FINE STRUCTURE AND FUNCTION OF THE CYTOPHARYNGEAL BASKET IN THE CILIATE NASSULA

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SUMMARY

The fine structure of the cytopharyngeal basket is described in detail. A circular palisade of cytopharyngeal rods is encircled at certain levels by a sheath and two annuli; crest-shaped structures project outwards from the basket. The rods, crests and sheath are largely composed of cytoplasmic microtubules of approximate diameter 240 Å. One of the annuli is mostly composed of densely staining inter-tubular material; the other consists mainly of fine fibres ranging in diameter from 40 to 90 Å.

Ingestion of algal filaments by Nassula and the sequence of movements of the rods which occurs during feeding are described. Evidence is presented which indicates that the crests and fibrous annulus both contract during feeding and act antagonistically to displace the apparently rigid rods. The initial displacement of the rods is spatially related to the orientation of the algal filament which is to be ingested. It is suggested that certain cilia respond to contact with the filament by transmitting some form of excitation to some of the crests along small bundles of microtubules which interconnect them. The suction force drawing the filament into the organism apparently acts only in the lumen of the basket. The basket seems to function as a fairly rigid structure which, acting in conjunction with the suction force, guides the filament into the organism and manipulates it to some extent. The basket also apparently grips the partly ingested filament to prevent it from slipping out of the organism during pauses in the action of the suction force.

INTRODUCTION

The class Ciliata is divided into two subclasses; the Spirotricha and the Holotricha. The Spirotricha are characterized by the presence of numerous compound ciliary organelles in the oral region. These organelles are generally smaller and less numerous in the Holotricha and they are usually absent altogether in the order Gymnostomatida. The ingestatory apparatus of the rhabdophorine and cyrtophorine gymnostomes consists almost entirely of a large and complex organelle, the cytopharynx, which is mainly composed of an elaborate association of filamentous elements. The cytopharynx is usually larger, structurally more regular, complex and compact, and more resistant to mechanical deformation in the suborder Cyrtophorina where this organelle is generally referred to as a cytopharyngeal basket (Corliss, 1959).

This paper deals mainly with the fine structure and function of the cytopharyngeal basket of the cyrtophorine Nassula. The basket is about 60 μ long and about 15 μ in diameter and consists of several distinct components. The major component is a

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circular palisade of slender rods (Ehrenberg, 1838). Two annuli encircle the palisade and pellicular thickenings radiate from the perimeter of the cytostome to form a collar around it and partly cover one end of the basket (Bütschli, 1887–9; Schewiakoff, 1889). The palisade is surrounded by a sheath for most of its length and crest-shaped structures project outwards from the sides of the basket (Wetzel, 1925). Several ribbon-shaped lamellae are associated with the palisade; each rod has one lamella attached to it (Rouiller, Fauré-Fremiet & Gauchery, 1957). The present study confirms and extends these descriptions. Most of the components consist mainly of closely packed cytoplasmic microtubules (Rouiller et al., 1957; Raikov, 1966). Microtubules are a common cytoplasmic component of many protozoans (Roth, 1958) and a wide range of other cells (Slautterback, 1963). Several functions have been suggested for microtubules in protozoans (see Grimstone, 1966) and in cells in general (see Porter, 1966). The present study indicates that some of these functions are performed by the microtubular components of the basket of Nassula; particular functions are apparently conducted by particular components which are structurally specialized to perform them.

The basket is favourable material for functional analysis because of its large size and occurrence in an easily cultured free-living protozoan. Nassula feeds on filaments of blue-green algae which enter the organism through the basket and are to some extent manipulated by the basket (Dragesco, 1962). Observation of living feeding organisms in the present investigation revealed a complex sequence of movements of the components of the basket and these are described. The possible interactions and functions of the components during feeding are discussed.

MATERIALS AND METHODS

The baskets of Nassula embedded in Araldite have been examined by electron microscopy of thin sections and by light microscopy of thick sections (0.5–1 μ) stained with methylene blue. The methods used for electron microscopy, for staining thick Araldite sections, and for culturing Nassula and the blue-green alga Phormidium inundatum on which it feeds, have already been described (Tucker, 1967). The basket and some of its components can be seen with the phase-contrast microscope in Nassula flat-embedded in Araldite, so that it has been possible to select individual organisms after embedding and cut sequences of transverse or longitudinal sections precisely orientated relative to the longitudinal axis of each basket. The organisms were impregnated with silver (Chatton & Lwoff, 1930) to ascertain the arrangement of their cilia.

Phase-contrast microscopy

Large numbers of organisms were examined after they had been concentrated by mild centrifugation. They were photographed with a Zeiss research microscope (Standard WL) using phase contrast, a green interference filter, a Zeiss microflash device and a Zeiss attachment camera loaded with Panatomic-X film (Kodak).

The feeding movements of the basket and the ingestion of algal filaments were observed after mixing starving Nassula from stationary phase cultures with a suspen-
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sion of filaments on a microscope slide. In these preparations the coverslip was supported at each corner by a fine smear of silicone grease so that slide and slip were separated by about 1 mm, thus permitting *Nassula* to swim freely.

Organisms were mixed with detergent (Teepol diluted to $10^{-4}$ with distilled water) on a microscope slide; this is a modification of the method of Child & Mazia (1956). Preliminary fixation in cold ethanol was omitted so that the pellicle of each individual ruptured more readily and released the highly refractile food vacuoles which hinder observation of the basket. In most cases a pellicular 'ghost' remained containing little visible material besides the basket and the macronucleus.

Baskets were squeezed out of living organisms after pipetting the organisms on to a microscope slide and covering them with a coverslip. Compression and shearing of the organisms by gentle manipulation of the coverslip often resulted in rupture of the pellicle of some individuals and extrusion of their baskets.

**DESCRIPTIVE CONVENTIONS**

Certain conventions will be used to describe the orientations, shapes and positions of the components of the basket.

One end of the basket (top) is attached to the pellicle around the cytostome; the other end (bottom) projects into the endoplasm. The basket is circular in cross-section; the inside or lumen of the basket is surrounded by a complex of several distinct components forming the cytopharyngeal wall. The term length will be used for the dimension of a component measured in a direction parallel to the longitudinal axis of the basket; depth for the same dimension of those components, such as annuli, the greatest dimensions of which are not parallel to this axis. The term breadth will be used for the dimension of a component measured along a radius of the circular cross-sectional profile of the cytopharyngeal wall; thickness for the dimension measured at right angles to its breadth and hence parallel to a tangent to the circular profile at a point co-radial with the component (Fig. 1). Every component has one of its surfaces closest to the lumen and another most distant from it: the inner and outer surfaces, respectively (Fig. 1). The inner ends of the pellicular thickenings of the collar border the cytostome and their outer ends are the portions most distant from the cytostome. Surfaces which are radial to the cytopharyngeal wall will be referred to as the sides of the components (Fig. 1).

