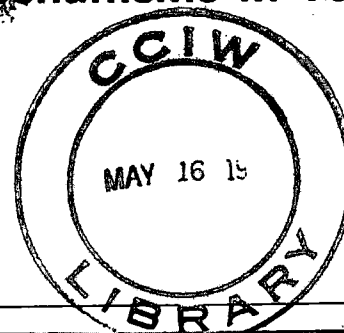■✦ Environment
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Feeding Mechanisms in Tunicates

D.B. Carlisle

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Abstract

In all tunicates the apparatus for generating and manipulating the mucus net which is used for trapping the food is essentially the same. The endostyle produces strands of mucus whose anterior end is passed dorsally by the peripharyngeal ciliated bands to the dorsal lamina where they are twisted into a rope and passed back into the oseophagus. In slow feeding the strands remain attached only by their anterior ends, while in fast feeding their hind ends are twisted into the rope before they can become attached to the peripharyngeal bands. In this way a funnel of mucus is formed whose anterior end is held open by the peripharyngeal ciliated bands. In ascidians, *Doliolida* and *Pyrosomatida* water is moved through this mucus trap by means of the action of cilia which line the gill slits. Evidence is presented that ascidians with spiral gill slits can move more water with less area of ciliated epithelium than can species with straight gill slits and it is suggested that the formation of major vortices reduces the shear forces needed to divide the water into the discrete streams which pass through the separate gill slits. Salpida and Larvacea in varying degree and by varying methods rely on muscular action to create the water currents required for feeding. A few aberrant deep sea ascidians rely on steady external water currents for feeding. Species of the Hexacrobylidae appear to feed on larger prey and may actively hunt for food by crawling over or under the sediment. The extinct Palaeozoic Calcichordata, which are probably ancestral to Tunicata, had the pharynx organized in a way comparable to that of the latter and probably fed in a similar manner.

Résumé

Dans tous les Tuniciers l'appareil pour générer et pour manipuler la nasse muqueuse, qui est utilisée pour attraper la nourriture, est essentiellement identique. L'endostyle produit des filaments muqueux dont la partie antérieure est passée dorsalement par arcs péricoronaux jusqu'au raphé dorsale où ils sont tordus pour produire le cordon nutritif et ce cordon est repassé dans l'aire oesophagienne. Pendant l'alimentation lente les bande-lettes de mucus sont attachées seulement par leurs extrémités antérieures, et pendant l'alimentation rapide leurs extrémités postérieures sont déjà tordues en cordon avant que les extrémités antérieures peuvent s'attacher aux arcs péricoronaux. Ainsi se forme un entonnoir muqueux. Dans les Ascidiacés, les Dolioles et les Pyrosomes, l'eau est passée à travers cette nasse muqueuse par les bâtiments ciliaires qui garnissent les fentes branchiales. Nous présentons évidence que les Ascidiacés qui possèdent des stigmates enroulés en spirale peuvent passer une plus grande quantité d'eau avec un plus petit nombre de cellules ciliées de fentes branchiales que les espèces qui possèdent des fentes branchiales droites; et nous suggérons que la formation de vortices majeures réduit les forces de cisaillement néces-saires pour diviser l'eau en courants séparés qui passent à travers les stigmates individuels. Les Salpes et les Appendiculaires tous en mesures variantes et par des méthodes diverses utilisent des contractions musculaires pour créer les courants d'eau requis pour l'alimentation. Un petit nombre d'espèces d'Ascidiacés aberrants de l'abysse dépendent d'un courant extérieur établi pour leur alimentation. Des espèces de la famille Hexacrobylides paraissent se nourrir de proie plus large, et peuvent chasser activement pour leur nourriture en rampant au-dessus ou au-dessous de la boue sédimentaire. Les Calcichordates, fossiles de l'ère paléozoïque qui sont, en toute probabilité, des ancêtres des Tuniciers, possédaient un pharynx organisé presque comme celui de ce groupe, et les auteurs proposent que leur mode d'alimentation était semblable.

List of Abbreviations

a,	anus		*lgb,*	longitudinal gill bar
ac,	anterior coelom		*lm,*	longitudinal muscle
am,	arched muscle		*lo,*	luminous organ
as,	atrial siphon		*lppb,*	left peripharyngeal band
at,	atrium		*lr,*	longitudinal ridge of pharynx
b,	brain		*lv,*	longitudinal vessel
bb,	branchial bar		*m,*	mantle
bc,	buccal cavity		*mc,*	mucus-secreting cells
bls,	blood sinus		*mf,*	mucus funnel
bm,	buccal muscle		*mn,*	mucus net
brn,	brain		*mr,*	mucus rope
bs,	buccal siphon		*ms,*	mucus strand
c,	cilia		*mu,*	muscle band
cc,	ciliated cells		*n,*	nucleus or visceral mass
cf,	ciliated funnel		*nc,*	neural complex
cg,	cerebral ganglion		*nch,*	notochord
cl,	cloaca		*no,*	nerves to endostyle
cm,	circular muscle		*oe,*	oikoplastic epithelium
co,	ciliated organ		*oes,*	oesophagus
cp,	ciliated pit		*oo,*	oesophaageal opening
d,	diaphragm		*ov,*	ovary
dl,	dorsal lamina		*p,*	papilla
dnc,	dorsal nerve cord of tail		*pc,*	posterior coelom
ee,	"emergency exit"		*ph,*	pharynx
ef,	entry filter or window		*plp,*	pleated part of left pharynx
end,	endostyle		*pp,*	primary papilla or pedicel
eo,	Eisen's oikoplast		*ppcb,*	peripharyngeal ciliated band
fc,	flagellated cells		*pr,*	eversible proboscis
ff,	feeding filter		*r,*	rectum
fo,	Fol's oikoplast		*ra,*	right atrium
g,	gonad		*rh,*	rhizoid attachment
gs,	gill slit		*rm,*	radial muscle or retractor muscle
int.	intestine		*rp,*	right pharynx
l,	lappet of dorsal lamina		*rpb.*	retropharyngeal band
la,	left atrium		*rpcb,*	retropharyngeal ciliated band
lbv,	longitudinal blood vessel		*rppb,*	right peripharyngeal band

s,	stomach	*tbv*,	transverse blood vessel
sbp,	smooth part of left pharynx	*te*,	testis
sp,	secondary papilla	*tgb*,	transverse gill bar
t,	tunic or test	*tr*,	trunk
ta,	tail	*v*,	velum
tap,	transatrial pedicel		

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D.B. Carlisle

Introduction

The majority of tunicates collect their food by filtering a current of water. It is perhaps incidental that this current may also serve for respiration. For the most part the work is done by the pharynx, and, despite a number of variations in the different classes of tunicates, there is a fundamental similarity in the basic mechanism and in the organization of the pharynx and of the gill slits. The pharynx in fact constitutes by far the largest part of the body in most tunicates, consisting of the greatly enlarged first part of the alimentary canal.

The side walls of the pharynx are perforated by gill slits, sometimes called stigmata. Primitively there are probably three pairs, but they may be reduced to a single enormous gill slit on each side, in the salps for instance, or may increase to many thousands by the cross division of gill slits and by the multiplication of rows in such forms as *Ciona*. The midventral line of the pharynx is occupied by the endostyle, which possesses a remarkable similarity in all classes of tunicates, and indeed in the larval lamprey. The endostyle consists of a midventral groove whose base is formed by a longitudinal row of flagellated cells. Each side of the groove consists of an alternation of ciliated and glandular cells, normally three zones of gland cells separated by two zones of ciliated cells. At the opening the two sides of the endostyle carry the marginal ciliated bands which run forward to the anterior end and then diverge to run around the anterior part of the pharynx as the peripharyngeal ciliated bands. These meet again on the mid-dorsal line inside the pharynx, just behind the ciliated funnel of the neural complex. From this point the dorsal lamina runs backward along the mid-dorsal line of the pharynx to the opening of the oesophagus. A further ciliated band, the retropharyngeal ciliated band, runs from the posterior end of the endostyle to the ventral side of the opening of the oesophagus.

The endostyle is the organ which secretes the mucus used to trap the food, the ciliated bands serve to distribute the strands of mucus to form the net which traps particles, while the dorsal lamina passes the strands back into the oesophagus so that food may be digested. Cilia inside the oesophagus twirl up the mucus strands into a rope by twisting them around and around, and this rope is drawn back down the oesophagus into the stomach where digestion begins.

As early as 1834 Lister watched ascidians draw in particles in the inhalent current. He followed the course of these particles as they passed into the pharynx and remarked on the presence of a membrane suspended across the pharynx. He suggested

that the water was perhaps filtered by this membrane. Fol (1872) was the first to suggest that the filter membrane was produced by the endostyle and consisted of mucus. A few years later Roule (1884) showed that in *Ciona* a current passes in through the mouth, thence through the gill slits of the pharynx into the atrial cavity and so out through the atrial siphon. He confirmed that the food particles in this current were trapped by a mucus membrane which was then spun into a rope and drawn back into the oesophagus, but he believed that the mucus was secreted by the neural gland. In all other respects he confirmed the outline of the mechanism as suggested by Fol. It was not until 1913 that Orton corrected Roule's misconception of the role of the endostyle.

The papers by Orton (1913), MacGinitie (1939), Jørgensen (1949-1955) and Werner and Werner (1954) have particularly contributed to our knowledge of the feeding mechanisms in ascidians. In the Thaliacea the processes have been described most particularly by Fedele (1921, 1923, 1933) and by Carlisle (1950). Fol (1872), Lohmann (1899), Körner (1952) and Fenaux (1968) have contributed to our knowledge of feeding in the Larvacea.

Ascidiacea

With the exception of some aberrant deep sea forms, all ascidians seem to utilize the same method of filtering water, whether they are solitary or colonial. The individual zooid at first glance is little more than a sac with two openings, the siphons (Fig. 1). The water flows in through the buccal siphon into the pharynx, through the pharyngeal walls, via the gill slits, into the surrounding atrial cavity, and then out through the atrial siphon (Fig. 2). Near its dorsal posterior corner the pharynx opens into the oesophagus.

Figure 1 (Opposite)
A feeding zooid of *Clavelina* from the right side. a, anus; as, atrial siphon; bs, buccal siphon; end, endostyle; gs, gill slits; l, lappet of dorsal lamina; mn, mucus net; mr, mucus rope; nc, neural complex; oes, oesophagus; ph, pharynx; ppcb, peripharyngeal ciliated band; r, rectum; s, stomach.
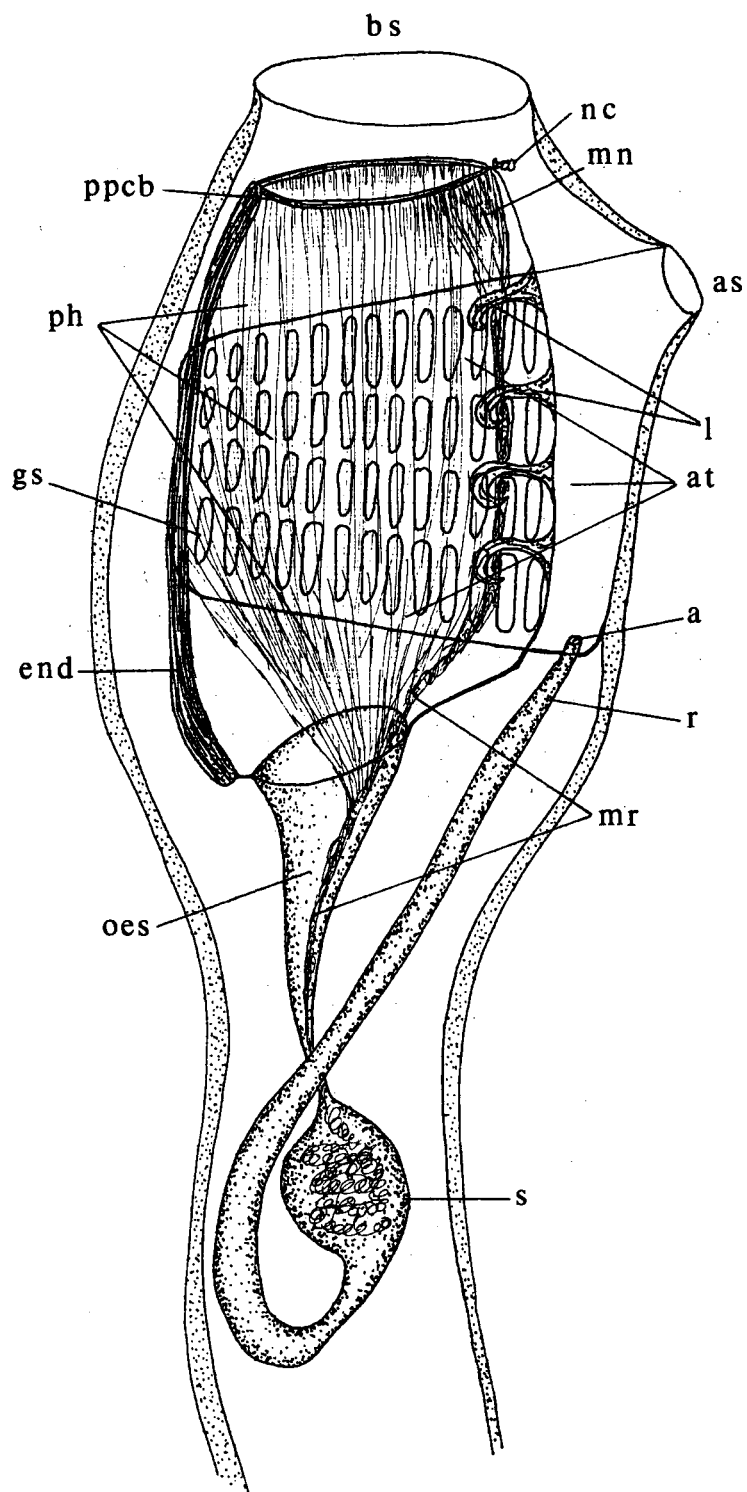
Figure 1.
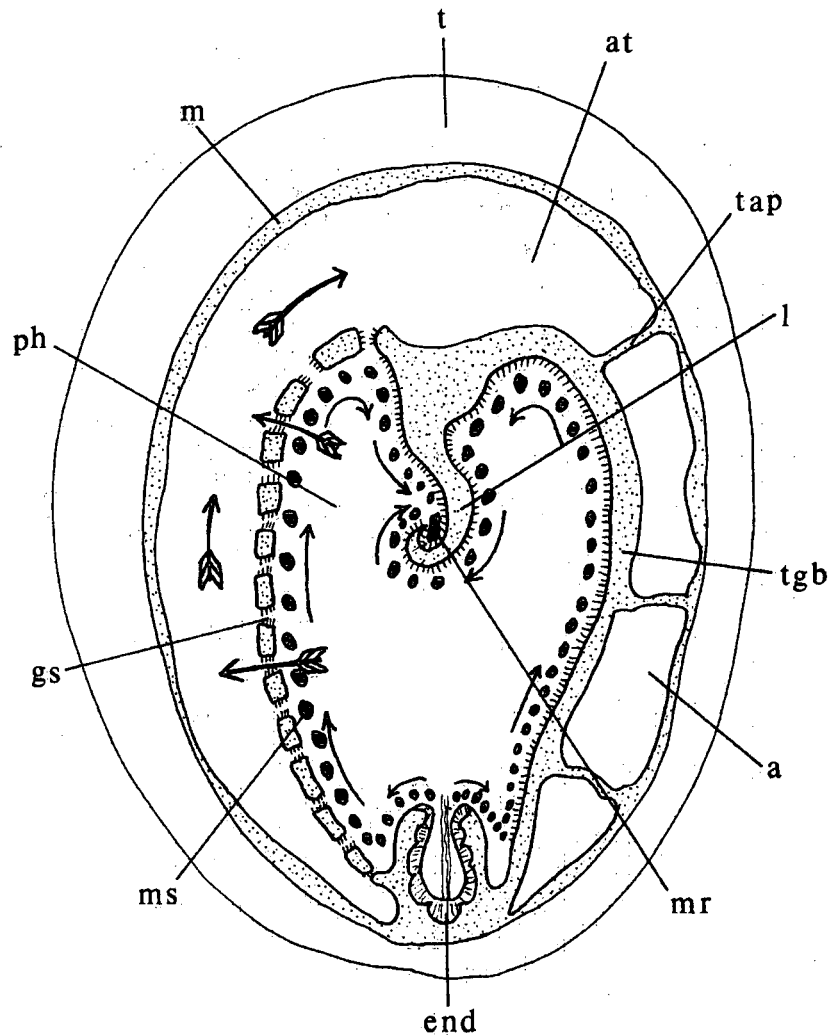
Figure 2. (above)

Diagrammatic transverse section through the phrynx and atrium of *Clavelina* while feeding.
Feathered arrows indicate water movements, tailless arrows movement of mucus strands.
On one side the section passes through the gill slits, or stigmata, on the other through
a transverse gill bar and the transatrial pedicels. at, atrium; end, endostyle; gs,
gill slits; l, lappet of dorsal lamina; m, mantle; mr, mucus rope; ms, mucus strand;
ph, pharynx; tap, transatrial pedicel; tgb, transverse gill bar. (After Werner, 1959).

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The mechanics of the water current

The current is produced by the action of the cilia which line the gill slits. Within each gill slit these cilia beat in a metachronal rhythm which serves to create an eddy so that the water is spun around inside the gill slit and passed through the wall of the pharynx from the inside to the outside. To the observer looking from the outside of the pharynx the metachronal wave passes around each gill slit in an anti-clockwise direction (MacGinitie, 1939).

Hoyle (1953) observed that in some ascidians, notably *Phallusia*, there are rhythmic contractions of the muscles of the mantle wall resulting in the forcible expulsion of the water from the pharyngeal and atrial cavities. He suggested that in *P. mammillata* at least, this squirting was the major mechanism for creating the water current. Carlisle (1966), however, concluded that less than five percent of the water current could be maintained by muscular contraction in this species, while more than 95 percent was the result of ciliary action.

Hecht (1916-1918) in *Ascidia atra*, and Goldberg, McBlair and Taylor (1951) in *Ciona intestinalis*, gave figures of around 80 millilitres per hour per gram wet weight for the filtration rate. Jørgensen (1949) reported a filtration rate of around 230 ml. $hr^{-1} gm^{-1}$ for *Ciona* and 540 ml. $hr^{-1} gm^{-1}$ for species of *Molgula*. In contrast, Hoyle (1953) has reported a figure of only 60 ml. per hour in "full-sized" *Phallusia mammillata*. He gives no weights, but from the internal evidence of the paper his animals cannot have been less than about 100 gm in weight. His figure then represents something less than 0.6 ml. $hr^{-1} gm^{-1}$, a figure less than 0.5 percent of the values obtained by Carlisle (1966) in the same species. Jørgensen (1955) has pointed out that the method which Hoyle utilized to measure the flow rate involved maintaining a head of water against which the animal must push its ciliary current. Since by Hoyle's own measurements an ascidian can only maintain a head of 2 mm of water, the slight head inherent in his technique is likely to be enough to counterbalance the ciliary flow almost completely. It seems more likely that the rate of ciliary flow in *Phallusia* is comparable to that in other ascidians, as found by Carlisle (1966), rather than that it should be so much lower. In fact, it seems probable that the real function of the muscular squirting which was demonstrated by Hoyle lies in clearing the branchial basket of waste material, rather like the method of producing pseudofaeces adopted by filter-feeding molluscs. Indeed, Jørgensen and Goldberg (1953) have pointed out that the muscular contraction of the squirting action deforms and tears the mucus film, bringing the mucus of the

A

1 mm
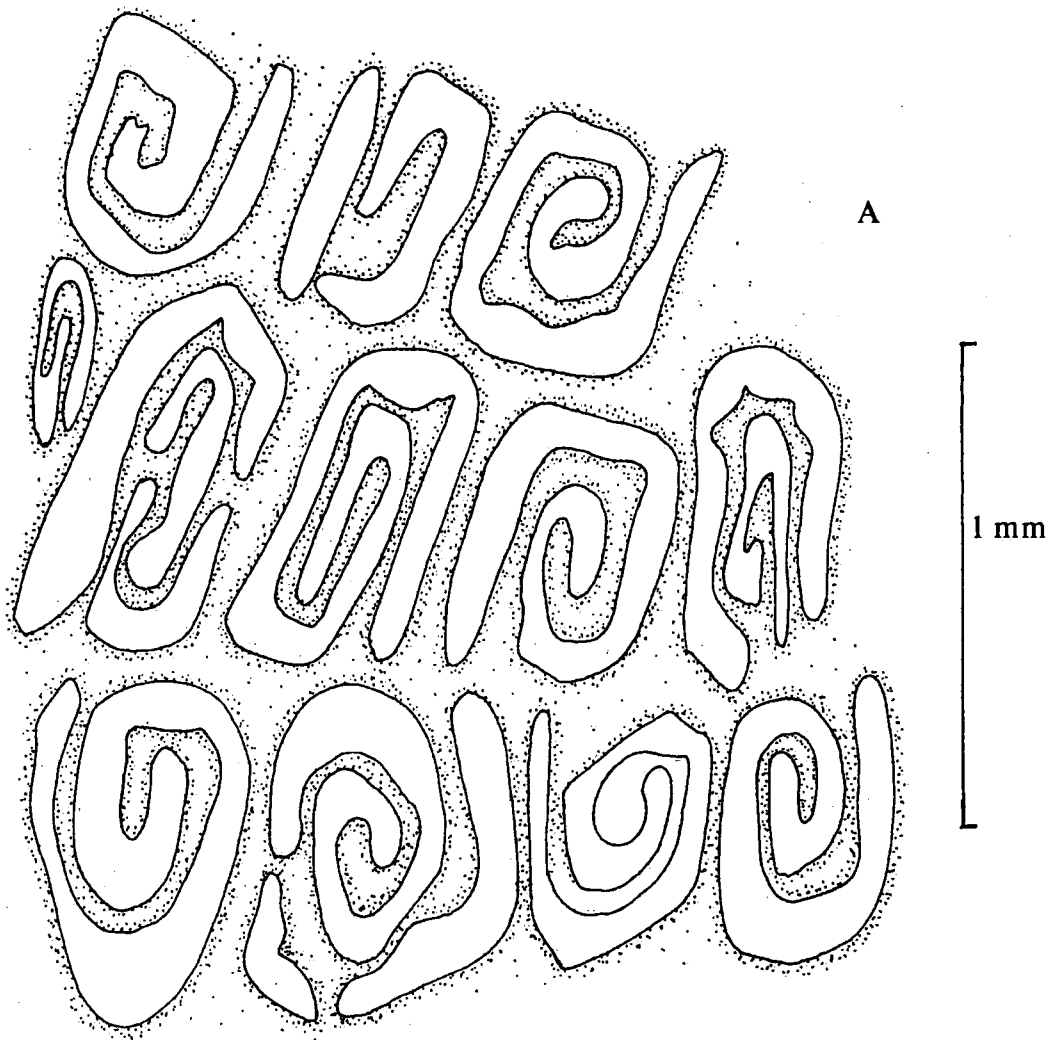
Figure 3.

