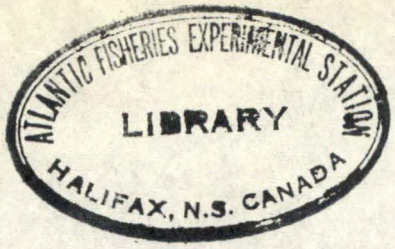

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Title

ON THE HISTOLOGICAL STRUCTURE OF THE DIGESTIVE TRACTS OF SOME CANADIAN PACIFIC COAST FISHES

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ON THE HISTOLOGICAL STRUCTURE OF THE DIGESTIVE TRACTS OF SOME CANADIAN PACIFIC COAST FISHES

The Ratfish	Hydrolagus colliei
The Starry Flounder	Platichthys stellatus
The Two-lined Flounder	Lepidopsetta bilineata
The Shiner	Cymatogaster aggregatus
The Blue Perch	Taeniotoca lateralis

by

Lloyd L. Bolton

INTRODUCTION

In connection with the work which is being done by the Biological Board of Canada on the life histories and particularly the feeding habits of fishes of the Canadian Pacific Coast, it was considered advisable that some attention be given to the structure of the digestive tracts.

Preparatory work on the structure of the tracts of some of the more common species was begun at the Pacific Biological Station, situated at Nanaimo, British Columbia, in July, 1924. The purpose of the work was to determine the structure of this important system, to seek a relationship between the structure of the tracts of various species and the type of food used by each, and ultimately to continue the investigation along physiological and biochemical lines, to determine the digestive reactions carried on in the various regions of the differently constituted tracts.

As a beginning, tracts of several species, including the five mentioned above were collected and preserved for histological sectioning during the summer of 1924. During the following winter, the sectioning and study of these five species was continued in the Biological Department of the University

of Toronto.

To the Biological Board of Canada, which made possible the collection of material, to the Honorary Advisory Council for Scientific and Industrial Research, which made possible the continuation of the work, and to the Department of Biology of the University of Toronto, in whose laboratories much of the work was done, I wish to express my indebtedness.

To Dr. W.A. Clemens, director of the Pacific Biological Station, who has aided in every way in the procuring of material, and to Dr. W.H. Piersol, who has directed my work while in Toronto and assisted me whenever possible, I wish to express my sincerest thanks.

METHODS

In the preservation of the tracts, the strictest attention was given to having the material absolutely fresh. In fact, in all cases the fish were obtained alive, killed, and the tract immediately excised and placed in the fixative. Thus no time was allowed for post-mortem changes to take place in the tissue, nor for autho-digestion of the mucous surface to begin. Precautions against the latter must always be taken in preserving digestive tissues, and particularly those of fishes, whose digestive secretions are, as a rule, extremely active.

Zenker's fluid was the fixative used throughout. The usual procedure of washing, removing the mercury salts and dehydration was followed, and the material kept till ready for sectioning in a mixture of equal parts of alcohol 95%, glycerin, and distilled water, which was found to be a very satisfactory medium.

The mucous surfaces were examined with the use of the binocular microscope. Fresh meterial was also teased and macerated when desirable. Transverse and longitudinal sections were cut throughout each of the tracts, particular care being taken to obtain longitudinal sections of all the transition regions. The sectioning was all done by the paraffin method.

The stains commonly used were Haematoxylin and Eosin or Orange G, Van Giesen's Acid Fuchsin and Picric Acid mixture, while for determining the distribution of mucus and mucus producing cells Mayer's Muci-Haematein or Toluidine Blue were found particularly satisfactory.

Histological work was done only on the regions from the oesophagus to the rectum inclusive.

HYDROLAGUS COLLIEI - The Ratfish

ANATOMY

The tract of Hydrolagus colliei consists of four clearly marked regions, viz. the oral cavity, the oesophagus, the intestine, and the rectum. Leydig, 1851, described the tract of the closely allied species, Chimaera monstrosa, as consisting of an oral cavity, a mid region, and an anal part. He did not consider the oesophagus as an entity, but described the mid region as consisting of stomach and intestine. Neuville, 1901, gives a diagram of the intestine of the same species, and calls that region of the tract leading into the intestine the "tube pylorique". In Hydrolagus, at least, this region is definitely oesophagus and leads directly into the intestine. Sections show nothing whatever to indicate the presence of a stomach.

The tract passes directly from the pharynx to the anus without coiling. In a male specimen 46 cm. long the lengths of the various regions were as follows: buccal cavity and pharynx - 7 cm., oesophagus - 2.7 cm., intestine - 9 cm., rectum - 2.5 cm.

The region of the junction of the oesophagus and the intestine is marked by a slightly thickened ring around the tract, as well as by a great increase in diameter. The pouch (Bursa entiana) found in the intestine of Chimaera just posterior to the junction region is slightly represented in Hydrolagus, but does not differ histologically from the wall of the remainder of the intestine.

The large bile duct enters the right side of the intestine about 8 mm. from its anterior end, and the smaller pancreatic duct enters about 6 mm. dorsad to it. The intestine is enlarged in its middle region. The region of the attachment of the spiral valve is clearly visible as a darkened depression on the outer surface of the tract. As in Chimaera, the anterior part (about two-thirds) of the valve is much like a simple typhlosole, but in the posterior region it becomes spiral, making about three complete turns. It ends abruptly with the end of the intestine.

The intestine narrows into the rectum which is much smaller in diameter, and leads directly to the anal opening.

HISTOLOGY

The Oesophagus.

The surface of the oesophagus shows numerous longitudinal folds. In the contracted state they may be quite irregular. The wall is comparatively heavy. Towards the posterior end the folds become deeper and much more regular. At the junction of the oesophagus with the intestine the folds gradually break up into villi.

The wall of the oesophagus consists of four coats, viz., mucosa, submucosa, muscularis and serosa.

