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The morphology and histology of the Virginia oyster, Ostrea Virginica, Lister

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THE MORPHOLOGY AND HISTOLOGY OF THE VIRGINIA OYSTER, *OSTREA VIRGINICA*, LISTER

by

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TABLE OF CONTENTS

PART I

INTRODUCTION

INTRODUCTION

The oyster has been known and used commercially since before the Roman and Greek civilizations, and reports of investigations pertaining to it can be found dating back almost to that period. Because of the commercial value of the oyster, most of the study of the animal has dealt with its economic importance. Definite information concerning the morphology and microscopic structure of this Mollusc, therefore, is scarce. To be sure, there is an abundance of miscellaneous literature, but no systematic microscopic study has been reported. T. C. Nelson (1938) states, "A critical survey of the abundant and scattered literature however reveals need for more fundamental investigations of the morphology of this pelecypod and of correlation of such findings with its ecology." Until recently few attempts have been made to gather all the material under one cover. For example, Philpots (1890) published two volumes entitled Oysters, and all about them, but he says little of scientific importance. Brooks, of Johns Hopkins

University, published his classic book, The Oyster, in 1905. This volume contains a vast storehouse of information on oyster growing and is of inestimable value to the layman, but unfortunately for this investigation Brook's work, like that of Philpots, was written for the oysterman. It is obvious therefore, that there is a definite need for more accurate work on the oyster. To satisfy this need, the writer has attempted an investigation to gather the existing literature on the subject, and to supplement this material with additional observations, thereby making, in so far as is practicable, a more complete morphological and histological study. Time and space do not permit a very detailed description of the anatomy, but an attempt has been made to summarize the existing information, in most cases after corroborating it in the laboratory at the University of Richmond. Since the original intent of this paper was a description of the digestive tract of Ostrea virginica, special attention will be devoted to it.

At the beginning our investigations were handicapped by a scarcity of literature pertaining to the histological technique. To remedy this sit-

uation, a systematic study of the histological methods that would best fix and stain oyster tissue was started in this laboratory by Wilson (1938) and Carsley (1938). Although the ideal method of fixation was never found by these students a great deal of information was obtained pertaining to the affinity for stains of different structures. This was of considerable value in working out the histology of many of the structures. Using the knowledge gained by these papers, this investigation of the histology of the digestive tract was begun.

PART II

REVIEW OF LITERATURE

REVIEW OF LITERATURE

One of the earliest investigations of the microscopic anatomy of the oyster was conducted by Peck (1887). His paper dealt primarily with a comparison of the gill structure of Arca, Mytilis, Arcodon, Dreissena, and Ostrea. He made note of the fact that Ostrea differed from Mytilis in having its gill plates fused to the pallium, whereas in Mytilis, the outer demibranchs are free. He discussed the epithelium of the gills in Mytilis, but made little mention of any of the similar structures in Ostrea. He did, however, in reference to Mytilis, describe three types of filaments, the cilia, and goblet cells. The interlamellar tissue of the gills was described as "lacunar tissue", probably because of the large empty cells.

Possibly the foremost authority on the morphology of oysters during the last century was John A. Ryder. He published many papers, one on anatomy being much quoted. In 1880, Ryder published a paper On the Course of the Intestine in Ostrea virginiana, Lister. His description of the stomach

is almost identical with the findings of this laboratory, and differs only slightly from the description of O. edulis by Yonge (1926-b). "The mouth is a wide opening between the upper median angles of the palps; so wide indeed that the animal can scarcely be said to have an oesophagus; immediately follows the stomach, which is seen to have pronounced folds internally, with a generally transverse direction, but two of these, which seem to lie in a somewhat ventral position, are a pair of inward projecting lobes, which are themselves lobulated." From here on, he describes the course of the intestine as it loops around the stomach and terminates in the rectum.

Ryder's Preliminary notice of some fine points in the minute anatomy of the oyster although an excellent paper, does not entirely agree with the findings of more recent investigators. He described the blood channels as being non-endothelial with the exception of the heart and the anterior and posterior aortae. The musculature of the heart is described as nonstriated, whereas Kellogg (1892) found that it was. Ryder found further that the

connective tissue is spongy and surrounds the gonads, the ducts to which are just beneath the mantle and lateral to the gonads. He found further that there are no muscle fibers in the digestive tract, which is ciliated throughout, while both Yonge (1926-b) and the writer find muscle in portions of the digestive tract.

Later in the same year, Ryder (1882-e) published the supplement to the above paper, in which he described at some length the culture, growth, and anatomy of the oyster. He also published two papers concerning the green color of oysters (1880 and 1882-f). His earlier paper (1880) which also discussed the breeding and food of the oyster stated that both the green and brown colors are due to diatoms. His supplementary paper dealt primarily with the coloration of the blood cells. He stated that the cells derive their color either directly from the plants or indirectly by "a hepatic coloring principle, which on account of some derangement of the normal metabolic processes of the animal has been dissolved and absorbed by the lymphohaemal fluids and then imbibed by the blood cells." MacMunn (1900) made a

similar observation, but found that the entero-chlorophyl" is the result of normal metabolism.

Still later in the same year, Ryder (1882-d) described the kidney, the organ of Bojanus. This structure was first described by P.P.C. Hoek (1882). According to Ryder, it is a "sickle-shaped" structure on the anterior side of the adductor muscle, composed of many tubules having a ciliated epithelium.

In his next paper (1883), Ryder discussed in some detail the morphology of the oyster. He described fairly completely the gills, palps, mantle, digestive tract, excretory system, and the nervous and vascular systems. His description was by far the most complete and accurate up to that time. It does not, however, altogether fit in with our present conception of the oyster anatomy. For example, Ryder's description of the circulatory system must necessarily be inaccurate since he did not recognize the existence of the accessory hearts described by Hopkins (1934) and later by Elsey (1935). However, any attempt at this point to discuss all his findings would be rather burdensome, and since they will be taken into consideration with the findings of other investigators,

it seems advisable to forego any further discussion of his paper at this point.

Mitsukuri (1881) published a paper on the significance of aberrant forms of Lamellibranch gills, but mentioned little of importance pertaining to the gill structure of Ostrea.

P.P.C. Hoek (1882) found that the generative organs of O. edulis are in the form of a lobulated gland that gives rise to both male and female gametes. The organ of Bojanus combines with the genital tract to form one urogenital canal.

Puysegur (1882) reported that the oyster was not the only mollusc suddenly to show a green color and that this color was due to green algae, and not copper.

Bourne (1889) in an abstract of a paper by P.P.C. Hoek, described the kidneys as opening into the pericardial cavity. In addition, there follows a description of the urogenital system and a discussion of the physiology of reproduction.

Transverse striations of the muscle fibers in the auricle and in the adductor muscle were first described by Kellogg (1892). The former were describ-

ed as being large and rather far apart. He also described the incomplete fusion of the right mantle to the visceral mass in O. virginica, the promyal of Nelson (1938). Kellogg also first recognized that the epithelium of the palps differed in its two surfaces: that on one side being ridged, and that on the other being comparatively smooth. The main body of the palps is composed of large cells, termed "fat" cells. His description of the digestive tract was rather meagre, with no mention of histological structure.

In his discussion of the nephridium, he stated, "It consists of large cuboidal vacuolated cells, without concretions." The nephridium is further divided into a glandular portion and a non-glandular portion, the non-glandular portion being posterior, while the glandular portion is anterior. The glandular portion has folds within its lumen. As yet, the author has found no evidence to warrant Kellogg's division. The cells of the nephridia sometimes contain a small round, deeply staining substance. None of the cells bear cilia.

Kellogg (1892) described three paired ganglia: the cerebral, which are connected by a supra-

esophageal commissure and are located lateral to the mouth; the visceral ganglia on the ventral side of the adductor muscle; and a pedal ganglia. In his description, he is not quite clear whether the pedal ganglia exists in Ostrea or in some of the other genera with which his paper deals. The gills are seen to be highly vascular, having a blood sinus connecting the filaments at their inner edges. The surface epithelium consists of columnar ciliated cells. The presence of many elongated glandular cells was also noted, as was the presence of chitinous supporting rods in the gill filaments.

Kellogg (1892) described the pedal muscle in Venus and then stated further that no similar structure exists in forms without a foot. Herdmann and Boyce (1899) describeda "protractor pedis" muscle in O. virginica and Ryder (1883) found a "pedal" muscle in the same species. While referring to the muscle, Kellogg proposed the idea that the large, dark portion, the adductor muscle, is the portion supplying the strength for the forceful closing of the valves while the smaller, light, non-striated portion is for quick closure of the valves.

Here again, his postulation conflicts with more recent investigators (Orton, 1935).

The description of the circulatory system by Kellogg was more complete than most of his work on the other structures. He described the presence of two auricles, a ventricle, and an anterior and posterior aorta. He also pointed out that the anterior aorta supplied the visceral mass and mantle, while the posterior aorta supplied the adductor muscle, running backward beneath the outer and upper wall of the pericardial chamber. Branches to the rectum, found by Elsey (1935) and the author were not mentioned. No capillaries exist in the oyster, their place being taken by sinuses, spaces in the connective tissue with no endothelial lining. However, the larger blood vessels in the gills and visceral mass are reported as having definite endothelial walls. The presence of this endothelium as a complete endothelial layer, in the opinion of the writer, is doubtful.

In referring to the gills, Kellogg described the lamellae as being divided into a number of folds, the plicae. The filaments of the plicae

are connected by a series of blood sinuses just above the wall of the water tube. This was also described by Ridewood (1903) and Elsey (1935). Frequent openings between filaments leading into the water tube were noticed. These have since been termed ostia. The epithelium of the gills is columnar and ciliated, the cilia from adjacent filaments touching each other to form a barrier-like structure. The water tubes are lined with cuboidal epithelium while the tissue of the gills consists of large circular cells interspersed with longitudinal and transverse muscle fibers.

John R. Philpots' two volumes, entitled Oysters, and all about them (1890) mentions little concerning anatomy or histology. The author gave a very complete history of the oyster, but approached his subject from a philosophical view point. It is however interesting reading.

In 1893, both Lankester and C. de Bruyne published papers on the phagocytes of oysters. Lankester pointed out that the large, highly granular cells in the surface of the epithelium, originally observed by him and described as secretory cells, are really wandering, phagocytic cells. De Bruyne, on the

other hand, suggested that the phagocytes are probably a part of the digestive system. From observations made in this laboratory, it seems to the writer that Lankester's first observations were probably accurate, but that some of the granular cells in the epithelium are phagocytes in the process of either engulfing or assimilating food material.

In 1897, Moore copied Brooks' and Ryder's accounts of the anatomy of the oyster verbatim in his description of the development, anatomy, and growth of the oyster.

Herdmann and Boyce (1899) described the histology of the gills, and to some extent, the blood. Their findings, in brief, are as follows: The epithelium of the gills is columnar with ellipsoidal nuclei. At the base of the filaments, the epithelium is cuboidal and squamous. Granular eosinophilous cells are found in the epithelium. Two triangular shaped skeletal bars (also described by Ryder, 1883; Kellogg, 1892; Ridewood, 1903; Leenhardt, 1926; Galtsoff, 1928 and Elsey, 1935) diverging to the exterior are found in each filament. The connective tissue is slightly fibrillated with fusiform or stellate cells. The

blood is composed of two types of cells, amoebocytes or eosinophilous cells, and smaller leucocytes. The amoebocyte wandering cells are found in almost all of the connective tissue and epithelium, a fact which is not altogether in accordance with the writer's observations. The liver is shown to have two main ducts leading from numerous small tubules (also described by Yonge, 1926-b on O. edulis). There are two types of cells in the liver, large granular "Kornerzellen" and dark "Fermentzellen." MacMunn (1900) described a granular cell of Frenzel that is similar to the "Kornerzellen" but goes further to state that the liver contains no ferment cells. MacMunn showed that there are cells in the connective tissue similar to the ferment cells of other molluscs. Yonge, (1926-a) showed that the gastric gland (liver) is composed of a single type of cell.

Herdmann and Boyce also described the protractor pedis muscle fairly completely.

MacMunn (1900) described the liver as a "folliculose" gland with ciliated tubules imbedded in a tunica propria. The cells of the terminal endings of the tubules are not ciliated, but contain entero-

chlorophyl either in the form of granules or dissolved in oil globules present in the cells. The entero-chlorophyl is a substance similar to chlorophyl, and later investigations seem to point to the fact that it might even be chlorophyl taken directly into the cell (Yonge, 1926-b). MacMunn (1900) stated that there is no glycogen in the liver. This statement is rather confusing. MacMunn apparently in referring to the liver meant not only the actual tubules of the digestive gland but also the "tunica propria" or surrounding connective tissue. If this conception is true, his statement is erroneous. According to MacMunn, the secretory cell of the gastric gland is the granular cell of Frenzel. Cells in the tunica propria (connective tissue) resemble the ferment cells in the gastric glands of other Molluscs in their manner of storing pigmented bodies colored by entero-chlorophyl or lipocrome. Looking at another investigator's work, we see that Yonge (1926-b) found the "liver to be actually a digestive gland used to assimilate soluble and to some extent insoluble substances. MacMunn described the gland ducts as being composed of columnar ciliated cells

with a striated border. He described the "amoebocytogenous" tissue in this manner: "We also find in the gastric gland of Ostrea very peculiar strands and islets of a tissue to which it is difficult to apply an appropriate name. In longitudinal, and indeed transverse sections, we find cells applied to each other forming a mosaic pattern which cells appear to be of epithelioid character. Where such strands approach the wall of the larger ducts, or that of the intestine, these cells seems to break away from each other and, being freed from mutual pressure, they become rounded, and look like leucocytes and amoebocytes." Nothing similar to this could be found in O. virginica, and it is the writer's opinion that MacMunn was describing a tangential section through the end of one of the gastric diverticula, because such a section adequately fits his description.

Ridewood (1903) described the gills of a number of Lamellibranchs, among which he included Ostrea edulis. He found that the distal principal filaments all have septa, but that the septa decrease in number as they get closer to the proximal edge, un-

til there are septa every fourth filament. The interlamellar septa have ciliated cuboidal tubes.

Mitra (1903), Van Rynhuk (1908), and T. G. Nelson (1918 and 1925) discussed the crystalline style. Its structure and functions have been enlarged on since these papers, but their descriptions are essentially accurate and complete.

In 1905, Brooks published The Oyster, an excellent and complete dissertation on the oyster and oyster growing, but one which does not emphasize all the details of morphological structure.

Kellogg (1915-16) described the ciliary movements of the gills, palps, and mantle. Material is collected on the gills and moved toward the two margins, and from these to the palps. The inner surfaces of the palps direct food orally, while the outer surfaces exhibit the reverse process. The mantle sends all material to a line parallel to the edge (Nelson (1924) described a series of these lines) and from there, the particles are pushed posteriorly and then out of the shell.

Mitchell (1915-16) showed that glycogen was apparently formed in the connective tissue from dex-

trose instead of dextrin. In his second paper, he discussed the seasonal change in the glycogen content of the oyster, and the fact that the so-called "fat" of an oyster refers to the glycogen. He does, however, find fat in the oyster as shown by ether extraction. From the writer's observations, it is probable that the extract contained general protoplasmic lipoids, and little substance comparable to the adipose or "fat" material found in vertebrate animals. Mitchell found further that, although glycogen is stored throughout the whole oyster, the highest percentage of glycogen occurs in the region of the gastric gland.

It was reported by Grave (1916), that the ciliary mechanism of the gills, mantle, and palps is capable of beating selectively, enabling the oyster to select food material from sand grains etc. The cilia are capable of reversing their beat, if necessary.

According to Churchill (1919), the nervous system consists of two ganglia just over the oesophagus. Two other ganglia, situated beneath the adductor muscle, are connected to the first ganglia by two

nerves. Peripheral nerves extend to all parts of the body from these two pairs of ganglia.

In 1922, Orton made a study of the blood of oysters. He found that the blood cells would live from 3 to 4 days in a sea water medium, a fact which perhaps has led many investigators to think that some of the blood cells found in the tissue were parasites. It is rather interesting to note that, according to Orton, the blood cells, when they are taken out of the animal will first congregate in clumps and then, after a few minutes will disperse. This phenomenon of congregation was also noted by the writer. Orton stated further that the origin of the blood cells is unknown, and that they have been seen to divide. They also were observed to send protoplasmic connections to each other, which according to Orton, is analagous to the clotting of mammalian blood.

In 1935, Orton published Oyster Biology and Oyster Culture, essentially a review of the modern knowledge of the oyster, with special reference to O. edulis. Although this book is not very detailed in its descriptions, there is little that Orton does not cover, including in it development, anatomy, habits,

feeding, growth, spawning, culture, enemines and pests, pollution, and purification.

Spärck (1924) gave us one side of a very much discussed question, in addition to a discourse on the physiology of sex. He stated that the green color of the oyster is not due to diatoms, but to an intracellular parasite. This view is directly opposed to those of Ryder (1882-a), Lankester (1893), and Puysegur (1882).

T.C. Nelson, also in 1924, discussed in more detail than did Kellogg (1892) the method by which food is carried to the mouth. When the food reaches the troughs in the gills that lead to the palps, it is in the form of "slime strings." Upon reaching the palps, it is taken to the mouth, provided that it is small enough to fit into the opening between the palps. Particles too large to enter are carried to the posterio-ventral portion of the animal by the pallial lobe, and forced out by a contraction of the muscle.

In the following year, Nelson (1925) published some interesting observations on the functions of the style. The style is shown to produce an

amylolytic enzyme, mechanically to separate food from non-digestible material, and to recover food that has escaped the stomach by building the food material into the core of the style. The style, constantly rubbing against the gastric shield, gradually becomes eroded, and thus returns the food material to the stomach. As the style is being worn on the gastric shield, it is constantly being replaced at its distal, posterio-ventral end.

In 1938, T.C. Nelson published a rather lengthy paper on The Feeding Mechanism of the Oyster, in which he discussed in considerable detail the functional importance of the promyal chamber, and the reduplications of the pallial border in O. virginica. The promyal chamber was first described by Kellogg (1892). Kellogg, however, did no more than just note the incomplete fusion of the right pallial lobe to the visceral mass. Nelson showed conclusively that the major portion of the water taken in by the right pair of demibranches passes to the outside through the promyal chamber. Thus, the promyal chamber, in addition to the cloacal chamber, is used to discharge water. The distal edge of the pallial

lobe is described as having three folds, (the reduplications mentioned above) two of which bear tentacles. The pallial curtain, the most medial, is extensible by virtue of the elastic fibers at the base of the epithelium. The epithelium of the pallial curtain is low cuboidal, interspersed with mucous cells. In addition, there are large mucous cells in the underlying connective tissue. The pallium itself consists of a series of small folds, running perpendicular to the large folds. These small ones make up the rejection troughs. The inner epithelium is a low columnar ciliated epithelium becoming cuboidal at its distal end, in the vicinity of the pallial folds. The epithelium rests on a band of collagen-like connective tissue. The median pallial fold is heavily ciliated and composed of low cuboidal cells. The external surface of the pallium has two distinct types of epithelium connected by a transition. One is smooth in appearance, and contains more mucous glands, while the other is composed of a higher epithelium thrown into many folds. Muscle fibers, both radial and concentric are also described.

To date, the most complete account of the morphology and histology of the digestive tract was written by Yonge (1926-b) from observations on O. edulis. The pallium is described as having a transversely ridged surface with a thickened margin bearing two rows of tentacles. This was described by Nelson (1938) for O. virginica and Elsey (1935) for O. lurida and O. gigas. The mantle cavity is divided into exhalent and inhalent chambers by the fusion of the four demibranchs and pallial lobes. This condition differs from that in O. virginica (Kellogg, 1892) and Nelson,(1938) as well as O. gigas (Elsey, 1935). The inner demibranchs are closer to the mouth than the outer ones. The gills overlap the palps for a short distance. The palps, of which there are two pairs, are ridged on their inner surfaces and smooth on their outer surfaces. This was described on O. virginica by Kellogg (1892). In the region of the mouth the two inner palps are fused. The mouth lies in the continuation of the groove between the two inner palps. The position of the mouth (Ryder, 1880 and Kellogg, 1892) in O. virginica is the same although its shape is slightly different. According to Yonge, the

mouth, a horizontal slit, empties into a short slit-like oesophagus which in turn empties into the sac-shaped stomach. Leading from the stomach is a large food sorting caecum that is also connected to the mid-gut, one of the other structures leading from the stomach. In addition, there are two main ducts leading from the digestive diverticula, one on each side of the stomach, a condition that is not exactly the same in O. virginica. The ventral portion of the stomach elongates into the style sac housing the crystalline style. The gastric shield, a cuticular structure against which the style revolves is situated on the dorsal wall of the distal half of the stomach. Yonge believes that the gastric shield is not a secretion, but is formed by the fusion of the cilia. Nelson (1925) apparently supports this theory by stating that the gastric shield is composed of chondrin. Berkeley (1935) found that the style is composed of mucin and chondrin while the gastric shield is composed of chitin. The gastric shield is bilobed in O. edulis and trilobed in O. virginica. The mid-gut is continuous with the style sac throughout its

entire length. From the midgut, the intestine doubles back dorsally, loops around the stomach and terminates in the rectum. This condition was found to be the same in O. virginica from our own observations and those of Ryder (1880) and Brooks (1905).

Yonge next described the histological structure of the digestive tract in O. edulis, and since this will later be compared to that of O. virginica, his description will be only briefly summarized here.

The gills are divided into plicae and these further divided into filaments, there being 9-12 filaments per plica. Similar observations were made by Ridewood (1903), Kellogg (1892) on O. virginica, and Elsey (1935) on O. lurida and O. gigas. Filaments are of three types: the principal filament found at the junction of the two plicae, the transitional filament along the side of the plicae, and the ordinary filaments found at the apex of the plicae. This description of Yonge's is based largely upon Ridewood (1903). Yonge does, however, enlarge upon it to some extent.

The inner surface of the palps is ridged and composed of ciliated cells with oval nuclei.

Phagocytes and unicellular mucous glands are found interspersed throughout the epithelium. The connective tissue consists of fine strands with many muscle fibers running through it. On the inner surface, the epithelium is more irregular, but still very similar. The mouth is composed of tall columnar cells with long cilia. The epithelium of the esophagus is similar to the mouth, except that the cilia are shorter. The stomach is composed of tall columnar ciliated, and in the region of the gastric shield, non-ciliated cells. Occasional mucous cells are found. The tubules of the digestive diverticula are composed of ciliated columnar cells, while the diverticula themselves are non-ciliated. The style sac is composed of columnar cells with large oval nuclei and long stout cilia. The midgut is composed of tall columnar cells interspersed with many mucous cells. Mucous cells are most numerous in the rectum which is composed, as is the rest of the digestive tract, of ciliated columnar cells.