Spiral gill slits; A (above), in *Agnesioposis translucida*; the gill vessels anad papillae
have been omitted for clarity (after Monniot, 1969); B (next page), in *Molgula occulta*; a
single infundibulum is shown in profile with four orders of spirals around it; C (page 8),
in *Corella parallelogramma*. lbv, longitudinal blood vessel; lv, longitudinal vessel;
pp, primary papilla or pedicel; tbv, transverse blood vessel.

B

Figure 3B.

lbv   lv   pp   tbv   C
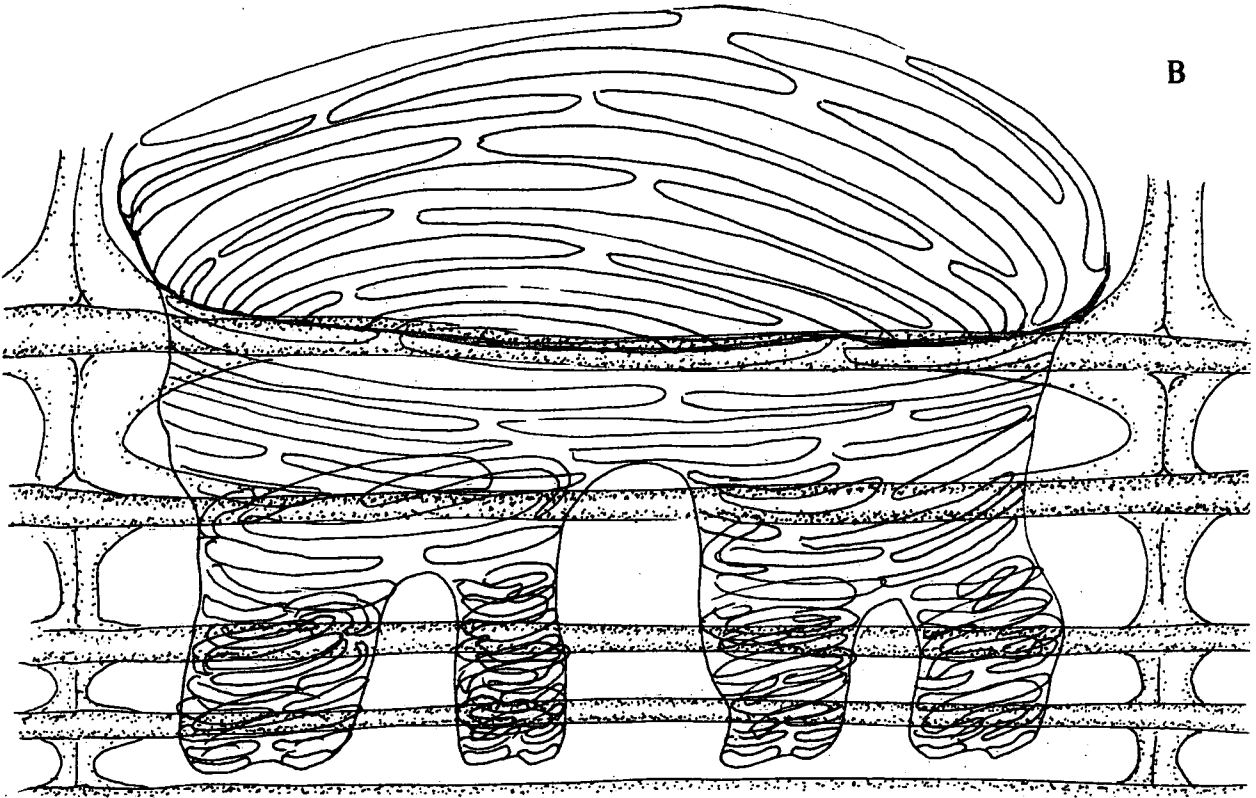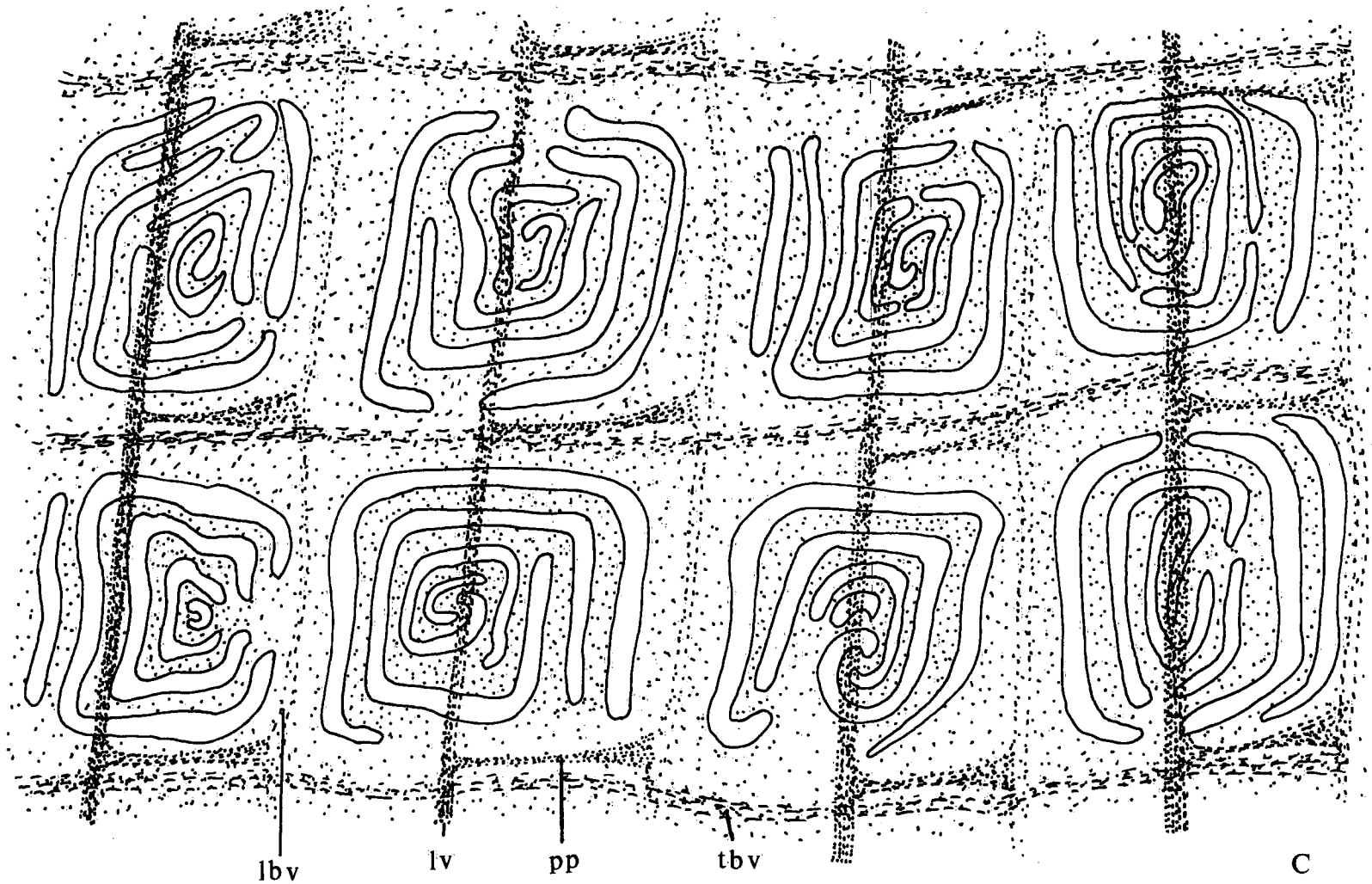
Figure 3C.

two sides together so that it will adhere, thus destroying the network. It seems probable that this fragmented network is expelled with the water ejected through the branchial siphon and that filtration cannot begin again until a new secretion of mucus is started.

The feeding current of ascidians, then, is produced by the cilia lining the gill slits which beat in a spiral manner around each slit and so create a swirl or vortex, thus drawing the water from the pharynx into the atrium, through the pharyngeal wall or branchial basket. In the most complex ascidians, the Molgulidae and to a lesser extent the Pyuridae, Corellidae and Agnesiidae, the vortex action of the gill slits is aided by their organization into spiral groups which help to create a larger vortex and presumably to increase the efficiency of filtration (Fig. 3). A mathematical treatment or a computer model might serve to elucidate this point, and we are currently, preparing such models of the flow through gill slits of various patterns.

Consideration of the ciliary patterns of the gill slits of *Phallusia* and other ascidians shows immediately that vortices must possess some selective advantage. The water flowing through any ascidian gill slit forms into a vortex. Indeed, careful observation shows that the metachronal rhythm of the cilia is by no means simple, but can be resolved into two components. As noted by MacGinitie (1939) the metachronal wave passes around each gill slit in a clockwise direction, as observed from inside the pharyngeal basket, but there is a second transverse component of the metachrony, so that each cilium beats a little behind the one nearer to the inside of the gill slit. The true metachronal wave-front is thus in fact diagonal. If one were to observe an isolated piece of the ciliated epithelium of a gill slit the general appearance would be something like that indicated in Fig. 4. The final effect of this diagonal metachrony is to impart a spiral swirling motion to the water passing through the slit. In terms of hydrodynamics this implies shear forces which must lead to energy losses of some considerable size. Presumably, however, these losses are lower than they would be if the water were passed straight through. The mere separation of the water into discrete streams through each gill slit would also be a source of loss of energy and we may suppose that the shear forces involved in such a simple separation must be higher than those involved in vortex formation. We may perhaps see a similarity between the pattern of metachronal waves and the diagonal placing of turbine blades, where the shear forces of the imparted vortex are lower than they would be for straight through flow.

The separation of water into discrete streams to pass through separate gill slits appears to be the major force impeding water flow and leading to energy loss. In essence, then, the more gill slits an ascidian has the greater are the energy losses. Since, however, it is the cilia lining the gill slits which provide the motive force for moving water in the first place, then it is necessary to have the gill slits no wider than can be spanned by cilia and no longer than mechanical rigidity will allow. Ascidians
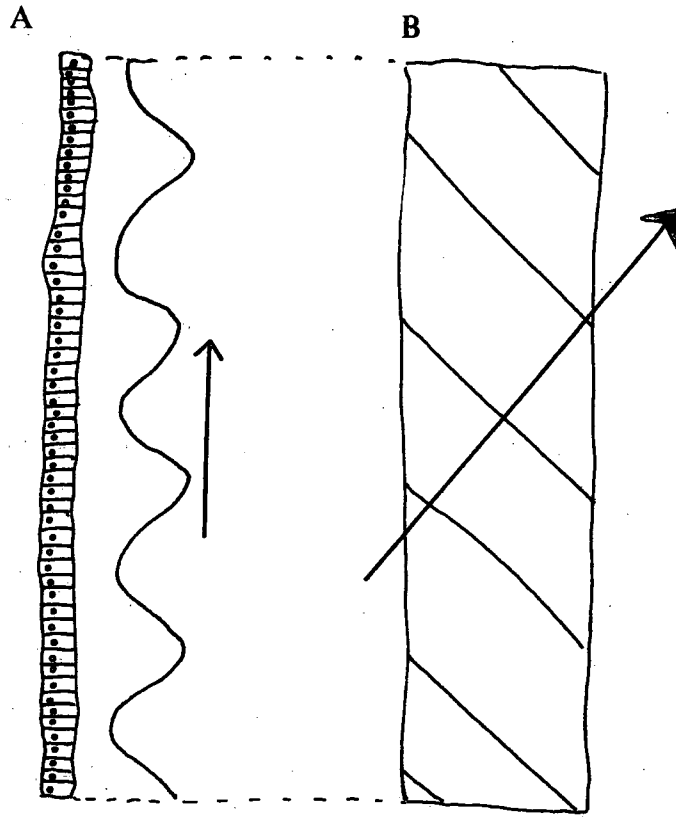
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Figure 4. (above)

Diagram of the ciliated epithelium of a gill slit, A, as seen from inside the pharynx, with metachronal waves of cilia, and, B, as seen in surface view, from inside the slit itself. The diagonal lines indicate the tips of cilia which are in synchrony, and show the metachronal waves. The arrows indicate resultant water movement.

are thus faced with the dilemma that they need many gill slits in order to move large volumes of water, but that the more gill slits they possess the higher are the associated energy losses. We suggest that vortex formation is the best way to minimize this loss, and that major vortex formation, involving a spirally arranged group of gill slits, is an improvement, in this respect, upon vortices involving only a single gill slit.

One way of testing this hypothesis is to measure the area of ciliated epithelium needed to move a unit mass of water in unit time. In practical terms this amounts to measuring the area of epithelium within a number of single gill slits and counting the total number of gill slits. We have measured the filtration rate by the method of Carlisle (1966) in ten specimens each of *Phallusia mammillata*, a species with straight gill slits, and of *Molgula manhattensis*, a species with spiral infundibula of gill slits (Fig. 3). All measurements were carried out at $15^{\circ}C$. *Phallusia* was capable of passing two cubic millimetres of water per second for each square centimetre of ciliated epithelium (actually 2.13 ± 1.02 mm^3 sec^{-1} cm^{-2}), while *Molgula* could pass thirty times as much through its branchial basket (73 ± 25 mm^3 sec^{-1} cm^{-2}). This enormous difference can only be ascribed to more efficient vortex formation.

Why then do all ascidians not possess spiral infundibula? We can only suggest that the problems of embryogenesis and of coordination of function appear more complex with infundibula than with a simple basket of straight gill slits, and that most species simply have not attained this degree of sophistication. Certainly spiral gill slits have evolved independently in different families of ascidians, though not to the degree of complexity found in the Molgulidae.

Formation of the mucus filter

The mucus film is produced by the glandular cells of the two sides of the endostyle. Each half produces a separate band of mucus which is moved towards the outer border by the activity of the ciliated cells. In some species (*Corella*, for example) these upper borders of the endostyle are relatively close to each other (Fig. 5). In such species the two mucus bands are held apart by the action of the long median flagella which extend from their supporting cells in the depth of the groove up to the top of the endostyle and undulate gently (Roule, 1884; Orton, 1913). In *Molgula*, however,

Figure 5.
Transverse sections of the endostyles of A (next page), *Corella parallelogramma*, with three bands of mucus-secreting cells (mc) and three of ciliated cells (cc) on each side, and with a single median band of flagellated cells (fc); and B (page 13), the wide open endostyle of *Molgula*, with reduced median flagella.
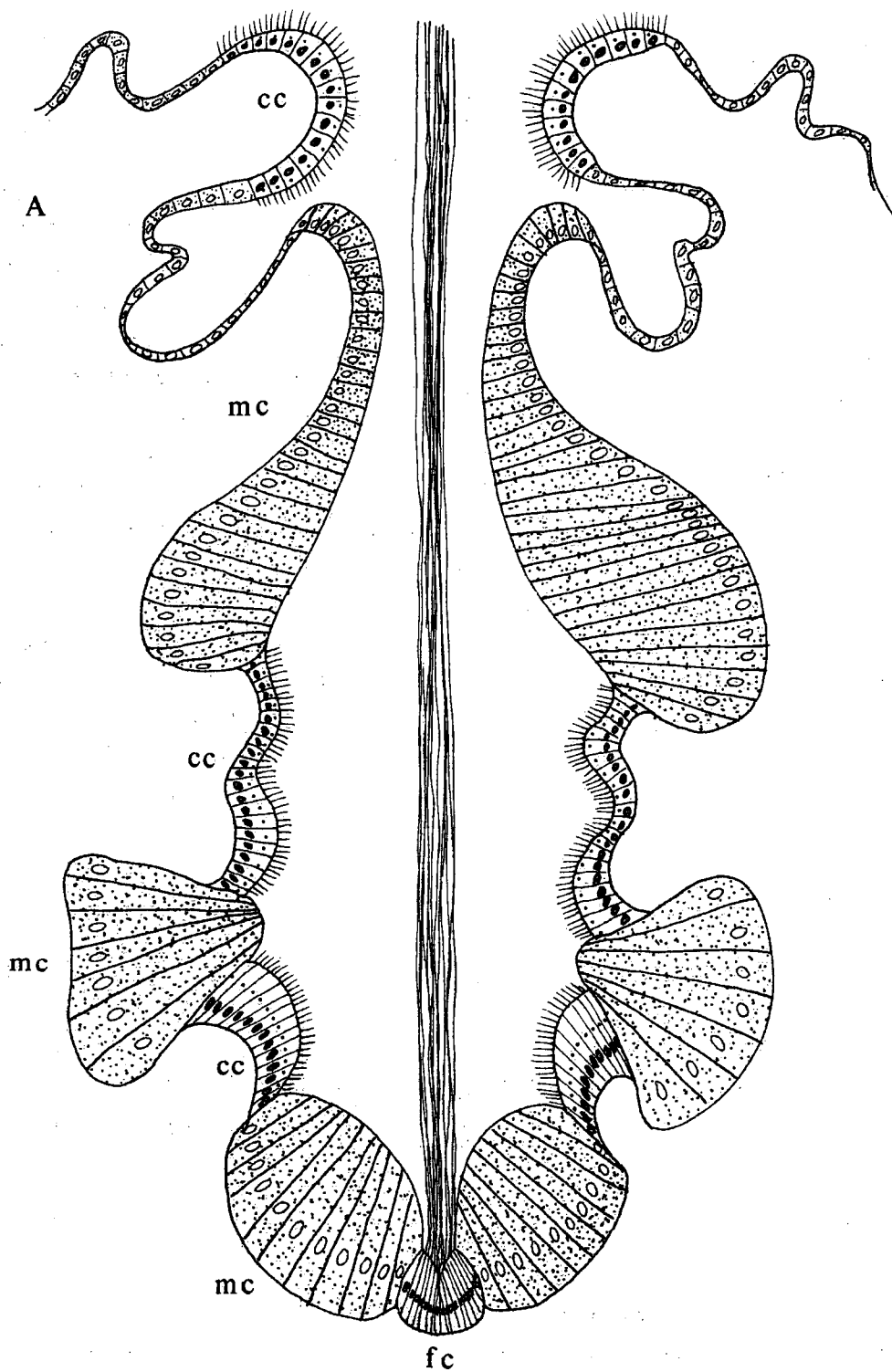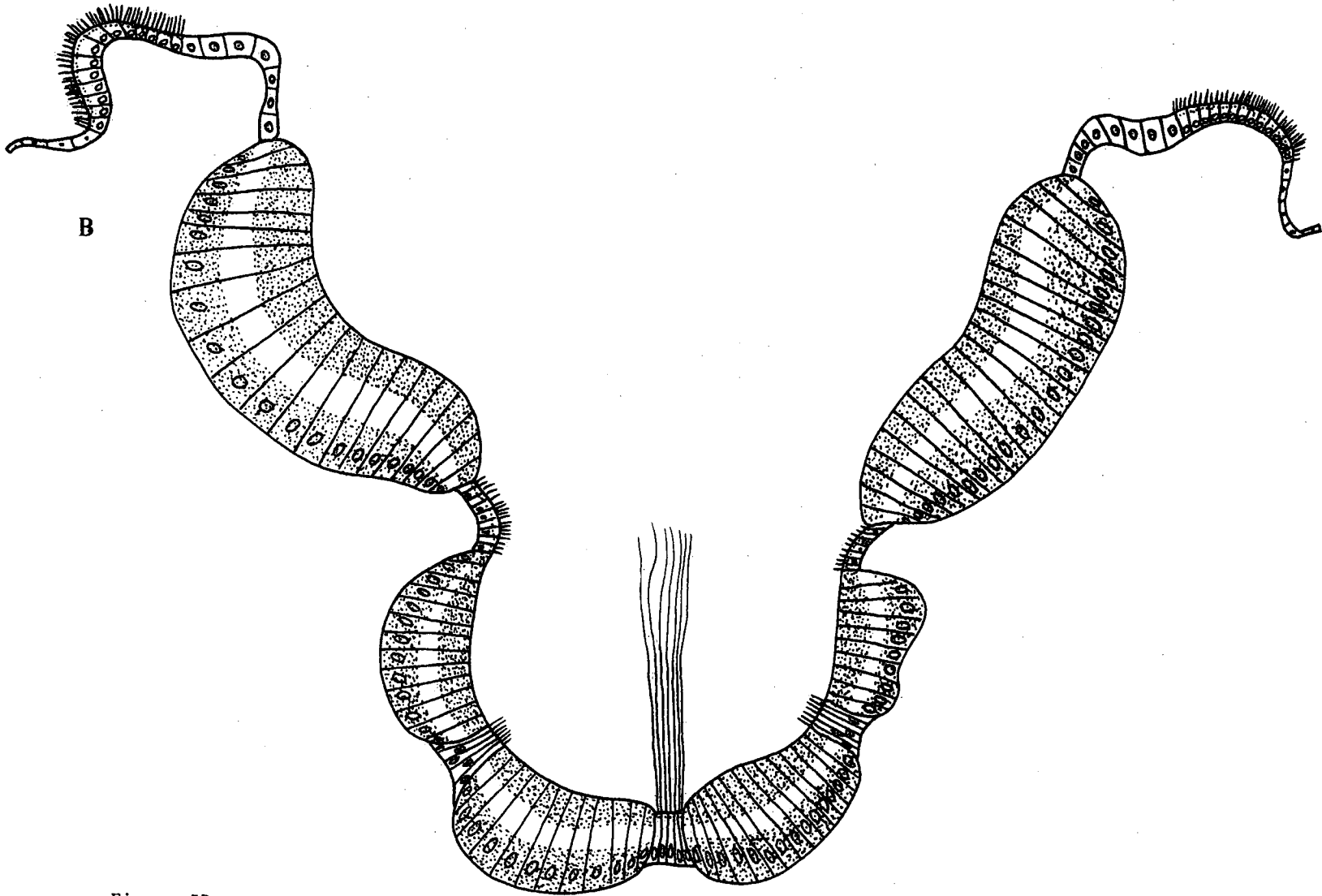
Figure 5A

12

B

13

Figure 5B.

and in many other species, the endostyle is broad and shallow, and the median flagella are correspondingly greatly reduced. In such species there may be less chance of the mucus bands of the two sides touching and so adhering to one another.

