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STUDIES ON THE CEPHALOCHORDATES OF MADRAS COAST III. STRUCTURE OF THE POST-PHARYNGEAL REGIONS WITH REMARKS ON WATER AND FOOD CURRENTS IN THE DIGESTIVE TRACT.*

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ORTON (1913) described the mode of feeding in amphioxus but he dealt mainly with the pre-pharyngeal region while Barrington (1937) drew attention to the whole question of the digestive mechanism in the adult. Later Azariah (1963) attempted to relate form and function of the pre-pharyngeal region in amphioxus. In this paper the structure and ciliary currents in the digestive tract are dealt with. The details of the material used and the methods followed in the present study are given in an earlier paper (Azariah, 1966).

OBSERVATIONS

OESOPHAGUS

At the posterior end of the pharynx the ventral wall obliquely rises and constricts the pharynx to form the oesophagus. This junction measures 95μ and can be made out by the nature of the lining cells. The epipharyngeal groove is continued through the junction ensuring the delivery of mucus into the oesophagus. The oesophagus measures 1.5 mm. in length and 228μ across in a specimen of 28 mm. in length and is lodged between the 28–31 myotomes. It is laterally compressed from side to side. The dorso-lateral walls of the oesophagus are very thick ,measuring 456μ. Posteriorly the oesophagial wall is relatively thinner and measures 266μ.

The epithelium of the oesophagus consists of a single layer of tall slender cells without vacuoles and pigments and is uniformly ciliated. Each cilium measures 57μ. The nuclei are elongated or oval and lie about the middle of the cells.

While this is the usual type of structure of oesophagus, in one specimen out of the 10 sectioned the author found that the oesophagus had two small lateral ' diverticulae ', the one on the left side being more dorsal in position than the right side. The sac on the right side is noticed only as a thickening of the outer wall of the oesophagus. The lumen of the oesophagus is, however, pushed forwards into a small narrow recess of 228μ long. The sac on the left side can be made out as a distinct pouch. The lumen within it is wider and longer and measures 418μ.

MIDGUT

As in all other temperate forms described by earlier authors, the midgut is a wide tube nearly twice as wide as oesophagus and commences about the 30th

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myotome in a specimen of 26 mm. length and leads into the ilio-colon ring at about the 33rd myotome. The midgut is divided into two approximately equal regions by a constriction caused by a thickened band of tissue on its wall. This front region can be further subdivided into a middle portion which extends upto the midgut diverticulum. The rest of the anterior portion (Fig. 1) is marked by a triangular ciliated patch (LCT) occurring on its left wall. Thus, the midgut can be divided into 3 definite regions : I. an anteriormost section into which the oesophagus opens and out of which is thrust forward the ventral diverticulum II, a middle region with its peculiar patch of cilia, and III. a region occurring behind the constriction.

I. *Anterior region of midgut :* The upper part of this region, into which the oesophagus opens, is lined by an uniformly ciliated epithelium. Each cilium is of 57 μ length. The cells bearing these cilia resemble those lining the oesophagus. However, the nuclei of the epithelial cells of the oesophagus, which are oval gradually give place to longer and slender nuclei towards the midgut. The nuclei are located in the upper ends of the cells of the midgut.

Midgut diverticulum : The midgut diverticulum commences from the junction of the oesophagus and the midgut in a median ventral position and extends forwards to a distance of 3.5 mm. through 23rd myotome along the right side of pharynx in a specimen of 17 mm. length. This diverticulum makes its appearance as an outpushing as early as in 5 mm. length lancelets and grows to a maximum length of 7 mm. in a specimen of 31 mm. length. Externally the diverticulum appears brownish yellow in colour. It is laterally compressed.

Transverse sections of diverticulum show that along the median dorsal and median ventral side the wall of diverticulum is thinner leading to the formation of a median dorsal and ventral grooves respectively. The cells lining these two grooves are smaller and more numerous and therefore, these grooves appear to have more cilia than the lateral walls of diverticulum. The lateral walls are thick and are formed of tall ciliated cells.

