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Anatomy and Histology of the Digestive Tract of a Deep-Sea Fish *Coelorhynchus carminatus*

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University of Nebraska - Lincoln

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CONTENTS

CONTENTS—*(Continued)*

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FIELD OF STUDY

THIS paper deals with the anatomy and histology of the digestive tube of one of the grenadiers or rat-tails, *Coelorhynchus carminatus* (Goode), as classified by Jordan & Evermann (1896).[1] Many studies on the structure of the digestive tracts of fishes have been made in the past, and pioneer work in the teleost group was done by Valatour in 1861, Pillet in 1894 on the Pleuronectidae, and Gulland in 1898 on the salmon. More recent studies have been made by Greene (1912) on the king salmon, Blake[2] (1930) on the sea bass, and Rogick (1931) on the minnow.

The species under consideration is very little known; the literature is scanty, and in most instances only the name is mentioned, with no appended description. It belongs, however, in the family Macrouridae and is a deep-sea fish, living at a depth of 200 to 315 fathoms. It is described as having an elongated, silver-gray body, a leptocercal tail ending in a long filament, scales with spines arranged in rows, fig. 1c, like wool-cards (carmen), a long, sharp, depressed triangular snout, and large eyes, fig. 1a.

Three specimens were used. One entire fish preserved in formalin was employed for dissection and anatomical study. Two digestive tracts fixed in Bouin's solution and preserved in 85 per cent alcohol were used for histological study. The

* Studies from the Department of Zoology and Anatomy, The University of Nebraska, No. 196.

[1] See Bibliography, p. 27.

[2] The writer wishes to acknowledge her indebtedness to Dr. Irving H. Blake of the University of Nebraska, under whom this work was done, for his encouragement and kind assistance during the course of the study.

paraffin method was used throughout. Sections were transverse, occasionally tangential, and were cut 7.5 microns thick. The stains used were Delafield's haemotoxylin, Mallory's triple stain, and Heidenhain's iron-alum haemotoxylin with eosin or orange G as counterstains.

The following parts of the digestive tract will be considered both anatomically and histologically: esophagus, stomach, in-

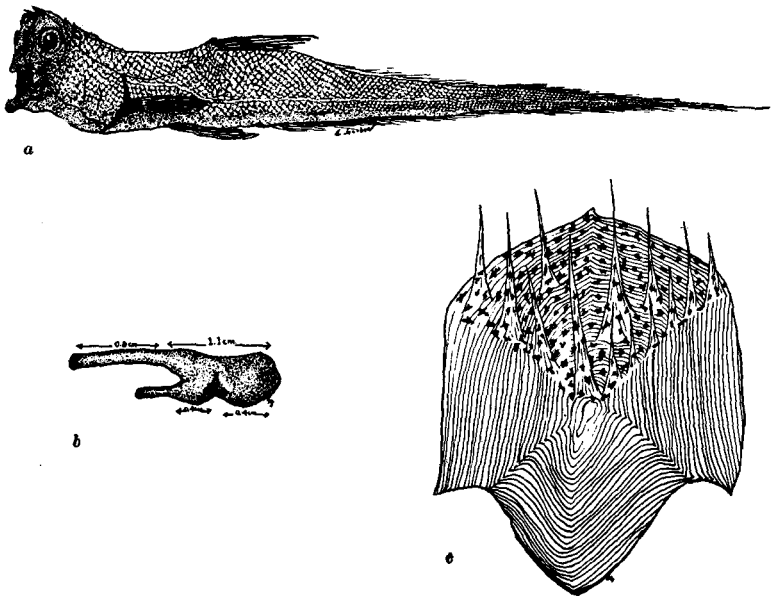

Fig. 1.—*Coelorhynchus carminatus.* *a*, lateral view; *b*, lateral view of esophagus and stomach; *c*, a scale.

testine, and pyloric caeca. The buccal cavity and pharynx will not be considered from the histological standpoint because of the poor condition of the material available for study. The tissue in this part of the tract was so badly mutilated, due to fixation and to the presence of a great amount of undigested food material, that no detailed description could be given with any degree of assurance.

BUCCAL CAVITY

Gross Anatomy.—The buccal cavity extends from the lips to the first gill slit, which marks the beginning of the pharynx. The initial width is about 4 mm., reaching a maximum of 9 mm. at the anterior boundary of the pharynx. The roof and

floor are essentially the same, possessing no peculiar structures. The surface of the roof has a wrinkled appearance due to the epithelium being thrown into a series of low longitudinal folds. These folds are more pronounced on either side of the cavity, no doubt due to the construction in this region. Low folds of epithelium are found also in the floor of the mouth. Teeth are present upon mandible.

PHARYNX

Gross Anatomy.—The pharynx extends from the anterior boundary of the buccal cavity to a region just posterior to the last gill slit. In the roof of the pharynx, just anterior to the first gill arch, are two rows of pharyngeal teeth. A callous pad, measuring 4 mm. in length and 3 mm. in width and having a raised portion in the center measuring about 1 mm. in diameter, is present in the roof of the pharynx in the region of the second and third gill arches. This pad is a bilobed structure, one element being found on either side of the median line; when examined under a dissecting microscope it is seen to consist of three folds of hardened tissue, the first being the most developed. Each fold is beset with teeth, which gradually diminish in size as the last fold is reached. On the inner side of the last gill arch, guarding the entrance to the esophagus, is a triangular area, the base toward the ventral surface of the pharynx, covered with pharyngeal teeth smaller than those previously described. Teeth are found also along the inner margin of the interbrachial septa, one tooth being present on each area of a septum. The gill slits are latero-ventral in position, and therefore there is a greater amount of uninterrupted surface in the roof of the pharynx than in the floor.

ESOPHAGUS

Gross Anatomy.—The esophagus of *C. carminatus* is a comparatively short tube of about uniform diameter, connecting the posterior part of the pharyngeal cavity with the stomach. Its length is .9 cm. in a fish measuring 15 cm. in length. The anterior boundary is just posterior to the last gill arch and the triangular area armed with pharyngeal teeth, previously mentioned. The point of junction between the esophagus and the stomach is marked anatomically by a definite change in the diameter of the digestive tube at this point, fig. 1b.

The diameter of the esophagus is about .2 cm. in dorso-ventral axis and about .25 cm. in dextrosinistral axis. Hand

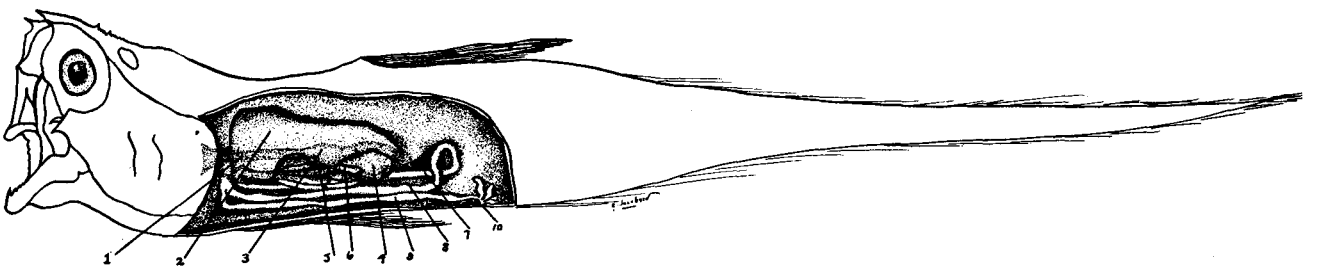

Fig. 2.—Lateral view of *C. carminatus* in outline with a portion of the left wall removed to show organs *in situ*: *1*, esophagus; *2*, liver; *3*, anterior cardia; *4*, cardiac "bulb"; *5*, pyloric stomach; *6*, pyloric caeca; *7*, first loop of intestine; *8*, second loop of intestine; *9*, third loop of intestine; *10*, anus.

sections show the inner lining of the esophagus to be thrown into a series of longitudinal folds of varying dimensions which are continuous with gastric folds of a similar nature, although less pronounced.

