dark color, due to the large central core of food material. On account of the irregularity in the arrival of this escaped food matter at the end of the caecum, this inner core may vary considerably in thickness throughout its extent (fig. 14). On the other hand, when an animal has had abundant food for a long period and has not been actively feeding just prior to examination, the style may be practically free from all food material and may possess a very thin core of whitish mucus.

Considerable variation in the length of the secretory portion of the typhlosoles occurs in different species. In some this region, instead of occupying most of the extent of the typhlosole, is quite restricted. As a result, the superficial layers of the style are applied much as a thick tape might be wound around a stick. Such a condition may occur in *Maetra* where, according to Kellogg ('92, p. 402), the outer lamellae of the style may actually be unwound from the core.

I have found a somewhat similar mode of accretion when feeding *Modiolus* a fine suspension of carmine grains. The coarse grains of color passed down the intestine to the diverticulum where they were built into the central core of the style. The finer grains, however, were carried across the faces of the typhlosoles at one point and applied to the surface of the style. As a result, this organ when removed much resembled a barber's pole, with the spiral band of bright red carmine grains wound around it.

One interesting anomaly may be described here as shedding some light on the manner in which the superficial layers of the style are laid down. A style of *Lampsilis anodontoides* was found to which an air bubble had become attached at some distance from the end of the sac. As the style was rolled round and round this bubble was flattened, and over it was deposited a thin layer of clear secretion cementing the bubble securely in place (fig. 17).

The description of the formation of the style as given above applies to all forms in which the style sac is incompletely separated from the intestine by the typhlosoles. In the more primi-

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five forms, where the style lies in the intestine itself and where there are no typhlosoles, the secretory activity seems to be located in the two ridges of cells which form a slight constriction between the style-bearing and the waste-carrying portions of the intestine. The styles of such species exhibit the same inner core and superficial layers found in the styles of *Anodonta*, *Modiolus*, etc.

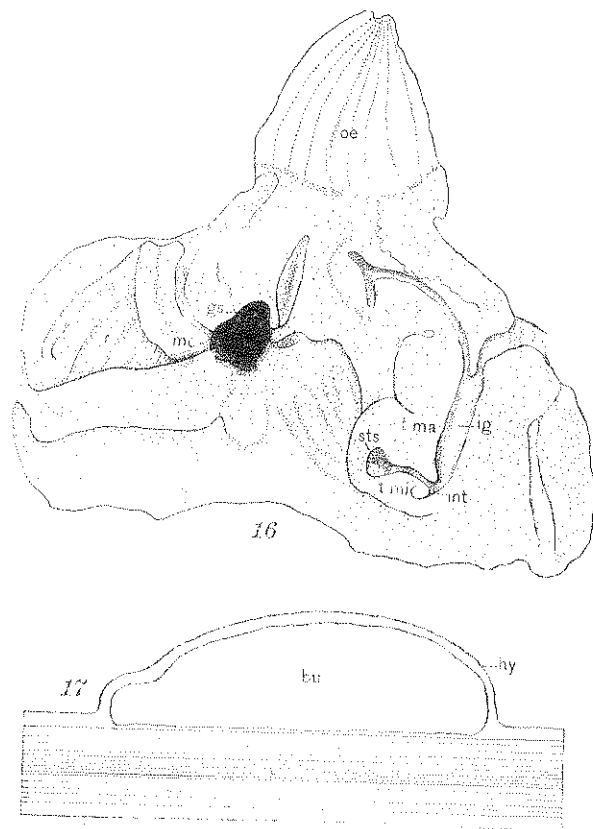


Fig. 16 *Anodonta grandis*; internal surface of stomach wall, dissected from right side, and spread out flat; *gs*, gastric shield; *ig*, intestinal groove; *int*, intestine; *me*, mouth of dorsal caecum shown in fig. 4; *oe*, oesophagus; *r*, one of transverse ciliated ridges lining the caecum and stomach wall adjacent to it; *sts*, opening of style sac; *t ma*, major typhlosole; *t mi*, minor typhlosole.

Fig. 17 *Lampsilis anodontooides*; optical section of crystalline style with attached air bubble. *bu*, bubble; *hy*, hyaline layers.

In those lamellibranchs where the style lies in a separate caecum, distinct from the intestine, there are marked differences from the type we have been considering. Since there is no connection between the style caecum and intestine, there is no means by which food particles could be incorporated into the structure of the style. Investigators who have examined the styles from this type of mollusc state that food particles are universally absent from them. In *Pholas*, for instance, following Barrois ('89), the style has a central core of bubbly mucous, entirely free from foreign particles. I find the same is true of *Martesia*.

Mitra ('01) noted the inner core of food matter in the style of *Anodonta*, but thought that it was due to some imperfection in the method of storing the ferment in the style sac. The absence of such an inner core in the styles of *Pholas* was held to be the result of a superior, more differentiated mechanism for storing the ferment and for passing the food material through the alimentary canal.

A further difference in this species lies in the fact that the style is comparatively short and thick and that the smaller and not the larger end projects into the stomach. I have found this condition in *Martesia*, where the style is of relatively enormous proportions, comprising as it does a large part of the visceral mass (fig. 8). In both of these forms the style resembles roughly a policeman's club in shape.

The relatively large style in *Teredo* and other boring molluscs was noted by Deshayes (48) and Quatrefages ('49).