The epithelium lining the diverticulum is similar to that of temperate species and consists of two types of cells, scattered in a mixed way. Barrington (1937) indicated these as type A and type B. The cells of type A are slender in form and possess a rod-like granular nucleus situated near the base of the cell and with a small prominent nucleolus. Vacuoles are also seen. The type B cells are broad with granular cell inclusions. The nuclei of these cells are spherical. As a rule type B cells are fewer than type A cells.

II. *Middle region of midgut :* Transverse sections show a considerable degree of specialization of ciliary disposition and histological differentiation in this region. On the left wall of the midgut ciliation is more conspicuous. It is concentrated in the region of a triangular area (LCT). The cilia in this area are longer and measure 133μ. The area has its apical portion turned forwards and towards the opening of midgut diverticulum. This area gradually broadens along the left wall of midgut. The broad basal part of this patch extends over the roof of midgut to very nearly the right side. The cells comprising this densely ciliated patch are more slender and numerous. The rest of midgut wall bears cilia measuring 57μ.

III. *Posterior region of midgut :* The posterior region of midgut starts from the constricted region described before and tapers into the ilio-colon ring at about the 33rd myotome. Sections through this region show that the epithelium lining the

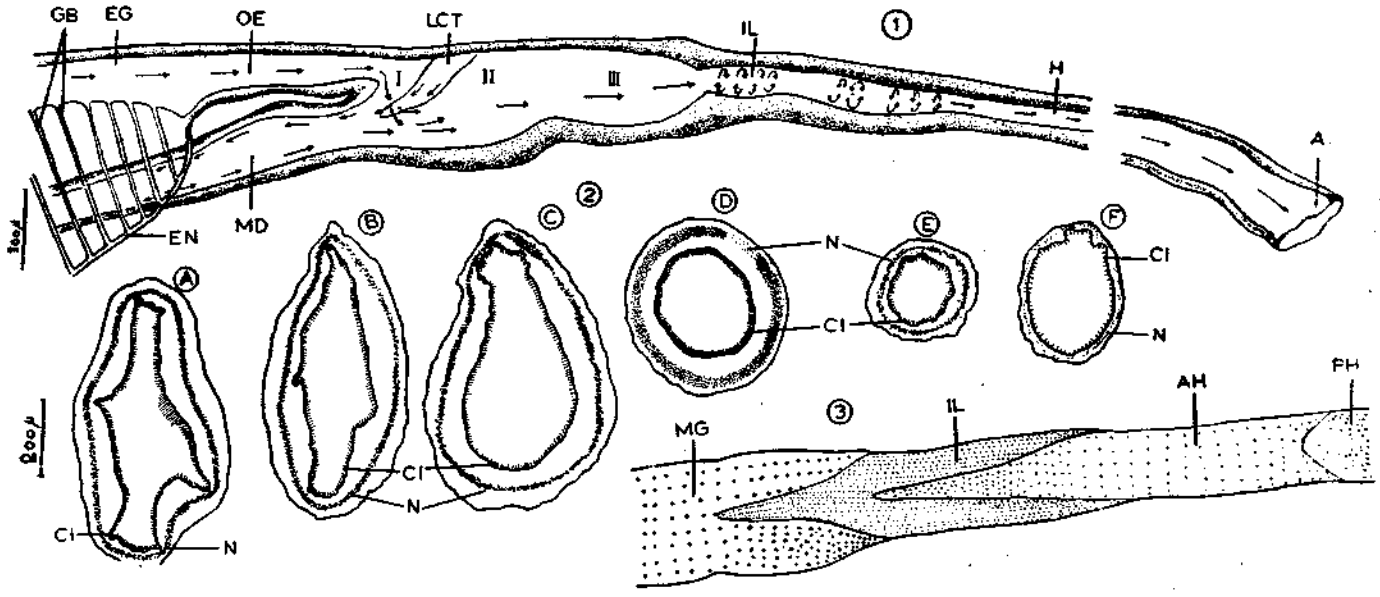

FIG. 1. Course of mucous cord with food and water in the gut. (*Camera lucida* drawing of a live specimen lying on its right side)

FIG. 2. Selected transverse sections of the gut.

