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Cephalopod Integument: The Ultrastructure of Kölliker's Organs and Their Relationship to Setae*

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Summary. Kölliker's tufts are transient epidermal bristles found on the external surfaces of late embryonic and juvenile octopods. The structure and growth of Kölliker's tuft is remarkably similar to that of polychaete setae. Each tuft is a fasicle of approximately 1500 distally tapered cannular rodlets located in an epidermal follicle composed of several lateral follicular cells and a single basal chaetoblast. The base of the follicle is associated with obliquely striated dermal muscle fibers. Together these elements comprise Kölliker's organ.

The rodlets, composed of longitudinally oriented filaments, are separated basally from one another by a layered meshwork of interstitial filaments. Microvilli on the apical concave surface of the scyphate chaetoblast insert into the base of each rodlet.

We infer that the tuft elongates by basal appositional growth and that glycoprotein secreted by the follicle cells is organized into filaments by the apical plasmalemma of the chaetoblast. Each microvillus serves as a template for the formation of a rodlet, therefore the number, size, shape, distribution and dynamic activity of the microvilli determine the morphology of the tuft.

Key words: Integument — Octopus — Setae — Secretion — Microvilli.

Introduction

The fine structure of epidermal bristles has been investigated in polychaetes (Bouligand, 1966, 1967; Gustus, 1973; Gustus and Cloney, 1973; George and Southward, 1973), echiuroids (Orrhage, 1971), pogonophorans (Gupta and Little, 1970; George and Southward, 1973; Orrhage, 1973) and brachiopods (Storch and Welsch, 1972; Gustus and Cloney, 1972; Gustus, 1973; Orrhage, 1973). These bristles, usually called setae or chaetae, are composed of longitudinally oriented filaments organized around longitudinal channels.

In polychaetes, each seta is secreted by an epidermal follicle composed of several lateral follicular cells and a single basal cell or chaetoblast. Apical microvilli on the chaetoblast are believed to act as templates on which the setae are cast. Growth is effected by the addition of material to the proximal (basal) parts of the setae. The specific shape of a completed seta and its contained longitudinal channels results from changes in the size, shape, number, distribution, and orientation of the microvilli on the surface of the chaetoblast during secretion (Gustus, 1973; O'Clair and Cloney, 1974).

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Among the cephalopod molluscs, members of the order Octopoda are unique in possessing cutaneous projections called Kölliker's tufts that in many ways resemble setae. These structures have been described from embryos of *Argonauta argo* (Kölliker, 1844; Joubin, 1892), juvenile *Octopus sp.* (Joubin, 1891) and *Bolitaena sp.* (Chun, 1902), older *Bolitaena diaphana* and *Eledonella* (Chun, 1904), *Octopus macropus*, *O. vulgaris*, *Scaeurgus*, *Ocythoe*, *Tremoctopus*, and *Eledonella* (Neaf, 1928). Boletzky (1973) has described some details of the fine structure of Kölliker's organs in *Octopus vulgaris* and *Eledone moschata* and has contributed important observations on their dynamic functions.

Kölliker's organs, each consisting of a tuft and associated ectodermal and mesodermal cells, are present in the integument of newly hatched octopods. The tufts are usually shed within a month (Boletzky, 1973).

Von Querner (1927) demonstrated with Schultze's reaction that Kölliker's tufts contain chitin. According to Hackman (1960), Rudall (1963), and Krishner (1973) chitin in skeletal elements is always associated with protein and is an example of a glycoprotein. It can also be thought of as a special form of glycocalyx according to the concept of H. S. Bennett (1969).

In this paper we describe the fine structure of Kölliker's organs in newly hatched *Octopus sp.*, compare them with setae and discuss some of the fundamental problems involved in their morphogenesis.

Methods

The eggs and attending female *Octopus sp.*[1] used in this study were collected at Tacoma Narrows (Puget Sound), Washington, in the spring of 1972 and subsequently placed in a marine aquarium where the female brooded the eggs until hatching occurred. The juvenile octopods were then collected and appropriately fixed for examination.

