ANATOMY AND HISTOLOGY OF THE ALIMENTARY TRACT OF THE KING SALMON

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INTRODUCTORY.

In the older literature one finds discussions of the anatomy, histology, and embryology of a number of species of trout.[a] More recently a brief histological study was made by Gulland on the Atlantic salmon, *Salmo salar*.[b] This study of normal structures is very brief and is given by the author incidental to the presentation of certain degenerative changes in the digestive tract in the Scottish species. Gulland's paper covers only about six pages of printed matter, including both the normal and pathological findings. Previous to the work of Gulland, Miescher[c] published certain gross findings in the Rhine representative of the Atlantic salmon, but he does not offer histological details.

No studies presenting the details of the anatomy and histology of the alimentary tract of the king salmon thus far have been published. In my studies on the function of the digestive tract of this salmon, and in similar studies of the changes that occur during the spawning migration of the species, it was found necessary to examine the normal structural characteristics of this interesting fish. The facts determined in these studies are set forth in the present paper.

The digestive tract of the king salmon is, on the whole, quite similar in its gross anatomy to that of the Atlantic salmon, *Salmo salar*, and also is comparable to the anatomy of the species of trout. The alimentary canal of the king salmon has the relatively simple structure which characterizes carnivorous fishes in general. It consists of the usual parts, i. e., the mouth, esophagus, stomach, and intestine. Of special interest are the numerous diverticula of the small intestine attached to that limb of the intestine which immediately follows the stomach, and known as the pyloric cæca. Figure 3 presents a salmon in outline in which the anatomy of the alimentary tract is shown in its normal relations.

The anatomy of the mouth is not presented in this paper.

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[a] Oppel, Albert: Mikroskopische Anatomie, bd. 1, p. 28, 70, etc., bd. 2, p. 7, etc.

[b] Paton, D. Noël: Life history of the salmon. Article 3, by Gulland, "Minute structure of the digestive tract of the salmon, and the changes which occur in it in fresh water." Report of the Fisheries Board for Scotland, 1898, p. 13.

[c] Miescher, Friedrich: Statistische und Biologische Beiträge zur Kenntniss vom leben des Rheinlachses im Süsswasser. Schweizerischer Fischerei-Ausstellung zu Berlin, 1880, p. 154. Also reprinted in Die Histochemischen und Physiologischen Arbeiten von Friedrich Miescher, 1897, p. 116.

HISTOLOGY OF THE ESOPHAGUS.

The esophagus in the king salmon is a very short muscular tube extending from the posterior part of the pharyngeal cavity to the stomach. The length of the esophagus is only 3 to 5 centimeters, even in the largest salmon.

A cross section through the esophagus shows three coats, of which the thickest and most important is the muscular coat. The lining of the esophageal tube is composed of epithelium which is complexly folded and which rests upon a subepithelial connective tissue foundation.

EPITHELIAL COAT OF THE ESOPHAGUS.

The epithelial coat of the salmon esophagus consists of columnar epithelial cells. In the anterior portion this epithelial layer is supported by one or two irregular layers of deeper cells. This region represents the transitional stages between the stratified epithelium of the mouth and pharynx and the single layer of epithelial cells lining the rest of the alimentary tract. The smaller basal epithelial cells are represented for some little distance down the esophagus, though they are relatively few in number except in the region just described.

Most of the epithelial cells have clear outer margins with a stainable protoplasm and the nucleus crowded to the base of the cell. This is interpreted as meaning that these cells are chiefly concerned with the production of mucus, that they are in fact mucous cells.

The mucous coat is complexly folded. In microscopic cross section the folds are often cut in such a way as to simulate simple tubular glands. When the gross folds are unusually large then these simple tubelike appearances may be located on the sides of the larger folds. This is a mechanical condition which appears later, i. e., especially in the mucous epithelium of the intestine, and in simpler form in the cæca. In all the sections available the esophagus is strongly contracted so that the epithelial coat is thrown into unusually rich folds. The study of cross sections leads one to the view that if the esophagus were fully distended most of these epithelial folds would be eliminated. No differentiation among the epithelial cells of the esophageal mucosa could be discovered.

SUBEPITHELIAL CONNECTIVE TISSUE OR TUNICA PROPRIA.

This coat in the esophagus consists of a simple type of areolar connective tissue. The nuclei are small and oval, the connective tissue strands are open and especially loose in arrangement. Occasional blood vessels are noted in the sections. Just at the base of the epithelial coat the connective tissue is a trifle more dense and the fibers and nuclei are arranged parallel with the inner surface of the epithelial coat. No evidence of smooth muscle fibers or other specially differentiated cells was found in the tunica propria of the salmon esophagus.

MUSCULAR WALL OF THE ESOPHAGUS.

The muscular wall of the esophagus consists of a single circular coat. This is a thick, heavy muscular mass which is composed wholly of striated fibers. These fibers are about 15 μ in diameter. Their length was not determined, but they are relatively long. Numerous nuclei are found along every fiber, each nucleus lying just within the sarcolemma. The nuclei of the striated fibers are oval, but vary considerably in shape and size, averaging 6 by 9 μ. The total thickness of the circular muscle coat amounts to about 200 μ in a young salmon 7 cm. long in which these measurements were made. In the adult fish of course the total mass of the muscle is very much greater.

SEROUS COAT.

The posterior end of the esophagus where it passes over into the stomach is free and therefore covered with a serous coat. The serous epithelial cells covering this coat are very thin, and are of the usual stratified type. No thick subserous connective tissue which characterizes the intestine and cæca was noted in the esophagus. It was noted, however, that numerous blood vessels are present just under the serous coat. This vascular factor characterizes the esophagus in all the salmon studied.

THE STOMACH.

FORM AND DIVISIONS OF THE STOMACH.

The stomach of the king salmon is a U-shaped organ. The first limb of the U extends from the esophagus straight posteriorly a distance equivalent to one-fourth the length of the fish. At this point the stomach makes a sharp bend and extends directly back toward the head for about one-third the length of the first division. In the adult the lengths of these divisions of the stomach vary in proportion to the total length of the fish, but in an average-sized salmon, 80 cm., the length of the first, or cardiac division is from 20 to 22 cm., and of the second or pyloric division, from 6 to 7 cm.

There is great variation in the size of the stomach in feeding salmon from Monterey Bay. In the market there are always individual fishes in which the stomachs are empty and contracted until the gastric cavity is obliterated, i. e., until the mucous folds are in contact and the cavity is a potential one only. In the empty condition the cardiac division of the stomach is a regular cylinder in form and shape. The contracted walls are firm and resistant to the touch, and the total diameter is reduced to 1.5 to 2 cm. The pyloric division is also contracted, is firm to the touch, and its diameter is a trifle less in any given salmon than that of the cardiac division. The mucosa is compressed into great longitudinal ridges or folds. When the stomach wall is split open for its full length and the cavity opened out, then these folds appear as prominent longitudinal ridges.

If only a small amount of food is present in the stomach—for example, a single small fish or a squid—the mucous lining is pressed firmly against this food mass and the gross shape of the stomach is much the same as in the empty organ. But in these instances the mucous membrane is quite red in appearance, as though filled with an unusual supply of blood.

When a large mass of food is present in the stomach and that in an advanced stage of digestion, then the muscular walls are greatly relaxed, the stomach is more flaccid, and its diameter is enormously increased. The mucous coat is then quite free from folds and the whole wall is relatively thin and pliable. In this condition the capacity of the stomach is at its maximum. In another connection I have called attention to the finding of as many as 18 squid besides several small fishes in one such dilated stomach. In such an instance the diameter of the stomach is greatly increased, but the length remains quite constant.

COATS OF THE STOMACH WALL.

The anatomical divisions that constitute the stomach wall are essentially the same in the king salmon as for carnivorous fishes in general, the details of the structure of the salmon comparing very closely with the carnivorous type. Beginning with the lining mucous membrane, one may record in their regular order the following coats:

- I. Mucosa.
  1. Epithelium.
  2. Tunica propria.
  3. Stratum compactum.
  4. Stratum granulosum.
  5. Muscularis mucosa.
- II. Submucosa.
- III. Muscularis.
  1. Muscularis circularis.
  2. Muscularis longitudinalis.
- IV. Serosa.

The stomach of the king salmon is characterized by the extensive differentiations of the mucosa, which possesses the five coats as outlined above. The structure is noteworthy for three features—first, the great differentiation of the gastric glands in the cardiac region; second, the strong development of the stratum compactum in this species; and, third, for the presence of a constant and definite layer of cells of special structure to be described more fully later as the stratum granulosum. This type of granule cell is present constantly, not only in the stomach but in all divisions of the alimentary canal. Its size and evident importance in the salmon physiology amply justify its description as a definite coat.

HISTOLOGY OF THE GASTRIC MUCOSA.

The epithelial coat of the salmon stomach presents a rather thick layer. A section through this coat will vary according to the location of the area in the stomach from which it is taken. In the cardiac region the coat is thickest, the thickness being due to the presence of the gastric glands. In the pyloric division of the stomach the structure is simpler, as will be described later.

Epithelium of the cardiac stomach.—The cardiac epithelial coat presents on the surface always a layer of slender cylindrical cells of the usual cylindrical or columnar epithelial type. Perhaps these cells are more slender than is noted in many animal species. Where the coat forms sharp folds the cylindrical cells will in cross section of the folds present great fan-shaped masses.