A. anterior midgut; B. middle midgut; C. posterior midgut; D. ilio-colon ring; E. anterior hindgut; F. posterior hindgut.

FIG. 3. Diagrammatic illustration to show the forward displacement of the cells.

A. anus; AH. anterior hindgut, CI. cilia, EG. Epipharyngeal groove, EN. endostyle, GB. gill bar, IL. ilio-colon ring, LCT. lateral ciliated tract, MD. midgut diverticulum, MG. midgut, N. nucleus, OE. oesophagus, PH. posterior hindgut.

region is similar to the rest of midgut except for the difference in ciliation. The cilia are of uniform length measuring 57μ. A specialised ciliary patch (Fig. 2, C), however, can be noticed on the left dorso-lateral wall—a region where cilia are densely arranged. The cells bearing the cilia are more slender and more in number, particularly at the specialised region. The oval nuclei of these cells take darker stain and are situated at the middle of the cells. The histology of the specialised ciliary region recalls that of ilio-colon region.

ILIO-COLON REGION

The region of the gut immediately behind the midgut labelled as the ilio-colon ring occupies the part of body between 33rd and 36th myotomes and leads to hindgut behind. The lumen of this ilio-colon region has a diameter which is half that of midgut (600 μ in a specimen of 26 mm. length) and has a uniform width and circular in cross section (Fig. 2, D). The epithelium is made up of slender and tall cells which are closely packed. As each cell bears a cilium measuring 57μ and as each cell is very slender, large number of cells occur, making the surface richly ciliated. These cells are mainly concerned with the transport of food cord. While this type of slender tall cells line the major part of ilio-colon wall, they are not found in a certain region of the dorso-lateral wall. This patch of cells, different from the rest of the cells, can be distinguished into two types of cells A & B which resemble type A & B of midgut and its diverticulum. This dorso-lateral patch of secretory and absorptive cells are quite distinct from the cells of the rest of the wall both in cell inclusion as well as the more peripherally located nuclei. When this patch of cells are traced section by section, the width of patch extends posteriorly, on either side to be finally confluent with the cells of anterior hindgut just as the specialised cells found in midgut are confluent with those of ilio-colon region. The cells of ilio-colon ring are fluorescent prior to metamorphosis similar to that of temperate forms (Wickstead, 1967).

HINDGUT

The hindgut is lodged between the 36th and 50th myotomes. The hindgut, as it proceeds backwards bends towards the ventral side over the region of atriopore and opens at the 50th myotomes as anus. The region of hindgut corresponding to the 37th and 38th myotomes is narrow (190μ, Fig. 2, E) and the rest of the gut increases to 295μ in depth of the lumen posteriorly (Fig. 2, F).

The epithelium of anterior hindgut has cells similar to type A & B cells of midgut. However, the cells are shorter than those found in midgut and its diverticulum. All cells bear cilia. In the posterior half of hindgut the epithelium has short and broad cells with a darkly staining oval nucleus. The ciliation is less when compared with that of anterior midgut. A distinct groove is seen on the dorsal side of the gut (Fig. 2, F). As the epithelium reaches the anus, the cells are shorter and slender with closely packed nuclei staining darkly. The anus is guarded on the ventral side by muscles which act as sphincter, making the anus closed as long as an hour.

The impushing of cells at various regions are shown diagramatically in Figure 3.

Remarks on the course of mucous cord and food currents in midgut and ilio-colon ring :