The convention for handedness in ciliates (Chatton & Lwoff, 1935) is widely used to aid description of the distribution of cortical organelles; a similar convention is applied here to the cytopharyngeal wall, as follows. When the portion of the cytopharyngeal wall nearest the observer is being examined and when top and bottom of the basket are orientated towards the top and bottom, respectively, of the observer's field of view, then the left side of this portion of the wall and of the components of this portion of the wall are on the observer's right and vice-versa. The convention pertains to the orientation of the components of the basket as they would appear if they could be resolved by direct examination of living organisms without any optical aid. Because the convention takes account of the orientation of the basket (top and bottom) it also
applies to the completely inverted images supplied by most compound microscopes. The convention, as stated above, defines the handedness of only some of the components of the basket. It does not define a left or right side of the basket as a whole.

Examination of living organisms has shown that the rods always slope from top right to bottom left. Longitudinal sections of baskets show the slope and orientation of the rods; hence their handedness, and the corresponding handedness of adjacent components, can be determined. These sections also show that lamellae are always attached to the left sides of the rods. When a transverse section is examined the handedness of the components can be assessed by reference to the asymmetrical association of rods and lamellae.

Fig. 1. Diagrammatic transverse section of the cytopharyngeal wall, as seen by an observer looking from the top to the bottom of the basket, showing the directions in which the breadth and thickness of a component of the wall (cross-hatched) have been measured. The right and left sides and inner and outer surfaces of another component (stippled) are shown.

RESULTS

General organization of baskets

The disposition of the main components in the basket will be outlined in this section to introduce the terminology used in the detailed description which follows. The names used for components of the basket in the original descriptions (see Introduction) have often been retained. Terms defined by Corliss (1959) have been used for oral structures where they are applicable.

Cytopharyngeal rods form a circular palisade (Figs. 2, 26, 33) which is encircled by a dense annulus (d) about 12 μ from its top (Figs. 2, 10) and below this by a sheath (Figs. 2, 3, 9). Between the dense annulus and the fibrous annulus which encircles the top of the palisade, the spaces between rods (Figs. 2, 15) apparently present, like the opening at the bottom of the basket (Fig. 3), no barrier to cytoplasmic movement or diffusion into and out of the lumen.
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At most levels, the cytopharyngeal wall consists mainly of rods, crests, rod lamellae and a sheath. The rods (r) are attached to the inner surface of the tubular sheath (s), the crests (c) project outwards from the sheath approximately at right angles and a rod lamella (x) is attached to the left side of each rod (Fig. 6). Each of these components is largely composed of closely packed microtubules; continuous lengths of the tubules of up to 3 μ have been observed in sections. The tubules are approximately 240 Å in diameter and circular in cross-section with a dense peripheral region or wall, radial thickness about 70 Å, and a less-dense central region or core. They are packed more or less hexagonally in the rods, crests and sheath but each rod lamella consists mainly of a single row of tubules (Fig. 6).

Fig. 2. Diagrammatic reconstruction of a basket (lateral view, top uppermost) drawn to scale, showing the shape and arrangement of its main components. The sheath encircles the palisade of rods below the dense annulus but has been omitted on the right side of the diagram to show the arrangement of rods inside it. A crest is attached to each rod above the dense annulus and spirals around the outer surface of the sheath at lower levels; for clarity only one crest has been illustrated.
Fig. 3. Schematic diagram of a median longitudinal section of a basket, top uppermost, showing the shape and arrangement of the main components of the basket. Rods on opposite sides of the basket are not contained in a single plane along their whole lengths and the crests are not planar (see Fig. 2) although represented as such in this diagram. For clarity, rod lamellae have been omitted and a cytostomal lamella (z) and a sub-cytostomal lamella (y) have only been drawn on the left side of the diagram. The rods (r) are unshaded except at their tops (black) where dense inter-tubular material (i) occurs. The thickened corrugations of the collar (i), the caps (p), the dense annulus (d) and the dense inter-tubular material (i') in the tops of the crests are also black. The lamellae are stippled, the sheath (s) is cross-hatched and the ciliary connectives (g) are represented by broken lines. The continuous lines drawn on the crests (c) indicate the paths followed by their microtubules. a, oral atrium; b, basal body; c, pellicular dense layer; f, fibrous annulus; k, ciliary fringe; l, lumen; m, cytostomal membrane; n, peri-cytostomal flange; o, oral opening.
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The top of the basket is situated at the bottom of an invagination of the pellicle, the oral atrium (a); the atrium is widest at its base and constricts to an elliptical oral opening (o) at its top which is about $4 \times 7 \mu$ across (Figs. 3, 25). The thickened corrugations (t) of the collar are situated on top of the palisade (Figs. 2, 22); they form the bottom of the oral atrium except for a circular central region of approximate diameter 3.5 $\mu$, the cytostome (m), covered by a thin cytostomal membrane (Figs. 3, 21, 25).

Cytopharyngeal rods

Baskets and their rods are usually about 60 $\mu$ long but their lengths vary considerably among individuals within a range of about 45–80 $\mu$. The number of rods in a basket is also variable, ranging from at least 23 to 34. Each rod (r) is straight for most of its length but about 5 $\mu$ from its top it bends inwards at an angle of about 20° to its longer lower portion (Figs. 2, 3, 18, 22). Not only does each rod slope from top right to bottom left (Figs. 2, 26) but it also slopes inwards from the top to the bottom of the basket (Fig. 3).

The external diameter of the palisade of rods at its top is about 12 $\mu$ but increases to a maximum of approximately 15 $\mu$ at the level of the bends in the rods. Below this the diameter decreases gradually to its minimum of about 8 $\mu$ at the waist approximately two-thirds of the way down the palisade. Finally the diameter again increases fairly uniformly to about 11 $\mu$ at the bottom. The lateral profiles of the palisades in Figs. 2 and 26 show the variations in diameters at the different levels in non-feeding organisms. The shape and diameters of the palisade change during feeding.

Each rod has a maximum, fairly uniform breadth of about 2.5 $\mu$ at levels between the annuli. Above the bottom of the fibrous annulus each rod tapers to a final breadth of about 1.5 $\mu$; below the top of the dense annulus rod breadth decreases abruptly at first and then each rod tapers gradually to a point at its bottom (Figs. 3, 18). Each rod has a maximum thickness of about 0.9 $\mu$ at its top and tapers gradually below this to a thickness of about 0.7 $\mu$ at its bend. Rod thickness does not vary appreciably between the bend and the dense annulus but decreases gradually below this, each rod tapering to a slender point at the bottom of the basket (Fig. 24) where the rods extend below the bottom of the sheath (s) for about 2 $\mu$ and splay apart slightly (Figs. 2, 3). At this level, the rod lamellae (x) often extend around the inner and outer surfaces of the rods as well as the left sides (Fig. 19).

The majority of rod microtubules are packed hexagonally at most levels; adjacent tubules, centre-to-centre spacing about 360 $\AA$, are usually linked by densely staining cross-connexions of approximate thickness 60 $\AA$ (Fig. 6, r). Longitudinal sections show the precise parallel arrangement of the tubules (Fig. 7); lightly stained regions occur in the spaces between tubules and apparently unite their walls. The lengths of these regions range between about 500 $\AA$ and 0.3 $\mu$; they are separated longitudinally by distances ranging between about 500 $\AA$ and 0.1 $\mu$. Fine cross-bridges, linking rod tubules, have also been detected in longitudinal sections, but they occur much less frequently than the lightly stained regions and are apparently not sufficiently numerous to account fully for the density and frequency of the cross-connexions seen in transverse sections. Therefore, rod tubules are probably mainly linked by long sheets of...
cross-connecting material, which have a lightly stained appearance in longitudinal sections, but when viewed along their lengths in transverse sections appear to be nearly as densely stained as the walls of the tubules.