General Histology.—The esophagus is made up of four distinct layers: (a) mucosa, (b) submucosa, (c) muscularis, (d) adventitia (and serosa). In a specimen in which the esophagus was greatly contracted, the mucosa was thrown into 10 to 12 longitudinal folds, thus forming a comparatively small lumen. In another specimen in which the esophagus was distended, the mucosa was but slightly folded.

ESOPHAGEAL MUCOSA.—Due to the fact that in the specimens studied the anterior part of the gut contained a considerable amount of food material, as was indicated by large pieces of muscle, connective tissue, branchial cartilages, etc., the mucosal lining was frayed and torn in most instances. Here and there were areas in which the mucosa was intact, and upon these the following discussion, as well as fig. 2, is based.

The mucosa is unusual in that it is a relatively thin layer, measuring only 63.7 microns in width. In the anterior part of the esophagus there are two types of cells: typical stratified epithelium with a basal columnar layer, and goblet cells. The latter predominate; they are flask shaped, being longer than wide and usually slightly constricted at the distal end. They typically border upon the lumen; although in a great many cases they are so closely packed that they are pushed below the superficial epithelial cells. The goblet cells become fewer in number as the simple epithelia of the cardia is approached.

The bottom layer consists of low columnar cells whose nuclei are slender and basal. Then follow the usual polygonal epithelial cells with comparatively large, centrally located nuclei containing chromatin granules. Resting upon these are the goblet cells described above; the epithelial cells between the goblet cells are more or less fusiform in shape due to the pressure exerted by the latter. Those epithelial cells which are on the surface are typically flattened, fig. 2.

The stratified epithelium of the esophagus extended but a short distance before it abruptly changes to simple columnar epithelium. This transition occurs just anterior to the point at which the pyloric caeca leave the intestine. Therefore the esophagus, histologically, is only about .5 cm. in length; whereas anatomically it appears to be .9 cm. long.

These simple columnar cells are not so tall as those found

in the anterior part of the cardia, to be described later, nor do they show the characteristic 'zoning' of the latter. They measure about 25.4 microns by 4.7 microns. Their nuclei are long and slender, measuring 7.1 microns by 2.4 microns, and containing a few chromatin granules.

SUBMUCOUS CONNECTIVE TISSUE.—The epithelial coat of the esophagus rests upon a comparatively broad layer of connective tissue of the simple areolar type. The layer itself measures approximately 118 microns in thickness where there are no folds present. At the folds it is about 283 microns in thickness. Immediately beneath the epithelium are numerous blood vessels, so numerous and consistent that they practically form a vascular layer. Below these blood vessels the connective tissue is very densely packed, and the heavy collagenous fibers run parallel with the epithelium. This dense layer measures 11.8 microns and might possibly be looked upon as the tunica propria. With Mallory's triple stain it stains a more intense blue than the remainder of the connective tissue, but blends almost imperceptibly with it. This remainder is much looser, the fibers not always running parallel with the mucosa, especially where it runs between the fasciculi. Small oval nuclei of the fibroblasts are numerous and are scattered among the fibers. Occasional blood vessels are to be found and also some elastic tissue, particularly next to the muscularis.

ESOPHAGEAL MUSCULARIS.—The musculature of the esophagus is entirely striated and consists of an inner longitudinal layer, whose fasciculi are intimately associated with the submucosa, and an outer circular layer. The former averages about 83 microns in thickness, the latter 142 microns. The fasciculi of the inner longitudinal layer are separated from one another by noticeable strands of collagenous fibers extending from the submucosa. Elastic tissue is prominent in the epimysium and is present also to some extent in the perimysium of the fasciculi.

The outer circular layer consists of fibers whose average diameter is 7.1 microns. The nuclei are oval and measure about 4.7 microns or more in length. Some areolar connective tissue with associated blood vessels is present between the fasciculi.

ESOPHAGEAL SEROSA.—The greater portion of the esophagus lies free in the body cavity and is covered with a serosa continuous with that of the remainder of the peritoneal organs. The serosa in the region of the esophagus consists of a layer of typically flattened oval peritoneal cells and a fairly thick

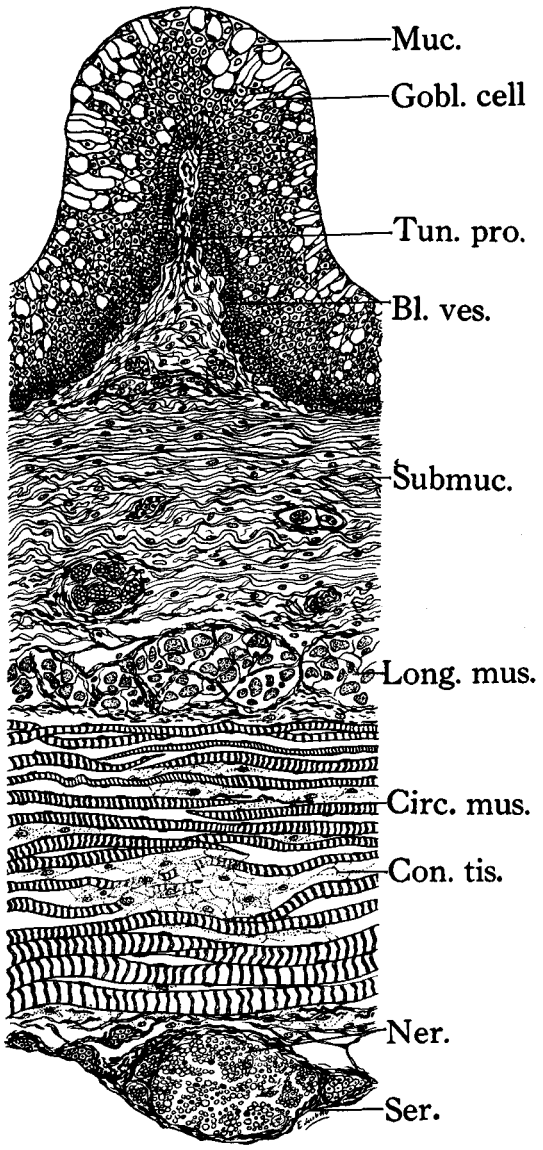

Fig. 3.—A narrow sector through a transverse section of esophagus. Medium to high-power figure, showing a fold of stratified epithelium covered with many goblet cells. *Bl. ves.*, blood vessel; *Circ. mus.*, circular muscle; *Con. tis.*, connective tissue; *Gobl. cell*, goblet cell; *Long. mus.*, longitudinal muscle; *Mus.*, mucosa; *Ner.*, nerve; *Ser.*, serosa; *Submuc.*, submucosa.

subserosal connective tissue layer containing numerous blood vessels and nerves.

STOMACH

Gross Anatomy.—The stomach is of the caecal type. It is J shaped, the longer limb extending straight caudad from the esophagus a distance of about 1.1 cm. At this point it turns abruptly forward; this first limb of the stomach ends in a bulb-like projection, fig 1b. The pyloric portion of the stomach comes off ventrally .4 cm. from the tip of the 'bulb.' The entire length of the pyloric portion is only .4 cm. The diameter of the pyloric portion decreases from .4 cm. at the point where it leaves the cardiac portion to .2 cm. where it joins the first loop of the intestine.

