

STRUCTURE AND FUNCTION OF THE GUT IN *SPADELLA CEPHALOPTERA* AND *SAGITTA SETOSA*

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(Text-figs. 1-14)

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INTRODUCTION

Beyond observations on the mode of feeding and type of food, little is known about nutrition and digestion in the Chaetognatha. This research represents an attempt to fill this gap in our knowledge. Originally it had been intended to work solely on *Sagitta*, but the greater ease with which *Spadella cephaloptera* was obtained and could be kept resulted in attention being largely transferred to this species.

The work was carried out at the suggestion and under the direction of Prof. C. M. Yonge, who has condensed and prepared the manuscript for

the press. The observations on living animals were made at the Plymouth Laboratory and the author is indebted to the Director and members of the staff, in particular Mr F. S. Russell, F.R.S., for interest and help. Thanks are also due to Dr J. A. Kitching for profitable suggestions and to Miss M. W. Jepps for help in identifying the ciliate parasite mentioned in the last section of this paper.

SPADELLA *CEPHALOPTERA*

Spadella cephaloptera is a bottom-living Chaetognath. It fixes itself to a suitable substratum by adhesive papillae on the ventral surface of the body (Fig. 1, *ap*). It was rarely seen to swim but makes occasional rapid movements from one point of attachment to another. John (1933) found it on

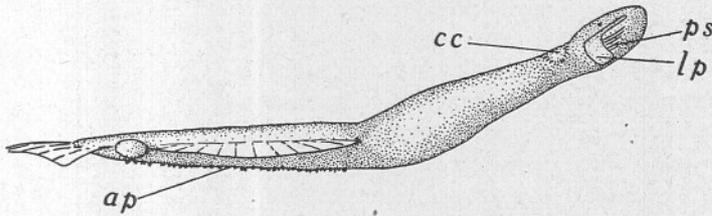


Fig. 1. *Spadella cephaloptera*, lateral aspect of living specimen showing normal resting position. $\times 18$. *ap*, adhesive papillae; *cc*, corona ciliata; *lp*, lateral plate; *ps*, prehendile spines.

the eastern shore of Plymouth Sound attached to weed and resting on mud in tidal pools. He quotes other workers who found it in similar environments.

The material for the present work was obtained from certain tanks at the Plymouth Laboratory. *Spadella* was reported to be infecting the tanks in 1911, and from this source John (1933) obtained much of his material in 1930. It was not, however, as abundant during 1937-8 as it was reported to have been a few years previously.

The animals were stored in finger-bowls and transferred to watch-glasses for close examination.

Morphology of the Alimentary Canal

Living animals were examined and drawn under the binocular microscope, the gut being clearly visible through the transparent body wall. They readily fed on small copepods while under observation.

For general histological work Bouin's fixative was used, except for the head where it caused considerable contraction. Flemming-without-acetic fixed the head well and penetrated adequately when the animal was cut transversely behind the anus. Sections were cut transversely and longitudinally at $4\ \mu$. Most of the staining was done with Mallory's triple stain, the slides being first treated with corrosive sublimate, followed by Lugol's solution and 'hypo'. This gave excellent results. Good results were also

obtained using Delafield's haematoxylin followed by erythrosin, and Heidenhain's haematoxylin followed by Biebrich scarlet. For the investigation of fat, material was fixed in Flemming's without-acetic and stained lightly with erythrosin. An unsuccessful attempt was made to detect glycogen, fixing with Carnoy and staining with Lugol's solution and also Best's carmine. Animals fed on copepods covered with iron saccharate were fixed in alcoholic ammonium sulphide and Bouin's fluid (Yonge, 1931), but iron could not be detected in sections. Material was fixed with food in the oesophagus and in the posterior region of the gut, and at $\frac{3}{4}$, 2, 5, 8 and 10 hr. and later periods after feeding.

Anatomy. The alimentary canal is divided into three regions: oesophagus, intestine and rectum (Fig. 2). The oesophagus runs obliquely over the bicornis muscle and then backwards through the head. When the animal is not feeding the mouth opening is laterally compressed, forming a narrow slit on the antero-ventral surface of the head. This slit occurs at the base of a groove, the 'vestibule' (John, 1933), the walls of which form thick lateral lips (Fig. 4, *ll*). The groove extends beyond the mouth anteriorly as a deep depression. John (1933) has described the lateral plates (Fig. 2, *lp*) lying in the dorsal region of the head supporting teeth at their anterior ends; and the prehensile spines (*ps*) on either side of the head, covered by the hood when the animal is not feeding.

In the region of the bicornis muscle the oesophagus is pear-shaped in transverse section, with the expanded region dorsal. It then becomes laterally compressed, expanding posteriorly to form a conspicuous bulb (Fig. 2, *b*). Beyond the bulb the oesophagus is laterally flattened and narrows rapidly as it leads into the intestine. As the latter is extended forwards on either side of the oesophagus as short diverticula (Fig. 2, *d*), the posterior half of the oesophagus forms a valve or nozzle which may prevent regurgitation from the intestine (see Fig. 2). When the oesophagus contains food it may be greatly distended, causing great displacement of the head musculature.

The intestine runs straight through the body and, apart from the lateral diverticula already mentioned, is without morphological differentiation (Fig. 2). The diverticula themselves are obliterated during the passage of food. In transverse section the intestine is circular or slightly elliptical and the lumen

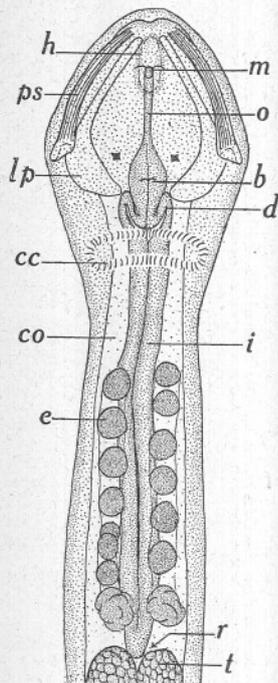


Fig. 2. *Spadella cephaloptera*, anterior half viewed from dorsal aspect. $\times 25$. *b*, bulb; *co*, coelom; *d*, diverticulum; *e*, eggs in ovary; *h*, edge of hood (on ventral surface); *i*, intestine; *m*, mouth; *o*, oesophagus; *r*, rectum; *t*, testis. Other lettering as before.

narrow, especially in the anterior region. When the gut contains food its wall is greatly distended, obliterating much of the coelomic cavity.