I have been unable to find any reference in the literature as to the exact location of the secretory activity in forms possessing a separate style caecum. We have seen that Mitra's supposition that the hepatopancreas is the source of this secretion is entirely opposed to the facts as determined for the species thus far considered. That this supposition is equally invalid in the type under consideration must be evident from the anatomical relations of the hepatopancreas to the style caecum, as found in *Martesia* (fig. 8). To my knowledge no channel exists through which a secretion from the hepatopancreas could be applied to the surface of the style, even if the effective stroke of the cilia of this

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portion of the alimentary canal were such as to carry a secretion to this point. Furthermore, I find at one side of the caecum itself a group of cells which form a structure very similar to the secreting epithelium of other forms. It may be concluded, therefore, that where the style exists in a separate caecum it is likewise the product of an epithelial secretion.

Embryology of the style and style-bearing organs

Concerning the ontogenetic development of the crystalline style the literature is singularly silent. Reference to it is found in the work of Drew ('01) in the passage quoted above (p. 81) and also in the following words. Speaking of the alimentary tract of the veliger larva of *Nucula*, he says (p. 349):

The cells of the epithelial walls of the stomach are of two kinds. Those at the anterior end of the stomach carry comparatively few cilia and those at the posterior end carry many cilia. At this stage some of the cells on the dorsal side of the stomach, near its anterior end, begin to secrete a mucus-like material that extends posteriorly in the lumen of the stomach as a small rod that probably represents the crystalline style. Later the posterior portion of the whole dorsal division of the stomach . . . is given over to secreting this material, but a definite rod may not be present.

In the same year Meisenheimer described the development of the crystalline style in *Dreissensia*, a form with a distinct style caecum. According to this investigator (p. 93), the anlage of the style caecum lies in the posterior wall of the stomach, to the right of the opening of the intestine. This region is characterized by the presence of very long bristle-like cilia (fig. 5). The epithelium of the stomach wall at this point evaginates to form a cone-like depression, the cells of which secrete a substance which is formed into a thick rod extending into the stomach (fig. 6). This blind sac continues to grow posteriorly until it finally forms a long caecum on the right side of the body. It opens into the stomach to the right of the intestinal orifice. The food of the larva of this species consists of plankton, but in consequence of the presence of hard particles in the stomach, incidentally acquired during feeding, Meisenheimer favors Bar-

rois' theory of the protective action of the dissolved style substance.

My own investigations of the embryological development of the crystalline style were carried on during three summers on the New Jersey coast, while engaged in a study of the food and feeding habits of the veliger larvae of *Ostrea* and *Venus*.

In the prodissoconch larva of *Ostrea* I find in the epithelium of the stomach the same two regions, differing in the cilia they bear, as were described by Drew for *Nucula*. Most of the stomach wall, like that of the oesophagus, is covered with short, even cilia which beat at a uniform rate. Just anterior to the middle of the stomach, and situated on opposite walls, are two groups of long cilia which beat much more strongly than the others. They coincide closely in position with those long cilia which have already been described in the adult (p. 77) which are situated on either side of the gastric shield and keep the alimentary matter in circular motion. As was pointed out by Meisenheimer ('01, p. 94), the food material in the stomach of the larva is in constant rotation under the action of the cilia. In *Venus* and *Ostrea* this motion is due largely to the action of the two groups of large cilia; it is always in a clockwise direction when viewing the animal from the anterior end. These cilia, both in position and in the direction of their effective stroke, correspond closely, therefore, to those found in the adult.

When these veligers are placed in water containing carmine grains in suspension, they feed upon it greedily and soon fill the entire alimentary canal. As the color grains enter the stomach they are caught by the large cilia and whipped into the mass of particles and mucus which is whirling in the cavity of this organ. This circular motion is not continuous, for the larvae exhibit spasmodic contractions of the velum and foot, as a result of which the stomach is compressed and the whirling motion ceases for a moment, to be resumed upon the relaxation of the surrounding organs. During the rotation of the alimentary mass the particles of carmine or food matter move slowly posteriorly into the opening of the intestine, where they are caught and carried downwards. At this stage there is no indication of the beginning of a separate intestine and style sac.

Such a condition is present in the oyster larva for the first week or ten days. As the free-swimming stage reaches its close, and the oyster larva attaches itself to some foundation, the mucous roll of the stomach is represented by a short rod extending backwards into the lumen of the intestine. Meanwhile the intestine becomes constricted lengthwise to form two tubes. In an oyster 4 mm. in diameter the organs are well differentiated and a distinct style is present. In a 9-mm. individual this differentiation is apparently complete. The style is comparatively short and thick and is as clear as crystal. It shows the same twisted inner core as is found in the adult. The gastric shield is likewise well developed at this stage. In a 9-mm. oyster it measures approximately 0.7 x 0.5 mm. The style of a 7-mm. oyster measures about 1.2 mm. long and 0.3 mm. in diameter at its widest extent.

At this time the young oyster has all of the organs for food-getting of the adult and eats the same food. We may suppose, therefore, that the style now serves it in the same manner as it does the adult.

3. NATURE

Description of the style

The length of the style is described by Mitra ('01) as fully three-fourths that of the animal. Gutheil ('11) gives a more conservative estimate, the style from an *Anodonta* 13 to 14 cm., measuring from 6 to 7.5 cm. Biederman ('10, '11) gives the length of the style of an adult *Anodonta* as 7 to 8 cm. My own observations agree closely with those of the last two investigators, though the length of the style varies greatly with the species and the feeding activity of the animal.

