

The endostyle and the feeding filter in salps (Tunicata)

Q. Bone*[‡], C. Carre[†] and K.P. Ryan[‡]

*Marine Biological Association, The Laboratory, Citadel Hill, Plymouth, PL1 2PB. [†]Observatoire Océanologique, LOBEPM-ESA 7076, BP 28 06234 Villefranche-sur-Mer, Cedex, France. [‡]Marine Biological Association, The Laboratory, Citadel Hill, Plymouth, PL1 2PB and the Department of Biology, University of Plymouth, Drake Circus, Plymouth, PL4 8AA.

[‡]Corresponding author: e-mail: q.bone@mba.ac.uk

This note examines the way the pharyngeal feeding filter is constructed within the salp endostyle. Three salp species (*Pegea confoederata*, *Thalia democratica* and *Salpa fusiformis*) have been examined: results from each are essentially similar. The endostyle is a deep ventral groove in the pharynx, divided by a line of long basal cilia. These are flanked by gland cells which secrete the ‘mucus’ which forms one of the sets of filaments of the filter. Beside the openings of this basal row of gland cells (marked by bumps of curious ‘mucus’ rodlets), there is a fence of two adjacent rows of cilia. The first of these two rows, (nearest the basal mid-line), whose tips lie next to the rodlet bumps, are regularly spaced 1.0 μm apart along the endostyle, whilst the cilia of the second row are at around half this spacing. Passing upwards in the endostylar groove, this first ciliary fence is followed by a ciliated zone, and then a second row of glandular cells is linked to a second ciliary fence with a single row of cilia only.

Filaments derived from the first set of gland cells pass obliquely over the ciliated zone, and continue over the second ciliary fence. The cilia of the second fence are often surrounded by very striking borders or curtains of rodlets which appear to dissolve, run down the cilia and pass off the tips as filaments, when they are joined by the first set of filaments to form a net with an elongate rhomboidal mesh. As this passes upwards, the mesh changes to become more or less square by the time it comes to overlie a striking asymmetrical band of cilia arranged in columns, when it is now composed of two sets of filaments running vertically and horizontally, spaced some 1.3 μm apart.

Various aspects of the formation of this filter net are considered.

INTRODUCTION

All tunicates except certain specialized abyssal forms (Monniot & Monniot, 1978) trap food particles on a net-like pharyngeal filter which is subsequently rolled up and swallowed. The filter is secreted by the endostyle, first named by Huxley (1851), when its function was unknown.

In a striking paper Fol (1876) showed that it was a ‘mucus’-producing gland, and that the ‘mucus’ produced was used to make a filter to trap food particles. Not only was Fol the first to figure the outlines of the salp feeding filter, but he gave the first accurate figure of a section of the salp endostyle (in *Pegea*).

Few later workers have studied the organ in salps. Garstang & Platt (1928) figured sections of the endostyle of *Cyclosalpa*, discussing its asymmetry, and since then Fredriksson et al. (1988) have examined the endostyles of *Thalia* and *Salpa* at the ultrastructural level, with special reference to iodine binding and peroxidase activity. Lastly, Goffinet & Godeaux (1992) have given a very brief description of the ultrastructure of the endostyle in *Thalia*.

The filter produced by the tunicate endostyle was first described in ascidians, by Monniot (1979a,b) and in more detail, by Flood & Fiala-Medioni (1979, 1981), who showed that it was composed of very fine fibres some 10–40 nm in diameter, forming a very regular rectangular or square mesh. Mesh size varied in different species, in *Ciona* it was some $0.4 \times 0.7 \mu\text{m}$. Flood & Fiala-Medioni

(1981) were also able to show ‘mucous’ filter filaments within the dorsal part of the endostyle in *Phallusia*, where it contrasted with the much more densely packed filaments in the more ventral region. In salps, the filter has only been examined in *Pegea* (Bone et al., 1991) where it is essentially similar to that of ascidians, though with somewhat thicker filaments and a larger mesh.

So far as we are aware, only one author (Holley, 1986) has considered how this remarkable feeding filter might be made within the endostyle. Holley examined the endostyle of *Ciona* (using transmission electron microscopy), and gave a detailed description of the ciliated and glandular zones, inferring the direction of beat of the cilia from the disposition of their basal apparatus. He did not examine the endostyle during secretion, but the morphology suggested which glandular zones might secrete the vertical and longitudinal filaments of the filter. He regarded the two filaments running at right angles in the filter as resulting from differential combing of the ‘mucus’ by special cilia operating in different directions.

The endostyle in salps has been examined, during the process of secretion of the filter, mainly using scanning microscopy. This has provided some insight into the mechanism of production, although a complete understanding remains elusive. As Fol (1876) observed, in salps the endostylar ‘mucous’ filter is drawn upwards from the front of the endostyle around the entrance to the pharynx to form a more or less conical net which is ingested together with entrapped particles (Figure 1).

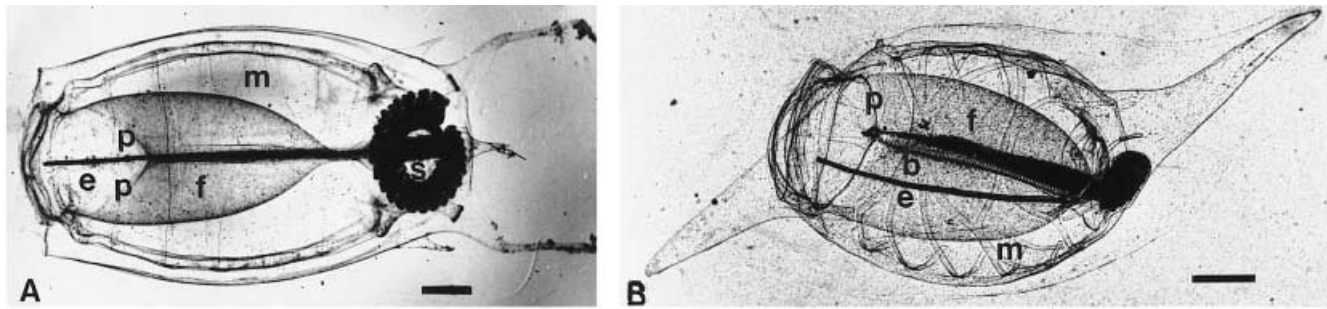


Figure 1. (A) *Thalia democratica*. Oozoid. Ventral view, anterior to left. The feeding filter (f) is maximally expanded by water entering the mouth as the locomotor muscle bands (m) relax. The endostyle (e) and peripharyngeal bands (p) are visible at the front of the pharynx, at the hinder end the beginning of the stolon (s). The mouth of the feeding filter is around the peripharyngeal bands, and the hinder end enters the oesophagus. (B) *Salpa fusiformis*. Blastozooid. Dorsal view, anterior to left. The muscle bands and endostyle are more clearly visible than in (A), and the transverse ciliated strips along the single gill bar (b) can be seen. Other lettering as for (A). Scale bars: 1 mm.

MATERIALS AND METHODS

Oozoids and blastozooids of *Thalia democratica* (Forskål) and *Salpa fusiformis* Cuvier, and blastozooids of *Pegea confederata* (Forskål) were collected in plankton tows from the Bay of Villefranche in the Mediterranean near Nice, and transferred to small crystallizing dishes of seawater in a constant temperature cabinet at 24 °C. Single salps were examined under a binocular microscope in smaller dishes (for the smaller *Thalia* in solid watchglasses) and algal suspensions added to initiate feeding. Usually suspensions of *Isochrysis galbana* Parke were used, but sometimes *Hymenomonas* sp. were also added. Under these conditions *Thalia* and *Pegea* usually began to form a filter within a few minutes, but it proved more difficult to persuade *S. fusiformis* to feed. As soon as the salp formed the feeding filter, fixative was injected into the pharyngeal cavity via the inhalent opening. Some salps were fixed with 5% seawater formalin, others with a formol–glutaraldehyde mixture, then rinsed

in seawater and post-osmicated, and stored in 70% alcohol. These were later dehydrated and embedded in Taab resin for transmission microscopy. Thin sections were examined in a Philips EM 300. However, most were injected with 2% osmic acid and then stored in 70% alcohol until they were dehydrated to acetone and critical-point dried for scanning microscopy in a JEOL 35C microscope.