The rectum is a short tube leading out of the postero-ventral region of the intestine (Fig. 3, *r*) and ending at the anus. Although narrow in the resting condition (Fig. 8), it is capable of accommodating the entire skeleton of a copepod during defaecation.

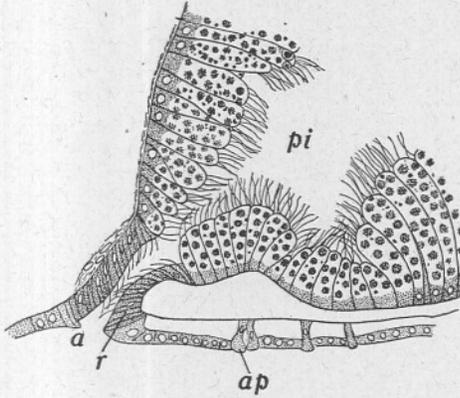


Fig. 3. *Spadella cephaloptera*, longitudinal, semi-diagrammatic section through posterior region of gut. $\times 50$. *a*, anus; *pi*, posterior intestine. Other lettering as before.

Histology. The epithelium on the ventral surface of the head is covered by a thick hyaline cuticle staining yellow or bluish with Mallory's triple stain and black with Heidenhain's haematoxylin. This cuticle lines the groove anterior to the mouth and covers the lateral lips which are formed by a thickening of the general epidermis (Fig. 4).

The epithelium of the oesophagus is composed of two types of cell, granular and vacuolated cells. The former are more abundant (Fig. 4, *grc*). They are columnar in form and packed distally with a granular material which is also found free in the lumen (Figs. 4, 5, *s*). This secretion is not mucus but consists of granules which stain with Heidenhain's haematoxylin and a matrix which takes cytoplasmic stains. These two constituents seem to be inversely related, and the granules may be the precursor of the matrix. When there is a copepod in the oesophagus the secretion can be seen among its appendages, and it is absent from the lumen after swallowing.

Although these granular cells appear to produce a similar secretion throughout the oesophagus, yet they differ, in different regions, in height and in the angle subtended to the basement membrane. Those forming the dorsal and lateral walls of the oesophagus immediately within the mouth are particularly tall and are directed downwards towards the mouth opening. Their secretion is very profuse and the granules frequently large. The ventral wall is composed of shorter cells similarly directed towards the mouth and in such a way that

in transverse sections the epithelium in that region appears very low. Their secretion consists of much finer granules.

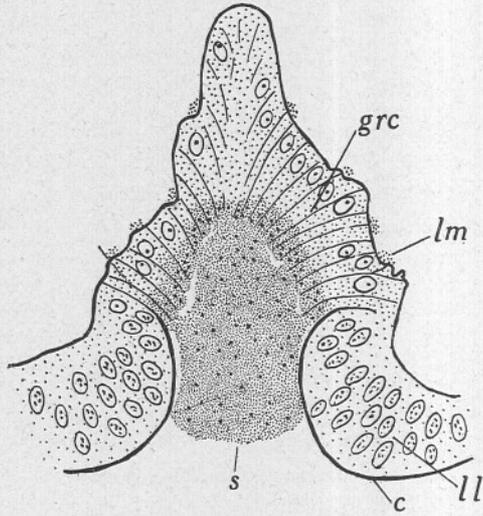


Fig. 4. *Spadella cephaloptera*, vertical section through mouth region. $\times 660$. *c*, cuticle; *grc*, granular cells; *ll*, lateral lip; *lm*, longitudinal muscle; *s*, free secretion.

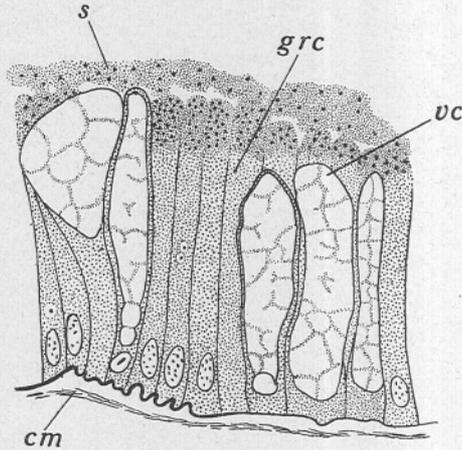


Fig. 5. *Spadella cephaloptera*, transverse section through epithelium of bulb. $\times 1320$. *cm*, circular muscle; *vc*, vacuolated cells. Other lettering as before.

Farther back along the oesophagus the cells have a medium height but become taller again at the bulb. This region is, however, especially characterized by the presence of the vacuolated cells (Fig. 5, *vc*). The basal cytoplasm of these cells possesses similar staining reactions to that of the granular

cells, but distally each contains a large vacuole sometimes faintly subdivided by thin spherical partitions. They vary in their degree of development, sometimes occupying almost the entire wall of the bulb, the granular cells forming thin streaks among them. They are clearly secretory, but it has not been possible to obtain preparations of them during, or immediately after, secretion. Behind the bulb the granular cells alone compose the epithelium: there they are low and indistinct.

The above account of the oesophageal epithelium differs from that of John (1933). He does not describe the vacuolated cells of the bulb, and considers the granular cells to consist of two types: tall and secretory in the dorsal half, short and probably not secretory in the ventral half of the oesophagus. He figures these two types as being very sharply segregated in the lateral region. The present investigation has failed to confirm this. Although the granular cells vary in height all produced a secretion with the same staining reactions.

The oesophagus is surrounded by circular muscle, the relation of which to the cephalic muscles is described by John (1933). His account of the presence of several small bundles of longitudinal muscle occurring laterally between the circular muscle and the thick basement membrane of the epithelium has been verified. Occasionally a fine strand of connective tissue may be distinguished outside the circular muscle.