Figure 14 shows the appearance of the style as taken from its sac, with a mass of food material attached to the anterior end. This figure gives an optical section through a style containing a thick core of food material, showing the spiral arrangement of this inner core and the layers of hyaline substance which have been secreted upon it.

In color the crystalline style varies from a clear, transparent structure, like a glass rod, to a deep brown, opaque rod, the color of chocolate. Aside from the presence of an inner core of food material, which may give more or less color to the styles of all species not having a separate caecum, the cortical layers of hyaline matter themselves may contain a diffuse yellow substance. In Anodonta and the adult Ostrea I have invariably found some trace of this color present, even in the clearest styles. In Lampsilis, Modiolus, Martesia, and the very young oyster, the cortical layers are usually as clear as crystal.

The composition of the style

Investigation of the composition of the style by the earlier workers—von Poli (1791), Hazay ('81), and Haseloff ('88)—revealed the following facts: gelatinous in consistency, the style dissolves readily in water, more rapidly in salt solution. It is coagulated by boiling water, alcohol, and other protein precipitants. It gives the delicate KCN reaction for albumen, and a violet color with the biuret test.

The most extensive investigation of the structure and chemical composition of the crystalline style which has yet appeared is found in the work of Barrois ('89). A chemical analysis of the style by Lambling is included in this paper. The conclusions of Barrois are based not only upon many different forms, but what is of equal importance, upon a large number of individuals of each species. In following the color variations of the style this investigator opened over 6,000 individuals of Cardium alone. Since this work has been given but scant attention by most subsequent writers, the results are given below in some detail.

The style was found to vary in size with the individual and with the relative proportions of the sac or caecum containing it. The longest style described by Barrois was 7 to 8 cm. in Anodonta anatina. As found in the marine species the style was usually of a pale yellow color, while in the Unionidae it was transparent and colorless. Great variation was found in the

color in *Cardium* during the year, being a deep orange in April, when it was most highly colored.

Much variation in the consistency of the style was observed in the different species, this difference being correlated with the rapidity with which the structure underwent dissolution. As found in the Unionidae, *Mytilus*, and *Ostrea*, it was soft and gelatinous, becoming much firmer in *Cardium*, *Solen*, *Donax*, and *Pholas*. The brown inner core of food material was held by Barrois to be abnormal. He noted that the style of *Pholas* never contained such a structure. The coaxial layers in the style of this species were thicker near the center and much thinner toward the periphery, grading from 9 to 35 microns, with an average of about 14 microns. The style of an adult *Pholas crispata* showed from one hundred to one hundred and ten successive layers.

In the cortical layers of the style Barrois found small crystalline bodies which were insoluble in water and resistant to acetic acid. These were undoubtedly the same structures described by Vulpian ('67) as crystals of calcium oxalate. Since the latter investigator also found uric-acid crystals in some styles, he concluded that the crystalline style must have some connection with the excretory function. Similar crystals were described by Hazay ('81), as semitransparent, ruby-colored rhombohedrons.

I have found such crystals in the styles of *Anodonta* and *Ostrea*. They are usually red or jet black, the former resembling in color the lipochrome pigment cells in the integument of the crayfish. These crystalline bodies are not normally present in the style, but appear after partial desiccation of the style substance.

A fairly complete chemical analysis, made by Lambling, is given by Barrois, '89. The style used was that of *Cardium edulis*, which in an adult specimen is about 26 mm. long and weighs approximately 0.026 g.

His analysis of fifty styles gave the following results:

Weight of fresh styles.....	grams 1.3225
Weight dried at 120°C.....	0.1705
Insoluble mineral matter.....	0.0025
Soluble mineral matter.....	0.0090

Expressed in percentage weight of the fresh styles gives:

Water.....		per cent	87.11
Solid matter {	organic.....		12.03
	inorganic.....		0.86

The albuminous nature of the style substance was shown by the following reactions. The styles were washed rapidly by decantation and dissolved in distilled water. The solution was neutral. Conc. HCl, Millon's reagent, biuret and Adamkiewicz tests were all strongly positive. Boiled with a 2 per cent solution of H_2SO_4 , neutralized and precipitated with alcohol, taken to dryness, and dissolved in water, the style substance gave a strong reduction with Fehling's solution, similar to that given by mucin and chondrin, but might have been due to the saccharification of a hydrocarbon. All attempts to find such a hydrocarbon were futile.

Maillard and Vles ('07) found a similar reduction when a solution of styles, containing one part to a hundred of sodium fluoride was tested with Fehling's solution. This reduction, though feeble, was constant. That it was not due to the presence of a sugar was shown by a negative reaction with phenylhydrazine.

Barrois tested this reducing action of the style and found it to be equal in reducing power to 0.059 gm. of glucose, per gm. of dry protein matter present. Whatever this reducing substance might have been, it was not optically active.

Passing a current of CO_2 through a solution of styles, and also saturation with $MgSO_4$ after the method of Hammarsten for blood serum, showed that practically all of the proteid matter of the style exists as a globulin.