Phagocytes, of which there are seven types, are found throughout the epithelium of the digestive tract and the connective tissue of the vis-

ceral mass.

In another publication, the same year, Yonge (1926-a) showed that the digestive diverticula is a system of blind tubes emptying into the stomach by means of ciliated ducts. Only one type of cell is present, which probably has in addition to its powers of absorption, the power to engulf small particles. "Injection of iron and pilocarpine fail to demonstrate the presence of secretory cells elsewhere than in the style sac." The digestive diverticula must, therefore, be organs of absorption and intracellular digestion.

In 1928, Yonge proved that the absorption of glucose took place only in the digestive tract, specifically in the gastric gland.

Galtsoff (1928) gave a description of the structures of the gills based on Peck (1877), Kellogg (1892), Janssen (1893), Ridewood (1903), and Yonge (1926-b).

Truitt (1931) gave a general account of the morphology and physiology of the oyster. Little new material on the anatomy or histology was presented.

Hopkins (1934) described the acessory hearts of the oyster. They are located on the inner surface of the edge of the mantle, at the posterior end of the cloacal chamber. He suggested that overlooking these hearts has caused an incomplete understanding of the circulatory system. His description of the circulatory system was taken from O. gigas, and differs from that of Elsey (1935) who independently discovered the accessory hearts a year later. Hopkins (1934) found that the blood from the accessory hearts goes to the mantle and the gills, thereby causing pressure in the circumpallial arteries from two sources, since they are directly continuous with the anterior aorta. According to Elsey (1935), the accessory hearts receive blood from the mantle, and then pump it to the heart by means of the pallial vein. In addition, part of the blood, (Elsey, 1935) from the gills, passes through the accessory heart and then goes to the auricle.

Two years later, Hopkins (1936) described the pulsating of the radial vessels in the mantle.

Berkely (1935) found that the style is composed of mucin and chondrin, a fact that explains

the red structures in the style sac after being stained with mucicarmine in this laboratory. The gastric shield is composed of chitin.

Elsey (1935) again described the incomplete fusion of the mantle to the visceral mass, described also by Kellogg (1892) and Nelson (1938), in his paper on O. gigas and O. lurida. He also mentions the pedal muscle described by Herdmann and Boyce (1899) on O. edulis. In addition, he takes up the histology of the mantle and gills in considerable detail.

PART III

MATERIALS AND METHODS

MATERIALS AND METHODS

Live specimens of O. virginica were brought from the York River and placed in a well aereated glass aquarium. The specimens seemed to thrive in their new environment.

Material for gross dissection of the digestive system was first injected either orally or through the rectum with a hot colored, gelatin mass. The mass was allowed to cool, and the whole specimen was hardened in 10% formaldehyde, and then dissected. Some of the specimens were injected orally with a plaster of paris mixture, but this did not prove so efficient as the gelatin mass. The observations made in this fashion were checked with two sets of serial sections. One was a serial made from a 1.5 cm. oyster, killed and fixed in Bouin's fluid for 18 hours, sectioned at 10 micra and then stained in Harris' modification of Delafield's haematoxylin and counterstained with eosin and orange G. The other serial was a large oyster, 7 cms. long, fixed in Bouin's fluid for 48 hours, dehydrated in alcohol and imbedded in

celloidin. Free hand sections were made, and examined under low magnification without staining. The position of the digestive tract was then graphically represented.

The nervous system was demonstrated by a modification of the Charles Sihler stain (Guyer, 1936). Another method employed was 10% nitric acid. The oyster was placed in the acid for 72 hours after first killing it in hot water. It was next washed in running water for 24 hours. Results of both these methods were disappointing. Use of serial sections was found to be the most practical method for determining the nervous system.

The circulatory system was studied both by reference to the serial sections mentioned previously and by injection. The injection mass consisted of a 10% solution of neutral red, and was injected directly into the ventricle. The heart was then tied off to prevent the stain's flowing out. The oyster was dissected without hardening. All other gross dissections were performed either on a fresh specimen or on one hardened in 10% formaldehyde.

Material for histological study was fix-

ed in Carnoy's fluid for 1 to 2 hours, Bouin's fluid for 8 to 12 hours, Flemming's-with-acetic for 6 to 8 hours, and in a few instances with Regaud's. Haematoxylin, eosin, orange G, as well as iron haematoxylin and Congo red were used to show the structure of the epithelium. Muscle tissue was stained with iron haematoxylin without a counterstain. Connective tissue was stained with Regaud's haematoxylin following Regaud's fixation. Connective tissue elements were fixed in Flemming's-with-acetic and stained with Bensley's (1939) aniline-fuchsin, methyl green technique.

Material to demonstrate glycogen was fixed in 9 parts of ethyl alcohol to 1 part of formalin, or by means of Regaud's technique. The glycogen was stained with sodium bisulphite, Bensley (1939). A saliva digested control was run in both cases.

PART IV

MORPHOLOGY

MORPHOLOGY

The oyster consists of two valves, left and right, connected by a hinge on the posterio-ventral side (figs. 1 and 2). There is a dark, red-purple ligament situated at the hinge, the leverage of which is such that the two valves will remain open if there be no force to close them. Although the oyster is considered bilaterally symmetrical, the left valve is usually larger than the right valve. The left valve is normally in the form of a deep cup, while the right valve is comparatively flat and acts as a cover to the cupped left valve. The oyster attaches itself by its left valve, leaving the right valve free to open. The shell has a laminated appearance, consisting of layers of calcium carbonate that are set down by the pallium as the oyster grows. The degree of concavity of the left shell depends not only on the species, but to a very great extent upon the type of substratum that the oyster is growing upon.

When the right valve is removed, the soft parts of the oyster can be seen attached to the shell

at four points: on the right and left sides by the adductor muscle, to some extent by the pallium where it is fused to the muscle, and the remnant of the retractor pedis muscle (Herdmann and Boyce, 1899). The large muscle, posterior and posterioventral to the pericardial cavity, is used for closing the shell. The smaller muscle at the anterior portion of the mantle is merely a remnant of the foot muscle that has no apparent function as such in the oyster. Herdmann and Boyce (1899) find that the retractor pedis muscle is capable of enlarging the opening underneath the fused portion of the pallial lobes, the oral hood. This is accomplished by raising the cucullus (the cowl-like fusion of the pallial lobes) with the contraction of the retractor pedis muscle.

In O. virginica the large muscle is situated somewhat on the dorsal side of the animal about two-thirds of the distance from the hinge to the opposite end of the shell. The muscle is attached directly to the left and right valves, leaving a purplish kidney-shaped scar. The muscle is fused to the mantle around its edges. There are two def-

inite parts to the muscle, the large, anterior, shield-shaped adductor muscle, and the smaller, posterior, crescent-shaped catch or ratchet muscle (fig. 3). The former is the larger and darker of the two, being a dull grayish color, while the catch muscle is a glistening white. The adductor muscle is the muscle used for a quick closing of the shell, while it is thought that the catch muscle is used to keep the valves closed over a period of time.

PALLIUM

The next most conspicuous structure is the pallium (mantle) which covers the oyster completely on its right and left sides, except for the region of the muscle. The pallium in the region of the muscle is fused not only to the muscle but also to the shell.

It might be well, in order to make the following descriptions somewhat clearer, to explain the angle at which the oyster was viewed. All dissections were made with the right valve of the oyster removed and with the animal resting on its left side. The mouth (just below the oral hood) will be considered anterior, the anus posterior, the region between the mouth and the rectum on the side toward the apex of the shell dorsal and the regions near the wider portion of the shell are roughly ventral (fig. 3). Earlier investigators were a little confusing in their discussions because they very often differed with each other in respect to the anterior and posterior axis of the oyster. Accordingly Yonge and Nelson submitted the question to Pel-

seneer in 1931 and arrived at this conclusion: "The principal axis in the oyster lies in the line drawn through the mouth and anus. The hinge is anterodorsal, while the palps and front portion of the gills project ventrad into the infra branchial chamber. The hinder part of the gills extend ventrad, ventroposteriorly and posteriorly." (Nelson, 1938).

The pallium is ridged very faintly, the ridges running radially from the region of the muscle. These ridges are only noticeable on the medial side of each pallial lobe. The left and right pallial lobes make up the two parts of the pallium. These are fused to each other on the dorsal side from a point about two-thirds of the distance from the anus to the mouth, to a point just a little anteroventral to the mouth (fig. 3). This fusion, the oral hood, forms a cowl-likestructure, the cucullus, over the mouth and the anteriodorsal portion of the labial palps. Both the left and right pallial lobes are fused to the visceral mass, the left lobe to a greater extent than the right. This condition was first observed by Kellogg (1892) on O. virginica. The space formed by the incomplete fusion of the right pallial lobe to the visceral mass has recently been

termed the promyal chamber (Nelson, 1938). In addition, the pallial lobes are fused to the posterior and posteriodorsal border of the gills, forming the exhalent or cloacal chamber, and the promyal chamber on the dorsal side and the inhalent chamber on the ventral and posterioventral sides (fig. 3).

The edge of the pallium is considerably thicker than the rest of the structure and is usually pigmented. At its border there are three small folds. These folds are not continuous all around the pallium, but start at approximately the ventral portion of the palps, continue posteriorly, getting thicker and more pronounced as they approach the posterioventral portion of the pallium where they reach a uniform size. They get smaller on the dorsal side of the pallium as they approach the point where the two pallial lobes are fused, finally disappearing a short distance posterior to the fusion.

The innermost fold is termed the pallial curtain and consists of a thick fold bearing large whitish tentacles (Nelson, 1938). When the tentacles are extended, this fold forms a curtain at right angles to the rest of the pallial lobe. This enables

the oyster very effectively to prevent any foreign material entering the inhalent chamber if it forces both the pallial lobes together. The distal edge of the pallium consists of two pigmented folds, one slightly larger than the other, the outermost, while the median fold is almost hidden. The median fold bears two types of tentacles, arranged in several rows. Elsey (1935) working on O. gigas and O. lurida found that the larger tentacles are more sensitive to hydrochloric acid than the others. In life, the outermost fold is usually found adjacent to the shell of the oyster.

Nelson (1938) described the pallial folds very completely. "The pallial curtains, innermost of the three reduplications of the pallial border, begins ventrally on each pallial lobe, approximately at the level of the central portion of the palps. It starts low and close to the edge of the pallium, gradually increasing in height and in distance from the border as it courses aborad to the pallio-branchial fusion. Dorsal and orad to this fusion the curtain extends to a point on a level with the anterior margin of the promyal chamber, beyond

which it decreases in height as on the ventral side, finally merging with the pallial surface near the anterior end." In a manner similar to the folds, the tentacles of the pallial curtain are smallest at its anterodorsal and anteroventral ends, but gradually get larger as the curtain extends posteriorly, finally becoming of uniform height at the posterior border of the pallial lobe. These tentacles are approximately 0.5 mm. apart. This description of the pallial curtain will also suffice for the other two folds, since they follow it rather closely.

At the point where the left pallial lobe is fused to the visceral mass, there is a small flap that remains free. This flap extends along the dorsal border of the visceral mass for a short distance. It is similar to the other portions of the pallial lobe in every detail, even having the three reduplications of its distal border.

GILLS

When the right pallial lobe is removed, five large structures come into view, the visceral mass, gills, labial palps, muscle, and heart. The visceral mass is a large structure of varying color, which may be described as a club-shaped mass with a small diverticulum coming off it posteriodorsal side; the rectum. The posterioventral extension of the visceral mass, containing the style sac and the midgut, has been named the "oral process" (P.P.C. Hoek, 1882). The visceral mass attached to the two pallial lobes laterally and dorsally, to the labial palps on its anteroventral side, and the gills on its ventral and posterioventral side, contains the digestive tract, part of the nervous and excretory systems, and the gonads.

Attached to the visceral mass are the four demibranchs of the gills. These demibranchs are attached to each other and to the mantle is such a fashion that they form by these attachments a water-tight wall. There are four demibranchs arranged in a crescent-shaped fashion, starting at the

base of the palps and terminating at a point immediately posterior to the muscle (figs. 3 and 9). The palps enclose the gills for a short distance (fig. 7), the right and left outer and inner demibranchs lying between the inner surfaces of right and left palps. The two inner demibranchs arise slightly more anteriorly than do the outer demibranchs.

Each demibranch consists of an outer and an inner lamella, connected to each other by interlamellar tissue. The outer lamella of each outer demibranch is united to the mantle, while the inner lamella of the outer demibranch is attached to the outer lamella of the corresponding inner demibranch. The inner lamella of the two inner demibranchs are fused, thereby completing the water-tight wall mentioned in the preceeding paragraph. The fusion of the right outer demibranch to the pallium in O. virginica is complete only at the aborad end of the gills (Nelson, 1938).

The demibranchs are divided into plicae, small ridges that give the gills a striated appearance. Theplicae are further divided into filaments,

small folds on the plica (Kellogg, 1892). There are three types of filaments, ordinary, principal, and transitional (Figs. 17 and 18). The ordinary filaments are found at the apex and along the lateral walls of the plicae. The principal filament is situated at the base of the cavity between the filaments, while the transitional filaments are found adjacent to the principal filament (fig. 18). Between the filaments are numerous pore-like openings, the ostia, that lead to the water tube on the inside of the demibranch. (Kellogg, 1892, Ridewood, 1903, Galtsoff, 1928, Elsey, 1935). The water tubes empty into the epibranchial chambers of which there are two, one for each pair of demibranchs. The left epibranchial chamber empties into the cloacal exhalent chamber situated just dorsal to the muscle (fig. 3). The right epibranchial chamber empties largely into the promyal chamber and probably to a very slight extent into the cloacal chamber. In the region just below the adductor muscle, there is a constriction of the epibranchial chambers, which is more pronounced on the right side than on the left. This is probably due to the presence of the

promyal chamber on the right side of the oyster. Nelson (1935) in reference to the promyal chamber of O. virginica states, "Correlated with the presence of this outlet on the right side, the suprabranchial (epibranchial) chamber on the right side has become correspondingly reduced, thus limiting water flow through it." It is thought that the development of this promyal chamber in certain species of the oyster has increased their propensity for feeding in comparatively turbid areas. It is a well known fact that O. edulis would die in turbid waters that O. virginica flourish in, so we have more than experimental and theoretical proof that the promyal chamber is a development to enable feeding in turbid waters.

PALPS

Anterior to and overlapping the gills are two paired labial palps (fig. 3). Primarily these are part of the digestive tract, and as such they should be described with it. Since the palps, with the gills and pallial lobes constitute the feeding mechanism of the oyster they are described with these structures. The palps are small triangular shaped, transversely ridged structures, just ventral to the mouth (fig. 9). The palps are ridged only on their inner opposing surfaces. They are smooth on their outer surfaces and the inner opposing surfaces of the two medial ones. The space between each pair of palps is continuous with the mouth, forming with it a sort of U-shaped structure (fig. 21). In edulis this is apparently not so pronounced, the space between the palps forming a ciliated groove that leads to the mouth (fig. 7). The palps are fused to the visceral mass by their thickened proximal border.

DIGESTIVE SYSTEM

The digestive tract is far more complex than one would expect of an animal as simple as an oyster. The mouth, a funnel shaped structure, situated at the anterior end of the visceral mass, is joined to the cavities between the two pair of labial palps. Actually, however, the mouth lies dorsally between the two pair of palps and is also continuous with the cavities between the two pairs of palps by means of small grooves (lateral oral grooves) that are found near the point at which the two pairs of palps are fused (fig. 7). The mouth empties into an esophagus that extends posteriorly for about one centimeter in a large oyster, and this in turn empties into a large sac-like structure, the stomach (fig. 9). The stomach is rather complex, consisting of many folds and diverticula. The food sorting caecum, with the exception of the style sac, is the largest of these. It starts at the posteriodorsal portion of the stomach on its right side, extends anterioventrally, finally ending in two blind pouches, one very small, pointing

anteriorly, and the other a fairly large pouch extending ventroposteriorly and to the left (figs. 4, 6, and 9). The food sorting caecum is connected to the stomach throughout its entire length, except for the diverticulum extending ventrally. It is also connected to the midgut at about the junction of the latter to the stomach. In addition to the caecum, there are two small evaginations on the right side. These evaginations apparently have not been described heretofore. They were not visible when a gelatin cast was made of the stomach, but they were rather obvious when traced out microscopically by means of serial sections (figs. 4 and 6). Yonge's drawing of a gelatin cast of the stomach of O. edulis (fig. 8), and a similar drawing of O. virginica (fig. 9), show nothing at all similar to this. It is possible that the gelatin mass distends the stomach to such an extent that these evaginations may not be seen.

There are a number of small ducts emptying into the stomach from the digestive diverticula. There were ten of these ducts in one specimen, only seven in another, while still another had five ducts. Most of these were very small and emptied into the

stomach at about the same region, but without first emptying into a common duct. Apparently the digestive diverticula are in the form of a series of compound tubular glands, the size of which is variable, as is the number of ducts leading into the stomach. There are two large main ducts, making up the greatest portion of the structure, one on the anterioventral side, and one on the posteriodorsal side. The others are very small and inconstant. These two large ducts are in accordance with the findings of Yonge (1926-b) on O. edulis, but he offered no description of the numerous small tubules emptying directly into the stomach, as is the case in O. virginica.

On its left side the stomach bears the gastric shield. In O. virginica it consists of a trilobed structure, the middle lobe being the largest. Yonge (1926-b) described a very similar structure in O. edulis. "On the dorsal wall of the stomach is borne the gastric shield, a cuticular structure of somewhat irregular shape, consisting of two broad lobes united by a narrow neck, the larger of the lobes being thin and smooth, while the smaller of the lobes is thicker and bears a number of teeth. It is against

this shield that the crystalline style bears."

At its base the stomach gives rise to two structures, the style sac and the midgut. The style sac starts on the left dorsal side of the stomach and extends posterioventrally in almost a straight line, except for its distal third which curves slightly more posteriorly than the proximal portion (fig. 9). The style sac extends between the gills and the muscle, finally ending in a blind pouch at the distal end of the visceral mass (Figs. 4, 6, 8, and 9). It is this blind sac that houses the crystalline style.

The midgut, on the other hand, starts at the base of the stomach on its right posterior side and extends anterioventrad until it crosses over the style sac on its right side, where it turns ventrally and runs parallel to the style sac for a short distance. The midgut gradually twists around the style until when it is about halfway down the style sac, it is on the left posterior side (figs. 4 and 6). It then proceeds to turn slightly ventrally until at the distal end of the visceral mass it has once again migrated to a position where it is on the

left side of the style sac. At its distal end, the midgut loops anteriorly and then extends anteriodorsally as the intestine. The style sac is connected to the midgut by a narrow isthmus, the major and minor typhlosoles. Actually, the major and minor typhlosoles are an essential part of the midgut, but in as much as their histological structures are dissimilar, it seems advisable to segregate them.

The intestine starts at the posterioventral end of the midgut and passes posteriodorsally along the anterioventral side of the style sac. It gradually extends diagonally across the style sac and midgut on their right side, until about the level of the anterior edge of the pericardial cavity. Here it turns dorsally rather sharply for a few centimeters and then turns anteriorly and to the left. The next loop of the intestine extends anterioventrally, passing to the left of the proximal portion of the stomach. It extends past the stomach for a few centimeters and then loops once more, doubling back in a posteriodorsal direction. This loop passes to the left of the style sac and the ascending loop of the intestine anteriodorsal to the pericardialcavity, ending finally in the rectum

which extends along the posteriodorsal border of the muscle and terminates in the anus.

CIRCULATORY SYSTEM

The circulatory system of the oyster is rather confusing and difficult to trace. The smaller vessels are merely sinuses in the connective tissue, and any attempt to trace them by dissection is almost impossible. Consequently, most of the following description will be limited to an account of the larger vessels. Former investigators were rather inaccurate in their observations because they failed to take into consideration the fact that there are two large pulsating vessels in the pallial lobes (Hopkins, 1934; and Elsey, 1935). Furthermore, many of the radial blood vessels in the pallial lobes of very young oysters have also been seen to pulsate (Elsey, 1935).

The heart is situated in the pericardial cavity just anterior to the adductor muscle and consists of three parts, two long, conical auricles, connected to each other by a thin layer of connective tissue, and a large, roughly spherical, thick walled ventricle. Both the auricles and ventricle are capable of vigorous contraction. When the ventricle is not

contracted, it becomes enlarged tremendously, almost completely filling the pericardial cavity (fig. 10). Upon contraction, it contracts in three dimensions elongating the relaxed auricles (fig. 11). (Kellogg, 1892, Orton, 1935, and others).

Leading from the ventricle are two large arteries, the anterior and posterior aortae. The anterior aorta runs into the visceral mass at the junction of the visceral mass and rectum (fig. 5). From here it runs anteriodorsally for a short distance and then gives off two branches, one small artery that runs posteriorly supplying the rectum and one large vessel that runs ventrad. This first large branch forks shortly after it comes off the anterior aorta giving off a fairly large artery supplying the gonads, digestive diverticula, and stomach, while the other branch of the fork extends posterioventrad into the smaller end of the visceral mass giving off about four or more small branches supplying the style sac, midgut, and gonads. The anterior aorta continues anteriorly giving off three small branches dorsally, two small branches ventrally, and one large ventral branch supplying the anterior

portion of the stomach, the esophagus, the gonads, the digestive diverticula, and the inner palps. From this point, the anterior aorta twists slightly ventrally and then gives off a large branch which itself branches and supplies both of the outer palps. The branch to the palps runs along their base giving rise to three main arteries supplying these structures. Each of these branches divides into smaller arteries extending in a sort of fan-shape (fig. 5). The anterior aorta continues anteriorly through the fusion of the pallial lobes until it reaches the termination of the cowl. Here it bifurcates giving rise to the right and left circumpallial arteries. These follow the edge of their respective pallial lobes, each giving off a large branch at about the level of the palps, that runs parallel to the edge of the pallium through the middle of the pallial lobe. The pallium is well supplied with blood by means of numerous radial branches from the circumpallial artery and branches from the artery running posteriorly through its middle. There are two main arteries supplying the pallium withblood, the circumpallial

arteries, and a smaller artery, a branch of the circumpallial artery, running down the middle of each pallial lobe. This vessel for the sake of something to call it, might be termed the median pallial artery. Branches of this supply mainly the pallial lobe in the region of the adductor muscle. The circumpallial arteries supply the distal border and the region in fairly close proximity to the edge of the pallium, eventually emptying into the acessory hearts. It might be well to point out here that in very young oysters the pallial lobes are more highly vascular than in adult oysters, a fact that seems to indicate that young oysters used the pallium to some extent as an aid to the gills.