The cells are almost always slender at the base and somewhat broader at the apex of the cell. They vary in size, but average in thickness 3 μ at the base and 6 μ at the free end. The length of the cells of the free surface varies from 33 to 50 μ. The nucleus is in the basal third of the cell. It is a slender oval mass showing a rather close chromatin network. The normal size of the nucleus is on an average 2 by 6 μ. The outer fourth of the superficial epithelial cell is usually paler, contains less stainable protoplasm, and is relatively larger. This zone is one that takes a most active part in fat absorption. The cylindrical cells are not granular. Gulland's[a] figure 8 represents a view of the cells for *Salmo salar*. The whole gastric epithelium is thrown into deep folds in the empty stomach, folds which are more or less obliterated in the actively digesting organ. When the walls are not greatly distended then the folds are deeper and more pronounced, and the superficial epithelial coat and the deeper seated gastric glands are rather distorted in position and appearance. A more normal relation of the parts of the mucosa is presented in the distended stomach, and in such a stomach, moreover, the relations are most readily determined. The most superficial of the cylindrical cells, the ones that form the free surface bordering on the lumen of the stomach, are the longest and most slender. From this surface, extending down into and lining the mouths of the crypts, the cylindrical cells become gradually and uninterruptedly smaller. The only change in type, however, is one of relative size of the cells. The crypts themselves vary greatly in depth, forming longer or shorter tubes into the bottoms and sides of which the gastric glands open. (See fig. 3, pl. XXVI.)

The epithelial cells lining the tubes of the crypts nowhere present abrupt change in structure from the most superficial type. Where the tubes are relatively long the lining cells, presumably the neck cells of Gulland, become short, cubical, and of uniform size. In the bottoms of the crypts the cubical type of cylindrical cell changes quite suddenly to the secreting type. There is no intermediate structurally different type of neck cell in the king salmon as suggested by Gulland[a] for the Atlantic salmon. The term, "neck cell," when it is used, must refer merely to the fore-shortened type of cylindrical cell.

The point at which the transition takes place from the cylindrical to the glandular type is rather difficult to observe, since it seldom happens that a vertical section cuts through this critical region. Gulland presents a figure of such a transitional region for the Atlantic salmon and calls attention to the change in cell type. In figure 3, plate XXVI, I show the relation as it exists in the adult king salmon. The opening between the crypt and the gland duct is distinctly constricted and rarely cut exactly through its lumen, except in stomachs taken at a stage of digestion in which the whole wall is decidedly relaxed. Then the gland mouth may be more distended or relaxed.

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[a] Paton, D. Noël, op. cit., p. 5, and fig. 9.

The superficial epithelial cells undoubtedly serve for the function of absorption throughout the full extent of their surface, even down into the depths of the crypts. This is proven in at least one instance, i. e., for the fats, as detailed in a later report of this series. Fat in process of absorption was found in the cylindrical cells down in the bottoms of the crypts, as well as in those cells on the free surface of the gastric epithelial coat.

The epithelial coat of the cardiac stomach is very thick because of the presence of the numerous gastric glands. The gastric glands are of the tubular type, though they are very irregular as to their position, size, and general arrangement. The glands are diverticula of the superficial crypts described above. Each crypt receives the openings from a number of the gastric glands. The openings are as a rule more numerous and more prominent over the bottoms of the crypts, but many glands open into the sides of and about the rims of the crypts. At the bottom of the average crypt there is a cluster of from three to six glands, the mouths of which open together or sometimes separately into the crypt. In the deeper lying crypts glands are more often to be found opening into the side of a crypt near its middle, or even near its superficial portion, than in those crypts located over the surface of a gross mucous ridge. These latter glands are very short and compact, and they are very irregular in shape and often oval in cross section.

The size of the gastric glands varies greatly, depending on their exact position. The more superficial glands are smaller and the deep ones larger and longer. The variation is due not so much to the size of the gland cells as to the number in the tube, yet there is greater variation in the size of the cells than noted for higher vertebrates.

The gland tubes are not always linear, on the contrary they are bent and very irregular in their extent. In the younger salmon the glands are more tubular as a rule, but older examples are often noted in which the tubes are bilobed at the blind end, or have one or more diverticula in the side walls. A cross section of such a mass of tubes presents very diverse and irregular outlines.

The gland cells are of a single type and are irregularly polygonal in shape, similar to the chief cells in the peptic glands of higher vertebrates. The cells are somewhat larger in the salmon, averaging 12 to 20 μ long by 9 to 18 μ in greatest width. In a cross section of a tube the cells do not approximate each other so closely as in the higher vertebrates, thus forming a wider lumen. (See fig. 4, pl. XXVI.)

The nuclei of the gastric secreting cells are slightly oval, almost spherical and rather large, with a nucleur diameter of from 5 to 6 μ. They are located near the bases of the cells. The cytoplasm of the gastric gland cells is highly granular, being filled with doubly refractive rather small zymogen granules. Special study has not been made of the chemical character of these granules. It has been noted, however, that the granules are relatively very small and that they stain in the characteristic way with eosin and with iron hæmatoxylin. The zymogen granules take these stains lightly and the technique must be followed with considerable care. The granules vary in numbers in the glands of different fishes from the feeding grounds, presumably, as Gulland suggests for *Salmo salar*, in relation to the stage of the progress of secretion and digestion. In certain young salmon [a] taken early in the progress of an active stage of digestion these gland cells are charged with granules throughout their entire cytoplasm. The granules are smaller and less refractive than the granules of the "granule cells" described below.

The vascular supply of the mucosa of the cardiac division of the stomach has not been especially studied by the methods of injection. Enough has been learned, however, from the study of histological preparations to determine that there is a comparatively rich blood supply to the gastric mucous membrane. Small blood vessels are found in the tunica propria just at the bases of the gastric-gland cells, also between the glands themselves. Numerous capillaries are found about the bases of the glands and in the connective tissue supporting the superficial epithelium in the regions lying just under the superficial epithelium and between the crypts, where there is a relatively high content of supporting connective tissue. Numerous capillary nets are noted. Ofttimes in relatively thick sections capillary nets are noted which resemble somewhat the arrangement of smaller blood vessels as demonstrated by Mall.

Epithelium of the pyloric stomach.—In the pyloric division of the stomach the mucosa forms deep folds arranged in a general longitudinal direction. These folds are covered throughout with the cylindrical type of epithelial cell. Those portions of the cells of the folds bordering on the cavity of the stomach are only slightly different from the cells in the deeper grooves between the folds. This difference consists largely in the fact that the surface cells are more slender and somewhat more club-shaped, comparable to the superficial cells in the cardiac region of the stomach. There are no proper gastric glands in this division of the stomach. The pyloric epithelium forms counterfolds with a number of shallow epithelial pits in the first two-thirds of this division of the stomach. These shallow pits in the mucous epithelium are present occasionally on the sides of the walls of the larger longitudinal folds mentioned above. The many apparent pyloric glands, as seen in the transverse sections of the pyloric gastric mucosa, are in reality only transverse sections of the longitudinal folds. Near the pyloric valve, marking the boundary between the stomach and the intestine, the gastric mucosa consists of a series of simple longitudinal folds, and no suggestion of pyloric gland-like pits occurs. The longitudinal folds are permanent ones, a fact indicated by the somewhat longer and more slender cells present on the free borders of the folds. (See fig. 3, pl. XXVI.) This type of structure extends to the circular constriction, the pyloric valve, marking the boundary between the pyloric stomach and the pyloric intestine.[b]

Tunica propria.—The connective tissue of the stomach mucosa, on which the epithelial coat rests, is called the tunica propria. The term is here used to designate all that structure between the basement membrane of the epithelium and the stratum compactum. The tissue forms an open network varying in general outlines and in thickness according to the degree of stretching of the stomach walls. Folds of the tunica extend up into the grooves among the gastric glands, forming a supporting net-

[a] Salmon no. 45 and 46, Pacific Grove, July 10 and 11, 1911. These are the salmon that were artificially fed as described in later paper (now in manuscript).

[b] A view of the pyloric valve is given in a figure in the report dealing with the gross anatomical relations in the salmon. This transverse section is through the body at a plane just anterior to the pyloric valve.

work. This network in certain regions forms strong strands quite to the bases of the superficial epithelial cells. The connective tissue is of the compact areolar type, the fibers of which run in a general way parallel with the stratum compactum beneath. This tissue forms a proper support not only for the epithelial coat, including its glands, but also for its blood vessels and nerves. Numerous small arteries and veins are present in the thicker portion of the tunica, and branches from these form a capillary network in the meshes among the glands, as previously stated. The type of connective tissue cell present here calls for no particular comment, yet there are a number of cells of the type described below as granule cells present just within the stratum compactum. In the normal tissue of the tunica these granule cells are not numerous, though in the degenerated tissue, as will be described in a later report, they become more numerous in this region.

Stratum compactum.—A structure of peculiar type and significance because of the fact that it is not uniformly present in the walls of the alimentary canals of vertebrates is the stratum compactum of Oppel. In the salmon stomach this tissue forms a well-developed sheath. It is not in so compact a mass as in the intestine and in the cæca described below. Gulland [a] says of *Salmo salar*, "This layer in whatever plane the stomach is cut is always found as a compact hyaline band lying rather nearer the muscularis mucosæ than it does to the fundi of the glands. It is, of course, pierced by blood vessels, etc., but I have never seen muscle strands from the muscularis mucosæ passing through it. It contains no nuclei, and no structure can be made out in it by ordinary methods. Nuclei lie upon it, however, and the fibers of the connective tissue on either side are directly continuous with it (fig. 10)." This characterization of the *Salmo salar* stratum compactum applies very well to the stratum of the king salmon. In the king salmon there is the one thick sheath, very heavy and well marked and of wavy outline. This thicker sheath lies toward the inner or epithelial side of the gastric wall. However, in transverse sections it is always noted that the main portion of the stratum bears a network of strands and fibers of the characteristic staining reactions located both on its inner and outer surfaces. The network on the outer surface is of heavier strands and is about twice as extensive as that on the inner surface. This network of fibers runs in a general circular direction.

The substance of the stratum compactum takes a Mallory connective tissue stain. It is shown by this stain to be a homogeneous, compact, nonfibrous mass, evidently of collaginous material. Iron hæmatoxylin stain also fails to reveal fibrous structure. Lying on the surface of the strands one notes here and there connective tissue corpuscles. The surface is sharply marked by a substance or structure which takes certain stains somewhat more sharply than the central portion of the stratum. Occasional nuclei are embedded in this superficial layer.