In conclusion, Barrois points out as most important the action of hot weak acids on the style, a reaction similar to that shown by the albuminoid matter of mucin and chondrin. These, he holds, are protein constituents, which in animals of higher organization, at least, appear to us as albuminoids which are more or less non-catabolized (*dégradés*), that is, no longer fill a biological rôle in the phenomena of general nutrition, and hence

ives:

per cent
87.11
12.03
0.86

cannot serve as a reserve of nutriment. List ('02) agreed with Barrois that the style is similar to mucin and chondrin.

Mitra ('01), ignorant of the work of Barrois, repeated most of the chemical analyses of Lambling, using the styles of *Anodonta*. His results in the main are of a confirmatory nature. He states that the style contains no cellular elements, but is composed of a colloid substance. Its solubility, according to him, is due to the presence of a minute quantity of salts. His tests for protein were similar to those of Lambling and revealed the same facts. His chemical analysis (p. 599) showed about 88 per cent water, about 12 per cent of protein (globulin), and about 1 per cent of salts, which is a close approximation to the results of Lambling.

The credit for first determining the presence of enzymes in the crystalline style has been given to Mitra ('01) by nearly all subsequent investigators. However, as van Rynberk ('08) points out, the credit for this discovery belongs to Coupin ('00), his work appearing a year before that of Mitra.

In a short note Coupin gives a summary of his results showing that the style contains no sugar or fat, but only an albuminoid substance. He concludes:

Le stylet Crystallin des acéphales est un suc digestif, un espèce de comprimé de diastase, contenant beaucoup d'amylase, et peu de sucrase, mêlées avec une substance muqueuse, destinée sans aucun doute à empêcher la trop rapide dilution du stylet dans l'eau de mer contient l'estomac, et peut-être aussi à agglutiner les particules solides qui nagent dans celle-ci.

He found, further, that the enzymes of the style were without action upon egg albumen.

Mitra showed that an aqueous solution of styles caused a rapid conversion of starch to sugar, with an intermediate product in the nature of dextrin. Its activity toward glycogen was found to be similar to that of ptyalin. No action could be demonstrated on egg albumen, fibrin, or muscle fibers.

An extract of the hepatopancreas showed the same activity as the style, while the extract of the mid intestine revealed hardly a trace of this digestive action.

The alcohol precipitate from an aqueous solution of styles was kept under alcohol for several months, and finding that the ferment power of this precipitate became increasingly less as it became more insoluble in water, Mitra concluded that the enzyme and protein of the style were identical.

Von Fürth, reviewing part of the literature, believed that it was more reasonable to suppose, with Coupin, that from the colloidal nature of the style it was really a mixture of enzyme and albuminoid material, rather than a solid mass of enzyme.

Van Rynberk ('08) repeated the experiments of Coupin and Mitra, and extended them to cover a comparison of the digestive activity of the style and the hepatopancreas on fats, proteins, and carbohydrates. This author found no action upon fats or proteids, nor upon cellulose, though starch was quickly digested, and raw sugar inverted by the enzymes present, in both the style and hepatopancreas.

My own investigations, as far as carried out, confirm those of Coupin and van Rynberk, and differ from those of Mitra only in so far as he regarded the style as exclusively a mass of enzyme. When digestion is proceeding slowly in *Anodonta*, as during the winter, the stomach contains a large amount of brown, ropy fluid, resulting partly from the dissolution of the style, and partly from secretions from the hepatopancreas. About 0.5 cc. of this liquid was drawn off and mixed with an equal volume of a 0.5 per cent starch solution, and the mixture placed at 32°C. In an hour the starch was completely digested. An extract of the hepatopancreas showed a similar activity, though the action was not nearly as rapid.

Mitra extracted the mid intestine and found a very faint amylolytic action of the filtrate. Van Rynberk repeated the work, but failed to confirm his result.

Since the secreting glands of the typhlosoles are all unicellular, it is evident that little enzyme could be stored in them. The following experiment was designed to avoid this possible source of error. The typhlosoles, together with the wall of the style sac, were carefully removed from an adult *Anodonta*. That part near the hepatopancreas was discarded, and the rest, after

rapid washing in distilled water, was placed in a vial with a small quantity of the mollusc's blood. A few drops of the glycerine and twenty drops of a 0.5 per cent starch solution were added and the vial placed at 32°C. At the same time a control containing blood and starch was also put in the incubator. The object of putting the tissue in the blood was to give the cells a chance to secrete a small amount of enzyme while bathed in fluid approximating that of the body. After twenty-two hours the control still showed the presence of starch, while in the vial containing the tissue all of the starch had been converted. It is evident, therefore, that the enzyme found in the style is secreted by the typhlosoles, and not by the hepatopancreas as was held by Mitra.

Apparently the molluscs which I have studied have no extracellular lipase. Van Rynberk ('08) could demonstrate no action upon fat, and I have found in the alimentary mass in the stomach of *Anodonta* great numbers of fat globules, as shown by their staining reaction with Sudan III. It is probable, furthermore, from the researches of Gutheil ('11) and the recent work of Churchill ('16), that fat is absorbed as such by the cells, and by phagocytes, there to undergo decomposition into fatty acid and glycerine.

The only addition which I have made to the chemical analysis of Lambling is the determination of the total and water-soluble nitrogen in the style.