The detailed structure of the mucus filter is not well known. It is clear that discrete strips of mucus are produced and that these together form a perforated network. We do not know, however, whether there is a definite pattern of production and of interlocking of these strips into a detailed geometrical network, or whether they interconnect more or less at random.

Transport of the mucus network

The strands of mucus are continuously transported from the margins of the endostyle across the inner face of the pharynx, and it is because of this constant renewal that the filter never has time to be clogged. MacGinitie (1939) demonstrated that the front end of each mucus strand is attached to the peripharyngeal ciliated band of that side. He thus confirmed the earlier observation of Fol (1872), who made the same observation in the thaliacean *Doliolum*. We still have no indication of the mechanism of this attachment. The peripharyngeal bands serve to move the anterior end of the mucus strands around from the mid-ventral to the mid-dorsal line of the anterior end of the pharynx. The posterior end of each mucus strand is carried back by the retropharyngeal ciliated band from the posterior end of the endostyle into the mouth of the oesophagus. An exception is *Clavelina*, in which the posterior end of the endostyle is in direct contact with the opening of the oesophagus and there is no retropharyngeal band (Werner & Werner, 1954).

In the youngest post-metamorphic juveniles, which may possess no more than two rows of gill slits, transport of the mucus net must depend entirely on the peripharyngeal and retropharyngeal bands. In older and larger individuals, cilia on the side walls of the pharynx aid the transport of the mucus strands from the endostyle to the dorsal lamina. A particularly simple arrangement of these cilia is to be seen in *Clavelina*, where, between each transverse row of gill slits, is to be found a ciliated circle running from the superior margins of the endostyle to the dorsal lamina. In *Ciona* the structure of the pharynx is more complicated, but the principle is the same. Where the transverse and longitudinal gill bars cross, branchial papillae are located (Fig. 9). These are bent over and point dorso-posteriorly. The convex side, which faces in towards the lumen of the pharynx, is covered with cilia which beat towards the apex of each papilla. The ventral papillae are small and the dorsal ones larger. The more anterior papillae are inclined more or less directly dorsally, while those set farther back on the wall of the pharynx point more towards the posterior. As a result the mucus film is stretched as it is transported; in the anterior part of the pharynx the papillae

14

A

—lbv

—gs

—lgb

tbv

B

—tgb

pp
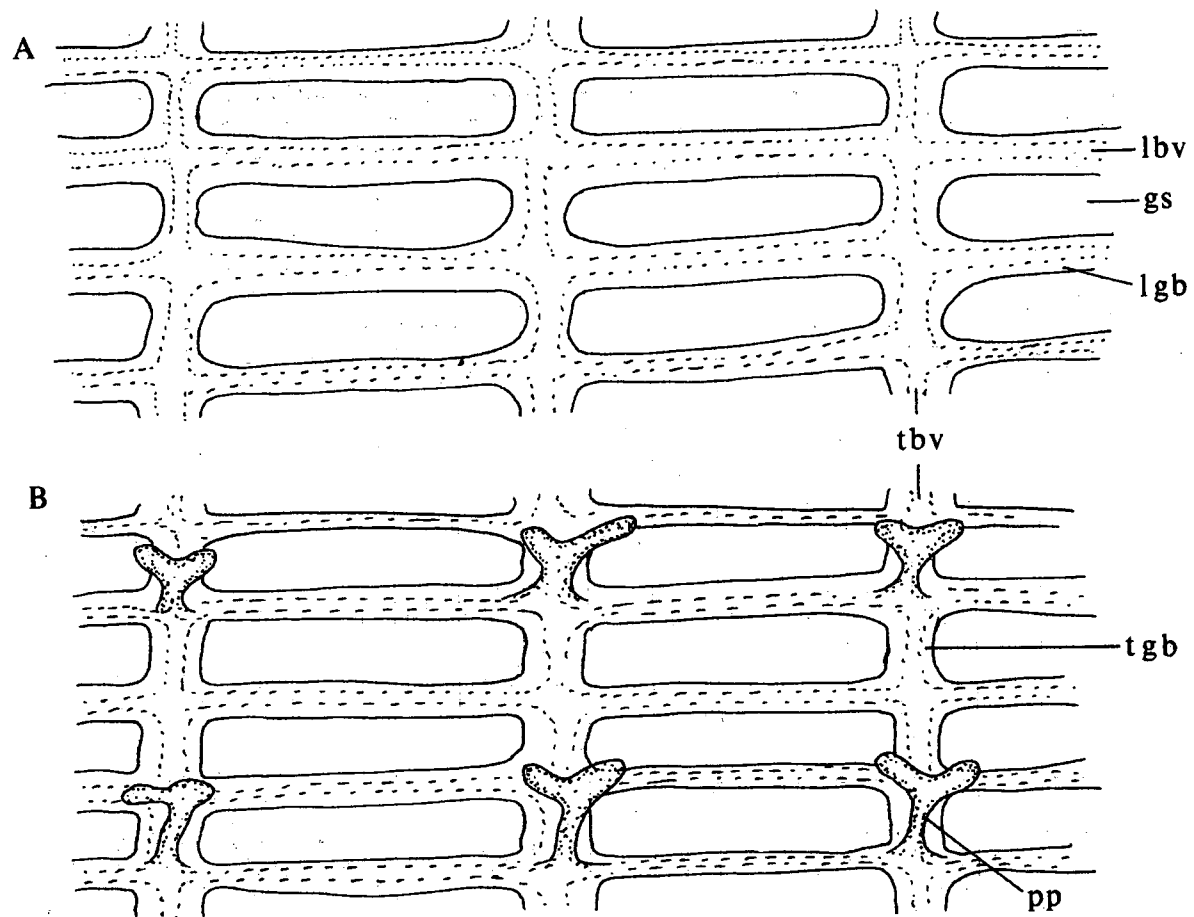
Figure 6.

Oblique views of the inner surface of part of the branchial basket of A (above),
Clavelina; B, *Perophora listeri*; C (page 16), Ascidiella; D, *Ascidia*.   gs, gill slit;
lbv, longitudinal blood vessel; lgb, longitudinal gill bar; lv, longitudinal vessel;
pp, primary papilla or pedicel; sp, secondary papilla; tbv, transverse blood vessel
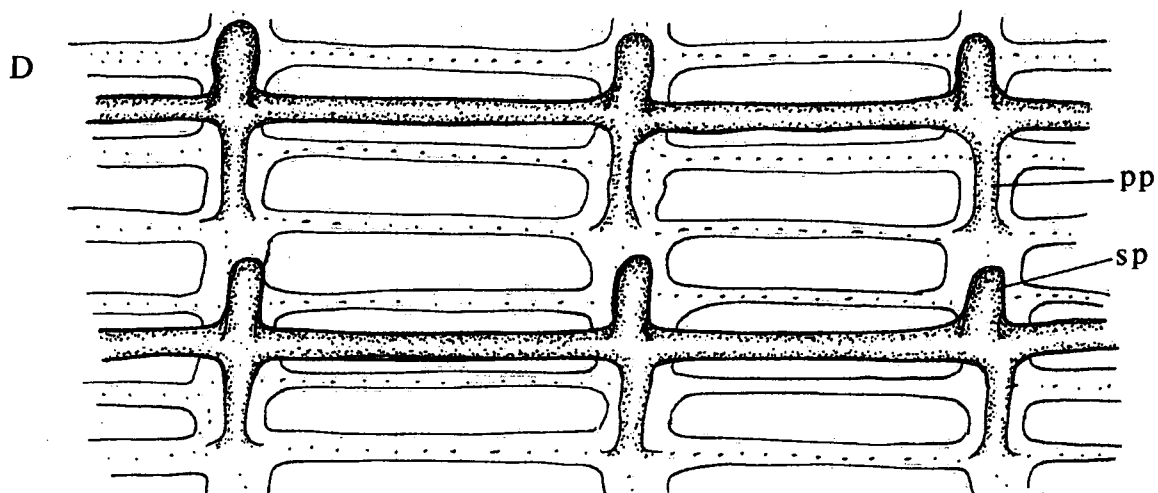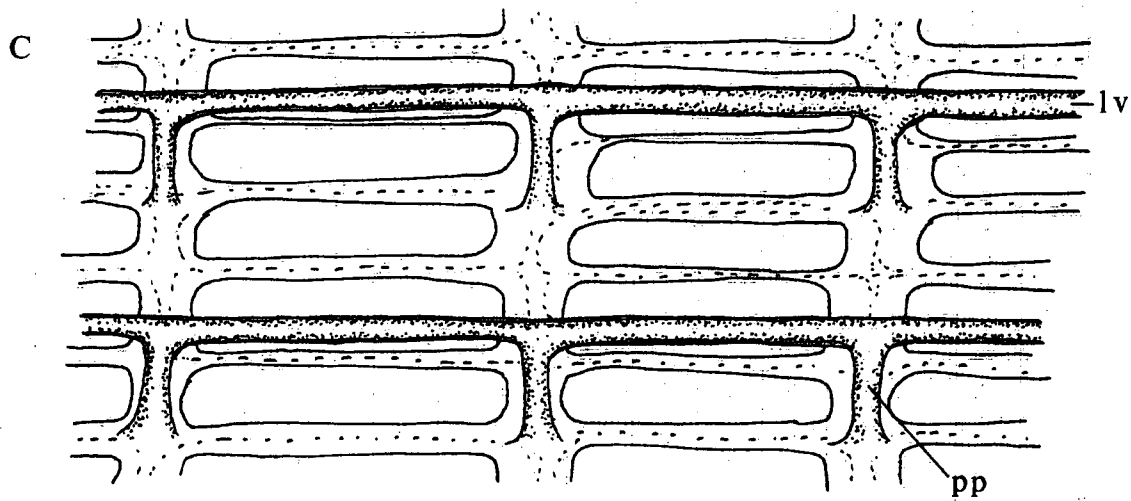
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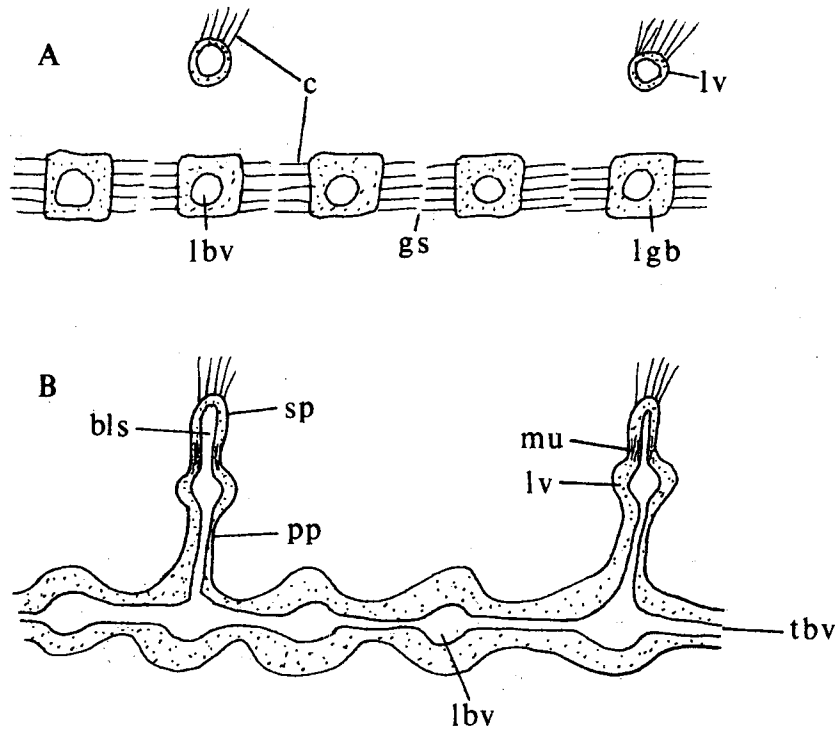
Figure 7. (above)

Diagrammatic transverse sections through the pharyngeal wall of *Ascidia*, with the inner surface towards the top. A, at a level between the transverse gill bars; B, at the level of a transverse gill bar. bls, blood sinus; c, cilia; gs, gill slit: note the cilia almost meeting across the width of the slit; lbv, longitudinal blood vessel; lgb, longitudinal gill bar; lv, longitudinal vessel; mu, muscle band; pp, primary papilla or pedicel; sp, secondary papilla; tbv, transverse blood vessel.
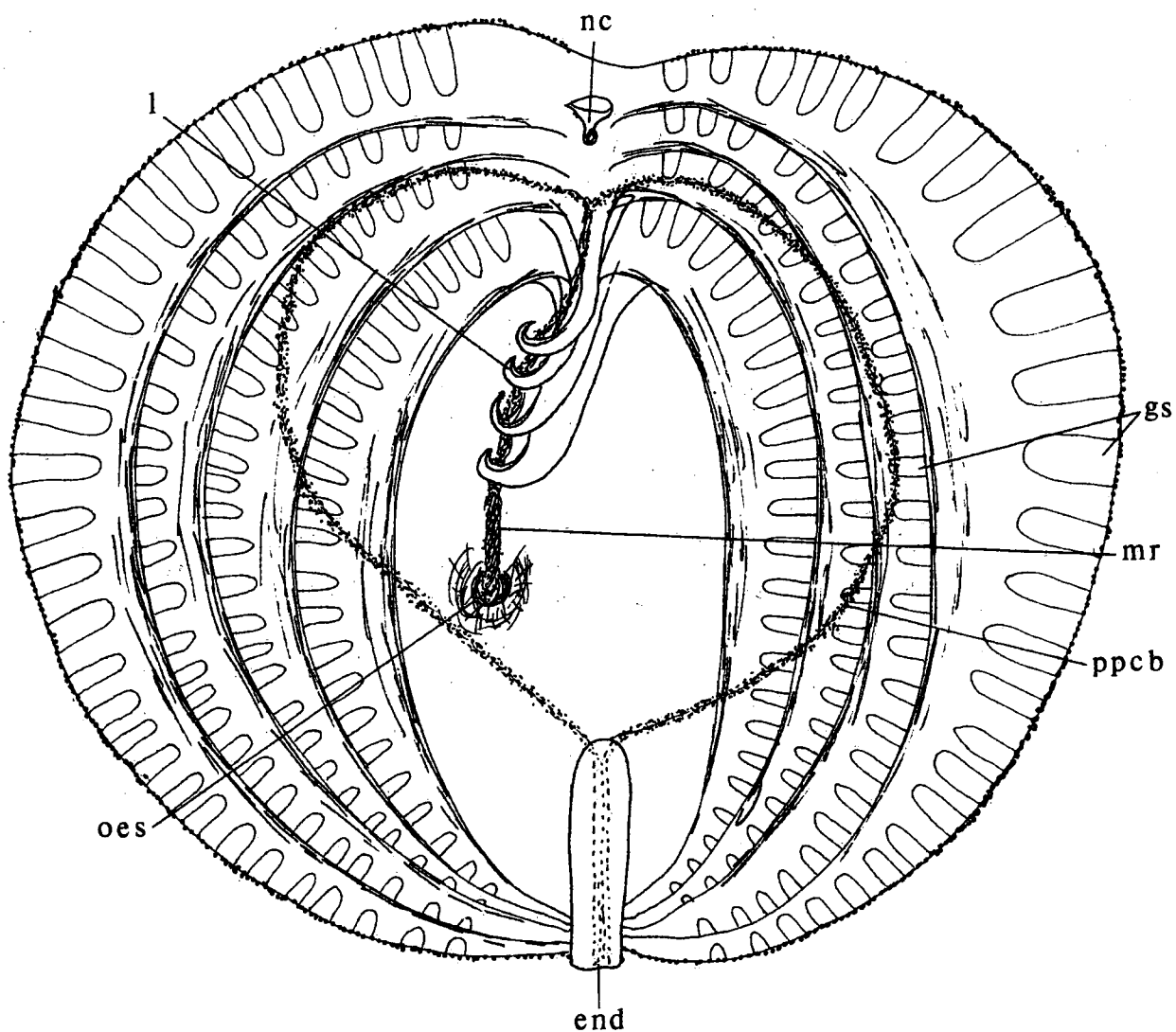
Figure 8. (above)
A view through the mouth of *Clavelina* while feeding. The mucus net has been omitted
for clarity. end, endostyle; gs, gill slits; 1, lappet of dorsal lamina; mr, mucus
rope; nc, neural complex with ciliated funnel; oes, oesophagus; ppcb, peripharyngeal
ciliated band. (Partly after Werner & Werner, 1954).
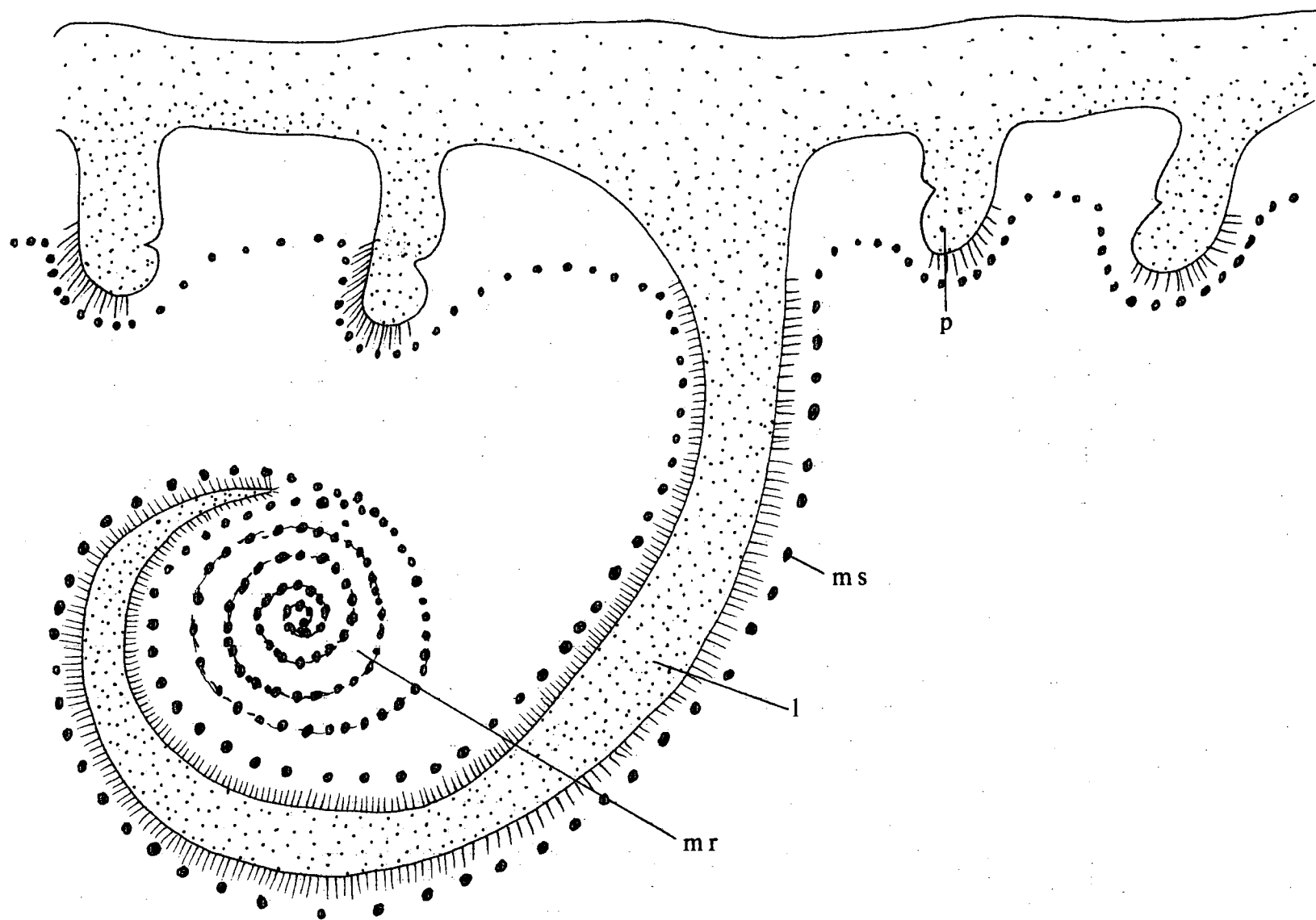
Figure 9.

aid the peripharyngeal ciliated bands in transporting the mucus network in a predominantly dorsal direction towards the dorsal lamina, while farther back in the pharynx the papillae convey the mucus strands more directly towards the opening of the oesophagus (Werner & Werner, 1954).