Prior to examination the juveniles were placed in sea water and kept at 0° C until activity subsided to facilitate photography of the whole animal and to obviate the discharge of ink upon fixation. If allowed to return to normal temperature, 10–12° C, the octopods would regain activity. For photography of the whole animals the chilled specimens were placed on a depression slide, flooded with filtered sea water and covered with a cover slip.

Whole mounts of the skin were fixed and mounted in Epon according to the method of Cavey and Cloney (1973) and subsequently examined with a Zeiss Standard Universal microscope equipped with polarization optics in order to determine the sign of birefringence of the Kölliker's tufts (see Gustus and Cloney, 1973). Specimens for scanning electron microscopy were fixed in Lillie's 10% buffered formalin, post-fixed in 1% OsO_4 buffered with 1.25% sodium bicarbonate, dehydrated in ethanol and then run through an ethanol-Freon TF series to 100% Freon TF. The specimens were then dried in a Bomar SPC-900 critical point drying apparatus and attached to freshly cleaved mica using silver paste. These preparations were coated with carbon and gold/palladium and examined with a Cambridge Mark II-A scanning electron microscope.

For light and transmission electron microscopy specimens were fixed for 1 hour in 2.5% glutaraldehyde buffered to pH 7.4 with 0.2 M Millonig's phosphate buffer and adjusted to 960 milliosmoles with NaCl. The specimens were then briefly rinsed in the buffer and post-fixed for 1 hour in 1% OsO_4 adjusted to pH 7.4 with sodium bicarbonate. The tissues were then rinsed in water, dehydrated in ethanol, transferred through three changes of propylene oxide and embedded in Epon according to the method of Luft (1961). One micron thick

1 Dr. Eric Hockberg, a specialist on the systematics of the octopoda at The Santa Barbara County Museum, Santa Barbara, California, has examined specimens of this species and determined that it is undescribed. It is his intention to describe it for publication.

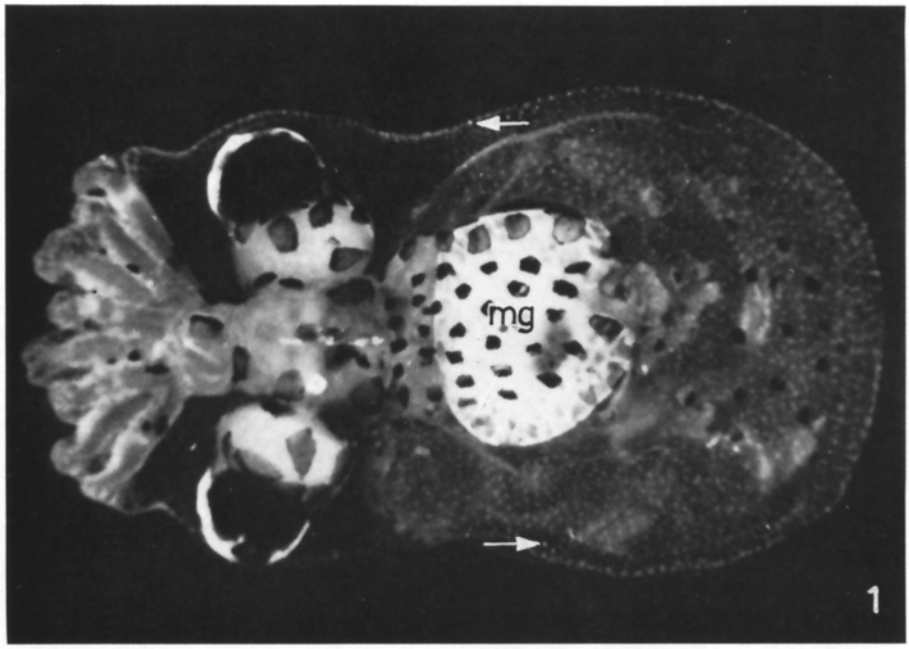


Fig. 1. Dorsal view of a juvenile *Octopus sp.*, approximately 6 days after hatching, illustrating Kölliker's tufts. These minute epidermal bristles are widely distributed over the surface of the mantle, siphon, arms and head and give the specimen a punctate appearance. Two tufts are indicated by arrows. The large white structure is the midgut gland (*mg*). 42.5×

