CHAPTER IV

MORPHOLOGY OF THE DIGESTIVE TRACT OF OPHICEPHALUS PUNCTATUS

It has been reported before that the isolated intestine of the Ophicephalus punctatus may suitably be utilised for assay of acetylcholine and serotonin as it responded nicely to the graded doses of those drugs. Therefore, it was considered desirable to describe briefly the general morphological features of the gut of the particular species of fish. The gross anatomical peculiarities e.g., size, shape, thickness, and histomorphological characteristics of the different portions of the gut of freshly killed Ophicephalus punctatus of varying sizes have been examined and reported in this section.

Material and Methods :

Full grown healthy specimen of O. punctatus weighing between 50 and 80 gms was anaethetised with clove oil (1 : 1000) in water within 10 to 15 minutes. (1 ml of clove oil was dispersed in a cylinder containing 1000 ml fresh water). The whole alimentary tract from the jaw to the anus was dissected out and displayed, and the measurement of each individual part was noted. Dissected portions of the gastro-intestinal tract were cut out with a razor blade and were placed individually in a fixative. The fixatives used were Zenker-acetic acid and Zenker-formol solution, or 4% formaldyhyde in a 0.05 mm sodium phosphate buffer at pH 7.2. The fixed tissues were dehydrated with graded alcohol, from 50% to absolute, within 48 hours, cleared in xylol-cedar wood oil mixture (1 : 1), embedded in paraffin (melting point 58° to 60°C), and sectioned at 3/u to 7/u thickness. Sectioned tissues were stained with Harris' haematoxylin-eosin and Heidenhain's iron haematoxylin methods. The presence of connective tissue collagen fibres was demonstrated by Mallory's phosphotungstic acid-haematoxylin and Van Gieson's staining method. A stable silver solution method (Naoumenko and Feigin, 1967) had been used for the identification of the nerve plexus.

The fixatives and staining reagents were prepared as per standard methods described in the book "Manual of Histologic and Special Staining Technics" (1960, McGraw-Hill Book company, Inc., second edition, New York, Toronto, London).

Results:

A) Gross Morphology

The general arrangement of the digestive system of O.punctatus was similar to that of common vertebrates as it bore many similarities in structural form also. The alimentary system of this particular fish comprised of (i) mouth or buccal cavity having teeth, tongue and glands; (ii) a pharynx from which the gill slit opened to (iii) oesophagus - a short but wide muscular tube; (iv) a dilated pouch or sac-the stomach; (v) intestine-an elongated tube starting from the upper lateral side of the stomach which was coiled upwards and after bending ended ventrally at the rectum; (vi) two blind slender tube-like structures-pyloric caeca - opening at the begining of the intestine near the stomach.

The liver, gall bladder and pancreas were all surrounding near pyloric region. The swim bladder opened through fine slender thread like ducts into stomach.

The roof and floor of the buccal cavity and pharynx are illustrated in figure 3. . The first gill cleft was taken as the dividing line between the buccal cavity and pharynx. Numerous teeth were present in premaxilla, prevomer and dentary. Mucosal folds might be seen on the roof and the floor of buccal cavity. In the pharynx there were two dorsal convex cartilaginous plates fitting into smaller ventral concave plates. The front of each ventral tooth plate was just behind the last gill arch. These plates were covered with sharp conical teeth similar to those on premaxilla. The tongue was supported below by hyoid.

The mouth or buccal cavity and the pharynx were lined with mucous membrane containing minute raised bodies which may be called the 'tastebuds'. The oral cavity was lined by the stratified squamous type of epithelium. The tongue was very thin and might be the covering of epithelial and connective tissue over the hyoid bone of the floor of the mouth.

The pharynx was the continuation of the buccal cavity as it was lined by stratified squamous epithelium.

The oesophagus was a short wide muscular tube continued from the pharynx and opened into the dilated pouch, the stomach. The taste buds were seen at the proximal end whereas little or no tastebuds could be seen at the lower end of the oesophagus. The opening of the oesophagus appeared to be guarded by sphincter like structure-a tough musculature.

The stomach appeared like a small blind pouch tapering caudally and more compact in shape and opened or continued through the pyloric region into the small intestine. The opening of the intestine started at some higher level near the upper portion of the stomach usually on the right side.