The mucosa consists of a layer of epithelium resting on a definite basement membrane, and a tunica propria. The epithelium is of the stratified squamous variety and is from 10 to 15 (or even 20) cells thick. The cells of which it is composed are, for the most part, large, heavy-walled, and polygonal in shape. They have large nuclei which are normally irregularly rounded or ovoid in shape, and comprise about half the volume of the cell. The cells of the basal germinative region are smaller, have smaller nuclei and thinner walls, and are elongated in their vertical diameter. Towards the surface the cells may retain their polygonal shape or they may become flattened, their shape evidently varying much with the degree of distension of the walls of the tract. The nuclei may become shrunken and flattened or saucer-shaped and pushed towards the basal side of the cell by the mucoid material formed in the cytoplasm of the upper side of the cell. This material gave an excellent mucus reaction with Mayer's Nuci-haematein and with Toluidine Blud. The exposed surface of the epithelium may be covered with a layer of cells which are evidently dead. They are much flattened and have heavy walls. The cytoplasm has disappeared, probably to become a part of the thickened wall. The nuclei are contracted and flattened, show no definite structure, and stain darkly. The result is a dense, more or less cuticular layer over the surface, composed of the remains of these dead cells, and affording additional protection to the underlying cells.

Beneath the epithelium is a definite, though thin, basement membrane. It appears quite clear and structureless, but neclei may be found at irregular intervals applied to its lower surface.

The tunica propria consists of a layer of fairly compact connective tissue under the basement membrane. Passing away from the epithelium it becomes looser in structure and gradually changes into the submucosa with no definite line of demarcation between the two.

There are no glands of any kind in the oesophagus.

The submucosa is composed mainly of loose connective tissue. It forms a framework for the folds of the mucosa, filling the region between it and the muscularis. In the submucosa are numerous large blood and lymph spaces, lined with very thin endothelium, or having no definitely cellular walls. Heavier walled blood vessels are also scattered throughout. Large bundles of nerve fibres may be found particularly in the region near the muscularis.

The muscularis consists of an inner longitudinal and an outer circular layer. Both layers are well developed and are about equal in thickness (6 - 12 cells). The bundles of fibres are commonly not packed closely together, but may have a fairly loose structure with connective tissue scattered between them. The fibres of the two layers are of the striated variety throughout.

Outside the muscularis, the serosa is a layer of loose connective tissue, carrying blood vessels and bundles of nerve fibres. These latter are very noticeable in all the sections and particularly those from the anterior part of the oesophagus where they may be so numerous as to form a discontinuous layer around a considerable part of the circumference of the tract. The bundles are arranged longitudinally, for the most part, and gradually become smaller towards the posterior part of the oesophagus. A very thin layer of peritoneal epithelium surrounds the connective tissue layer.

The Junction of the Oesophagus and Intestine.

The transition from the oesophagus to the intestine, while it appears to be more or less gradual to the unaided eye, is in reality quite abrupt, as a longitudinal section of this region shows. The stratified epithelium of the oesophagus ends abruptly and the columnar epithelium of the intestine is continuous with its basal layers. The upper layers overhang the transition point as a projecting shelf.

The submucosa is quite continuous, showing little change other than the gradual appearance of a few non-striated muscle fibres scattered through it.

The striated muscle layers of the oesophagus pass posteriorly slightly beyond the point of transition in the epithelium. The inner longitudinal layer tapers off quite gradually in this region and disappears. The outer circular layer is continued slightly beyond the longitudinal layer and tapers off to end somewhat more abruptly. The smooth muscle of the intestine appears gradually as scattered fibres in the outer part of the submucosa. Sections of the extreme anterior end of the intestine, then, will show an outer circular and inner longitudinal layer of striated muscle fibres outside the regular smooth muscle layers of the intestine.

The Intestine

The internal surface of the intestine is covered anteriorly with closely packed villi varying in size from .5 to 1.5 mm. in length, and from .5 to .5 mm. in diameter. Posteriorly, particularly where the course of the valve is spiral, the surface of the wall becomes arranged in a series of grooves or fine folds, having a direction transferse to the longitudinal axis of the tract. These grooves are quite regular and uniform for a distance

of 1 to 5 cm. from the region of the attachment of the valve to the wall. They become zig-zag, then, and gradually break up into villi.

In section the intestine shows four coats comparable to those of the oesophagus.

The mucosa consists of a single layer of ciliated columnar epithelium, in which are scattered many mucous goblet cells, a basement membrane which is not always distinct, and a tunica propria, which like that of the oesophagus gradually merges into the submucosa. The epithelial cells are very tall and narrow and appear polygonal or cylindrical in cross section, those from the posterior end of the intestine being considerably taller than those from the anterior end. The lateral walls are very indistinct, and are perceptible only with difficulty even under great magnification. The walls of the exposed ends of the cells are comparatively heavy and ciliated, and the terminal bars show clearly. The cilia are extremely fine and short and can be seen clearly only under very high magnifications. Cilia are to be found over the entire surface of the intestine. The basal ends of the cells are usually tapered and pass to the basement membrane between the smaller cells of the germinative region of the epithelium. The nuclei of the epithelial cells are cylindrical in shape, with rounded ends and show a definite nuclear membrane. They lie in the lower part of the cell, their upper ends usually reaching to about the mid region of the cell.

The mucous goblet cells appear at the very anterior end of the intestinal epithelium and are scattered throughout. In the anterior and middle regions of the intestine they usually show the typical goblet shape when distended with secretion, but towards the posterior end where the epithelium becomes heavier, the pressure of adjoining epithelial cells is evidently much greater and the mucous cells show little lateral distension even when filled with

secretion.

The submucosa forms a bed on which the mucosa rests, and a framework for the villi, and serves to carry the nerves and blood vessels supplying the mucosa. It is composed of connective tissue with occasional smooth muscle fibres scattered through it. The blood vessels in the outer part of the submucosa are mostly definitely walled, but in the villi they break up into extremely fine vessels, many with no definite walls other than those formed by the closely netted fibres of the connective tissue.