In the open meshes of the stratum there are always found a number of cells of the type designated in this paper as granule cells. These cells form a part of a special coat, the stratum granulosum. (See fig. 3, pl. XXVI.)

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[a] Paton, D. Noël, op. cit., p. 14.

Stratum granulosum.—There is present throughout the alimentary tract of the king salmon a special type of cell forming a distinct layer or coat. Throughout the preliminary studies this layer has been designated the granule cell layer, on account of the distinctive characteristic of the cells composing it. Oppel has described a stratum granulosum for the mucosa of certain animals, and while the homologies are not certain in the king salmon, I propose to use this designation as a permanent name for the structure in question present in *Oncorhynchus*.

The granule cell layer is a part of the mucosa. In the salmon stomach its cells lie between the stratum compactum and the muscularis mucosæ and in the meshes of the stratum compactum itself. The granule cells form quite a dense and well-marked coat, not only in the stomach but in the intestine and cæca. However, the boundaries of this coat are not sharp on either the inner or outer margin. On the inner margin cells of the coat are found scattered among the inner meshes of the network of the stratum compactum and occasionally in the tunica propria. The outer margin or boundary of the zone is sharper. The inner margin of the muscularis mucosæ marks this boundary. Yet a few scattered granule cells are found in the connective tissue of the submucosa outside the muscularis proper.

The arrangement of the cells about the surface of the layer, also the occasional displaced granule cells, suggest that these cells may be amœboid and migratory. This I believe to be true to a very limited extent. But I have not yet succeeded in observing amœboid activity in teased living cells. In fasting salmon, however, I find extensive migration of the cells into the tunica propria on the inner border and into the muscular coats on the outer border of the stratum granulosum. This observation is especially striking in the degenerating cæca. The granule cells, however, are never found far removed from the proper location of the stratum. They have not been observed in the blood vessels, in the liver, or in any skeletal muscle tissue.

The granule cells are supported by a delicate meshwork of fibrils of the histological type and staining properties of the stratum compactum. There is no distinct pattern or type of arrangement of the cells. That portion of the layer just within the muscularis is from four to eight cells thick. Many blood vessels pierce this coat on the way to the deeper structures. But ordinary histological methods do not reveal any definite vascular supply to this structure. It seems to derive its nutritive materials from the capillaries of the adjacent muscles and of the tunica propria.

The granule cells are irregularly oval in outline, varying in size between 6 by 9 μ and 7 by 12 μ in diameter. The nucleus is always eccentric in position and relatively small in size, 2.5 by 4 μ in diameter.

The special and characteristic feature of the structure of these cells is the presence of the cytoplasmic granules that always crowd them, uniformly filling the cell body. The only differentiation noted in this regard is that those cells most free and isolated from the stratum proper have somewhat fewer granules.

The granules stain deeply with a number of dyes, such as eosin, orange G, acid fuchsin, etc. The sharpest differentiation is obtained by staining with iron hæmatoxylin, preferably after Bensley's corrosive bichromate fixative. The iron hæmatoxylin, differentiated to just the right degree, stains the granules an intense bluish black, while the rest of the cytoplasm is clear or may be counterstained with eosin. The cell granules are relatively uniform in diameter. In many preparations one can distinguish only a slight variation in the size of the granules. In certain preparations, however, there is considerable variation in the diameters of the granules within a single cell, a variation that is uniform throughout the cells of a given stomach. The size of the granules in a series of specimens measured varied around 0.8 to 1 μ in diameter. The extremes noted in these preparations were 0.4 and 1.2 μ, respectively. In several samples where the granules were especially uniform in diameter and in which the granules crowded the cell cytoplasm for its full extent the size ran from 0.4 to 0.5 μ in diameter.

The salmon pancreatic cells and the gastric gland cells also contain granules, zymogen granules. But the number and arrangement of granules in each of these two types of cells is characteristically different from that of the granule cells proper. Measurements show that the zymogen granules of the pancreatic cells also vary through a rather wide range. In sections from the same salmon from which the measurements of granule cells given above were taken the pancreatic zymogen granules measure from 0.6 to 1.5 μ in diameter. The number of granules in the individual cells was small and the granules were located chiefly on one side of the cell, presumably the side next the lumen. In another fish the granules of the cells of the pancreas measured from 0.4 to 1 μ in diameter. The loading of granules was somewhat greater in number than in the cells with the larger granules.

The granules of the granule cells take stains somewhat differently from the granules of either of the two types of secreting cells under comparison. The pancreatic cells and the granule cells of the pyloric cæca present in the same microscopic field are always in sharp contrast. The stain of the granules of the granule cells is sharper, more distinct and brilliant, and of a different shade of color when both are stained with the same dye.

Evidence of solution of the granules of these peculiar cells has been observed in the preparation of tissues by certain fixatives. Unfortunately this factor has not been investigated sufficiently for final report.

The character of the distribution of the cells, the staining properties of the granules, and the persistence of these cells during marked degenerative changes of much of the structure of the stomach and other portions of the alimentary canal all point to some function of special nature. This function, I believe, is that of internal secretion. The granules present are, therefore, true zymogen granules. In another connection I have presented the view that this function is one of lipase production. The absence of any tubular arrangement or ducts, and the apparent lack of an adequate capillary net would suggest that the granule cells must discharge their products directly into the surrounding tissue spaces from which distribution takes place.

The granule cells were observed by Gulland.[a] He figures a bit of the stratum compactum and adherent granule cells in its meshes. Gulland calls these cells "large

[a] Paton, D. Noël, op. cit., pl. 4, fig. 10.

eosinophile leucocytes." This identification will not apply to the cells of the king salmon for the following reasons: First, the salmon leucocytes are very much smaller than the cells in question. Also one never finds a cell of the granule-cell type inside a blood vessel, though diligent search has been made. Second, the granule cells form a distinct and persistent structure in a definite region of the stomach and alimentary tract. It is out of the question that the eosinophiles of the blood should form such a cell grouping in every animal of whatever age.

Muscularis mucosæ.—The muscularis mucosæ is present in the salmon stomach. It consists of a rather thick double layer of muscle fibers, relatively much thicker than the corresponding coat in the mammalia. The fibers run both in the circular and in the longitudinal direction. There is much irregularity in the direction of the fibers, due in part to the fact that the muscularis mucosæ follows the folds of the mucosa rather than the outlines of the outer and heavier coats of the stomach. There are many free bundles of fibers more or less irregularly disposed in the folds of the submucosa and on the external or outer surface of the muscularis.

In the contracted stomach, in which the mucosa is thrown into deep longitudinal ridges, a cross section shows that the muscularis mucosa takes a somewhat sinuous or wavy course in its position between the mucous epithelium and the outer muscular coats. In places the muscularis will be in contact with the inner or circular muscle coat, where its thickness is shown to be from 40 to 60 per cent as great as that of the muscularis circularis itself. In the loops of connective tissue between two such points of contact between the two muscular coats there are always found the free or isolated bundles of muscles mentioned above. These isolated bundles unquestionably belong to the muscularis mucosæ.

HISTOLOGY OF THE GASTRIC SUBMUCOSA.

The submucosa of the king salmon forms an irregular connective tissue coat between the muscularis mucosæ and the muscularis circularis. In places the submucosa seems quite obliterated and the two muscular coats are in contact. Under the deeper longitudinal folds of the mucosa the submucosa forms quite extensive masses of loose areolar connective tissue. Evidently in the salmon stomach this layer forms an adaptable coat for allowing the mucosa to slide over the inner surface of the muscularis circularis or vice versa.

The submucosa contains in its meshes numerous blood vessels of different sizes. There are also isolated bundles of muscle fibers from the muscularis mucosæ, and a sprinkling of granule cells that have wandered from the stratum granulosum.

HISTOLOGY OF THE GASTRIC MUSCLE COATS.

The proper muscle coats of the salmon stomach are two in number, the circularis and the longitudinalis. Between the two, and rather more deeply embedded in the longitudinalis, is the plexus of Auerbach, which should be mentioned in this connection.

Muscularis circularis.—The inner of the two muscle coats is the circular layer. It is a well-developed sheet of muscle in the salmon stomach, as shown in every cross section of the gastric walls. It is about twice the thickness of the longitudinal muscle sheet.

The circular muscle is composed of smooth muscle fibers in the pyloric and lower three-fourths of the cardiac division of the stomach. Striated muscle cells extend down from the esophagus for some little distance, about one-fourth the length of the cardiac end. The point has been determined only in the young, where the striated fibers extend well below the esophageal boundary of the gastric gland region. There is no special peculiarity of structure of the gastric smooth muscle fibers. A rich vascular supply permeates this muscle coat, the blood vessels being for the most part small branches of the vessels lying between the two muscle coats.

Muscularis longitudinalis.—This is the outer of the two muscle coats of the stomach. It is well separated from the circularis by the numerous blood vessels and bundles of nerves of the Auerbach plexus, together with the connective tissue supporting these structures. These smooth fibers are rather less compact in arrangement than the circularis, perhaps due to the numerous blood vessels that are found in its substance. This coat is of course pierced by every vessel penetrating to the deeper structures. The striated muscle fibers of the upper end of the stomach are not found in the longitudinal muscle. This coat is very thin over the esophageal end of the stomach.

Plexus of Auerbach.—This nerve complex has not been sufficiently studied. It may be noted here, however, that the plexus is strongly developed in the king salmon stomach and in the other parts of the alimentary canal. Relatively large bundles of fibers are to be found between the two muscle coats and embedded in the longitudinal coat.

HISTOLOGY OF THE GASTRIC SEROSA.

The serosa of the salmon is formed by the single layer of plate-like epithelioid cells that is found in all the vertebrates of the higher series. Under certain conditions these cells become much thicker, i. e., quite cubical in shape. This form has been especially noted in the retrogressive changes that occur during the migration fast of the king salmon.