Two blind pyloric caeca, bilaterally placed around the pyloric end, opened in the gut, and the small intestine perhaps started from this point. Figure (4) shows the whole of the gut as it remained in the body cavity and figure (5) shows the diagramatic view of two pyloric caeca where the portion of the liver is displayed and this shows the opening of bile duct into the intestine.

The intestine formed a U-shaped loop at the mid region of the whole tube. The width of the intestine was more or less uniform throughout the gut. After forming a U-shaped loop distally the intestine passed downwards caudally and opened into anus, anterior to the point of entrance of the genital and excretory ducts.

As the anus was approached the rectum or terminal portion could be distinguished from the rest of the intestine by its straight course to the anus and by the pigmentation darker than the other parts. Figure 6 shows the gut removed from the body cavity with some connective tissues cut so that it can be better displayed. The mucosa of the oesophagus, stomach and intestine exhibited characteristic folds (Fig.7). The fine recticulate folds of oesophagus changed abruptly into the coarse folds in the stomach. The upper portion of intestine had five or six longitudinal folds while the internal surface of the rectum had a reticular appearance. The pneumatic duct of the swim bladder opened into left side of the cardiac end of stomach. Bi-lobed liver covered the oesophagus, the left liver being larger than the right one. Prominent gall bladder with duct was attached to the right liver. The scattered pancreas covered with connective tissue beneath the stomach and liver was highly vacularized and opened into the upper portion of the gut just below the opening of the pyloric caeca. A sphincter-like musculature was present at the pyloric region of the stomach and at the rectum also.

Different measurements of the gut in relation to body weight and size were taken and it was found that although the body weight varied widely with the length of the body the total length of the intestine did not vary in that proportion. The size of the caeca and stomach did not vary from each other inspite of wide variation in body weight and length. As the O.punctatus is larvivorous in habit its intestine was also comparatively smaller than other herbivorous fishes. Table -II shows the gross measurement of the body and the various parts of the intestine of O.punctatus.

B. Histology

It has already been told that the gross pattern is the same allthrough the gut i.e. the gut is composed of mucous coat, submucous coat, muscular coat and serous coat. While there were

Table - II

The gross measurements of the body and various parts of the digestive tract of O. punctatus

Length (from tail fin to mouth) in cm.	Body weight in gms.	Length of intestine in cm. (when uncoiled) mean ± S.D.	Length of Stomach in cm. mean ± S.D..	Length of caeca in cm.
15 (5)	40 - 50	4.8 ± 0.2	2.2 ± 0.3	0.8 - 1
16 (5)	42 - 55	4.9 ± 0.4	2.3 ± 0.4	0.7 - 1
17 (5)	51 - 62	5.1 ± 0.3	2.3 ± 0.3	0.7 - 1
18 (5)	55 - 75	5.2 ± 0.5	2.3 ± 0.4	0.7 - 1

Number of animals used are given in parenthesis.

many variations in the thickness distribution and the types of cells at the different portions of the gut of the fish, the fundamental architecture of the whole tube remained more or less constant. The size and thickness of the oesophagus, stomach and intestine varied widely, while after exposing the inside, the mucosa of these parts exhibited characteristic folds. The fine reticulate folds (13 to 14 in number) of the oesophagus changed to coarse folds (6to 8 in number) in the stomach. The intestine had fine longitudinal folds fused with villi like projection. Near the pyloric region and anal opening there were some valve like flaps of the tissue. The mucosal layer lining the lumen of the intestine consisted of glandular epithelial cell with underlying tunica propria containing loose cellular connective tissue. Beneath this there was sub-mucosal layer containing blood vessels, nerves, muscle strands composing the muscularis mucosae and some multicellular glands. The muscular coat was very often divided into inner circular and outer longitudinal, although in oesophagus there was profuse muscular strands inside the submucosa. The serous coat was composed of connective tissue surrounded by peritoneal layer. Nerve plexuses and fibres often appeared around the wall beneath the serous coat. However, the characteristic peculiarities of the different regions as observed microscopically in the different tissues stained by different staining methods as already mentioned are given below under separate headings. (Fig 8a,b,c)

Mucous coat :