Abbreviations for figures. *bl* basal lamina, *c* chaetoblast, *ep* epidermal cells, *f* filaments, *gb* Golgi body, *h* haptosome, *if* interstitial filaments, *kt* Kölliker's tuft, *lc* lateral cell, *m* obliquely striated muscle fiber, *md* macula densa, *mf* membranous fragments, *mg* midgut gland, *mi* mitochondria, *mv* microvillus, *r* rodlet, *rer* rough surfaced endoplasmic reticulum, *v* vesicle.

sections were stained with azure II and methylene blue (Richardson *et al.*, 1960) and examined by light microscopy. Thin sections approximately 600 Å thick were stained with uranyl acetate and lead citrate (Reynolds, 1963) and examined with either a Philips EM-300 or an RCA EMU-3G electron microscope.

Results

Each Kölliker's organ is composed of three principal parts: a follicle of specialized epidermal cells, an extracellular fasicle of cannular rodlets (Kölliker's tuft) and a group of obliquely striated dermal muscle fibers (Fig. 12).

Kölliker's tufts are widely distributed over the surface of the body but are more abundant on the mantle than on the head, siphon or arms (Figs. 1 and 2). The principal axis of each erupted subulate (awl-shaped) tuft is obliquely oriented with reference to the surface of the skin and is directed toward the anterior end of the animal. The tufts develop within epidermal follicles during late embryogenesis; later they erupt through the surface of the epidermis (Fig. 3).

An errupted Kölliker's tuft is approximately 13 μ in basal diameter and 50 μ in length (Fig. 6). Each tuft is composed of a fasicle of approximately 1500 distally