In *Perophora* (Fig. 6) similar papillae are branched. In *Ascidiella* longitudinal bars or "vessels" are formed, apparently from fusion of the tips of branched papillae, during organogenesis (Fig. 6C). In *Ascidia* these longitudinal vessels (Fig. 6D, Fig. 7) bear secondary papillae. In other genera and families the arrangement of the longitudinal ridges and papillae within the pharynx may be increasingly more complex, but little is known of the functional significance of this complexity. Fenaux (1968) has reported observing waves of muscular contraction in *Phallusia* bringing rows of papillae together and then moving them apart, so aiding in the transport of the mucus film. We have observed similar waves of muscular contraction in the folded walls of *Corella parallelogramma*. We conclude that the papillae, ridges, folds, bars and vessels which adorn the inner face of the pharyngeal basket of so many ascidians serve to aid the transport of the mucus network in some undefined way, perhaps by combining muscular action with ciliary action.

Formation of the mucus rope

Along the mid-dorsal line of the pharynx the mucus network is gradually gathered up into a twisted cord or rope of mucus with adherent food particles. Werner & Werner (1954) have given an excellent account of the formation of this rope and of the role played by the dorsal lamina and the dorsal languettes or lappets (Fig. 8). In its simplest form the dorsal lamina is a long unbroken fold of tissue extending down from the mid-dorsal line of the pharynx. It does not, however, hang straight down, but always bends over to one side, usually to the right. In the majority of ascidians it is partially or completely broken up into a series of lappets or languettes. In *Clavelina* the lappets are not completely separate but are joined by lower parts of the dorsal lamina. In the anterior part of the pharynx the languettes are small, but increase in size towards the posterior end. Each lappet lies directly opposite one of the transverse bars between the rows of gill slits and its ciliation is a direct continuation

Figure 9. (page 19)
A diagrammatic transverse section, at the level of a transverse gill bar, through the dorsal lamina and part of the pharyngeal wall of *Ciona* while feeding. l, lappet of dorsal lamina; mr, mucus rope; ms, mucus strand; p, papilla. (Modified from Werner & Werner, 1954).

of the peripharyngeal ciliated circles which run along these bars. We have seen that these ciliated circles transport the mucus strands from the margins of the endostyle towards the dorsal lamina; the cilia of the languettes continue in the same direction so that the strands from the two sides meet at the tip. During feeding these languettes are curved towards the right side of the animal in such a way that the convex side almost touches the surface of the gill bars. As early as 1884 Roule observed that their concave surface thus forms a sort of canal. In this canal the cilia, meeting on both sides of the rolled lappet, create a rotary movement which twists up the two mucus films like a rolled carpet (Fig. 9). The posterior end of each mucus strand has already been conveyed back to the opening of the oesophagus by the retropharyngeal ciliated band, and the cilia of the oesophagus, beating in a spiral in the same direction (clockwise as observed from the front end), further twist this mucus rope and draw it back into the oesophagus and then into the stomach.

Deep sea ascidians

Some deep sea genera, while possessing all the apparatus required for forming the mucus filter and moving it around, show a great reduction of the branchial basket. Gill slits may be few in number, large in size or devoid of cilia in such forms as *Culeolus*, *Fungulus* or *Hypobythius*. Deep sea species of genera whose shallow water species are perfectly normal in this respect may also show such reduction; *Boltenia elegans*, for instance, shows an almost embryonic condition, with mere protostigmata such as are found in the newly developed zooids of *Boltenia ovifera*. This reduction is carried to its greatest extent in *Pterygascidia*, which no longer possesses true gill slits, merely a single large opening on each side between the pharynx and the atrium.

Such species, which seem to have evolved independently and belong to diverse families, appear to be characteristic of the continental slope, not of the abyss, and are known especially from regions where the slope is dissected into submarine canyons.

Figure 10. (page 22)
Sketch of the deep sea ascidian, *Bolteniopsis*, an inhibitant of the continental slope. Note the hypertrophied buccal siphon, which, it is believed, normally faces upstream into the steady external water current so that food particles are swept into the pharynx whose gill slits are atrophied. Only about one quarter of the length of the stalk is included in this sketch. as, atrial siphon; bs, buccal siphon.
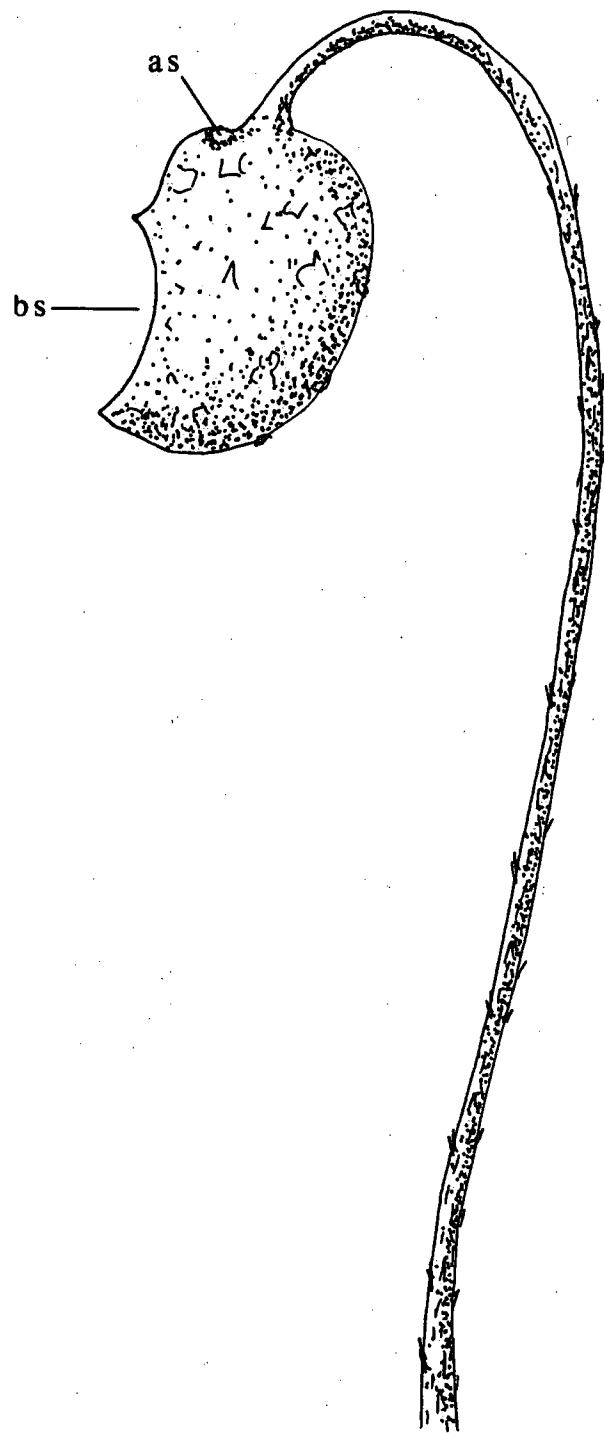
as

bs

Figure 10.

We suggest that they have evolved to take advantage of the steady if slow bottom currents which run along such canyons and that they simply sit facing upstream with their mouths open (Fig. 10). In such a situation they have no need to create their own feeding current but instead rely on an external current; hence the loss of the apparatus for moving water.

The abyssal plain contains an assemblage of ascidian species of a more normal form, though generally of small size, often no more than one or two millimetres. Thus Monniot and Monniot (1968) reported such common genera as *Styela*, *Cnemidocarpa*, and *Polycarpa* in four out of six hauls with the epibenthic dredge at abyssal depths. The species involved were, though tiny, perfectly comparable with shallow water species of the same genera, and presumably fed in the same way. Alongside these species, however, they found highly aberrant species of the genera *Hexacrobylus* and *Gasterascidia*, which they believed to be macrophagous. Indeed, we may conclude that the whole family Hexacrobylidae, to which both genera belong, is macrophagous. The concept of a predatory ascidian stalking its prey is appealing.

In *Gasterascidia* there is neither pharyngeal cavity nor gills. The oesophagus opens directly from the buccal siphon, which has been converted into a protrusible proboscis (Fig. 11). The greater part of the body is occupied by the stomach which opens from the thin-walled, non-muscular oesophagus. Monniot and Monniot are of the opinion that the oesophagus serves to secrete digestive enzymes, and that the stomach is primarily a region where the food is broken down by these enzymes. They conclude that the stomach secretes no enzymes of its own and that the major region of absorption of the products of digestion lies in the dorsal part of this organ.

Gasterascidia appears to capture its prey, consisting apparently of *Foraminifera* and minute crustaceans, by crawling over or just under the surface of the sediment and engulfing them with its proboscis.

Hexacrobylus is slightly larger - sometimes seven or eight millimetres long - and probably does not move about. Like *Gasterascidia*, it totally lacks any pharyngeal apparatus. The buccal siphon is large and muscular, but not elongated into a proboscis. Nevertheless, *Hexacrobylus* appears to be able to ingest relatively large prey, presumably trapped by lying in wait, like a sea anemone.
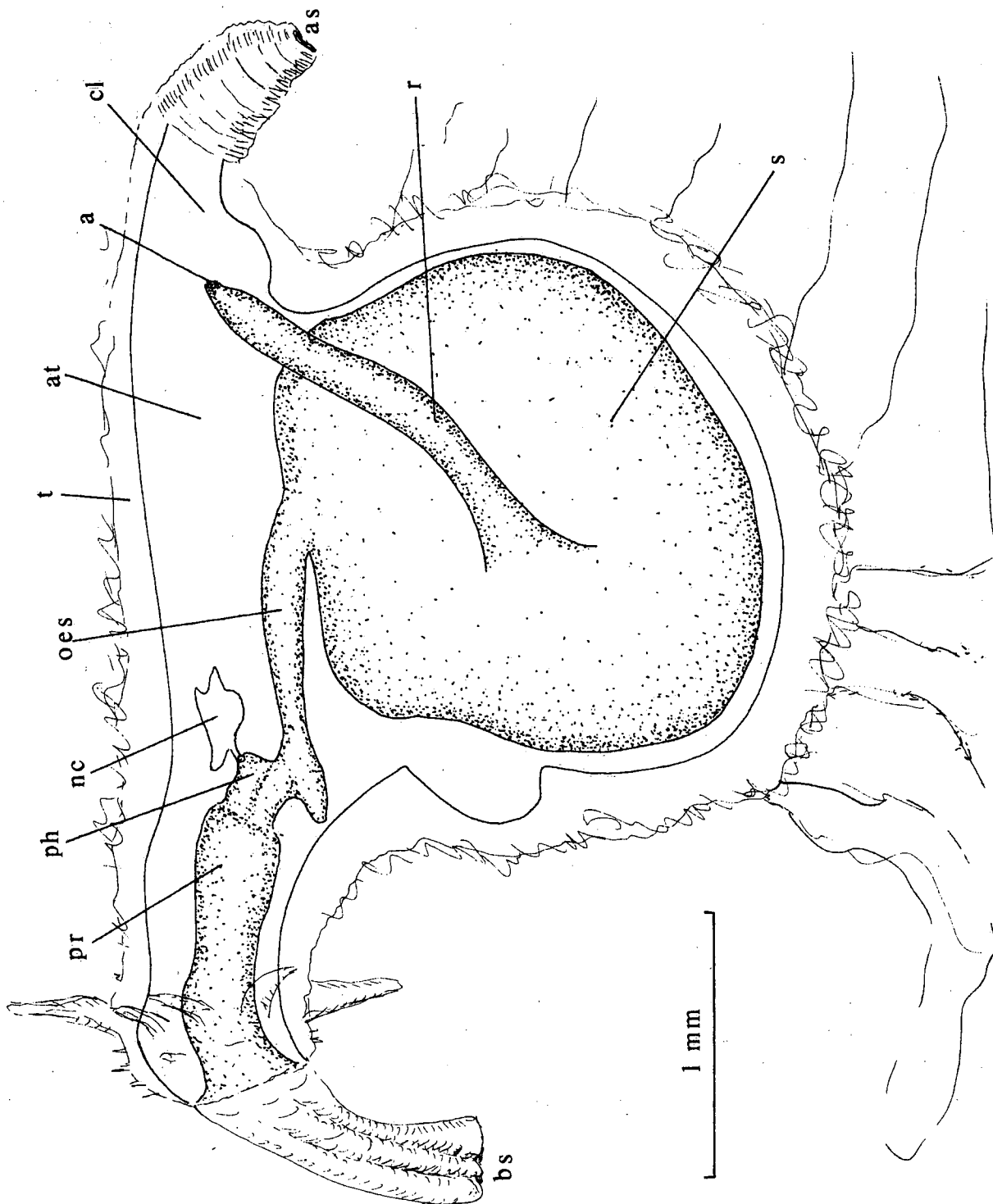
Figure 11.
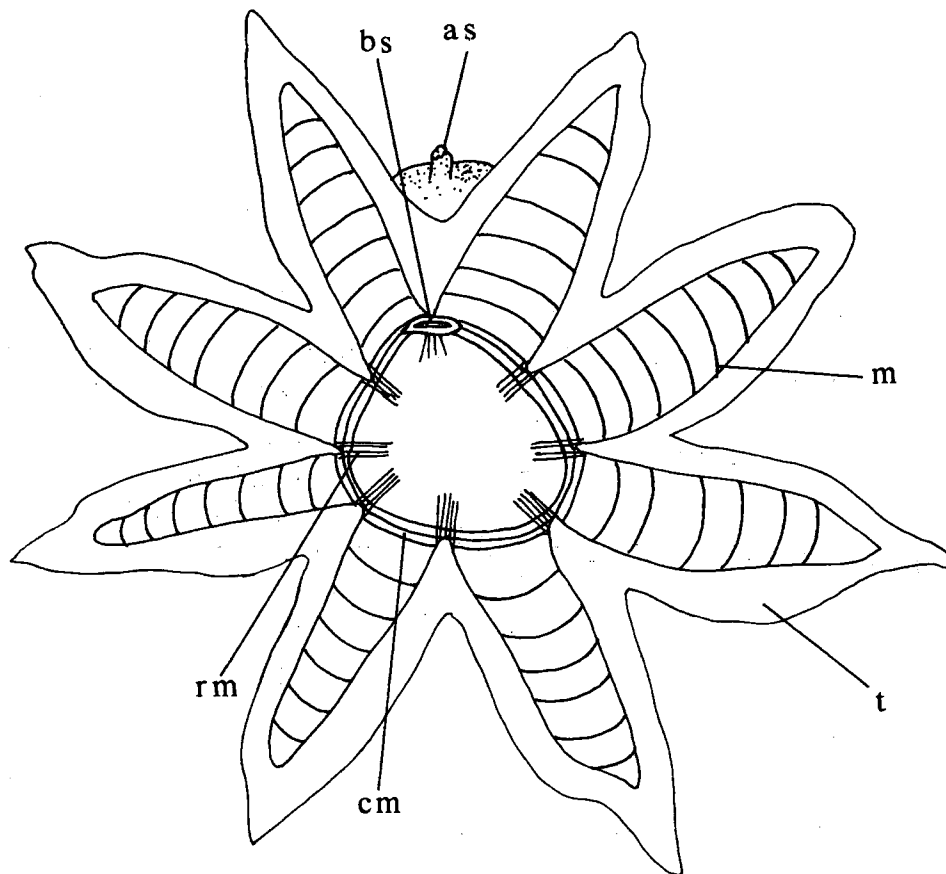
Figure 12. (above)

Sketch of the oral or upper surface of *Octacnemus*, an aberrant deep sea ascidian. The animal is flattened and the tunic and mantle drawn out into eight "petals". as, atrial siphon; bs, buccal siphon; cm, circular muscle; m, muscular mantle; rm, radial muscle; t, tunic or test.

Figure 11. (page 24)

Gasterascidia sandersoni: a sketch of a dissection from the left side of the animal. a, anus; as, atrial siphon; at, atrium; bs, buccal siphon; cl, cloaca; nc, neural complex; oes, oesophagus; ph, pharyngeal rudiment; pr, eversible proboscis formed from the muscular walls of the buccal cavity; r, rectum; s, stomach; t, tunic.

(A composite compiled from the drawings and descriptions of Monniot & Monniot, 1968).

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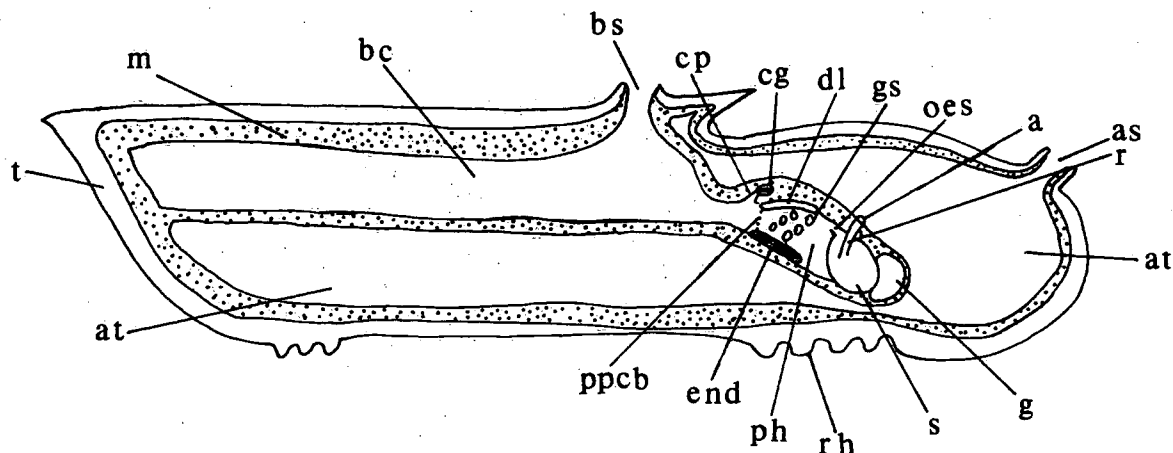
Figure 13. (above)
Diagrammatic longitudinal section through the body of *Octacnemus*. a, anus; as, atrial
siphon; at, atrium; bc, buccal cavity; bs, buccal siphon; ct, cerebral ganglion; cp,
ciliated pit; dl, dorsal lamina; end, endostyle; g, gonad; gs, gill slit; m, mantle;
oes, oesophagus; ph, pharynx; ppcb, peripheryngeal ciliated band; r, rectum; rh, rhizoid
attachment process of tunic; s, stomach; t, tunic.

More enigmatic is *Octacnemus*, another abyssal genus which is often stated to be
macrophagous. In this genus the whole pharyngeal apparatus is present, though in a
much reduced form (Fig. 12). The animal is flattened, with the buccal siphon offset
a little from the centre of the upper side. The disc formed by this flattening is
drawn out into eight petal-like processes which are highly muscular. The buccal siphon
opens into an enlarged buccal cavity (Fig. 13), which occupies the whole upper part
of the animal, extending into the "petals" and separated from the atrial cavity by a
horizontal membrane. The pharynx, gut, gonads and other organs of the body, which all
have a recognizably ascidian form, are located near the centre of the disc. The pharynx
possesses six miniscule gill slits on each side, arranged in two diagonal rows and
opening into the extensive atrium, which, like the buccal cavity, extends into the
petals. The under surface, both of the central disc and of the eight petals, is fur-
nished with rhizoidal processes for the attachment to the substrate. One may suppose
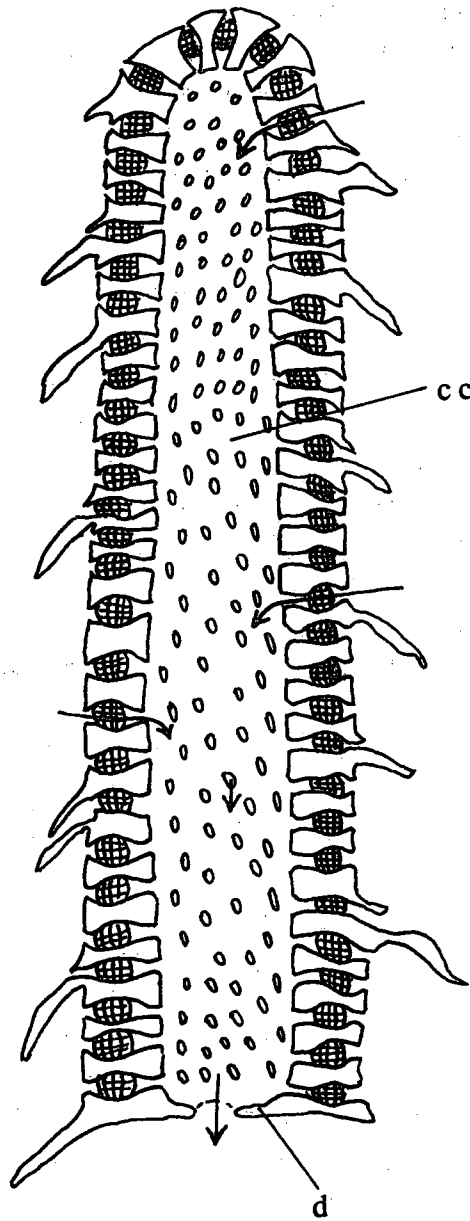
Figure 14, (above)

A longitudinal half section of a colony of *Pyrosoma* showing the zooids embedded in the thick test. Arrows indicate the flow of water through the buccal siphon of a zooid and out through the atrial siphon into the common cloacal cavity. cc, common cloacal cavity; d, diaphragm.

that the "petals" in some way serve to catch the prey and to stuff it into the buccal siphon. Specimens have been taken with copepods in the buccal cavity, but we are at a loss to suggest any details of the mechanism by which food is caught or to speculate in any way upon the role played by the obviously functional endostyle, dorsal lamina and the rest of the pharyngeal apparatus. Indeed, macrophagy in *Octacnemus*, remains mere supposition, for the copepods could be commensals, or their presence could be artifacts of collection or of preservation. Macrophagy cannot be substantiated, though it does seem more plausible than any other mode of feeding that we can imagine for this strange animal.