The oesophagus showed flattened squamous epithelial cells arranged in two or three layers (or even four near the base of folds) in such a way that a definite strata could not discretely be isolated as if the rounded cells were closely packed one after another. There were numerous mucus-secreting cells, oval or rounded in shape, with nuclei placed eccentrically. The underlying basal cell layers were flattened. In between the mucous cell layers there were few taste-buds containing papillae

of the connective tissue and nerve endings which tended to occur on the midway and the top of the mucous folds, surrounded by goblet cells. Near the basal end of each taste-bud there were 8 to 9 nuclei or more. The sensory cells having such large number of nuclei gradually tapered distally to protrude through the epithelium. Beneath the nuclei of the sensory cells could be seen nuclei of rounded cells which did not reach the epithelial surface. The basal cells of the stratified epithelium were mostly flattened or columnar, as if the columnar cells became more flattened as they reached this surface and their shape was destorted by the goblet cells. There were no glands in the connective tissue beneath the epithelium but there were some columnar embryonic epithelial cells at the basal region. (Fig.9)

The surface epithelium of the mucous coat of the stomach was abruptly changed from that of the oesophagus. The stratified squamous epithelium of the oesophagus was replaced in the stomach by tall columnar epithelial cells along with profuse compound glands. Numerous crypts or gastric pits sank down into mucosa and at their bottoms there were openings of the gastric glands. The connective tissues beneath the epithelial layer were less compact than in the oesophagus. (Fig 10)

The columnar epithelial cell had a clear border. These lining epithelial cells were changed to secretory cells where the clear border was lost. Among tall columnar epithelial cells there were rounded cells with large nuclei. The cells of the compound tubular glands were of one type only. At the pyloric end of the stomach the compound tubular glands were lost but the epithelial folds became deeper forming tubules which were branched giving glandular appearence. The cells in these tubules did not contain granules like compound gland cells; The epithelium of the surface of the stomach consisted of single layer of high columnar prismatic cells but in between the crypts there were numerous peculiar honeycomb like structures probably denoting mucus, secreted by underlying glands. The product

of secretion from the numerous glands differed in its staining characteristics from the product of goblet cells of the oesophagus. There were numerous gland cells beneath the surface epithelium. The gland cells, though quite alike each other, differed in size and arrangement pattern at different places. The gland cells of stomach might be of two types, fundic and pyloric. Fundic glands were found at the mucosa of the body of the stomach, whereas the pyloric glands were confined to the pyloric part. Pyloric gland cells were shorter and their epithelium clearly resembled the surface of mucosa. Numerous fibrous connective tissues were often found beneath the epithelium by ensheathing the tubular glands, but whether they were elastic or collagenous could not be definitely stated. The muscularis mucosae or the muscle of mucosa could be found in smooth muscle layer along with the lining of the tubular glands, ultimately ending beneath the mucous layer and they were disposed mostly longitudinally.

The mucosal villi in the ileum were much more slender and finger like processes with longitudinal folds forming a rather complex pattern of arrangement. Each of the villi was a finger like process of mucosa consisting of epithelial covering and a core of connective tissue containing blood and lymph capillaries.

The lining epithelial cells of intestine were mainly simple columnar with nucleus placed at basal side (Fig 11) the free surface of the cell some 'brush' border like appearence could be seen but it was difficult to determine with light miscroscope whether these brushes were cilia or not. Some of the epithelial cells were tapering and most of them were dilated with mucus distally while the proximal part of the cell was narrow. Numerous gland cells opened into intestinal cavity at intervals. These gland cells were characterised by being rounded in shape without brush border appearence. There were frequent goblet cells at the lower portion of the ileum and these intestinal goblet cells were comparatively smaller than those of the stomach. Large pear-shaped cells were often found both at the lining epithelium and

in between the gland cells. The height of these cells was about half the height of the columner cell, but the cells were much wider with one end extending to the free border. The oval nucleus at the base of these cells often contained a large number of eosinophilic granules. The connective tissue layer consisted of loose collagenous fibres and there was no distinct lamina propria; possibly it merged into submucosa (Fig. 11). The mucous lining of pyloric caeca was more or less like intestinal mucous layer but there were numerous mucus secreting cells. The villi in the caeca were finger like processes of mucosa consisting of columnar epithelial covering and a core of connective tissue containing numerous blood vessels.