Ten styles of *Lampsilis* were washed rapidly in distilled water, drained on filter paper, and weighed. Two cc. of distilled water were added and, when the styles had dissolved, 2 cc. of a mixture of tannic and hydrochloric acids were added to precipitate the protein matter. The precipitate was filtered off through a small, dry, nitrogen-free filter paper, and the filtrate analyzed for the water soluble nitrogen. The residue on the filter was washed free of all traces of the filtrate, and, with the filter paper, was digested in boiling conc. H_2SO_4 , and the amount of nitrogen determined by the micro-Kjeldahl method. The results of this experiment follow:

Ten styles, fresh.....	<i>gram</i>
Nitrogen, per g. of fresh style.....	0.16
Water soluble.....	0.0011
Precipitable.....	0.0061
Total nitrogen.....	0.0072

In criticism of Mitra's conception of the style as a mass of pure enzyme, it may be urged that the evidence which he advances is inconclusive. The mere fact that the style substance, kept under alcohol, lost in ferment power, proves nothing, for it is well known that enzymes in general lose their activity on long standing under such conditions.

Furthermore, examination of the substance of a freshly formed style with the dark stage condenser shows it to be composed of small globules of fairly uniform-size, interspersed with many very minute globules or particles, in the nature of colloid particles. It is probable that we have to do with an adsorption phenomenon, the enzyme being borne on the surface of the globules of albuminoid substance.

It may be concluded, therefore, following Coupin ('00), that the crystalline style is a structure of colloid nature, resembling mucin, and containing an enzyme, or enzymes, of strong amyolytic power.

Nature of the gastric shield

Finally, a few observations are necessary regarding the nature of the gastric shield. Subjected to the biuret test, it rapidly disintegrates under the action of the strong caustic. On the addition of the copper-sulphate solution a purple color forms on the surface, grading into a pink toward the interior. This would indicate the presence of such substances as proteoses and peptones. From the zonation of the colors it is probable that this reaction is due to products of digestion absorbed by the shield. From its consistency and action toward the common reagents it is probably in the nature of chondrin.

The shield is very resistant to the action of the digestive juices of the stomach. I have invariably found it present in all living specimens that I have examined. If kept in a test-tube with a

small amount of digestive juice, it slowly softens and, after several days to a week, disintegrates. It is therefore, a structure, which, unlike the style, is not renewed at the commencement of feeding activity.

The Spirochaetes of the crystalline style

As is well known, the crystalline style in many species of lamellibranchs harbors a large spirochaete, which has been put by Gross ('10) in a separate group, the *Cristispira*. My own observations have been on *Cristispira balbiani* Certes, from the oyster, and *C. anodontae* Keysselitz, from *Anodonta grandis*, one of the largest spirochaetes known. Why they occur in the style and whether or not they are harmful to the host are questions which cannot be considered in the present work. These organisms are mentioned here only because they throw some additional light on the consistency of the style substance.

Examination of a fresh style of *Ostrea* or of *Anodonta* shows the spirochaetes in great numbers, moving back and forth with their characteristic corkscrew motion. The majority of these Protozoa occur at or near the surface of the style, but apparently they have no difficulty in passing inward to the core and back again through the substance of the cortical layers. Fixed sections show them in all parts from the core outward.

In *Anodonta*, at least, these organisms are usually found in the greatest numbers in the bubbly mucous in the end diverticulum of the style sac, becoming much fewer toward the stomach. It is probable that their presence near the anterior end of the style and in the stomach is due to the continual forward movement of the former, and not to choice on the part of the spirochaetes.

A striking peculiarity in their distribution lies in the fact that the styles of some species appear to be wholly free from them. Such is the case in the styles of *Lampsilis luteolus* and *L. anodontoides* that I have examined, though in one instance twelve spirochaetes were discovered in a style of *L. anodontoides*.

Also, I have never found any spirochaetes in the styles of *Modiolus*, though oysters attached to them are heavily infected.

Finally, the spirochaetes, unlike most protozoans, are able to resist the action of the digestive ferments of the alimentary canal, and are apparently unacted on by the juices in the stomach.

4. FUNCTION

The conclusions of former investigators regarding the function of the crystalline style have been considered in the historical summary (p. 57). The inadmissibility of most of these must be evident at once, and they will be dismissed without further comment. A critical examination of the others is reserved until after the presentation of my own conclusions and the evidence upon which they are based.

Realizing that the misconceptions of many former workers have been due largely to abnormal and unfavorable conditions surrounding the mollusc under observation, my own endeavors have been to study the style in its physiological relationships while the animal was still in or near its natural environment.

In this I have been greatly aided by the opportunity afforded by the floating laboratory for oyster research of the New Jersey Agricultural Experiment Station. As this laboratory is stationed over the natural beds of the oyster, with clams and mussels within a few feet, it was possible to remove a bivalve from its natural environment and within two or three minutes to have it opened and under observation in the laboratory. Too much emphasis cannot be laid on the necessity for study of this nature in determining the physiological significance of the style.

The first investigations covered a determination of the factors in the normal environment which bring about the secretion or dissolution of the style.

The ribbed mussel, *Modiolus*, occurs most commonly in bunches along the banks of tidal creeks and estuaries. Natural oysters also may be attached to these clusters. From their position on the bank, the molluscs are necessarily exposed from three, to as much as six or eight hours between successive high tides, during which time, of course, no feeding takes place.

Upon examining mussels which had thus been exposed for about six hours, it was found that the style was invariably present. In many instances, however, it was quite soft and had begun to undergo dissolution.