Pyrosomatida

The Pyrosomatida are free-swimming colonial tunicates in which the colony is shaped like a hollow cigar (Fig. 14). The individual zooids are located in the thickness of the wall of this cylinder, which opens at one end only. The buccal siphons are on the outside of the cylinder and the atrial siphons open into the central cavity of the colony. Thus water taken in through the buccal siphon is passed through the atrial siphon into the central cavity of the colony and ejected through the single opening which thus defines the posterior end of the colony. The combined currents of all the zooids making up the colony may transport quite a large volume of water, so that the colony may move comparatively rapidly. The swimming ability of the colony as a whole is increased by a constriction in the posterior part which narrows down the opening and so creates something of a jet effect.

The structure of the individual zooids is rather similar to that of ascidians, except that the siphons are at opposite ends of the body, instead of side by side at the same end (Fig. 15). The greater part of the zooid is taken up by the enormous pharyngeal cavity whose lateral sides are perforated by the gill slits. Whereas in ascidians the gill slits are normally arranged parallel to the endostyle (except in those forms where a spiral arrangement is adopted), in the Pyrosomatida the gill slits are arranged perpendicularly to this organ. On each side lies a peribranchial cavity into which the gill slits open. These cavities on the two sides are separated from

Figure 15. (opposite)
A zooid of *Pyrosoma atlanticum* seen from the left side. as, atrial siphon; at, atrium; bs, buccal siphon; cl, cloaca; end, endostyle; gs, gill slit; l, lappet of dorsal lamina; lo, lumous organ; m, mantle, nc, neural complex; oes, oesophagus; ph, pharynx; ppcb, peripharyngeal ciliated band; s, stomach; t, tunic.
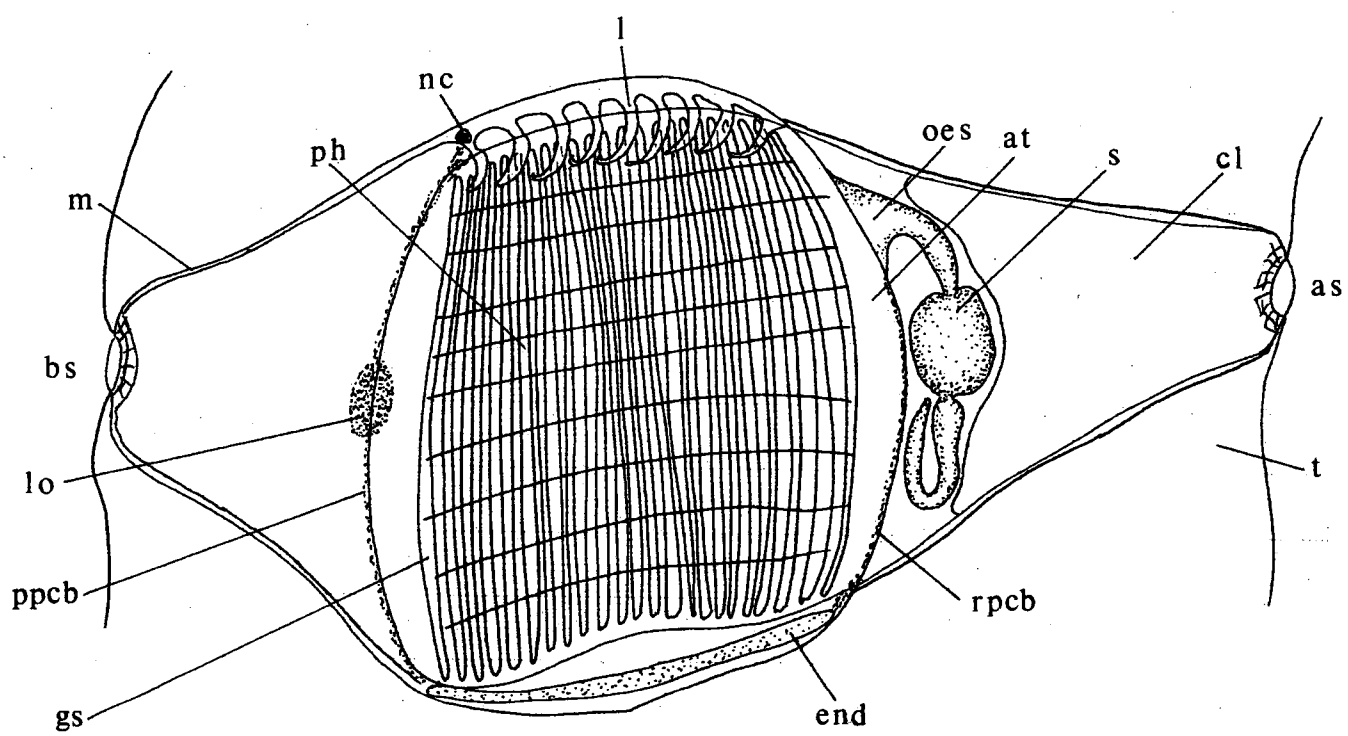
Figure 15.

one another and only join posteriorly behind the stomach to form the rather small atrium, which in turn opens through the atrial siphon into the central cavity of the colony.

The oseophagus opens from the pharynx at the dorsoposterior corner, and the alimentary canal is located in the median plane between the peripharyngeal cavities and in front of the atrium.

In transverse section the endostyle of Pyrosomatida is very similar to that of *Ciona*, being deep and narrow, with long flagella on the median cells. Fenaux (1968) has, however, shown that the lateral ciliated bands are broken up into discrete patches of ciliated cells. The proximal part of the side walls of the endostyle is made up of a fine double membrane ornamented on its lower surface by little groups of ciliated cells regularly spaced out. The distal part is thickened by a continuous cellular rib. According to Fenaux this complex occupies a fixed position and its orientation does not vary during the period of activity. The mucus film is spread in close contact with the distal cellular rib Fenaux noted different modalities of feeding which seemed to be associated with variable rhythms, with the speed of the inhalent current and with the intensity of secretion of mucus by the endostyle. These modalities were comparable with those observed earlier by Fedele (1921, 1933), in *Doliolum*, and by Carlisle (1950), in *Salpa*. As in ascidians, the strands of mucus are attached to the peripharyngeal ciliated bands at their front end, and passed around the pharynx to be picked up by the lappets of the dorsal lamina. The posterior end of the strand is passed back into the mouth of the oesophagus by the retropharyngeal ciliated band. Fenaux has noted that the mucus network is very often not in contact with the sides of the pharynx through-out its whole length. It touches the gill bars along a variable part of the anterior pharynx, while a significant distal portion takes on the form of a funnel, as first described by Fedele (1928) and by Carlisle (1950) in other Thaliacea.

The mucus rope may be formed by two different methods in *Pyrosoma*. The first is exactly like the process described for ascidians, with the formation of a single twisted rope by the lappets of the dorsal lamina. In the second mechanism the mucus strands from the two sides of the pharynx are twisted up separately to make two separate strands; back in the posterior part of the pharynx these are twisted together into a single rope. During a period of a few minutes one may see both mechanisms at work, one after the other. Fenaux suggested that in the second mechanism the twisting of the two cords into one single rope is due to the action of the cilia of the oesophagus.

The buccal siphon is lined with a glandular epithelium which, as in ascidians, produces mucus filaments which may trap certain particles in the inhalant current. Under some circumstances this secretion is particularly abundant, and it is possible to observe larger particles trapped by this anterior mucus filter before the water enters
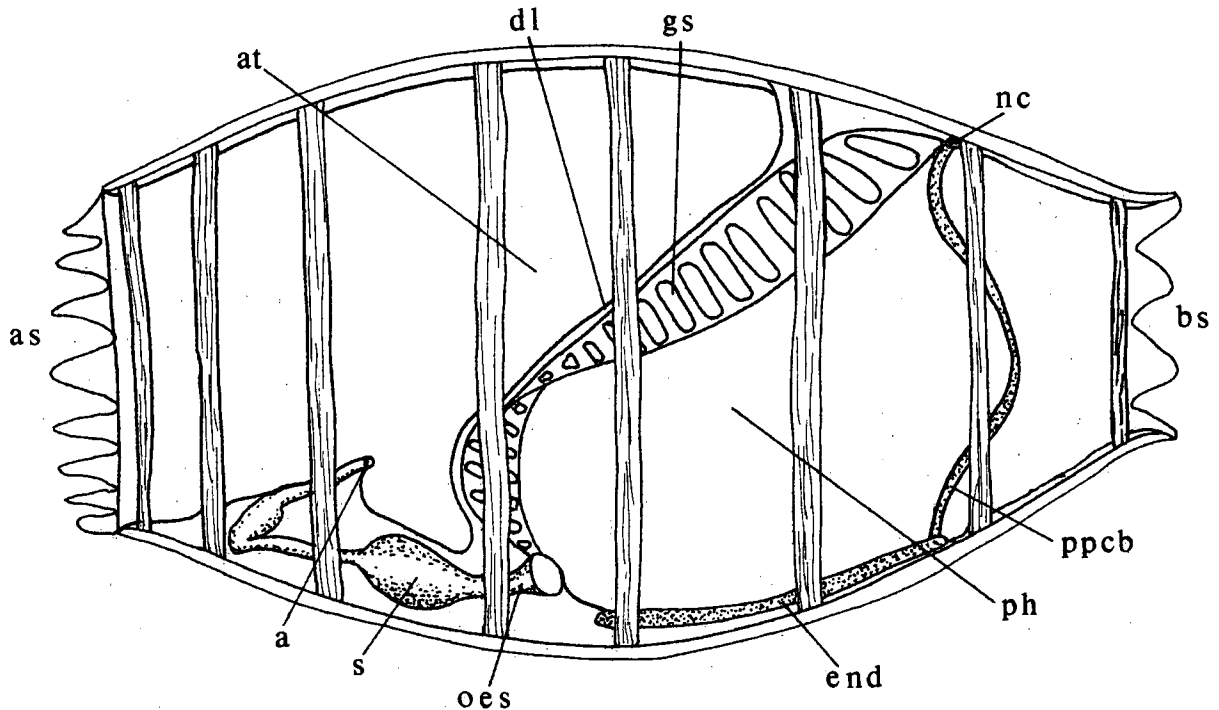
Figure 16. (above)

A zooid of *Doliolum* from the right side. a, anus; as, atrial siphon; at, atrial cavity; bs, buccal siphon; dl, dorsal lamina; end, endostyle; gs, gill slit; nc, neural complex; oes, oesophagus; ph, pharyngeal cavity; ppcb paripharyngeal ciliated band; s, stomach.

into the pharynx proper. The particles trapped in this mucus net are never ingested, but instead *Pyrosoma* reverses the water current from time to time (according to Fenaux) and blows out the whole of this anterior mucus network and attached particles through the buccal siphon. Fenaux gives no indication of the mechanism of this current reversal, but our own observations indicate that it is analogous to the squirting of ascidians, that is to say, that it is produced by a sudden contraction of the muscles on the mantle wall. This is not of course a true reversal of current, but merely causes the water in the pharynx to be expelled through the buccal siphon, while that in the peripharyngeal cavities and atrium is expelled through the atrial siphon. In any case, the larger particles, trapped in the mucus of the buccal siphon, are expelled as a form of pseudo-faeces.

Doliolida

The zooid of *Doliolum* takes the form of a small barrel, in which the hoops are represented by the circular bands of muscle, eight in number. The wide buccal siphon (Fig. 16) opens into the anterior pharyngeal cavity which is separated from the atrial cavity by an obliquely transverse membrane pierced by the single row of gill slits on each side. The oesophagus opens from the ventral side of this membrane in the median line and the gut extends back, lying below the atrium. The endostyle lies along the midventral line of the front half of the body, generally starting about the second muscle band and extending to about the fourth or fifth, according to the species. The peripharyngeal ciliated bands run from the anterior end of the endostyle around the two sides of the pharynx to meet dorsally in the form of a spiral node (Fig. 17). From this node the much reduced dorsal lamina, consisting of little more than a ciliated band, runs down the mid-line of the transverse membrane to the opening of the oesophagus. The role of the lappets of the dorsal lamina of ascidians in twisting the mucus network into a rope is taken over by the spiral node, where the peripharyngeal ciliated bands meet.

The feeding mechanisms in *Doliolum* were first described by Fol (1872) and then more completely by Fedele (1923) and by Neumann (1935). More recently Fenaux (1968) has pointed out that *Doliolum*, like *Pyrosoma*, has alternative feeding mechanisms comparable to those first described in *Salpa* by Carlisle (1950).

The current which brings the water and the suspended particles in through the buccal siphon is created entirely by the cilia of the gill slits, as in ascidians; it is completely independent of the contraction of the muscular bands, quite unlike what happens in salps. The contraction of the muscle bands, unlike the contraction of the muscles of the mantle of ascidians, serves two quite distinct functions. It may serve to expel faeces from the atrium and pseudofaeces from the pharynx (as in ascidians), or the contraction may serve as an escape reaction, violently propelling the barrel along. If *Doliolum* is disturbed when swimming in an aquarium tank we have observed that it will contract the musculature of whichever siphon is farthest from the disturbance, and then the rest of the muscle bands of the body. In this way, only the siphon towards the disturbance is open, so that contraction of the muscle bands results in a jet of water projecting the zooid away from the source.
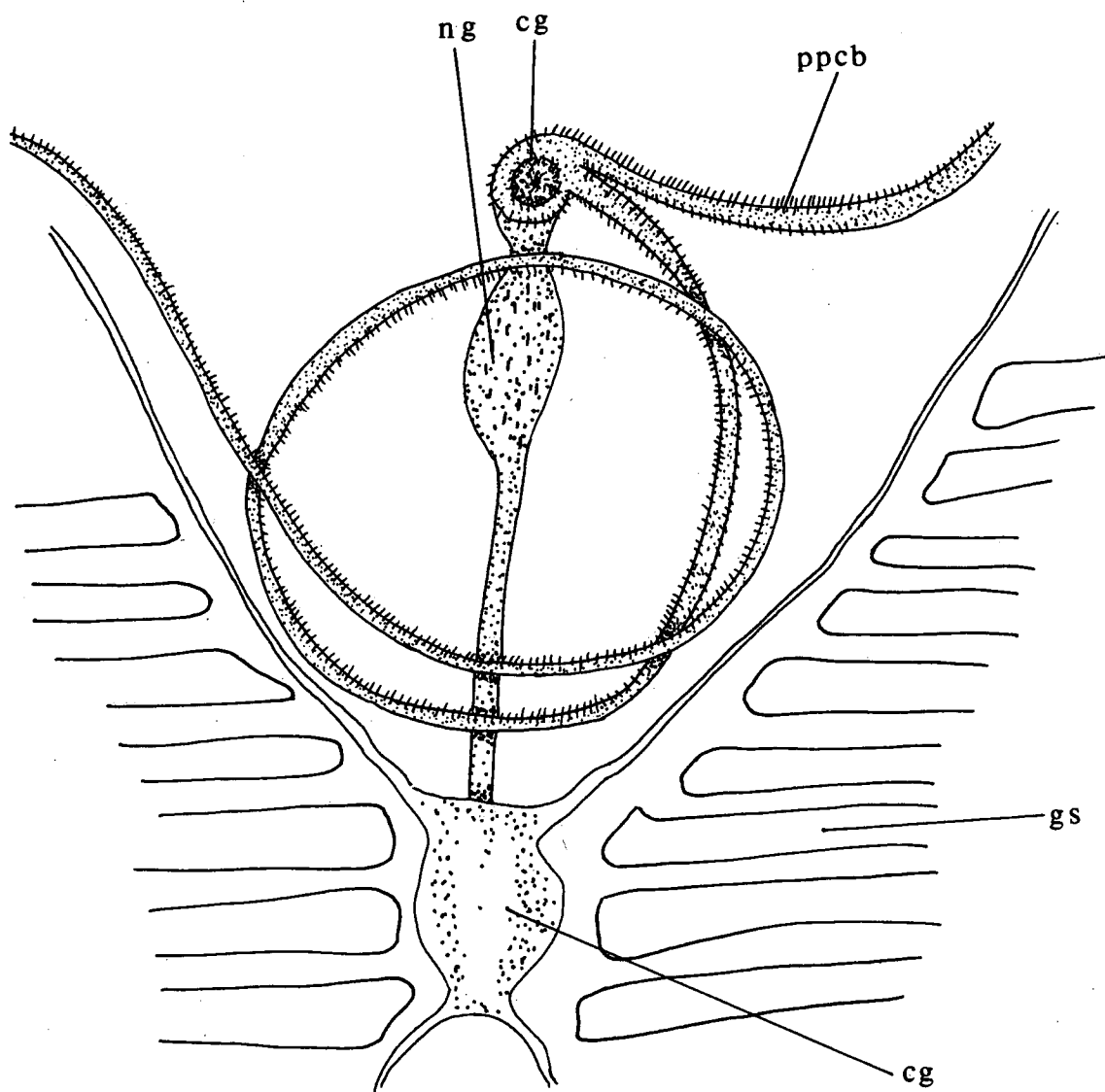
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Figure 17. (above)

The spiral node of *Doliolum* viewed from inside the pharynx. cf, ciliated funnel; cg, cerebral ganglion; gs, gill slit; ng, neural gland; ppcb, peripharyngeal ciliated band.

The front end of the mucus strands secreted by the endostyle are attached to the peripharyngeal ciliated bands and carried dorsally towards the spiral node. When the water current through the pharynx is fast (relatively speaking) the posterior end of the mucus strands is carried back by the retropharyngeal ciliated band to the opening of the oesophagus. In this way a continuous curtain of mucus strands is formed attached at the front end to the peripharyngeal bands and running back over the surface of the branchial basket to terminate in the oesophagus. As the anterior ends of the strands reach the spiral node they become twisted up into a rope which is further twisted and pulled back by the spirally beating cilia of the oesophagus. Under conditions of slow feeding, when the mucus production and the water currents are both at low levels, the strands of mucus are attached only by their anterior ends to the peripharyngeal ciliated bands, and are unattached posteriorly. In this way they form a fringe surrounding the pharynx. When they arrive at the spiral node these strands become twisted into a rope which is then passed backwards down the dorsal ciliated band (the remnant of the dorsal lamina) and pulled into the oesophagus by the oesophageal cilia. In whatever manner it is formed, the final mucus rope ultimately enters the stomach.

The same two methods of feeding are found in blastozooids and also young oozooids, phorozooids and gonozooids. The old oozooids, also termed "old nurses", have lost all trace of the internal organs and are incapable of feeding themselves. Except for a rather imprecise drawing by Fedele (1923) there have been no observations of the feeding of gastrozooids, but our own observations have indicated no significant departures from the mechanics found in blastozooids which is outlined above.

Salpida

Salps are amongst the biggest of the planktonic animals, and individual zooids may be as much as 20 cm long. The body is approximately cylindrical or prismatic and is surrounded by a complex arrangement of muscle bands which may completely surround the body or may be interrupted ventrally. The greater part of the body is occupied by one single large cavity which is barely divided into separate pharyngeal and atrial