THE INTESTINE.

FORM AND DIVISIONS OF THE INTESTINE.

The intestinal portion of the alimentary tract of the salmon is, like the stomach, a U-shaped tube. In this case the first limb of the U is relatively short. It includes that portion of the intestine which has the numerous diverticula, the pyloric cæca. This limb begins at the pyloric valve which marks the limit of the pyloric end of the stomach. It extends anteriorly to about the level of the point where the esophagus and stomach join. At this level there is a sweeping bend in the intestine from the ventral to the dorsal position in the celomic cavity. The intestine here takes a position along the dorsal lateral side of the stomach and runs a straight posterior course to the cloaca and vent. This straight stretch of the intestine forms the second limb of the U, and is considerably longer than the first limb. In the adult salmon the two divisions of the intestine vary in length in proportion to the length of the fish. But for an 80-cm.

salmon the average length for the first limb is from 9 to 10 cm., that of the second limb from 28 to 30 cm. The last 4 or 5 centimeters of the intestine form a somewhat prominent enlargement, the cloaca.

There is a considerable variation in the size of the intestinal tube, even in fishes of the same length. This variation is greatest in the pyloric portion where the numerous cæca are attached. The variation in size is, however, dependent upon the degree of distension by food materials. It is largely an adaptation of the tube to its content, in so far as the diameter is concerned. If the tube be widely dilated the structural walls are correspondingly thin.

APPENDAGES OF THE INTESTINE.

Along the full length of the pyloric intestine there are numerous diverticula, the pyloric cæca. There is considerable variation in the number of cæca in different individuals of the genus *Oncorhynchus*. Jordan and Evermann, in "Fishes of North and Middle America," state that the number of cæca in the king salmon varies from 140 to 185. The larger number would seem to be more nearly the average.

The cæca are absent from the line of the wall of the pyloric intestine represented by the inner curvature. This line is marked by the attachment of intestinal blood vessels and of the gall and pancreatic ducts. All other portions of the wall of this limb of the intestine are studded thickly with pyloric cæca. The cæca, as a rule, arise as individual diverticula coming off at right angles to the surface and then bending posteriorly to lie like a fringe about the end of the pyloric stomach, and overlapping each other from this point for the full extent of the pyloric intestine. (See fig. 1.) The diameter of a cæcum at the base is usually somewhat less than in the main body of the diverticulum, averaging from 5 to 6 mm. Just outside the bases the cæca are somewhat larger—from 7 to 8 mm. in diameter. From this point to their tips they taper very gently to terminate in a blind sac.

The lengths of the cæca vary greatly in different regions of the pyloric intestine. The longest cæca are found at the origin of the intestine and just within the pyloric valve. At this point the appendages reach a length of from 6 to 8 cm., even longer in larger fishes. In any given segment those cæca that border on the line of the inner curvature are apt to be slightly shorter than the others in this segment. The lengths diminish progressively from the pyloric valve to the most anterior portion of the pyloric intestine. The last cæca are found on the ventral surface of the dorsal portion of the pyloric intestine, just where the anterior bend ceases and the straight limb of the intestine begins. These cæca are often 1 cm. or less in length, and the last two or three usually stand in a single row along the extent of the intestinal tube.

The pyloric cæca in their number, size, and arrangement form one of the most striking characteristics of the intestinal tract of this entire genus. In fact, the number is sufficiently constant to be given by systematists as a specific character. In the past the physiological function of these organs has been somewhat in doubt, but in the course of this work we have, fortunately, been able experimentally to establish certain points in their physiological economy to the salmon.

INTESTINAL COATS.

The coats of the intestinal tube are not so numerous as in the stomach. Certain parts present in the latter organ are not present in the intestinal tract, either in the intestine proper or in the pyloric cæca. Those structural coats that are present in the intestine are the following, beginning with the mucous membrane:

- I. Mucosa.
  1. Epithelial coat.
  2. Tunica propria.
  3. Stratum compactum.
  4. Stratum granulosum.
- II. Muscularis.
  1. Muscularis circularis.
  2. Muscularis longitudinalis.
- III. Serosa.

HISTOLOGY OF THE MUCOSA.

Epithelial coat.—The epithelial coat of the intestinal mucosa is very simple as to structure, but rather complex as to its folding. A section at right angles through this coat will always show a single layer of columnar epithelial cells. I have been unable to show any differentiation in the structure of these cells in the different regions of the coat. The foldings of the mucous epithelium, however, are so complicated that it is difficult to secure right-angled sections through any great extent in any single section. In a cross section of the middle portion of the intestine which is uncomplicated by the origin of pyloric cœca, it will be noted that the mucosa is thrown into relatively complex longitudinal folds (figure 5). Occasionally in a widely distended intestine these folds are somewhat smoothed out, so that the arrangement seems less complex. If, however, the intestine be sharply contracted, then there are not only general longitudinal folds but many smaller collateral folds. Edinger[a] in one of his plates presents a series of figures to show the character of the folding of the mucosa in fishes. The type of folding that he figures holds for the intestine of the salmon. In another place[b] I have given a brief description of this folding as it appears in the pyloric cæca. There, however, the mucosa is more simple in its arrangement than in the intestine.

In a transverse section through a longitudinal fold in the intestinal mucosa the fold or ridge itself will have secondary pits or grooves on its lateral extent. Very often it happens that the section will present the appearance of definite tubes, or be through what looks like simple tubular glands, or sections of wide oval loops of epithelium will be shown. The tubelike structures do, indeed, suggest definite intestinal glands in the mucosa, but there is absolutely no histological differentiation between the cells of these narrow pitlike folds and the cells of the free epithelial surface. Although the folding of the epithelium is rather complicated one must accept the fact that the complexity of folding is not accompanied by histological differentiation of structure.

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[a] Edinger, L.: Ueber die Schleimhaut des Fischdarmes, nebst Bemerkungen zur Phylogenese der Drüsen des Darmrohres. Archiv für mikroskopishe Anatomie, bd. XIII, 1877, p. 651.

[b] Greene, Chas. W.: "The absorption of fats by the alimentary tract with special reference to the function of the pyloric cæca in the king salmon, Oncorhynchus tschawytscha." Transactions of the American Fisheries Society, 1911, p. 261.

The mucous epithelium is constantly marked by the presence of two common types of cells in addition to the typical cylindrical epithelium; first, mucous cells, and second, wandering cells presumably of the leucocyte type. In a normal epithelium the number of mucous cells varies through wide extremes. In certain sections they are relatively rare, and there are long stretches of epithelium without a single goblet cell. In other material the number of mucous cells is relatively great, and certain studies made on fasting salmon strongly support the view that the number of goblet cells is greatly increased during the early fasting period when the mucous membrane as a whole is just beginning its retrogressive changes. Certainly the number of mucous cells is greater if the fasting be somewhat prolonged. The observations on the salmon support the current view that cylindrical epithelial cells may be transformed into goblet cells.

The wandering cells are indicated by the small round nuclei interstitially arranged with reference to the normal epithelial cells. The nuclei are found chiefly near the basal portion of the epithelial coat, but they may also be found between the outer limbs of the cylindrical cells. In the normal digesting fish taken from among those feeding naturally on the banks at Monterey there are comparatively few of the wandering cells. This observation is of special significance in connection with certain theories concerning the part these cells are assumed to take in the process of absorption. Certainly in the salmon the small number present is strongly against the theory that fat absorption is accomplished by the wandering leucocytes. Indeed no evidence supporting this view of their function has been found in connection with numerous fat-absorption experiments.

I have been unable to show any particular differentiation in different limbs of the intestine, in so far as the mucous epithelium is concerned. The only differences noted are limited to complexity of the folding. In the pyloric intestine where the spaces between the cæca are small and limited, the intestinal epithelium has very simple folds. In the middle of the straight limb of the intestine these folds are more complex.

Tunica propria.—The tunica propria of the salmon intestine is defined as the connective tissue support extending from the bases of the epithelial cells to the stratum compactum. The tissue is composed of white fibrous connective tissue of the areolar type, carrying cells and nuclei of the connective tissue varieties. It is a very thin layer except in those places where the mucosa is folded into deep ridges. In the latter case the tunica propria extends into these ridges, forming a connective tissue foundation for the support of the epithelial coat.

The strands of the tunica propria support the blood vessels lying under the epithelium. These blood vessels consist of relatively small arteries penetrating from the muscle coat and forming a rich capillary network under the epithelium proper.

Stratum compactum.—This peculiar structure which has been described in some detail for the stomach is also present throughout the intestine. It forms a thick, heavy sheath, lying midway between the epithelium and the circular muscle coat of the intestine. The stratum compactum is not so complex as in the stomach in that it has a thinner mass of strands over the surface of the main sheet. A transverse section of the intestinal wall will usually show a heavy wavy line of dense nonfibrous connective tissue, which stains the characteristic blue with Mallory's connective tissue stain. Its composition in the intestine is apparently the same as in the stomach. The inner surface of this stratum, that is, the surface toward the middle of the lumen of the intestine, is marked by a few smaller strands of the same peculiar type of connective tissue. The outer surface of the stratum has two or three times as many of these smaller strands. A distinct network is formed by the strands extending over to the circular muscle coat. In the intestinal region the number and complexity of arrangement of the strands composing the stratum compactum is about 40 to 50 per cent as great as in the stomach.

Nowhere does there seem to be any direct opening or break through the thicker portion of the stratum compactum, except where blood vessels penetrate this coat, a point which Gulland has made in his study of the stratum compactum of the stomach of *Salmo salar*. It may very well be, as Oppel suggests, that the stratum compactum is a protective supporting membrane. It certainly acts as such for the stratum granulosum in *Oncorhynchus*, whether or not it forms a supporting structure for the whole intestine.