The intestinal epithelium is composed of gland cells and absorptive cells. These are not sharply segregated, but, whereas one type is distributed throughout the length of the intestine, the other is largely restricted to that region anterior to the ventral ganglion.

The gland cells may be recognized at all stages of activity by their affinity for nuclear stains. In animals which have not fed for some hours or days the cells are tall and columnar, expanded distally by large, clear vacuoles (Fig. 6, *gc*). Ten minutes after food has been taken the vacuoles present every appearance of disruption (Fig. 7 A, *gc*). Three-quarters of an hour after feeding, the cells, identified by their affinity for nuclear stains, are small and inconspicuous. Three hours after feeding they are larger (Fig. 7 B, *gc*), but possess only small vacuoles embedded in the distal parts of the cell. Proximally can be seen clear eosinophilous inclusions (*ei*) which are probably the precursors of the vacuoles. At this stage of development the gland cells appear to be ciliated, though cilia could not be found at the end of restitution.

The description just given of these vacuolated cells leaves little doubt that they secrete digestive enzymes. When actively secreting, with the vacuolar wall ruptured (Fig. 7 A), there is no evidence that the basal cytoplasm and the nuclei pass into the gut. Further, throughout this investigation no mitotic figures have been found among the cells. They therefore appear to be polyphasic according to the definition of Hirsch (1931). They are most abundant in the anterior region of the intestine and in the diverticula; at the level of the ventral ganglion and posteriorly they are rare.

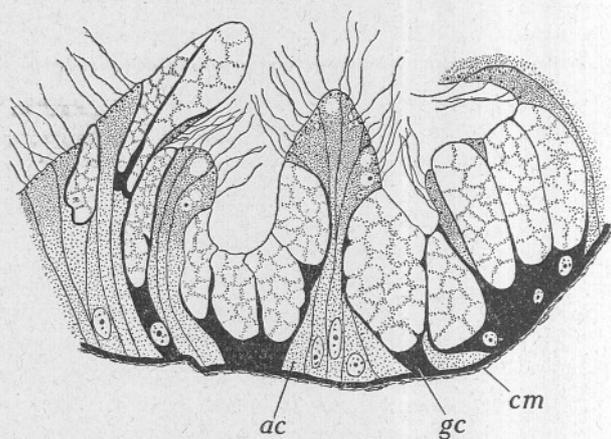


Fig. 6. *Spadella cephaloptera*, transverse section of intestine, anterior region. $\times 1000$.
ac, absorptive cell; gc, gland cell. Other lettering as before.

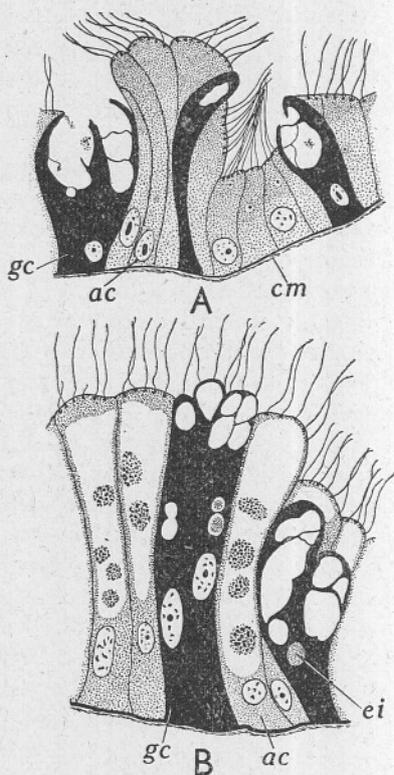


Fig. 7. *Spadella cephaloptera*, transverse sections of intestine, anterior region: A, 10 min. after feeding; B, 3 hr. after feeding. $\times 1000$. ei, eosinophilous inclusion. Other lettering as before.

The absorptive cells may first be described as they appear after the animal has fed (Fig. 7 B, *ac*). The greater part of each columnar cell is occupied by a vesicle containing one or more large concretions of material which stain black with Heidenhain's haematoxylin and yellow or reddish with Mallory. The cytoplasm is largely restricted to the basal part of the cell where the nucleus is embedded; distally it merely forms a narrow wall enclosing the vesicle. This cytoplasm stains lightly with the ordinary cytoplasmic stains. In the extreme distal wall of the vesicle are the basal granules of long flagella-like cilia. These cells are invariably in the condition just described between $\frac{3}{4}$ and 5 hr. after feeding. Later, 8 and 10 hr. after feeding, the vesicle becomes subdivided into smaller, spherical vesicles each containing an inclusion. The cytoplasm round these smaller vesicles has a characteristic, finely granular, appearance and stains a bluish grey with Mallory. In animals fixed at later stages after feeding every gradation was found from the condition described above to cells containing few, small vesicles with indefinite inclusions, and finally cells the distal parts of which consist entirely of finely granular cytoplasm staining bluish grey with Mallory (Fig. 6, *ac*). Examination of material fixed with Flemming-without-acetic, up to 8 hr. after feeding, shows that the cell vesicles also contain masses of fat.

In the living animal, observed by reflected light just after feeding, large numbers of globular bodies can be seen within the intestinal epithelium.

It will be noted that these ciliated cells acquire a maximum development of vesicles with included concretions a short time after the gland cells have secreted, and that the vesicles become progressively reduced later. Of two animals sectioned a few minutes after feeding one had these cells in a non-vesicular condition while in the other the vesicles were small. It is concluded that this type of cell is absorptive in function, and the concretions described within them constitute some sort of digestive product. They gave negative tests with Best's carmine and Lugol's solution, and therefore are not glycogen. Fat has been found to occur independently of them in the vesicles. It is therefore suggested that they consist of nitrogenous material, possibly amino-acids.

The only other possible interpretation of these cells is that they are excretory. However, it is *a priori* very unlikely that this small marine animal excretes nitrogenous material in solid form, and it is almost inconceivable that such a large amount would be produced so rapidly after feeding. Further, the concretions were found to be insoluble in K_2CO_3 solution and they gave a negative Murex test (using the method of Howland, 1924).