Submucous Coat :

The submucosa of the different parts of gut was composed of areolar connective tissue and was less cellular, although the dimension of this layer varied greatly at different regions. Beneath the epithelial covering of the mucous layer of oesophagus there was often dense connective tissue which could be nicely visualised by VanGieson's stain. This connective tissue at the submucous layer of oesophagus formed numerous septa ensheathing the longitudinal muscles. The submucous coat of oesophagus was much wide at two sides and narrow at certain regions. There were numerous longitudinally arranged muscles, mostly striated, which were of particular characteristics to this layer only. The muscle bands here were not densely packed as in the outer circular muscle coat (Fig. 12). The oval nuclei were placed peripherally. In between the fibrous septa numerous blood vessels and nerves were present.

The submucous coat in the stomach was composed of fibrous connective tissue, but it was less compact than in the oesophagus. The collagenous fibres were denser immediately under the epithelium running downwards in the submucous coat. Numerous muscularis mucosae composed of longitudinal muscle fibres were present, but they varied considerably in extent becoming well defined near the pyloric region.

In the submucosal layer of stomach numerous tortuous blood vessels, nerve plexuses and nerve bundles were present. Near the circular muscle coat the connective tissue ran in wavy bundles closely simulating muscle fibre and could well be identified by Van Gieson's staining. There was no muscle tissue in between the wavy bundles but the opening of the swim bladder tubes at this region could be demonstrated near the lower end of stomach; the wide dilated opening lined by cubical epithelium, just beneath the circular muscle layer and inside the submucosal fibrous band was the charecteristic feature of the stomach. (Fig. 13,14,15).

The submucous coat of intestine was much smaller and below the villi-like projections there were wavy bands of collagenous connective tissue as identified clearly by Van Gieson's stain. In the submucosal layer there were scattered muscle fibres and glands which opened into the intestinal cavity. The connective tissue layer was of ordinary loose type and consisted of both collagenous and elastic fibers. This layer was very narrow at the pyloric caeca. No distinct lamina propria could be seen but in the submucosal layer nerve plexuses could be identified by silver staining. Small oval lymph glands often ensheathed by connective tissue could be found beneath the circular muscle layer at the base of submucous layer as well as in the mid-region . The glandular structures found in the submucous coat were little. (Fig. 16).

The muscularis mucosae were not seen in oesophagus, but longitudinal band of muscle fibers oriented along the long axis and often inclined towards a plexiform arrangement near the stomach could be identified. No muscularis mucosae could be seen in the intestine and in most regions glands were limited within the mucous layer.

Muscle coat :

In general, the muscle coat of the gut, except the oesophagus, was arranged in two layers, outer longitudinal and inner circular, and almost in all the regions it formed a continuous investment of regular thickness, although it varied in the different regions like stomach, upper portion or foregut, hindgut and rectum.

The muscle coat of oesophagus consisted of somewhat incomplete inner longitudinal layer varying in extension between the mucous coat and outer circular layer of striated muscles. There was a faint or trace of longitudinal outermost coat but very often it was incomplete. The innermost longitudinal coat was closed by interspaced connective tissue septa enclosing blood vessels. Within the muscle bundles the muscle fibres were often enveloped by collagen fibre network lying in an intercellular matrix in which connective tissue cells and fine nerve bundles and blood vessels were enclosed. The muscles of both innermost longitudinal and outer circular layer were mostly of striated type with elliptical large nuclei placed peripherally. Strands of longitudinal muscle fibre often extended to the submucous layer. Individual muscle bundles in longitudinal arrangement were less thick than the circular layer, but the total girth including the fibrous septa inside the submucous layer appeared thicker (Fig. 12). The longitudinal muscle coat seemed to be continuous with the upper part of pharynx but abruptly ended in its opening with stomach. There was no inner longitudinal muscle coat in the submucous layer of stomach. But there were two clear layers of muscle coat, inner circular and outer longitudinal, in the stomach. The outer longitudinal muscle coat of the stomach was often incomplete and interrupted by faint longitudinal muscle bands beneath the serous coat. The inner circular muscle coat was much thick and compact and appeared to be continued down from the outer muscle layers of oesophagus. A third incomplete oblique muscle layer often lay in between the circular and the

longitudinal layers at the pyloric region of stomach which appeared as criss-cross arrangement. The circular and longitudinal muscles were mostly of smooth variety with large ellipsoid nuclei (Fig.13) placed inside the muscle but some striated varieties could be seen in between the circular muscle bands. At the pyloric end of the stomach the circular muscle layer became well developed to form a sphincter. The longitudinal muscle fibres at the pyloric region became continuous with the foregut.