Stratum granulosum.—The meshes of the stratum compactum directly support a layer of the special type of cells which has been described in connection with the stomach as the granule cells. This layer, the stratum granulosum, is most dense in its arrangement in the outer network of the stratum compactum There are a few cells more or less scattered, lying on the inner surface of the same connective tissue supporting membrane. The granule cells of the intestine are characterized by the same form and size and structural arrangements as described for the granule cells of the stomach. Here, also, they possess the uniformly distributed, highly refractive granules which take stains in the specific way already described.

These granule cells form a very definite and characteristic structure in the intestine and their appearance and presence indicate some function of a significance which one can not escape ascribing to the presence of the granules in the cells, a suggestion that has been briefly discussed in connection with the stomach.

There is no muscularis mucosæ present either in the intestine or in the pyloric appendages.

The submucous coat, which characterizes the structure of the walls of the stomach, is also absent in the intestine.

HISTOLOGY OF THE MUSCLE COATS.

The muscle coats of the salmon intestine consist of an inner circular and an outer longitudinal coat with the plexus of Auerbach and numerous blood vessels between.

Muscularis circularis.—The inner muscle coat of the intestine consists of fibers which run in a circular direction. It is a relatively well-developed muscle sheath about twice as thick as its fellow, the longitudinal muscle. It is composed wholly of smooth muscle fibers, the detailed structure of which does not vary in any particular way, so far as noted, from the usual type of smooth muscle. A rich vascular supply, consisting of smaller blood vessels and capillaries, is present in this coat, the capillaries coming quite largely from arterioles present between the two muscle coats.

Muscularis longitudinalis.—The outer intestinal muscle coat consists of longitudinal fibers. It is a rather thin coat composed of smooth fibers and carrying numerous small blood vessels within its mass or between it and the inner coat. The presence of the small blood vessels tends to break up to some extent the compactness of arrangement of the muscle fibers.

Plexus of Auerbach.—This nervous structure is quite prominent in all sections through the alimentary canal. It, however, has not yet been studied in detail for the salmon. The ganglia and the bundles of fibers of the plexus lie between the two muscle coats but rather largely imbedded in the inner wall of the outer coat.

HISTOLOGY OF THE SEROSA.

The serous coat of the salmon intestine is composed of a single layer of epithelia cells and the subserous supporting connective tissue. These cells are not so flat and attenuated as is usually the case for this coat in the intestines of mammals, but are more nearly cubical in shape, often quite cylindrical. Especially when the intestine is contracted are the cells of the serosa deeper than wide.

The subserous connective tissue is rather prominent in the salmon intestine. It consists of white fibrous connective tissue about twice as thick in total mass as the epithelial coat proper.

HISTOLOGY OF THE PYLORIC CAECA.

Oppel says, in volume II of Mikroskopische Anatomie, that "the pyloric cæca, when they exist, possess the structure of that portion of the alimentary tract on which they are attached." Gulland says of the cæca of the Scottish salmon that "in structure they exactly resemble the upper part of the intestine, so much so in fact that but for the difference in size it would be impossible to say whether a section came from one or the other." In the king salmon, too, the pyloric cæca are diverticula of the pyloric limb of the intestine, and one would naturally expect them to have a structure built on the same plan as the intestinal region from which they arise. This in general is true, though the pyloric portion of the intestine is so broken up by the origin of the numerous cæca that the intestinal structural constants are not altogether simple in this region.

The coats described above for the intestine are all present in the walls of the pyloric cæca. The only difference is one of relative complexity, or one might rather say simplicity, of arrangement. The parts enumerated from the lumen toward the outer wall are:

- I. Mucosa.
  - 1. Epithelium.
  - 2. Tunica propria.
  - 3. Stratum compactum.
  - 4. Stratum granulosum.
- II. Muscularis.
  - 1. Circularis.
  - 2. Longitudinalis.
- III. Serosa.
  - 1. Serosa proper.
  - 2. Subserosa.

MUCOSA.

Epithelial coat.—The epithelium of the pyloric cæca is a coat of one uniform type throughout, and consists of a single layer of slender cylindrical cells with interspersed goblet cells and wandering leucocytes. The epithelial coat reaches its greatest development in the normal adult animal, in which its total surface extent is eight times the outer surface of the cæcum. This relatively enormous extent is secured by deep folds of the coat, which extend out into the lumen of the appendage. Cross sections of the pyloric appendages show all possible varieties of section of the epithelium. There are transverse sections of simple tongue-like folds extending from near the stratum compactum to approximately the center of the lumen. These are not villi, though they appear so. On the other hand there are regions where the epithelium is cut obliquely, or even in a plane parallel with the surface of the epithelial coat for some extent. There is in such a picture necessarily a great amount of irregularity in the boundary outlines of the epithelial surface.

The picture just described is due to the fact that the epithelial coat of the mucosa is built on a simple but extremely flexible plan. There are no projections of its surface comparable to the villi of the small intestine in the mammalia, but there are many clefts and folds. If a king salmon's cæcum were completely relaxed as to its musculature and distended from the inside to its maximum extent, both as to length and as to circumference, its epithelial coat would be greatly smoothed out and comparatively simple. Its irregularities of surface still observable would be chiefly low longitudinal ridges broken here and there by transverse furrows and partial clefts. Relaxation of the muscular walls and dilation of the cæcum to this extent probably seldom occur in nature though ofttimes closely approximated. In a maximally stretched cæcum the proportion between the surface extent of the mucosa and the outer wall should be lower than the minimum given in the preceding paragraph. If in such a case the cæcal wall should contract in diameter without changing its length the epithelial coat would be thrown into deep longitudinal ridges and clefts that would extend from the base of the mucosa to the center of the lumen of the cæcum. These ridges would appear in transverse section as simple uncomplicated folds, i. e., very long slender finger-like projections of mucous epithelium into the lumen of the cæcum. These projections show, as might be expected, a uniform type of cell from tip to base, as shown in figure 8, plate XXVIII.

Now if the cæcum contracted in length it would bend and twist these longitudinal folds, and at the same time interrupt their continuity by producing transverse ridges and pockets in the epithelial coat, giving rise to a net-like appearance as seen from a surface view, a view not unlike that presented by Edinger[a] in his figures 11 and 13b, and by Eggeling[b] in figure 156. A cross section of a cæcum in this state always shows an extraordinary complexity of section throughout the epithelial coat, a complexity that was found extremely confusing until interpreted in the manner presented above.

[a] Edinger, L.: op. cit., pl. 41.

[b] Eggeling, H.: Dünndarmrelief und Ernährung bei Knochenfischen, Jenaische Zeitschrift, bd. 43, 1908, p. 416.

In the young salmon of fingerling size or smaller the extent of epithelium is relatively less; therefore the folds are simpler than has been described for the adult above. This is shown in a number of colored figures illustrating the process of fat absorption in young salmon, presented in a paper to follow. A similar epithelial folding has been described for other fishes, i. e., for the herring, *Murœna*, and the sturgeon.[a]

The cells of the epithelial coat are of two types, and these are fairly uniformly distributed, i. e., the usual cylindrical cells, and the goblet cells. It is not desired to discuss here the question of the origin of goblet cells from the cylindrical cell, though the current views are supported by these observations.

The cylindrical cells are very slender in the adult, though less so in the younger fish, i. e., under 1 year old. Their size in a number of measurements made on paraffin sectioned material was from 45 to 63 μ long by 5 to 6 μ in diameter. They have slender oval nuclei located at the basal two-fifths of the cells. The cytoplasm of these cells presents no special structural features under ordinary conditions. If rapid absorption of fat is taking place it is shown by the special fat staining of fresh material to be present in the cylindrical cells. In paraffin preparations the fat is dissolved out and open empty spaces will appear in the cytoplasm (fig. 11, pl. XXVIII). No granules have been found and nothing comparable to the cells of Paneth have appeared in any of the sections.

The superficial borders of the cylindrical cells have a striated border marked by a homogeneous ground substance set with numerous fine striæ running vertical to the surface of the cell.

The goblet cells appear at tolerably regular intervals, though they are not numerous in the normal material. Their cell bodies seem a trifle larger on the average than the cylindrical cells. If the mucous of the goblet cell has not completely formed, then the striated border will be continuous over its surface. Many of these cells are present with the mucous content just beginning to discharge, in which state they are most prominent in appearance. The nuclei of the goblet cells lie nearer the bases of the cells than in the adjacent cylindrical cells. They also contain a greater amount of chromatin, hence stain more deeply with chromatin dyes.

The cells at the bottoms of the epithelial clefts or folds do not show any structural traits different from those on the folds projecting farthest into the lumen. This leads to the conclusion that there are no gland vestiges in the cæca of the salmon homologous with the glands of Lieberkühn, such as figured by Brass[a] for the human vermiform appendix. Neither have any rudiments of these glands of the type described by Kingsbury[b] for the small intestine of *Necturus* been found.

A certain number of wandering leucocytes are always found in the normal epithelial coat. In all the salmon material examined these white corpuscles are very small in size and are greatest in number in the basal region of the epithelial coat. They are not regularly distributed, but sometimes are present in groups of two or three. Their nuclei

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[a] Brass, A.: Atlas der Gewebebelehre des Menschen, bd. I, 1896, Göttingen. Reference from Oppel, Mikroskopische Anatomie, bd. II.

[b] Kingsbury, Benjamin F.: The histological structure of the enteron of Necturus maculatus. Proceedings American Microscopical Society, vol. 16, 1894, pp. 19–64.

are to be distinguished from the nuclei of the cylindrical epithelial cells by the fact that they are smaller in size and more round in outline and stain more intensely. The diameter of these cells is from 3 to 4 μ, while the long diameter of the oval nuclei of cylindrical cells is 5 to 6 μ, or even 10 μ in younger salmon. Occasionally through the epithelial coat scattered leucocytes are found in the outer third of the layer. In fact, these cells may be located at any level from the general nuclear region to the surface border of the coat. They do not appear to be, at least have not been observed, escaping from the epithelial coat into the intestinal cavity.