Densely staining material occupies some of the interstices between rod tubules (r) in a region near the centre and outer surfaces of each rod at the lowest level at which the material occurs, a level approximately half-way between the annuli (Fig. 15). Progressively more of the interstices are filled with dense material as the top of a rod is approached, until about 2 μ from the top almost all the interstices are occluded by it (Figs. 3, 17, r). Differences in the packing of the tubules, compared with the hexagonal arrangement lower down, are spatially correlated with the presence of dense material. In addition, tubules are packed differently in different regions where the dense material occurs; they are packed irregularly near the outer surface, and approximately rectilinearly in the centre and near the inner surface of the top of each rod (Fig. 17). The rods gradually shorten and finally disappear as they disintegrate from their bottoms upwards when organisms are mixed with detergent. Under these conditions, the cohesion of rod tubules is apparently greatest where the tubules are embedded in dense material because the rate of shortening is lowest in the region where the dense material occurs.

A dense cap is situated on the outer portion of the top of each rod; the caps are mainly composed of densely staining material which is slightly less dense than the interstitial material in the rods and apparently does not enclose any tubules. Caps (p) appear crescent-shaped in transverse sections of the basket (Fig. 20); longitudinal sections show that each cap is plano-convex with the convex surface facing upwards (Figs. 3, 25). The flat lower surface is separated from the top of the adjacent rod by a distance of about 200 Å which is traversed by numerous dense strands of variable thickness.

**Sheath and dense annulus**

The breadth of the sheath (s) is fairly uniform, about 0.5 μ, between the waist and the dense annulus (d), but decreases gradually from the waist downwards to the bottom of the sheath, which is about 2 μ above the bottom of the palisade (Fig. 3).

Transverse sections of the sheath show that its microtubules are less regularly packed than those in the rods. They also show that cross-connexions between adjacent tubules are less frequent, less dense and less regularly arranged than those in the rods (Fig. 6). The thickness of the cross-connexions ranges from about 40 to 80 Å. Numerous cross-connexions are also apparent in longitudinal sections of the sheath; they are apparently spaced irregularly at intervals ranging from about 100 to 900 Å, and project at a variety of angles from the walls of the tubules (Fig. 8). Longitudinal profiles of the walls of sheath tubules are not as straight and smooth as those of rod tubules. Adjacent sheath tubules run more or less parallel to one another but the distances between them do not remain as constant as the distances separating adjacent rod tubules (Fig. 8; compare Fig. 7). Each sheath tubule slopes, for most of its length, less steeply from top right to bottom left than adjacent rods and spirals around the outside of the palisade.
The rods splay apart everywhere except at their tops when the basket is squeezed out of the organisms or when organisms are mixed with detergent (Fig. 24). The structural integrity of the sheath apparently breaks down more rapidly than that of the rods under these conditions. This is further evidence, in addition to the fine-structural differences described above, that the composition of the sheath differs from that of the rods.

Thick Araldite sections stained with methylene blue show that the dense annulus forms a complete circle around the palisade. It is mainly composed of densely staining material in the interstices between the tubules at the top of the sheath (Figs. 11, 12, d). This material has the same appearance as the dense material in the tops of the rods (Fig. 12; compare Fig. 17). The annulus (d) consists of alternate broad and narrow segments; the broad segments are positioned between the sides of the rods and the narrow segments pass around their outer surfaces (Figs. 9, 12). Thus the bulk of the annulus lies between the rods, where it has a breadth of about 0.5 μ and a depth of about 1 μ. The bottom of the annulus is fairly flat but the top is serrated and the annulus is deeper near the sides of the rods (Figs. 4, 11).

Tubules emerge from the top of the annulus (d), converge towards, and form part of adjacent rods (r), in which they exhibit all the detectable characteristics of the other rod tubules (Fig. 11). Since the total number of rod tubules below the annulus plus the total number of sheath tubules is about the same as the total number of rod tubules above the annulus, the tubules of the sheath appear to consist exclusively of continuations of some of the rod tubules. Sequences of longitudinal and transverse sections...
through the dense annulus indicate that the tubules which pass through the annulus bend away from the sides of the outer portions of the rods and contribute to the segments of the sheath positioned between the rods (Fig. 4). Tubules from the centres of the outer portions apparently contribute to the segments immediately adjacent to the outer surfaces of the rods. These tubules do not bend so far from their longitudinal paths in the rods to reach their positions in the sheath; some of them retain the characteristics of rod tubules down to a level below the annulus and do not pass through it (Fig. 4).

**Crests**

The number of crests (c) is equal to the number of rods (r) in a basket (Fig. 9). The bottoms of the crests are near the waist. Each crest projects outwards for increasingly greater distances at higher levels (compare Figs. 6 and 12), reaching a maximum breadth of about 3 µ at a level approximately half-way between the annuli (Fig. 3). The inner surfaces of the crests (c) are attached to the outer surface of the sheath (s) below the dense annulus (d) and to the outer surfaces of the rods (r) above it (Figs. 2, 3, 6, 15). The crests are thickest near their inner surfaces and taper gradually towards their outer surfaces (Fig. 15). Below the dense annulus each crest and its tubules slope from top right to bottom left, but less steeply than adjacent rods, and follow a left-handed helical path around the outer surface of the sheath (Fig. 2). Above the annulus most of the crests bend outwards to their left (Fig. 15); from the annulus to a level about 10 µ below it they bend outwards to their right (Fig. 12). Then the bending reverses once again and they bend to their left just above the waist. Below this they are so small that no bending is apparent (Fig. 6).

Crest microtubules are often linked by cross-connexions which are more regularly arranged than those in the sheath. Packing of crest tubules varies between regular hexagonal arrangements and irregular distributions of unevenly spaced tubules (Figs. 6, 12, 15, 16, e). The cross-connexions and walls of the tubules closely resemble those in the sheath but tubules are usually more widely spaced in the crests. Small dense granules of irregular shape and distribution, with diameters ranging between about 50 and 100 Å, are situated between the tubules, often apparently in contact with the cross-connexions (Fig. 16). Similar granules occur less frequently in the sheath.

Most of the tubules curve inwards at the tops of the crests (c), pass through the fibrous annulus (f), and contact the outer surfaces of the rods (r) (Figs. 3, 13). Where they pass through the annulus the interstices between them are filled with dense material which is continuous with the dense material in the tops of the rods (Figs. 3, i; 17). Certain tubules continue straight upwards from along the whole breadth of each crest. They are more compactly arranged in bundles (g) above the tops of the crests (Figs. 13, 14); these ciliary connectives fuse with the proximal ends of certain basal bodies (Figs. 3, 13). Most of these basal bodies are packed together rectilinearly to form part of groups of cilia (Fig. 20, k) which in turn form part of the ciliary fringe (Fauré-Fremiet, 1959). One end of the fringe (k) is close to the oral opening (o) (Figs. 3, 25, 36). Most of the ciliary connectives join to cilia near this end of the fringe but some of them attach to other cilia bordering the oral opening (Fig. 3).
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Lamellae

Three distinct types of lamellae are associated with the basket: rod lamellae, subcytostomal lamellae and cytostomal lamellae. Each lamella consists mainly of a single straight row of microtubules in which adjacent tubules are closely apposed. The longitudinal axes of the tubules are parallel to the longitudinal axes of the lamellae.