The foregoing measurements refer to a fish measuring 15 cm. in length. In a second specimen, the first limb of the stomach measured 1.3 cm. The pyloric portion, again, came off ventrally .4 cm. from the tip of the bulb. The distance covered by the pyloric portion was greater, however, measuring .8 cm., as it extended anteriorly and slightly ventrally.

The pancreas lies between the intestine, at one side, and the esophagus and the stomach on the other, being diffusely scattered, especially among the pyloric caeca. The ducts enter the intestine just anterior to the point of attachment of these caeca.

The liver is comparatively very large and consists of one main lobe and of what seems to be a very small subsidiary lobe. The organ is rather pyramidal and measures 1.8 cm. in length, .7 cm. in maximum width, and .5 cm. in maximum thickness. The greater part of the organ lies upon the left side of the stomach, fig. 3; the remainder, described in the foregoing as a subsidiary lobe, rests upon the dorsal surface of the stomach. The single duct leading to the gall bladder emerges from the liver in the connective tissue septum occupying the slight crease or fold between the large lobe and the subsidiary one. The gall bladder is small.

General Histology.—The coats of the gastric wall are similar, in general, to those found in the stomach of other fishes. Beginning with the inner mucosal lining, they are: (a) mucosa (epithelium and tunica propria), (b) submucosa, (c) muscularis, (d) serosa.

The mucosa, however, is rather peculiar in that it is very similar at both ends of the stomach, being simple, columnar epithelium, with typical glandular cardiac mucosa intervening.

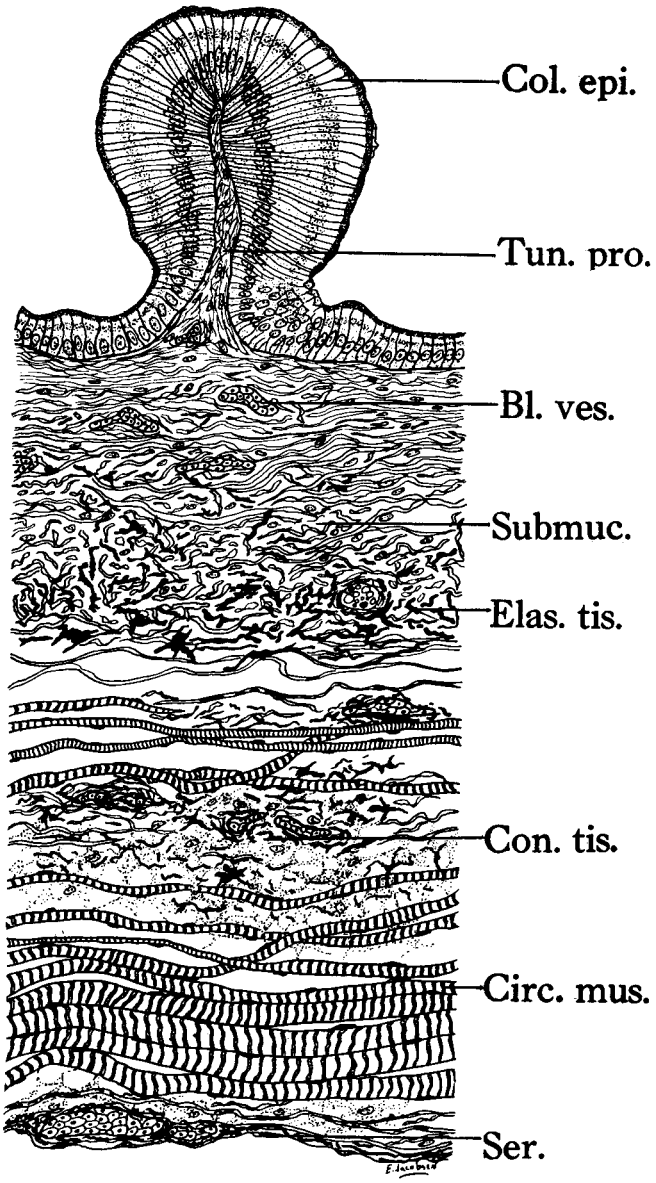

Fig. 4.—A narrow sector through a transverse section of anterior nonglandular portion of cardia. *Bl. ves.*, blood vessel; *Cir. mus.*, circular muscle; *Col. epi.*, columnar epithelium; *Con. tis.*, connective tissue; *Elas. tis.*, elastic tissue; *Ser.*, serosa; *Submuc.*, submucosa; *Tun. pro.*, tunica propria.

There is not the usual transition from stratified esophageal epithelium to typical glandular cardiac mucosa or from cardiac glands to pyloric, for the latter do not occur. Since there is such marked difference in the various regions of the stomach, each region will be described separately. The cardiac mucosa is comparatively thick, due to the presence of the gastric glands, but is not, as will be shown later, of uniform thickness throughout. The most anterior portion of the cardiac mucosa is very thin, consisting only of a simple columnar epithelium, thrown, even in a fully distended stomach, into a regular series of low folds and supported by comparatively little connective tissue. The folds become larger and more numerous just anterior to the pyloric portion of the stomach. The supporting connective tissue likewise becomes more evident, and a gradual change is observed from a much folded but nonglandular mucosa to a typical glandular gastric mucosa consisting of a thick layer of tubular glands. Thence, through the region of the cardiac bulb the mucosa is a uniformly thick layer until the posterior portion of the bulb is reached. Here the mucosa is much thicker on the ventral wall, and the glands are cut entirely in cross section in this thicker area, as they also are at the very tip of the bulb, due to the fact they so project into the lumen as to be cut transversely instead of vertically in a transverse section of the organ.

Anterior Nonglandular Portion of Cardiac Stomach.—GASTRIC MUCOSA.—The gastric epithelium is a single layer of slender cylindrical or columnar cells, resting upon a tunica propria which runs up into the folds of epithelium in very thin strands, fig. 4. The epithelium is thrown into folds which average about 135 microns in height and are about 5 mm. apart in a fully distended stomach; minor folds may occur between the major ones. In a contracted stomach these folds are present, but they are seated upon gross epithelial folds into which the submucosa projects. The cells vary in their micrometer measurements with respect to their location upon these folds. The cells upon the ends of the major folds measured on the average 44.6 microns in length and 2.9 microns in width at the exposed end, tapering only slightly toward the base. The nuceli of these cells are oval and quite slender, measuring 12.1 microns by 3.5 microns. Their chromatin is arranged in granules which are peripherally placed for the most part, with one or two centrally located. There is an outer region, measuring about 2 microns, of darkly staining cyto-

plasm at the distal ends of the cells. It resembles somewhat the cuticular border found on the intestinal cells of the higher vertebrates, but is not visibly striated. Just within this outer dark band of cytoplasm is a band of clear, nonstaining cytoplasm of about the same width. These two bands together occupy about the distal third of the cells. The cells between the folds are much shorter, being of a low columnar type and not tapering toward the base. They measure 23.6 microns by 4.7 microns, and their nuclei are oval, measuring 7.1 microns by 5.9 microns. Their cytoplasm is like that of the taller cells on the folds, with the exception that the bands described above are not quite so wide and the outer dark band is not quite so distinct.