In the intestine two distinct and complete muscle layers appeared throughout the length of entire intestine including hind gut and rectum. The outer longitudinal and inner circular were separated by loose connective tissue enclosing in between numerous lymph nodes or glands, nerve plexuses and blood vessels (Fig.16 & 17). The circular muscle layer was mostly smooth but intermixed with striated variety. Strips of striated muscle bands ran along the smooth muscle layers. The majority of the muscle fibres in both the circular and longitudinal layers were oriented alike inspite of their varying depth of extent at different regions.

The relative thickness of muscle layers at the different regions of the gut is given in table (III). But the approximate length of individual muscle fibre could not be delineated in the present study. The nuclei of the muscle varied in size and shape at different regions. These nuclei of smooth muscles were mostly alike and were oblong in shape, approximately 25 - 40/u in length and 4 - 8/u in thickness (Fig. 18). The nuclei contained chromatins scattered throughout the nucleus. Nucleoli were very often prominent and varied in number from 1 - 3. The nuclei of striated muscles were smaller but ellipsoid in shape, 15 to 20/u in length and 8 to 12/u in thickness. They were mostly placed peripherally underneath the connective tissue covering. Most of the nuclei of striated muscles contained large number of nucleoli, usually 3 to 5 in number.

In the pyloric caeca the muscle arrangement was closely alike that of intestine and no morphological difference could be

Table — III

Relative thickness of the muscle layers at differet regions of the gut (measured from six different regions from outside to inside)

	Outer coat in microns	Inner coat in microns
Oesophagus	690 ± 85	860 ± 160
Stomach	320 ± 35	453 ± 65
Intestine	296 ± 42	746 ± 132

Each figure is an avarege of 12 sections from the same fish with standard deviation (±).

determined except the relative thickness.

Serous coat :

The serous coat of the different regions of the gut did not show any particular morphological peculiarity except it was pierced by blood vessels and nerves at different places. Although the continuity of the serous coat could not be seen, it was mostly composed of connective tissue fibres.

Innervation :

Like all other fishes, the nerves of the digestive tract in Ophicephalus punctatus consisted of vagi and splanchnic nerves. The former were unequally developed. The vagus was smaller and limited to the distribution of its own side of the stomach; the larger left vagus branched extensively on the wall of the stomach and swim bladder and might perhaps also send branches into the intestine though it was not actually traced anatomically. The splanchnic nerve, also unequally developed from spinal sympathetic ganglia, remained close to the origin of coeliac mesenteric artery. Nerves could be traced along all the major branches of arterial system to stomach, intestine, gall bladder, spleen etc.

Microscopically, it had been observed that many nerve plexuses at the submucous and muscular coat were present throughout the whole gut. (Fig. 19,20,21,22). Nerve fibres or fasciculi united together to form plexuses in adjacent layers could be seen both in the submucous and muscle layers. Most of the nerves were unmyelinated and had often been confused with the connective tissue strands but with silver staining method they could be delineated. (Fig. 20, 22). In the subserous layers of stomach and fore-gut could be seen the same nerve plexuses, some of which were aggregated together along with the mesentery to form ganglia. Nerve plexuses were observed

in between the circular and longitudinal muscle coats of intestine and in the submucosa beneath the circular muscle coat. Bundles of unmyelinated fibres were intermingled to form ganglionated knob-like swellings of variable sizes and cell content. These appeared to be myenteric plexuses. The submucosal network of nerve fibres could be seen in certain slides, but no ganglion-like structure could be demonstrated. The meshwork in the submucosal plexuses was not so dense or prominent and nerve cell could not be found. Some nerve fibres forming fine reticular branches could be seen under the mucosal layer, particularly near the taste buds of oesophageal mucosa,. They formed a fine network or anastomosing branches accompained by small cells.