The posterior aorta starts dorsally and then twists abruptly posteriorly along the dorsal border of the pericardial cavity, where one or two small branches are given off, supplying the distal portion of the rectum. At the anterior edge of the adductor muscle, the posterior aorta gives off a large ventral branch that extends along the anterior edge of the muscle. This artery gives off a few small branches, the largest of which penetrates

the middle of the muscle, and then becomes lost in the muscle tissue along its ventral edge. The posterior aorta then continues as a rather small artery to the catch muscle, where it branches once.

Blood from the digestive tract, palps, and digestive diverticula is carried into the median branchial vein by means of large sinuses. This medial vessel is the largest of three large vessels that run along the gills, each one running parallel to a point of fusion of the gills. Blood from the gonads, adductor muscle, and rectum, after being collected in sinuses, passes first into the excretory tubules, which empty finallyinto the median branchial vein, and from there directly to the auricle through the two lateral vessels (Elsey, 1935).

In the gills, blood from the medial branchial vein must first pass through the gill lamellae before it can be taken up by the two lateral branchial vessels. The lateral branchial vessels are directly connected to the auricles by a branchial vein. In addition, there is still another pair of vessels run-

ning parallel to the fusion of the two outer demibranchs and the mantle. These two are connected to the accessory hearts by means of a small vessel at their anterior end. They are also continuous with the auricles by means of the pallial vein, so that blood from the accessory hearts goes through the two outer demibranchs and then into the auricles through the pallial vein. It is interesting to note that many of the blood sinuses in the gills end as blind sacs (Elsey, 1935). "At the level of each interfilamentar shelf, the vertical vessels communicate with lacunae, which in turn open into the tubes of the ordinary and transitional filaments. The filaments end blindly at both ends. Consequently all of the blood which enters the interfilamentar tissue from the vertical vessel must return by the same path." (Elsey, 1935) Exactly how this might be accomplished with pressure forcing the blood cells in one direction is not quite clear to the writer, unless the blood cells go through the interfilamentar tissue and then return through a different vessel. In so far as the blood cells exhibit no dislike for going through walls, it appears rather log-

ical to expect them to go through the interfilamentar tissue instead of resisting a steady pressure and reversing their path. The vertical vessels of the gills are connected by sinuses in the interlamellar tissue, so that blood vessels of the two lamellae of one demibranch are connected. It seems obvious, after the writer's cursory observations on O. virginica and after reviewing the excellent work by Elsey (1935) on O. gigas and O. lurida, that the circulation of the blood between the visceral mass, the mantle, and the gills is by no means definitely established. There are still a few points that are confusing. For example, the writer finds from his observations on O. virginica that the branches from the median branchial vessel usually extend into the inner lamellae of the two inner demibranchs. The two lateral branchial vessels apparently receive branches from the outer lamellae of the two inner demibranchs. Although no difinite connections between the two lamellae were demonstrated with any degree of regularity, it is logical to expect that they should be there so that the blood from the inner demibranchs would pass

into the lateral branchial vessels and then be carried to the heart. This should be investigated in greater detail.

The circulation of blood in the oyster is briefly this: The anterior aorta supplies the visceral mass, palps, mantle, and rectum with blood, while the posterior aorta pumps blood to the muscle and, to some extent, to the rectum. Blood from the deeper portions of the visceral mass and the muscle is taken to the median branchial vessel, while the blood from the gonads and the outer portion of the visceral mass passes first to the renal organ and then to the median branchial vessel. Blood from the pallium is pumped into the vessels of the outer lamellae of the two outer demibranchs and then to the pallial vein. In the gills, the blood from the median branchial vessel is passed through the gill lamellae and then into the two lateral branchial vessels. The two lateral branchial vessels empty into the pallial vein, which in turn empties into the auricles.

EXCRETORY AND REPRODUCTIVE SYSTEMS

The excretory system consists of a paired structure, the organ of Bojanus (P.P.C. Hoek, 1882) or as it has more recently been termed, the renal organ (Orton, 1937). It consists of two sets of branching tubules on the right and left sides of the visceral mass, emptying into a larger tubule on the ventral edge of the adductor muscle (fig. 3). There are also a few branches emptying into the main duct from the posterior border of the catch muscle (Orton, 1937 on O. edulis). Numerous blood sinuses in the visceral mass empty into the renal organ throughout its entire anterior portion. Posteriorly, the renal organ connects with the median pallial vein.

P.P.C. Hoek (1882) described a urogenital opening on the anal process, into which both the renal organ and the gonad ducts empty. Such a structure was not observed by the writer on O. virginica. The genital duct, was however seen to be in communication with the left suprabranchial chamber.

The genital organs are paired structures, one on the right and one on the left side of the visceral mass. They are situated just medial to the renal tubules. The two parts are connected by numerous anastomosing and branching tubules that increase in complexity as they approach the posterioventral portion of the animal (Bourne, 1889-90). The genital organs of both male and female are identical, consisting of two main ducts collecting from a series of small, branching tubules. The terminal ends of these tubules are enlarged during the breeding season to form lobes of gonadal structures. The active proliferation of the sperm and the egg cells occurs at these terminal portions. At the points where the gonadal cells are being formed, there is no epithelial wall as there is in the actual gonad ducts. The condition of these tubules is very similar to that found in the mammalian testis. The portion actively proliferating the sexual gametes has no epithelial wall other than the spermatagonia or oögonia. In the oyster, however, there very often is a portion of the tubule, near one of the ducts, that has ciliated cuboidal epithelium on one side and the

germinal epithelium on the other. At no point along their course do the gonad ducts extend into the deep tissue of the visceral mass. They are situated just beneath the surface, medial to the renal tubules, and lateral to the lobes containing the gonad cells. The gonad ducts and lobes extend posteriorly into the oral process.

NERVOUS SYSTEM

The nervous system of the oyster consists of two large paired ganglia; the cerebral, situated ventral to the esophagus, and the visceral ganglia between the muscle and the oral process. These two ganglia are connected by two commissural fibers, the cerebro-visceral connectives (Orton, 1935). The cerebral ganglia supply the visceral mass with numerous small branches, and give off the two large circumpallial nerves that follow the circumpallial arteries. The nerve along the posterio-ventral border of the catch and adductor muscles is a branch of the visceral ganglia. The visceral ganglion also gives off a large branch that passes laterally to the oral process and extends to the gills. Here it forms a large nerve trunk running parallel to the median branchial blood vessel. In addition, there is a large nerve that extends along the dorsal border of the visceral mass. Its origin was not definitely ascertained, but it appears to be a branch of the visceral ganglia.

The nervous system of the oyster is not

very complex, but it is rather difficult to demonstrate. Neither nitric acid nor chloral hydrate-glycerin-haematoxylin penetrated deeply enouth to show all the nerve bundles. Since other authors give only a limited description of the nervous system, apparently they encountered the same difficulty. A more detailed description is needed, but it is dependent upon the development of a suitable technique for staining nervous tissue fibers in toto.

PART V

HISTOLOGY

PALLIUM

Excellent accounts of the histology of the pallium have been published by Rawity (1888) on O. edulis, Kellogg (1892) on O. virginica, Leenhardt (1926) on Gryphea angulata, Elsey (1935) on Ostrea lurida and Ostrea gigas, and Nelson (1938) on O. virginica. Little further information can be added to these papers, but a brief resume will be made of the microscopic anatomy of the pallial lobes.

The major portion of the pallium consists of a mixture of loose vesicular connective tissue, interspersed with blood vessels, muscle fibers, and nerves. Most of the blood vessels are branches of the circumpallial artery, while at the distal edge of the pallium, the nerves are branches of the circumpallial nerve. The other pallial nerves, of which there are four pairs, are branches of the visceral ganglia. Branches of the circumpallial nerve can be seen extending to the tips of the larger tentacles (Elsey, 1935). Kogita (1932) found five pairs of pallial nerves branching from the visceral ganglia of O. circumpicta. Elsey (1935)

found only four pairs supplying the pallial lobes of O. lurida and O. gigas. These nerves branch repeatedly, their branches anastamosing with each other, forming a network of nerve fibers. At their distal ends, they join to the circumpallial nerve. This is continuous around the free edge of the mantle (Elsey, 1935). Muscle cells can be seen to run radially, transversely, and concentrically. The concentric muscle is confined to the edge of the pallium and runs parallel to it. The transverse muscle runs through the connective tissue, apparently connecting the two opposite surfaces of the pallial lobe. The radial muscle has its origin on the ventral border of the adductor muscle and is inserted at the distal edge of the pallium (fig. 12). About midway along its course it branches repeatedly, one series of branches going to the lateral surface, and the other going to the medial surface (Nelson, 1938). Large groups of muscle fibers can be seen to have a large nerve imbedded in their mass. The muscle fiber bundle is enclosed in a large blood sinus (Elsey, 1935). The ridges seen on the mantle are caused by these blood sinuses and groups

of muscle fibers (Rawitz, 1888).

The pallium of the young oyster is more highly vascular than that of an adult (Elsey, 1935). Furthermore, in an oyster about two centimeters long, the right pallial lobe is very much more vascular than that of the left. Since young oysters are known to use their pallial lobes as respiratory organs (Hopkins, 1934; Elsey, 1935), this fact seems to bear out Nelson's (1938) contention that the promyal chamber receives a large part of the water passing through the oyster.

The epithelium of the lateral surface of the pallium is divided into two regions. In the portion nearest the distal edge, the epithelium is heavily ciliated and thrown into a series of folds (fig. 15). Numerous mucous gland cells are present (fig. 13). The epithelium of the lateral surface, nearer the median portion of the animal is somewhat higher and thrown into a great many very small folds. The epithelium has a slightly cuticular surface and a faint basement membrane. It rests on a layer of elastin-like connective tissue, under which is a layer of longitudinal muscle. The layer of con-

nective tissue is heavier near the central portion of the pallial lobe.

The epithelium of the medial surface has a very different appearance from that of the lateral area (fig. 14). It is folded into a series of very small troughs or ridges. These run concentrically around the pallium and constitute a series of small channels running parallel to the inner rejection trough, a small shelf of tissue just at the base of the pallial curtain. The epithelium at the proximal border shows low columnar cells. These get smaller distally, finally becoming cuboidal on the pallial curtain. The medial epithelium shows fewer mucous cells than the outer epithelium except in the region of the palps. Here there is a wealth of mucous cells, rivalling that of the outer pallial surface. The epithelium has a cuticle and basement membrane, and rests on a thin layer of connective tissue.

The epithelium of the medial surface runs into the pallial curtain. On the inner surface of the pallial curtain, the epithelium becomes a low columnar to cuboidal. The cilia are much longer

than those on the rest of the pallium. Mucous gland cells are numerous, both in the epithelium and in the connective tissue just beneath the basement membrane. The epithelium on the outer surface of the pallial curtain is not so heavily ciliated and does not contain as many mucous gland cells as does the inner surface. At the base of the pallial curtain, on its outer side, there is a large gland, probably mucous-secreting in function (Nelson, 1938). The cells referred to as mucous gland cells stain a brilliant red with mucicarmine (fig. 13), showing that mucin is definitely their secretion.

GILLS

The microscopical structure of the gills has been and is a favorite subject for investigation. This structure, more than any other in the oyster, has been often and completely described; so much so that there has been much repetition of material. In order to make this paper a complete summary, a little further repetition will be necessary. More complete details of gill structure in different species will be found by reference to Kellogg (1892), Ridewood (1903), Yonge (1926-b), Galtsoff (1928), and Elsey (1935).

Histologically, the gills are composed of three parts: the outside epithelium, the supporting tissue (connective tissue, chitinous rods, blood vessels, etc.), and the inner lining of the water tubes (fig. 16). The outside epithelium is composed of columnar cells from 15 to 20 micra in height and 4 to 6 micra in width. It covers completely the outside surface of the gills. There are three distinct types of filaments, (folds involving the epithelium and connective tissue) found on each plica: the

principal, transitional, and ordinary filaments (figs. 17 and 18). There are usually two transitional and nine ordinary filaments per plica in O. virginica. The grooved principal filament is situated between adjacent plicae. Ridewood (1903) reports 9 to 12 filaments for O. edulis, while Elsey (1935) reports 11 to 17 in O. gigas and 4 to 10 in O. lurida. O. virginica was seen to vary from 9 to 12 filaments per plica, but the majority of plicae had 11. The principal filament in O. virginica is broad and flat, usually about 100 micra wide and not more than 30 micra high. This condition is similar to that found in O. gigas (Elsey, 1935), but differs vastly from the condition in O. edulis (Yonge, 1926-b and Ridewood, 1903). In O. edulis, the principal filament is very tall, and not short as in O. virginica and O. gigas. In O. virginica it is characterized by a pronounced groove in its center. The whole surface is heavily ciliated. The cells have rougly spherical nuclei which rarely exceed 10 micra in diameter. The chitinous bars just under the epithelium of the principal filament are short, thick, and triangular in shape. Muscle fibers run

between the two chitinous bars, between the bars of the principal and transitional filaments, and occasionally, from the bars into the underlying vesicular connective tissue. Just beneath the principal filament, bordering the water tube, or imbedded in the connective tissue, there is, sometimes, a small bundle of vertical muscle (Yonge, 1926-b). There are also chitinous bars extending from the principal filament to the transitional filament, termed by Elsey (1935) "skeletal cross-bar." Similar cross-bars can be observed between opposing lamellae of adjacent demibranchs.

Two zones of cilia are visible on the principal filament: the frontal cilia, in the region of the frontal groove, and the lateral cilia at the edges of the filament. With the exception of the small cells constituting the grooves of the filament, the epithelium is from 15 to 16 micra in height. No secretory cells were observed. Beneath the chitinous rods and the epithelium, there is no visible layer of connective tissue fibers. Instead, the chitinous rods are imbedded directly in the

vesicular connective tissue. They apparently take the place of the connective tissue fibers usually found beneath the epithelium.

The transitional filaments, the filament adjacent to the principal filaments, are long and triangular (fig. 18). They are normally as long as their width at the base, usually about 60 micra. Their chitinous rods vary from 30 to 60 micra in length and from 5 to 10 micra in thickness. Muscle fibers connect the two rods to each other and to rods of the adjacent filaments. The epithelium varies in height from 10 micra at the base of the filament to 20 micra at the apex. Frontal cilia and lateral cilia (fig. 46), are very short, rarely exceeding 5 micra in length, although some observed were almost 8 micra long. Mucous gland cells rarely occur on the transitional filaments.

The ordinary filaments, making up the rest of the plica (fig. 18), are about the same length as the transitional filament, but a little narrower. There is a slight tendency for these filaments to become slightly elongated toward the apex of the plica, but this variation, if present, rarely ex-

ceeds 10 micra. The apical ordinary filament, unlike the others, is considerably broader at its apex than at its base. This gives it a club-shaped appearance, its base being 30 micra wide, while its apex is often 80 micra wide (fig. 18). The epithelium of the ordinary filaments is similar to that of the transitional filaments. The chitinous rods are very slender, always under 4 micra wide. In addition to the frontal and the lateral cilia, the latero-frontal cilia along the angular portion of the filament can be distinguished (Ridewood, 1903). The cilia are short, usually considerably under 10 micra in length. Mucous glands are numerous at the apices of the ordinary filaments, but more prevalent on those filaments at the apex of the plica.

Between the filaments, there are numerous openings, the ostia (fig. 18), leading into a small tube within the filament. This tube is lined with simple cuboidal epithelium. This occasionally appears to be ciliated, but no definite ciliation could be demonstrated with any degree of certainty. The tube leads into a water tube between the gill lamellae. Here again, the epithelium is simple cuboidal and in

isolated spots, apparently, ciliated. Ridewood (1903) and Yonge (1926-b) reported abfrontal cilia in the epithelium of the water tube. These cilia are found opposite the principal filaments. They are probably the structures that cause the ciliated appearance at various points. If there are cilia in O. virginica, they are small, not over 5 micra in height.

PALPS

The histology of the palps, although relatively simple, has never been described for O. virginica. Yonge (1926-b) described it in O. edulis. The two are very similar. There are two surfaces to the palp, differing from each other, morphologically and histologically. The inner surface consists of a series of radiating folds that appear as faint striations to the naked eye. In O. virginica, the aborad side of these folds have small microscopic channels running concentrically, with the base of the palps as the center and the larger folds as radii (fig. 19). These channels, often as much as 60 micra deep, are formed by groups of columnar cells that are lower than those surrounding the excavations. The channels are deeper and more numerous as the folds approach the mouth. (Yonge (1926-b) reports one such channel per fold in O. edulis, but, in O. virginica there are often two and occasionally three on a single fold. The large folds lean in the direction of the mouth. The epithelium on the oral side is fairly regular, ranging from 30 to 40 micra in height.

The cells are approximately 3 to 4 micra wide. At the base of the fold, the epithelium becomes somewhat lower and is often less than 15 micra in height. The nuclei range from spherical to ellipsoidal and are seldom over 8 micra long. Cilia are small and numerous, ranging from 10 to 15 micra in length. On the aborad side and the apex of the fold, the cells, excluding those in the pits of the epithelium, are varying in height, ranging from 40 to almost 80 micra. In the epithelial pits, the height is often less than 20 micra. Nuclei and cell width are similar to that of the orad side, but the cilia are much longer, often 40 micra in length. The epithelium on both sides of the palps has a thin cuticle and basement membrane. It rests on an extremely thin layer of elastin-like connective tissue, which is interspersed with very delicate, spindle-shaped, smooth muscle cells. Yonge (1926-b) makes it a particular note (on O. edulis) "that there are no muscle cells within the folds such as could cause it to contract downwards." The author, however, observed numerous very slender, spindle-shaped cells with an elongated, finely chromatic nucleus. This was assumed to be

smooth muscle. These same cells are also found in almost all parts of the digestive tract. Just below the epithelium of the furrow between two folds is a small bundle of longitudinal smooth muscle (Yonge, 1926-b). Phagocytes are numerous, and mucous cells, although present, are sparse and scattered. Beneath the epithelium, the palps are made up of the typical loose, vesicular connective tissue found in the interlamellar tissue of the gills and in many other regions of the oyster.

The outer surface of the palps is very different from the inner surface. No folds are present, the cilia are shorter, and the cills are smaller and more regular, with an occasional variation in cell height (fig. 20). The cells range from 18 to 20 micra high and from 3 to 4 micra wide. Nuclei are oval and about 4 micra long. Cilia are very delicate and somewhat under 5 micra in height. Their presence was doublted at one time, until Yonge (1926-b) demonstrated their existence on living material. A slight cuticle and basement membrane are visible. The epithelium rests on a layer of elastic connective tissue

fibers. Beneath and running through this layer, is a layer of longitudinal smooth muscle (Yonge, 1926-b). Underneath this is the vesicular connective tissue described above. Mucous cells in the epithelium are extremely numerous, more so than in any other region of the body.

DIGESTIVE TRACT

The epithelium of the digestive tract is typical, there being no similar structure at any other place in the animal. The cells are, without exception, tall columnar, being very much taller than they are wide. All cells, including those under the gastric shield, are ciliated and have a more or less cuticular distal surface. The epithelium rests on a layer of connective tissue that may or may not be interspersed with smooth muscle cells. Throughout most of the epithelium, it is possible to see numerous phagocytes in the act of going through the epithelial wall, either to or from the lumen (fig. 23).

The mouth in Ostrea virginica differs somewhat from O. edulis in that it is continuous with the cavities between the palps for a short distance. At the point where it ceases to be continuous it leads into a slit-like esophagus. There is a definite break in the epithelium at the junction of the mouth and palp surfaces (fig. 21). The epithelium of the

palps is not so columnar as that of the mouth and is thrown into numerous folds and ridges. In addition to this differentiation, the two surfaces of the mouth differ slightly. The portion continuous with the medial surface of the inner palp is sparsely ciliated in the region adjacent to the palp. The ciliation of this surface becomes heavier, and the cilia much longer toward the median portion of the mouth. The cilia of the latter portion are very long, ranging from 75 to 100 micra in length, while the cilia in the region nearest the inner palps are only 30 to 50 micra in length. The ciliation of the opposite side of the mouth is exactly the reverse. At the junction of the mouth and the outer palps, the cilia range from 80 to 100 micra in length, while in the median dorsal region of the mouth they are very sparse and somewhat under 60 micra long. The mouth being continuous with the cavities between both palps, any reference to its proximity to a palp surface, means both the left and right palps. Apparently, although this has not been determined experimentally, the lateral cilia in region near the palps beat toward the median portion of the mouth.

Here, the cilia beat in the direction of the esophagus, thus establishing a current directly to the digestive tract.

From a cursory examination, the epithelium of the mouth, as most of the epithelium of the digestive tract, appears to be pseudostratified (figs. 21 and 22). This, however, is not the case. The cells are so thin that it is almost impossible to obtain a section where there is only one layer. The epithelium is actually composed of simple columnar cells, some of which are so narrow that there is a definite bulge in the region of the nucleus (fig. 23). Because of this, the nuclei of adjacent cells are seldom at exactly the same level, usually being staggered, thus enhancing their pseudostratified appearance.

The epithelium of the mouth adjacent to the outer palps, at the point where ciliation is the heaviest, is composed of tall comummar cells (fig. 21), ranging from 80 to 100 micra high and about 3 micra wide. The nuclei of these cells stain darkly with haematoxylin and appear to be composed of very fine chromatin granules. Nothing that could possibly be identified as a nucleolus was visible, although many

of the nuclei had a clump of chromatin that strongly suggested such a structure. The nuclei are ellipsoidal in shape and vary somewhat in size. The average, however, is about three micra wide and six micra long. The nuclei are usually situated in the proximal two-thirds of the epithelium, at varying levels. As the epithelium of this, the dorsal side of the mouth (portion continuous with outer palps), approaches the medial region, it becomes considerably lower, measuring only about 30 to 50 micra. It is at this point that the cilia on this side of the mouth are the smallest. These measurements were taken at the anterior end of the mouth. It was found that as the mouth approached the esophagus, the epithelial height and the cilia length of the median and lateral portions became more nearly equal in size.

Referring again to the anterior region of the mouth, the portion continuous with the two inner palps (ventral portion) is found to be composed of a fairly uniform epithelium, about the same size as the opposite side at its highest point (fig. 21). There are regions where the epithelium dips down due to clusters of smaller cells. These small cup-like

excavations do not affect the basement membrane and are not folds. They are scattered throughout the epithelium at irregular intervals, on both the dorsal and ventral sides of the mouth.