No eosinophile granule cells of any kind have been found in the alimentary epithelial coat of the king salmon. Such cells are described in this region for *Proteus* by Oppel. This observation is of importance in association with the fact that granule cells of a type already described above compose the stratum granulosum.

Tunica propria.—The tunica propria of the cæca extends from the epithelial layer to the stratum compactum. Its extent varies greatly, and its outlines are very irregular. On the epithelial surface the tunica must follow the irregularities of that coat, while next the stratum compactum it is equally sinuous, owing to the great irregularity of surface of the stratum. The whole outline is not unlike that in the intestine, though simpler.

The tunica propria is a connective tissue structure carrying numerous blood vessels and supporting a number of special types of cells in its meshes. The connective tissue fibrils form an open meshwork of the usual areolar type. (See fig. 9, pl. XXVIII.) At its epithelial boundary it forms a very definite limiting membrane, the basement membrane, on which the epithelial cell layer rests. From this basement membrane the more or less open meshwork of fibrils begins. Numerous oval nuclei appear in the ground substance. The compactness of the tissue and the relative number of nuclei vary extremely in the various specimens. Where the tunica propria extends up into a fold of epithelium its tissue usually appears somewhat more compact. Definite loops of small blood vessels and capillaries are present in these projections. Open tissue spaces are found both in the loops and in the broader expanses of the coat just within the stratum compactum, in the normal tissue these spaces being small in size. Beside the proper nuclei of the connective tissue there are relatively large numbers of nuclei of the white corpuscle type belonging to cells that have not yet been comparatively studied. These cells vary extremely in number in different areas and in different salmon.

The small arteries and veins, as well as the capillaries, are readily distinguished by the red corpuscles, which possess characteristic oval nuclei that take certain dyes in a differential way. Here and there are present certain large cells with a lightly stained cell body and a characteristic large nucleus, the identity of which has not yet been determined.

Lying just within the stratum compactum are scattered granule cells. These granule cells usually lie next the stratum compactum, sometimes in its loops, but occasionally may be found near the deeper loops of basement membrane, never in the epithelial coat. In fasting salmon this group of granule cells is more prominent.

Stratum compactum.—The stratum compactum is always present in the cæca of the king salmon. It is a striking structure and always stands out prominently in every microscopic section. Under the low magnification it appears as a broad wavy band extending around the circumference of the cæcum and lying within the muscular and external to the tunica propria coat.

The stratum is wavy in appearance, whether the section be transverse or longitudinal, but the extent of the folds which give rise to this phenomenon varies with the distension of the cæcum. Apparently the more contracted the organ as a whole the deeper the sinuosities of the stratum compactum. The stratum is a fairly uniform band, i. e., its thickness is relatively constant, varying between 12 and 16 μ.

Its structure has been described in other forms by several investigators, notably Mall.[a] Gulland[b] says that "a well-marked stratum compactum" is present in the Atlantic salmon. In the king salmon the stratum compactum is a homogeneous layer of fibrous connective tissue. It gives a characteristic staining with Mallory's connective tissue stain, but shows no fibrillar structure. On its surface the stratum of the cæca gives rise to a few smaller strands which extend out into the tunica propria on the one hand and form the network extending to the circular muscle coat on the other. These strands, especially in the latter case, form a supporting network for the special granule cells of the region. No nuclei are present in the body of the stratum compactum, but lying along its surface, especially on the surface next the tunica propria, a few nuclei are found. These nuclei are irregularly ovoid in shape, and appear to be embedded in a thin layer of tissue lying on the surface of the homogeneous portion of the stratum compactum. This layer stains a trifle more intensely than does the main body of the layer.

The stratum compactum is not present in the alimentary tract of all fishes, and why it should be so prominent a structure in the case of the king salmon remains a question. The coat is dense and it forms an uninterrupted layer (except where blood vessels penetrate from the outer to the inner structures of the cæcum). When a cæcum is widely distended the stratum is comparatively even and smooth in appearance. When the cæcum is contracted it is thrown into deep sinuous folds, no matter in what plane the section may lie. Its function is probably best explained by Oppel,[c] who suggests that this is the supporting membrane for the structures of the entire organ. The fact that the stratum compactum is a homogeneous uninterrupted sheet seems to have further significance than that assigned by Oppel. The fat studies described in a later paper suggest that this structure may be concerned in the process of the absorption of the food products by the cæcum.

Stratum granulosum.—The cæca have a characteristic layer of granule cells bearing the usual relations to the stratum compactum and its network, i. e., the relation described

[a] F. P. Mall: Reticulated tissue and its relation to the connective tissue fibrils. The Johns Hopkins Hospital Reports, vol. 1, 1896. Baltimore.

[b] Paton, D. Noël: Investigations of the life history of salmon, art. 3, The minute structure of the digestive tract of the salmon, and the changes that occur in it in fresh water, by G. Lovell Gulland. Report of the Fisheries Board for Scotland, 1898.

[c] Oppel, A.: Beiträge zur Anatomie des Proteus anguineus. I. Vom Verdauungstraktus. II. Von den Lungen, Archiv, für mikroskopische Anatomie, bd. 34, 1889, p. 511-572.

for both the stomach and the intestine. This layer of granule cells is very prominent in the pyloric cæca of the king salmon. Normally the layer is from two to four cells deep between the outer surface of the stratum compactum and the circular muscle coat. These cells are supported by the delicate strands of the outer surface of the stratum compactum. A few granule cells are located on the inner wall of the stratum compactum and occasional ones are found in the tunica propria, as mentioned above.

The numerous relatively large granule cells suggest Oppel's granule cells of *Proteus* in structural characteristics. The granule cells of the king salmon, however, have not been found in the epithelial coat, though under pathological conditions they undoubtedly do wander into other near-by regions. Gulland says, speaking of the pyloric appendages and the intestine: "In both structures the eosinophile leucocytes are numerous, but are to be found mainly in the connective tissue about the stratum compactum." In discussing these cells in connection with the structure of the stomach attention was called to the fact that Gulland figured cells of undoubtedly the same type as present in the king salmon. But I must again assert that these can not be blood leucocytes of the eosinophile or any other type.

The granule cells of the king salmon cæca have small round nuclei placed more or less eccentric with reference to the center of the cell. The cell body itself varies extremely in outline. In the majority of instances this outline is that of a broad oval, but where a cell is located in a restricted mesh its shape is adapted to conform to the space. This is especially to be noted in those cells present in the tunica propria.

In a section of a cæcum of a Monterey salmon taken June 23, 1911, the following measurements of granule cells are noted: 8 by 12, 8 by 10, 5 by 13, 8 by 14, and 9 by 14 μ. The special characteristic of the granule cells is the presence in the cytoplasm of large refractive granules. These granules stain with eosin, with Mallory's stain, taking especially the Bismarck brown but occasionally the acid fuchsin. They stain sharply with iron hæmatoxylin. The granules are very evenly distributed throughout the cytoplasm. The cells outside the stratum compactum seem more uniformly crowded with granules by whatever method of staining they be studied, and more densely granular than in the granule cells of the stomach. Those scattered cells that are present in the tunica propria are the ones that present the greatest irregularity in the distribution of the granules. A greater irregularity in outline suggests that the cells are amœboid. In one or sometimes both poles of these irregularly shaped cells the granules are fewer in number, though the difference is only slight. Also in the smaller cells there are on the average fewer granules than in the larger. The number of granule cells in the normal tissue is such that the connective tissue meshes seem about one-half filled with the cells. In the relatively distended cæcum the total thickness of the layer is less. These points are mentioned for comparison with the structural condition found in degenerated cæca in which the number and relations of the granule cells differ sharply from the normal Monterey salmon type, as will be described in a paper to follow.

MUSCULAR COATS.

The muscular coats are the inner circular and the outer longitudinal muscles. The two coats are not so thick as in the intestinal wall or in the thick muscular walls of the stomach. But they are on the whole very regular and uniform.

Muscularis circularis.—The circularis is thicker than the longitudinalis in the salmon cæcum in the approximate ratio of 3 to 2. The coat is composed of relatively long, smooth muscle fibers. When the cæcum is distended the number of fibers present in the total diameter of this coat in a transverse section is only from 8 to 10, often less. Numerous capillaries are always present. Very rarely in the normal animal will one find a granule cell in the spaces of the muscle fibers of the circular muscle coat.

Just at the base of the cæcum the muscle is a little thicker and the lumen usually smaller. The structure shows all the appearances suggesting a degree of valvelike action capable of influencing the filling or emptying of the cæcum from or into the cavity of the pyloric intestine out of which it originates.

Muscularis longitudinalis.—The longitudinalis is a thin sheet of smooth muscle, the outermost of the muscle coats. In the king salmon the longitudinal coat varies in thickness according to the distension of the tube, but on an average it is from 5 to 8 fibers thick.

This coat is chiefly distinguished by its vascularity. Relatively large veins are found at intervals in the coat and occasionally a section will show arterioles entering the cæcum from the exterior, penetrating this coat to distribute branches out in the space between the two muscle coats. In the neighborhood of these blood vessels there is connective tissue in relatively large amounts, an arrangement which tends to break up the continuity of the muscular sheet.

On the inner surface of the longitudinal muscle coat between it and the circular muscle fibers, sections reveal the presence of numerous nerve cells and fibers of the plexus of Auerbach. In the individual ganglia of this plexus there are only a few nerve cells.

SEROUS COAT.

The serous coat of the cylindrical cæca of the king salmon consists of a single layer of squamous epithelial cells resting on a subserous connective tissue base. In the normal tissue these cells are extremely thin and attenuated. Their boundaries are difficult to measure in transverse section, but the extent is from 10 to 12 μ. The nuclei are spindle shaped, having a diameter of about 1 μ and a length of about 5 μ. In many specimens the serous cells are more cubical in outline and the coat is correspondingly thicker.

DESCRIPTION OF FIGURES.

The 11 figures presented in illustration of this paper were drawn for me by Mr. George T. Kline, biological artist of the University of Missouri, to whom I am indebted for skill and painstaking care in the interpretation of the specimens represented.