Tunica propria.—The epithelium rests directly upon a thin homogeneous membrane, the membrana propria. The tissue underlying this basement membrane is made up of tightly packed collagenous fibers running parallel with the outer surface of the epithelium. In spite of its noticeably heavy and compact fibers, it is abundantly vascularized, especially at the bases of the folds, where comparatively thin strands of connective tissue push upward, supporting the epithelium. Capillary knots are present immediately beneath the epithelium and in the connective tissue between its folds. There is no distinct line of demarcation, such as a muscularis mucosae, between the tunica propria and the submucosa; in fact, the tunica propria is greatly reduced and in some areas appears to consist of only a few strands of collagenous fibers contiguous with the basement membrane.

GASTRIC SUBMUCOSA.—The submucosa of the anterior portion of the cardiac stomach forms a comparatively uniform layer of white fibrous connective tissue between the tunica propria and the muscularis circularis. Toward the epithelial layer the fibers are closely packed and run parallel, for the most part, with the mucosa, but toward the muscularis circularis the tissue assumes more the appearance of characteristic loose areolar connective tissue. Blood vessels are numerous in the submucosa and vary in size, some attaining a considerable diameter. The characteristic nuclei of the fibroblasts, some of which contain a distinct nucleolus, are numerous, both closely adhering to the fiber bundles and in the meshes of the looser fibers. Elastic fibers and plates are present in that portion of the submucosa which consists of loose areolar connective tissue and is adjacent to the muscularis. This elastic element, quite

conspicuous even here, reaches its maximum development in the glandular region proper and forms a characteristic element in the subepithelial layers of the cardiac stomach.

GASTRIC MUSCLE LAYERS.—In general, it may be said that there is but one layer of muscle in the anterior part of the cardiac stomach, a muscularis circularis; however, at the junction of the esophagus and the stomach where the stratified epithelium of the former becomes simple columnar, the inner muscularis longitudinalis characteristic of the gut anterior to the stomach is continued for a short distance. It soon disappears, leaving only the muscularis circularis, which is of uniform thickness about that of the submucosa or a little more. It consists entirely of striated muscle with white fibrous connective tissue scattered between the muscle fibers. Striated muscle fibers with a diameter of 9 microns or more are to be found in noticeable bundles next to the serosa, especially on the ventral and left walls of the stomach; toward the submucosa the diameter of the striated muscle fibers is less, and they are more scattered, with considerable amounts of loose white fibrous connective tissue between them. Blood vessels are quite abundant in the connective tissue meshes between the muscle fibers. The whole muscle layer, in fact, is characterized by its rather loose nature and its relatively large amount of white fibrous connective tissue as well as its being composed entirely of striated muscle. Elastic tissue similar to that found in the submucosa occurs locally in considerable amounts where white fibrous connective tissue is present, between groups of muscle fibers to some degree, especially in areas of vascularization, and as an occasional strand or two between individual muscle fibers.

GASTRIC SEROSA.—The serosa forms a distinct layer in the anterior part of the cardiac stomach. It consists of an outer layer of very flat and elongated peritoneal cells with oval, slender nuclei and of a fairly thick subserosa composed of white fibrous connective tissue, numerous capillaries, and occasional larger blood and lymph vessels, and nerves.

Region of Transition between Nonglandular and Glandular Mucosa.—GASTRIC MUCOSA.—The region of transition between the simple, nonglandular, folded epithelium of the anterior portion and the typical glandular mucosa occurs just dorsal to the pylorus, fig. 1b. The mucosa consists of areas of nonglandular epithelium previously described and of areas of low tubular gastric glands, the two regions alternating. In this

particular region can be seen the manner in which the connective tissue septa or trabeculae, so characteristic of the typical gastric mucosa, fig. 5, to be described later, are formed. The strands of connective tissue, which were described as pushing up into the epithelial folds to support them, become heavier and much longer so that they fold over and anas-

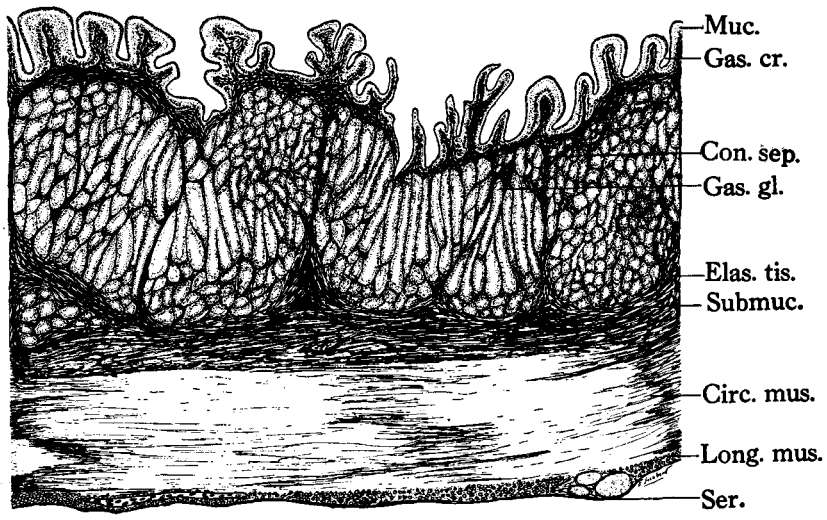

Fig. 5.—A low power of portion of transverse section of typical glandular portion of cardia, showing the depth and regularity of the mucosa and the characteristic connective tissue trabeculae of the tunica propria. *Cir. mus.*, circular muscle; *Con. sep.*, connective tissue septa; *Elas. tis.*, elastic tissue; *Gas. cr.*, gastric crypt; *Gas. gl.*, gastric gland; *Long. mus.*, longitudinal muscle; *Mus.*, mucosa; *Ser.*, serosa; *Submuc.*, submucosa.

tomose with strands from neighboring folds and come to lie just under the epithelial cells. Thinner strands of connective tissue, arising from the loose areolar tissue of the submucosa, extend upward between the glands. Thus there are formed groups of 12 to 15 glands enclosed by major septa derived from the outpushings of connective tissue supporting major epithelial folds, and within the enclosed group are smaller, thinner strands of supporting tissue between the glands themselves.

Tunica propria.—The tunica propria is the same as that for the nonglandular region with the exception that where it occurs in association with the glands it no longer runs parallel with the outer surface of the epithelium but follows the contour of the glands which it supports. It is extremely well

vascularized, even the thinnest strands being abundantly supplied with capillaries. The elastic tissue becomes quite evident in the supporting connective tissue associated with the glands.

GASTRIC SUBMUCOSA.—The submucosa is similar to that previously described as associated with the nonglandular epithelium, but in the glandular areas it loses its inner closely packed layer of fibers and is much thinner, consisting only of loose, areolar connective tissue. Elastic tissue is more prominent.

GASTRIC MUSCLE LAYERS.—The musculature still consists of but one layer, the muscularis circularis, and is, as previously stated, made up entirely of striated muscle fibers with white fibrous connective tissue and some elastic tissue.

GASTRIC SEROSA.—The serosa is similar to that already described.

Region of Typical Cardiac Mucosa Possessing Gastric Glands.—GASTRIC MUCOSA.—That portion of the cardiac stomach which is posterior to the junction of the cardiac stomach and the pyloric presents a typical gastric mucosa, consisting of long tubular glands which reach their maximum development just anterior to the cardiac bulb. The epithelium is of the simple columnar type which covers the entire surface of the gastric mucosa and extends down into the pits. The gastric wall presents a very uniform appearance, due to the fact that there are no gross folds in the stomach lining in this region. The mucosa is very deep, occupying almost two thirds of the entire wall, fig. 5.