These absorptive cells occur throughout the intestine, being least numerous in the anterior region where the epithelium is largely composed of gland cells. The possession of cilia by these cells will be discussed later.

In dividing the intestinal cells into glandular and absorptive cells, the above account agrees with that of John (1933). In that paper, however, the glandular cells are described as 'granular' and the absorptive cells as 'non-granular', the two types of cell varying in size to form longitudinal ridges throughout

the gut. In the absence of detailed figures it is not possible to correlate John's two types of cell with those described here. Further, both from observation of the living animal and from longitudinal sections, it appears that the taller epithelial cells form irregular papillae and not continuous ridges.

The ciliation of the gut is described here for the first time. Surrounding the intestine and closely applied to the basement membrane is a membrane which is continuous with the dorsal and ventral mesenteries. External to this is a very thin layer of tissue running round the intestine, presumably circular muscle. Longitudinal muscle has not been found. In the posterior region the mesenteries are absent and the intestine is supported dorsally by the transverse septum, a sheet of tissue inserted into the ventral body wall just posterior to the anus and running obliquely forwards and upwards to the dorsal body wall.

The epithelium of the rectum is composed of very regular columnar cells devoid of vacuoles or inclusions of any sort. They bear short cilia (Fig. 8). They are bounded by a very thick basement membrane (*bm*) which is thrown into folds and capable of considerable expansion. John (1933) mentions that the rectal epithelium appears to be ciliated. Like the intestine, the rectum is surrounded by circular muscle fibres. It is supported dorsally by the transverse septum.

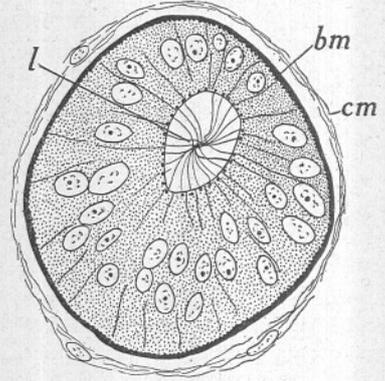


Fig. 8. *Spadella cephaloptera*, transverse section through rectum. $\times 1000$. *bm*, basement membrane; *l*, lumen with cilia. Other lettering as before.

Physiology of the Alimentary Canal

Feeding. As already mentioned, *S. cephaloptera* is usually found adhering to the substratum by its adhesive ventral papillae. These papillae are situated on the more posterior region of the body, to which the head and anterior region are inclined at an angle (Fig. 1). It is in this position that the animal awaits the approach of its food. When a copepod swims past, there occurs a very rapid upward and backward jerk, and at the same time the hood is thrown back and the prehensile spines extended. During this movement the animal maintains a firm grip of the substratum: it has never been seen to chase its food.

If the copepod is secured, the head of the *Spadella* remains directed almost vertically upwards during swallowing (Fig. 9 A). The prehensile spines play an important part in manipulation, turning the copepod until either head or tail is worked into the mouth opening, and then clawing it down the oesophagus. Usually the struggle is short, but may be prolonged for 10 min. or more, during which time a particularly large copepod will be turned round repeatedly, now the head and now the tail being worked into the mouth. Usually such prolonged struggles end in the prey being relinquished.

As pointed out by John (1933) the mouth becomes terminal during feeding; immediately after the prey has passed into the oesophagus it is possible to observe this (Fig. 9 B). A considerable lip, supported by the lateral plates, then extends above and beyond the mouth. This lip is presumably formed of the extended walls of the vestibule of the resting animal. The prey is apparently clasped by the prehensile spines (*ps*) against this lip, and the movements of the latter, due to the supporting lateral plates (*lp*), help to work the copepod down into the mouth. The ends of the lateral plates move repeatedly towards the mouth, gripping the prey with the teeth (*te*) which they support at their extremities.

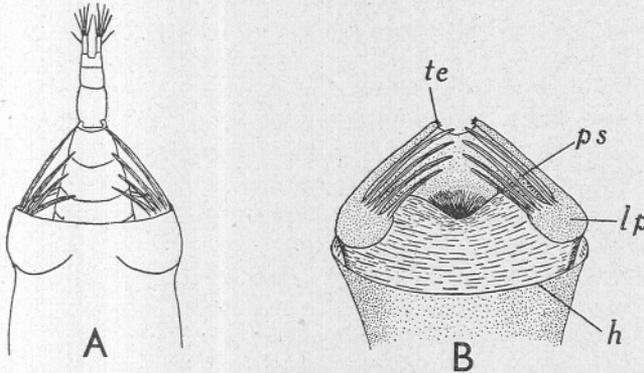


Fig. 9. *Spadella cephaloptera*, ventral view of head showing: A, prey being swallowed; B, position of mouth after swallowing. *te*, teeth. Other lettering as before.

The above account is in general agreement with that given by John (1933). The consideration given in that paper to the head muscles and their probable functions is beyond the scope of the present investigation.

Sections have shown that copepods swallowed by a *Spadella* are coated with the secretion produced by the granular cells. Application of carmine powder and manipulation with needles leaves no doubt that a sticky secretion is present in the mouth region. As the prey is clasped against the mouth region it is probably coated with secretion produced by the granular cells. By this means the appendages would be entangled and the copepod prevented from escaping. That the prey is not killed is shown by its circulating blood and gut peristalsis, which can be observed for some time after swallowing. On the other hand, a copepod which has been relinquished is unable to move away.

During this work small copepods (especially *Acartia clausi*) were used as food. No selective feeding was observed; the animal reacted in the usual way to any copepod moving past its head but failed to catch those above a certain size. As it is generally considered (cf. John, 1933) that the corona ciliata on the dorsal region of the head (Fig. 1, *cc*) is a sensory organ, it may be significant

that it appears to be copepods just above or behind the head which elicit the feeding response.

Spadella readily catches two or even three copepods in quick succession; on the other hand individuals could be starved for three or four days without apparent ill-effect. It is probable that under natural conditions the animal eats as opportunity arises, and is capable of subsisting on stored material for a long time when food is not available. The above account shows that *Spadella* possesses the characteristics of an animal adapted for seizing and swallowing active prey (Yonge, 1928).