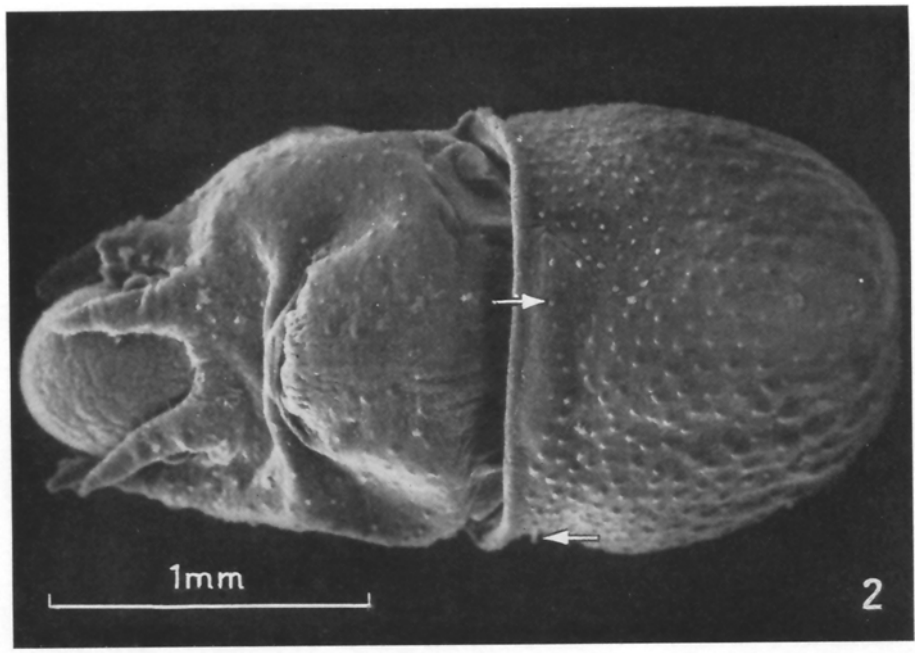


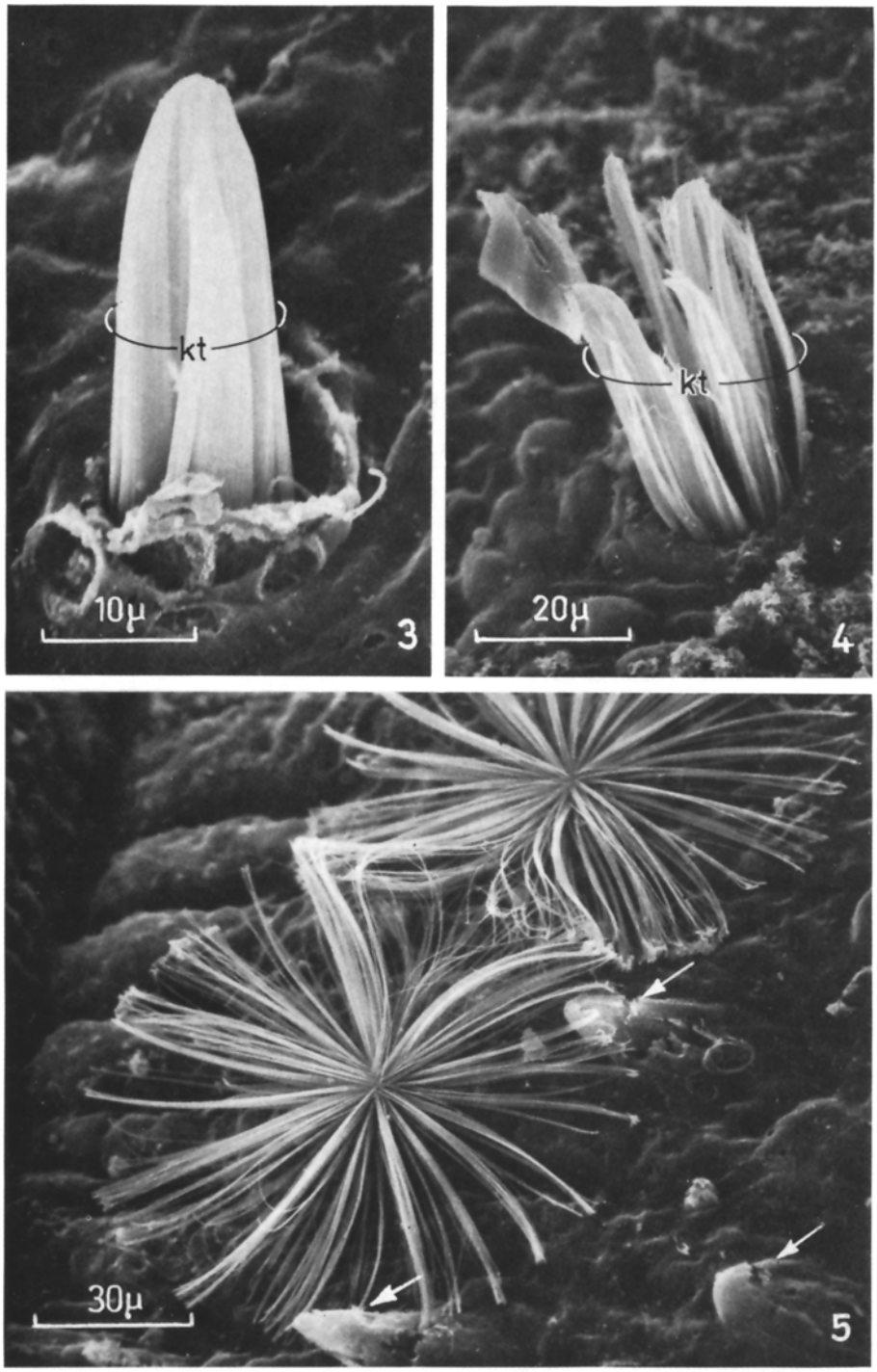
Fig. 2. Scanning electron micrograph of the ventral surface of a 1-day-old juvenile *Octopus sp.* Kölliker's tufts (arrows) on the mantle, siphon, arms and head have erupted and protrude from the surface of the epidermis. 42.5×

tapered cannular rodlets, each with a basal diameter of about 350 nm. The rodlets each have six flattened facets and appear hexagonal in transverse section. The rodlets are composed of longitudinally oriented filaments and are surrounded near their base by a layered meshwork of interstitial filaments (Figs. 6, 8 and 9).