The epithelial cells of the cardiac are very long, slender, columnar cells. These cylindrical cells are so arranged upon the low gastric folds as to form fan-shaped masses. The cells vary in their micrometer measurements with respect to their position. At the outer ends of the fan-shaped masses where they are the longest they measured on the average 58.2 microns in length, 4.8 microns in width at the exposed end, and 2 microns or less at the basal end, some tapering to a process. The nuclei are in the basal third of the cells. They are slender oval masses whose chromatin is conspicuously in the form of granules, for the most part placed peripherally. The average size of the nuclei is 11.8 microns by 2.4 microns. Some of these cells measured only 47.2 microns in length; others were as long as 61.7 microns. These apical cells of the fan-shaped masses give way to cells which are shorter, measuring 23.6 microns in length, 2.4 microns in width at the apical end, and less than 2 microns in width at the basal end. The latter in turn gradually

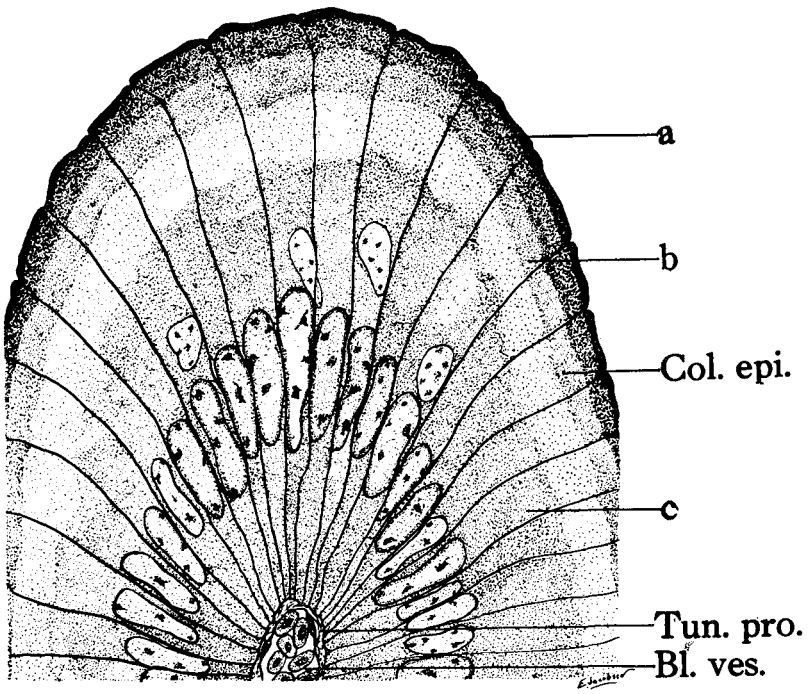

Fig. 6.—A highly magnified portion of field, showing the top of a gastric fold in the glandular region of the cardia. (This figure also illustrates the epithelium of the nonglandular portion of the cardia.) It shows the characteristic "zoning" of the cytoplasm of the epithelial cells. *Bl. ves.*, blood vessel; *Col. epi.*, columnar epithelium; *Tun. pro.*, tunica propria; *a*, narrow blue-stained zone at periphery; *b*, red-stained zone below it; *c*, broad blue-stained zone, which is paler in region adjacent to red-stained zone.

decrease in size until they are transformed into the cuboidal cells of the basal portions of the crypts. The cytoplasm of the superficial cells, both of those on the gastric folds and of those lining the upper portions of the crypts, reacts to certain stains in a rather striking manner. Mallory's triple stain differentiates cytoplasm in the following manner: (a) a narrow blue-stained zone at the periphery, measuring about 2 microns in width; (b) below it, a red-stained zone, measuring about 2.5 microns in width; (c) a broad blue-stained mass in the middle portion of the cell, measuring about 18 microns, which is slightly paler in the region just above the nucleus; (d) and the remainder of the cell below the nucleus stains pale blue, fig. 6.

With Heidenhain's iron-alum haemotoxylin and with haemotoxylin and eosin stains, the peripheral band of cytoplasm resembles very closely the cuticular border found in the intestinal cells of higher vertebrates. Below it the cytoplasm forms a band of pale, nonstaining cytoplasm, measuring about 7 microns in width. The remainder of the cytoplasm, both above and below the nucleus, stains uniformly. This staining reaction is very similar to that described for the plaice (Dawes 1929), and it is probably indicative of the fact that the superficial cells are mucus-producing and that the various zones represent different stages of activity.

The gastric crypts vary in depth, but all are deep, the majority of them extending about half the depth of the mucosa. The gradual change from the tall slender cells of the free surface to the distinctly cuboidal cells of the lower portions of the crypts has already been mentioned. These deeper cells have the same characteristic staining reaction as do the superficial ones. The cuboidal cells, however, show no characteristic staining, and they extend down through the stroma a distance of about 130 microns. They differ structurally from the superficial cells lining the epithelial depressions and therefore are probably comparable with ducts. They measured 7.1 microns by 5.9 microns. The nuclei are oval, almost spherical, measuring 2.7 microns by 2.4 microns and the comparatively little chromatin is in the form of one or two small masses. Several of these ducts of cuboidal cells may open into a single epithelial pit. The cuboidal cells change abruptly to a region of very low cuboidal cells, almost squamous in their appearance, which measured about 4.7 microns by 2.7 mcirons. Their nuclei are oval, measuring 2.7 microns by 2.1 microns. These flat cells present a decided change in structure from the most superficial type and are characteristic throughout the glandular region of the cardia. The region where the change from cuboidal to flat epithelium takes place is noticeably constricted. There may be some question as to whether the cuboidal cells or the flat cells are synonymous with the 'neck cells' described by Gulland for *Salmo salar*. The squamous shape of these cells may be due to pressure since there are usually two, and sometimes more, glands opening into a single duct of cuboidal cells. In either case, both are constant throughout the glandular region and both represent a distinct change in structure from that of the superficial epithelial cells of the surface and of those lining the pits. The flat cells change abruptly to the secreting