Digestion. Yonge (1937) has put forward strong evidence in favour of the view that, primitively, digestion is an intracellular process, and that specialized animals have either retained this method in correlation with a particular type of food, or have evolved a process of extracellular digestion. Extracellular digestion usually involves the distinct processes of: (1) food conduction, usually accompanied by lubrication; (2) enzyme secretion; (3) absorption of digested material; (4) formation and conduction of faeces. Frequently, specialized regions of the gut are associated with each of these processes.

Spadella is an animal of considerable morphological specialization and digestion is extracellular. However, there has been very little specialization of the gut in relation to the constituent processes of digestion mentioned above.

Food, once inside the oesophagus, passes down to the posterior region of the intestine (Fig. 10). This movement is clearly brought about by peristalsis and may take from 2 to 8 min. The secretion of the oesophageal granular cells, the function of which during feeding has already been mentioned, may also serve for lubrication.

The intestinal gland cells are chiefly situated in the anterior region of the intestine through which the food quickly passes. Their secretion must, therefore, be passed backwards, and this is borne out by observation of peristaltic movements and of fluid globules in the gut lumen.

Little evidence has been obtained as to the function of the vacuolated cells in the oesophageal bulb, which closely resemble the intestinal gland cells in appearance. However it was noticed that after a copepod had been swallowed, the *Spadella* almost invariably took gulps of sea water. This water passed into, and distended, the bulb and was then forcibly expelled from there into the intestine. Should the vacuolated cells be liberating a digestive secretion, this water would effectively carry it down into the intestine.

An attempt was made to estimate the pH of the lumen of the gut during

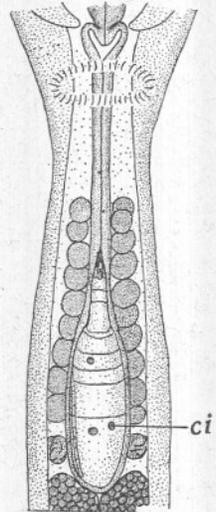


Fig. 10. *Spadella cephaloptera*, dorsal view showing copepod in the intestine. $\times 25$. *ci*, ciliates within copepod.

digestion. A copepod, to which granules of neutral red were adhering, was swallowed by a *Spadella*, and the colour of the dye, seen through the gut wall, was compared with that of standard solutions. The estimated value was approximately pH 6.4.

Absorptive cells occur throughout the intestine, and probably also act as storage cells. It is not uncommon for digestive and absorptive cells to occur together in the intestine (Yonge, 1937).

The rectum is concerned with defaecation, consisting of a small tube in the resting condition, and probably little more than a large pore when the faecal pellet passes out. This latter consists of the empty exoskeleton of the copepod.

The whole process of digestion, from feeding to defaecation, occupies about three or four hours.

Function of the Cilia. The presence of long flagella-like cilia, borne by the absorptive cells, and possibly by the gland cells at one phase of restitution, is somewhat surprising in an active carnivorous animal, the gut of which is capable of peristaltic movements. Nevertheless, the cilia are clearly seen in preparations and have been detected in the living animal in the following manner. An animal was decapitated and a suspension of carmine applied to the cut surface. A large piece of carmine, followed by several small particles, entered the gut and moved slowly down to the anus. This movement was unaccompanied by peristalsis.

Thus it appears that the cilia maintain a current in the gut lumen. This may be concerned with respiration or with the removal of dissolved excretory matter liberated by the intestinal cells. Neither circulation of body fluids nor excretory organs have been described in *Spadella* so it may be concluded that the intake of oxygen and excretion of katabolites takes place at the surfaces of the body. If these processes occur at the surface of the gut, then some means would be necessary to renew the water in the lumen. The cilia would provide this means.

There exists a third possible function of the cilia. Recent work has emphasized the importance of excretory organs in osmoregulation. John (1933) draws attention to the littoral habitat of *Spadella* and demonstrates that the animal can survive immersion in hypotonic sea water. In the absence of excretory organs the possibility arises that in *Spadella* the gut may be concerned in osmoregulation, acting like a large flame cell. In this connexion, animals were placed in 80 and 75 % sea water and observed carefully. The body became visibly turgid and difficulty seemed to be experienced in adhering to the glass dish. Water was repeatedly swallowed and the intestine became greatly distended. Carmine suspension applied to the anus and vaginal openings failed to reveal any currents. When these animals were examined in section the distension of the intestine and oesophagus was still seen, but the epithelial cells showed no unusual features.

It thus appears that the gut cilia are not concerned in osmoregulation. Although further investigation would be required finally to establish this

point, the above observations suggest that in *Spadella* a decrease in external osmotic pressure leads to a passage of water into the coelom until a new equilibrium is established owing to the increased turgor of the body wall.¹

SAGITTA SETOSA

The genus *Sagitta* has received considerably more attention than has *Spadella*. Burfield's monograph (1927) contains detailed anatomical and histological descriptions of '*S. bipunctata*'; Kuhl (1928) gives an account of the North Sea species; John (1931) has studied the anatomy of the head of '*S. bipunctata*'; and Kuhl (1932) has investigated in great detail the various movements concerned in feeding.

There has been considerable confusion in the past concerning the different species found off the British coasts. At one time they were all referred to as the species '*S. bipunctata*' but now it is established that *S. bipunctata* itself is a warm-water form not found off Britain, and that the British forms are *S. setosa*, *S. elegans*, together with the oceanic form, *S. serratodentata*, which is only occasionally driven inshore (see Russell, 1935, for the distribution of these three species). The present work concerns *S. setosa* only.

One difficulty has confronted several workers: that of keeping *Sagitta* alive and healthy in the laboratory for any length of time. During the present work it was found that only a few individuals out of any catch would survive for as long as 24 hr. Further, it was only very occasionally that *Sagitta* would feed under these conditions.

These represent very real obstacles to the interpretation of the histology of the gut, where it is so important to be able to examine material at all stages after feeding. There exists, however, another line of approach. In the first part of this paper a description has been given of the gut of *Spadella*, based on material at many stages of feeding and digestion. An attempt will now be made to interpret the gut of *Sagitta*, as examined in freshly caught animals, by a comparison with that of the closely allied *Spadella*.