Some specimens were returned to water, infused with 2.3 M sucrose, mounted on support pins for a Reichert FC4 cryo-ultramicrotome, frozen in liquid nitrogen or by cooling in the FC4 chamber, and planed with a glass knife. When sufficient material had been removed by the knife, the specimens were thawed in water, rinsed to remove the sucrose, dehydrated in acetone, dried in a critical point drier and mounted for scanning microscopy.

To examine living endostyles, the smaller salps were confined in small compressoria and examined by interference contrast microscopy. The larger salps were

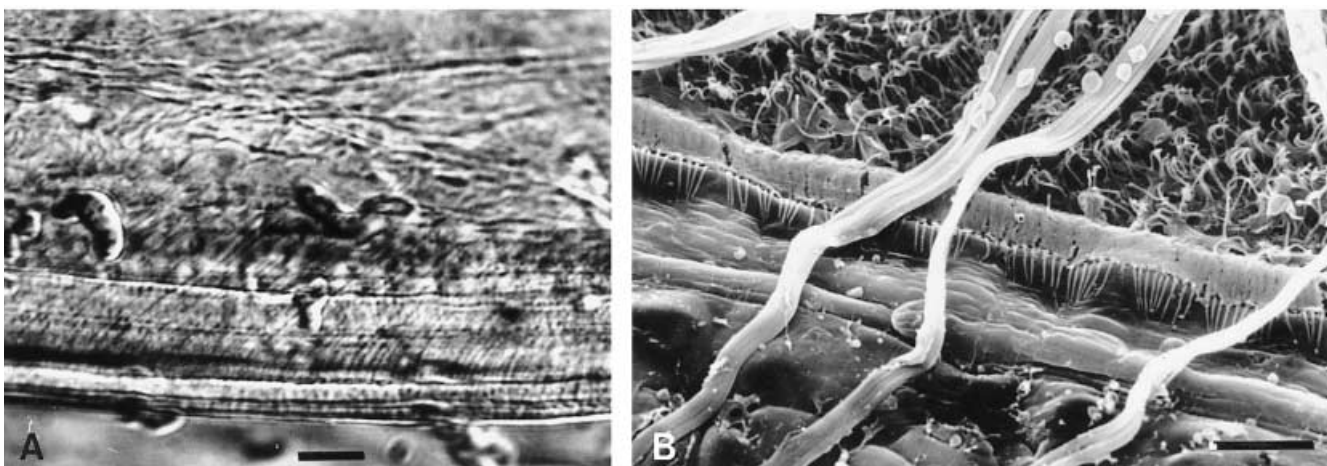


Figure 2. (A) *Thalia democratica*. Young blastozooid, anterior to right, living, Nomarski optics. Lateral view of base of anterior region of endostyle showing basal long cilia inclined forwards. (B) *Pegea confederata*. Blastozooid, scanning microscopy. Ciliary zone and upper fence cilia (partly covered in 'mucus') with long basal cilia aggregated in bundles. Base of endostyle to upper right. Scale bars: A, 100 µm; B, 10 µm.

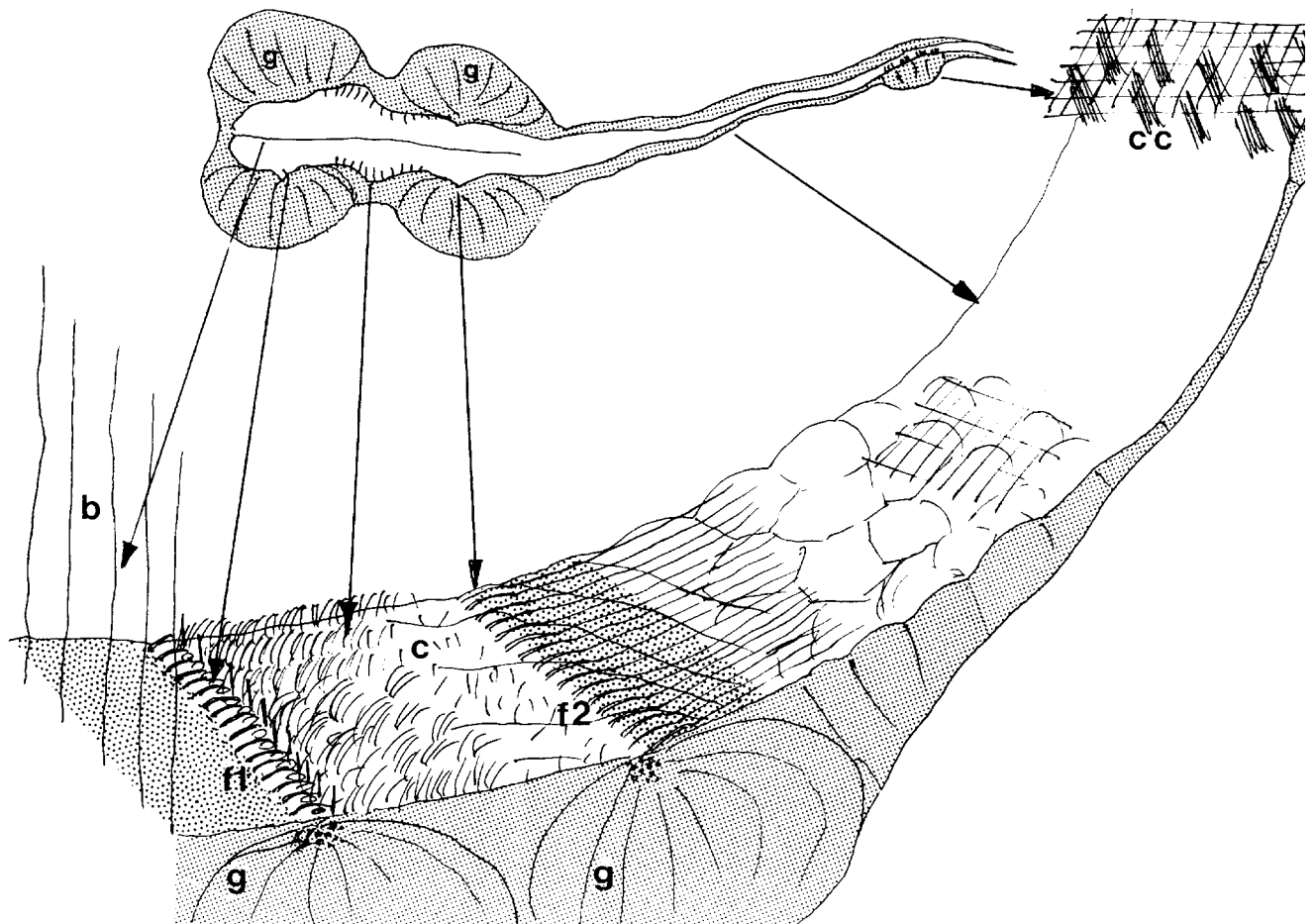


Figure 3. Schematic view of the inner surface of the salp endostyle, showing the different regions, with the portions of the filter observed in different preparations. At upper left, schematic transverse section of mid-region of endostyle, showing the onion-like glandular bulges of the two rows of gland cells (g) in section. Coarse dots: surfaces normally covered with 'mucus'. b, basal long cilia; c, ciliated zone between gland cells (g); cc, columnar cilia; f1, first ciliary fence; f2, second ciliary fence.

dissected and portions containing the endostyles examined similarly.