In all regions of the mouth, the epithelium has a cuticular structure at its distal border and a definite basement membrane resting on a layer of connective tissue fibers. These fibers somewhat resemble the elastic fibers found in mammalian tissue. R. A. Carsley, another investigator in this laboratory, is at present investigating their structure in more detail. Smooth muscle cells are scattered throughout this connective tissue layer, usually running in a circular fashion, as does the connective tissue fibers. Occasionally, one of the connective tissue fibers can be seen to extend into the epithelial layer and apparently anchor itself between the cell membranes of two adjacent cells.

At the distal end of the cells, the basal filaments of the cilia extend from the blethroplasts giving the cells the appearance of having a striated border (fig. 22). The basal filaments come together to form a thick, thread-like structure a short dis-

tance from the cuticular border. Thus, there is formed a fan-shaped structure of cytoplasmic fibrillae with the wider portion starting at the blethroplasts, just under the cuticle, and terminating a few micra distad of the cuticle. The thread formed by these filaments extends down to the nucleus and appears to coursd along the edge of the nuclear membrane. It extends deep into the cell, terminating in a small fan-shaped ciliary root extending into the basment membrane. The cilia are long and slender, tending to clump together after fixation. This gives the appearance of a flagellum rather than a row of cilia (figs. 22 and 23).

Scattered throughout the epithelium are a few large granular cells, apparently secretory in nature. These granular cells stain a bright red with eosin and correspond to the eosinophilous cells of Herdmann and Boyce (1899). The exact function of these cells is not definitely understood. Their structure, and possible function will be discussed later in this paper.

The epithelium of the esophagus is composed of alternate groups of high and low cells (fig.

24). The wavy outline of the peripheral edge is independent of the basement membrane. This condition is very similar to that seen in the mantle (Nelson, 1938), but the excavations are far more numerous (fig. 24). This differs considerably from the condition in O. edulis (Yonge, 1926-b) where the lumen of the esophagus is perfectly smooth and lacks the crypt-like excavations found in O. virginica. O. virginica possesses a prominent basement membrane resting on a layer of connective tissue fibers interspersed with both longitudinal and circular muscle. Yonge (1926-b) makes no mention of such a connective tissue layer in O. edulis.

The cells in the epithelial excavations range from 45 to 60 micra in height, while the rest of the cells are from 60 to 100 micra high. The cells in both cases average from 3 to 5 micra in width, at their widest point. The shape of the nuclei varies considerably, ranging from a small round structure about 3 micra in diameter to a large ellipsoidal structure almost 8 micra long. They are situated in the lower half of the epithelium (fig. 25), leaving the peripheral portion clear and hyalin.

The cilia are very similar in structure to those found in the region of the mouth. They are, however, considerably smaller, being approximately 30 micra long. The ciliary mechanism is identical. The basal filaments and blethroplasts although not so pronounced, occupy the same position in the cell. The free surface of the epithelium shows little evidence of any modification, although Yonge (1926-b) described a cuticular surface for all the ciliated cells in O. edulis.

Granular secretory cells are present in the esophagus, but not in any great quantity. There are, however, numerous goblet-like cells that appear to be either vacuolated or devoid of their secretion (fig. 25). Similar cells are described by Gutheil (1912) on Anodonta cellensis and Yonge (1926-b) on Ostrea edulis. In Ostrea virginica these goblet-like cells seem to be more prevalent in the excavations of the epithelium, although a great many are found among the taller cells. These cells resemble very closely the goblet cells found in the rectum of mammals. Some of the secretory cells have bright red granules, while others have dark brown or black

basophilic granules. Yonge (1926-b) found that these are merely different stages in the same type of cell.

In the region of the stomach, there are two distinct epithelial types. One is found just underneath the gastric shield, while the other is found surrounding the rest of the lumen. The typical epithelium of the stomach is uniform and regular, about 50 to 60 micra in height and about 4 to 5 micra thick (figs. 26 and 27). The cells have a very definite cuticular border approximately 3 micra thick. The nuclei are ellipsoidal and situated approximately in the center of the cell. In O. virginica they are not in a straight line as Yonge (1926-b) illustrated them in O. edulis. Possibly the best way to describe them is to say that they are clustered or bunched in a straight line in the middle of the epithelium (figs. 26 and 27). The cilia are short and slender, being about 20 to 25 micra in length. The ciliary mechanism, although not so clearly visible as that of the mouth and the esophagus, is very similar. A definite basement membrane is present, resting on a layer of elastin-like connective tissue fibers. This layer of connective tissue is interspers-

ed with circular muscle cells. A similar observation is made by Yonge (1926-b) on O. edulis. The layer of connective tissue fibers is variable. At some points it is so thin as to be almost negligible, and at other places it is almost 10 micra thick. No definite distribution of it was ascertained, except that it is usually thickest under the gastric shield.

The epithelium under the gastric shield is very different from that described above. The cells are large, usually over a hundred micra in height, and very narrow, seldom over 2 to 3 micra thick (fig. 28). The nuclei, apparently following the shape of the cell, are about 3 micra wide and almost 12 micra long. The cytoplasm of the cell is much clearer (almost hyalin in nature) than that of the adjoining epithelium. There is a cuticle and a basement membrane present, as in the other epithelium. In addition, in O. virginica there are cilia. Yonge (1926-b) noted the absence of cilia under the gastric shield in O. edulis. Instead, he described "fine strands having the appearance of cilia and arising from basal granules at the edge of the cells."

These fine strands are described as connecting the epithelium to the gastric shield. In O. virginica, there appear to be definite cilia, imbedded in the laminated gastric shield. The cilia are very long, extending into the shield for quite a distance. Berkeley (1935) found that the gastric shield is composed of chitin. If such is the case, it is apparent that the chitinous substance is secreted by the elongated cells and imbeds all the cilia in that region. The imbedding of the cilia acts as a means of anchoring the shield to the epithelium. The space between the gastric shield and the epithelium (fig. 28) is about 2 micra thick. This space might possibly be due to cytoplasmic shrinkage caused by fixation, but measurements were made on tissue fixed in Bouin's fluid, a fixative that gives a minimum of shrinkage in oyster tissue. The cilia seen in this space are clumped in exactly the same fashion that we find those of the epithelium previously described.

Scattered throughout the epithelium are

numerous eosinophilic secretory cells. Yonge (1926) described these as mucous cells, but they do not stain either with mucicarmine or thionin, two stains specific for mucin. With mucicarmine, they exhibit a slight reaction, turning slightly pinkish, but not enouth to consider the results valid. Herdmann and Boyce (1899), taking another side of the issue, described a similar cell in the epithelium of the mantle as a wandering cell. These cells in the mantle and the gills give a very definite reaction with mucicarmine, staining a bright red, showing that they contain mucin. Some of the eosinophilous cells in the stomach epithelium can be seen to protrude a little beyond the cuticle, thus giving the impression that they are going through the epithelium. On the other hand, most of the evidence points to the fact that they are stationary elements of the epithelium and not wandering cells. First, no nucleus can be seen in the granular portion of the cell. Second, there are no cilia visible in such a cell, pointing to the fact that it is modified for a definite purpose. Third, many of these cells can be observed actively pouring

their secretion into the lumen. The last, and possibly the most conclusive evidence is the distribution of these cells. They are found in definite parts of the digestive tract, and not found in others. In the stomach, they are found in all parts of the epithelium, except under the gastric shield usually appearing in groups.

The epithelium of the food sorting caecum is similar to that of the stomach. It consists of tall columnar cells about 60 to 90 micra in length and not more than 4 to 5 micra wide. The nuclei, situated approximately in the middle of the cell, as in the stomach, are ellipsoidal. The cilia in the caecum are small, ranging from 10 to 15 micra in length. The cuticle is even more definite than in the stomach, appearing as a faintly striated border, just distad to the blethroplasts, about 3 micra thick. Scattered throughout the epithelium are many eosinophilic, a few basophilic, and no vesicular secretory cells. As in the stomach, these secretory cells tend to appear in clumps.

Extending from the stomach are two large

ducts that communicate with the digestive diverticula by means of a series of smaller branching tubules (fig. 29). The epithelium of the two large ducts is identical to that of the stomach. As the large ducts branch a few times, changes begin to appear in the epithelium. In the larger tubules, little difference can be noted, except a shortening of the cilia and either the absence of or scarcity of smooth muscle cells. In such a tubule, the epithelium is under 60 micra high and about 4 to 5 micra wide. The nuclei are jammed together in the proximal third of the cell layer. They are not, however, so close together as they usually are in the stomach. Cilia about 10 micra long extend through a cuticle about 2 micra thick. The lumen is regular without any excavations in the epithelium. In cross-section it appears round. Numerous secretory gland cells can be found scattered throughout the epithelium.

Following the tubule, until it branches several times, other changes are seen to take place. The most conspicuous of these is the size of the cells. The height of the epithelium has decreased,

until it now averages approximately 30 micra in height. The cuticle remains constant in thickness at 2 micra, while the cilia remain at 10 micra in length. The nuclei are round to ovoid, being usually about 4 micra in diameter and have a very prominent nucleolus. They are situated a little distad from the basement membrane and are arranged in a straight line. The basement membrane is not very prominent and rests on a thin layer of connective tissue fibers. No muscle cells can be observed in this layer of connective tissue. Sprinkled throughout the epithelium of all the tubules are a great many eosinophilic, granular, secretory cells. They are very large, often over 20 micra long and 10 micra wide. In a few instances, the secretory portion can be seen to have pushed a round, darkly stained nucleus to the bottom of the cell. There are occasionally empty goblet cells, similar to those described in connection with the esophagus. There are few, if any, basophilic granular secretory cells.

At the ends of these tubules are the digestive diverticula (fig. 29), that constitute the functional part of the whole structure (Yonge, 1926-

a and 1926-b). The lumen of the diverticula have a rather singular appearance, being shaped somewhat like an "X" or a "T". This shape is caused by the distribution of two different types of cells. There are large clear cells with oval granules, staining yellowish with orange G, and smaller, darker cells that have a slight tendency to take basic stains. Both cells contain numerous vacuoles. The large cells, termed the "old" cells, make up the high points of the epithelium, while the smaller, darkly staining "young" cells are found in the crypts of the epithelium (Yonge, 1926-a and 1926-b). There are four large crypts and four high spots in each of the tubules, giving it an "X"-shape in cross-section. The basement membrane does not follow the outline of the epithelium, but is almost round (fig. 29). The cells have no cuticle, but occasionally, one has the appearance of being ciliated. Yonge (1926-b) found that no cilia can be seen in sections of the digestive diverticula. Similar observations were made by Carrazzi (1896, 1897), MacMunn (1900) and Vonk (1924). Both Potts (1923) and Yonge (1926)

have observed the beating of cilia in other lamellibranchs and since retractile cilia have been observed in Ostrea and other related genera, Yonge (1926-b) concluded that retractile cilia are found in the digestive diverticula of all lamellibranchs. These digestive diverticula are imbedded in a loose, vesicular connective tissue, the properties of which are being further investigated by workers in this laboratory.

Upon staining the visceral mass with Bauer-Feulgen technique (Bensley, 1939), a modification of Schiff's reagent, it was found that not only did the vesicular connective tissue surrounding the tubules contain glycogen (figs. 30, 31, 32, and 34), but also the tubules themselves (fig. 33). The glycogen was present in both the diverticula and tubules. It appears in small thin pockets usually at the periphery of the cells. Glycogen pockets are more prevalent in the larger cells, the so-called "old cells" (Yonge, 1926-b). Yonge (1926-b), using the iodine method for the detection of glycogen, reported no glycogen in the epithelium in O. edulis. It was found in this laboratory that io-

dine gave negative results in cases where Bauer-Feulgen would give positive tests. Bensley (1939) suggests Bauer-Feulgen stain as superior to either Best's carmine or iodine. In addition, the fixative used was one that would allow the least glycogenolysis to take place. It is altogether possible, after considering all these factors, that the test used by Yonge in 1926 was not quite sensitive enough. When a saliva digested control was stained, it was found that no red staining substance was present in the epithelium. Instead, there was a red substance in the lumen of the tubules (fig. 31). The former showed a confirmatory test for glycogen, while the latter demonstrated a substance, the chemical composition of which the writer does not know. Although a red stain was demonstrated in the tubules before salivary digestion, it was not nearly so definite nor so profuse as that material stained after digestion. The Bauer-Feulgen technique is essentially a modification of the Schiff reagent for aldehyde tests. It was at first thought that the saliva was digesting the plant material in the tubules to an aldose sugar, but the Schiff reagent

does not react with sugars. It does however, react with amino acids, phenols, and a few other compounds. Apparently, there is some smino-carbohydrate (glucosamine, aminohexose, or some similar compound) that is present in the tubules. It is possible that the ptyalin, present in the saliva, hydrolyzes the compound, freeing the amino group from the carbohydrate carbon chain, thus causing the color formation with Schiff's reagent.

The epithelium of the style sac has a very different appearance than the epithelium of the rest of the digestive tract (fig. 37). It is composed of very regular columnar cells, from 50 to 60 micra in height and 5 to 6 micra wide. The nuclei are oval shaped and about 4 micra wide. They are arranged in a comparatively straight line in approximately the middle of the cell (fig. 36). Nuclear and cell membranes are very delicate, as they are in the rest of the digestive tract. The cilia are short and stout, approximately 25 to 30 micra long on the sides of the style sac adjacent to the typhlosoles of the midgut. On the portion farthest from the midgut, the cilia are slightly lon-

ger, being from 30 to 50 micra in length. The epithelium has a very pronounced cuticle and basement membrane. It rests on a thin layer of connective tissue fibers. No muscle cells are visible at any point around the style sac. The epithelium of the style sac, although continuous with that of the midgut, is very dissimilar, and at the point where they join, there is only a slight transition, if any (fig. 39). The blethroplasts in the cells of the style sac instead of being in a straight line, as they are in the rest of the digestive tract (fig. 23), describe an arc at the distal end of the cell. In addition, these blethroplasts are over twice the size of any other similar structure in other regions of the digestive tract. The basal filaments are much thicker than those previously described and join to form a single filament much deeper in the cell. Aside from these few differences, the ciliary mechanism is essentially the same.

There are very few mucous cells in the style sac; so few indeed, that it was at first thought there were none at all present. However, upon staining

with mucicarmine, a great many of the cells took on a slightly pinkish hue, showing that mucous was probably present, but in very dilute quantities. Upon staining with Regaud's haematoxylin, following Regaud's fixative, numerous basophilic bodies are seen, not only in the cell, but also congregated in definite lines outside the cell, imbedded among the cilia. Although List (1902), Nelson (1918), Edmondson (1920), and Mackintosh (1925) are of the opinion that the major typhlosole secretes the style, this evidence of a secretion in the style sac itself seems to support the contention of Gutheil (1912), and Yonge (1926-b) that the style is secreted by the cells of the style sac. Furthermore, Berkely (1935) has shown that the style is composed of a mixture of chondrin and mucin, the proportions of these varying with the species. The fact that the cells of the style sac react positively to a specific mucin stain, without giving the typical mucous reaction seems further to prove Yonge's theory. Examing the other points of proof: Gutheil (1912) describes clear vesicular granules above the nuclei. Yonge (1926) demonstrated the secretory nature by

injecting iron saccharate into the muscle of the oyster and then staining with potassium ferrocyanide followed by hydrochloric acid. This showed very clearly the same granules observed by Gutheil (1912) and the writer. Another method used by Yonge (1926-a) was the injection of pilocarpine. This showed that the style sac had the only cells giving evidence of active secretion. Yonge (1926-b) also found, as was observed in O. virginica, that these granules are being actively passed into the lumen, and are confined to the region of the style sac. Viewing the evidence just presented, it appears likely that the epithelium of the style sac secretes the style, rather than the major typhlosole of the midgut.

At the base of the epithelium of the style sac, there are numerous round, lightly staining spaces, apparently in the cells themselves (fig. 38). The cell membranes are so delicate and indefinite, that it is extremely difficult to distinguish between something inside a cell and something between two cells. Inside of these canals is an area of lightly staining chromatin-like material, arranged in the form of a spireme (also described by Mack-

intosh, 1925, and Yonge, 1926). Mackintosh (1925) also described smaller radial, or more likely, circular canals connecting the larger longitudinal canals. The writer was unable to demonstrate such structures in O. virginica. Mackintosh (1925) suggested that these canals are similar to connective tissue and aid in strengthening the epithelium. The canals are also found to a lesser extent in the major and minor typhlosoles of the midgut, but soon disappear before they reach the gut itself. Phagocytes can also be seen in the epithelium of the style sac, but not in any large quantity.

The midgut is considered by Yonge (1926-b) and other contemporary investigators as the portion connected to the style sac, and the portion that loops around the stomach. It is proposed here, to consider as the midgut only the region that is continuous with the style sac (figs. 4 and 6). The part of the gut looping around the stomach will be termed the intestine, and its terminal portion will be considered the rectum (figs. 3, 4, and 6). The midgut can be further divided into the major and minor typhlosoles and the midgut proper. In order to

make the descriptions clearer, the portion of the midgut farthest from the style sac, referred to above as the "midgut proper", will be referred to as either the distal region of the midgut or the midgut proper. The epithelium of the two typhlosoles is slightly different from that of the distal region (fig. 40), and so the writer feels justified in making this distinction.

The epithelium of the typhlosoles consists of very tall columnar cells, usually well over 80 micra in height (fig. 41). The cells of the minor typhlosole are slightly smaller than those of the major typhlosole. The distal region of the midgut is composed of comparativelylow columnar cells, usually under 50 micra in height (fig. 40). In all cases, these cells range from three to four micra in width. The cilia are similar to those found in the stomach, and are 10 to 15 micra long. All cells have a thick cuticle and basement membrane. The epithelium rests on a thin layer of elastin-like connective tissue fibers. No muscle cells can be seen around the epithelium. Phagocytes are present in both the epithelium and the lumen. They are particularly abundant in the

typhlosoles. Mucous cells may sometimes be found in the epithelium of the distal region, but they are usually found in great abundance in the typhlosoles (figs. 40 and 41). Intra-epithelial canals are found in the typhlosoles of the midgut, but tend to disappear in the epithelium of the distal region. Numerous pits are present in the epithelium of the three portions of the midgut, usually more prevalent, however, in the minor typhlosole.

At the junction of the style sac and the midgut there is a definite break in the cilia from the long brush-like structure of the style sac to the smaller, thinner cilia of the midgut. The ciliary mechanism is the same as that found in the stomach.

Dorsally, the midgut appears as a small diverticulum of the style sac, but it increases in size, finally becoming as large as, and often larger than the style sac itself.

The epithelium of the intestine is composed of tall columnar cells about 60 micra in height and about 4 micra wide (figs. 42, 43, and 44). Numerous excavations can be found in the epithelium,

similar to those of the esophagus, but not nearly so numerous (fig 44). The lumen of the intestine has a distinctive shape due to the presence of the broad, grooved typhlosole. The lumen has the shape of a "3" (fig. 43). This is a distinct difference from the lumen of the rectum (fig. 46), which is not so flat. In addition, the lumen of the rectum is much larger than that of the intestine (figs. 42, 43, 46 and 47). Numerous secretory gland cells, the three types of which have been previously mentioned, can be found in the intestine. These are especially numerous in the groove of the typhlosole. Both cuticle and basement membrane are present. The epithelium rests on a rather heavy layer of connective tissue fibers. A few circular muscle fibers, are present. Phagocytes are numerous.

The epithelium of the rectum is more uniform and regular than that of the intestine (figs. 45 and 46), and a little lower, being somewhat under 60 micra in height. In the rectum, the folds of the typhlosole have come together making the medial groove deeper and less "V" shaped. The nuclei are

found bunched together in the proximal two-thirds of the epithelium. Three types of secretory cells are found. They are more numerous here than in any other region of the gut (Yonge, 1926-b). Phagocytes are present, both in the epithelium and in the lumen.

Throughout the digestive tract, in the various places noted, there are three types of secretory cells. These have been considered somewhat in detail at different places in the histological discussion, but the various authors' viewpoints are so confusing that further consideration seems justified. They have been termed mucous cells by some investigators (Yonge, 1926-b), and even considered to be "wandering cells" by others (Herdmann and Boyce, 1899). Their regular distribution in various parts of the gut points to the fact that they must serve a definite function as a part of the epithelium, but just what this function is, is unknown. Although Yonge (1926-b) designated them as mucous cells in O. edulis, similar structures in O. virginica show little evidence of a mucous secretion. They give a negative reaction with thionin and a very slight re-

action with mucicarmine. These facts would seem to indicate that they are not mucous secreting cells. As a matter of fact, while there is very little pointed evidence that they are secretory cells, these cells have been observed in the act of apparently pouring their secretion into the lumen of the gut. In addition, the cells in question very closely resemble the mucous gland cells of the pallial lobes and the gills. These stain a bright red with mucicarmine. Thionin was not used on these because of their positive reaction with mucicarmine, a specific mucous stain. Admittedly, this is flimsy evidence on which to base a conclusion, but it is the best available. Assuming then, that they are secretory cells, they may be divided into three definite types. One is empty and devoid of its contents. This resembles a typical mucous secreting cell, from which the contents have been removed. Secondly, there is a tear-shaped cell, filled with large red eosinophilic granules, usually about 1 to 2 micra in diameter. These granules stain with most acid stains, but most intensely with eosin. The third and last type is com-

posed of small darkly staining basophilic granules. These granules vary somewhat in size, often attaining the same size as the eosinophilic granules, averaging about 1 micron in diameter. The goblet and eosinophilic cells are about the same size and shape, often almost 30 micra long and 15 micra wide. The basophilic cells on the other hand, although usually as long or longer than the other two types, are seldom wider than 8 micra at their widest point, and often they are much smaller. Singularly enough, the size of the basophilic granules has a definite correlation with the size of the whole secretory cell, the larger the granules, the larger the cell. There are also numerous transitory stages between the three types. Some cells have red-brown granules of varying shades of color; still others appear to have lost some of their granules. All of which points to a conclusion that Yonge (1926-b) has deduced: that all three cells are different stages of the same type of cell. It appears that the cell starts as a long thin cell, extending almost to the basement membrane. The first granules secreted are small, darkly staining, and basophilic. As the granules become larger, so

does the cell. The larger granules tend to group themselves near the distal border of the epithelium, although sometimes the cell does extend through the whole height of the epithelium. As the granules become larger, the basophilic tendency becomes correspondingly reduced, until they finally are acidophilic. The next stage is the actual secretion of the granules. This leaves behind an empty, distended cell, corresponding to the goblet cell described above.