Advantage was taken of the fact that *Sagitta* can be obtained in large numbers to make extracts and test for the presence of enzymes.

Morphology of the Alimentary Canal

The animals used were taken by tow-net off Plymouth and brought into the laboratory in the late afternoon. Many were already in a flaccid condition, but healthy ones were actively swimming near the surface. These were most successfully kept in shallow dishes standing in circulating water. Only active animals were fixed. These were caught by pipette, straightened out on a glass

¹ Note on the female reproductive system. This has been described by several workers for *Sagitta* (see Burfield, 1927). In *Spadella*, according to Vasiljev (1925), conditions are similar, but John (1933) states that the ovary opens direct into the seminal receptacle. Examination of sections has confirmed the findings of Vasiljev, the chief difference between conditions in *Spadella* and *Sagitta* being the presence in the former of a dorsal pouch in the seminal receptacle. Possibly this contains the sperm when eggs are passing down the oviduct and so occluding the main lumen of the 'samentasche'.

slide, and rapidly flooded with fixative. If they curled up during this they could readily be straightened again before fixation was complete. They were allowed to remain in a minimum amount of fixative on the slide until no tendency to curl remained, and then were removed to a larger volume of fluid if necessary. This is essentially the method of Burfield (1927). Bouin's fluid, Carnoy's fluid and Flemming-without-acetic all gave satisfactory results.

Material was embedded in paraffin wax and sectioned transversely and longitudinally at $4\ \mu$. Satisfactory sections of the head were obtained by this method, using a very sharp knife and moving it after every few cuts. Two stain combinations were used: Mallory, preceded by corrosive sublimate, Lugol's solution and 'hypo'; and Heidenhain's haematoxylin and erythrosin. For whole mounts, borax carmine was used.

Anatomy. The account given by Burfield (1927) of the alimentary canal of '*S. bipunctata*' applies to *S. setosa* with the exception of one or two particulars. The oesophagus of *S. setosa*, after expanding to form a slight bulb behind the complex lateralis muscle, becomes laterally compressed, and passes directly into the intestine; there are no lateral diverticula. Like the most posterior portion of the oesophagus, the intestine also is laterally flattened so that the lumen is almost or entirely obliterated (Fig. 13). Especially in the anterior region the intestinal epithelium is very low, the gut appearing as little more than a thickening of the median mesentery.

Histology. Histologically, the oesophagus is divided into four regions: from the mouth to the level of the dorsal pit; from the pit to the bulb; the bulb itself; and from the bulb to the commencement of the intestine. Three types of epithelial cell can be recognized: granular cells, vacuolated cells, and compound granular cells.

Immediately within the mouth the epithelium consists of granular cells, columnar in form and packed distally with a granular secretion (Fig. 11, *grc*). This secretion also occurs free within the lumen of the gut. Stained with Heidenhain's haematoxylin and erythrosin it consists of black granules embedded in a reddish matrix. With Mallory it appears either blue or purple. The cells lining the roof and sides of the oesophagus are very tall and produce large granules, while those occurring in the ventral region are shorter and the secretion finer. Intermediate conditions occur. The dorsal wall of the oesophagus is raised as a small projection corresponding to the dorsal pit in the epidermis, and the granular cells cease abruptly at this projection.

The second region of the oesophagus is composed of the vacuolated cells (Fig. 12 A, *vc*). Apart from the nucleus within the basal cytoplasm, the entire cell is occupied by a large vacuole, sometimes subdivided by spherical partitions. Occasionally small hyaline globules staining bright red with Mallory occur within the vacuoles and possibly represent the precursors of these. In transverse section the oesophagus in this region is in the shape of an inverted pear, and the narrow ventral region consists of a low epithelium of granular cells similar to those occurring farther forward.

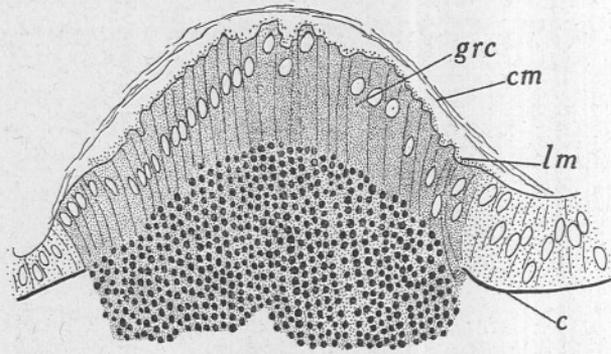


Fig. 11. *Sagitta setosa*, vertical section cutting mouth region obliquely. $\times 500$. Lettering as before.

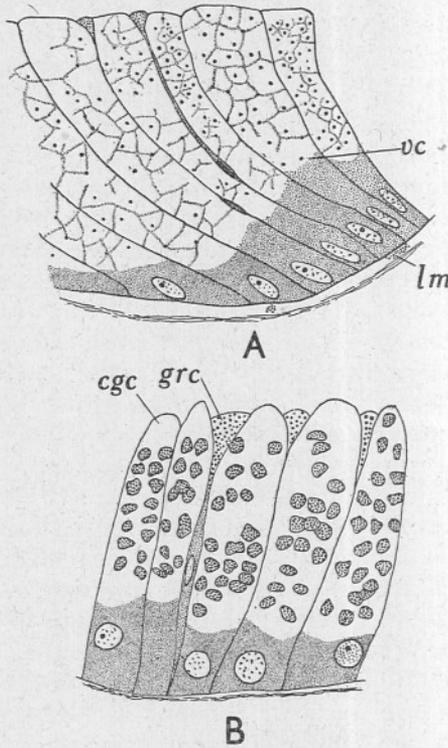


Fig. 12. *Sagitta setosa*, transverse section of oesophagus: A, in region of vacuolated cells; B, through bulb with compound granular cells. $\times 1000$. *cgc*, compound granular cells. Other lettering as before.