Simply because they were of the most convenient size, our observations were made almost entirely upon oozoids of *T. democratica* and (chiefly) upon both oozoids and blastozoids of *S. fusiformis*. However, no essential differences have been found between the structures of the endostyles of the different species and stages, and the following account refers to all. It is however only in *S. fusiformis* that we have been able to observe the filter within the endostyle in any detail.

OBSERVATIONS

The form of the salp filter and its deployment

The feeding filters of salps produced by the endostyle lie freely within the pharynx, depending from the peripharyngeal bands around the entry of the pharynx, and increasing in diameter slightly downstream, then tapering to the base of the gill bar and drawn steadily into the mouth of the oesophagus. Figure 1 shows the filters of an

oozoid of *Thalia democratica* and a blastozoid of *Salpa fusiformis*. When the filter is in place, it remains expanded as the locomotor muscle bands contract and relax regularly, so causing water to flow through the pharynx and the salp to move forwards. Although in nature, salps presumably normally always deploy the filter as they swim forwards, this was not the case in our laboratory experiments. Under the artificial conditions of a dense algal suspension and confinement in a small dish, the salps usually fed with the filter deployed for a minute or so and then stopped pumping water through the pharynx. As soon as the flow of water through the filter ceased, the filter first shrank to a conical shape, and then collapsed. It seems clear that the feeding filter is to some extent elastic (see Discussion), and that as water flows through it, the filter mesh is expanded in consequence of the pressure drop across the filter.

Filter production is controlled by the salp, and even after overnight 'starvation', salps placed in dense algal suspensions do not always produce a feeding filter immediately. Nerve bundles run along the length of either side

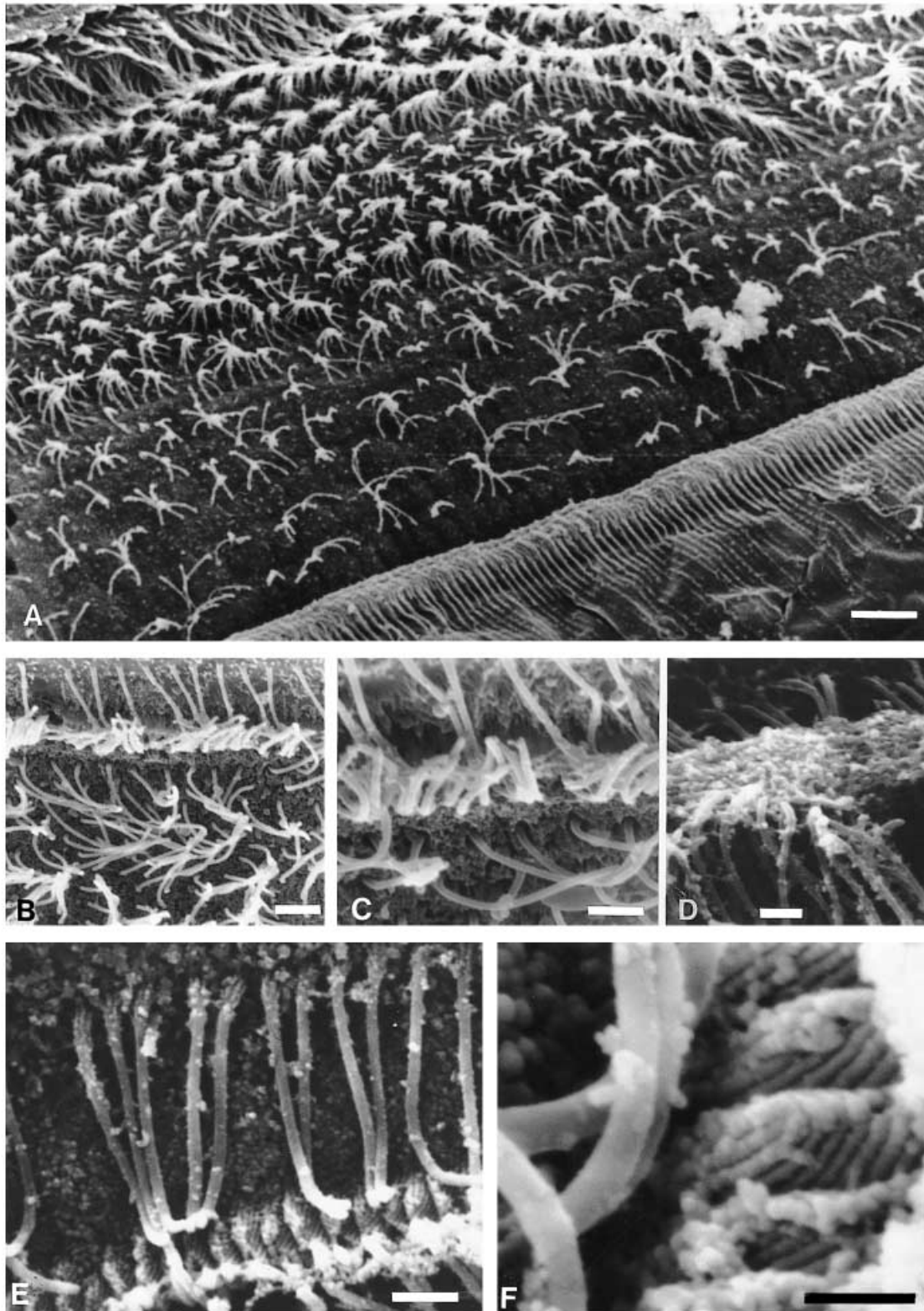


Figure 4. *Salpa fusiformis*. (A) Similar oblique view as in Figure 3, of the regions of the inner surface of the endostyle that are involved in production of the filter. The basal centre line is out of the figure shortly before the top left corner where the first ciliary fence is just visible. The major part of the field is the ciliary zone in which there are dense cilia initially which become fewer and fewer as the second (upper) fence of cilia is approached. This shows the very regular spacing of this second fence, and the vertical filaments of the filter passing upwards (towards bottom right) from the tips of these cilia. (B) Non-secreting endostyle. The first ciliary fence (centre-line of endostyle upwards in this and in C and D) showing first ciliary fence with cilia lying against endostylar surface, and adjacent to second row of more closely spaced cilia. (C) Enlargement of same endostyle as in A. Note that the cilia next to the ciliated zone are closer spaced than the first fence of cilia. (D) 'Mucus' covering the first ciliary fence in a secreting endostyle. (E) First ciliary fence showing tips of cilia between a line of ridged protuberances, and grooved ciliary bases surrounded by short stubby microvilli. (F) Detail of the protuberances. Scale bars: A, 10 μm ; B & E, 2 μm ; C & D, 1 μm ; F, 0.5 μm .