Assuming that the above explanation is correct, the question ofwhether these cells die after eliminating their secretion, or whether they return to normal epithelial cells, or whether they immediately form more granules still needs to be determined. Although Yonge (1926-b) assumes that the secretion is mucous, this point of view needs further confirmation.

VASCULAR SYSTEM

The heart consists of three chambers, two auricles and a ventricle. The ventricle is further divided, although incompletely, into two small chambers. Since the only histological difference between the auricles and ventricle is the thickness of the wall and the amount of muscle fibers (figs. 48 and 49), their microscopical structure will be discussed together. The heart consists essentially of an outer layer of simple columnar epithelium, a medial layer of muscle fibers and connective tissue, and an inner layer of thin endothelium-like cells.

The epithelial layer is thrown into a great many folds upon contraction of the muscle fibers, but when the heart is distended, the epithelium is smooth and regular. It consists of low columnar cells, 10 to 12 micra high and about 4 to 6 micra in width. The tendency of the auricles to show higher folds than the ventricle upon contraction is very pronounced. This is probably due to the thinner walls of the auricle. The nuclei of the epithelial cells are irregular in shape, ranging

from spherical to ellipsoidal, sometimes even being tear-shaped. They rarely are larger than 4 micra across their largest dimension. They are composed of fine chromatin with a prominent nucleolus. The epithelium has no cuticle and only a faint semblance of a basement membrane. It appears to rest directly upon the vesicular connective tissue. Kellogg (1892) described small cytoplasmic loops extending from the epithelium, but such structures were not visible on a heart fixed in an incompletely contracted state. In fact, it became apparent after studying a heart in the state of contraction, that the structure he observed was probably a cell forced into the condition he described by the forceful contraction of the muscle.

The muscle fibers in the heart run in almost every conceivable direction. No attempt was made to map the distribution or direction of the fibers, but they are so arranged that contraction of the muscle causes the heart to contract in three dimensions. The muscle fibers are imbedded in the connective tissue at either one or both ends. Those imbedded at only one end have their origin at the

base of the epithelium (fig. 50). The fibers are composed of anastamosing, multinucleated, striated cells (First described by Kellogg, 1892, in the auricle.). These cells range from 2 to 5 micra in width, and although their length could not be definitely ascertained, some were observed to be over 200 micra long. The striations are comparatively thick and close together, about 2 micra thick and about 2 micra apart (fig. 51). They are unlike mammalian tissue, consisting of a solid, darkly staining band instead of a series of thickenings on the myofibril. As a matter of fact, no myofibrils were visible, an observation that is difficult to explain. In addition, no glycogen could be demonstrated by either the Iodine or the Bauer-Feulgen technique. The nuclei of these cells are elongated and vesicular. They are situated in the center of the cell. In cross section, the muscle cells appear as hollow tubes, with the striations running around the periphery and the nucleus in the center. The nuclei are scattered and hard to find.

The endothelial layer consists of long flat cells. These are so thin that the cytoplasm ap-

pears to be an elongation of the two ends of the ellipsoidal nucleus. Rarely are these cells found to be continuous around the lumen. This fact leads the writer to believe that they possibly are not endothelial cells, but a modified connective tissue element. Of course, the very dimensions of the cells make them almost impossible to see, unless the section goes through the nucleus. This might account for their appearance of being non-continuous. However, whichever viewpoint is taken, the cells function as an endothelium. These same cells are also found in all arteries, rerely completely lining the lumen.

The blood channels may be placed in two classes, the arteries and the sinuses. The arteries, regardless of size, have definite walls (fig. 52), while the sinuses are merely spaces in the connective tissue. The major portion of the artery wall is composed of connective tissue fibers. Radiating from the fibers encircling the vessel are chords of connective tissue. These add strength and rigidity to the artery. Sprinkled through the circular connective tissue are

the same delicate spindle-shaped smooth muscle cells, described in connection with the palps. Lining the lumen are the endothelial cells. These, as stated before, rarely can be seen completely encircling the lumen. Occassionally, one of these cells appears to extend into the connective tissue layer. Their nuclei are about 5 micra long and less than 3 micra wide and stain heavily with haematoxylin. The cells at their widest point are no wider than the nucleus, at which point they seem to bulge in order to make room for it. They are tapered at both ends and extend from 11 to 15 micra in length.

The sinuses have no definite connective tissue. wall. They are merely spaces in the connective tissue. Occasionally, one of the lining cells, similar to those of the arteries can be seen. Sinuses are as large as the arteries, and often larger.

URINARY SYSTEM

The renal organ of the oyster consists of a series of coiled branching tubules, the histology of which is comparatively simple. The epithelium, making up these tubules is composed of very low columnar, almost cuboidal cells about 15 micra high and from 8 to 10 micra wide. The distal half of the cells are vesicular, giving them the appearance of being secretory in nature. The cytoplasm, encasing a large oval, vesicular nucleus, is contained in the proximal portion of the cell (fig. 53). The cells are irregular in shape, giveing the epithelium a hilly appearance. In the larger tubules, the epithelium may be as high as 30 or even 40 micra. In such cases, the nuclei are situated in the lower third of the cell, the peripheral two-thirds being vesicular. The proximal border of the cell is rounded and not like anything heretofore described. No cuticular structure is visible, but there is a definite basement membrane. The cells are non-ciliated. The epithelial layer of the smaller tubules rests on a thin layer of smooth muscle. In the larger

tubules, there is also a layer of connective tissue fibers. Numerous eosinophilic secretory cells are present, apparently confined to the larger tubules.

GENITAL DUCTS

Any attempt to describe the germinal epithelium would involve considerable investigation of oogenesis and spermatogenesis; so the following description will be confined to the ducts themselves.

The terminal ends of the ducts are characterized by two types of epithelium, the germinal epithelium and the epithelium of the genital duct. The genital ducts at their extreme ends are merely spaces in the connective tissue, lined with connective tissue fibers. These run into larger ducts that possess a definite epithelial lining. Still further ventrally, the connective tissue tubules, still partially composed of germinal epithelium, possess a layer of simple cuboidal cells (fig. 54). The cells constituting this layer are about 5 micra long with cilia almost the same length. Small spherical nuclei are found in approximately the middle of the cell. These stain intensely with haematoxylin and other basic stains. The epithelium rests on a thick layer of elastin-like connective tissue fibers. No muscle cells are present. In the larger tubules, the description

of the epithelium is identical, except of course, that it is continuous completely around the tubule.

MUSCLE

Little can be said in reference to the large shell muscle except that a part, the adductor muscle, is striated and the other portion, the catch muscle, is not. The striations of the former are broad and far apart (Kellogg, 1892). The nuclei are ellipsoidal and confined to the periphery of the cell. The muscle fibers are multinucleated, very large, and are not branched.

The above paragraph sums up the knowledge of the microscopical structure of the muscle. A study of this structure presents a peculiar problem. It is very difficult to fix and section satisfactorily the muscle, together with the surrounding soft parts. Even using a very small portion of the tissue, good results are obtained only after careful fixation. Additional microscopical investigation of the muscle is needed.

PART VI

SUMMARY AND CONCLUSIONS

SUMMARY AND CONCLUSIONS

Little absolutely original material has been offered by this paper. True, many of the histological investigations have been on species other than Ostrea virginica, but after all, the microscopical structures of the various species of Ostrea are very similar. The primary aim of this paper was to gather the existing material, corroborate it where necessary, and to fill in unknown parts (if practical), in order to make a more complete summary of the morphological and histological structure of O. virginica than was to be had up to the present time.

Some of the new material and conclusions advanced are as follows:

The heart consists of an outer layer of regular columnar epithelium, that becomes folded upon contraction of the heart; a medial layer of striated, branched muscle, the fibers of which run in many directions; and an inner, often incomplete, layer of thin squamous cells. Arteries have the same endothelium-like layer, surrounded by smooth muscle and connective tissue. Sinuses are merely spaces in con-

nective tissue.

The renal organ is composed of low columnar cells with a vesicular peripheral edge. The cells are non-ciliated.

The genital ducts are composed of simple cuboidal cells with long cilia.

In very young oysters, the right pallial lobe is more highly vascular than the left, showing that the promyal chamber must be used to as great an extent as the cloacal chamber, or even greater.

The labial palps have two surfaces that differ histologically and morphologically. The outer smooth surface is composed of simple columnar epithelium, while the inner folded surface is composed of an epithelium slightly higher than the former. Each fold has one or more grooves on its aborad side. The epithelium on both sides of the palps rests on a layer of smooth muscle.

The digestive tract is composed of simple columnar ciliated epithelium. The style sac epithelium differs from that of the rest of the gut in structure and function. Excluding the style sac, the only differences between the epithelia of the differ-

ent structures are: height, width, cilia height, and the underlying connective tissue (text fig. I).

Granular secretory cells are found throughout the digestive tract, with the exception of the style sac. These, although they assume a variety of forms, are different phases of the same cell. If these cells do secrete mucous, it is probably not in the form of mucin. All the cells of the style sac are secretory in nature, secreting the substance of the style.

The gastric shield is a chitinous secretion of certain cills in the stomach that imbeds the surrounding cilia upon solidification. It thus firmly anchors itself to the epithelium.

Glycogen is found to some extent in most of the connective tissue cells, and also in the epithelium of the hepatic diverticula.

Smooth muscle is found to surround the digestive tract at almost every point.

This paper has shown several points that need further investigation. A survey of the Golgi net and mitochondria under normal and experimental

TEXT FIGURE I

Structure	Height micra	Width micra	Cilia length micra	Secretory cells	Connective tissue
Mouth	80-100 30-50	3-4	75-100 30-40	Few	Medium
Esophagus	60-100 45-60	3-5	30	Few	Thick
Stomach	100 50-60	3 4-5	25	Several	Thick
Food Sorting Caecum	60-90	4-5	10-15	Several	Thick
Digestive Tubules	30	4-5	10	Many	Thin
Digestive Diverti-cula	10-30	6-8	retra-ctile	None	Thin
Style Sac	50-60	5-6	25-30	Few if any	Very thin
Midgut	40	3-4	10-15	Few	Medium
Typhlosole	80	3-4	10-15	Many	Medium
Intestine	60	4	10-15	Many	Thin
Rectum	60	4	10-15	Abundant	Medium

The above table is a comparison of the epithelial structures in the various regions of the gut in a single oyster. The figures are the result of a measurement of typical epithelium. There is variation, not only in different specimens, but even in a single individual. The figures above are therefore given, not as a means of identification, but essentially as a method of comparison.

conditions would certainly throw a great deal of light on the processes of digestion, assimilation, and secretion. The histology of the adductor and catch muscles should be investigated. Still another interesting cytological and physiological problem is the method of storing glycogen and fatty material in the connective tissue and in the hepatic diverticula. Morphologically, it would help considerably to have a good detailed account of the nervous and circulatory systems. Returning to physiology, an investigation of the renal organ might yield some interesting results. The exact nature of digestion and assimilation in the digestive tubules is still rather confusing and the same is true of the cells in the digestive tract. The functional importance of the food sorting caecum needs to be elucidated. There are many other problems far too numerous to mention here, but they all are necessary for a more complete understanding of the oyster. If this paper stimulates any such research, the author's labors will have been justified.

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PART VII

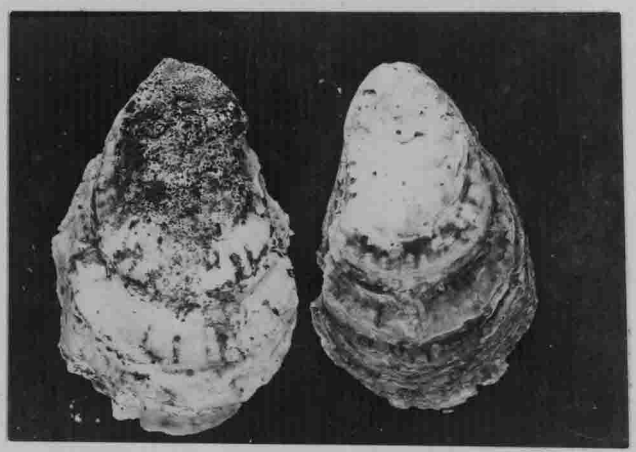
ILLUSTRATIONS

Figure 1

External view of the left and right valves. ½ natural size.

Figure 2

Internal view of left and right valves showing (a) hinge ligament, (b) scar for attachment of retractor muscle, and (c) scar for the attachment of adductor and catch muscles.



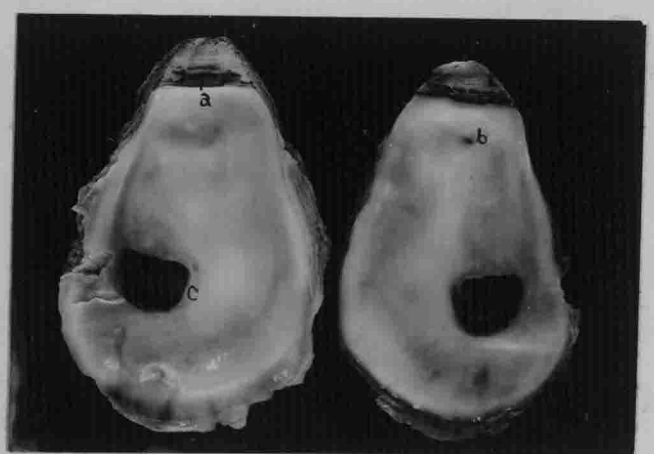

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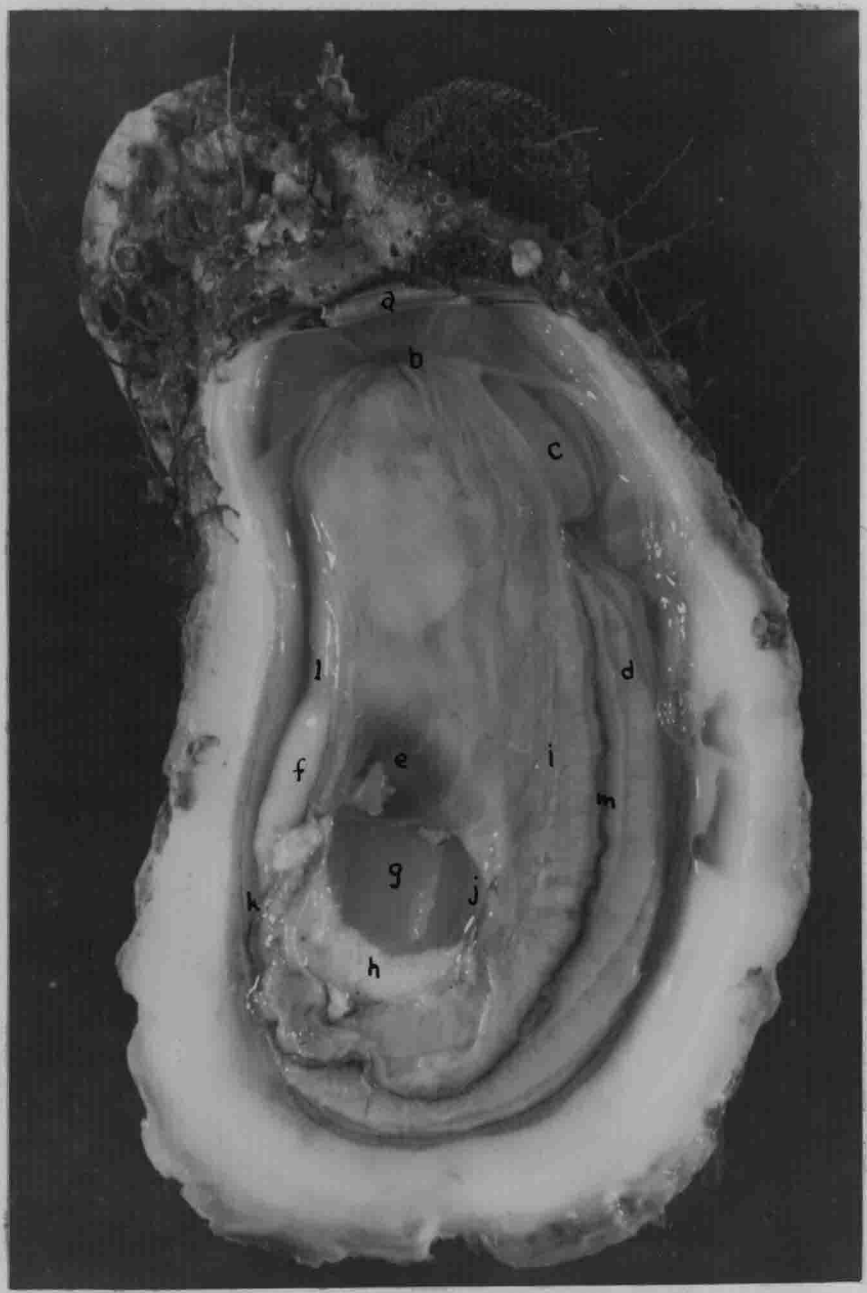
Figure 3

Whole oyster, right valve removed. (4x). (a) hinge ligament, (b) oral hood, (c) labial palps, (d) gills, (e) heart, (f) rectum, (g) adductor muscle, (h) catch muscle, (i) pallium, (j) region of renal organ, (k) cloacal chamber, (l) promyal chamber, (m) tentacular border of pallium.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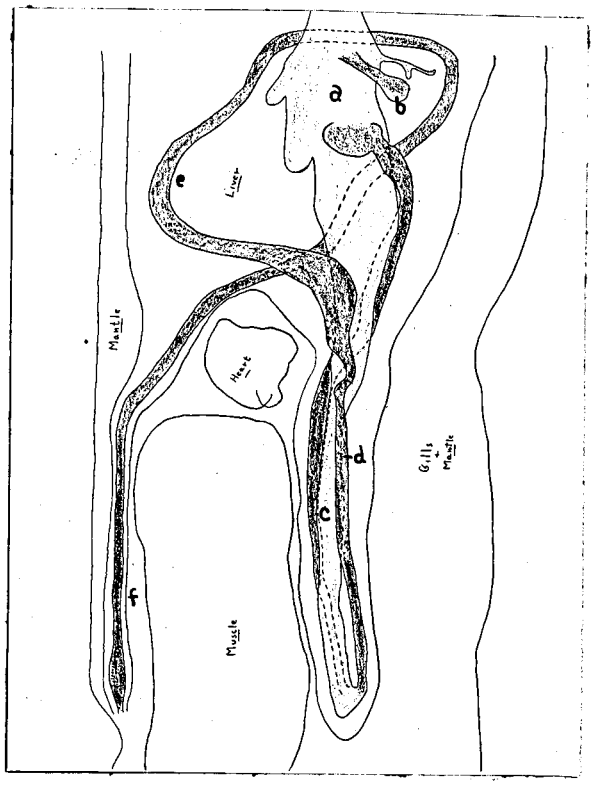
m

Figure 4

Drawing of the digestive tract of a large oyster taken from free hand celloidin sections. (a) stomach, (b) food sorting caecum, (c) style sac, (d) and (e) intestine, (f) rectum, unlabelled dark portion is midgut.

Figure 5

Arterial system of oyster, (natural size). (a) median pallial artery, (b) palps, (c) circum-pallial artery, (d) branches of anterior aorta supplying the visceral mass, (e) branch of anterior aorta supplying the rectum, (f) anterior aorta, (g) posterior aorta, (h) artery to adductor muscle, (i) artery to catch muscle, (j) pallium, (k) gills, (m) ventricle, (n) auricle.


a
b
e
Liver
Mantle
Heart
d
Gills
Mantle
c
f
Muscle

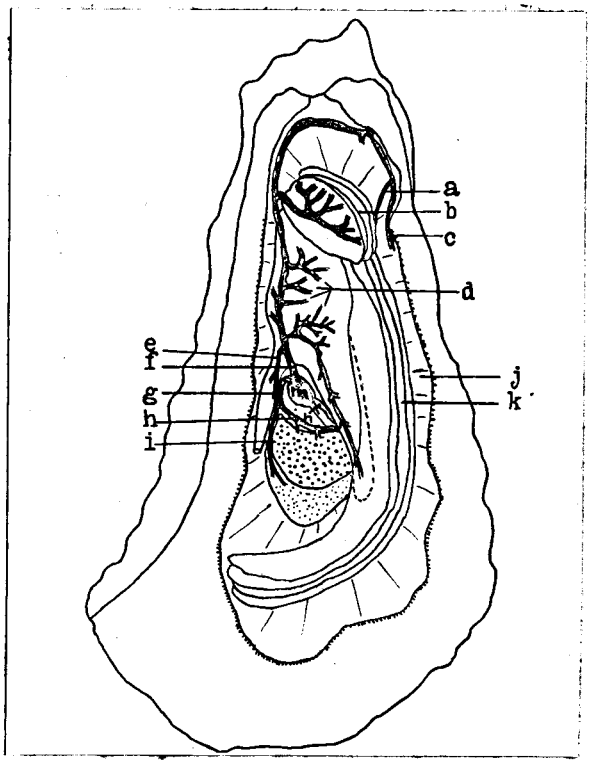
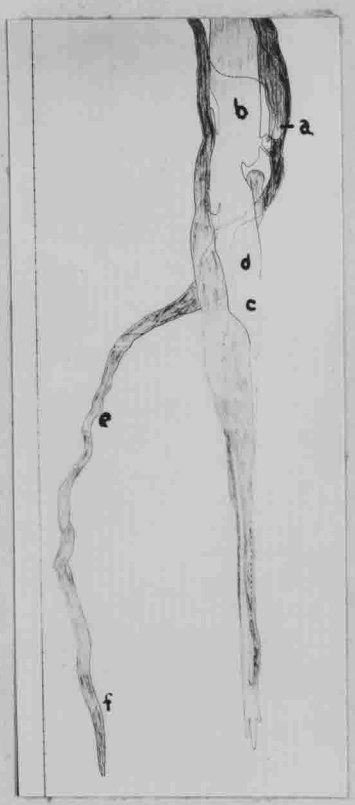

a
b
c
d
e
f
g
h
i
j
k

Figure 6

Graphic representation of the digestive tract of a small oyster, taken from serial sections. (a) food sorting caecum, (b) stomach, (c) style sac, (d) midgut, (e) intestine, (f) rectum.

Figure 7

Junction of palps and gills in O. edulis. Copied from Yonge (1926-b).