The food particles embedded in mucous cord collected in the epipharyngeal groove reach midgut through oesophagus. It takes 20-25 seconds to reach midgut from the anterior part of oesophagus. The ciliary action present in gut is responsible for further progress of food material. The course of food and ciliary currents of water in Madras forms are exactly as described by Barrington for the temperate

form. The course of food currents is shown in Text Figure 1. Although this is the course in normally feeding lancelets, under certain conditions leading to occlusion of the opening of ilio-colon ring different types of water currents may be observed. The observations reported by Barrington were made on a single specimen which happened to be transparent. At Madras, even the larger forms are transparent and the author was able to observe the course of food in several specimens. The ventral groove which Barrington reports for temperate specimens is lacking in Madras forms (Text Figure 2, C). In both ' the emission of particles from the cord and then return to it ' and ' their passing towards a little distance after leaving the cord ' and being involved in a reverse eddy set up by backward currents in ventral groove ' require comment. It is clear from the anatomy that the mucous cord is narrower than the cross section of ilio-colon ring. Further, there is no constrictor muscles between midgut and ilio-colon ring. In normal position of ilio-colon ring within the body of amphioxus the mucous cord may not be hampered in its course. Hence, there may be no occasion for constriction at the mouth of ilio-colon ring and its pressure against the mucous cord and the cessation of passage of food cord. Such pressure could be built up on various parts of gut, pharynx or ilio-colon region when the specimen is left in high concentrations of particulate matter. The steady flow of food matter and its accumulation may block the passage. In fact, the reverse eddy and forward movement of particles are on the increase when lancelets are left in high concentration of food matter.

The food cord is then sent to ilio-colon ring. The food cord moves backwards in a screw-like motion traversing the inside of the lumen in a rotating manner. During a period of one hour observation, the direction of the rotation was clockwise 72 times, alternated by 102 times anti-clockwise rotation and 78 times neither clockwise nor anti-clockwise but the cord was oscillated both ways and the particles were finally sent backwards. Similar such kinds of rotations were common in all the lancelets examined. Hence, it is evident that the cilia beat backwards and slightly in an inclined way to the side to bring about rotation and the beating of cilia may not be always in one direction. The ilio-colon ring is transportary in character since it has only cilia bearing cells and serves to mix the particles of food with digestive enzymes.

Remarks on digestion and absorption in the gut

Schneider (1879) found, on feeding the lancelets with blue litmus powder, that the diverticulum and its junction with midgut were red. Barrington (1937) confirmed his observation and stated that the diverticulum is more acidic (pH 6.2). Qualitative work of Barrington revealed the amylaclostic activity of diverticulum, midgut, and hindgut and also showed that starch, glycogen, sucrose, salicin, maltose, lactose, triacetin, gelatin and casein can be digested but not inulin, sawdust and gumarabic. Positive results were shown with regard to lipoclastic and proteolytic activity in diverticulum, midgut, and hindgut. The pharynx showed no sign of enzymatic activity. Absorption takes place in midgut and its diverticulum and hindgut.

In Madras forms the time taken for the passage of food in different sizes of lancelets are as follows :

4 mm. (post-larval form)	.. 6.55 minutes
5 mm.	.. 20.00 minutes
6 mm.	.. 30.00 minutes
10 mm.	.. 1 hr. 3 minutes
25 mm.	.. 1 hr. 20 minutes,

Earlier studies have shown the total time as 1 hr. and 2 hrs. 4½ minutes (see Barrington, 1937). Since the actual length of specimens is not known, it needs only to be said that the passage of food depends on the size of animal and not on the duration of digestion and absorption. It will be seen that the time is proportionate to the length. It will be evident from the remarks of previous authors that the ilio-colon ring is of no digestive and absorptive significance. From the account in this paper it may, however, be inferred that the enzymes which have been acting on the food particles in the diverticulum and midgut are brought to bear on particles of food remaining attached to the cord. This inference can be drawn from the fact that the water currents as well as the cord are subjected to rotatory motion of the anti-clockwise, clockwise and rocking types of movements.

Summary

The histological study of the gut is described and the possible functions of different tissues of the different regions suggested.

In the light of more detailed knowledge on morphology specially what is gained from fresh live material, the improbability of there being a continuous dorsal ciliated tract and of the forward displacement of cells at different regions are discussed. Similarly, observations of the movements of food in ilio-colon region aided by the use of carmine particles show not only anti-clockwise movements as noticed by Barrington and others, but also clockwise and rocking movements.

A survey of the total time taken for food passage in alimentary canal was made and found that the duration of passage of food depends on the size of animal and not on the duration of digestion and absorption.

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