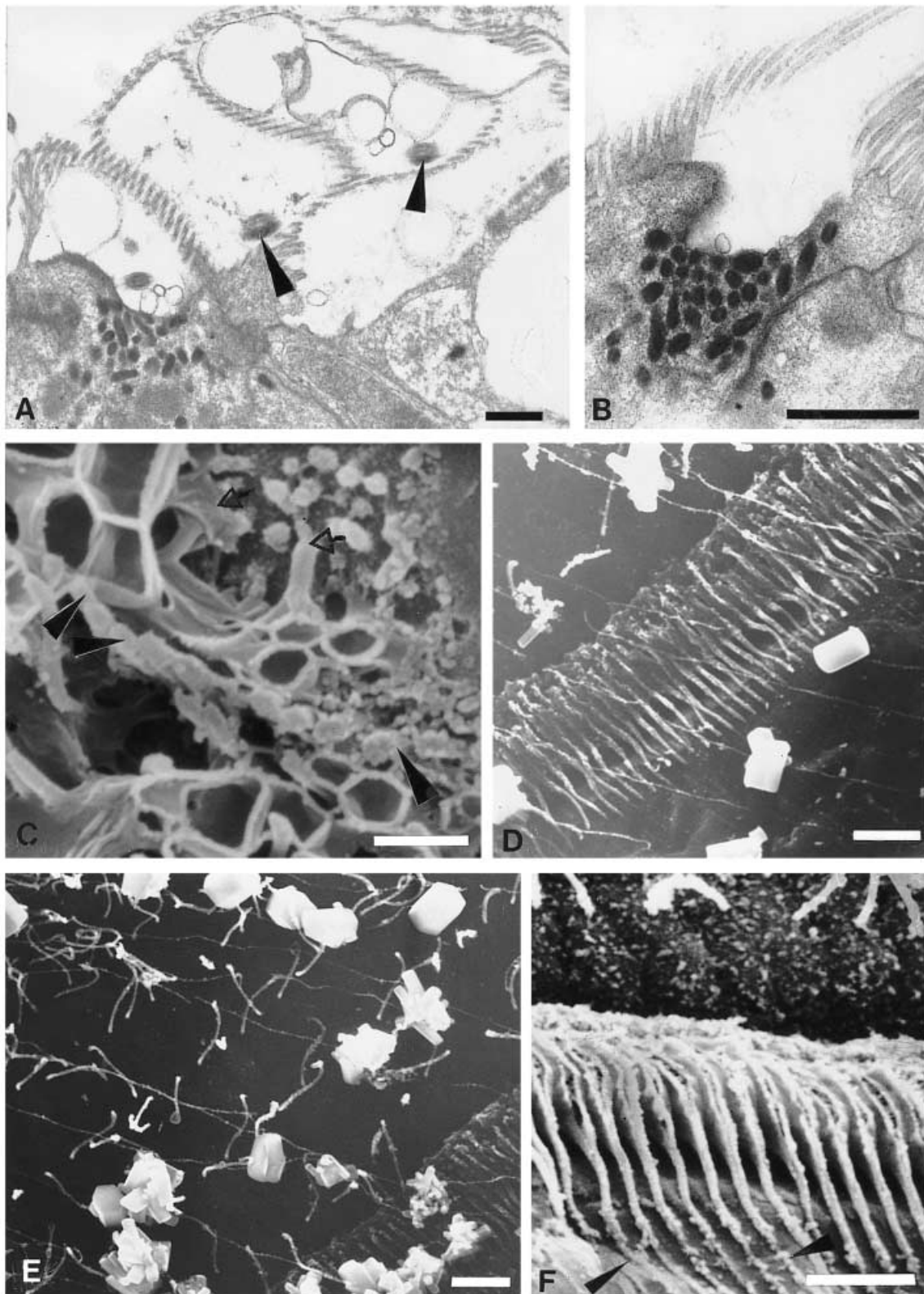


Figure 5. *Salpa fusiformis*. (A & B) Sections of top of first (basal) glandular cells. Dense inclusions are seen near the tips of the cells, and extensive fine tubular elements resembling greatly extended thin microvilli of similar diameter to the rodlets seen in scanning images. Fragments of the 'microvilli' lie around expanded vacuolar material surrounding ciliary cross sections (arrows). (C) Frozen planed preparation. Higher power view of ciliary tips as seen in Figure 4B–E, showing sections of vacuolar material, short microvilli surrounding base of first row of cilia (open arrows), and line of sectioned upright cilia in the second row (closer together than the first row; long arrows). The cut face of the preparation is seen at upper right. (D) Oblique first filaments passing over the cilia of the second fence (in this preparation there are no second filaments seen from the tips of the cilia of the second fence). (E) Similar view to D, oblique first filaments seen at the upper end of the ciliary zone (to left), where they are approaching the second ciliary fence seen at bottom right. (F) 'Mucus' filaments (arrows) passing from the tips of the cilia of the second fence. In this case, no first filaments are seen. Scale bars: A, D, E, 10 μm ; B & C, 0.5 μm ; F, 1 μm .

of the endostyle, and branches from these pass to the sides of the basal regions, although synapses with endostylar cells have yet to be observed. Since salps anaesthetized with MS222 never secrete filters, the endostylar innervation is at least partially excitatory.

Endostylar structure

The endostyles of all tunicates seem much alike in general structure, although as Garstang & Platt (1928) pointed out, the ciliated regions of the salp endostyle are somewhat less complicated than those in the endostyle of sessile ascidians, whilst in doliolids the endostyle is relatively simpler (Godeaux, 1981).

Essentially, the endostyle is a deep V-shaped groove in the pharyngeal floor with thicker walls basally, and dorsally thinning to merge into the extremely thin inner pharyngeal epithelium. The arrangement is seen schematically at top left of Figure 3. It is unclear whether the top of this endostylar groove is in life open to the pharynx. Garstang & Platt (1928) concluded from their serial paraffin sections of the endostyle in *Cyclosalpa* that the top of the endostyle was closed from the pharynx by the apposition of the very thin endostylar epithelial walls below the asymmetrical upper ciliated band. It is obviously difficult to process and section this delicate region of the endostyle in its natural position, for the thin walls collapse together and become much folded, but in our semithin sections of *S. fusiformis*, although the two thin upper walls of the endostyle are folded, they do not seem to be apposed as Garstang & Platt (1928) suggested.

Figure 3 illustrates the inner surface of the endostyle schematically, where the portions of the filter observed in our scanning electron microscopy (SEM) observations are also indicated. This diagram is based on scanning microscope views of the endostylar surface involved in secreting the filter such as that seen in Figure 4A. The basal midline lies to the left, the dorsal part of the groove to the right. The upper part of the endostyle has not been examined in detail since the structure of the filter has already been formed before this level, although its composition may there be altered (as for example by iodination). At the base of the endostyle, as in the ascidian endostyle, a row of central cells produces very long cilia (Figure 2A), which in our scanning preparations are often aggregated in bundles (Figure 2B) much as spaghetti joins in bundles in the pan unless stirred initially. They may reach so far as to overlie the second ciliary fence some way up the side of the endostylar groove. These cilia are however much less conspicuous than they are in scanning views of *Ciona* or *Pyrosoma* (Q.B., personal observations), and in our preparations do not form the same kind of continuous frieze as in both these other tunicates. In lateral Nomarski views of small living blastozooids of *Thalia* the long cilia are visible as a row along the endostyle (Figure 2A), but this row is much less densely compact than, for example, in small transparent specimens of *Ciona* similarly viewed. It is difficult to know whether they are motile in life (as Berrill, 1950 supposed, and as Fedele, 1934, seems to suggest), for except in very small blastozooids, to see them *in vivo*, it is necessary to stretch open the endostyle, and when opened out in this way, they are not motile, but conditions are far from

those in the normal endostyle. They did not beat in the intact small blastozooids of *Thalia* we examined by Nomarski microscopy, and it seems most probable to us they are non-motile, simply acting as a central division to separate the filter sheets formed on either side. However, Holley (1986) stated that the basal flagella in the endostyle of the ascidian *Ciona* 'undulated slowly in waves propagated from their bases upwards with lateral displacements along the axis of the endostyle'.