The oysters attached to these mussels, provided they had been exposed for an hour or more, failed to show any style present. What, then, is the relation of the style to the feeding activities of the oyster? As is well known, oysters occur naturally in reefs, or barriers, built up through successive generations attaching themselves to those already present. Since the oyster is able to exist for some time out of water, the upper limit of the reef comes to lie between the mean low- and high-water levels. Consequently, large numbers of individuals near the top are exposed for several hours at each ebb tide.

Toward the close of ebb tide I went out on such a reef and opened oysters at the rate of one a minute for two and a half hours, a period extending over the close of the ebb and the early flood of the tide. Began at 9.05 a.m., at which time the oysters around me were all or partly submerged. A style was present in all that were opened.

By 9.45 a.m. most of the reef about me was exposed, and the oysters opened for the next hour were devoid of styles. Shortly after 10.00 a.m. the tide began to return, and when it had risen sufficiently to cover the oysters they opened and began siphoning vigorously. By 11.00 a.m. the reef was practically covered, and from then on till 11.35 a.m. when the experiment was concluded, a style was present in every oyster opened. All the bivalves taken during the entire period came from a spot less than 20 feet square and nearly level, hence all were subjected to practically the same period of exposure.

Subsequent laboratory experiments confirmed these results and revealed the fact that the style of the oyster may be dissolved within an hour, often in much less time. Furthermore, a new style may be built up within fifteen minutes from the time the animal begins active feeding.

There is, however, a great variation in the length of time required for these processes, depending on the previous feeding

activity of the mollusc, as well as its general physiological condition. External conditions, principally temperature, also cause considerable variation in the time required.³

Upon dissolution the style forms a brown viscous fluid, most of which remains in the style sac until the animal again begins active feeding, when it is carried forward into the stomach by the active beating of the cilia.

From the above experiments it may be concluded that the crystalline style is a structure intimately connected with the feeding activities of the mollusc, and which, in oysters exposed at low tide, is completely dissolved and renewed again at least twice every twenty-four hours.

The style of *Modiolus* is much more resistant and ordinarily remains intact during the normal periods of inactivity. If mussels are kept in the laboratory without food, the style begins to disappear after about twelve hours, and is usually completely dissolved after twenty-four hours of inactivity, though here again there is great variation between individuals.

The fresh-water genera, *Anodonta* and *Lampsilis*, exhibit a condition similar to that of *Modiolus*. Absence of food or inactivity of the animal due to cold or adverse conditions brings about a gradual dissolution of the style, occupying from a few hours to several days.

The disappearance of the style in forms which had long been out of water was noted by Meckel ('29) and by a number of other workers. Mitra ('01) believed this disappearance and subsequent renewal to be a periodic function. As Allen ('14, p. 136) has justly pointed out, this periodicity was due to the periodic emptying and filling of the aquarium in which the animals were kept. The latter investigator also gives the correct interpretation of the presence or absence of the style, and showed that the addition of food to the water containing starved mussels soon caused its partial regeneration.

We have already seen (p. 80) that the cilia of the style sac put the style into rotation while pushing it forward into the

³ The rapid dissolution of the style of the oyster was noted by Möbius ('83) and later by Barrois ('89). Garner ('41) even went so far as to hold that with but few exceptions the style was lacking in all of the *Monomyaria*.

stomach. Though surmised by several former workers from the co-axial layers of which the style is composed, I am unable to find any statement in the literature indicating that this rotation has actually been observed.

After nearly two years of experimenting, in which many individuals, both marine and fresh-water, were opened, I was able finally to cut through the stomach wall and expose the head of the style in such a way that the animal did not contract unduly, with consequent displacement of the alimentary canal.¹

As a result the style is not put under tension, but is free to rotate in the style sac, in spite of the fact that it forms a U-shaped bend in following the course of the intestine. To my surprise, I found this rotation to be quite rapid. In *Anodonta* kept at a constant temperature of 11.5°C. prior to, and during the experiment, the maximum number of revolutions per minute was eleven. In *Modiolus*, at 25°C., the maximum number per minute was thirteen. The direction of rotation in both species is clockwise when viewing the animal from the anterior end. (Compare this with the direction of rotation of the food particles in the stomach of the veliger larvae of *Ostrea* and *Venus*, p. 88.)

The food material in the stomach, entangled in mucus, becomes wound about the head of the style, and whirled around in the lumen just posterior to the gastric shield. So strong is the tractive force of the rotating style that strings of mucus from any part of the body, if led to the stomach cavity, are at once drawn in and wound up in the food mass.

While rotating the style in this manner, the cilia of the style sac push it anteriorly against the gastric shield, with force enough to cause the style to bow out when the stomach walls are drawn apart. In *Modiolus*, at 25°C., this forward move-

¹ The technique employed is as follows: An individual which has been siphoning vigorously is removed from the water, and the right valve removed by passing a sharp, thin scalpel between the shell and the adductor muscles, leaving the latter intact. The mantle and gills are then laid back, and a small incision is made in the body wall over the dorsolateral region of the stomach. At this point the stomach wall comes very close to the surface in those species I have studied. By means of fine hooks, the cut edges of the stomach are pulled apart to expose the head of the style where it bears against the gastric shield.

ment, when the end of the style is unopposed, is approximately 1 cm. per minute.