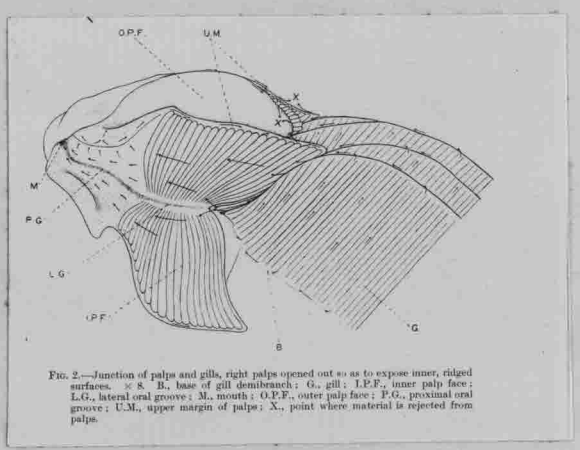


FIG. 2.—Junction of palps and gills, right palps opened out so as to expose inner, ridged surfaces. × 8. B., base of gill demibranch; G., gill; I.P.F., inner palp face; L.G., lateral oral groove; M., mouth; O.P.F., outer palp face; P.G., proximal oral groove; U.M., upper margin of palps; X., point where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

Figure 8

Gelatin cast of the stomach and style sac of O. edulis. Copied from Yonge (1926-b).

Figure 9

Drawing of whole oyster, right valve and pallial lobe removed. Visceral mass dissected to show gelatin cast of digestive tract. (a) labial palps, (b) food sorting caecum, (c) midgut, (d) intestine, (e) style sac, (f) gills, (g) pallium, (h) mouth, (i) esophagus, (k) stomach, (k) rectum.

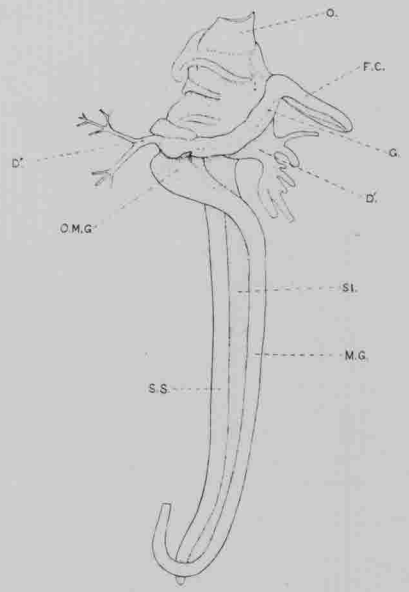


FIG. 3.—Gelatin cast of stomach with style-sac and first part of mid-gut and portion of œsophagus, from ventral aspect. × 4. D', larger, left duct of digestive diverticula; D'', smaller, right duct of same; F.C., food sorting cæcum; G., ventral groove; M.G., mid-gut; O., œsophagus; O.M.G., opening of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

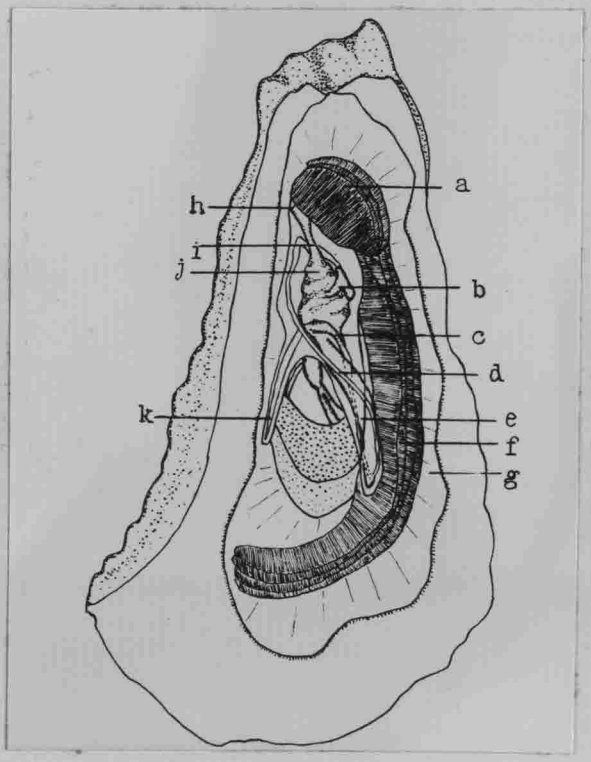
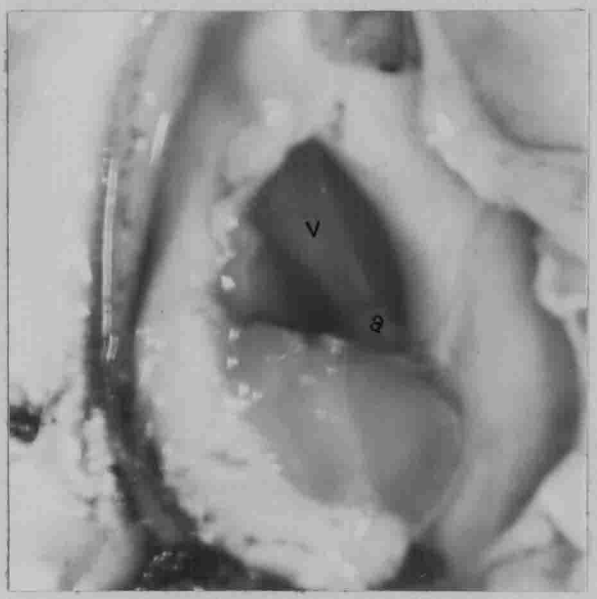


Figure 10

Pericardial cavity (4x) showing auricle (a) contracted.

Figure 11

Pericardial cavity (4x) showing ventricle (v) contracted.


V
a

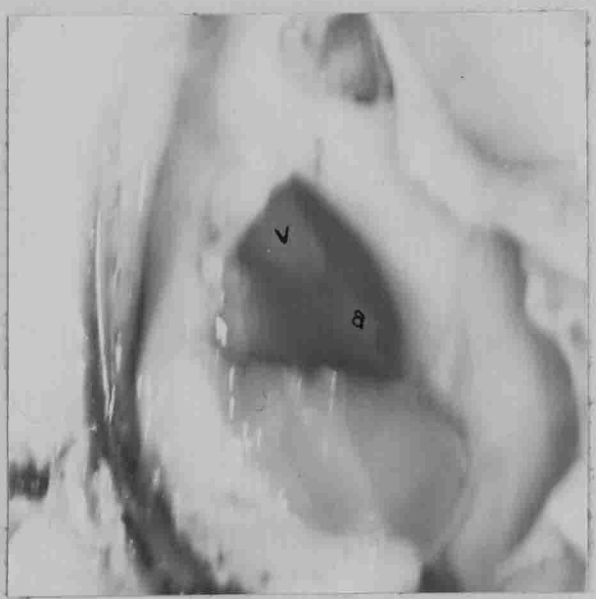
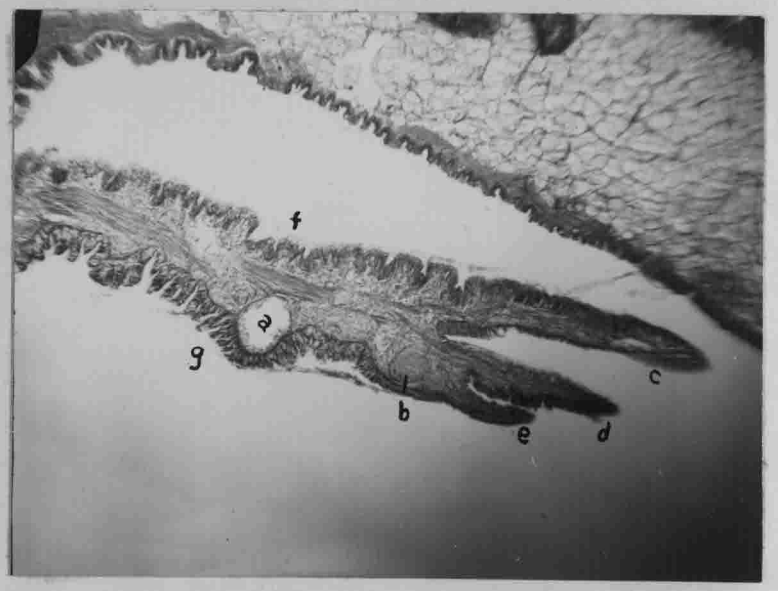

V
a

Figure 12

Pallial border (80x). (a) circumpallial artery, (b) circumpallial nerve, (c) external pallial fold, (d) median pallial fold, (e) pallial curtain, (f) outer pallial surface, (g) inner pallial surface.

Figure 13

External pallial surface stained with mucicarmine to show mucous gland cells (360x).


f
a
g
b
e
d
c

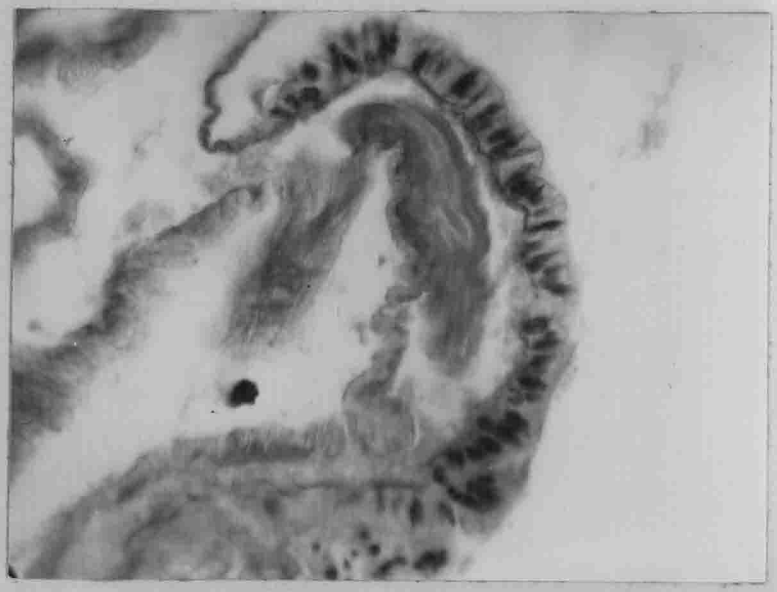
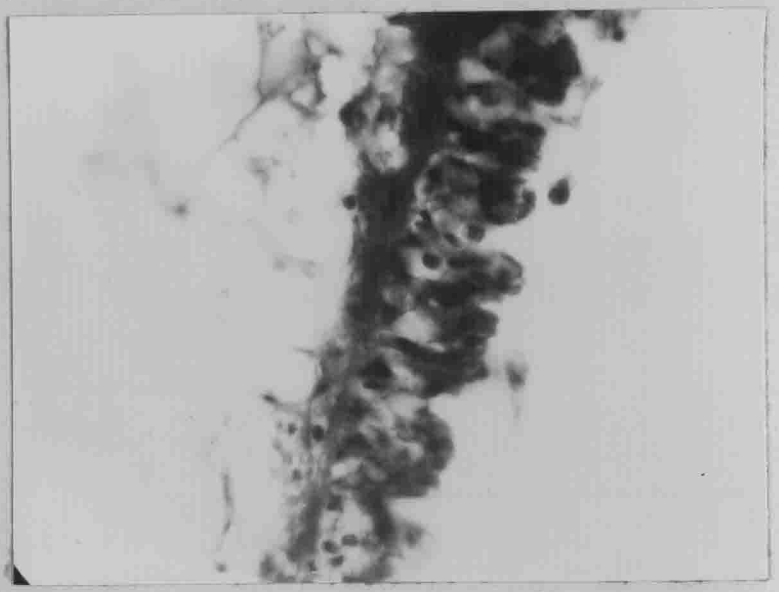


Figure 14

Internal pallial surface (800x).

Figure 15

External pallial surface (800x).



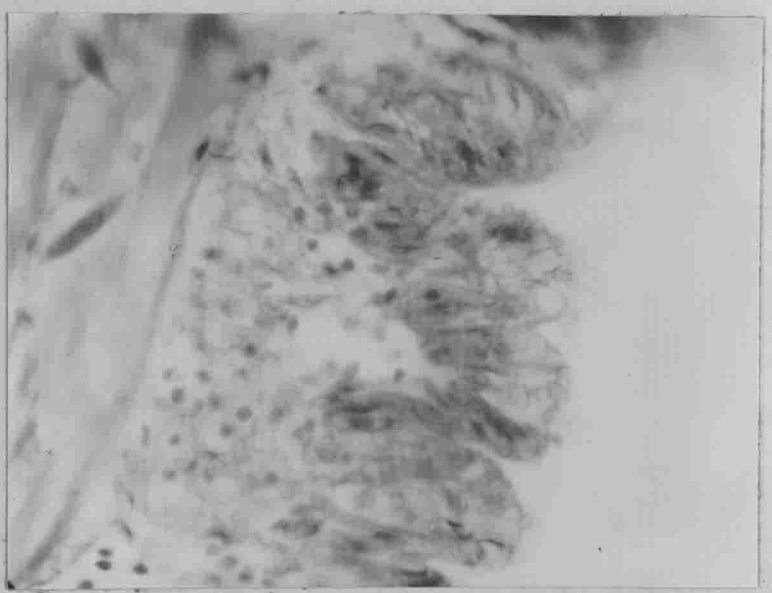
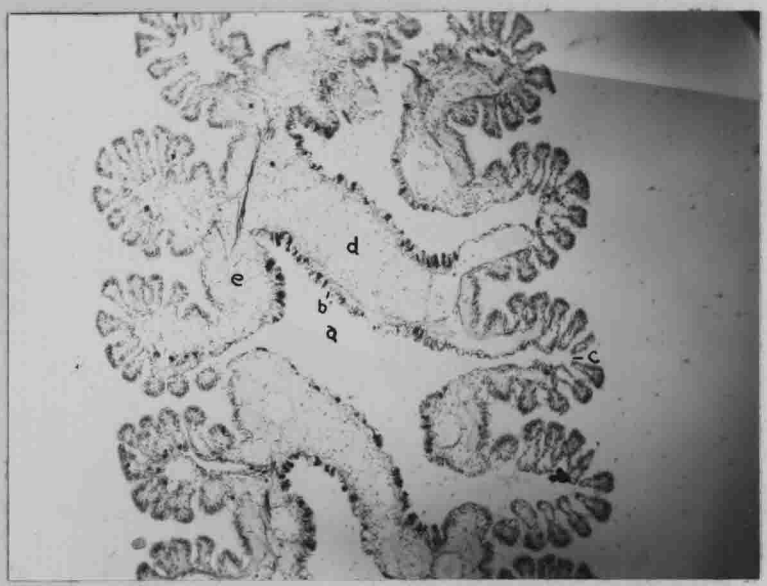


Figure 16

Cross-section of demibranch (80x). (a) water tube, (b) lining of water tube, (c) ostia, (d) interlamellar tissue, (e) interfilamentar tissue.

Figure 17

Cross section of gill plica (360x), (a)frontal cilia, (b) latero-frontal cilia, (c) lateral cilia, (d) chitinous rod, (e) outer epithelium, (f) muscle connecting two rods, (g) underlying connective tissue.


d
e
b
a
c

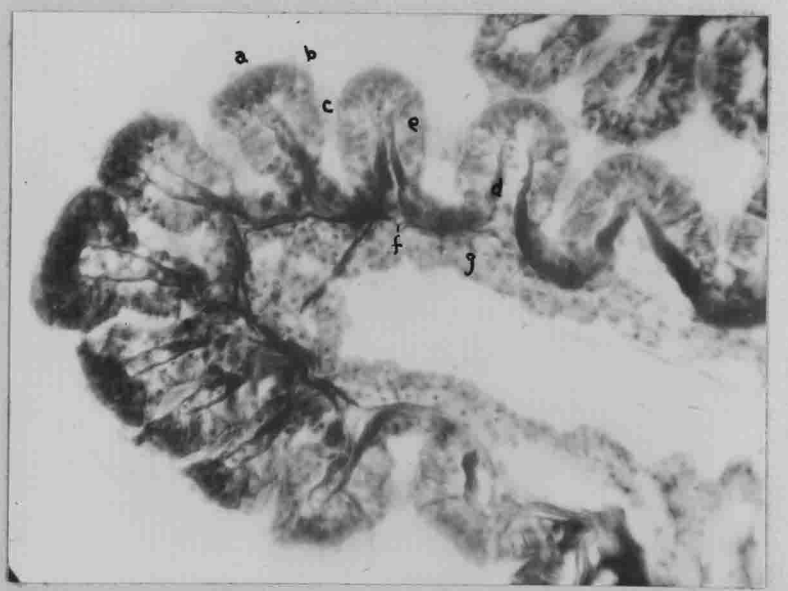
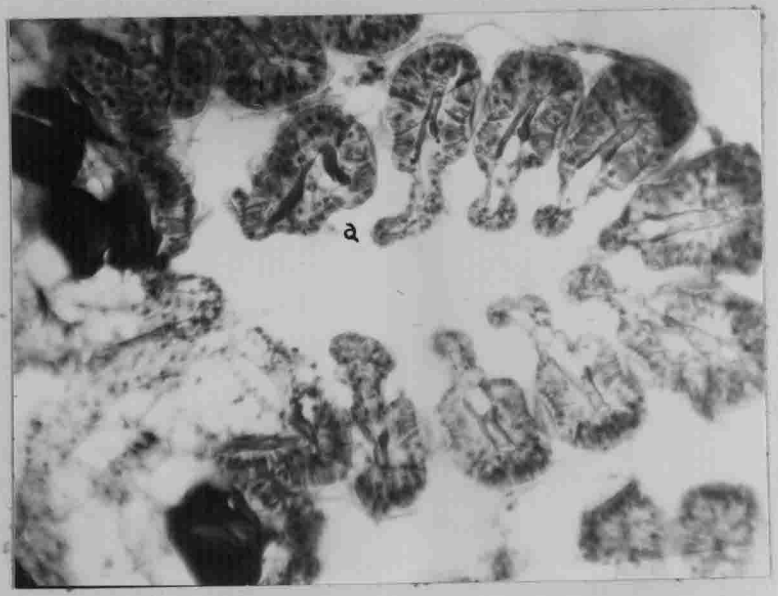

a
b
c
e
d
f
g

Figure 18

Cross section of gill plica (360x) showing ostia (a). (b) principal filament, (c) transitional filament, (d) ordinary filament, (e) frontal cilia, (f) latero-frontal cilia, (g) lateral cilia.

Figure 19

Inner palp surface (800x). (a) cilia in aborad groove, (b) aborad side, (c) orad side.


a

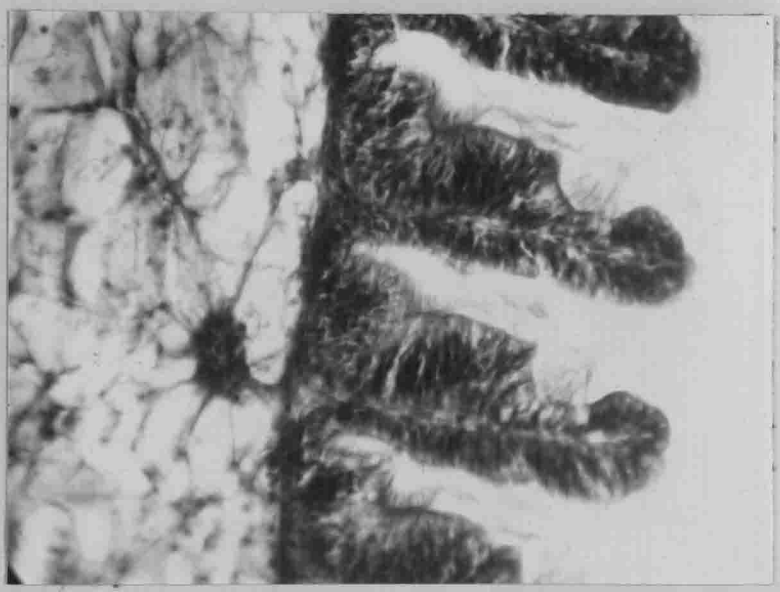
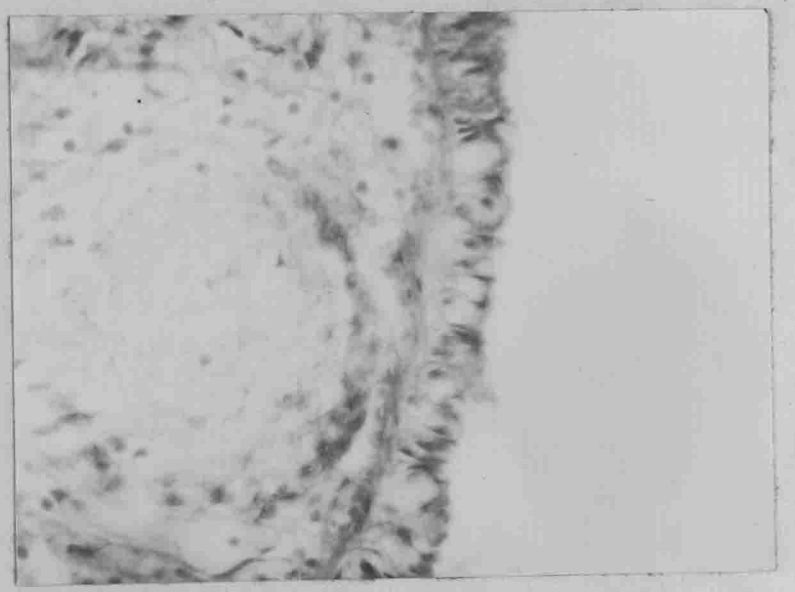


Figure 20

Inner palp surface (800x)

Figure 21

Junction of mouth and palps (80x). (a) palps and (b) mouth.



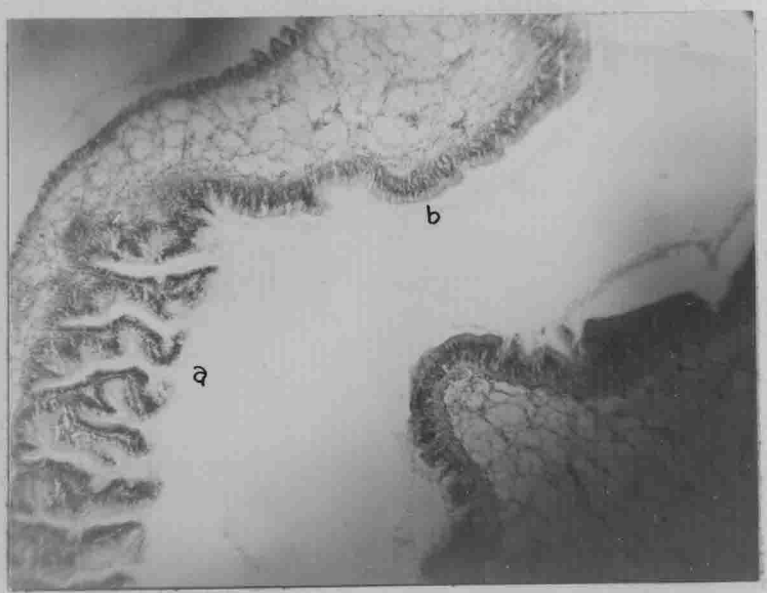

b
a

Figure 22

Mouth (800x).