The basal cilia are flanked by glandular cells, which form a conspicuous onion-like bulge in transverse sections of the endostyle (Figure 3, upper). Very similar appearances are seen in ascidian and appendicularian endostyles in section (e.g. Godeaux & Firket, 1966; Fredriksson et al. 1988). These cells have obvious rough endoplasmic reticulum, mitochondria with tubular cristae, and vacuolated inclusions of various kinds, including electron-dense granules some 250 nm in diameter which are found close to the apices of the cells (Figure 5A,B). At their bases, they are much infolded. Between the central long cilia and the apices of the glandular cells, there is often a more or less smooth 'mucus' layer showing occasional 'bubbles' at the surface. Since this layer does not apparently play any role in filter production, it is presumably simply the 'incontinent' result of 'mucus' secretion when the filter itself is not actually being formed.

Close to the tips of the basal glandular cells there is a row of cilia spaced very regularly some 1.0 μm apart, forming a sort of ciliary ruler or fence (Figure 4B–E), usually lying against the endostylar surface. In our scanning microscope preparations these cilia are invariably bent over away from the centre of the endostyle (that is, pointing upwards in the endostylar groove). Just centrally to their insertions and around them there are short microvilli, which surround their curiously grooved bases (Figure 4E).

Their tips are usually curved away from the cell surface and in endostyles which are not secreting, lie against a series of small rounded protuberances which are composed of rodlets or tubes some 95 nm in diameter (Figure 4E,F). The status of these small rodlets will be considered later. In sections of secreting endostyles, (Figure 5A,B), what seem to be equivalent to the rodlets (of similar diameter) are seen as elongate microvilli that apparently become detached from the cell surface around 'bubbles' of 'mucus'. No elongate microvilli are observed in SEM views of this region, nor anywhere else in the salp endostyle.

Although the fence cilia are variously curved towards their tips, (Figure 4E,F), the impression gained from our SEM views is that in life they simply flex upwards and downwards towards and away from the the rodlet protuberances. In endostyles fixed during or just after secretion (i.e. when the filter is being deployed), the tips of the fence cilia are covered with an irregular mass of 'mucus' (Figure 4D) which also covers the row of cilia on just the other side of the protuberances to the first row. This second row consists of cilia contrasting with those of the first row because they are more or less upright and closer spaced, (at intervals of 0.5 μm rather than 1 μm , Figure 4C). It seems clear from Figure 4C, and from the same row sectioned after impregnation with sucrose, seen in Figure 5C, that this is in fact a separate row, and not simply the first cilia of the succeeding ciliated zone which

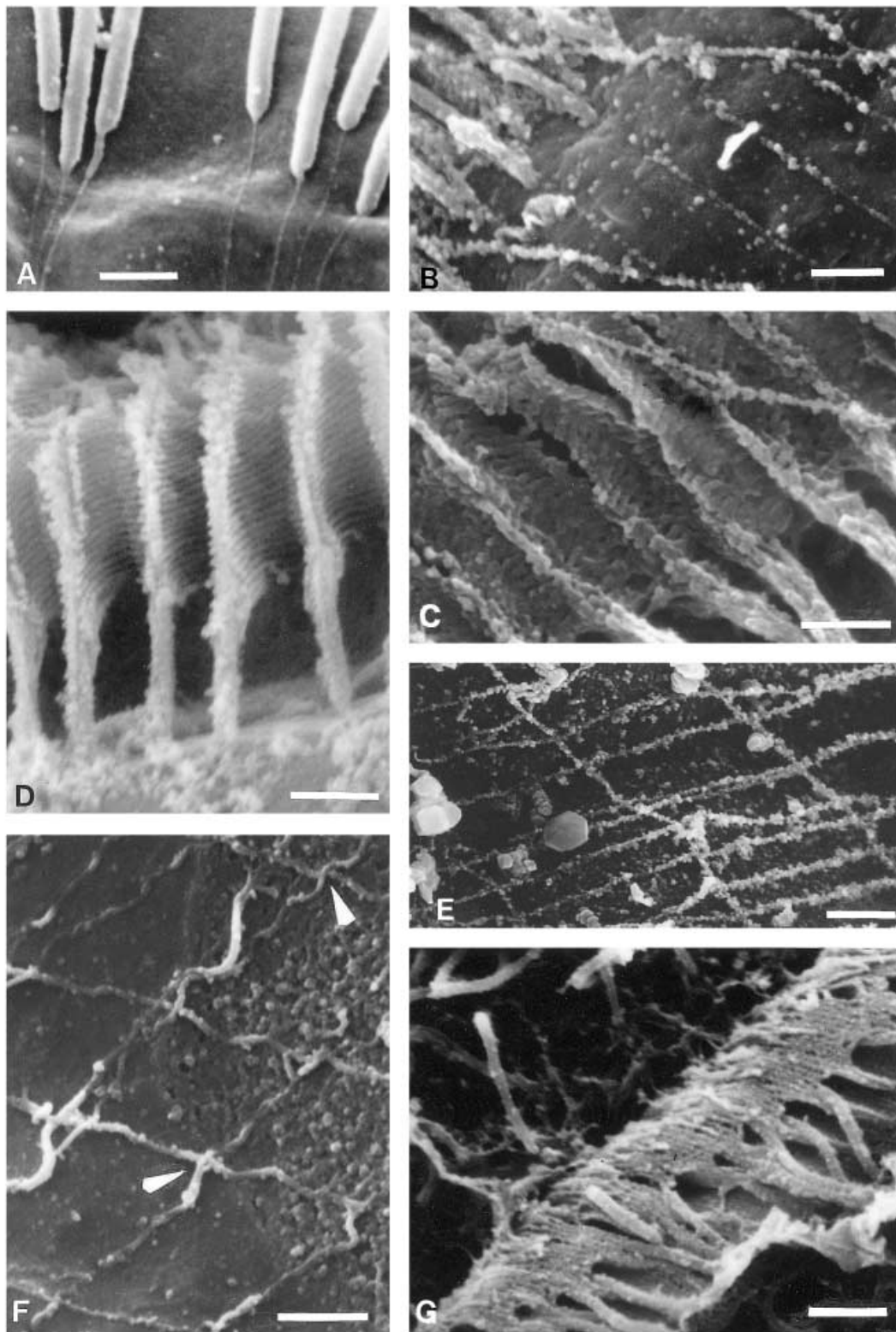


Figure 6. (A) *Pegea confoederata*. Blastozooid. 'Mucus' filaments passing upwards in endostyle from tips of cilia of second fence. (B) *Salpa fusiformis*. Second fence showing widely spaced first filaments passing over the second filaments coming from the tips of the second fence of cilia at upper left. Top of endostyle to bottom right. (C) *Salpa fusiformis*. Similar to (B), to show second fence cilia bordered by lines of patterned rodlets. The rodlets appear to pass down the ciliary shafts towards their tips breaking down into smaller particles as they do so. The ciliary tips are just off the bottom right of the figure. A single first filament overlies the fence. (D) *Salpa fusiformis*. The secondary fence of cilia bordered by curtains of rodlets around their bases, 'mucus' beads passing from their tips (below). (E) *Salpa fusiformis*. Filter close above second fence, showing elongate rhomboid mesh. First filaments run from 10 o'clock to 7 o'clock. (F) *Salpa fusiformis*. Slightly higher up the endostylar groove away from the second fence, the filter mesh has now near-equal sides. The first filaments (thicker and running from 10 o'clock to 4 o'clock) can be seen to overlap the thinner second filaments (arrows). (G) *Pyrosoma*. Tetraxooid. Second fence showing grooved 'mucus' rodlet like appearance over the bases of the cilia. The top of the endostyle is to bottom right. Scale bars: A, 0.5 μm ; B–D, F, 1 μm ; E & G, 2 μm .