Usually a single rod lamella (x) is connected to the left side of each rod by an uneven layer (j) of dense material (Figs. 6, 7). The lamellae extend from the bottoms of the rods to a level just below that at which the tops of the rods are enclosed by the fibrous annulus. Adjacent tubules are so closely apposed that their centre-to-centre spacing is usually the same as their diameters.

The top of each subcytostomal lamella (y) is attached to the inner end of a thickened corrugation of the collar (t), where there is a small process (n) dipping downwards and outwards for a short distance into the lumen (Figs. 3, 25). Each process is composed of two dense pericytostomal flanges; the space between the flanges (n) is mainly occupied by the top of a subcytostomal lamella (y) (Figs. 21, 23). The longitudinal axes of the lamellae and their tubules are aligned with the longitudinal axis of the basket at this point of contact with the collar. The lamellae (y) and their tubules bend gradually towards the periphery of the basket down to a level about 5 μ below the collar until they slope at an angle of about 60° to the longitudinal axis of the basket (Fig. 3). Each lamella (y) extends downwards and outwards below this and passes closely alongside a rod lamella (x) (Figs. 15, 17). Subcytostomal lamellae (y) eventually emerge outside the palisade, continue straight downwards and outwards near the left sides of the crests (c) and terminate near the level of the dense annulus (d) (Figs. 3, 25). The walls of adjacent tubules are sometimes in direct contact as in the rod lamellae; equally often they are separated by a distance of about 80 Å and linked by cross-connexions of approximately 50 Å thickness.

Each cytostomal lamella (z) is also attached to the inner end of a corrugation (t) but at a point which is just interior to and above the top of the subcytostomal lamella (y) connecting to the same corrugation (Fig. 3). Thus there is an equal number of corrugations, subcytostomal lamellae and cytostomal lamellae in the basket; the range of these numbers is at least 60–90 in different individuals. The longitudinal axes of these two lamellar systems and their tubules are at right angles where they meet the corrugations (Fig. 23). The cytostomal lamellae (z) are positioned immediately underneath the cytostomal membrane (m) (Figs. 23, 25) and radiate from the centre of the cytostome to the inner ends of the corrugations (t) (Fig. 21). The centre-to-centre spacing of adjacent tubules is about 300 Å and their walls are apparently connected by dense material in the spaces between them.

Fibrous annulus

The fibrous annulus (f) encircles the top of the palisade and covers the sides and surfaces of individual rods (r) near their tops (Figs. 2, 20). It is about 4 μ deep at its periphery but becomes gradually shallower as it extends inwards beneath the collar nearly as far as the inner ends of the thickened corrugations (t) (Figs. 3, 25). The
bottom of the annulus (f) is divided into discrete segments separated by the rods (r) and crests (c) (Fig. 17). The annulus is mainly composed of fine fibres which are either loosely packed or closely apposed in dense bundles (Fig. 17). The diameters of individual fibres range between about 40 and 90 Å; sections give no clear indication of their lengths but show that most of the fibres and bundles follow approximately circular courses around the annulus (Fig. 20).

Collar, cytostome and oral atrium

The collar, cytostome and oral atrium are modified regions of the pellicle, which is a complex multi-layered structure over most of the surface of the organism. An inner dense layer consists of at least seven distinct laminae of different densities and thicknesses. A vesicular layer (h), composed mainly of a single layer of closely packed vesicles, is usually positioned between the dense layer (e) and the cell membrane (Fig. 25). Each vesicle contains an irregular reticulum of densely staining material and is bounded by a unit membrane. The vesicular layer is not apparent in living organisms but as soon as a fixative is applied a translucent surface layer appears which is visible with the light microscope. Hence, the vesicular layer is probably a thin compact layer in living organisms which swells and becomes distorted during fixation.

The inner surface of the cell membrane is closely applied to the dense layer, the vesicular layer being absent, at the sides (w) of the oral atrium (a) where the dense layer is thicker than in most other portions of the pellicle (e) (Fig. 25). The dense layer (e) is folded where it forms the lips of the oral opening (o); the folds run down the sides of the atrium (a) for about 1 μ and also radiate outwards from the perimeter of the opening for about 2 μ across the ventral surface of the organism (Fig. 25). Microtubules also radiate, for about 3 μ, from the perimeter of the opening. Most of the tubules form a single layer just beneath the dense pellicular layer; they are irregularly spaced and are apparently not cross-connected. An annulus of fine fibres encircles the perimeter of the opening; the diameter of individual fibres ranges between about 40 and 90 Å.

The collar (t) and cytostome (m) form the dome-shaped floor of the oral atrium (a) (Figs. 21, 25). The thickened corrugations of the collar are specialized portions of the pellicular dense layer; in cross-section each corrugation has a hook-shaped profile. The arms of the hook point downwards; the arm on the right side of a corrugation is usually about half the length of the arm on the left side and curves towards it along most of the length of the corrugation (Fig. 23). At the inner ends of the corrugations the arms are of approximately equal length and it is these which project downwards and outwards to form the two pericytostomal flanges (n) of each corrugation (Fig. 23). The cell membrane covers the corrugations and lines the furrows between them (Fig. 23).

The cytostomal membrane (m) is the portion of the cell membrane which covers the cytostome, where the membrane is the only pellicular layer separating the cytoplasm in the lumen of the basket from the external environment (Figs. 23, 25). The membrane is slightly invaginated at the centre of the cytostome (Figs. 3, 21). Its structure is
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apparently identical with that of the triple-layered unit cell membrane of a wide range of cells (Robertson, 1959).

Cytopharyngeal vesicles

The vesicles are mainly concentrated in the lumen and in the immediate environs of the basket, although a few apparently identical vesicles are distributed sparsely and apparently at random throughout the remainder of the cytoplasm. They are about \(0.2-0.4\) \(\mu\) in diameter, are bounded by a unit membrane and contain an irregular reticulum of densely staining material (Fig. 12, v).

Feeding

Nassula comes to rest with the top of its basket closely alongside an algal filament before feeding. The distance between the tops of the rods and the filament is much too small for the top of the basket to be at the bottom of the oral atrium; the atrium probably evaginates because the top of the basket is at the summit of a small pellicular bulge which rises above the surrounding pellicle.

Once the top of the basket is closely applied to the filament the basket performs a sequence of feeding movements. In the first feeding movement the palisade becomes elliptical in cross-section as the tops and bottoms of the rods bordering the longer sides of this ellipse become more widely spaced (Figs. 5B, 33-35). Simultaneously these rods slope less steeply from top right to bottom left than they do in the resting baskets of non-feeding organisms (Fig. 5B; compare Fig. 5A). Constriction of the mid-level of the palisade raises and narrows the waist (Figs. 5B, 27). The tops of the rods at the ends of the ellipse move apart slightly and move downwards and outwards (Figs. 5B, 27), but remain less widely spaced than those at the sides. The major axis of the elliptical transverse profile at the top of a palisade which is in the first feeding position is always parallel to the longitudinal axis of the algal filament (Fig. 35), although the filament may be oriented at any angle to the longitudinal axis of Nassula and is still outside the organism at this stage.