Figure 23

Drawing of mouth (1000x). (a) cilia, (b) cuticular distal border, (c) blethroplast, (d) basal filament, (e) cell membrane, (f) ciliary root, (g) nucleus of phagocyte.

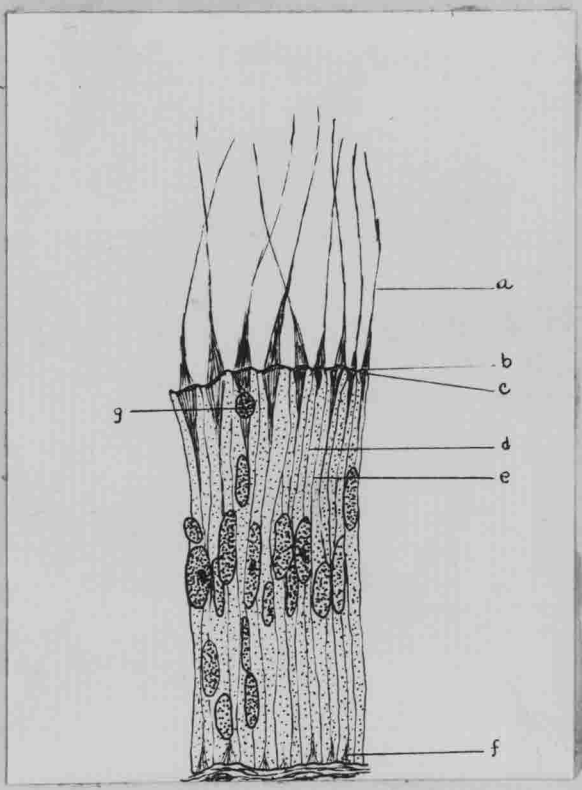

a
b
c
g
d
e
f

Figure 24

Esophagus (80x) showing the irregularity of the epithelium.

Figure 25

Esophagus (800x). (a) goblet cell, devoid of secretion, (b) excavations in epithelium, (c) cuticle.

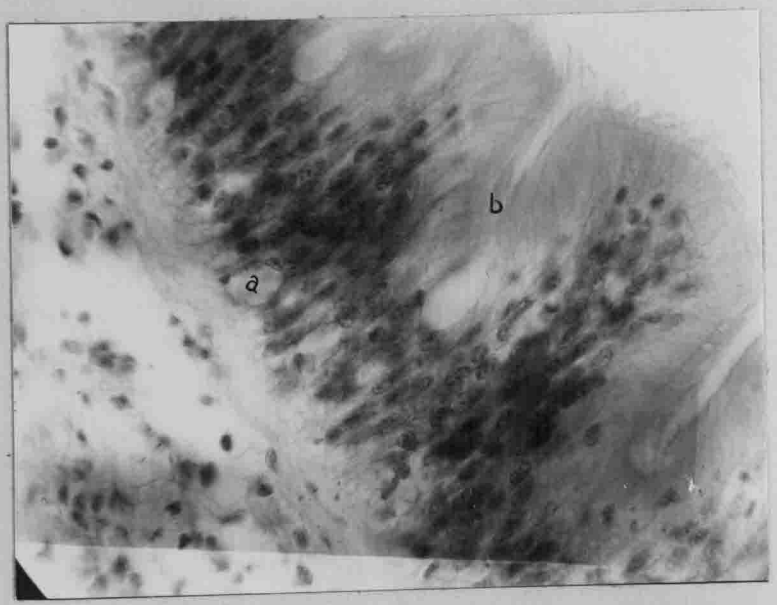
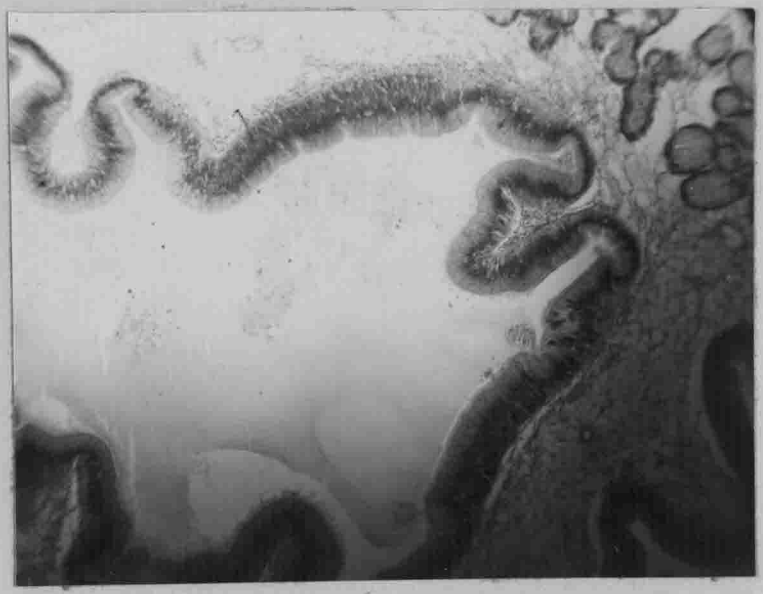

a
b

Figure 26

Stomach (80x).

Figure 27

Stomach (800x). (a) basement membrane, (b) line of blethroplasts, (c) cuticle, (d) cilia.



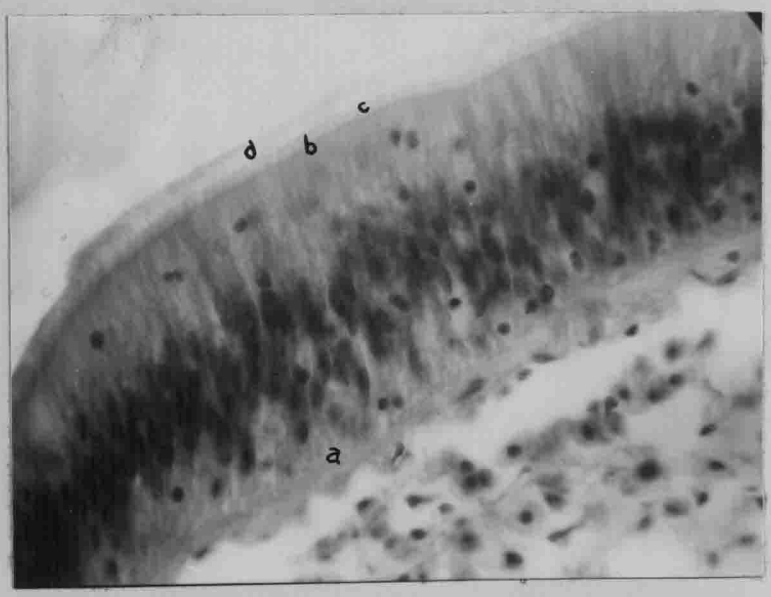
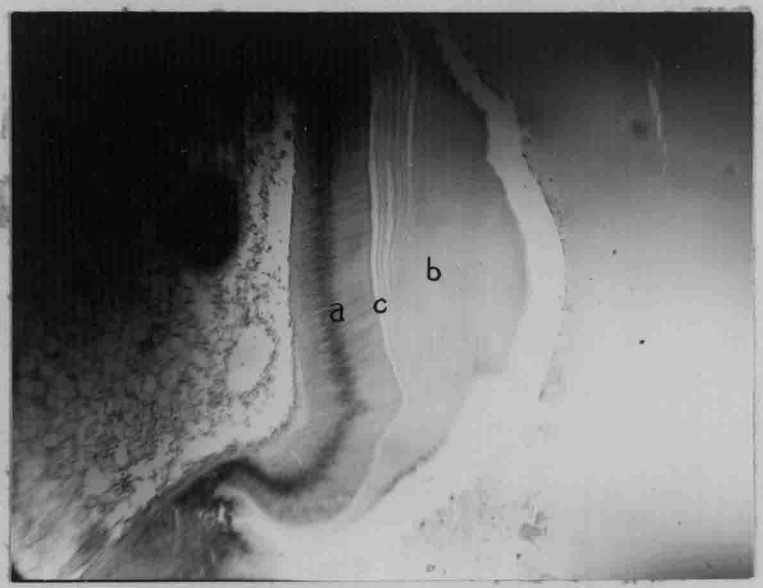

c
d
b
a

Figure 28

Stomach, showing (a) gastric shield, and (b) epithelium under the shield (80x).

Figure 29

Digestive diverticula (360x). (a) digestive duct, (b) digestive diverticulum.


a
c
b

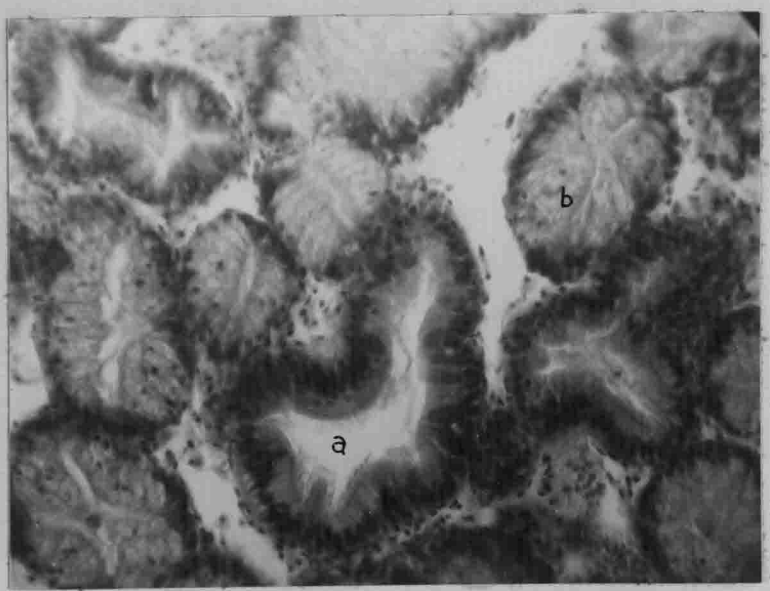
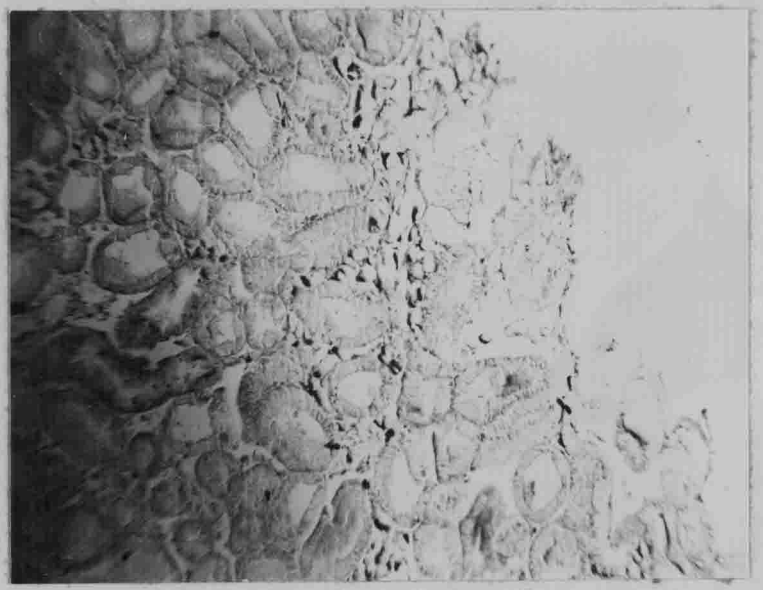

b
a

Figure 30

Glycogen in connective tissue surrounding the digestive diverticula (80x).

Figure 31

Saliva digested control of Fig. 30 showing the red-staining substance inside the ducts that wasnot present before digestion.



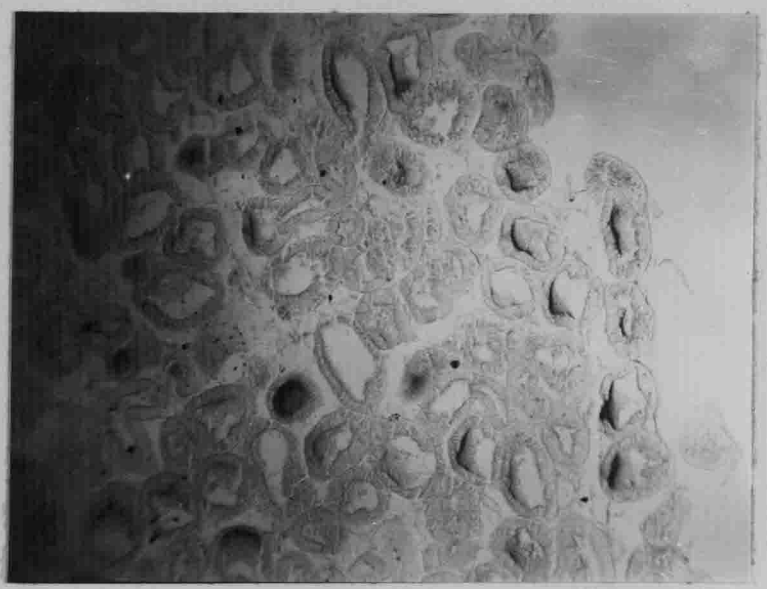


Figure 32

Cross section of connective tissue surrounding the rectum stained with Bauer-Feulgen technique to show glycogen.

Figure 33

Same section as Fig.32,but digested with saliva before staining (80x).

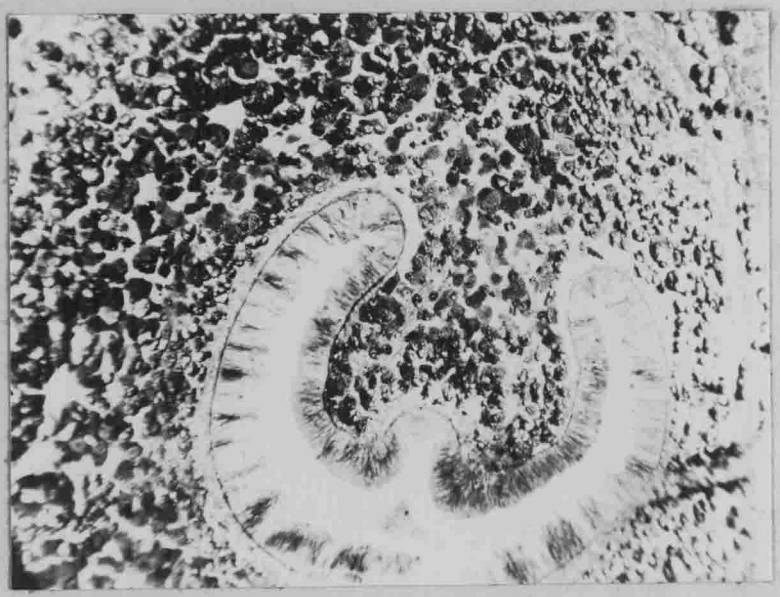


Figure 34

Glycogen (b) in epithelium of digestive duct (a). Stained with Bauer-Feulgen technique. (360x).

Figure 35

Glycogen in connective tissue (800x).

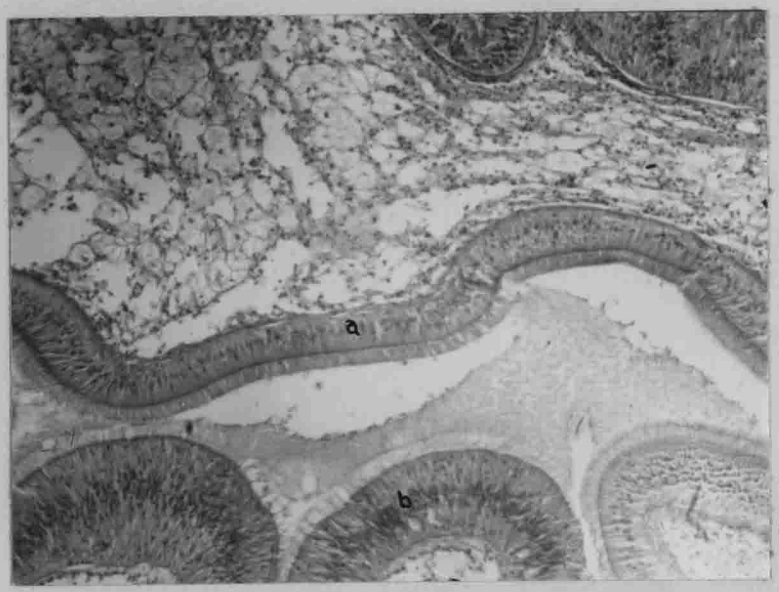

b
a

Figure 36

Style sac and midgut (80x). (a) style sac and (b) midgut.

Figure 37

Style sac epithelium (800x).


a
b

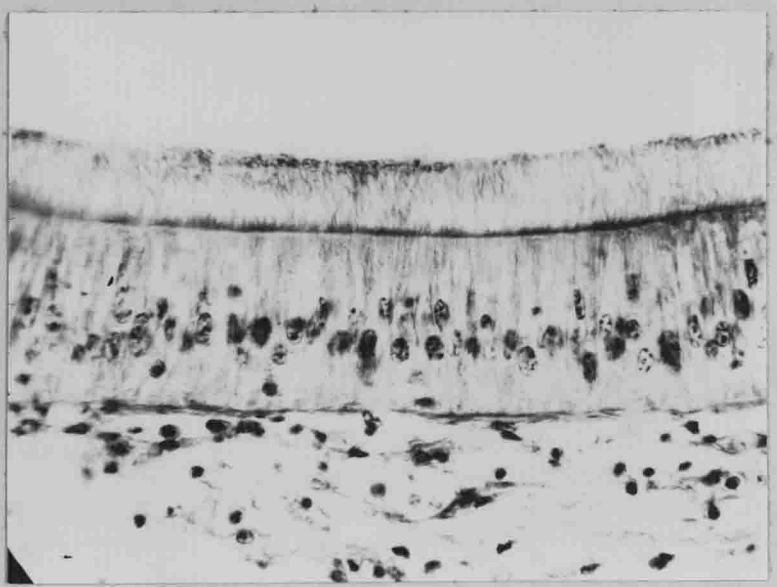
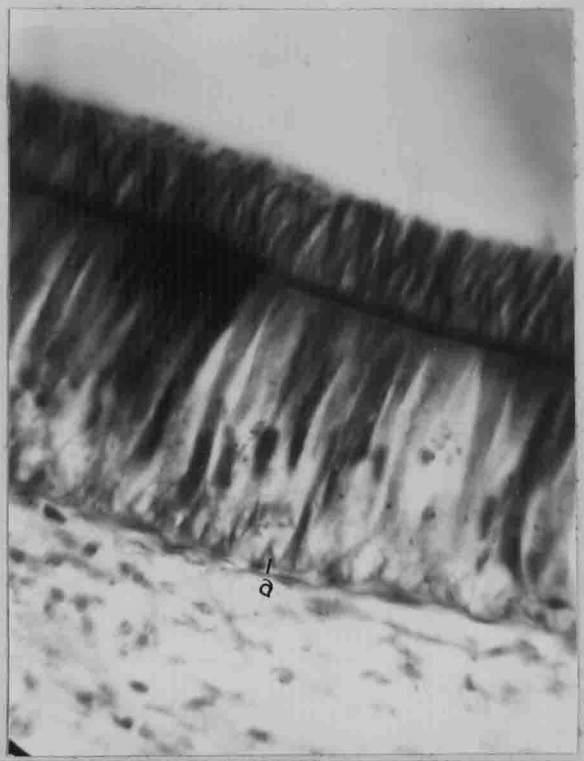


Figure 38

Style sac epithelium (1000x) to show intra-epithelial canals (a).

Figure 39

Junction of style sac and midgut (80x). (a) style sac, (b) midgut, (c) major typhlosole, (d) minor typhlosole, (e) sinus, (f) secretory cell.


a

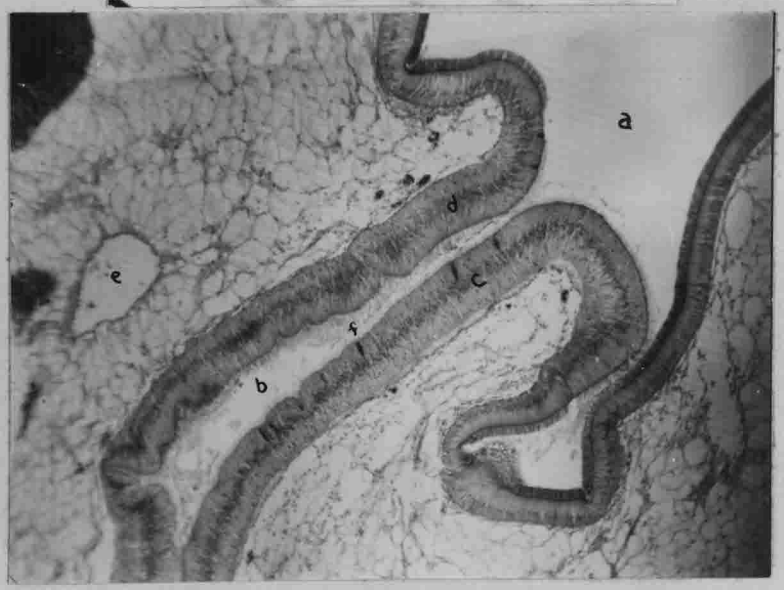
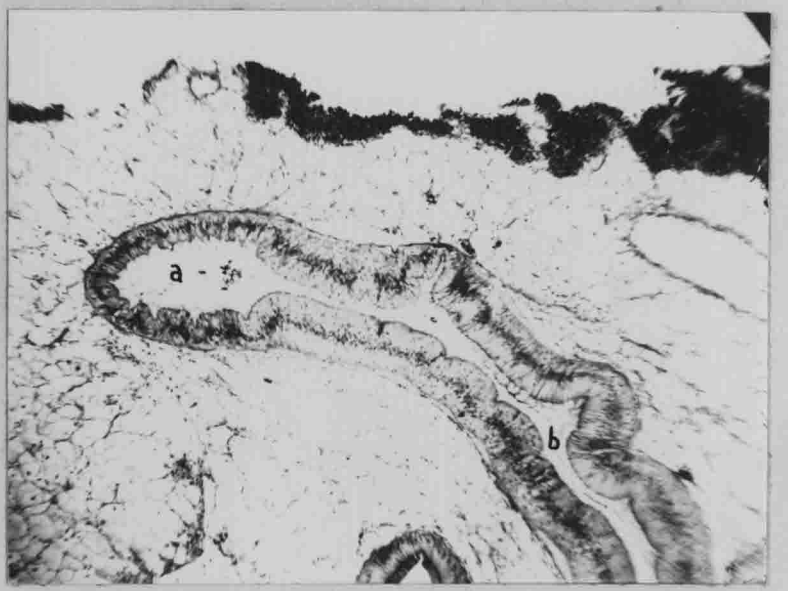

a
d
e
c
f
b

Figure 40

Midgut (a) and isthmus (b) connecting the midgut and the style sac (80x).