The muscularis is composed of three layers, viz. an inner longitudinal, a middle circular, and an outer longitudinal layer. The inner longitudinal layer is thin, and in many transverse sections does not form a continuous coat, but may be represented in parts by only a few small bundles of longitudinal fibres inside the circular layer. Occasional fibres may be seen branching from its inner side into the submucosa. On the outer side it is separated from the circular muscle by a layer of connective tissue about equal in thickness to itself.

The middle layer is the most uniformly developed. It is quite continuous and complete throughout the length of the intestine, though it may vary much in thickness. In the posterior part of the intestine it divides into two layers of which the outer one is the heavier. Between the two parts there is a wide band of loose connective tissue in which is embedded the distal parts of a series of glands, of which mention will be made later.

The outer longitudinal layer, again, varies greatly in its degree of development. It may be a complete continuous layer, usually not as heavy as the circular layer, or it may be quite irregularly developed, being in one part of its circumference as thick as or thicker than the circular layer, while

in another part there may be only a few scattered fibres, or gaps may be found where it is entirely absent.

The serosa consists of a layer of loose connective tissue, carrying the blood and nerve supply for the tract, and is branded on its outer side by a thin peritoneum.

The spiral valve is formed by the infolding of part of the wall of the intestine. In this the mucosa, submucosa, and two inner muscle layers take part. The outer muscle layer takes no part whatever in the valve structure. The mucosa and submucosa are continuous and identical with those layers of the wall of the tract. In the region where the valve joins the wall, the direction of the muscle fibres may become somewhat confused, but in most sections it may be seen with little difficulty that the inner longitudinal and middle circular layers continue into the valve, keeping their same relative positions with respect to the mucosa, and their same directions with respect to the axis of the tract.

In the valve the two parts of the circular layer are separated by a layer of loose connective tissue, which in the thickened region along the free edge of the valve becomes almost mucous in nature. This connective tissue layer carries the blood vessels which supply the valve, and shows also many large lymph spaces and scattered muscle fibres. What appears to be a large lymph sinus passes the entire length of the valve through the centre of the thickened free edge.

The Junction of the Intestine and Rectum.

In this transition region the villi gradually disappear, as also do the folds or grooves of the mucosa, and the larger longitudinal folds of the rectum appear.

There is again an abrupt change from the columnar to stratified epithelium, just as the opposite was the case in the transition from the oesophagus to the intestine. In this case, however, the stratified epithelium, as would be expected, does not overhang the columnar, but rises gradually from it.

The submucosa is continuous with little or no perceptible change. Posterior to the point of the transition in the epithelium the inner longitudinal and the inner portion of the circular muscle layers become scattered and gradually disappear. The glands which will be described later in connection with the rectum are continuous throughout this region. The outer part of the circular muscle gradually becomes much heavier, and the outer longitudinal layer shows a lesser and more gradual increase in thickness.

The Rectum

In the rectum the epithelium is stratified. Posteriorly it is continuous with that of the external surface of the body. The submucosa becomes somewhat thinner. The circular muscle layer is heavy, particularly posteriorly where it forms the anal sphincter. The longitudinal layer disappears towards the posterior part of the rectum.

The stratified epithelium is not of the ordinary squamous type, as the superficial cells do not become flattened but rather remain cuboidal, with the exposed wall thickened and staining darkly.

A series of about 5 or 10 groups of elongated and many-lobuled glands lie embedded in the wall of the tract and form a more or less continuous glandular layer just inside the circular muscle layer of the anterior part of the rectum. They extend anteriorly from their ducts, so that approximately their distal half lies in the wall of the intestine where it divides the

circular muscle layer into an inner and outer portion. The glandular region is about 15 mm. long. The structure of one of these groups is as follows:

At the base of a fold of the mucosa is a pit into which a number of ducts, usually about 4 to 6 empty. The stratified epithelium of the rectum descends into the pit. Its upper layers are continuous with, and gradually change into the epithelium of the ducts, which are composed entirely of mucous-secreting cells. The lower layers underlie this mucous epithelium for some distance, then gradually disappear. This pit, then, may be considered as the primary duct of the gland and the ducts which enter it as the secondary ducts. These latter at once turn anteriorly and proceed more or less parallel to one another, branching, or rather dividing irregularly, and it soon becomes impossible to consider any one of the branches as the main duct for the group. They may all, then, be considered as secondary ducts. These in turn again break up into fine branches which radiate in all directions, and ultimately lead into, or terminate in pits lined with serous secreting cells. The serous pits in turn, may also consist of 2 or 3 or even more forks.

The structure, then, is a series of long ducts, lined with mucus-secreting cells, branching profusely and irregularly, and ultimately serving to collect the secretion produced by the many lobules of serous glandular tissue.

The serous tubules in cross section show about 6-12 cells around their circumference. The cells are cuboid in shape, with fairly large round nuclei placed centrally. The lateral walls of the cells are scarcely perceptible, and those of the basal and exposed ends of the cell are very thin. Adjacent tubules are separated by a thin connective tissue membrane.

The cells of the ducts are elongated and have definite, clearly visible walls. The nucleus is, as a rule, pressed to the base of the cell by the droplets of secretion which fill the greater part of the space within the cell.

PLATICHTHYS STELLATUS - The Starry Flounder

ANATOMY.

The tract of P. stellatus shows five definite regions, of which the lengths in a specimen 48 cm. long were as follows: bucco-pharyngeal cavity - 11 cm., oesophagus and stomach - 10 cm. (3.5 + 6.5) intestine - 38 cm., rectum - 6 cm. The measurements of the oesophagus and stomach are given together, as it is impossible to detect any definite point of junction when viewing the external surface of the tract. Internally a region of gradual transition is distinguishable, and in the specimen measured this showed the oesophagus to be about 3.5 cm. in length, and the stomach therefore, about 6.5 cm. The oesophagus and stomach pass posteriorly in the dorsal part of the body cavity. The junction with the intestine is marked by a constriction and the wall of the intestine immediately becomes much thinner and the tube greater in diameter. From the pylorus the intestine passes ventrally and turning dorsally becomes considerably coiled before joining the rectum. The large bile duct is short and enters the ventral side of the intestine (about 1 cm. from the pylorus in the specimen measured). The junction of the intestine and rectum is also marked by a slight constriction and the diameter of the rectum is again greater than that part of the intestine to which it is joined. In keeping with the lateral compression of the fish, the rectum is also flattened and the anus

is an elongated slit. In the specimen measured the anus was 11 cm. from the mouth.