A hemispherical hyaline extrusion bulges out of the cytostome as the first feeding movement is completed and engulfs the filament (u) where it lies across the top of the basket (Fig. 32). Occasionally the filament is released without ingestion proceeding further; the portion which was surrounded by the extrusion appears darker (phase contrast) than the remainder of the filament (Fig. 31).

The first feeding position is usually maintained for up to 10 sec, until the filament bends into a hair-pin shape as it moves down the lumen. Simultaneously the second feeding movement occurs; the rods become more widely spaced near the mid-level of the palisade, which dilates, and they also slope more steeply until they are nearly parallel to the longitudinal axis of the basket (Figs. 5C, 29). In this second feeding position, which is usually maintained for less than 1 sec, the basket is still elliptical in cross-section and the perimeter of the palisade is greater at all levels than in the resting basket, therefore the annuli and sheath are extensible. The third feeding movement begins as soon as the bent portion of the filament has passed through the lumen; the palisade again becomes circular in cross-section and constricts at all levels, pressing the
two strands of the filament together. In this *third feeding position* the tops of the rods are less widely spaced, and are closely positioned around the two strands of the filament, but their bottoms are more widely spaced than in the resting basket (Fig. 5D; compare Fig. 5A).

Occasionally *Nassula* encounters a tip of a filament with the top of its basket. Then

![Diagram of Nassula feeding positions](image)

Fig. 5. A series of scale drawings showing the arrangements of the rods (black) in the near side of a basket (lateral view, top uppermost) as they appear in living *Nassula* at different stages in the ingestion of an algal filament. The basket is elliptical in cross-section in B and C and orientated with the major axis of the elliptical cross-section at any level in the plane of the drawing. The shapes and positions of the portion of the filament in direct contact with the basket are also shown.
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the tip is drawn into the lumen and the filament initially enters the basket as a single strand; it is not bent into a hair-pin shape as it is when ingestion starts from somewhere along its length. When ingestion begins from one end of a filament, the basket passes from its resting position directly to the third feeding position by constricting slightly at all levels above the dense annulus and dilating at levels below it, omitting the first and second feeding movements.

No movement of the rods has been detected while the filament enters Nassula once the palisade is in the third feeding position, except when the shorter arm of a bent filament has passed through the basket. Then the palisade dilates slightly at all levels below the dense annulus and constricts at levels above it until its top is again closely applied around the remaining longer uningested arm of the filament (Fig. 28). This position is maintained for several minutes while the remainder of the filament is drawn in as a single strand. The tip of the last portion of the filament to enter the basket moves all the way down the lumen and stops moving only after it has passed through the basket. Then the fourth feeding movement occurs; the palisade dilates at all levels above the dense annulus and constricts at all levels below it as the rods return to their resting positions. The rods seem to be fairly rigid as changes in their shapes or lengths have not been detected when the feeding movements occur or at any other time during ingestion. Each of the four feeding movements is usually undertaken in about 1 sec.

The first portion of the filament to be ingested emerges from the lumen and proceeds across Nassula until it strikes the pellicle on the opposite side of the organism, the dorsal surface, which bulges outwards momentarily at the point of contact. The end of the loop of the hair-pin-shaped filament slides around underneath the pellicle towards the posterior end of Nassula and continues to curve around towards the oral surface again (Fig. 30). The filament may make up to three complete circuits around the inside of the pellicle as it is drawn in. It is often contained as a plane coil deforming the shape of Nassula, which is approximately a prolate spheroid before feeding, to a disc with convex sides.

Nassula may ingest a length of up to 1.6 mm of a single filament, about 8 times its own length, in periods ranging from about 4 to 15 min. Shorter lengths are ingested in correspondingly shorter ranges of time. The filament usually enters more rapidly, up to 20 μ/sec, at the beginning of ingestion than during the final stages, usually less than 7 μ/sec. It often stops moving completely, sometimes for a few seconds and at other times for as long as 2 min, and then continues inwards. There are sometimes as many as six pauses before ingestion finally stops. The rods remain in the third feeding position during the pauses and frequently the filament uncoils slightly and slips out through the cytostome for a short distance. If filaments are released shortly after ingestion by squashing and bursting Nassula, they immediately straighten out, demonstrating that they elastically resist coiling.

A long filament is usually not ingested completely; when ingestion finally stops, Nassula swims in small circles until the tip, or tips if the uningested portion is double-stranded, of the filament tangles in clumps of other filaments. Nassula bends the filament by swimming for a short distance and then permits it to straighten elastically. This is repeated and the pendular motion continues until the filament breaks, usually
somewhere in the lumen, probably as a result of the combined effects of stress and the preliminary action of digestive enzymes. The portion of the filament distal to the break slips out of the top of the basket; the other portion which is still attached to the ingested coils moves down the basket and out of its bottom. The basket remains in the third feeding position throughout these manoeuvres until portions of the filament no longer occupy the lumen, then it returns to its resting position.

It has not been ascertained whether the freshly ingested intact filament is contained in a membrane-bounded sac as it enters, or immediately after it enters, or whether it lies 'naked' in the cytoplasm until it is enclosed in several discrete food vacuoles. Each filament consists mainly of cells arranged end to end in a single linear array (Fig. 34). Portions of the coiled filament start to break at several points, apparently by the separation of adjacent cells, about 3 min after entering *Nassula*. Most of the cells have lost their linear arrangement about 10 min after entry and are grouped together in spherical clumps which are distributed throughout the cytoplasm; each clump is apparently contained in a food vacuole. Electron microscopy of starving *Nassula* indicates that digestion of intracellular structures is not completed for several hours.

**DISCUSSION**

**Feeding movements**

Dragesco (1962) described the ingestion of algal filaments by *Nassula ornata* and noted several of the features described above, but did not mention any movement of the rods; he concluded that the basket did not seem to play an active role in ingestion.

Bending of the filament into the lumen indicates that while the basket is in the first feeding position, and throughout the periods of inward movement of the filament, a force (the suction force) is acting on the filament tending to draw it down the lumen. The tops of those rods which move outwards and downwards during the first feeding movement might be pushed to their new positions by the suction force. However, during at least part of the time that the first feeding position is maintained, the hyaline extrusion bulges out of the cytostome, suggesting the absence of a suction force at this time. If this suggestion is correct, the first feeding movement would appear to be an intrinsic accomplishment of the basket.