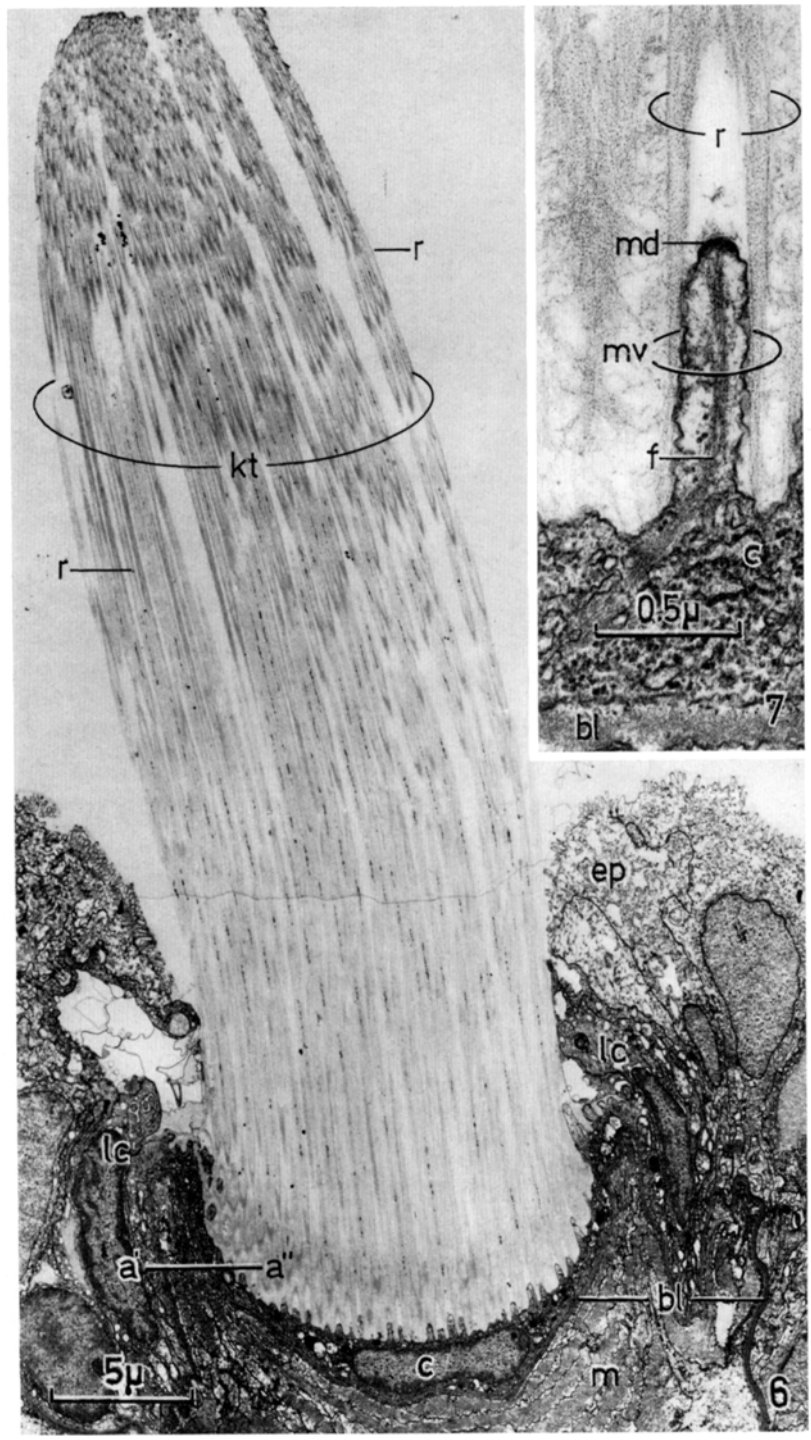
The tufts are strongly anisotropic; when rotated 360° between crossed polarizer and analyzer each tuft was bright at angles of +45°, +135°, —45°, and —135° and dark at angles of +90°, +180°, —90°, and —180°. When the long axis of the tuft was aligned parallel to the slow axis (γ axis) of a quartz first order red retardation plate (addition position) the tuft appeared blue against a red background; rotation of the tuft to a position orthogonal to the slow axis of the red plate (subtraction position) caused it to become yellow against a red background; therefore the tuft is positively birefringent with respect to its long axis.