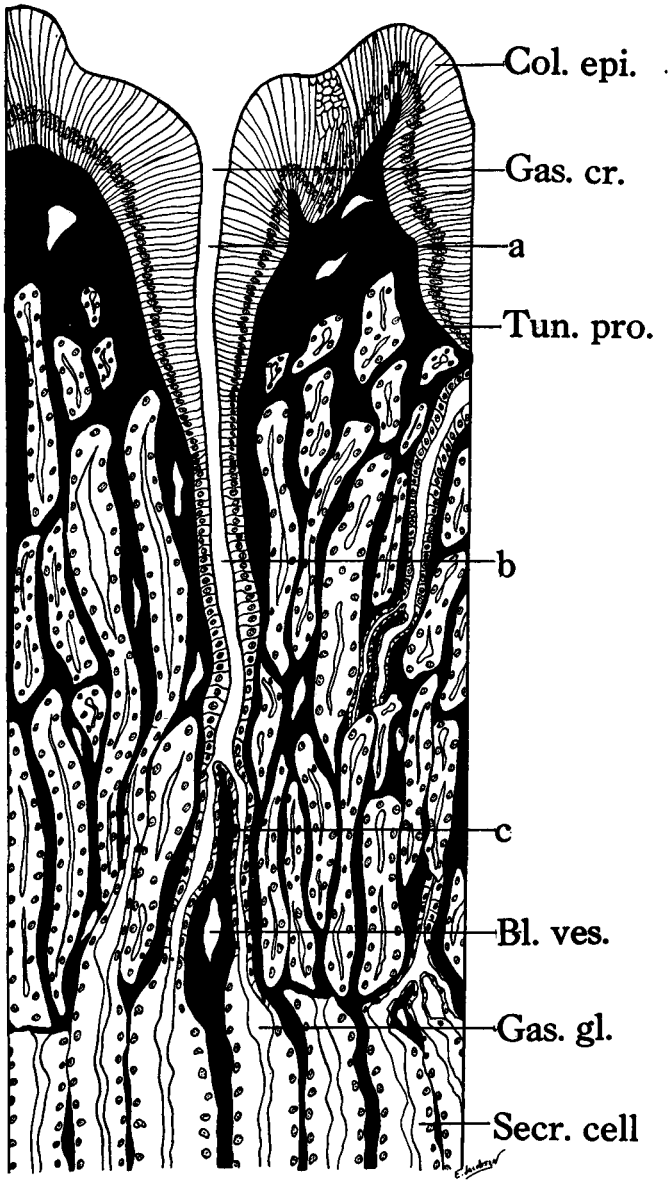

Fig. 7.—A semidiagrammatic high-power figure of portion of glandular mucosa of cardiac stomach, showing arrangement of ducts. *Bl. ves.*, blood vessel; *Col. epi.*, columnar epithelium; *Gas. cr.*, gastric crypt; *Gas. gl.*, gastric gland; *Secr. cell*, secreting cell; *Tun. pro.*, tunica propria; *a*, epithelial pit lined with superficial epithelium; *b*, "duct" region lined with cuboidal epithelium; *c*, region of flat "neck cells."

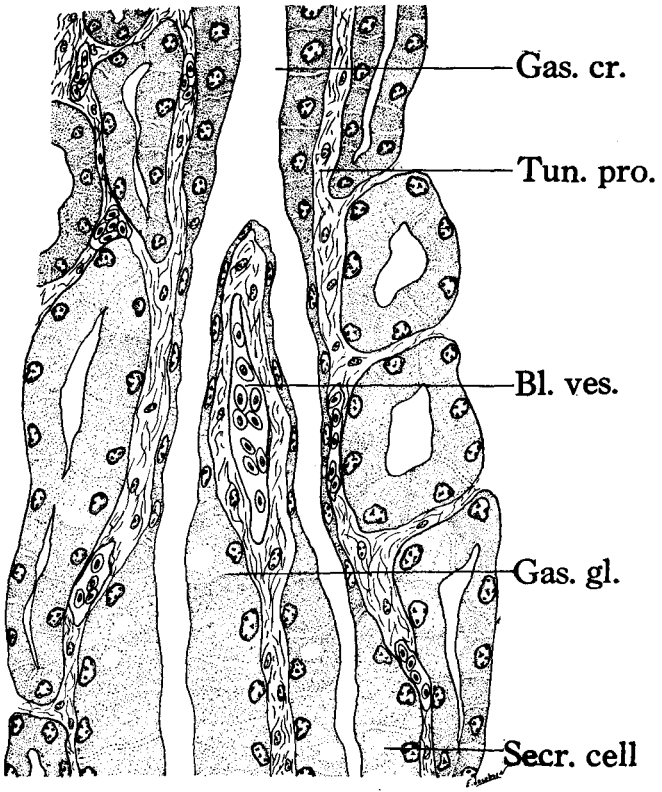

Fig. 8.—A highly magnified portion of glandular gastric mucosa, showing cuboidal, "neck," and secreting cells. *Bl. ves.*, blood vessel; *Gas. cr.*, gastric crypt; *Gas. gl.*, gastric gland; *Secr. cell*, secreting cell; *Tun. pro.*, tunica propria.

cells of the gastric glands. Therefore there seems to be an intermediate, structurally different type of cell between the superficial epithelium and the secreting epithelium of the glands. Thus crypts may be said to consist of three regions: (a) an epithelial depression formed by the continuation of the superficial epithelial cells of the surface into the pit for a distance of 142 microns; (b) a duct region formed by cuboidal cells, measuring 127 microns; and (c) a relatively short region, measuring 28 microns, of flat neck cells, fig. 7.

The glands are long regular tubes, rarely bifurcated at the blind end, and with comparatively wide lumina; they therefore present a regularity of arrangement which gives a general

uniformity of thickness to the mucosa, since there are no major gastric folds. As stated previously, two or three gland ducts may open into a single epithelial pit, the usual number being one or two; moreover, two or more glands may open into a single gland duct, the usual number being two.

The secreting cells of the glands are of a low columnar type, often pyramidal in shape with apices toward the lumen, fig. 8. They average 9.5 microns in length and 11.8 microns in their greatest width. Their nuclei are round and basal in position, measuring 4.7 microns in diameter. They do not contain a great deal of chromatin, and what is present is usually concentrated in one or two irregular masses in the center. The cells in all specimens studied appeared clear and pale, the cytoplasm being only faintly granular. No zymogen granules were demonstrable. This fact is probably due to the disintegration of the granules upon fixation.

As previously stated, the lumina of the glands are notably wide, measuring from 7.1 microns to 14.1 microns, the former being about average. Even in cross-sections of the glands, the pyramidal cells do not approximate each other very closely, and a wide lumen is preserved.

Tunica propria.—It is in this region that the tunica propria is developed to its greatest degree. Next to the submucosa it is very difficult to make out a sharp line of demarcation; the two layers intermingle so as to give the appearance of a continuous layer. However, where it penetrates upward and between the gastric glands it is very prominent. Bands of fibers 7 to 23 microns in width mark off the groups of glands, while thin strands occur between the individual glands. Beneath the superficial cells of the epithelium is a band of fibers very closely packed, about 11 microns wide. The entire tissue is very abundantly vascularized. Corpuscles can be distinguished in noticeable numbers in even the thinnest strands between the glands. Elastic tissue is present in the widest portions of the penetrating strands of connective tissue near the submucosa, but is does not extend very far between the glands.

GASTRIC SUBMUCOSA.—The submucosa of the glandular region of the cardiac stomach forms a fairly uniform layer of 83 microns in thickness between the tunica propria (which merges imperceptibly with it) and the muscularis circularis. It is of a loose areolar structure heavily impregnated with fibers and plates of elastic tissue. The elastic tissue stains heavily, usually black, with all stains used and gives to the

layer a very striking appearance. In some instances it completely obliterates the areolar connective tissue.

The submucosa contains numerous blood vessels, some of considerable size, small isolated groups of smooth muscle fibers, a nerve plexus, and the nuclei of the fibroblasts.

GASTRIC MUSCLE LAYERS.—In the region of the cardiac stomach just dorsal to the point at which the pyloric portion of the stomach comes off, the muscularis is seen to consist of two layers, an inner circularis and an outer longitudinalis. The latter is only about a seventh as wide as the former and is not a continuous layer. The circularis consists of a mixture of striated and smooth muscle fibers, the latter predominating, and considerable amounts of connective tissue. It is to be noticed that as the smooth muscle becomes more prominent and as the longitudinalis becomes a more distinct layer, the amount of connective tissue present in association with the muscle tissue becomes less.

In the region of the cardiac bulb where the two muscle layers are developed to their greatest degree, the circularis is about five times as thick as the longitudinalis. Connective tissue is prominent only between the two muscle layers, although a small amount does occur in the circularis associated with small blood vessels. Numerous blood vessels and nerves of the myenteric plexus, with their supporting connective tissue, occur between the two muscle layers.

GASTRIC SEROSA.—The serosa is as previously described.