Shortening of the crests would probably pull the tops of the rods downwards and outwards because the tops of the crest tubules curve inwards and fuse with the tops of the rods. The increasing distances separating the tops of the rods along the long sides of the elliptical transverse profile of the palisade (Fig. 35) might result from stretching of the fibrous annulus along these sides when the rods at the ends of the ellipse move outwards. The rods are apparently fairly rigid and hence such displacements of their tops must alter the positions of the rods along their entire lengths and may be mainly responsible for the observed changes in the shape and diameters of the palisade at lower levels. For example, the rods will pivot on the dense annulus and their slope will change as observed when they become more widely spaced in the upper part of
the basket if the dense annulus offers greater resistance to stretching than the fibrous
annulus. Contraction of any component or components of the basket other than the
crests could probably not produce the first feeding movement. It is difficult to see how
individual rods could propel themselves to their new positions as their shapes remain
unaltered. Rods become more widely spaced at the top and bottom of the basket, but
contraction of either of the annuli would draw them together in the upper part of the
basket; contraction of the sheath would draw them together at all levels below the
dense annulus because sheath tubules spiral around the outside of the palisade. Since
the crests also spiral around the palisade below the dense annulus but do not extend
below the waist, their contraction would constrict the palisade between the dense
annulus and a level near the waist as observed, but it would not prevent the rods from
becoming more widely spaced at the bottom of the basket.

The algal filament must touch some of the cilia bordering the oral opening when it
lies across the opening and while the rods move to, and stay in the first feeding position.
The particular cilia contacted will depend on the orientation of the filament. Perhaps
they respond to contact or deformation, as suggested for kinocilia in the vertebrate
labyrinth (Lowenstein & Wersäll, 1959), and transmit some form of excitation through
the ciliary connectives to particular crests. If connectives make appropriate connexions
between cilia and crests, so that when particular cilia or groups of cilia contact the
filament only certain crests in two regions on opposite sides of the basket are stimulated
to contract, this might produce an elliptical arrangement of rods at the top of the basket
with its major axis parallel to the longitudinal axis of the filament.

Omission of the first feeding movement when *Nassula* starts ingestion at one end of
a filament is in agreement with the interpretation outlined above, because the longi-
tudinal axis of the filament is always nearly perpendicular to the oral surface of *Nassula*
so that the filament does not touch the cilia around the oral opening. Omission of the
first and second feeding movements under these circumstances reveals that they are
not essential for the generation of the suction force and indicates that they are mainly
related to the bending of the filament. The first feeding movement is apparently an
active movement of the basket, but no active change by the basket need be assumed
for the second feeding movement, which can be accounted for by the rods being pushed
to the second feeding position by the bent portion of the filament as it moves down the
lumen (Fig. 5 C).

The function of the hyaline extrusion may be to initiate digestion of the portion of
the filament it encloses and thereby reduce the rigidity of this portion of the filament
until the suction force can fold it against the cytopharyngeal wall and pull it into the
lumen. Constriction of the palisade at all levels during the third feeding movement,
when the bent portion of the filament has passed out of the lumen, may be partly due
to elastic recoil of the annuli and sheath. However, constriction of the top of the pali-
sade until its diameter is less than in the resting basket shows that the third feeding
position is not only the result of elastic recoil. A sphincter-like contraction of the
fibrous annulus would constrict the top of the palisade.

Complete cessation of suction cannot alone account for the pauses in the inward
movement of the filament because the elasticity of the filament would then result in
uncoiling and reverse movement outwards. Although the filament sometimes briefly slips outwards for a short distance it usually stops moving completely during the pauses, which often last for several minutes, when conceivably the elastic restoring force of the filament could be exactly balanced by the suction force. However, such an exact balance of forces need not be assumed. The top of the palisade is closely applied to the filament when the basket is in the third feeding position (Fig. 28). The tightness with which the top of the palisade is clamped around the filament may be subject to variation by the action of the fibrous annulus and might suffice to prevent uncoiling of the filament if suction decreases or ceases altogether during the pauses. While the filament is moving inwards, the fibrous annulus may be poised, partly constricted, with an internal diameter just sufficient to permit the filament to pass through it, but ready to clamp the top of the palisade around the filament as soon as the suction force decreases. The decrease in the rate of entry of the filament as ingestion proceeds may be mainly due to the increasing frictional resistance of the increasing length of coiled filament to its continued movement around the inside of the pellicle while pressed against it. The pauses in ingestion may allow additional time for enzymes to reduce the rigidity of the ingested portion of the filament, and to break it into short lengths, so that there is less resistance to further inward movement. Algal cells start to separate about 3 min after they enter Nassula, indicating that digestive enzymes are either rapidly synthesized or are present in considerable amounts prior to feeding. In the latter case, these enzymes may be stored in the cytopharyngeal vesicles, which are structurally similar to vesicles resembling primary lysosomes and exhibiting acid phosphatase activity in the hymenostome Tetrohyemenia (Elliot, 1965).

The palisade dilates at all levels above the dense annulus and constricts at all levels below it during the fourth feeding movement; this might be mainly due to elastic recoil of the sheath and/or one or both annuli. The diameter of the palisade remains unchanged only at the level of the dense annulus during this movement. The rods apparently act as rigid levers and pivot on the dense annulus; their tops swing outwards and their bottoms inwards. This should result in greater changes in the diameters of the palisade at the bottom than at the top since the annulus is nearer the top, and this is what has been observed. The arrangement of the rods in the third feeding position can be similarly interpreted; constriction of the fibrous annulus draws the tops of the rods inwards so that their bottoms swing outwards. This increases the diameter of the lumen at the waist, which would otherwise not be great enough for two strands of the filament to pass through simultaneously.

The crests and fibrous annulus are antagonists if the interpretations suggested above are correct; contraction of the crests dilates the top of the basket and constricts the waist while contraction of the fibrous annulus has the opposite effect.

Suction

The feeding movements of the basket occur just before and just after, but not during the main period of inward movement of the algal filament; thus there is no evidence for the performance of a sustained 'gobbling' action by the basket.
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*Nassula* is often stationary while it is feeding so that it cannot be pushing the filament into itself by swimming on to it. Fauré-Fremiet & Dragesco (1949) considered the possibility that surface tension might be responsible for driving filaments into the cyrtophorine *Nassulopsis*. Kitching (1952) and Hull (1961) both suggest that the suction force applied by the suctorian *Podophrya* is due to a general negative pressure inside the organism. However, the last tip of the filament to enter *Nassula* stops moving only after it has passed through the basket. Thus the filament moves about 60 μ after complete inclusion by *Nassula*. Therefore, the action of surface tension or a general negative pressure inside *Nassula* cannot be the factors responsible for filament movement at this time. As this movement is resisted by the elastic tendency of the filament to uncoil, it seems unlikely that it can be fully accounted for by the momentum of the filament at the moment its tip enters *Nassula*. Cessation of filament movement when the filament has passed through the basket indicates that the suction force only acts on the filament while it is in the basket.

Suction might be generated by a stream of cytoplasm actively directed down the lumen and replaced at levels above the dense annulus by cytoplasm flowing into the lumen through the slits between the rods. Small cytoplasmic particles usually cannot be detected near the basket of living feeding *Nassula* examined by phase-contrast microscopy. This is mainly because the rods and filament are much more refractile than such particles. Hence it has not been possible to gain any indication of cytoplasmic streaming in the immediate vicinity of the basket during ingestion by following the movement of small particles. To account for the movement of prey cytoplasm down the tentacles of the suctorian *Tokophrya*, Rudzinska (1965) has suggested that waves pass down the microtubular walls of the inner tubes of the tentacles. However, waves apparently do not pass down the rods or sheath of the basket in *Nassula*, because the sheath is in contact with the rods and no movement of the rods has been detected during the majority of the period of ingestion.