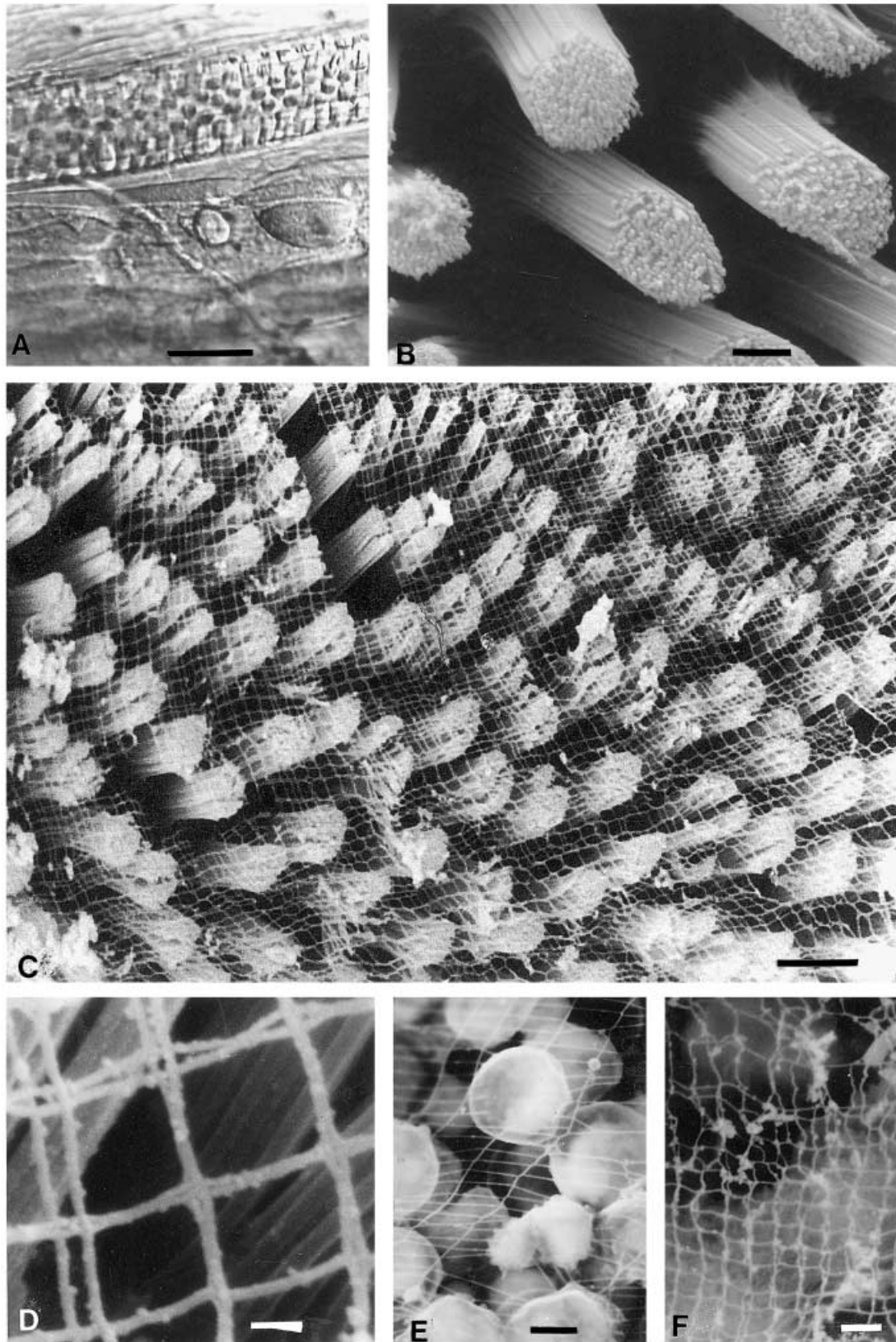


Figure 7. (A) *Thalía democratica*. Young blastozooid. Living, Nomarski optics. The asymmetric band has many fewer columnar ciliary rows than in larger specimens. (B) *Salpa fusiformis*. Adult oozoid. Columnar cilia. (C) *Salpa fusiformis*. Adult oozoid. Filter net above columnar ciliary band. Note that whilst mesh is more or less square over most of the area shown, at some sites it is rectangular. (D) *Salpa fusiformis*. Portion of filter of different individual to (B), showing two layers of filter superposed over the columnar cilia. Note square mesh. (E) *Pegea confoederata*. Blastozooid. Filter deployed in pharynx under tension having collected numerous *Isochrysis* cells. Note that dimensions of mesh vary (especially the lengths of the sides formed by the thin filaments) indicating elasticity of filaments. In other views, the mesh may be nearly square as in (D) and (F). (F) *Pegea confoederata*. Area of filter deployed in pharynx presumably not under tension, showing more or less square mesh. Scale bars: A, 50 μm ; B,C,E,F, 2 μm ; D, 1 μm .

are separated by the same distance as the cilia of the first fence row.

Next, there is then a wide zone of ciliated cells which sometimes exhibit metachronal waves apparently passing obliquely along the endostylar axis (Figure 4A). We are unable to say in which direction these cilia beat, but presumably from the rear of the endostyle forwards, since the filter passes out of the endostyle anteriorly. The oblique direction of beat is of importance in forming the filter, as will be seen below. Curiously, within this ciliated zone, the outer third consists of cells with fewer and fewer cilia until eventually the zone ends with cells bearing single cilia only. In several endostyles examined, the earlier inner part of this ciliated zone was wholly or partially covered with a sheet of 'mucus' grooved rather like the rodlets mentioned above. In endostyles which are secreting filters, we have observed filaments passing obliquely upwards towards the upper part of the endostylar groove, across the upper part of the ciliated zone (Figure 5D,E). Presumably these are secreted by the lower group of gland cells, whose apices lie just adjacent to the cilia of the fence seen in Figure 4B–E, but in none of our numerous preparations have we succeeded in detecting the connection of these filaments with the ciliary fence. The filaments are seen first only just prior to the upper border of the ciliated zone. They cross the ciliated zone (Figure 5D,E) to pass over the fence and join the second series, are more widely spaced than are the cilia of the first two regularly spaced rows, being around $2\ \mu\text{m}$ rather than $1\ \mu\text{m}$ or $0.5\ \mu\text{m}$ apart. It is therefore disappointing that we have not been able to see how these wider spaced filaments are produced.

The end of this ciliated zone is marked by a second row or fence of cilia (spaced $1\ \mu\text{m}$ apart) taking origin from a line along the long axis of the endostyle often partially obscured by 'mucus' secretions. Once again, the cilia of this fence lie against the surface in scanning microscope preparations, that is, they point vertically upwards in the normal position of the endostyle, lying against its wall. Details of secretion of the filter net will be considered later, but it is from the tips of these cilia that 'mucous' strands are formed which make one of the elements of the filter as seen in Figures 3, 5F & 6A.

Various remarkable appearances are presented by these cilia in our scanning micrographs, sometimes they are more or less free from adhering particles, at other times there are ridged protuberances between them, and at yet other times, they bear on each side a sort of veil or sheet of rodlets similar to those described above (Figures 5F & 6C,D). Over the top of these curious features pass the first filaments secreted (Figure 6B & D) and these come to overlie obliquely the filaments passing from the tips of the second ciliary fence at angles around 35° – 45° . Sometimes, as in Figure 6B, the first formed filaments are thicker than the second which they overlie and are stuck to, and although this is not always so obvious as in this figure, it is possible to recognize in the fully formed filter outside the endostyle (Figure 7E) the sites of origin of the two filaments now disposed at right angles to each other. The first views of the two filaments of the filter just after the second fence (Figure 6E) thus show a rhomboidal mesh formed by two sets of filaments with very different spacing. Whilst the filaments are sometimes very 'clean' as

in Figure 6F, more usually they are more or less beaded, as in Figure 6E. Since the filaments and in the pharynx when it is deployed are not beaded, such beaded appearances are perhaps artefacts of fixation.