Figure 18. (opposite)
Sketch of a salp, *Thalia democratica*, from its left side. This is a drawing of a preserved specimen in which the region of the ganglion is contracted, thus pulling the branchial bar out of its normal position, almost parallel to the endostyle. at, atrial cavity; bb, branchial bar; bs, buccal siphon; cg, cerebral ganglion; cm, circular muscle; end, endostyle; n, nucleus or visceral mass, consisting of gut, heart, reproductive organs, etc; ph, pharyngeal cavity; ppcb, peripharyngeal ciliated band; t. tunic or test.
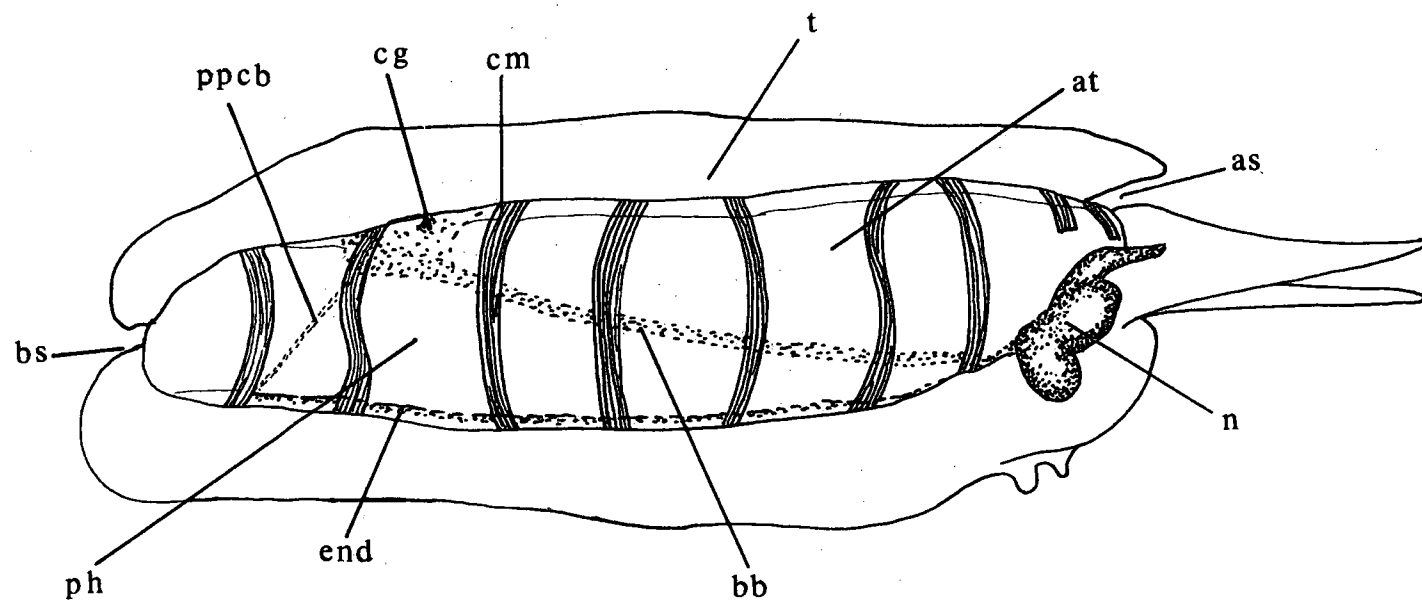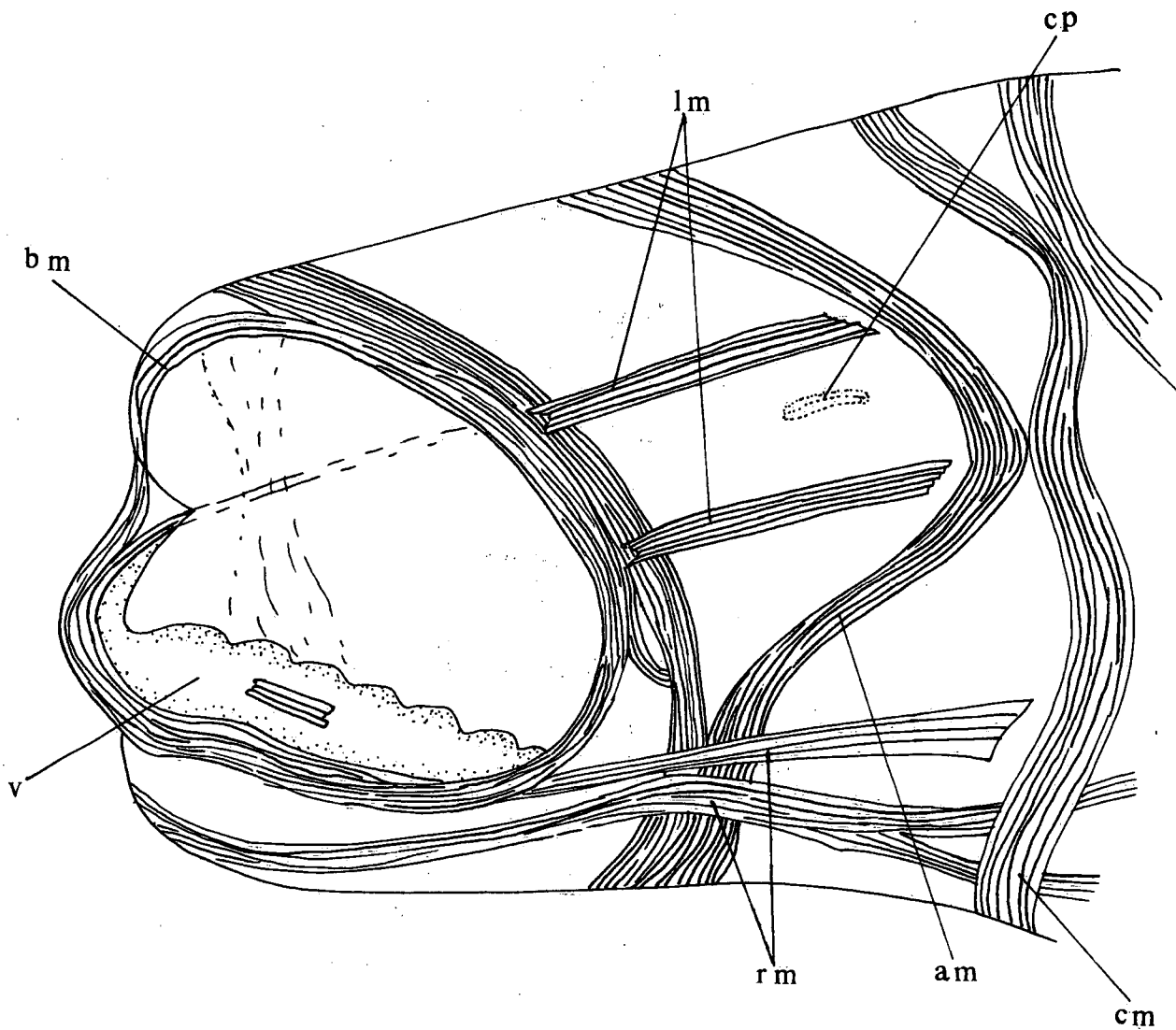
Figure 18.

Figure 19. (above)

Buccal siphon of a salp viewed from the left and somewhat from the front. am, arched
muscle; bm, buccal muscle; cm, first circular muscle; cp, ciliated pit; lm,
longitudinal muscle; rm, retractor muscle; v. velum.

cavities by the branchial bar which runs down the sagittal plane of the animal (Fig. 18).
The pharyngeal cavity lies in front of and below this bar, which runs from a mid-dorsal point
just inside the mouth backwards and downwards to the mid-ventral posterior region. The
atrial cavity lies above and behind the branchial bar, and the stomach and gut lie at
its hind end in the ventral region.

The animal swims by means of rhythmical contractions of the circular
muscles, which produce a dorso-ventral flattening of the body and a consequent expulsion
of water through one or both of the siphons. Relaxation of the muscles allows the
elasticity of the tunic to expand the body and so to draw water in again. The buccal
and atrial siphons are themselves provided with muscles, which appear to be serially
homologous with the circular muscles of the body, and which by their constriction serve
to close the siphons partially or completely (Fig. 19). During contraction of the body
muscles those of the buccal siphon normally contract also, while those of the atrial
siphon relax. Water is thus expelled primarily through the latter. During relaxation

Figure 20.
Diagram of the movement of water and of mucus in *Salpa maxima* during feeding. All the
drawings represent living animals viewed from the right side. The branchial bar is
shown in its normal position in life, almost parallel to the endostyle, instead of in
the diagonal position which it assumes in preserved specimens after the region of the
cerebral ganglion and ciliated pit have contracted in death.

A (page 38), the movements of mucus during slow feeding;

B (page 39), the movements of water during slow feeding. The water is
moved by muscular action and the siphons function as imperfect valves, allowing a great
deal of water to pass in the "wrong" direction, as indicated by the two short arrows
at each siphon. Water is moved to and fro at each cycle of contraction. The numbers
represent the position of a particle which has evaded capture during the course of eight
successive cycles of muscular contraction. The Arabic numerals represent its position
at the finish of each expansion phase and the Roman numerals its position at the end
of each succeeding contraction.

C (page 40), diagram of rapid feeding. The valves are more efficient
and the water passes through the body in the course of a single cycle, as, atrial siphon;
at, atrial cavity; bb, branchial bar; bs, buccal siphon; cg, cerebral ganglion; cp,
ciliated pit; ph, pharynx; ppcb, peripharyngeal ciliated band; r, rectum; rpcb, retro-
pharyngeal ciliated band; s, stomach (after Carlisle, 1950).
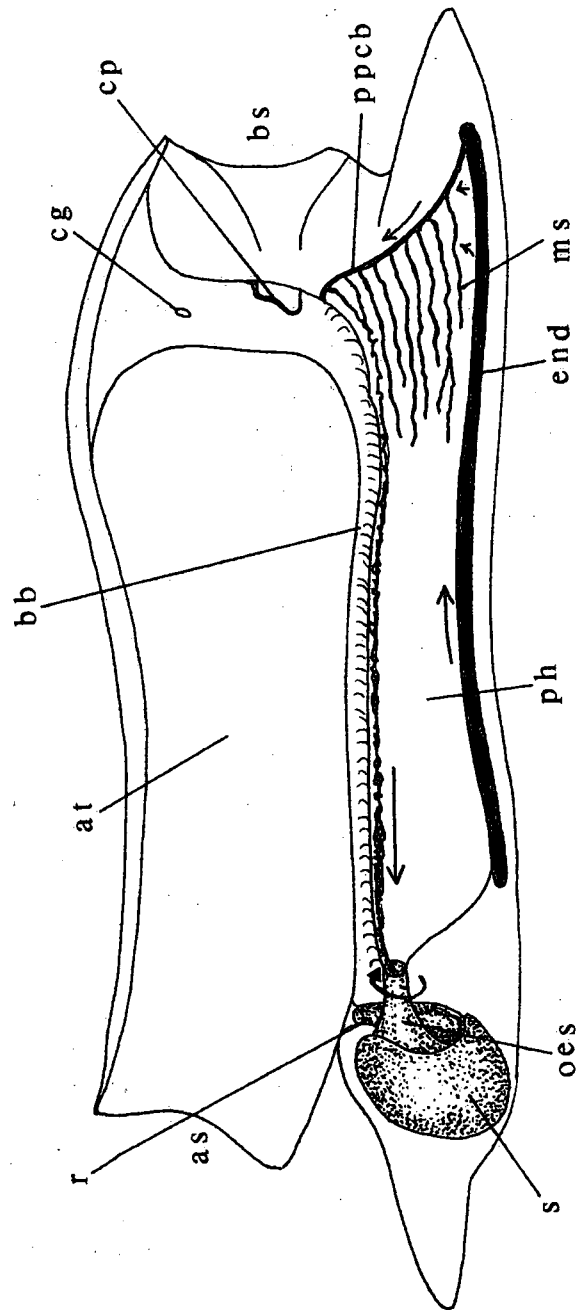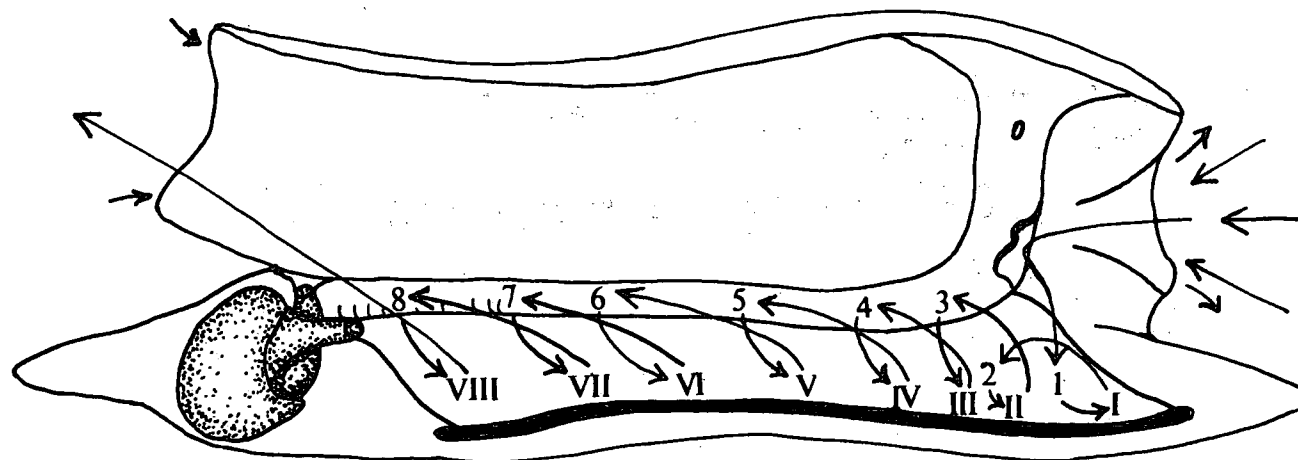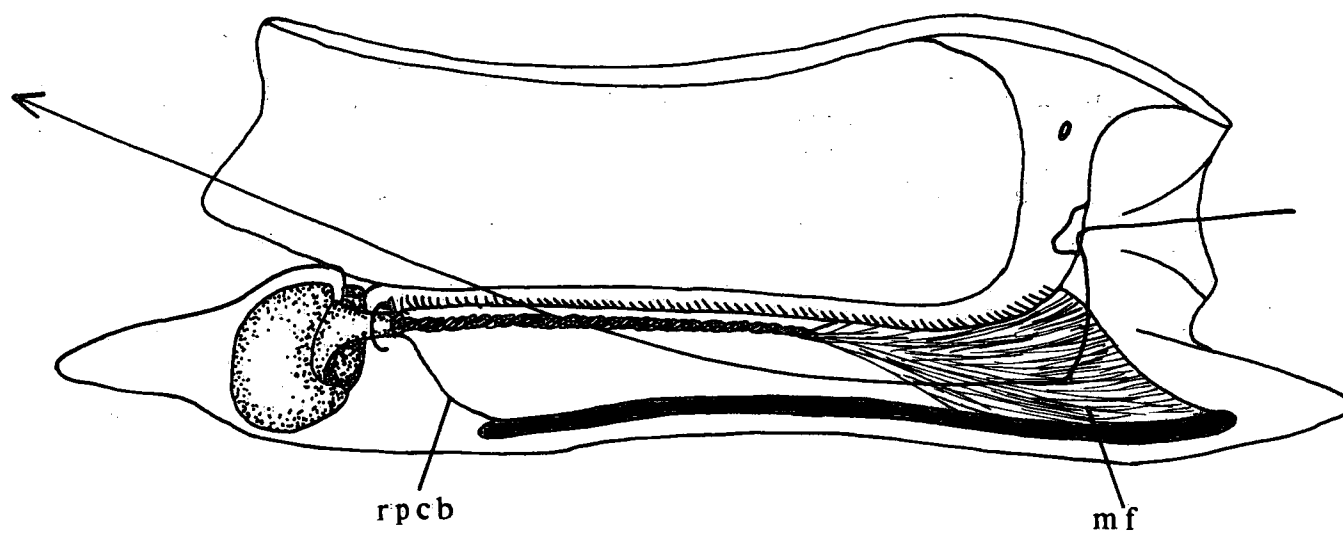
Figure 20A.

Figure 20B

Figure 20C.

of the body muscles, those of the buccal siphon also relax, while those of the atrial siphon contract, thus closing the latter and ensuring that fresh water is drawn in through the buccal siphon. In this way a current of water is drawn through the animal and serves both for propulsion and to bring in a supply of food particles for filtration.

The first account of feeding in salps was that of Fedele (1933), while Carlisle (1950) was the first to describe the two modes of feeding, fast and slow, which seem to be characteristic not only of salps but also of other tunicates. The description which follows is based upon that of Carlisle (1950) with additional observations incorporated.

Slow feeding, which is characteristic both of well-fed animals and of salps in an environment low in filterable particles, is accomplished by a slow progression through the water. The contraction of the circular muscles is imcomplete and the rhythm is rather rapid. Only a small part of the water contained in the pharyngeo-atrial cavity is expelled at each contraction (Fig. 20B) and the muscles of the siphons do not close completely. In consequence of this imperfection in the valve action of the siphons water enters by both, though more abundantly by the buccal siphon, and is likewise expelled through both. The small amplitude of contraction and the imperfection of the valves make for slow swimming on the part of the animal and lead to a to-and-fro circulation of the water in the pharyngeal and atrial cavities (Fib. 20B).

These cavities are completely continuous with one another, except in the mid-line; in effect there is one enormous gill slit on each side. In the pharynx, except at the extreme posterior end, during contraction the water moves forward towards the buccal siphon. In the atrial cavity and the posterior end of the pharyngeal cavity, contraction of the muscles causes the water to move backwards towards the atrial siphon. During expansion, water moves more freely in the pharyngeal cavity, while during contraction there is greater water movement in the atrial cavity, since more water enters through the buccal and more goes out through the atrial siphon.

During slow feeding any particle which enters through the buccal siphon performs a sort of "hesitation waltz", to use the colourful expression of Fenaux (1968), moving first two steps back and then one step forward. As muscles relax the dancing particle is swept in through the buccal siphon and hurled against the ciliated pit (Fib. 21) which faces it. Some particles get no farther and are ultimately phagocytosed there. Most of them, however, turn downwards with the incoming current and enter the first part of the pharynx. The succeeding contraction expels some water; that in the pharynx moves forwards and somewhat downwards, carrying the particle with it. A second expansion moves it dorsally and backwards just a little farther. It continues in this way (if it is not trapped by the strands of the mucus filter), so that at the end of each complete
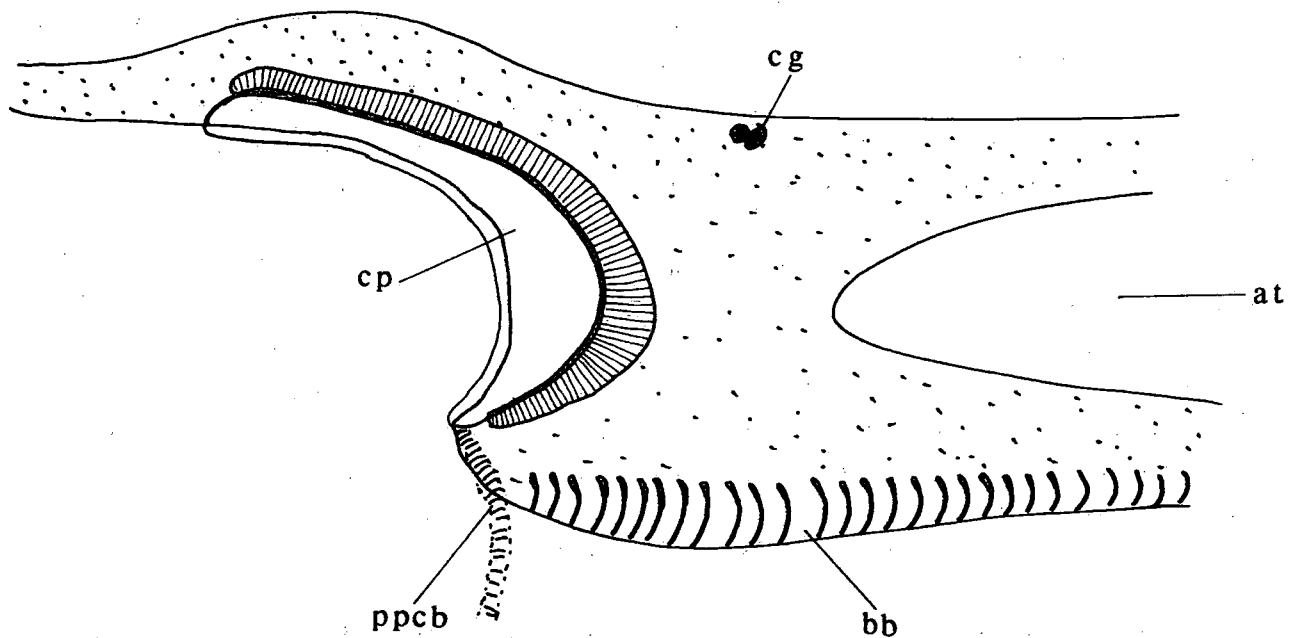
Figure 21. (above)

Sketch of the ciliated pit of *Salpa* viewed from the left side in a preserved specimen.
at, atrium; bb, branchial bar; cg. cerebral ganglion; cp, ciliated pit, ppcb, peri-
pharyngeal ciliated band.

cycle it is a little farther back than at the end of the preceding. Finally it enters the region where water runs towards the atrial siphon during contraction, and is thus expelled. This does not usually happen until it has reached the posterior end of the pharynx; just occasionally does a particle pass into the atrial cavity from farther forward and is expelled at an earlier stage. The process from entry to expulsion may take from three to twenty cycles of contraction, but usually from six to ten suffice. In actual fact very few particles escape and most are trapped by the mucus filter. We have estimated the clearance rate during slow feeding to be better than 99 percent; less than one particle in hundred is expelled.

It is to be noted that the water is moved entirely by muscular action. Cilia serve only to move mucus. The pharynx has come to consist of little more than the supporting structures for the ciliated bands, and the network of gill slits, so characteristic of ascidians, has been lost, since it is no longer needed for moving water. As in other tunicates, the endostyle provides the mucus which is secreted in discrete ribbons along the length of this organ. After it is formed (Fig. 20A) the ribbon is moved forward by the cilia of the endostyle. In the anterior third, cilia push the ribbons out to the side, and for a time they remain attached by both extremities. Finally the hind end is released and a pennant of mucus is formed which is attached only by its front end. This now lies in the region where the marginal ciliated bands of the endostyle run into the two peripharyngeal ciliated bands. The front end of the streamer of mucus is carried slowly up one of these bands to the point where the two bands join again and run into the ciliated band of the dorsal lamina. Here the ciliated crest of the branchial bar collects the pennants of mucus and passes them back towards the oesophagus. Part of the motive force for this movement comes from the cilia of the dorsal lamina, while part is furnished by those of the oesophagus which beat spirally and so spin the mucus into a rope and drag it backwards into the stomach. Since the water in the pharynx flows to-and-fro, the pennants or streamers of mucus are wafted back and forth, and sometimes lie behind and sometimes in front of the band. We may surmise that this increases the chances for a food particle to hit and be trapped by one of these mucus strands.

At first glance rapid feeding looks quite different. The differences are, however, only of degree, not of kind, and there is a whole gamut of gradation between the two forms of feeding. No new mechanism comes into action. The current of water is produced just as in slow feeding, but more efficiently. The circular muscles contract much more completely, so that the dorsal lamina and the endostyle are brought almost into contact, and the greater part of the water is expelled at each stroke. The rhythm of contraction is consequently slower, since each contraction is more complete. The muscles which regulate the siphons (which are serially homologous with the circular muscles of the body) also contract more strongly, completely closing the siphons so that the valve

action is now much more efficient. Accordingly the ingested particles no longer move to-and-fro, since there is no longer any water running in the "wrong" direction through the siphons (Fig. 20C). Water enters through the buccal siphon during expansion, and pauses for a moment in the anterior part of the pharynx. Then, when the animal contracts, the water is expelled violently through the atrial siphon.

The secretion of mucus by the endostyle is also more rapid. The mucus is carried forward along the endostyle, and in the anterior third (just as in slow feeding) it is pushed off sideways in ribbons. In slow feeding these ribbons remain attached for some time at both extremities before being freed at the hind end. In fast feeding there is no time for the hind end to be freed before the ribbon is collected by the branchial bar, which, during contraction, almost touches the endostyle at every stroke. In this way, the hind ends of the streamers are collected together while their front ends are carried around the peripharyngeal ciliated bands. A funnel of mucus is thus formed, which, like a plankton net, strains out the particles from the water which passes through it. The cilia which beat spirally around the oesophagus (clockwise when regarded from the front) twist the hind end of this funnel into a rope and draw it back into the stomach. Neither the dorsal lamina nor the retropharyngeal ciliated band takes any part in the passage of the mucus rope into the oseophagus.