Fine structure and function

Crest microtubules are embedded in dense material and fused to the tops of the rods; this would seem to preclude contraction of crests by sliding of crest tubules over one another but not by shortening of tubules. However, the gradual decrease in the breadths and thicknesses of the crests below the dense annulus might be due to interdigitation of the lower ends of their tubules between those of the sheath. Shortening of the crests might then result from sliding of their tubules between those of the sheath. Sheath tubules presumably do not slide over each other as their tops are embedded in the dense material of the dense annulus, but crest tubules might slide down between the static ones of the sheath as they pull the tops of the rods downwards. Cross-connexion of tubules in the crests and sheath is similar, but the cross-connexions are apparently not as regularly spaced and as frequent as the cross-bridges linking myofilaments in striated muscle (Huxley, 1957).

The numerous sheets of cross-connecting material which link tubules in the apparently rigid rods might provide considerable resistance to sliding or shearing of
the tubules. Interpretation of the structure and function of the rods is further aided by comparison with the human femur. The latter, like a cytopharyngeal rod, is a rigid column bent inwards near its top. At certain times, the line of action of the load transmitted through each is directed downwards and slightly outwards; the load being due to the weight of the trunk for the femur and due to contraction of crests for rods. Koch (1917) concluded that the greatest shearing stresses occur in the head and neck of the loaded human femur. Dense intertubular material occurs in the corresponding portion of a rod, between its top and its bend, and might bind tubules together and increase resistance to shearing of tubules on each other. The dense annulus has a similar composition and, as discussed above, there are indications that this is also a region of rigidity where considerable shearing stresses occur. The function of the dense material between crest tubules at their points of application to the tops of the rods can be similarly interpreted and suggests an analogy with the tendonous attachments of skeletal muscles to bones in the vertebrates.

Direct continuity between sheath tubules and some rod tubules implies that the wall of an individual microtubule can have a different structural appearance at different points along its length and that the packing, cross-connexion and cohesion of microtubules may vary along their lengths. Behnke & Forer (1967) consider the possibility that the differences between microtubules in spermatids of the crane-fly Nephrotoma may not reside in the walls of the tubules but in the regions immediately surrounding the walls. Variations in the structural appearances, packing, cohesion and suggested functions of microtubules in the basket are often spatially correlated with the presence of substances with different appearances in the spaces between the tubules, such as cross-connexions and the dense intertubular materials.

Fibres with an appearance and thickness similar to those in the fibrous annulus occur in certain ascidian epidermal cells (Cloney, 1966), the spasmoneme of the peritrich Vorticella (Randall & Hopkins, 1962) and vertebrate smooth muscle (Panner & Honig, 1967). Like the fibrous annulus, these cells or parts of cells sustain prolonged contractions. By contrast, microtubules are often situated in parts of cells which exhibit brief but frequent movements; for example, the axostyles of certain flagellates (Grimstone & Cleveland, 1965) and cilia and flagella. The duration of the contraction apparently maintained by the microtubular crests of the basket is brief compared with that suggested for the fibrous annulus. Insect fibrillar flight muscle performs extremely brief and frequent contractions; correlated with this, the thick myofilaments appear tubular in cross-section (Auber, 1962; Shafiq, 1963; Ashhurst, 1967). While fine fibres are often spatially associated with prolonged contractions and thicker tubular fibres with more rapid and more frequent contractions, this does not necessarily imply a detailed similarity of all those contractile mechanisms where mainly tubular fibres are present or of all those where mainly fine fibres occur.

This study of the cytopharyngeal basket of Nassula indicates the extent to which the several components of a large and complex organelle may be specialized to perform different but co-ordinated functions. As noted above, some basket components structurally resemble certain individual cytoplasmic components and organelles occurring in a wide range of other cells and, in addition, some of the suggested functions of
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Basket components, inferred from feeding movements, are similar to those suggested by other investigators for the structural counterparts of basket components in other cells. Such correlations of structure and function indicate that studies of large, complex and relatively accessible organelles of protozoans may be pertinent to our understanding of organelles in general.

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REFERENCES


PLATES

Unless otherwise stated, all figures are electron micrographs of Nassula fixed with glutaraldehyde, post-fixed with osmium and stained with uranyl acetate and lead citrate. All micrographs of cross-sections of the basket show components of the cytopharyngeal wall as they would appear to an observer looking from the top to the bottom of the basket; where a portion of the wall is shown, the lumen is always towards the bottom of the micrograph, so that the inner surface of the wall is nearest the bottom of the micrograph and the left sides of components of the wall are the sides nearest the left sides of the micrographs. Figs. 6, 9, 14, 15, 17, 19 and 20 are micrographs of cross-sections from a sequence through the same basket. In phase-contrast micrographs, baskets shown in lateral view are top uppermost; all phase-contrast micrographs have been taken using microflash.

Fig. 6. Part of the cytopharyngeal wall in cross-section near the waist showing the packing of the microtubules in the rods (r), rod lamellae (x), sheath (s) and crests (c). A layer of dense material (j) is positioned between each lamella and its adjacent rod.

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Fig. 7. Median longitudinal section of part of a rod cut at right angles to its breadth, showing the parallel arrangement of microtubules. The tubule (x) on the left side of the rod is part of the rod lamella; dense material (j) is situated between it and the adjacent rod tubule.

Fig. 8. Longitudinal section of part of the sheath, showing the fine structure and parallel arrangement of the microtubules which are linked by cross-connexions (arrows).
Fig. 9. Cross-section of a basket at the level of the dense annulus (d), passing through the top of the annulus in the upper right-hand quadrant of the micrograph. A crest (c) and a rod lamella (x) are attached to each rod (r).

Fig. 10. Lateral view of a basket in living *Nassula*, showing the position of the dense annulus (d). The lower part of the basket passes out of the plane of focus near the waist Phase contrast.
Fig. 11. Longitudinal section cutting part of the cytopharyngeal wall near its outer surface at levels near the dense annulus (d). Some microtubules (arrows) bend away from the closely packed assemblage of tubules in the rods (r) and enter the annulus. Tubules extending above the top of the sheath (s) slope towards adjacent rods in the annulus.

Fig. 12. Part of the cytopharyngeal wall in cross-section at the level of the dense annulus (d), showing the dense material between microtubules at the top of the sheath. Numerous cytopharyngeal vesicles (v) occur in the cytoplasm in the environs of the basket. A crest (c) bending outwards towards its right, a rod lamella (x) and a rod (r) are also shown.
Fig. 13. A section near the top of the basket and parallel to the longitudinal axis of the basket. Some microtubules at the top of the crest (c) curve inwards towards the top of the adjacent rod (r) at the level of the fibrous annulus (f). Other tubules form part of the ciliary connectives; they continue straight upwards (arrow) and at higher levels are grouped together in small bundles (g). The attachment of one of these to the proximal end of a basal body (b) is shown.

Fig. 14. A ciliary connective in cross-section. × 190,000.