HISTOLOGY.

The tract throughout its length shows four layers, viz., mucosa, submucosa, muscularis, serosa. Considerable variation in the arrangement and structure of these parts is found in the different regions of the tract.

The Oesophagus.

The oesophagus when contracted, is thick-walled. It is, however, capable of great distension and to provide for this the mucosa is thrown into deep longitudinal folds. These primary folds, in turn, have their sides again thrown into a number of smaller irregular secondary folds (usually 5 - 8 on each side of the primary fold).

The necessity of this provision for great expansion is readily understood because the food of this species consists chiefly of crabs and clams or other molluscs, often large in comparison with the size of the fish, and which are as a rule ingested whole. This fact also accounts for the highly mucous nature of the oesophageal epithelium.

The epithelium of the oesophagus is an active mucus-producing tissue. When fully functioning it appears, under a low magnification, to be composed almost completely of goblet cells. To correctly interpret the structure, a portion of the epithelium which is not actively secreting must first be observed. In such a region it is seen that the structure is pseudo-stratified in nature. The cells are tall and closely compressed. Their lateral and basal walls are very thin, but that of the exposed end is heavy. The nuclei are normally ovoid, but may be spindle-shaped, due to the lateral pressure. They

are scattered at various levels, usually being so arranged as to allow the cells to fit together as closely as possible. Clearer spaces appear in the middle and upper parts of the cell where the secretion is being formed. In a region which is in an active state of secretion, the structure is more difficult to determine, owing to the fact that the cell boundaries are very indistinct and the nuclei are irregularly scattered and mis-shapen and may be more or less obscured by the droplets of secretion forming within the cells. However, by following along the epithelium from a portion which is quiescent to a portion which is secreting actively, it will be seen that as mucus is formed in the middle and upper parts of the cell, it forces the nucleus to the lower part and causes it to become flattened. The mucus forming in this way and rising to the exposed surface in a number of cells in close proximity to one another, forces the intervening cells downward and distorts them into irregular shapes. The nucleus is usually involved in the distortion, becoming more or less rounded or irregular in shape.

The submucosa is quite typical, consisting mainly of fibrous connective tissue carrying blood vessels, and also small scattered groups of longitudinal muscle fibres which have evidently wandered away from the muscularis. The blood vessels are small and scattered and mostly comparatively heavy walled, but a few sinuses may be observed which are bounded only by the network of connective tissue fibres.

The submucous tissue in the folds of the tract is not very extensive, being merely enough to support the mucosa and carry the necessary blood and nerve supply. The basement membrane is very slightly developed. A denser region immediately under the epithelium may be considered as representing a tunica propria. It gradually merges into the typical submucous structure

without showing any definite line of demarcation.

The muscularis consists of an inner longitudinal and an outer circular layer. The fibres are of the striated kind. When the oesophagus is contracted both layers appear comparatively heavy. The outer circular layer is quite uniform in development, but the inner longitudinal is more irregular. It may be heavily developed in parts of its circumference, where its bundles may extend in a scattered manner through the overlying submucosa, reaching, in some cases, into the longitudinal folds. In other parts it may be very thin or even discontinuous.

The serosa is a very thin connective tissue layer - little more than is necessary to support the blood vessels and nerves which supply this part of the tract. The peritoneum consists of a single layer of thin, flattened cells.

The Junction of the Oesophagus and the Stomach.

Towards the posterior end of the oesophagus the secondary longitudinal folds decrease and disappear, and the primary folds are continued into the much heavier longitudinal folds of the stomach.

The transition point from the oesophagus to the stomach is marked chiefly by the cessation of the actively secreting epithelium of the oesophagus and its replacement by the columnar epithelium of the stomach. Occasional large goblet cells may be found in the anterior part of the stomachic epithelium, so the transition is not altogether an abrupt one.

The gastric glands appear almost immediately after this transition region.

The submucosa is continuous with little or no perceptible change, other than a slight increase in the blood supply, particularly in the number of sinuses, and the appearance of the gastric glands in the upper part

of the tissue.

In the muscularis, on the other hand, there are definite changes in structure. The muscle fibres change from the striated to the smooth variety and the relative position of the two layers becomes reversed. Both layers also become less heavily developed.

The outer circular muscle of the oesophagus continues as a heavy layer for some distance beyond the transitional point of the epithelium and ends abruptly. The striated fibres of the inner longitudinal layer in this region gradually become replaced by smooth fibres, the two kinds mingling considerably in the transition. Posterior to the cessation of the striated circular muscle of the oesophagus, the longitudinal layer is left as the outer muscle layer of the stomach, and the circular smooth muscle layer appears inside.

In this transitional region the longitudinal muscle layer may split and surround a small quantity of circular muscle which is composed as a rule, of intermingled smooth and striated fibres.

In short, there is in the muscularis a region where there is an intermingling in regard to both the kind and the direction of the fibres.

The Stomach.

The internal surface of the stomach has a darkened brownish tint in its anterior and middle regions, but this is not evident in the pyloric part. The whole inner surface of the stomach is thrown into longitudinal folds, much heavier than those of the oesophagus, and which may have a zig-zag course when the organ is contracted. In the pyloric region they become smaller. To the unaided eye their surfaces appear quite smooth, but under a medium nagnification they are seen to have a netted appearance. This is due to the presence,

over the whole surface, of innumerable small pits or furrows. They are very narrow and usually 2 to 6, or even more, times as long as they are wide, and may give off short branch furrows on either side. The gastric glands empty into the bottom of these pits.