It is evident that the ciliary mechanisms which participate in the transport of mucus are identical in fast and slow feeding. Only the time-relations are different. The muscular mechanisms adopted for driving water through the body likewise differ only in the degree of speed and contraction, both in the body muscles and in the siphonal muscles. In all, three differences can be recorded between the two modes of feeding:

1.   the speed of beating of the cilia;
2.   the speed of secretion of the mucus;
3.   the speed and degree of contraction of the circular muscles.
These are all differences of degree, not of kind.

Observations of the mucus rope show that roughly the same proportion consists of food particles, whatever the speed of feeding. The ratio of food to mucus remains constant, in other words. This is true whether the particles are genuine food particles, such as algal cells, or non-nutritive particles, such as graphite. It thus seems likely that the quantity of particles in suspension is the chief stimulus to altering the rate of mucus production and the form of feeding; the chemical nature of the particles seems immaterial. The state of hunger of the animal, however, has an effect. A salp which has fed abundantly will produce copious faeces, sometimes defaecating every five or ten minutes. Such an animal can rarely be induced to commence fast feeding whatever the stimulus to which it may be exposed. A constant response, however, can be obtained

from an animal which has been starved for 24 hours, and in which the rate of production of faecal pellets has dropped to less than one in two hours. Such an animal will always begin fast feeding when exposed to a high concentration of particles. The stimulus appears to be entirely mechanical, for not only will a cloud of inert particles provoke the response, but a filtered homogenate of crab flesh or of seaweed will not, even though the unfiltered homogenate will do so.

Carlisle (1950) suggested that the ciliated pit (Fig. 21) might be involved in sensing the concentration of particles in the incoming current. A fraction of the particles can be seen to accumulate in this pit, at which point the incurrent is diverted abruptly downwards. Histological examination of this region in animals which has been fed with graphite particles showed that amoebocytes from the blood eventually phagocytose some at least of these particles. Experimental evidence to support Carlisle's suggestion is, however, lacking.

Larvacea

The Larvacea are small planktonic tunicates which retain a chordate tail through-out life. In appearance they somewhat resemble the tadpole larva of ascidians, hence the name Larvacea. Most of them are minute, with a trunk no longer than one millimetre and a tail three to seven times the length of the trunk. A few are larger; *Oikopleura vanhoeffeni* (Fig. 22), for instance, has a trunk up to eight millimetres long. The pharyngeal apparatus resembles that of other tunicates. Except in *Kowalevskaia*, there is a short endostyle which consists of a very few cells (Fig. 23). From its front end a pair of peripharyngeal bands reach around the pharynx to meet dorsally. Their cilia, however, are modified and fused into undulating membranes. There is no dorsal lamina (Fig. 24) and the mucus rope seems to be formed by the peripharyngeal ciliated bands. The gill slits are reduced to a single pair, each of which has two rows of cilia to drive water out into the atria. The atria retain the paired condition found in larval ascidians; there is in fact a separate short atrium on each side instead of the single all-eveloping atrium of adult Ascidiacea (Fig. 25). The buccal siphon, or mouth, lacks muscles and is rigid and permanently open. In sum, the pharyngeal apparatus of Larvacea functions in the same general way as in other tunicates: the cilia of the gill slits draw water through the mouth; the endostyle secretes the mucus strands which are formed into a food trap by the peripharyngeal bands and twisted into a rope by these bands aided by the cilia of the oesophagus (Fig. 26). The overall feeding process of Larvacea differs, however, in the use of external filters and methods of concentrating the food before it enters the mouth; once in the mouth the mechanism is essentially familiar.

The main organ for creating the external current is the tail, which is broad and long, and attached to the ventral side of the trunk between the rectum and gonads.

Figure 22.
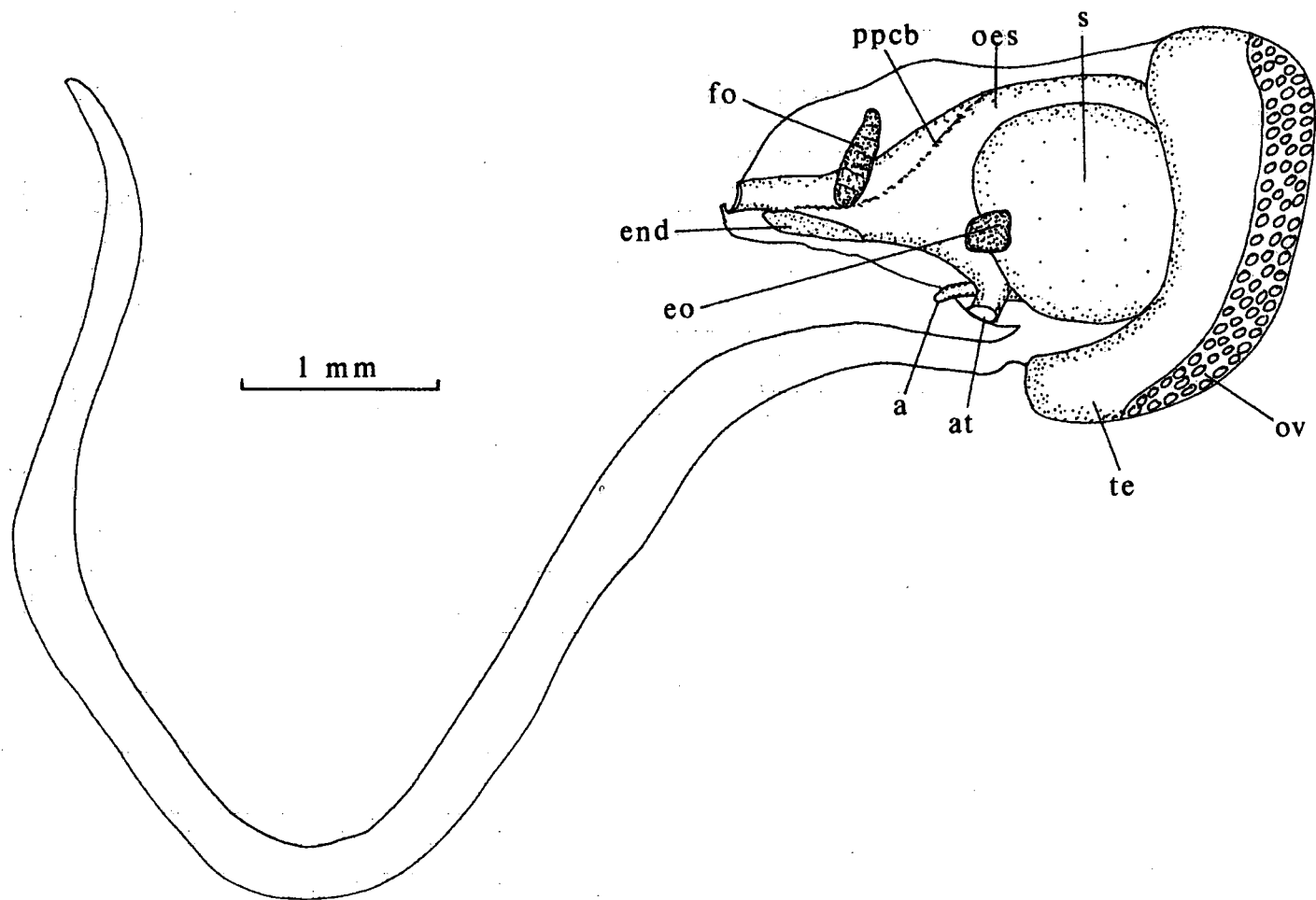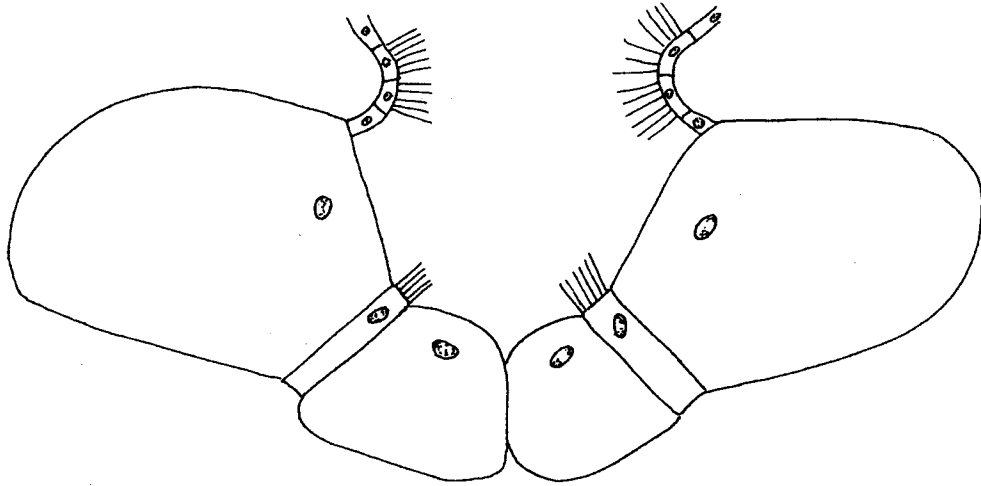
Figure 23. (above)

Transverse section of the endostyle of *Oikopleura labradorensis*, showing four mucus-secreting cells and six ciliated cells.

Figure 22. (opposite)

Sketch of *Oikopleura vanhoeffeni* from the left side. a, anus; at, atrium; end, endostyle; eo, Eisen's oikoplast; fo, Fol's oikoplast; oes, oesophagus; ov, ovary; ppcb, peripharyngeal ciliated band; s, stomach; te, testis.
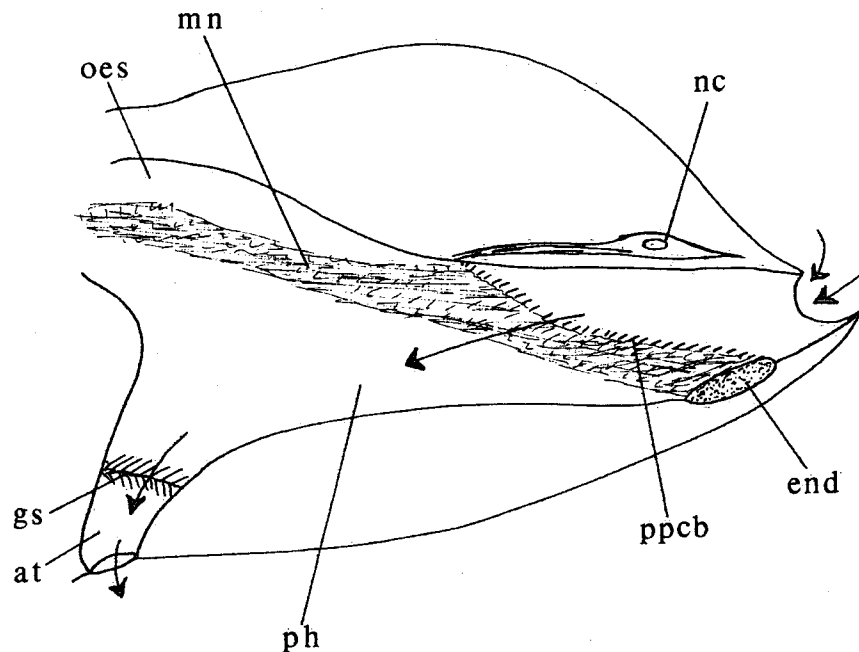
Figure 24. (above)

Diagram of the anterior part of the trunk of *Oikopleura* from the right side during feeding. at, atrium; end, endostyle; gs, gill slit; mn, mucus net moving back into oesophagus; nc, neural complex; oes, oesophagus; ph, pharynx; ppch, peripharyngeal ciliated band.

During development the tail twists through ninety degrees into a horizontal position with the nerve cord lying on its left side (see Fig. 25). Its undulations serve not to draw water through the pharynx (a task carried out by the gill slits), but to drive water through the system of external filters secreted by the specialized oikoplastic epithelium of the trunk.

Larvacea in fact live in a complex disposable house secreted by this epithelium. Lohmann (1896) first showed that in the earliest stage of secretion the house consists of a continuous sheet of cuticular secretion. Körner (1952) showed that it consists

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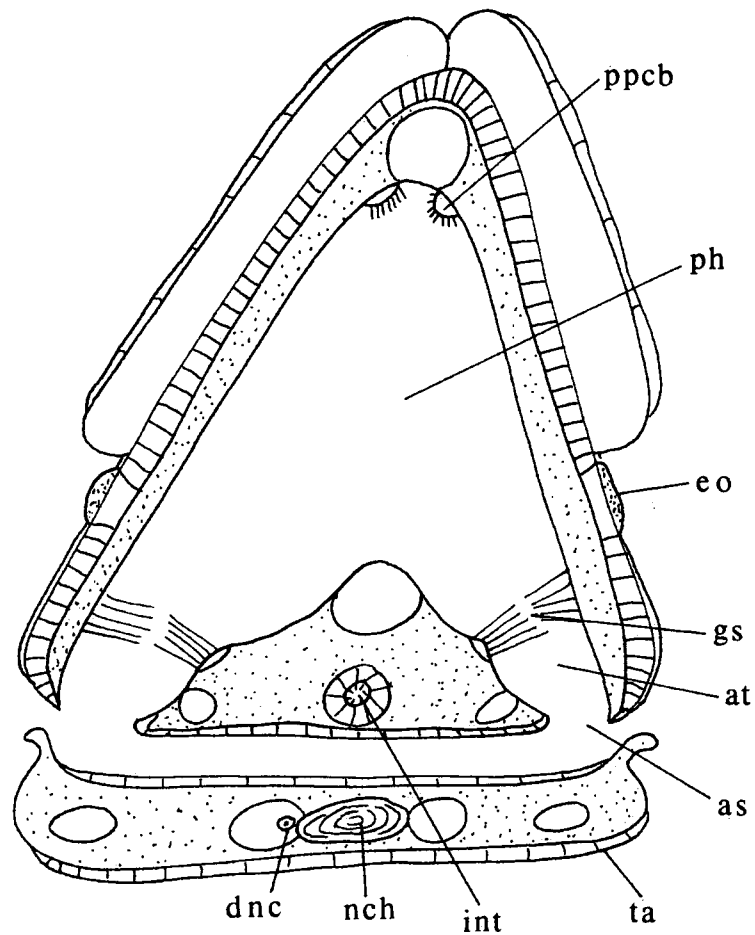
Figure 25. (above)
Transverse section of *Oikopleura* at the level of the gill slits. as, atrial siphon
or branchial opening; at, atrium; dnc, dorsal nerve cord of tail; eo, Eisen's oikoplast;
gs, gill slit; int, intestine; nch, notochord; ph, pharynx; ppcb, peripharyngeal ciliated
band; ta, tail.

Figure 26. (above)

Sketch of the trunk of *Oikopleura albicans* from the right side. a, anus; as, atrial siphon or branchial opening; at, atrium; bc, buccal cavity; bs, buccal siphon or mouth; end, endostyle; gs, gill slit; nc, neural complex; oes, oesophagus; ov, ovary; ph, pharynx; ppcb, peripharyngeal ciliated band; r, rectum; s, stomach; ta, tail; te, testis.

of mucopolysaccharides and proteins, and is lacking in the cellulose which is so charac-
teristic of the tunic of other tunicates. Nevertheless it appears to be homologous with
the tunic. The oikoplastic cells of the trunk are not all alike, but are arranged in
complex patterns and secrete at different rates. The result is that the simple con-
tinuous cuticular sheet soon becomes a most complex formation whose precise structure
differs from species to species. The functioning of the house is best understood in
the family Oikopleuridae, and most of what follows refers primarily to that family.

The simplest house is that of *Oikopleura longicauda*, which simply has two openings,
one behind for entrance of water and the other in front for exit. In the majority of
species the entrance takes the form of a pair of windows, which are furnished with screens
to exclude larger particles. Like the rest of the house, these screens, formed of criss-
crossing fibres, are secreted by oikoplasts. In *O. albicans*, the species best known
from the studies of Lohmann (1899), the house is pyriform and divided into a number of
chambers. The animal itself is lodged in two median chambers, the trunk in one and
the tail in the second (Fig. 27). The paired entry chambers, opening from the outside
by their screened windows, communicate with the tail chamber, so that undulations of
this organ draw in water through the windows and pass it back and finally upwards into
the paired chambers of the feeding filters. The water passes through these filters
and enters the tail chamber once more. Here partial baffles prevent too much mixing
with the unfiltered water entering from the entry chambers, and instead it is passed
backwards and finally ejected through the mid-ventral exit port (Figs. 27 and 28).

Figure 27. (page 52)
Diagram of *Oikopleura* from the left side, showing its position in the house and the
feeding currents. ee, "emergency exit"; ef, entry filter or window; ff, feeding filter;
ta, tail; tr, trunk. (Partly after Lohmann, 1899a).

Figure 28. (page 53)
Sketch of *Oikopleura albicans* from above to show its position in the house and the feeding
currents. ee, "emergency exit"; ef, entrance filter or window; ff, feeding filter; ta,
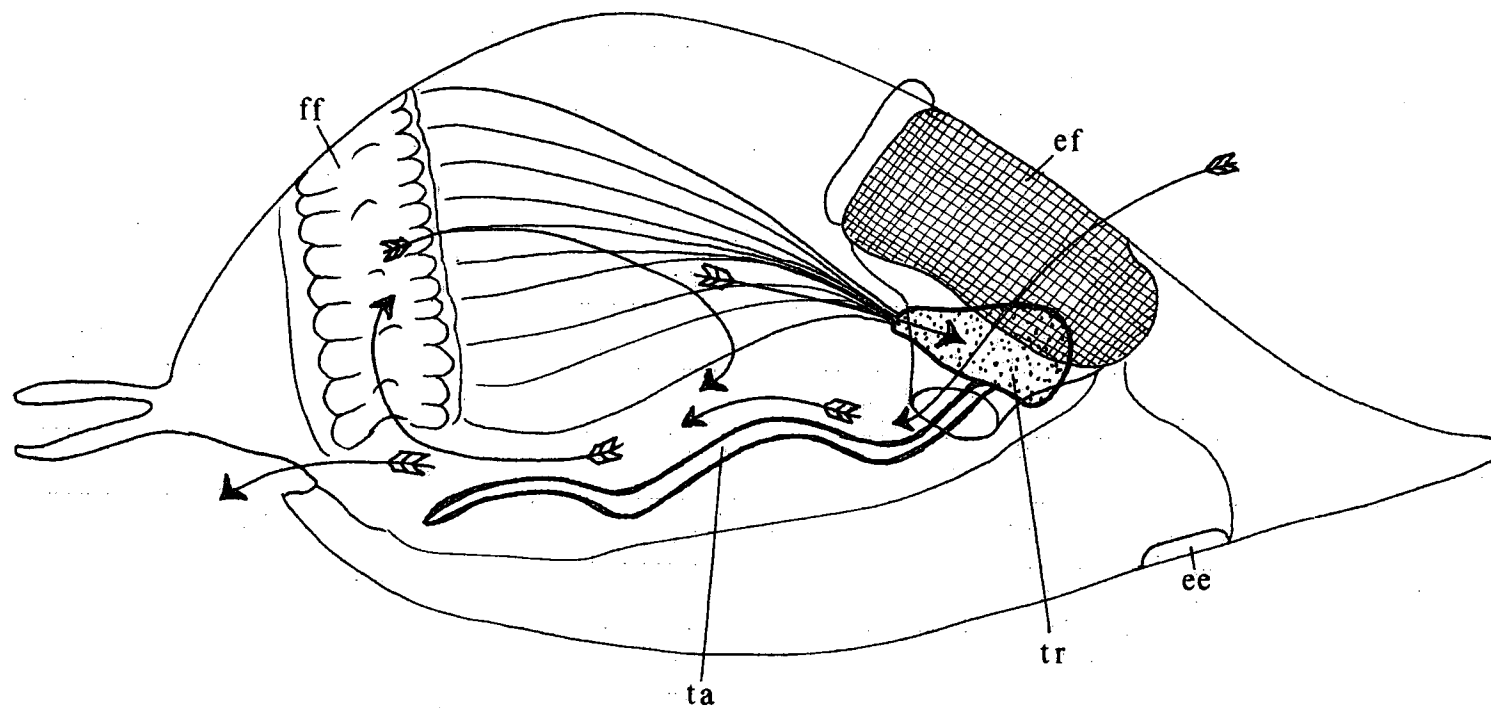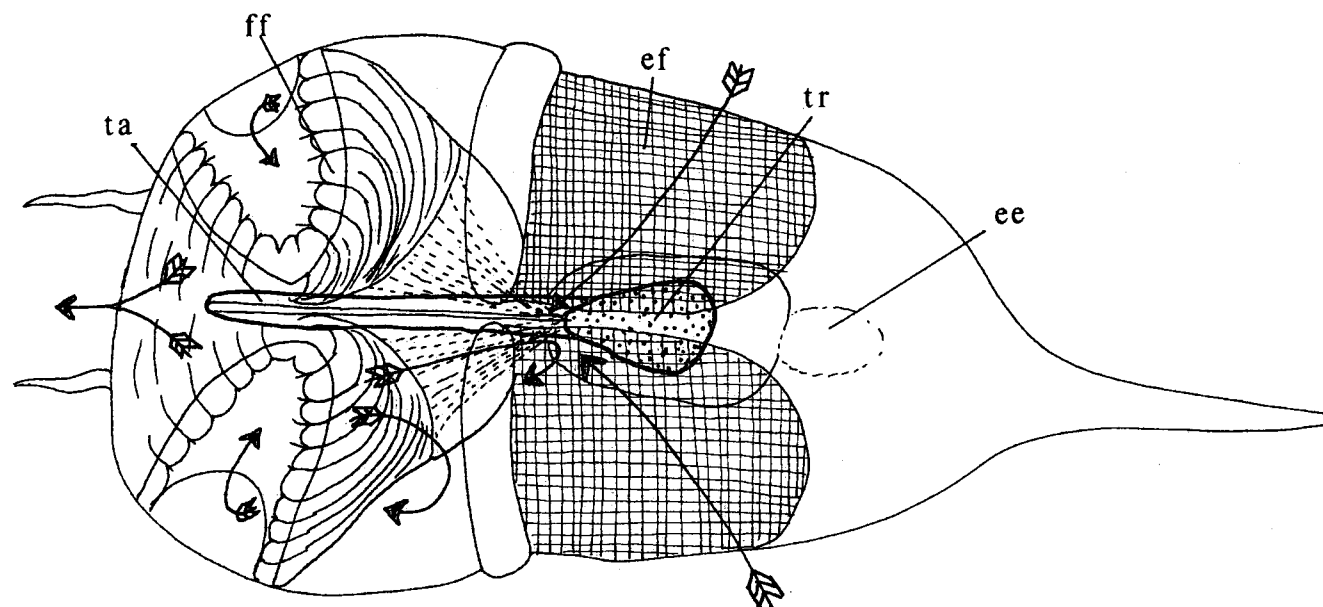tail; tr, trunk. (Partly after Lohmann, 1899).