Figure 41

Cross section of typhlosole (800x). (a) basement membrane, (b) underlying connective tissue, (c) secretory cell, (d) cilia, (e) empty secretory cell.


a
b

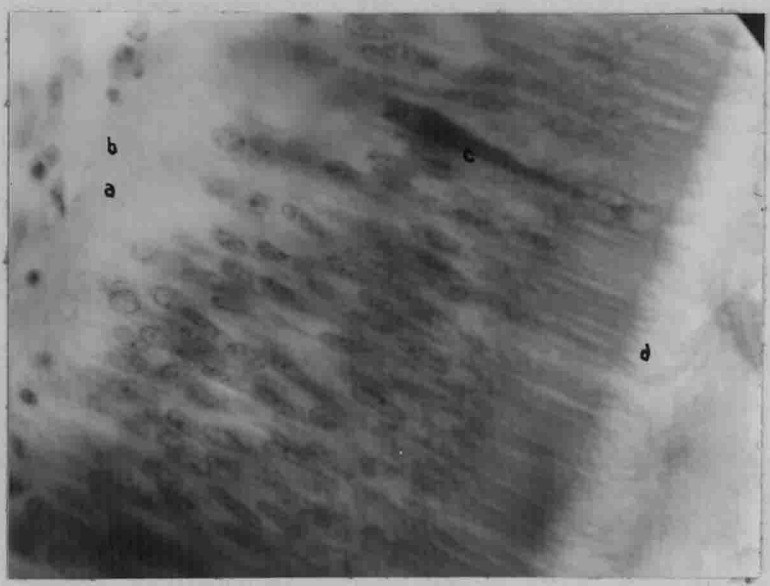
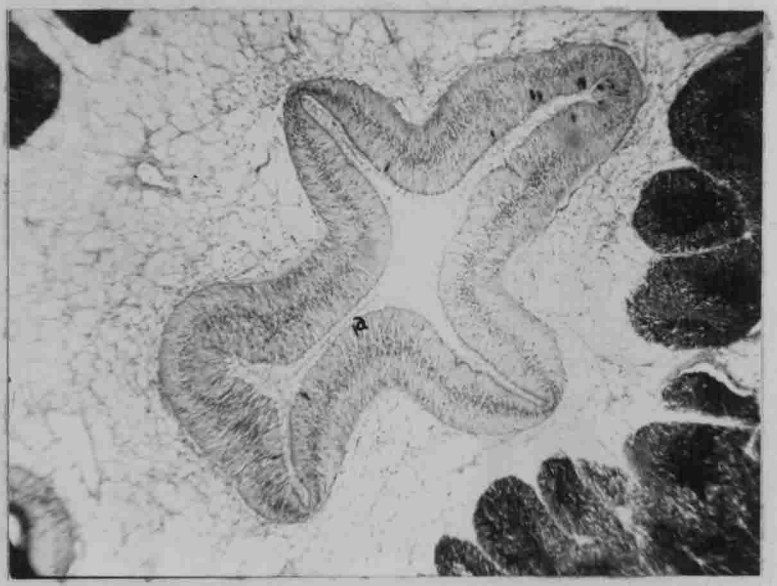

b
a
c
d

Figure 42

Cross section of intestine shortly after it comes off the midgut, showing (a) secretory cell (80x).

Figure 43

Intestine at about the level of the stomach (80x). (a) food material, (b) groove of typhlosole.



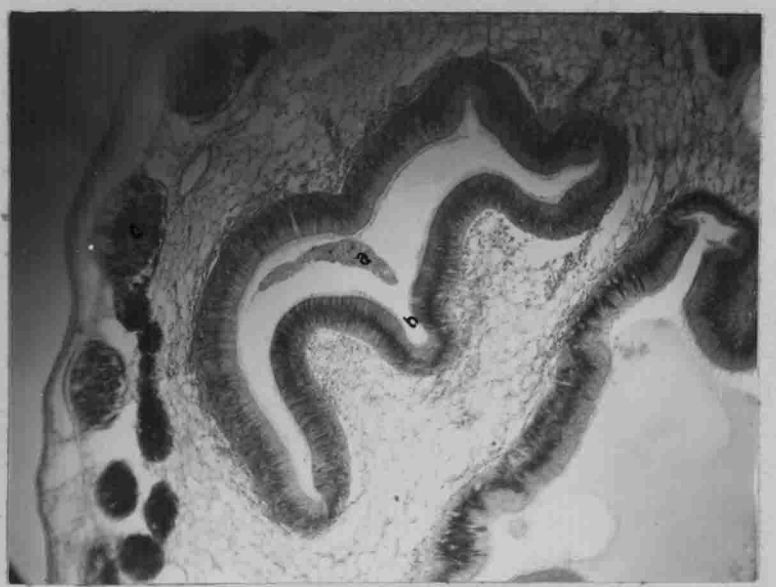
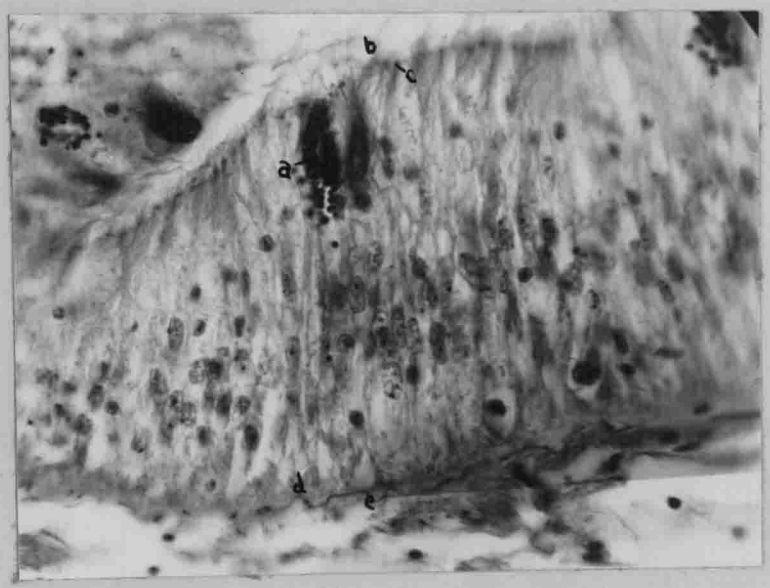


Figure 44

Cross section of intestine stained with iron haematoxylin (800x). (a) basophilic sedretory cell, (b) cilia, (c) blethroplast.

Figure 45

Cross section of rectum stained with haematoxylin, eosin and orange G (800x). (a) cilia, (b) cuticle. (c) food material


b
c
a
d
e

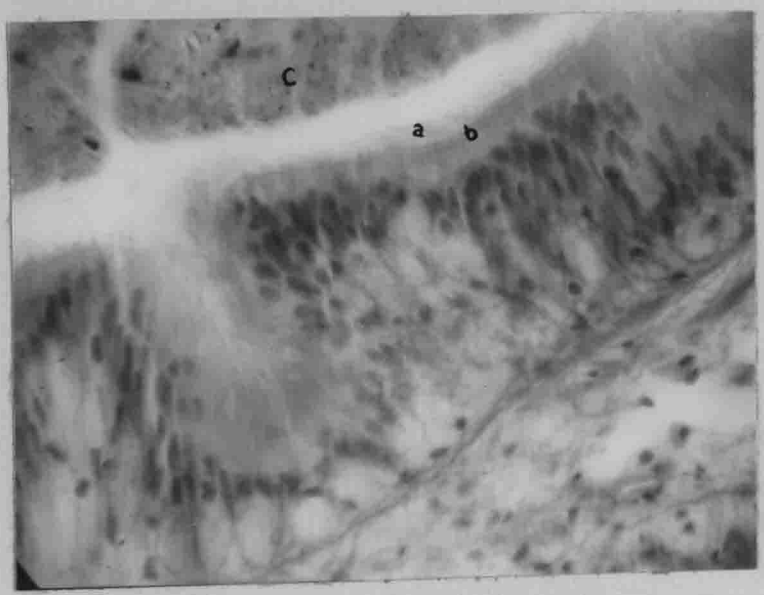

C
a
b

Figure 46

Cross section of the middle portion of the rectum (80x).

Figure 47

Cross section of the terminal portion of the rectum (80x).

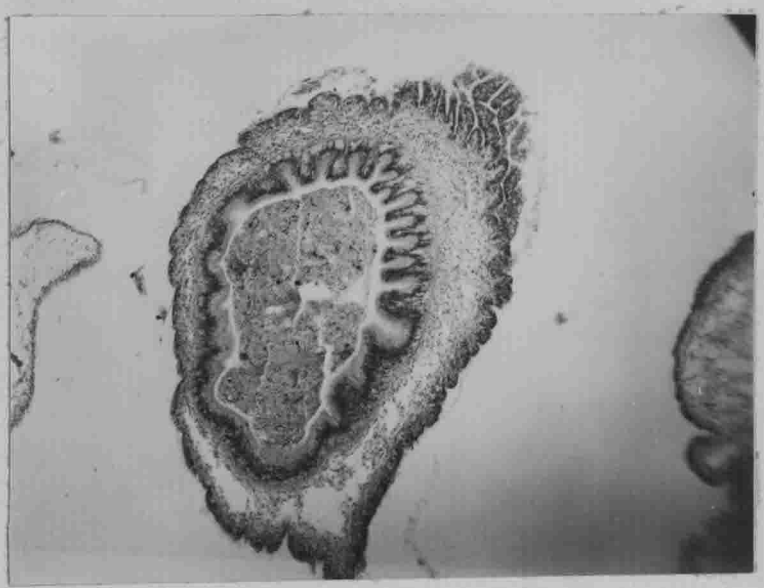
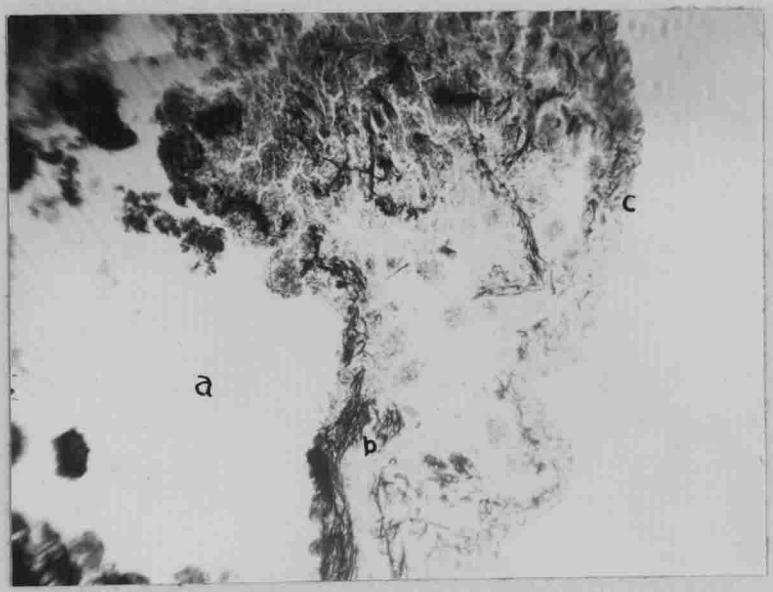


Figure 48

Cross section of auricle (80x) showing distribution of (m) muscle fibers, (a) auricular cavity, (b) connective tissue, (c) epithelium. Throughout the connective tissue there are numerous muscle cells appearing in cross section. These are too small to indicate.

Figure 49

Cross section of ventricle (80x) showing distribution of (m) muscle fibers, (a) connective tissue, (b) epithelium.


c
a
b

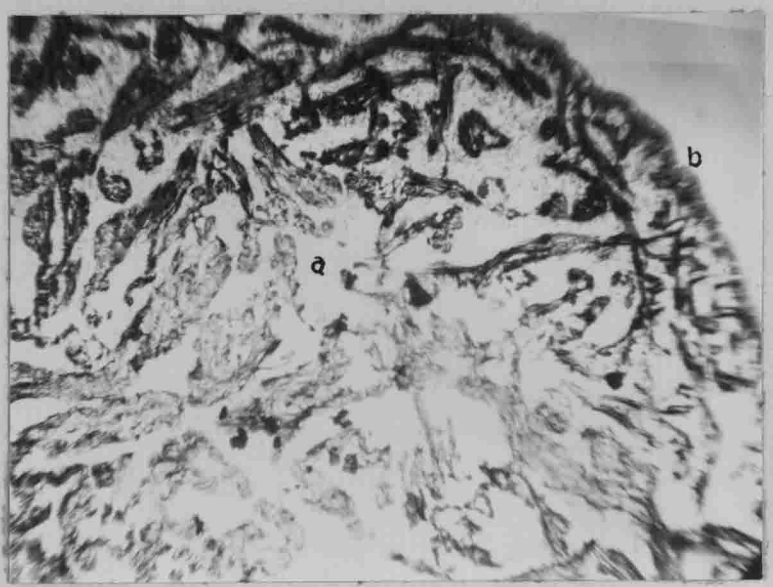
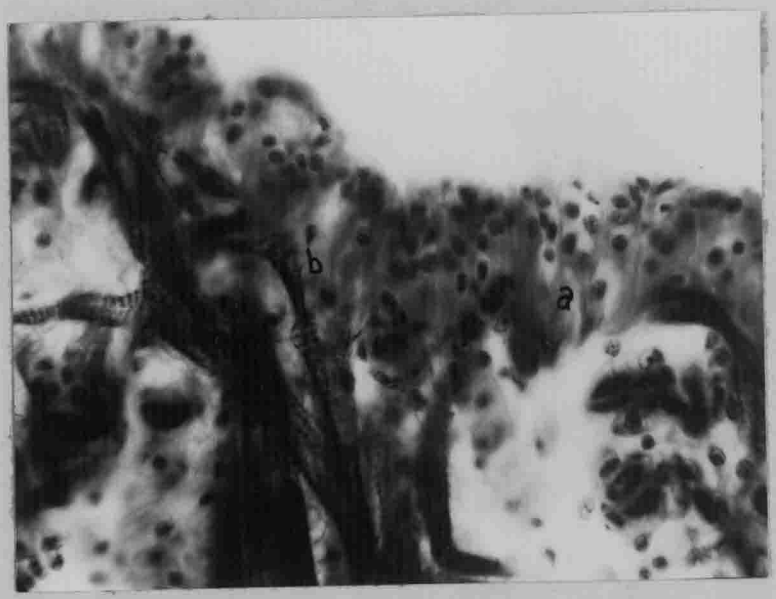

b
a

Figure 50

Epithelium of the ventricle (800x). (a) epithelium, (b) attachment of muscle connective tissue underlying the epithelium.

Figure 51

Cross section of ventricle stained with iron haematoxylin to show striations (800x). (a) branching fiber, (b) phagocyte.


b
a

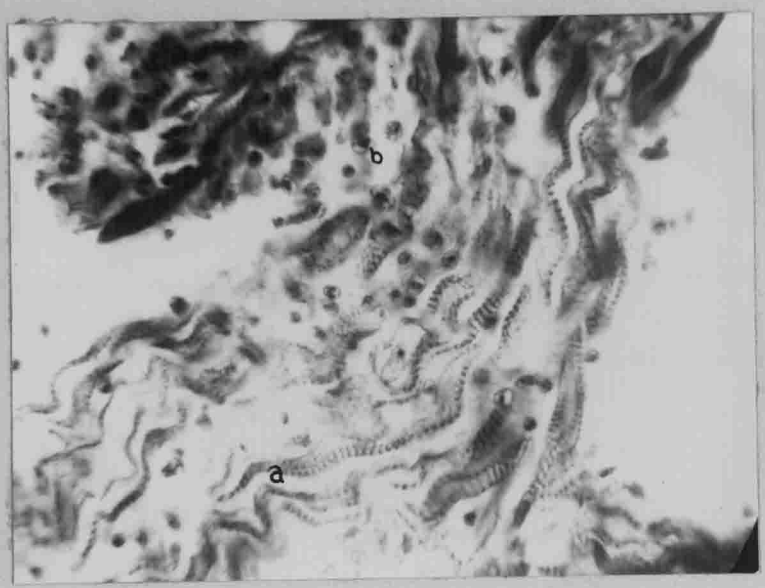

b
a

Figure 52

Cross section of large artery (360x). (a) lining cell, (b) layers of connective tissue interspersed with muscle.

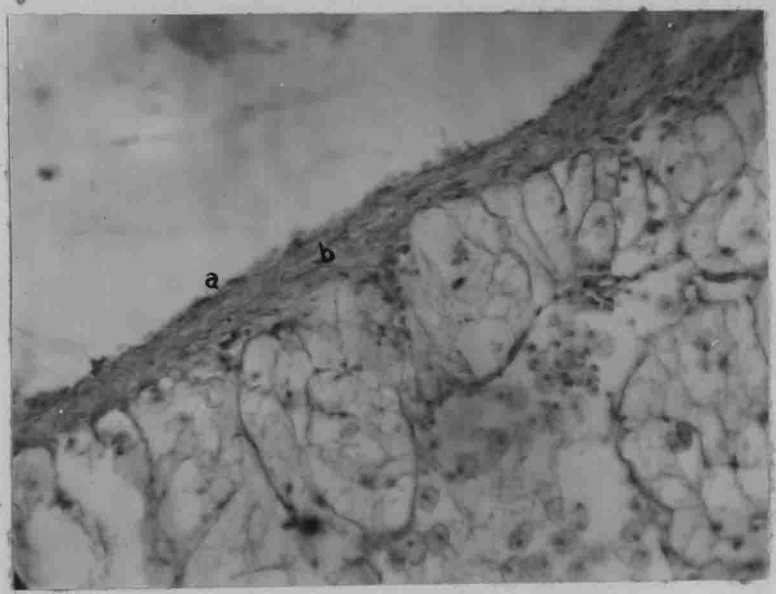
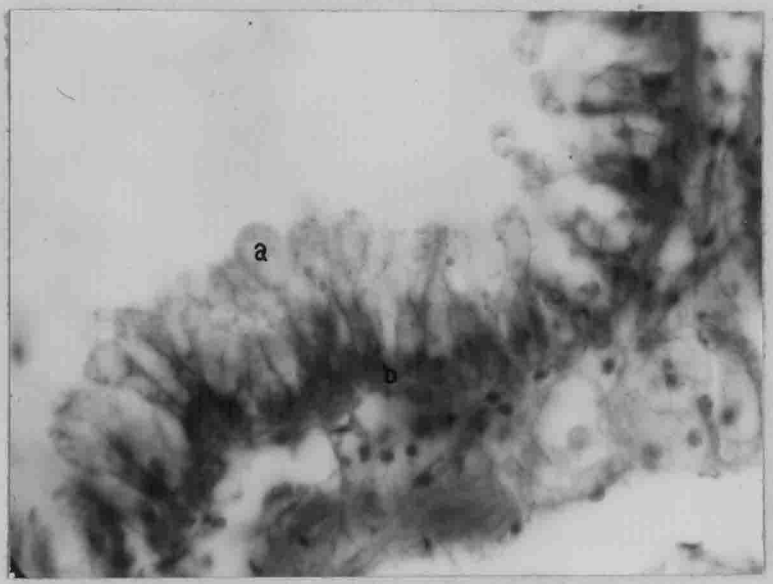

a
b

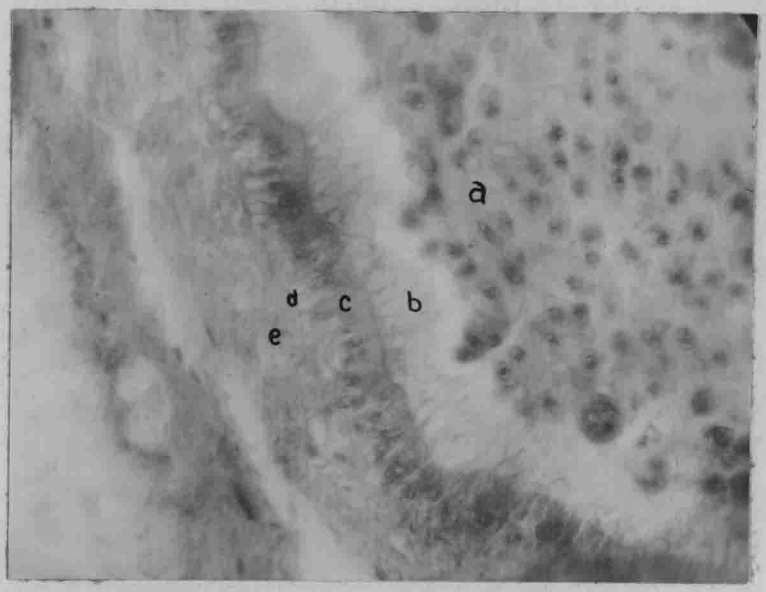
Figure 53

Cross section of nephridia tube (800x). (a) vesicular distal portion, (b) basement membrane.

Figure 54

Cross section of gonad duct (800x). (a) ova, (b) cilia, (c) cells, (d) basement membrane, (e) underlying connective tissue.


a
b


a
d
c
b
e

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Lewis C. Goldstein

AUTOBIOGRAPHY

I was born in Paterson, N. J., December 31, 1917. I attended Grammar School 13 and Eastside High School in Paterson, graduating from the latter in June, 1934.

I entered Seth Low Junior College of Columbia in September 1934 and transferred to Richmond College in September 1935. I received the degree of Bachelor of Science in June 1938 Biology being my major subject. During the session of 1937-38, I served as laboratory instructor in Comparative Anatomy.

From September 1938 until June 1940, I served as graduate assistant in Zoology. During this time I completed my work toward the degree of Master of Science.

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