The epithelium of the stomach is simple and columnar. The cells are tall and narrow and closely pressed together. The lateral and basal walls are distinct, but extremely thin. The exposed wall is heavy and very finely ciliated. The nuclei are ovoid or more elongated, depending on the lateral pressure on the cell. The cytoplasm is quite uniformly granular, or may be more dense near the free end of the cell.

The gastric glands are of the compound tabular type. Their ducts empty at the bases of the pits of the epithelium, there being usually from 2 to 6 to each pit. The columnar epithelium of the surface lines the pits and may descend a short distance into the duct of the gland. It is shortly replaced, however, by clear mucous cells. The ducts may divide into from 2 to 4 branches. The distal end of each duct is continued into a small number of tubules (usually 2 to 4) which are lined with serous secreting cells.

The mucous cells of the ducts are comparatively large and cuboid. When filled with their mucous secretion, they stand out very clearly in the sections. Their walls are quite definite and the nucleus can usually be observed flattened against the base or corner of the cell.

The lumen of each tubule is very small in diameter. The serous cells forming their walls are much smaller than the mucous cells of the ducts. They are cuboid or slightly flattened and have extremely thin walls. The cytoplasm is finely granular and stains densely with eosin. Their nuclei are comparatively large and rounded and rest in the lower part of the cells. Each tubule is

surrounded and separated from its neighbors by a very thin sheet of connective tissue.

The submucosa is quite typical of this kind of tissue. Thin walled blood and lymph sinuses are somewhat more numerous than in the oesophagus. Smooth muscle fibres are scattered throughout, particularly in the region near the mucosa.

The two muscle layers are continuously developed throughout the stomach region. Anteriorly the two layers are of about equal thickness. The outer longitudinal layer is fairly uniform over the whole organ, but the inner circular layer gradually increases as it passes towards the pyloric and where it may be several (6 to 8) times as thick as the longitudinal layer, and where it takes an important part in the structure of the valve of the pylorus.

Junction of the Stomach and the Intestine.

The junction of the stomach and the intestine is marked by a well developed pyloric valve. The large folds of the stomach cease at the beginning of the valve structure, and are replaced by much smaller ones, which pass over the valve and on its posterior side graddially break up into the zig-zag willow-like structure of the intestine.

The gastric glands disappear shortly before the beginning of the pyloric valve. The valve itsels is a simple elevation of the wall of the tract in which the mucosa, submucosa and circular muscle take part. The epithelium is continuous over the valve, the beginning of the intestinal mucosa being marked by the appearance of goblet cells near the distal end of the valve. The submucosa is continuous without change. The circular muscle, which is already a heavy layer at the posterior end of the stomach, is still more in-

creased under the base of the valve and gives off a branch which extends nearly to the distal end of the latter and acts as a sphincter for its control. Posterior to the base of the valve the heavy muscle layer changes very abruptly to the much thinner intestinal layer, which continues from its outer part. The outer longitudinal muscle is continuous throughout the region, but becomes somewhat reduced in thickness. A portion of the inner side of the longitudinal layer may be carried a short distance into the valve and probably acts as a dilator to open the valve and allow the passage of food material.

The Intestine.

The wall of the intestine is much thinner than that of the oesophagus and the stomach. Its mucosa is thrown into numerous narrow longitudinal folds which follow a regular zig-zag course throughout its length. The fact that the folds are partially interrupted at each of the zig-zag turns gives the impression at first, that the structure is really that of leaf-like villi. These inter-ruptions, however, do not usually pass to the base of the folds which, therefore, are continuous, and the structure is not one of villi, though it no doubt represents a step towards their development. The sides of the folds are again much folded in the plane perpendicular to the wall of the intestine. The result is that a maximum amount of surface is presented to the food material in the tract. The folds gradually decrease in size as they pass towards the posterior end of the intestine.

The mucosa of the intestine consists of simple columnar epithelium with many goblet cells scattered throughout. As in the oesophagus a region of the sub-epithelial tissue immediately under the epithelium is more compact than that farther removed, and may be considered as having the function of the tunica propria of the higher vertebrates. The epithelial cells are closely compressed

when the tract is contracted and the nuclei are arranged between the middle and basal portions of the cells. The cytoplasm is quite uniformly granular. The lateral cell walls are extremely thin, while the basal walls are more distinct. The wall of the exposed end is heavy and very finely ciliated.

The submucosa is quite typical of this kind of tissue. It forms a thin framework for the folds of the mucosa, and is a comparatively thin layer between it and the muscularis.

The muscularis consists of an inner circular and an outer longitudinal layer, and is comparatively heavy. Both layers are developed quite uniformly throughout the length of the intestine. The inner circular layer is much the heavier of the two. The two layers of muscle are separated by a thin layer of connective tissue in which are imbedded the larger nerves supplying the intestine.

Junction of the Intestine and the Rectum

The transition from the intestine to the rectum is also marked by a well developed circular valve. At the beginning of the valve structure the zig-zag folds of the intestine become replaced by numerous narrow, straight, longitudinal folds, which come to an end at the free edge of the valve. The surface of the posterior side of the valve is similar to that of the rectum.

Longitudinal sections of the region show that the structure is nearly identical with that of the pyloric valve. The mucosa is continuous over the whole surface with no change in its intimate structure. The rest of the structure resembled so closely that of the pyloric valve that further description is unnecessary.

The Rectum.

The surface of the rectum is slightly folded or undulating, and has a

"pebbled" appearance due to its being covered with short, closely packed villi. At its posterior end there is again a valve developed.

The mucosa and submucosa of the rectum are practically identical with that of the intestine. The circular muscle, which is comparatively heavy throughout the rectum, is much increased at its posterior end to form the anal sphincter. The outer longitudinal muscle does not extend as far as the circular, but towards the posterior end it becomes scattered and disappears.

Posterior to the valve the goblet cells disappear from the epithelium of the mucosa and this columnar epithelium is shortly replaced by an abrupt change into the stratified mucous epithelium of the epidermis.