Figure 27.

Figure 28.

The trunk chamber does not lie on the route of currents set up by the undulations of the tail, but possesses a separate opening to the outside through which a little waste water normally flows and which is generally called the "emergency exit", since it is through this exit that the animal will normally leave the house if it is damaged or if the filters become clogged. Indeed, under conditions of heavy algal growth, these may become clogged in as little as 15 minutes, and *Oikopleura* has been observed to secrete a fresh house every 20 minutes under such conditions.

The windows serve to exclude all particles over three micrometres long, and many in the range of one to three micrometres. Only particles below this size reach the feeding filters, whose functioning is still incompletely understood. Lohmann (1896-1934) laid the foundation of our knowledge, though some of his views were disputed by Garstang (1928). More recently Jørgensen (1966) has described the structure of these filters and Fenaux (1967, 1968) has added to our knowledge of their functioning. The account which follows is largely based upon Fenaux's descriptions.

Each filter consists of two leaves held apart by a membranous network of fine rectangular meshes, each about 800 x 100 nm. The parts are so exactly placed that the two free borders of these leaves, located one above the other, open into separate chambers. The lower opening emerges into the trap or tail chamber, and the upper into the dorsal chamber which houses the trunk. Only the upper portion is furnished with a highly complex network of meshes. Fenaux suggests that this complex functions as follows. The water which is in the process of being driven by the tail into the trap enters between the free edges of the leaves in the filter. It moves upwards through the filters, passes into the dorsal chamber, and rejoins the tail chamber laterally. It is then expelled. The suspended particles accumulate at the base of the filter and a momentary stoppage of the undulations of the tail allows the particles to sediment. The animal then sucks this concentrate through the buccal siphon, using the cilia of the gills for this sucking.

Fenaux appears to have misunderstood the processes involved. We have never observed any momentary cessation of tail beats, such as is required by his postulate, while it is easy to calculate that the time required for the "sedimentation" of particles of the order of size involved (800 x 100 nm as a maximum) is far too long. Several minutes would be required, even if Brownian movement did not totally prevent such sedimentation, or indeed, if the swimming actions by the organisms (most of which are flagellated) did not also counter any such gravitational effect. Moreover, such "sedimentation" would have to take place in an upward direction. We suggest instead the following mechanism, which is more consonant with our own observations. The water enters the trap or feeding filter, exactly as described by Fenaux. Essentially this trap consists of two narrow passages, one more or less unobstructed and leading finally

to the tail chamber for ultimate expulsion, while the other, which leads towards the buccal siphon, is obstructed by the meshwork. At all levels these communicate with one another, so that it is perhaps better to regard the whole as a single passage whose upper part is obstructed by a meshwork hanging from its roof. As water and suspended particles enter this passage, or passages, the particles tend to bump repeatedly against the meshwork and so are slowed down, while the water tends to follow the more direct path. There is, as it were, a chromatographic separation. The particles, by purely hydrodynamic means, are diverted into a slowly moving current, while the faster moving current ends up relatively free of particles. This latter current is directed towards the tail chamber for expulsion, while the former is directed towards the buccal siphon, where cilia of the pharyngeal apparatus serve to draw in the highly concentrated suspension and effect the final trapping in a mucus net in the usual tunicate manner. The food particles, of course, consist of the smaller and smoother types of unicellular algae, mostly below 800 nm in size. Organisms above 3 μm are excluded by the windows, while those between 800 and 3000 nm which succeed in passing through the windows cannot be sorted into the slow stream in the feeding trap since they are too big to pass through the meshes there and instead remain in the fast current and are expelled. Some of the smaller particles in this size range may indeed become stuck in the trap and so clog the filter.

In the Fritillariidae the house is a capsule consisting of two valves from which the animal remains suspended by the mouth, without being lodged inside (Fol, 1872). Tail wagging induces a current through the filters in these valves, and the capsule appears to function like the house of *Oikopleura*, though it has not been observed in any detail.

The house of *Kowalevskaia* has been observed even more rarely. Fol (1872) describes it as an ellipsoid of revolution inside which the animal lies. It has only a single large opening, and the animal lies in fact in a groove. Fol described the water currents in this groove which were set up by the tail, but made no reference to any filters. No one since has reported observing the house of this genus, but from the complexity of the oikoplasts on the trunk one must conclude that the house is moderately complex and possesses filters of some kind.

All in all, the feeding mechanisms of *Kowalevskaia* present a number of unsolved problems, not the least of which is the lack of endostyle. Fenaux (1967) has described the structure of the pharynx, and observed that it is divided longitudinally into three chambers by right and left ciliated ridges, both dorsally and ventrally (Fig. 29). The posterior end of the pharynx is much flattened and furnished with a veritable carpet of cilia leading into the oesophagus. The stomach possesses undulating membranes formed of fused cilia. Fenaux (1968) has observed feeding (in animals deprived of a house) to take place in the following manner. The cilia of the gills pull a water current

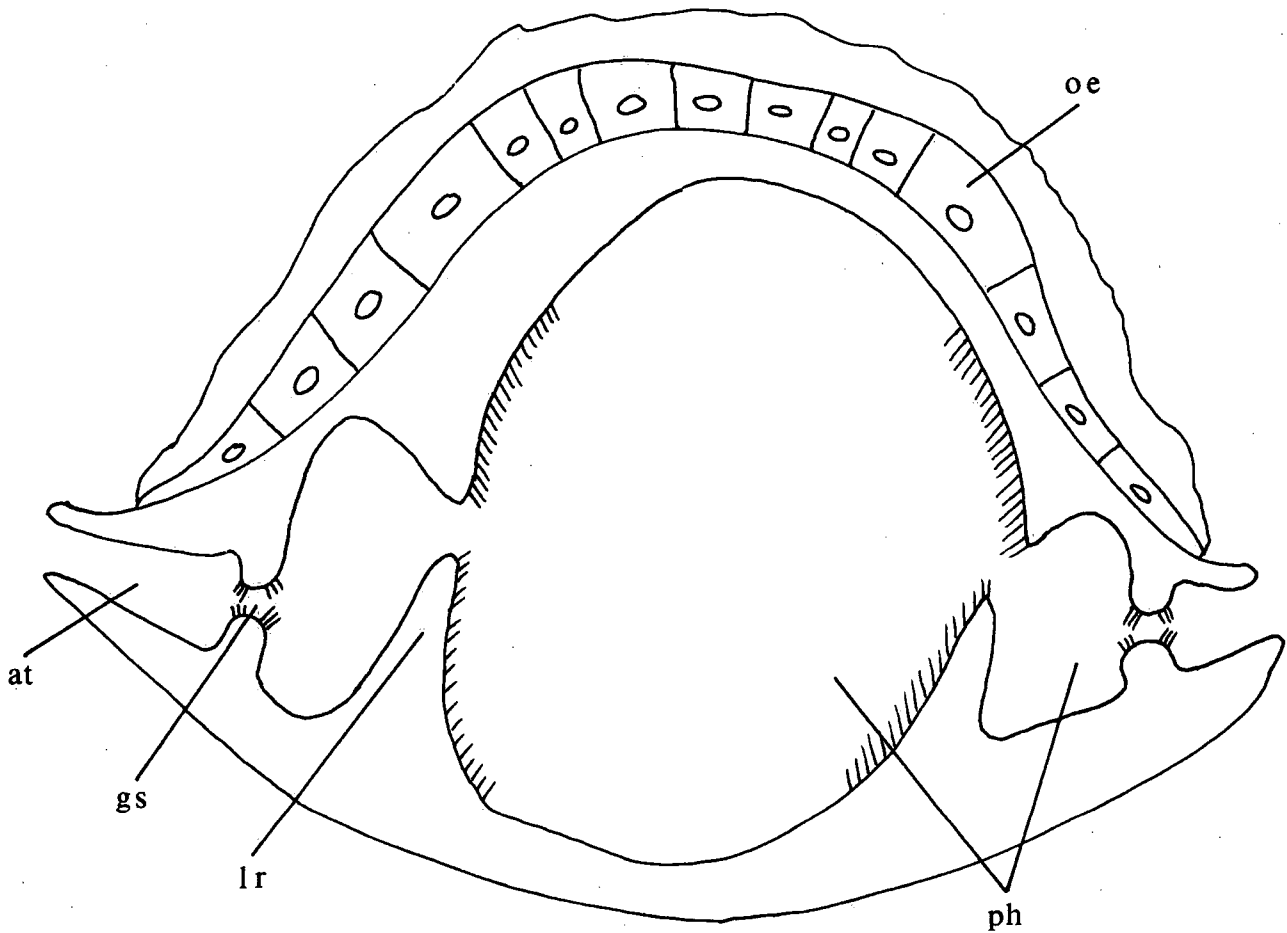Figure 29. (above)

Transverse section through the trunk of *Kowalevskaia* at the level of the gill slits.
at, atrial cavity; gs, gill slit or spiracle; lr, longitudinal ciliated ridge of pharynx;
oe, oikoplastic epithelium; ph, pharyngeal cavity.

through the pharynx where the lateral ciliated ridges hold back any suspended particles. The cilia of these ridges convey such particles backwards towards the oesophagus. The total movement of particles is governed by the cilia of the gill slits, those of the ridges and those of the entrance to the oesophagus, so that they are sloshed to-and-fro until they are trapped in mucus secreted by the wall of the oesophagus. He suggests that, as in Thaliacea, there are slow and fast modes of feeding. We must confess that his description is far from clear to us and does not seem to provide a mechanism which we can accept as correct or at least as complete. There is room for much further observation on this genus, which is so aberrant.

Calcichordata

The Calcichordata is an extinct Cambrian taxon which Jefferies, in a series of papers (1967-1978), has shown to be chordate with echinoderm affinities. He has argued that they are ancestral to the tunicates. They are divided into the Mitrata, which do not depart far from bilateral symmetry, and the more aberrant Cornuta, in which the body is shaped like a boot.

Dr. Jefferies has homologised parts of the mitrate body with tunicate structures (Fig. 30) and suggests that they may have fed in a similar manner. We are grateful to him for the following description which he has kindly written for inclusion in this paper.

"The pharynx of mitrates was divided into right and left parts, and judging by its morphological relations in the adult, the right pharynx appeared later in ontogeny than the left, or primary, pharynx. There is evidence in certain mitrates that the whole of the internal wall of the right pharynx was longitudinally pleated. Also the left pharynx was divided into an anterior smooth part and a posterior part with longitudinal pleats like those of the right pharynx. This probably corresponds to a functional difference between the smooth and the pleated parts of the pharynx. On tunicate analogies it is most likely that the smooth pharynx was anterior to the mucous bag, while the pleated pharynx was posterior to the front margin of the mucous bag. Possibly the pleats acted, like the intrabranchial structures of tunicates, to hold the mucous bag away from the pharyngeal wall proper, so that the bag could filter more effectively. The peripharyngeal bands, on this assumption, would coincide with the places where the pleated and smooth parts of the pharynx, came in contact with each other, i.e. with the anterior limit of the right pharynx, and the anterior limit of the pleats in the left pharynx. The ciliated organ, on this assumption would be situated near where the peripharyngeal bands met dorsally, and in the mitrate *Placocystites* there is evidence of its possible position in the appropriate place.
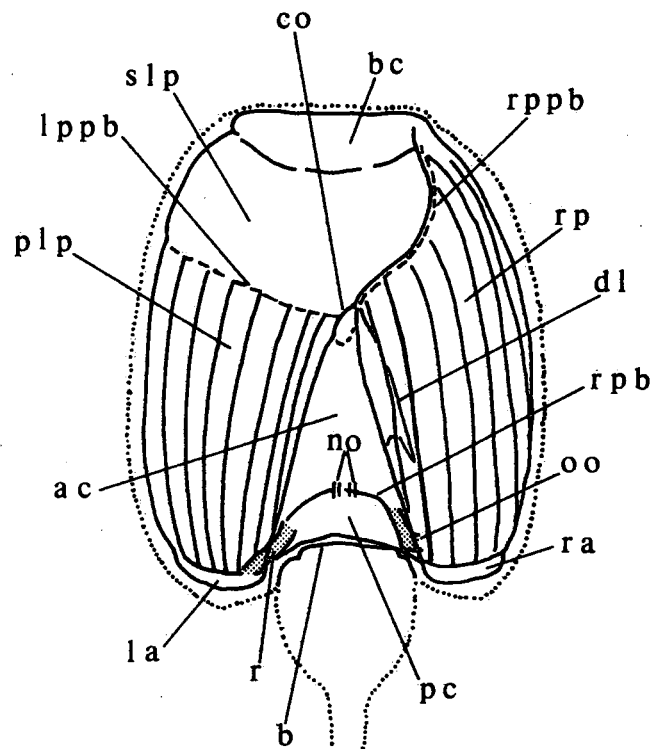
Figure 30. (above)

The pharynx and related organs of a mitrate in dorsal aspect (diagrammatic). The rectum and oesophagus are stippled. The anterior coelom was dorsal in position and the left pharynx continued beneath it to meet the right pharynx. ac, anterior coelom; b, brain; bc, buccal cavity; co, ciliated organ; dl, dorsal lamina; la, left atrium; lppb, left peripharyngeal band; no, nerves to endostyle; oo, oesophageal opening; pc, posterior coelom, or right and left epicardia; plp, pleated part of left pharynx; ra, right atrium; r, rectum; rpb, retropharyngeal band; rppb, right peripharyngeal band; rp, right pharynx (pleated); slp, smooth part of left pharynz. (R.P.S. Jefferies, unpublished).

"The line of contact between right and left pharynxes in mitrates does back right to the anterior coelom from near the assumed position of the ciliated organ. When the chambers are reconstructed near this line of contact it becomes necessary to postulate an elongate structure in the soft parts, hook-shaped in section, with the hook sloping downwards and rightwards, like a dorsal lamina. This structure could well have carried the mucous rope. If this were the case, then the opening of the oesophagus should be situated near the posterior end of the dorsal lamina. In an appropriate place there is in fact a semicircular hole in the skeleton, which probably carried the oesophageal opening. This hole is well to the right of the mid-line and excavated in the wall of the posterior coelom. Its position right of the mid-line can be compared with the similar situation of the oesophageal opening in salps.

"A pair of nerve canals (no) pass out of the posterior coelom, through the ventral skeleton near the mid-line. The nerves in these canals, from their position, probably supplied the endostyle. There is sometimes a right one of this pair of nerves, and in one specimen of *Placocystites* this process curves rightwards dorsally, towards the assumed position of the oesophagus. Assuming that the nerves (no) were symmetrical to the endostyle, then this process probably carried the retropharyngeal band which passed, as is usual in tunicates, from the right posterior extremity of the endostyle to the opening of the oesophagus. This conclusion is confirmed by the presence of two nicks near the base of this retropharyngeal process. These nicks would have put the right and left pharynx in communication with the posterior coelom, like the pharyngo-epicardial openings on either side of the retropharyngeal band of *Ciona*. It follows from this that the posterior coelom of mitrates is homologous with the left and right epicardia of tunicates, and there is evidence in some mitrates that the posterior coelom was in fact not single, but formed from a pair of chambers.

"The whole situation in mitrates, therefore, suggests that their pharynx was organized like that of modern tunicates, and must have functioned in essentially the same manner.

The situation in cornutes is more difficult to reconstruct. These forms has no right gill slits or right pharynx. Consequently the right epicardium, right peri-pharyngeal band and the right half of the endostyle could not have existed as such". (Jefferies, in Litt.).

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Conclusions

The mechanisms for moving water in tunicates and those for moving mucus are quite distinct and separate systems. In the majority of ascidians, both water and mucus are moved by means of cilia, but separate sets of cilia are used for the two processes. The cilia which line the gill slits are used to generate water currents, while those which are found in patterns of bands inside the pharynx are used for the movement of mucus.

The mucus-carrying apparatus shows the least variation in fundamental pattern. The mucus itself is secreted by the endostyle in separate strands which are then attached at the front end to the peripharyngeal ciliated bands and thus conveyed in a dorsal direction to the dorsal lamina where they are collected and twisted into a rope. This mucus rope, with trapped food particles, is pulled back into the oesophagus by the cilia which are located on its walls. In slow feeding, which is probably quite widespread amongst ascidians as well as in the Thaliacea, the strands of the mucus attached to the peripharyngeal ciliated bands remain free at their hind ends so that they may wave about. In fast feeding the hind ends are caught up into the beginnings of the mucus rope and so a mucus filter funnel is formed. The rate of secretion of mucus and the rate of transport are related to the concentration of particles in the incoming current, so that the mucus rope always has roughly the same proportion of attached particles as it enters the oesophagus. Economy of mucus may be the root cause of the two modes of feeding. The mucus is ultimately lost to the body, since it is not digested, and represents wasted organic material. A mechanism to limit the rate of production of mucus when food particles are scarce may be important in the economy of the animal, and when little mucus is being produced a complete filter funnel cannot be formed from the small amounts of mucus present in the pharynx.

The variations we may observe in the mucus secreting and transporting apparatus are relatively minor. The endostyle of an ascidian does not differ significantly from that of a larvacean, except in the number of cells. There is, however, much greater variability in the apparatus for moving water.

In the simplest system the rectangular gill slits are lined with cilia which, by their beating, drive the water through the slit and create at the same time a swirl or vortex. The amount of water moved is obviously proportional to the area of the ciliated epithelium. Mechanical requirements of strength, hydrodynamic requirements of the range over which cilia can exert an effect upon the water, and restrictions

imposed by supplying the ciliated epithelium with blood and nutrients, limit the size
of each gill slit. Thus for a single gill slit there is an optimum size. If its width
is much greater than twice the length of a cilium there is a central dead space. If
its length is too great the bar between adjacent gill slits is impossibly fragile.
Accordingly the water is constrained to flow through a narrow space. If an ascidian
is to move a large volume of water it must do so by increasing the number of gill slits
rather than by increasing their size. Typically, the gill slits are about five to seven
times as long as broad and are arranged in rows, sometimes as many as 700 - 800 rows.
Each half-row may contain up to 500 gill slits so that the total number may be in excess
of 500 000. Frictional losses are minimized by keeping the branchial wall as thin as
possible, consistent with adequate strength of the gill bars and with providing enough
thickness to optimize the area of ciliated epithelium in each slit.

Numerous lines of evolution seem to have led to more efficient ways of moving water.
The simplest seems to be adoption of spiral gill slits, an event which has occurred
more than once. By increasing the size of the vortex these spiral groups of slits appear
to reduce the shear forces needed to separate the water into the numerous distinct streams
going through the separate gill slits. As a result a smaller area of ciliated epithelium
is required by an ascidian with spiral than with straight gill slits. *Molgula*, for
instance, moves water through its gills perhaps thirty times more efficiently than does
Phallusia. We presume that a cost has to be paid for this greater hydrodynamic efficiency
in greater complication of control mechanisms required for coordination. Nevertheless,
spiral gill slits have evolved in at least three distinct evolutionary lines of ascidians.

A second approach to the problem of moving more water lies in the adoption of
muscular action for the process. In ascidians, muscular action is confined to squirting,
the process by which accumulations of undesirable particles are expelled from the body
in the form of faeces (from the atrium) or pseudofaeces (from the pharynx). In the
pelagic tunicates, however, we see varying degrees of adoption of muscular action for
producing the feeding current. *Pyrosoma* and *Doliolum*, like ascidians, rely on the cilia
of the gill slits for the feeding current, using the muscles of the mantle wall for
squirting and for the escape reaction. In *Salpa* the gill slits are totally lost, except
as wide open spaces, and the entire current is maintained by muscular action, by means
of the circular muscles of the mantle. The Larvacea have solved the problem in quite
a different manner. They use the tail, otherwise characteristic of the larval stages
of tunicates, to create a current, again by muscular action. The hydrodynamics of
the filtering system would well repay study, and computer modelling of the filtering
system of *Oikopleura* is currently underway in our laboratory.

Along a different line of evolution, deep sea ascidians have adopted other methods
of feeding. Some simply face upstream in regions of steady current, and rely on such
an external current to sweep food into their mucus filter. A few species of the abyss

appear to have abandoned filter-feeding altogether in favour of macrophagy, and either lie in wait to seize passing prey in some way, or crawl about actively hunting for food. Such creatures, however, are quite tiny and seem to feed on Foraminifera and small crustaceans.

Many problems remain unsolved in the realm of the filter feeding of tunicates. Some of these will respond to closer observation, while the complex hydrodynamic problems may yield to mathematical analysis or to computer modelling. The mathematical techniques available seem inadequate for the purpose at the moment; biological systems are too complex, and it could well be that the major advances in theoretical mathematics over the next few decades may stem from the struggle of mathematicians to develop and apply new techniques for the mathematics of biology, in the same way that the great flowering of our present day mathematics took place in the seventeenth century with the struggle to apply mathematics to astronomy and then to physics.

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