We have already seen (p. 77) that the cilia on the stomach walls in proximity to the gastric shield beat so as to rotate in a clockwise direction any matter in contact with them. They thus aid the movement of the style in keeping the food material in quite rapid rotation.

The tip of the style, enclosed in a mass of alimentary matter is constantly dissolving away as it is pushed up against the gastric shield. This dissolution is, however, confined to the cortical layers, the central core of bubbly mucus remains undissolved and is wound up with the rest of the mass around the head of the style.

The causes for the dissolution of the style consist, first, in the action of the juices of the stomach; second, the wearing against the point of the shield, and, third, the tendency of the style to dissolve spontaneously within a certain time after formation.

Figure 12 shows the oesophagus, stomach and style-bearing organs of *Modiolus*. In two favorable specimens I was able to cut through the stomach wall and observe the movement of the style with the surrounding alimentary mass, while strings of food and mucus from the gills and palps were passing up the oesophagus. It was therefore possible to observe the action of the various parts while they were functioning in an approximately normal manner.

The food particles, with large numbers of sand grains, entangled in mucus, pass up the oesophagus in a single string and, bridging the groove at the entrance to the stomach, are carried to the base of the gastric shield. Here they are caught in the revolving alimentary mass and wound around it. The mass, in turning, comes in contact with the spiral cavity described on page 78 and shown in figure 12. As this mixture of mucus, food particles, and foreign matter sweeps across the highly ciliated grooves of this structure, sand grains, together with much of the newly arrived matter from the oesophagus, are caught out of the mass and carried down the tract and into the right side of the caecum (p. 78 and fig. 15).

Here the mechanism described above (p. 78) comes into play, and by means of this most of the sand grains are separated from the food particles and are carried into the intestinal groove, while the latter pass out of the left side of the caecum. From here the ciliary currents carry the food across the ventral wall of the stomach to the right side of the gastric shield, where it again enters the revolving alimentary mass (fig. 12).

In the two mussels which I had under observation a steady stream of sand and food particles was entering the right side of the caecum, while a stream of practically pure food particles was issuing from the left side. At the same time pellets composed almost exclusively of sand grains were passing along the intestinal groove and into the intestine. From time to time small portions of the three streams just described were removed and examined microscopically, and revealed the fact that practically complete separation of food and sand was taking place in the caecum.

By means of this mechanism the ingested material in the stomach is gradually sorted over and purified, until, when feeding has ceased, the stomach contains a mass of pure food material.

In all cases where I have had the revolving style under observation the motion is not continuous, but is interrupted by periods of inactivity, preceded by a gradual slowing down of the style. During these periods of cessation the cilia of the style sac themselves become greatly reduced in activity. After a period of rest the cilia again begin to beat actively, followed by a resumption of motion of the style. What the stimuli may be that cause the cessation and resumption of motion on the part of the style I have been unable thus far to determine. 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 possible that the large bristle-like cilia of the style sac may be under the control of the nervous system and respond to certain internal reflex stimuli called forth by the stage of digestion of the animal.

We have seen that the ribbed mussel lives under conditions such that its feeding periods are usually restricted to a few hours. As a result, it makes the most of every opportunity, and if one examines a cluster of these molluscs after the rising tide has just covered them, the surface of the water will be seen boiling like a miniature spring, due to the very active siphoning.

When removed to a dish containing sea-water with much fine sand in suspension, the water is soon completely filtered by the activity of these molluscs. Comparatively little discrimination is made by the palps and gills, save against the larger particles; everything else is taken into the stomach and sorted there. Within about an hour after placing a mussel in roily water it will begin to void faeces in a continuous ribbon, composed almost wholly of sand grains held together by thick mucus.

In a recent paper on the ciliary mechanism of lamellibranchs, Kellogg ('15, p. 660) describes the peculiar 'sand-eating' genus *Macoma*. He states that the entire digestive tract of this form is filled with debris. Since the backwardly directed currents of the palps and mantle are as well developed in this as in other species, Kellogg is unable to account for the ingestion of such large quantities of silt. In view of the fact that *Modiolus*, though possessing well-marked outgoing currents, ingests much sand along with its food, it is probable that *Macoma* will show upon investigation some similar means of sorting out the food materials from foreign matter in the alimentary canal. A further point of similarity between these two species lies in Kellogg's observation of the extraordinary power of the cilia in this form. My own observations on *Modiolus* indicate that the outgoing tracts are for the purpose of removing the larger particles which do not enter the mouth.

As we have seen above (p. 71), *Anodonta* possesses a diverticulum which somewhat resembles the 'sand-sorting' caecum of *Modiolus*, though less well defined. Space will not permit of a description of analogous structures in the various forms studied. Suffice it to say that *Lampsilis*, *Ostrea*, and *Venus* all show some modification of the stomach wall in connection with the intestinal groove and extension of the major typhlosole, by means of

which a certain amount of selection and separation of the material in the stomach does take place.

In artificial feeding experiments I have found that the selective action of the palps of *Ostrea* is very great as compared with that of *Modiolus*, even the most finely divided carmine grains being rejected. Coincident with the increase of selective power on the surface of the body there is a corresponding lack of specialization of the wall of the stomach for sorting over the alimentary matter.