LIST OF ABBREVIATIONS USED.

Bl. v	Blood vessel.	M. muc	Muscularis mucosa.
Col. ep	Columnar epithelium.	Neck c	Neck cells.
Fat vac	Fat vacuoles.	Str. comp	Stratum compactum.
Gast. gl	Gastric glands.	Str. gran	Stratum granulosum.
Goblet c	Goblet cell.	Submu	Submucosa.
M. circ	Muscularis circularis.	Tu. pr	Tunica propria.
M. long	Muscularis longitudinalis.	Wh. corp	White corpuscle.

PLATE XXV.

FIG. 1.—This outline figure of the adult salmon is presented especially to show the external appearance of the viscera and the space relations of its parts. A segment of the left ventral wall is removed, exposing the organs in their natural position. The drawing is a semitransparency. The parts to which special attention is called are:

1. The liver, filling the anterior portion of the body cavity.

2. The cardiac loop of the stomach. The index figure is placed over the anterior portion of the posterior limb of the intestine shown as a transparency through the thickness of the stomach.

3. The pyloric limb of the stomach. The numeral is placed over the extreme anterior end and very near the constriction which is the location of the pyloric valve.

4. The pyloric intestine.

4′. The extreme posterior end of the intestine.

5. The pyloric cæca.

5′. Marks a cæcum that is almost surrounded by pancreas.

6. Pancreatic gland.

7. The spleen.

8. The long strap-like testes.

9. The ventricle.

Note that the pancreas is of the diffuse type of gland and is attached to the surfaces of, and lying among, the pyloric cæca. The gland is omitted over the cæca of the anterior loop. One-fourth natural size.

PLATE XXVI.

FIG. 2.—Cross section of a normal empty stomach from salmon no. 21, Monterey, Cal. This low-power figure shows the relation of the gross parts, also the compact folding of the glandular portion of the stomach into longitudinal rugæ when it is empty. Magnification, $\times 4$.

FIG. 3.—A low-power drawing of a transverse section of the stomach wall, showing the histological relations of the various coats. For the interpretation of the abbreviations used to designate the parts, refer to the list of abbreviations above. Camera outlines. Magnification, Leitz ocular 1, objective 3.

FIG. 4.—A representation of a highly magnified gastric gland. To the left of the figure are three transverse sections of the gland tubes, one showing a deep oval. To the right of the figure is a longitudinal section of a short loop of gland cells just at the point where transition to neck cells takes place. The gastric-gland cells are loaded with granules from base to free margin. The granules vary considerably in size, as discussed in the text. The neck cells are free of granules. Camera lucida outlines. Magnification, Leitz ocular 2, objective 1/12.

PLATE XXVII.

FIG. 5.—A quadrant of a transverse section of the intestine taken from a salmon (no. 117) from Ilwaco, Wash. This salmon was in the first stage of the migration fast, hence the relative thickness of the different layers is undoubtedly somewhat different from the normal. However, it shows the relative position of the layers and the relative complexity of folding of the mucous epithelial coat. Camera outlines. Magnification, Leitz ocular 2, objective 3 (lower lens removed).

FIG. 6.—A superficial sketch of a single cæcum chosen from the longer group at the beginning of the pyloric intestine of a feeding salmon from Monterey, Cal. This cæcum is 8 by 160 mm. The smaller cæca have the same relative outlines as the larger. Magnification, $\times 1$.

FIG. 7.—A transverse section of a normal cæcum from salmon no. 22, Monterey, Cal. This section shows the relation of the different coats, also is especially good to show the enormous extent and complex outlines of the mucous epithelial coat. The wavy band is the stratum compactum which characterizes not only the cæca, but the intestine and the stomach as well. Camera lucida outlines. Magnification, Leitz ocular 1, objective 3 (lower lens removed).

PLATE XXVIII.

FIG. 8.—A portion of the wall of the cæcum of a normal feeding salmon (1911 series, no. 11) from Monterey, Cal. The magnification in this figure is chosen to bring out the general histological relationships of the various parts. When the section is at exact right angles, as shown in the folds at the left, it presents an almost diagrammatic regularity. The folds to the right of the figure are complicated, hence many of the cells are cut at oblique angles. The muscular walls are relatively thin in this section, a condition that exists when the cæca are distended with food material. Magnification, Leitz ocular 1, objective 3.

FIG. 9.—A highly magnified section through the wall of a pyloric cæcum taken just at the bottom of a deep fold. The legend is sufficient to identify the parts. Particular attention is called to the relations of the stratum compactum. This dense connective tissue structure is marked on its external surface by a network of strands inclosing the cells of the stratum granulosum. These cells are characterized by the uniform and dense loading with highly refractive granules which usually completely obscure the small eccentric nucleus. Camera outlines. Magnification, Leitz ocular 1, objective 7.

FIG. 10.—A portion of the cylindrical epithelium and the substratum of the tunica propria highly magnified from a feeding salmon (no. 22) from Monterey, Cal. The figure presents a camera lucida outline of the cells, showing their relative size, structure, the relations of the nuclei, etc. Two goblet cells in different stages of maturity are shown. In the tunica propria there is a capillary in which lies the cross section of a red-blood corpuscle cut somewhat excentric to the nucleus. Camera lucida outlines. Magnification, Leitz ocular 4, objective 1/12.

FIG. 11.—Showing a highly magnified group of columnar epithelial cells from the pyloric cæcum of a Monterey salmon in the active stage of absorption. The fat vacuoles are relatively large and numerous in the outer third of the cells. They are present, but more scattered, in the inner limbs of the cells. In preparations made with a positive fat stain applied to fresh material these vacuoles are proven to contain fat droplets. Magnification, Leitz ocular 4, objective 1/12.

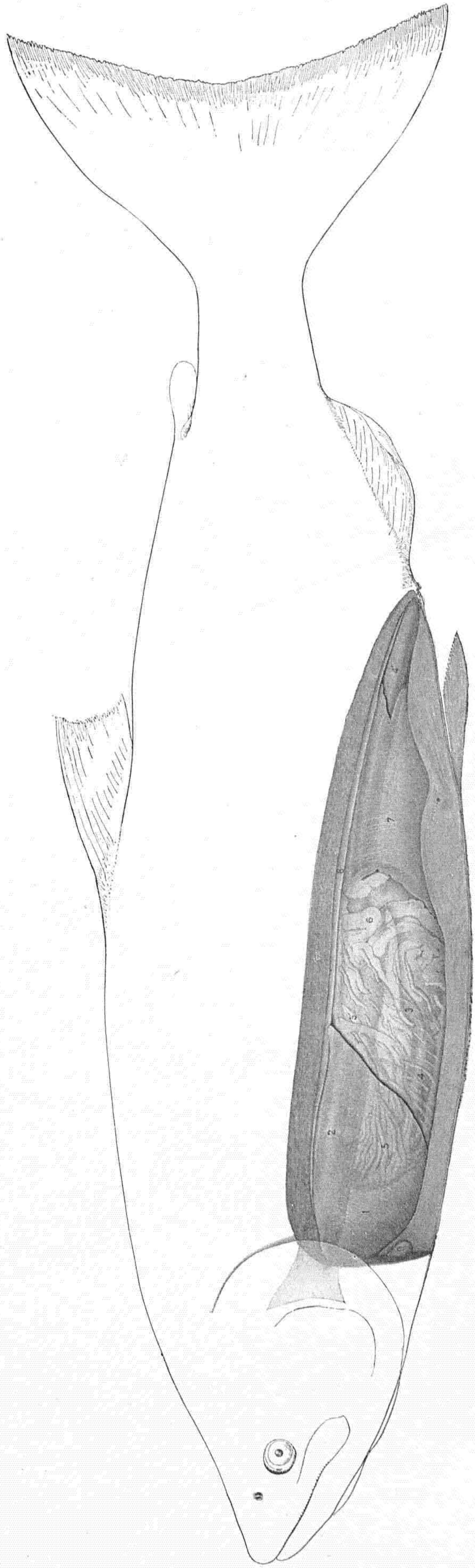


FIG. 1.

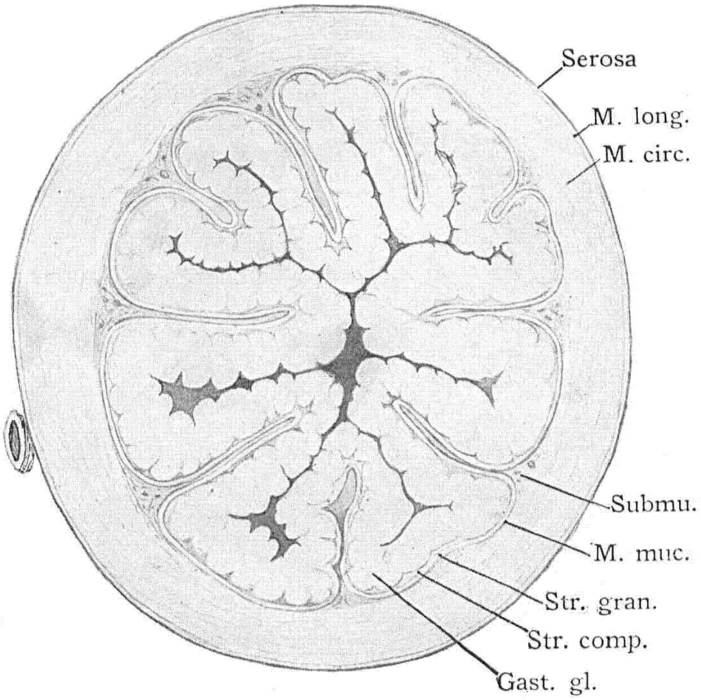
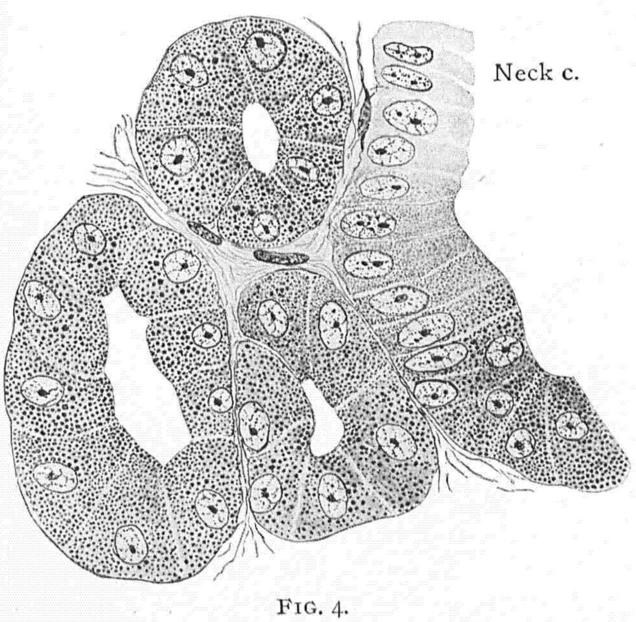


FIG. 2.