The epithelium of the bulb is chiefly composed of the compound granular cells, tall columnar cells closely packed with large inclusions which themselves contain small granules (Fig. 12 B, *cgc*). These inclusions stain reddish with Mallory and take up erythrosin slightly. It should be made clear that these cells are quite distinct from the vacuolated and the granular cells already described. They take the place of the former abruptly on a level with the anterior limit of the transversus dorsalis muscle.

Among the compound granular cells occur cells similar to those described round the mouth. Here they are very narrow, occurring as thin streaks among the others (Fig. 12 B, *grc*). Distally they contain the usual granular secretion, staining blue with Mallory's triple stain and black with Heidenhain's haematoxylin. They are chiefly found in the extreme dorsal and ventral regions of the bulb. Beyond the bulb granular cells entirely replace the compound granular cells and form a rather low epithelium. They end abruptly with the beginning of the intestine.

The description given above differs from that of Burfield (1927) for '*S. bipunctata*'. He states that the epithelium consists of 'one type of cell of a narrow and high cylindrical form'. These cells, according to him, contain closely packed granules staining with eosin and being either very small, or large and containing smaller granules within them. This description would apply exactly to the epithelium of the bulb as described above. The small granules would be those in the granular cells, while the large granules clearly correspond to the 'compound granules' described above. The oesophagus of *S. setosa* thus appears to include the types of cell referred to by Burfield but to be of a more complex nature than that described by him for '*S. bipunctata*' in that it contains at least one other type of cell (the vacuolated cells) and is divided into four histologically distinct regions.

The intestine may be roughly divided histologically into anterior and posterior regions. The anterior region occupies about three-quarters of the entire intestine. The epithelium is very low, being composed of large, flattened cells (Fig. 13 A) which give to the intestine of the living animal a curious scaly appearance. It is characterized by cells containing a large vacuole with 'compound granules' (*cgc*) indistinguishable from those contained within the cells of the oesophageal bulb.

The posterior region of the intestine is composed of a very regular columnar epithelium with basal nuclei and bearing short cilia (Fig. 13 B). These may be seen when the walls of the intestine have been drawn slightly apart; otherwise their presence is indicated by a row of basal granules (Fig. 13 B, *bg*). The division between the two types of cell is not abrupt, and granular cells occur among the columnar epithelium in the posterior region (Fig. 13 B, *cgc*).

Burfield (1927) described the intestine of '*S. bipunctata*' as oval or rectangular in cross-section and figures it as consisting of a tall epithelium, while the most striking characteristic of the intestine of *S. setosa* is its lateral compression and low epithelium. On the other hand, Burfield's recognition of

two types of cell, glandular cells with granules of secretion and non-granular, occasionally ciliated, cells, would agree with the above account.

Meek (1928) describes the epithelium as forming a thin layer anteriorly where the lumen is large, and becoming vacuolated posteriorly and thus reducing the lumen. During the present investigation many healthy living *Sagitta* were examined and only when the gut contained food were the walls ever found not applied together and obliterating the lumen.

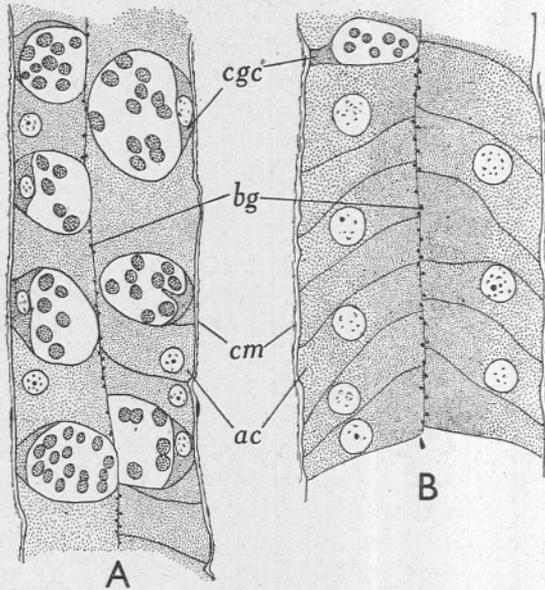


Fig. 13. *Sagitta setosa*, transverse section of intestine showing close application of lateral walls: A, in anterior; B, in posterior region. $\times 1000$. *bg*, basal granules. Other lettering as before.

Comparison with Spadella. The resemblance between the granular cells occurring round the mouth in the two animals is very exact. It is therefore concluded that in *Sagitta* these granular cells produce a glutinous secretion which entangles and lubricates the prey. As in *Spadella* these granular cells are also scattered throughout the oesophagus.

Beyond the granular cells there occur in *Sagitta* the vacuolated cells, followed by the compound granular cells of the bulb. In *Spadella* the only other type of cell is the vacuolated cell characteristic of the bulb. The two types of cell in *Sagitta* are presumably both secretory, but no purpose would be served in speculating whether either or both correspond functionally to the vacuolated cells of *Spadella*.

In *Spadella* there occur glandular secretory cells in the anterior region of the intestine and ciliated absorptive cells throughout. It seems probable that

the flat granular cells of the anterior region of the intestine in *Sagitta* correspond to the glandular cells of *Spadella*, and the columnar ciliated cells in *Sagitta* correspond to the absorptive cells of *Spadella*.

Physiology of the Alimentary Canal.

Feeding. *Sagitta* is carnivorous, feeding upon copepods, herring larvae, other *Sagitta*, etc. (Lebour, 1922, 1923). It rarely feeds in captivity. Experiments were conducted to estimate the effect of light on feeding. Of forty-seven animals kept in the dark, six fed; of twenty-seven kept in light, none fed. In so far as these data are significant, it appears that *Sagitta* feeds only under certain conditions, one of which is a low light intensity. This agrees well with the diurnal migration normally undergone (Russell, 1927). Animals were kept in a cool place under natural conditions of illumination and fixed at 3-hourly intervals throughout day and night. No periodic secretion in the gut was noted in the sections. This would imply secretion on the stimulus of food as might be expected with a predacious carnivore. After being seized, food passes steadily down to the posterior region of the intestine where digestion occurs. As shown in Fig. 14, the intestinal walls remain opposed except in the region occupied by the prey. Digestion probably takes about 5 hr. Immediately after defaecation the animal swallows water thereby distending the gut anterior to the prey. The actual passage of the faeces is not accompanied by any obvious peristalsis, but 'flickering' of cilia was detected.