PYLORIC STOMACH

PYLORIC MUCOSA. — The change from cardiac to pyloric stomach is as abrupt histologically as anatomically. Where the pyloric portion ventrally leaves the cardiac, the mucosa abruptly changes from a typical thick glandular mucosa to a low much-folded nonglandular epithelium which so resembles the mucosa of the anterior part of the cardia that one hardly would be able to tell them apart without the associated tissues to identify them. Practically the only difference between the two is that the pyloric mucosa is more folded, the folds being 130 microns high.

The cells on the tops of the folds average 47.2 microns in length by about 2.4 microns in width at the exposed end and 2 microns or less at the base. Their nuclei are very slender oval bodies measuring 11.8 microns by 2.4 microns. The chromatin is in the form of small scattered granules. The cells on

the sides of the folds and between the folds are shorter, thicker, and nontapering. They measure 23.6 microns by 3.5 microns. Their nuclei are more nearly spherical, measuring 7.1 microns by 4.7 microns. These cells show the same characteristic zoning in their cytoplasm as did the cells of the cardia.

Tunica propria.—This layer forms a very densely packed substratum of collagenous fibers about 30 microns in thickness, except where it penetrates the epithelial folds where it is five times as thick and is accompanied by abundant vascularization. The thickness and density of the fibers and the fact that they penetrated into the repeatedly branched folds of the epithelium affords proof of its supporting character.

PYLORIC SUBMUCOSA.—The pyloric submucosa is similar to that already described for the cardia and forms a layer of rather loose areolar connective tissue about 72 microns in thickness.

PYLORIC MUSCLE LAYERS.—The musculature consists of an inner circularis and an outer longitudinalis. The former is about six times the thickness of the latter. Both are made up of very closely packed smooth muscle fibers with little connective tissue or vascularization. The circularis increases greatly in thickness at the pyloris, being twice its normal width.

PYLORIC SEROSA.—The pyloric serosa consists of a single layer of flat peritoneal cells with little or no connecting tissue except where the larger blood and lymph vessels and nerves penetrate.

INTESTINE

Gross Anatomy.—The intestine extends forward from the pylorus about .2 cm. to the point where numerous diverticula, the pyloric caeca, are given off, this point being directly ventral to the esophageal-gastric junction. It then bends ventrally in a rather wide curve, the length of which is approximately .5 cm., and extends caudad through the lower portion of the coelom a distance of 2.1 cm. It then loops upward and over itself in a very short loop, measuring about .2 cm., to go cephalad, ventral to the first loop and dorsal to the last, fig. 2, not yet described, extending forward about 2.3 cm. and reaching the anterior extremity of the coelom. Here, it turns downward in a very slight loop to extend caudad in the most ventral portion of the coelom a distance of 2 cm. Posteriorly the tube narrows slightly. The diameter varies slightly throughout the length of the tube, depending upon the degree of distention by

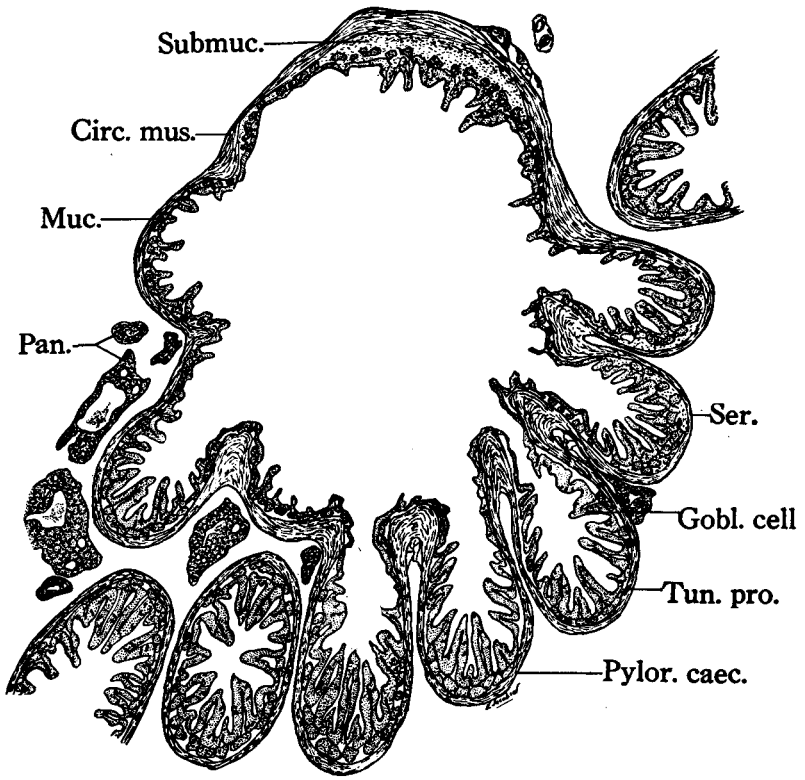

Fig. 9.—A low power of region of intestine, showing pyloric caeca as diverticula of the same. They will be seen to have the same structure as the intestine with the exception of the absence of the submucosa and probably the longitudinalis also. *Cir. mus.*, circular muscle; *Gobl. cell*, goblet cell; *Muc.*, mucosa; *Pan.*, pancreas (diffuse); *Pyl. caec.*, pyloric caecum; *Ser.*, serosa; *Submuc.*, submucosa; *Tun. pro.*, tunica propria.

food materials. The walls are thin and semitransparent and are therefore colored by the food matter contained within.

Pyloric Caeca.—There are 15 pyloric caeca lying on the left side of the stomach. They are apparently in two groups, bound together by membranes, nine being in the outer group (toward the peritoneum), averaging .4 cm. in length and .1 cm. in thickness, and six underneath this outer group, averaging .6 to .7 cm. long and .1 cm. thick. In addition to these 15 caeca, there are six more, dorsal and slightly lateral to the stomach, three of them being longer than the others. The former average .6 cm. in length and .2 cm. in thickness; the latter, .4 cm. long and .2 cm. thick. The caeca taper slightly in diameter as

the blind ends are approached, measuring approximately .25 cm. at their bases and .1 cm. or less at the blind ends. They are given off in an area which measures about .1 cm. in length encircling the intestine. The caeca, as was found in the sea bass (Blake 1930), are absent on the inner dorsal curve of the pyloric intestine, this being the region of the blood supply, and they originate laterally, mostly sinistrally, and ventrally from the small intestine just anterior to the pylorus.

Without exception, the caeca run directly caudad, covering the major portion of the stomach, fig. 2. The number of caeca is not constant. In specimens examined the number was found to vary from 19 to 21, but the general arrangement is the same.

GENERAL HISTOLOGY.—It has been stated that the pyloric caeca, being diverticula of the intestine, have the same structure as that portion of the intestine where they are given off. This also may be said to be true of *C. carminatus,* fig. 9, but there is a variation in the relative thickness and in the degree of development in several of the coats. The layers, proceeding from the lumen, are: (a) mucosa (epithelium and tunica propria), (b) muscular layers (circularis and longitudinalis, the latter being discontinuous), (c) serosa. There is no submucosa present.

CAECAL MUCOSA.—*Epithelium.*—When cut in transverse section, the pyloric caeca demonstrate a very regular folding of the mucosa into 7 to 12 major folds about .33 mm. high, and 8 to 13 minor folds about .13 mm. high. Usually the major and minor folds alternate. In sections these appear as fingerlike projections having a uniform histological structure, the cells being the usual tall cylindrical and goblet cell types.