Following the second fence position, there is a zone of cells invariably covered with a 'mucous' sheet in secreting specimens, and then a pavement consisting of large rounded cells bearing small microvillar projections. These cells have exposed surfaces and are free of 'mucus'. Over them views of the filter are seen, closer to the fence still rhomboidal but now the filaments lie at angles around 60° to each other (Figure 6F). Further away from the fence, the filter mesh is more or less square, or sometimes rectangular, as it is in the filter deployed outside the endostyle (Figure 7E,F). The pavement continues, and the walls of the endostyle become thinner and thinner until similar to the inner pharyngeal epithelium elsewhere, some 1 – $1.5\ \mu\text{m}$ thick. Finally, on the *right* side (seen from above) of the endostylar groove only, there is a thicker band of cells bearing remarkable multiple cilia fused into columns, each column being formed of 100 – 150 separate cilia lying closely together (Figure 7A–C).

In small blastozoids of *S. fusiformis* and *T. emocratica*, some 3 – $5\ \text{mm}$ long overall, there is but a narrow band bearing many separate cilia, not aggregated into columns as they are in mature blastozoids. In more mature blastozoids ($1\ \text{cm}$ pharyngeal length, but still not bearing embryos), there are three or four lines of ciliated cells and each now has a single ciliated column upon it (Figure 7A). In life, these beat as a single unit, upwards towards the top of the endostyle. In secreting endostyles, this band of columnar cilia is overlain by the filter, which now has a largely square mesh of some $1.3\ \mu\text{m}$ (Figure 7C). There are some rectangular portions ($0.4 \times 1.3\ \mu\text{m}$) not unlike views of it seen outside the endostyle (Figure 7E). Sometimes, two filter layers are superposed over the columnar cilia, perhaps an artefact of fixation (Figure 7D).

As Garstang & Platt (1928) observed, it is not easy from sections to know where this band of cilia lies with regard to the opening of the endostylar groove. Their transverse sections suggested that the walls of the endostyle were so closely apposed below the ciliary band, that the band lay where the endostyle was open to the pharynx. It seems more probable to us that the columnar ciliary band (which beats upwards) lies below where the two walls of the endostyle are close together even above, but are not apposed so closely as to seal the top of the endostyle completely from the pharynx.

In a general way, this description of the different endostylar zones in the salps we have examined accords with that given from sections of the endostyles of the ascidians *Ciona* (Holley, 1984) and *Molgula* (Godeaux, 1989), and with that for the salp endostyle by Fredriksson et al. (1988) except that in the ascidians, the asymmetrical dorsal band is apparently present on both sides of the endostyle, to judge from the figures of Holley (1986), Godeaux & Firket (1968) and Godeaux (1989). According to Garstang & Platt (1928) the same is true in *T. democratica* (see also Goffinet & Godeaux, 1992) and *Pegea confederata*, but not in *S. maxima*, whilst Fol (1876) states that both bands are present in most salps. So far as we have been able to observe, in *S. fusiformis* and *T. democratica* there is only a single band. The functional

significance (if any) of the presence or absence of two bands is not known.

A striking feature of the salp endostyle is the remarkable and distinctive appearances of the rodlets and the 'mucous' sheets upon the second ciliary fence has not been observed in ascidians. We have yet to examine ascidian endostyles, but have made some preliminary observations upon *Pyrosoma atlanticum* tetrazooids which seem to show essentially similar grooved 'mucus' secretion (Figure 6G).

DISCUSSION

The secretion of the filter net

The upper glandular cell group secretes the thinner filaments lying at right angles to the long axis of the endostyle. These pass vertically upwards from the tips of the cilia forming the second fence, which determine their spacing. The basal or lower glandular cell group must secrete the other filaments of the net (lying along the long axis of the endostyle), seen first as they pass over the upper part of the ciliated zone and the second fence (Figure 5E). Since we have so far been unable to see in any of our preparations filaments depending from the tips of the cilia of the first fence as they do from the second (upper) fence we cannot say how their spacing is determined, but the strands are fairly widely spaced in comparison to those of the fence cilia. Thus there is apparently a difference in the way that the two kinds of filament are spaced. Importantly, the filaments produced by the lower group of gland cells (however they may be spaced), are *oblique* to the longitudinal axis (shown by the alignment of the upper ciliary fence to the bottom right in Figure 4A). They cross over this ciliary fence above a second row of filaments. We have also confirmed that the spacing of the vertical filaments is determined by the spacing of a ciliary fence, as Holley (1986) suggested for *Ciona* depending from the tips of the cilia forming the fence (Figures 5F & 6A). The wider spaced row of filaments overlies the other narrower row obliquely and becomes attached to the vertical filaments, (Figure 6B), hence at this stage they are not at right angles to each other as they later become, and the filter mesh is rhomboidal (Figure 6E) rather than square or rectangular. Higher up the endostylar groove, the filaments form a more or less rectangular net, and still closer to the opening of the endostylar groove into the pharynx, over the asymmetrical band of ciliary columns (Figure 7A,B) the net openings are often square.

Nothing is known of the properties of the net within the endostyle, but the different rectangular and square images suggest that it is probably elastic, as it almost certainly is outside the endostyle when it forms the feeding filter. For instance, in the filter which has captured *Isochrysis* cells (Figure 7E), the rectangular mesh of both thick and thin filaments varies apparently according to the cells captured. In contrast in Figure 7F, where the filter mesh is more or less square rather than rectangular, it is presumably not under tension, and certainly seems much like the filter seen above the columnar cilia in Figure 7C,D. Here too it is presumably not under tension, and so represents the basic form of the

filter, with a square mesh of 1.3 μm in the adult oozoid of *Salpa fusiformis*.

Although the net is usually single at the level of the ciliary columns, on occasion it appears double, as if the filters formed on either side of the endostyle overlay each other over the ciliary columns (Figure 7D).

Deployment

The deployment of the salp filter is a more complex process than in ascidians or pyrosomas. In both, the filter is produced along the length of an endostyle which lies from top to bottom of the gill basket, and as it issues from the top of the endostylar groove it is draped over the gill bars, and supported by them. In contrast, whilst the salp filter is presumably produced along the length of the endostyle, it must be moved forwards along the endostyle and then transported upwards around the peripharyngeal bands of either side, to be joined together at the mid-line top and bottom as it expands to form the filter.

It must be admitted that although we have been able to determine which parts of the endostyle secrete the two sets of filaments making the filter, we have not succeeded in making any further progress in understanding its deployment, nor how the spacing of the first formed filament set is determined. One difficulty is that although we fixed endostyles when the feeding filter was in place, and the endostyle was in principle secreting the filter, it is likely that in different specimens, the fixative took more or less time to interrupt filament production within the endostyle. Again, although the filter was in place, secretion (which is under nervous control) may have stopped just prior to fixation.

The changing angles of the two sets of filaments to each other after they join, and the change from a rectangular to a square mesh indicates that the filaments are elastic, and that they are under some tension until they reach the level of the columnar cilia. The fully formed filter deployed to collect food particles is also elastic, to judge from the different appearances it presents (see Figure 7E,F). What underlies this tension, and how are the filaments moved up the endostyle?