LEPIDOPSETTA BILINEATA - The Two-lined Flounder

ANATOMY.

Anatomically Lepidopsetta bilineata differs from Platichthys stellatus chiefly in having a series of four caeca given off from the anterior end of the intestine.

In a specimen 27.5 cm. long the various regions measured as follows: bucco-pharyngeal cavity - 4 cm., oesophagus - 1.7 cm., stomach - 5 cm., intestine - 29 cm., rectum - 1.8 cm., caeca - each about 2 cm.

The short oesophagus, leads into the larger stomach, which passes backward and turns ventrally to conform with the shape of the body cavity. The junction of the oesophagus with the stomach is marked externally only by an increase in diameter of the latter. At the pylorus the stomach again narrows and leads into the intestine, the junction being marked by a slight constriction.

Almost immediately three of the intestinal caeca are given off - one

dorsally and two ventrally. Between the two ventral cases the bile duct enters. The fourth caecum is given off venttally behind (1.3 cm. in the specimen measured) the others.

The junction of the intestine with the rectum is again clearly marked by a slight depression encircling the tract. The rectum is slightly greater in diameter than the intestine.

HISTOLOGY.

The Oesophagus.

The mucosa of the oesophagus is longitudinally folded as in Platichthys, but the secondary folds are not so marked.

The epithelium resembled that of Flatichthys in that it is pseudo-stratified and mucus-secreting. Since the longitudinal folding is not so great as in Platichthys, the cells are not so much compressed laterally, when the tract is contracted. The cells are heavier-walled than those of Platichthys, and the mucus-secreting function is not nearly so well developed, consequently the pseudo-stratified appearance is more regular throughout.

There is no definite basement membrane. The tunica propria is, as in Platichthys, composed of closely-packed connective tissue fibres and it gradually merges into the looser structure of the submucosa.

The arrangement and structure of the layers of the muscularis is similar to that of Platichthys, except that the inner longitudinal layer is less developed and disappears before reaching the posterior end of the oesophagus.

The serosa, like that of Platichthys, is very thin.

Junction of the Oesophagus and the Intestine

The folds of the oesophagus pass gradually into the larger longitudinal folds of the stomach. The secondary folds disappear and the mucosa becomes typically pitted.

Microscopically the transition from oesophagus to stomach is not clearly marked but is more or less gradual. It is true there is an abrupt change from the pseudo-stratified mucous epithelium of the oesophagus to the columnar epithelium of the stomach, but mucous cells are scattered for some distance into the latter.

The gastric glands do not appear for some distance beyond this region of transition in the epithelium, then they appear gradually. The submucosa is, of course, continuous throughout with little or no change.

The inner longitudinal muscle disappears, as before mentioned, before the stomach is reached. The outer circular layer on the other hand, is continued a considerable distance into the stomach, forming the only muscular coat for about the first quarter or third of its length. It tapers off more or less gradually, and with a slight mingling of the two kinds of fibres, the thinner circular smooth muscle of the stomach continues from it. The outer longitudinal layer of smooth muscle appears as scattered fibres in the connective tissue of the serosa, slightly anterior to this region, and from the point where the circular smooth muscle begins it forms a definite, though thin layer.

The Stomach.

The stomach differs little in structure from that of Platichthys. Throughout the greater part of its wall are imbedded glands practically identical in structure with those of Platichthys. These, however, do not extend to its extreme anterior end, but appear some distance back, first as scattered

groups of ill-developed glands, gradually increasing in number and size till the normal development is reached. Towards the posterior end of the stomach the glands gradually disappear in a similar manner.

The submucosa presents no notable difference from that of Platichthys.

The muscle layers are quite uniformly developed throughout the greater part of the organ, the inner circular layer being from twice to three times as thick as the outer longitudinal layer (the latter having an average thickness of 8 to 10 cells). Towards the pyloric and the circular muscle increases more abruptly than in Platichthys.

The larger nerves supplying the organ are imbedded in the connective tissue layer which separates the two parts of the muscularis.

Junction of the Stomach and the Intestine.

The stomach becomes much narrowed posteriorly where it passes into the intestine. There is no fold in the mucosa to form a pyloric valve as in Platichthys, but the wall of the tract becomes much thicker and firmer, due, as will be shown later, to a great increase in the thickness of the circular muscle layer. The large longitudinal folds become correspondingly smaller and probably gradually change into those of the intestine.

The pits of the stomach are continued to the transitional region, although the gastric glands disappear before this point is reached.

The transition from stomach to intestine is marked chiefly by the appearance of a great proportion of goblet cells in the epithelium and a great increase throughout the region, in the development of the circular muscle, which forms a pyloric sphincter. The longitudinal layer gives off from its inner side, fibres which pass into the circular layer and serve to dilute the tract when necessary for allowing food material to pass. Posterior to this region

it is a much thinner layer. The submucosa becomes much reduced.

The Intestine.

The mucosa of the intestine is arranged in zig-zag folds, much as in Platichthys. The folds themselves, however, are comparatively heavier and deeper than those of Platichthys.

The epithelium of the intestine is practically identical in structure with that of Platichthys. It is ciliated and highly mucous. The submucosa is reduced almost to a minimum. Of the two muscle layers, the inner circular is again much the heavier. This layer is particularly well developed from the beginning of theintestine to the region just beyond the posterior ventral caecum. Its thickness in this anterior region is about equal to the height of the folds of the mucosa when the tract is wholly contracted. Posterior to the caecum mentioned, it becomes reduced to about half this thickness. The outer longitudinal layer is very thin , being for the most part only 2 - 4 cells in thickness. It forms, however, a continuous unbroken coat.

The Caeca.

The surfaces of the caeca are like that of the intestine, except that the folds are comparatively narrower and deeper, and show more tendency to branch and join with one another.

Histologically they do not appear different from the anterior part of the intestine from which they branch.

Junction of the Intestine and the Rectum.