Summing up what has been said of the origin of the style in the developing embryo (p. 88), together with the above description of the process of feeding in the adult *Modiolus*, it is evident that one of the primary and important functions of the crystalline style throughout the life of the lamellibranch is to aid in keeping the alimentary mass in the stomach in motion.⁵ This movement is necessary, not only as a means of sorting out foreign matter in the stomach, but also as a substitute for muscular peristalsis in keeping the food in motion.

Since the head of the style with sand grains and other rough objects attached to it bears against the stomach wall, there is necessarily developed at this point a resistant structure to prevent injury to the epithelium. The gastric shield therefore arose as such an organ of protection, as was first believed by Barrois, who compared it to the 'Trichter' of insects and some other arthropods.

Barrois, followed by Pelseneer ('91) and others, believed that the function of the style, when dissolved, consisted in surrounding sharp particles and thus preventing injury to the epithelium. Against this interpretation I would urge the following objections: First, the style, allowed to dissolve of itself without the addition of any fluid, forms, with the exception of the inner core, a liquid of such a thin consistency as to be of little or no possible value as a protective covering; second, the cells of the alimentary tract are covered with a heavy carpet of large cilia, so that no sharp object would be likely to come in contact with

⁵ Milne-Edwards ('59) was the first to advance this theory, but without the least evidence in its support.

the surface of the cells; third, the streams of food and foreign matter entering from the oesophagus are largely composed of heavy mucus secreted by the gills and palps, and this is in sufficient quantity and of a consistency capable of acting as a protective covering if such were needed; fourth, an examination of the pellets of sand passed down the intestinal groove of *Modiolus* shows them to be composed of sand grains compactly held together by a small amount of thick mucus, such as that secreted by the body surface or oesophagus.

A second function of the crystalline style, though probably a minor one, has been referred to above (p. 83) in describing the formation of the style, where it was shown how undigested food materials, passed down the intestine along with the waste matter, are caught and incorporated into the inner core of the style.

During the winter I have found individuals in which practically the entire style was composed of the brown particles which form so large a part of the alimentary mass of the stomach. The crystalline style thus serves incidentally, except in those forms in which it lodges in a separate caecum, as a means of restoring to the stomach nutriment which might otherwise be lost.

One is greatly tempted at this point to consider the endo-style of *Amphioxus*, in so far as it acts in the transfer of food, as possibly an analogous structure. However, as I have been unable to examine living specimens of this animal, this similarity is merely suggested here.

Finally, we have seen that the crystalline style of the lamellibranchs contains a very active amyolytic ferment, as was first held by Coupin ('00), followed by Mitra ('01), van Rynberk ('08), and others. From the evidence presented by these workers, together with the facts shown in this paper, it is probable that the enzyme is held in a viscous matrix of a mucin-like substance which gives the reactions of a globulin.

Since the food of the majority of lamellibranchs consists mainly of unicellular plants, and since these animals have no salivary secretion, an abundant supply of starch-splitting enzymes in the alimentary canal is a necessity.

The presence of a style of great dimensions has been noted by several workers in *Teredo* and other boring molluscs, and I have found the same in *Martesia*. The relatively great development of the style in these forms is probably connected with their mode of feeding and living.

Some of these molluscs bore into objects close to the high-tide level, and are hence unable to feed for long periods. All of them communicate with the exterior by means of a comparatively small opening. It is probable that their periods of feeding are further disturbed by deposits of mud over these openings, so that it is necessary to use to the best possible advantage every opportunity for feeding. Such being the case, the presence of a large amount of enzyme, ready for immediate use, could not but be of great advantage to these molluscs.

5. SUMMARY AND CONCLUSIONS

In the present paper the attempt has been made to bring under one head the results of all former workers regarding the crystalline style of lamellibranchs and to settle important questions concerning its origin, nature, and function.

A survey of the literature shows that much of the credit given to Mitra ('01) rightfully belongs to Barrois ('89, '90) and Coupin ('00).

Anatomical features of the stomach and intestine of some common lamellibranchs, not previously noticed, are pictured and described.

The mode of secretion and formation of the style has been studied in marine and fresh-water forms. It arises as a thin core of bubbly mucus, upon which are deposited co-axial layers of a gelatinous protein, containing enzymes.

Its embryological development is followed in *Ostrea* and *Dreissensia*.

The ciliary mechanism of the stomach, intestine, and style sac has been traced out in some detail. It was found that the molluscs studied possess the ability of separating food from foreign particles in the stomach by ciliary action. This mechan-

ism was found best developed in those species, like *Modiolus*, in which little discrimination is shown by the gills, palps, and mantle.

The actual rotation of the style was observed, and is here described for the first time. This movement of the style is of great importance in separating the food from foreign particles and in serving as a substitute for peristalsis.

The gastric shield is shown to be an organ for the protection of the gastric mucosa against the abrasive action of the head of the revolving style.

In those species in which the style sac is incompletely separated from the intestine, the style serves as a means of restoring to the stomach undigested food particles which might otherwise be lost.

The conclusions of Coupin ('00), that the style contains strong amyolytic ferments, have been confirmed. These enzymes are held in a stiff gelatinous matrix of a globulin-like substance.

In some of the boring molluscs the style attains a relatively great size, thus representing a large mass of enzyme for immediate use.

The conclusions of Mitra ('01), regarding the style as solely a mass of pure enzyme secreted by the hepatopancreas, and the theory of the protective action of the style substance, held by Barrois, Pelseneer, and others, are hereby rendered untenable.

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