Digestive Enzymes. Some 1000 specimens of *S. setosa* were ground up with cleansed silver sand moistened with glycerine. The whole was diluted with 10 c.c. of 50% glycerine, a few drops of 5% thymol added and kept at a temperature below 4° C. until required.

Digests were carried out with a variety of carbohydrates, the glucose produced being estimated by the method of Hagedorn & Jensen as modified by Boyland (1928). With soluble starch and glycogen appreciable quantities of glucose were produced the pH optimum being in both cases about 6.4. No appreciable digestion of sucrose, maltose or lactose was obtained. Attempts to detect the presence of proteoclastic enzymes also gave negative results.

The very small amounts of extract obtainable with even large numbers of animals probably explain the failure of the bulk of the experiments but in the case of proteoclastic enzymes it is possible that the enzymes are not liberated in the active form until food enters the gut and so would not be

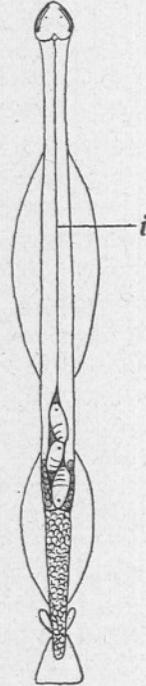


Fig. 14. *Sagitta setosa*, complete animal with three copepods in posterior region of intestine, lumen of anterior region (*i*) occluded. $\times 7$.

extracted. It is a fact that most of the secretory cells in the gut of *Sagitta* contain granules which are usually regarded as pre-secretion. The pH optimum of the sucroclastic enzyme agrees closely with the pH of the gut of *Spadella* as estimated colorimetrically by feeding with food coloured with neutral red. It has been shown by Yonge (1937) that the optimal pH of the enzymes usually corresponds closely with the pH of the gut.

DISCUSSION

In respect of food, structure of the gut and physiology of digestion, *Sagitta* and *Spadella* closely resemble one another. On the other hand, they differ widely in their habits and habitat and these differences seem to be reflected in their behaviour in the laboratory. *Spadella* is a shallow-water animal found in shore pools where it will be exposed to a wide range of light intensity, salinity and temperature. It is sedentary in habit, waiting for its prey to approach within catching distance. It is, therefore, not surprising that it lives well under laboratory conditions, feeding under bright illumination and in various suspensions, e.g. iron saccharate and neutral red. *Sagitta setosa*, on the other hand, is an active member of the plankton of open waters living in a much more uniform environment. Further, in common with other members of the plankton, it is probably adapted for life at a particular light intensity maintaining this by diurnal vertical migrations (Russell, 1927). It is clearly unable to live for long or behave normally under laboratory conditions.

NOTE ON AN APOSTOMOUS CILIATE

Small ciliates were frequently observed moving actively about within copepods (*Acartia clausi*) which were being digested in the gut of *Spadella* (Fig. 10, *ci*). These were the trophont stage of an Apostomous Ciliate (probably *Vampyrophrya pelagica*). The group has been investigated in detail by Chatton & Lwoff (1935), but as *Spadella*, owing to its transparency, provides a particularly good opportunity of observing the trophont stage while within a predator, the following details are recorded.

The copepod, with encysted ciliates on its terga or pleopods, is swallowed by *Spadella* and usually reaches the posterior region of the gut in 5-7 min. By this time the ciliates have decysted and may be already inside the copepod or can be seen actively swimming among its appendages. Entrance seems to be made from the ventral, anterior, region, possibly by way of the maxillary gland. Once inside, the ciliates at first move slowly among the tissues, but, as these are gradually digested, movement becomes more rapid. Ten minutes after entering they are already appreciably larger and when the copepod 'shell' is defaecated they are greatly distended and move slowly. This movement persists for a few hours, but no attempt is made to leave the copepod.

The ciliates may also be seen in copepods eaten by *Sagitta setosa*. Considering the abundance of this species and the numbers of copepods they must eat, it will be realized that it must play a very large part in the life of

the protozoan. Chatton and Lwoff's method of pricking infested copepods so as to activate the ciliates was successfully repeated and the tomites obtained. In one experiment a copepod was only slightly damaged and lived for over an hour after the ciliate had entered its body.

SUMMARY

Spadella cephaloptera

1. The oesophageal epithelium is composed of granular and vacuolated cells. The former produce a glutinous secretion which appears to immobilize and lubricate the prey. The function of the vacuolated cells remains undetermined.
2. The intestinal epithelium is composed of gland cells and absorptive cells. The gland cells contain large vacuoles which disrupt when food is taken, restitution taking several hours. The absorptive cells accumulate large concretions of fat and other material as digestion proceeds; later this gradually disappears.
3. The absorptive cells in the intestine are ciliated. The cilia may be concerned with respiration and excretion; there is no evidence that they are concerned with osmoregulation.
4. The rectum has a ciliated columnar epithelium.
5. The general course of feeding and digestion is discussed.
6. It is stated in a footnote that the reproductive system of *Spadella* is similar to that of *Sagitta*.

Sagitta setosa

7. The oesophagus is divided histologically as follows: (i) a region of granular cells round the mouth; (ii) a region of vacuolated cells; (iii) a region of compound granular cells; (iv) a second region of granular cells. The granular cells produce a profuse secretion with probably the same function as the glutinous secretion in *Spadella*.
8. The intestinal epithelium is composed of compound granular cells and simple columnar cells. By comparison with the intestine of *Spadella* the former are regarded as enzyme-secreting cells and the latter as absorptive.
9. Extracts of many whole *Sagitta* digest soluble starch and glycogen with an optimum at pH 6.4.
10. In captivity *Sagitta* feeds more readily in the dark than in the light.
11. The frequent occurrence of an apostomous ciliate (probably *Vampyrophrya pelagica*) in copepods eaten by Chaetognaths is noted and certain observations recorded.

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