The junction of the small intestine and the rectum is marked as in Platichthys, by a well developed valve. The folds of the intestine are continued over the valve into the rectum.

In the valve structure the mucosa, submucosa and circular muscle take part. There are no fibres passing from the outer longitudinal layer into the valve structure with those of the circular muscle as was the case in Platichthys. The circular muscle, however, passes into the valve, but quickly disappears as such and gives rise to a structure composed of intermingled groups of fibres, some having a circular, others a radial direction with respect to the longitudinal axis of the tract.

The Rectum

The rectum, when contracted, shows a few large longitudinal folds which are in turn thrown into smaller folds, continuous with those of the small intestine. When distended, the large folds, of course, disappear.

The epithelium of the rectum does not differ from that of the small intestine. At the posterior end the goblet cells gradually disappear from the columnar epithelium, which also loses its cilia and is replaced by the mucous epithelium of the skin.

The submucosa presents nothing exceptional.

Both layers of the muscularis are much increased in the rectum, each being 2 to 3 times as thick as in the small intestine. Posteriorly the longitudinal layer gradually disappears, while the circular layer, as in Platichthys, is much increased, forming an anal sphincter.

CYMATOGASTER AGGREGATUS
The Shiner

ANATOMY.

The tract of Cymatogaster aggregatus is characterized by the absence of a stomach. In a specimen of average size (14 cm.) the lengths of the various regions were as follows: bucco-pharyngeal cavity - 3 cm., oesophagus - .7 cm., intestine - 16 cm., rectum - 2 cm.

From the pharynx the tract makes a single loop and leads to the rectum which passes postero-ventrally to the anal opening.

The junction of the small intestine and rectum is marked exteriorly by a slight constriction.

HISTOLOGY.

The Oesophagus.

The inner surface of the oesophagus is folded longitudinally. The folds are numerous but not deep, and their free edges are arranged in small lobules, or pimple-like elevations.

The wall consists of the usual layers, viz: mucosa, submucosa, muscularis and serosa.

The mucosa consists of an epithelium with a definite basement membrane and a tunica propria. The epithelium is thick and highly mucus-secreting, and appears, when active, to be composed almost entirely of goblet cells. It is pseudo-stratified in nature. The cells are large and elongated, with clearly marked walls. The nuclei are comparatively small and usually crowded into the lower part of the cells by the secretion. It is evident that there must be much erosion of the epithelium by the food material. The vast production of mucus

for the lubrication of the surface suggests that the food must be very rough in nature - which, in fact, is the case. The fact that new epithelial cells are produced in great numbers in the germinative region at the base also suggests that constant renewal is necessary. In addition to the destruction of cells by erosion, it is also quite probable that the cells do not regenerate after they have produced and discharged their secretion. In many places the epithelium presents a more or less stratified appearance, but without the flatenning of the surface cells that is usually found in this kind of epithelium. The explanation would seem to be that the epithelium here has discharged its secretion and been eroded, then has been replaced from the germinative region without sufficient time having elapsed for the production of secretion.

Scattered throughout the mucous epithelium are small islands of non-mucous, low columnar (in places almost cuboidal) epithelium. The boundaries of the cells in these regions are quite distinct, though the cell membrane is extremely thin. The wall of the exposed side of the cells is slightly heavier. The cytoplasm is uniformly finely granular, the nucleus round or ovoid and situated in the lower part of the cell. The boundaries of these islands are sharply marked, there being no indication of a gradual transition from their columnar structure to the normal pseudo-stratified structure of the oesophagus.

A definite basement membrane supports the epithelium.

The tunica propria may be considered as being represented by the more compact connective tissue immediately underlying the epithelium. It gradually becomes looser in structure and merges into the submucosa without any definite line of demarcation.

The submucosa is quite typical of this kind of tissue. It is highly vascular, due, probably, to the need of a rich supply of materials from which to build the enormous amount of mucous secretion produced. A noticeable feature

is the great number of leucocytes scattered throughout the meshes of the connective tissue.

The muscularis consists of an inner longitudinal and an outer circular layer. The inner layer is comparatively thin, but is a continuous though not a compact coat. The outer circular layer is also somewhat loosely constructed. Anteriorly it is a very heavy coat, being several times as thick as the remaining part of the wall. In passing posteriorly it rapidly decreases, till at the posterior end it is only about double or equal in thickness to the rest of the wall. The fibres are of the striated variety throughout.

The serosa is a very thin connective tissue layer, covered with a thin peritoneum of much-flattened cells.

Junction of the Oesophagus and the Intestine.

In this region the folds become smaller and continue into the folds of the intestine.

Microscopically there is no sharp transition involving all the layers. In longitudinal sections of this region the epithelium shows an abrupt change from the pseudo-stratified structure of the oesophagus to the simple columnar epithelium of the intestine. In the epithelium of the extreme posterior part of the oesophagus there is little mucus produced. The tunica propria and submucosa are continuous throughout. Both layers of striated muscle continue some distance beyond the region of the transition in the epithelium. The inner longitudinal layer disappears gradually, while the outer circular shows a marked increase at its posterior extremity. The thin outer longitudinal layer of smooth intestinal muscle appears immediately after the termination of the circular muscle of the oesophagus, and for a short space is the only muscle layer. The inner circular smooth layer begins abruptly a short distance posteriorly.

The Intestine.

The folds of the intestinal mucosa are not as heavy as those of the oesophagus. In the anterior region they are very irregularly arranged, but towards the middle region of the intestine they take a very regular zig-zag course, which is continued to the posterior end. The wall consists of the four usual layers. It is thin - so thin, in fact, that the epithelium constitutes fully half (and in many places more than half) of its total thickness.

The mucosa consists of a columnar epithelium resting on a well-defined basement membrane. The structure underlying this, and which is ordinarily to be considered as representing the tunica propria and the submucosa, is so thin that it seems of doubtful value to subdivide it into two separate regions.