The cylindrical cells are very slender and taper but little. They measured on the average 50 microns in length and 2.5 microns or less across. The cytoplasm shows no particular structural features, staining uniformly throughout. The nuclei are slender oval bodies lying in the basal third of the cells and containing scattered masses of chromatin. They measured about 7.1 microns by 2.4 microns. There is a cuticular border or "top plate" 5.7 microns in width. This top plate shows very definite vertical striations. In vertical sections of the cells terminal bars appear immediately beneath the cuticular border. In areas where the cells are cut tangentially, as at the base of the folds, is seen the typical honeycombed or hexagonal appearance of terminal bars in surface view. The top plate is

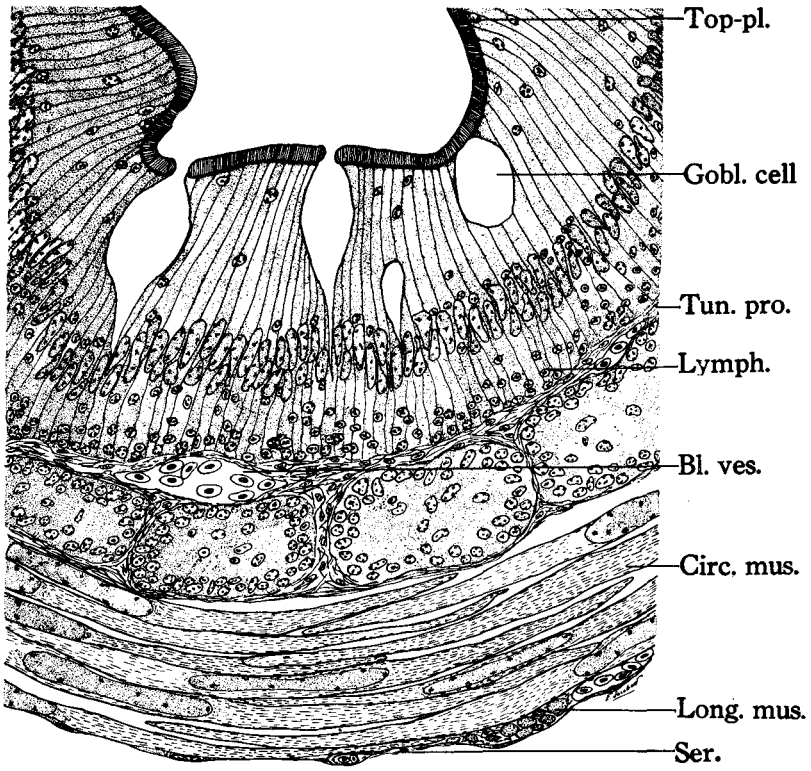

Fig. 10.—A highly magnified portion of caecal epithelium, showing a portion of the bottom of a mucosal fold. (This figure illustrates also typical intestinal epithelium.) The tubular-appearing structures in the tunica propria at the base of the epithelial fold are not glands but transverse sections of the lower portions of mucosal folds of epithelium. *Bl. ves.*, blood vessel; *Cir. mus.*, circular muscle; *Gobl. cell*, goblet cell; *Long. mus.*, longitudinal muscle; *Lymph.*, lymphocytes; *Ser.*, serosa; *Top-pl.*, top-plate; *Tun. pro.*, tunica propria.

ruptured occasionally to allow for the escape of mucus from the goblet cells. These are scattered irregularly throughout the epithelium, and in Mallory's triple-stained sections they appear characteristically blue in contrast to the pink-staining columnar cells. There are present no granular cells which might be indicative of glands.

Lymphocytes, smaller in size than the columnar cell nuclei and staining much more deeply, are very numerous, forming layers along the bases of the columnar cells and also penetrating interstitially into the epithelium, coming to lie just under

the top plate. They form a very conspicuous element of the epithelium, fig. 10.

Tunica propria.—The tunica propria is a very narrow layer about 2 to 3 microns in width between the epithelium and the circularis. This layer is made up of compact fibers running circularly, parallel to the circularis. From it, however, looser strands of fibers, in whose meshes are blood vessels and numerous lymphocytes, penetrate the epithelial folds to their very tips.

CAECAL MUSCLE LAYERS.—The inner circularis forms a definite layer about 35 microns in thickness, but the outer longitudinalis, when present, seems to be only about 5 microns in thickness. The longitudinalis, even in direct cross-section of the caeca, appears to be discontinuous. It is a question as to whether this is actually the case or whether it is due to shrinkage of the layer during the fixation of the tissue. There is very little connective tissue associated with either layer and only a few small blood vessels.

CAECAL SEROSA.—The serosa is composed of the usual single layer of flattened peritoneal cells, and where a portion of the pancreas scattered diffusely between the caeca is attached, it is continuous with the peritoneal covering of that organ. Very little connective tissue is present and usually only where an occasional blood vessel of rather small caliber penetrates the layer.

Histology of the Intestine.—The intestinal coats are not constant throughout the length of the organ. The most anterior part of the intestine (in region the pyloric caeca) is relatively thick walled and is made up of the four typical intestinal coats: (a) mucosa, (b) submucosa, (c) muscularis (circular and longitudinal), and (d) serosa. This is also true for the middle and posterior portions of the intestine, with the exception that the wall as a whole is very thin and transparent, allowing fecal material to show through; the layers, therefore, are correspondingly thinner. The submucosal layer is apparently absent in the diverticula of the intestine as is also the longitudinal muscle layer. In the posterior part of the intestine the submucosa is relatively thin and very loose, containing a great deal of adipose tissue, but it increases in compactness as the anus is approached; the inner circular muscle layer also increases greatly in thickness.

INTESTINAL MUCOSA.—The mucosa, as in the case of the pyloric caeca, is thrown into a number of folds. There is also

the same characteristic appearance of what at first glance appear to be cross-sections of glands in the tunica propria at the bases of the folds, but which are actually extensions of the lower portions of the epithelial folds which are cut in cross section. The epithelium is of the same nature as that described for the pyloric caeca. Tall, columnar cells are constant throughout the intestine. Goblet cells are found in various stages of formation and are no doubt modified columnar cells, but they are fewer than the latter, and their number does not seem to vary appreciably in any portion of the intestine. 'Wandering cells' or lymphocytes are numerous at the bases of the epithelial cells as well as interstitially, as noted in the case of the caeca.

Tunica propria.—The tunica propria, consisting of rather densely packed white fibrous connective tissue with numerous scattered nuclei of fibroblasts, forms a definite supporting layer at the bases of the epithelial folds, around their cross-sections and between them. It is richly supplied with blood vessels.

INTESTINAL SUBMUCOSA.—When present, the submucosa consists of a loose areolar type of connecting tissue, containing numerous fat cells, small blood vessels, and nerves. It forms a layer between the tunica propria and the inner circularis.

INTESTINAL MUSCLE LAYERS.—The inner circularis is the thicker of the two muscle layers. It is of the nonstriated type and is very densely packed. Small blood vessels are to be found between it and the outer longitudinalis, which is also nonstriated and is about a third as thick as the circularis. Near the anus the circularis is greatly thickened to form the anal sphincter and is very abundantly vascularized, containing blood vessels of considerable size and a meshwork of small capillary vessels. The longitudinalis is not present at this point. As in the case of the salmon (Greene 1912), the plexus myentericus (of Auerbach) is imbedded in the inner wall of the longitudinalis.

INTESTINAL SEROSA.—Seemingly contrary to what has been described in other fishes, the serosa in *C. carminatus* is more like that found in the intestine of mammals than the cuboidal, almost cylindrical type described by Greene (1912) and Blake (1930). On the contrary, the cells are of the usual flattened peritoneal cells found in higher forms, and there is little or no subserosa present. The serosa is absent in the terminal portion of the alimentary tract, being replaced by a fibrous connective tissue layer which connects it with the surrounding tissues.

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