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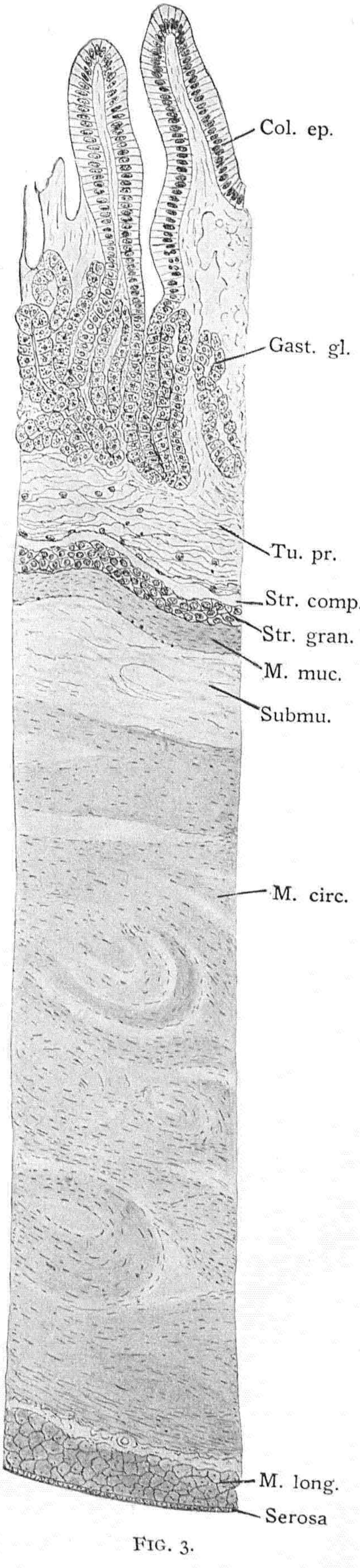

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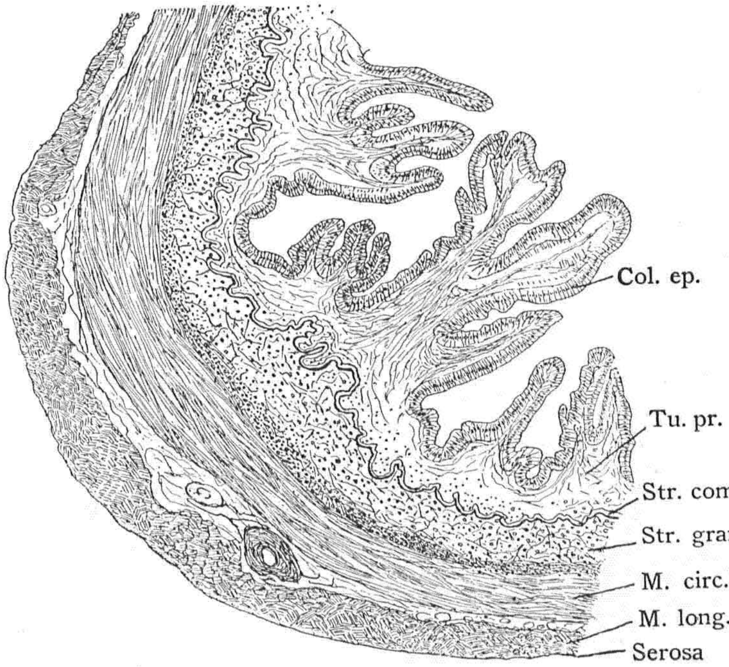

FIG. 5.

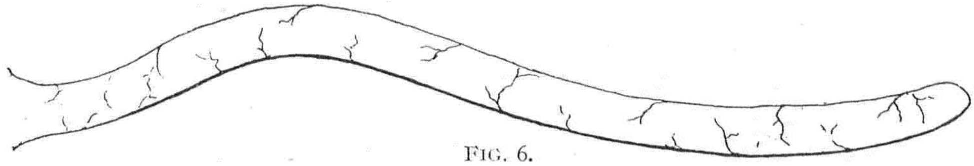

FIG. 6.

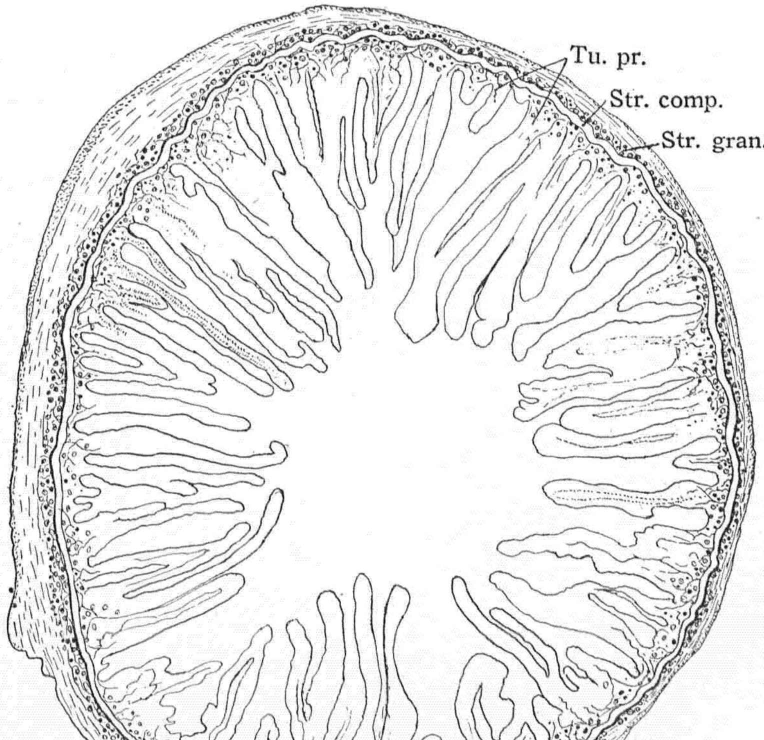

FIG. 7.

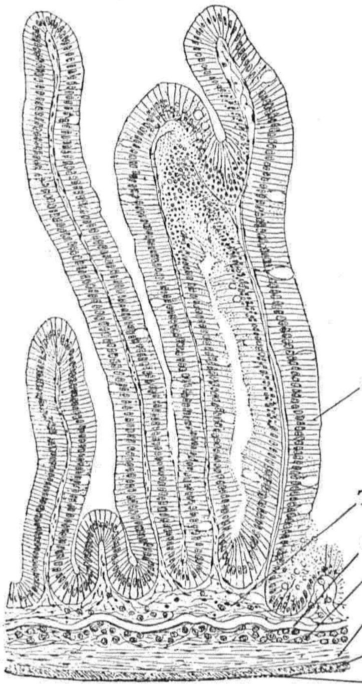

FIG. 8.

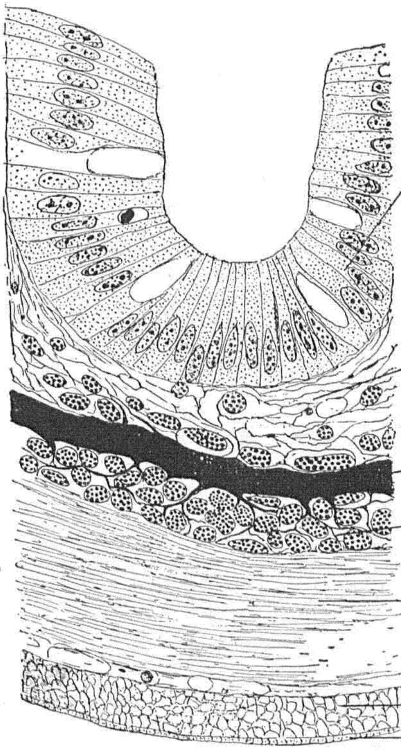

FIG. 9.

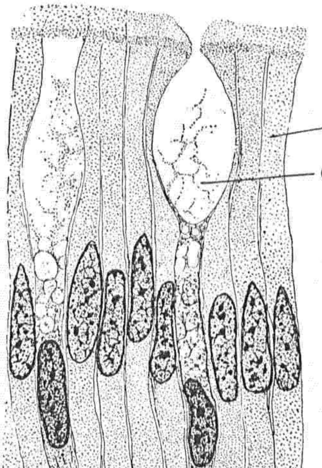

FIG. 10.

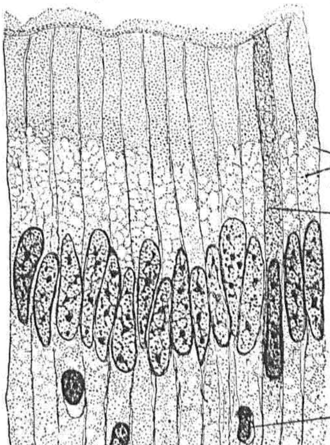

FIG. 11.