The epithelium is of the simple columnar ciliated variety, with goblet cells scattered throughout. The cells are tall and narrow, with oval nuclei lying in a regular order in the lower halves of the cells. The upper halves of the cells are quite uniformly finely granular. The lateral cell walls are extremely thin, while those of the exposed ends are heavier and provided with very fine, short and uniform cilia.

In numerous regions throughout the intestine, the epithelium, in the sections examined, was separated from the basement membrane, and pushed some distance away from it by protein food material which had been absorbed by the epithelial cells and passed through them much more quirkly than it could be absorbed and carried away by the lymph stream. So great was this "ballooning" frequently, that the epithelium was ruptured and the food material could be seen passing back into the lumen of the tract through the rupture. This phenomenon is evidently identical with that observed by Macallum (1924) in guinea pigs and rabbits. The absorbed material was distinctly visible as small, closely packed droplets, filling the

space inside the "balloon". Immediately under the base of the epithelium the material could be seen exuding from the cells, that from each cell remaining seperate from that of its neighbors and producing the appearance of a very fine honeycomb.

As stated before, it is of doubtful worth to try to distinguish tunica propria from submucosa, as this sub-epithelial layer is very thin and uniform in structure. It is highly vascular, the larger blood vessels and sinuses causing a local expansion of the layer, and indeed of the whole of the surrounding wall of the tract, in order to accommodate them. The meshes of the connective tissue are more or less completely filled with leucocytes. The two muscle layers are together slightly thicker than the submucosa. Both layers are quite completely and uniformly developed throughout, the inner circular layer being slightly the heavier of the two.

The serosa differs little from that of the region of the oesophagus.

Junction of the Intestine and Rectum.

The junction of the small intestine and the rectum is marked by a definite valve. The longitudinal folds are continued over the surfaces. In the structure of the valve all the layers take part. The epithelium is continuous over the surface without change. The submucosa is, of course, continuous. The muscle of the valve is mostly circularly directed, but at the base of the structure there is a considerable intermingling of both longitudinal and circular fibres. The muscle structure is continuous with the circular layer of the wall of the tract. A few fibres branch off from the outer longitudinal layer to the valve structure.

The Rectum.

The folds of the mucosa of the rectum are practically identically the same as those of the intestine, except that they are slightly larger.

Histologically the rectum is like the intestine, except that the layers, with the exception of the epithelium, are all slightly increased in thickness. The epithelium becomes thinner in the posterior part, the cells gradually becoming shortened and cuboidal. There is a definite point of transition from this to the stratified epithelium of the outside of the body. The goblet cells gradually disappear, as does also the previously well developed basement membrane. The submucosa becomes thicker and there is a slight increase in the circular muscle, which, however, becomes thinner again before it ends more or less abruptly near the point of transition in the epithelium. The outer longitudinal muscle spreads out and disappears gradually.

TAENIOTOCA LATERALIS - The Blue Perch

ANATOMY.

Anatomically the tract of Taenioteca lateralis is practically identical with that of Cymatogaster, though, of course, much increased in size.

HISTOLOGY

The Oesophagus.

The oesophagus is much folded longitudinally and the folds also have the pimple-like elevations along their free edges. Histologically it is practically the same in structure as that of Cymatogaster, the epithelium, however, being comparatively heavier. Islands of columnar epithelium are also

present in the regular pseudo-stratified epithelium of the oesophagus, and in these the cells are comparatively taller and narrower than in those of Cymatogaster, and their nuclei are not so uniformly arranged. The other layers are arranged as in Cymatogaster.

Junction of the Oesophagus and the Intestine.

In the region of the junction of the oesophagus and the intestine the longitudinal folds of the oesophagus disappear and for a distance of 3 or 4 cm. are replaced by a band of finger-like villi projecting into the lumen of the tract. These villi are about .25 mm. in diameter and 1 mm. in length. Histologically they do not differ from the normal structure of this region. Posterior to this band of villi the smaller folds of the intestine appear.

The transition from oesophageal to intestinal epithelium is abrupt. The basement membrane which is extremely well developed in the oesophagus becomes much less so here. There is a slight increase in the sub-epithelial tissue. The inner longitudinal muscle disappears as in Cymatogaster and the outer circular layer is thicker at its posterior extremity. The inner circular muscle layer of the intestine appears immediately following, or just within the posterior end of the circular striated muscle. The longitudinal muscle of the intestine appears as smooth fibres scattered through the posterior part of the circular oesophageal muscle, and which collect to form the layer.

The Intestine.

The surface of the intestine is in very regular zig-zag folds - so regular as to give it the appearance produced by a piece of cloth of "herring bone" weave.

The cells of the epithelium are comparatively taller and narrower than those of Cymatogaster, and are also ciliated. The basement membrane is not nearly as pronounced as in the oesophagus. The sub-epithelial tissue is thicker and a denser portion near the epithelium can be considered as the tunica propria, gradually merging into the submucosa.

In the actively absorbing intestine the epithelium may be separated from the basement membrane not only in small "balloons" but over large areas, and the intervening space filled with absorbed food material.

The inner circular and outer longitudinal muscle layers are both developed uniformly throughout. The inner circular layer is three to four times as thick as the outer longitudinal, and the two together are not as thick as the epithelium, throughout the greater part of the intestine.

Junction of the Intestine and the Rectum.

The junction of the intestine and the rectum is marked by a valve and an increase in the thickness of the wall of the rectum. The folds of the mucosa are continuous and become slightly heavier in the rectum. The arrangement of the layers in the junction region is as in Cymatogaster.

The Rectum.

The anterior part of the rectum is like the intestine in structure. The epithelium of the rectum may also be separated from the basement membrane by absorbed food material.

Posteriorly, in the anal region, the goblet cells disappear from the epithelium, which becomes thinner, due to the cells becoming cuboidal. It is replaced abruptly by the stratified epithelium of the outer surface of the body. The sub-epithelial tissue gradually disappears. The circular muscle is much increased at its posterior end to form an anal sphincter, and the

outer longitudinal layer becomes scattered, the fibres spreading through the tissues of that part of the body wall, and disappearing.

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