There are only two ciliated zones in the endostyle, which could provide the motive power. The strongly ciliated zone between the first and second glandular regions and their associated ciliary fences appears to produce a current directed obliquely forwards and upwards, and it seems that it is this that draws the first filaments obliquely towards and over the second set of filaments. In life, the columnar cilia beat strongly upwards, so drawing the first and second sets of filaments, now joined, upwards to the top of the endostyle. There remain two problems which this picture of the production of the filter by the endostyle omits. First, after it has risen to the level of the columnar ciliary band, how is the filter moved forward along the endostyle? Secondly, the curious problem of how is the filter joined top and bottom once it has left the endostyle and passed around the peripharyngeal bands?

The structure of the filter in ascidians and salps is very similar, as is the structure of their endostyles. It seems therefore very probable that the secretion of the filter must be similar in both. That is, in salps and ascidians,

and probably in all tunicates both sets of filaments are related to ciliary fences. Certainly in *Pyrosoma*, the second fence is linked to grooved 'mucus' profiles (Figure 6G) similar to those of salps. We do not know enough in detail about the endostyles of different species to know whether the spacing of the fence cilia (which are found in *Ciona*, Holley, 1986), as well as in salps and *Pyrosoma* differs in different species. For instance, Flood & Fiala-Medioni (1981) have observed that the ascidian, *Styela plicata* has a filter with a very different mesh size (almost double in length, and 20% wider) to that of *Ciona* and it will be interesting to compare their endostylar fences.

The rodlets

It seems likely the rodlets are the first stage in the secretion of the 'mucus' which will form the filaments of the filter. We have eschewed consideration of the composition of the filter, or of the rodlets, and as noted earlier, the term 'mucus' is simply used descriptively, rather than indicating any particular chemical composition (see next section). It seems to us that the most likely scenario is that the secretions of the two groups of gland cells consist mainly of the structural basis of the filaments and that these secretions emerge as groups of rodlets. These are transformed into filaments, for at the upper row of fence cilia, a sheet of rodlets lying against the upper part of each cilium, breaks down into particles and these seem to become fluid and flow outwards from the tips of the cilia as filaments. That such filaments lie on top of a 'mucus' layer without fusing with it suggests that they are different in composition to the layer covering the zone of cells following the upper ciliary fence. However, these vertical filaments have to fuse with the oblique filaments formed earlier, and so cannot be entirely non-adhesive. Since similar rodlets are also in found in the endostyle of *Pyrosoma*, it seems probable that they are characteristic of the tunicate endostyle.

It is not easy to harmonize the striking scanning microscope views of rodlets at various sites with views of transmission microscope sections of secreting endostyles, for in the latter it appears that there are extremely elongate microvilli near the apices of the glandular cells (as well as around the adjacent cilia). Sometimes it seems that the microvilli are linked to 'bubbles' of mucous material. Such microvilli are not observed in our scanning views of the area in endostyles that are not secreting. We interpret (with some hesitation) the microvilli seen in sections of secreting endostyles as equivalent to the rodlets of scanning preparations but this remains to be demonstrated definitively. We know of no arrangement of 'mucus'-secreting cells comparable to those producing the rodlets in the salp endostyle, though Compère & Godeaux (1997) observed somewhat analogous products from gland cells in the endostyle of a new species of doliolid.

Other aspects

We have not considered anything other than the secretion of the strands of the filter. It is evident from the comparative studies by Frederiksson et al. (1988) that in the endostyles of all tunicates there are homologous regions concerned with iodination and as Godeaux

(1989) found, several enzyme types are secreted. Nor have we considered the chemical composition of the fine filaments forming the filter. Flood & Fiala-Medioni (1981) concluded from histochemical tests on ascidian endostyles that the net filaments consisted of a peptide core ensheathed by polysaccharides. Deep-etched replicas of the filter in *Thalia* kindly made for us by Dr P. Chang show that central repeating structures form a core within the filaments, but we have yet to examine these in detail.

Q.B. is grateful to the Royal Society for a grant for consumables, and once again, thanks the Director and staff of the Station Zoologique for their kind and courteous hospitality. C.C. is in the plankton group LOBEPM-ESA 7076.

REFERENCES

- Berrill, N.J., 1950. *The Tunicata, with an account of the British species*. London: The Ray Society.
- Bone, Q., Braconnot, J.-C. & Ryan, K.P., 1991. On the pharyngeal feeding filter of the salp *Pegea confederata* (Tunicata: Thaliacea). *Acta Zoologica (Stockholm)*, **72**, 55–60.
- Compère, P. & Godeaux, J.E.A., 1997. On endostyle ultrastructure in two new species of doliolid-like tunicates. *Marine Biology*, **128**, 447–453.
- Fedele, M., 1933. Sulla nutrizione degli animali pelagici III. Ricerche sui Salpidae. *Bolletino della Società di Naturalisti di Napoli*, **45**, 49–118.
- Flood, P.R. & Fiala-Medioni, A., 1979. Filter characteristics of ascidian food trapping 'mucous' films. *Acta Zoologica (Stockholm)*, **60**, 271–272.
- Flood, P.R. & Fiala-Medioni, A., 1981. Ultrastructure and histochemistry of the food trapping 'mucous' film in benthic filter feeders (Ascidiacea). *Acta Zoologica (Stockholm)*, **62**, 53–65.
- Fol, H., 1876. Ueber der Schleimdrüse oder den Endostyl der Tunicaten. *Morphologisches Jahrbuch*, **1**, 222–242.
- Fredriksson, G., Öfverholm, T. & Ericson, L.E., 1988. Iodine binding and peroxidase activity in the endostyle of *Salpa fusiformis*, *Thalia democratica*, *Doliioletta gegenbauri* and *Doliolum nationalis* (Tunicata, Thaliacea). *Cell and Tissue Research*, **253**, 403–411.
- Garstang, W. & Platt, M.I., 1928. On the asymmetry and closure of the endostyle in *Cyclosalpa pinnata*. *Proceedings of the Leeds Philosophical Society*, **1**, 325–334.
- Godeaux, J.E.A., 1981. Etude au microscope électronique de l'endostyle des doliolides (Tuniciers cyclomyaires). *Annales de la Société Royale Zoologique de Belgique*, **111**, 151–162.
- Godeaux, J.E.A., 1989. Functions of the endostyle in the tunicates. *Bulletin of Marine Science*, **45**, 228–242.
- Godeaux, J.E.A. & Firket, H., 1966. Ultrastructure de l'endostyle de *Molgula manhattensis* Kay (Ascidie stolidobranchie). *Comptes Rendus de l'Académie de Sciences. Paris*, **262**, 488–490.
- Goffinet, G. & Godeaux, J., 1992. Ultrastructure de l'endostyle des salpes. *Rapports et Procès-verbaux des Réunions. Commission Internationale pour l'Exploitation Scientifique de la Mer Méditerranée*, **33**, 250.
- Holley, M.C., 1986. Cell shape, spatial patterns of cilia and 'mucus'-net construction in the ascidian endostyle. *Tissue and Cell*, **18**, 667–684.
- Huxley, T.H., 1851. Observations upon the anatomy and physiology of *Salpa* and *Pyrosoma* together with remarks upon *Doliolum* and *Appendicularia*. *Philosophical Transactions of the Royal Society*, **251**, 567–605.

- Monniot, C. & Monniot, F., 1978. Recent work on the deepsea tunicates. *Reviews in Oceanography and Marine Biology*, **16**, 181–228.
- Monniot, F., 1979a. Microfiltres et ciliatures branchiales des Ascidies littorales en microscopie electronique. *Bulletin du Museum National d'Histoire Naturelle de la France, 4me. Série, A, Zoologie*, **1**, 843–859.

- Monniot, F., 1979b. Mise en evidence d'un filtre 'micropore' naturel chez les Ascidiacea. *Comptes Rendus de l'Académie des Sciences Paris. Série D*, **289**, 927–929.

Submitted 4 February 2000. Accepted 16 March 2000.