Fig. 15. A rod and associated structures in cross-section at a level approximately half-way between the annuli and near the lowest level at which dense inter-tubular material occurs in the rod (r). Subcytostomal lamellae (y) are situated closely alongside the rod lamella (x). The crest (c) bends outwards towards its left and its inner surface is attached directly to the outer surface of the rod. There is apparently no barrier to cytoplasmic movement or diffusion between the lumen and the outside of the basket through the slits (asterisks) between adjacent rods.

Fig. 16. Part of a crest in cross-section showing the arrangement of its microtubules, cross-connexions and small dense granules (arrows). × 170,000.
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Fig. 17. A rod ($r$) in cross-section about 2.5 μ below its top, showing the dense material between most of its microtubules. Some tubules are packed approximately rectilinearly in the lower half of the micrograph; they are all irregularly packed near the outer surface of the rod in the upper half of the micrograph. A subcytostomal lamella ($y$) lies closely alongside the rod lamella ($x$). Microtubules of the crest ($c$) are cut in oblique longitudinal section because they curve in towards the outer surface of the rod at this level. They are embedded in dense material (arrow) where they pass through the fibrous annulus ($f$) and then pass out of the section. Other sections show that this dense material is usually continuous with the dense material in the rods.

Fig. 18. Lateral view of a rod (top uppermost) in a basket squeezed out of Nassula; the rod is slightly shorter than its original length because under these conditions rods disintegrate from their bottoms upwards. Variations in the breadth of the rod at different levels and the bend at its top are shown. An abrupt change in breadth (arrow) occurs at a level corresponding to that of the dense annulus.

Fig. 19. Cross-section of a rod ($r$) within a few microns of its lower extremity and below the bottom of the sheath. The rod lamella ($x$) extends around the inner and outer surfaces of the rod as well as its left side. $\times 110000$. 

Fig. 20. Cross-section of a basket just below its top at the level of the fibrous annulus (f) cutting through the tops of some of the rods (r), the outer ends of some of the thickened corrugations of the collar (t), the lower edge of the oral atrium (a), the radially arranged subcytostomal lamellae (y) and some of the caps (p) on the tops of the rods. The rectilinearly packed group of cilia and basal bodies (k) are part of the ciliary fringe.
Fig. 21. Cross-section of the top of a basket at the level of the collar showing its thickened corrugations (t) radiating from the perimeter of a circular region, the cytostome, the centre of which is slightly invaginated (arrow). The section cuts more deeply into the collar in the upper half of the micrograph, where it passes through the tops of some of the subcytostomal lamellae (y); each lamella is positioned between two pericytostomal flanges (n). The section cuts through some of the radially arranged cytostomal lamellae (z) in the lower half of the micrograph. A dense pellicular wall (w) forms the sides of the oral atrium (a).

Fig. 22. Lateral view of the top of a basket showing the position of the thickened corrugations of the collar (t) on top of the circular palisade of rods and the bend in each rod (arrows) near its top. Detergent treatment and phase contrast.
Fig. 23. Longitudinal section through part of the top of a basket at the level of the collar, cutting transversely through the inner ends of some of its thickened corrugations (t) where they border the cytostome. The oral atrium (a) and the uppermost portions of the basket are towards the left of the micrograph. A unit membrane (arrows) covers the corrugations. The tops of the outer ends of the cytostomal lamellae (z) are situated inside the pellicular thickenings of the inner ends of the corrugations. The top of each subcytostomal lamella (y) is positioned between a pair of pericytostomal flanges (n). The microtubules of the cytostomal lamellae are cut transversely and those of the subcytostomal lamellae in oblique longitudinal section.

Fig. 24. A basket squeezed out of *Nassula*; the basket has split longitudinally into two nearly equal parts and flattened out. The rods are still held together at their tops but they have splayed apart below this level. They taper to slender points at their bottoms, where they are normally straight or slightly curved. The wavy shape is induced by the isolation procedure. Phase contrast.
Fig. 25. A median longitudinal section of the upper portion of a basket; the top of the basket is towards the left of the micrograph. The cytostome (m) is at the centre of the collar where the top of the basket is not covered by thickened corrugations (t). The section passes close to one side of the oral opening (o). The wall (w) at the sides of the oral atrium (a) is continuous with the dense layer (e) of the pellicle but unlike the latter it is not covered by the vesicular layer (b). This micrograph gives a good indication of the linear extent of the subcytostomal lamellae (y). The bottoms of the lamellae (l) are positioned near the crests (c) and pass closely alongside rod lamellae (x) at higher levels (z). They extend towards the centre of the lumen above this (j) and terminate near the pericytostomal flanges (n) at the top of the basket (4). Dense caps (p) on top of the rods (r), the dense intertubular material (i) in the tops of the rods, cytostomal lamellae (z) immediately underneath the cytostomal membrane, the fibrous annulus (f), the dense annulus (d) and the ciliary fringe (k) are also shown.
Figs. 26–29. Phase-contrast micrographs of four different baskets shown in lateral view in living Nassula. The baskets in Figs. 27 and 29 are elliptical in cross-section and oriented with the major axis of the elliptical cross-section at any level in the plane of the micrograph. All × 2000.

Fig. 26. Resting basket, showing the sloping arrangement of its rods; the level of the waist is indicated by the arrow.

Fig. 27. A basket in the first feeding position, showing the spreading of the rods at the top of the basket and the constriction of the palisade near the level of the waist (arrows).

Fig. 28. The top of a basket in the third feeding position; the top is constricted compared with its diameter in the resting condition. The algal filament is being ingested as a single strand.

Fig. 29. The upper half of a basket in the second feeding position; the rods (r) are almost parallel to the longitudinal axis of the basket. The bent algal filament is about half-way down the lumen; ingestion has started close to one end of the filament (arrow).

Fig. 30. Living Nassula starting to coil an algal filament which is entering through the top of the basket (arrow) as a double strand; the filament has deformed the shape of the ciliate until it is nearly circular in lateral profile. Phase contrast.
Fig. 31. Part of a living algal filament shortly after it lay across the top of a basket in a hyaline extrusion. The darker portion of the filament (arrows) had been surrounded by the extrusion. Phase contrast.

Fig. 32. Lateral view of the top of a basket in the first feeding position. The algal filament (u) is surrounded by a hyaline extrusion (arrow) from the cytostome where it lies across the top of the basket. Living Nassula, phase contrast.

Figs. 33–35. Phase-contrast micrographs of the tops of three different baskets viewed from above. Living Nassula, all × 2000.

Fig. 33. The circular arrangement of the tops of the rods in a resting basket.

Fig. 34. A transient stage during the first feeding movement as the tops of the rods start to become more widely spaced. Algal cells have a linear end-to-end arrangement.

Fig. 35. The rods are in the first feeding position. The major axis of the elliptical transverse profile of the top of the palisade is parallel to the longitudinal axis of the algal filament.

Fig. 36. Light micrograph of the ventral surface of Nassula impregnated with silver. The anterior end of the organism is towards the right of the micrograph and its left side is towards the bottom. The numerous small dots show the position of basal bodies. The larger but less intensely impregnated circular areas are probably regions where the pellicle has broken. The intensely impregnated areas (k) are regions occupied by groups of closely packed basal bodies attached to cilia of the ciliary fringe. The circular region (o), devoid of small dots and immediately anterior to one end of the fringe, is the site of the oral opening.