The base of each rodlet is occupied by a microvillus (Figs. 6, 7, 8, 9, and 12). Extensions of the core of each rodlet beyond the tip of the microvillus are narrow and invariably contain irregularly spaced membranous fragments (Figs. 6, 8, 9, and 10). The longitudinal arrays of filaments at the bases of the rodlets are less densely packed and less oriented than those in the central and distal parts (Figs. 7 and 8).

The follicle consists of a basal cell which we call a *chaetoblast* and several lateral cells (Figs. 6 and 12); each of these cells rests on a 100–200 nm thick basal lamina (Figs. 8 and 9).



Figs. 3—5. Scanning electron micrographs showing erupting and everted tufts. Fig. 3 shows an everted subulate tuft (*kt*). The rodlets of some tufts separate (Fig. 4) and radiate into patulous arrays on the epidermis (Fig. 5). Three erupting Kölliker's tufts (arrows) are shown in Fig. 5. 2100×, 1100×, 750×



Figs. 6 and 7

The scyphate (cup-shaped) chaetoblast (Figs. 6, 8, and 12) has a large flattened nucleus containing clumps of condensed chromatin. The cytoplasm contains free ribosomes, abundant rough surfaced endoplasmic reticulum, Golgi bodies, mitochondria and many vesicular elements (Figs. 8 and 9). Approximately 1500 microvilli are present on the concave apical surface of the chaetoblast (Figs. 6, 7, 8 and 9). Each microvillus (ca. 0.8 μ in length and 0.2 μ in diameter) contains a terminal macula densa associated with the plasmalemma and a bundle of longitudinally oriented 50–70 Å diameter filaments (Figs. 7 and 8). These filaments are inserted distally in or near the macula densa and extend deeply into the cytoplasm. Many of these filaments attach to the basal plasmalemma (Fig. 8). Both of these areas of attachment between the filaments and the plasmalemma will be referred to as haptosomes (Cloney and Florey, 1968) because they are evidently filament-to-membrane *intracellular* attachment devices and do not closely resemble hemidesmosomes which serve to hold cells to extracellular components.

The microvilli also contain ribosomes and many small vesicles (Figs. 7, 8, and 9). Occasionally small discrete pieces of the plasmalemma were found alongside the microvilli. The conspicuous irregular profiles of the plasmalemma on the microvilli may be a consequence of both exocytosis (fusion of vesicles with the plasmalemma) and apocrine secretion (separation of fragments from the surface).

The organelles of the lateral follicular cells (Figs. 6 and 10) are similar to those of the chaetoblast but the free surfaces of these cells apposed to the Kölliker's tuft lack microvilli. The follicular cells are easily distinguishable from neighboring epidermal cells. The latter are cuboidal and have extensive lateral interdigitations, spherical or ovoid nuclei devoid of conspicuous clumps of heterochromatin, a comparatively electron translucent cytoplasm containing the usual organelles and abundant microvilli on their apical surface (Figs. 6 and 12). The basal lamina of the surrounding epidermal cells exceeds that of the follicular cells in thickness, approaching 300 nm in some regions.

The base of the follicle is associated with obliquely striated muscle fibers of the dermis (Figs. 6, 8, 9, and 12). These cells are arranged in 4 to 5 orthogonal layers and adjoin the basal lamina of the chaetoblast and lateral follicular cells.

Eversion of the tuft is accompanied by an outward displacement of the follicle and the chaetoblast becomes saucer-shaped. The rodlets of everted tufts in fixed specimens were often seen to be spread out in patulous arrays on the surface of the epidermis (Figs. 4 and 5). The rodlets of each chaetoblast are ultimately shed leaving small pits in the epidermis. The fate of the follicular cells is unknown.

Fig. 6. Longitudinal section of a Kölliker's organ. The fasicle of rodlets constitutes Kölliker's tuft (*kt*). Each rodlet (*r*) arises from a microvillus on the apical concave surface of a basal chaetoblast (*c*). The tuft is surrounded by several lateral cells (*lc*) and epidermal cells (*ep*). Obliquely striated muscle fibers (*m*) attach to the basal lamina (*bl*) of the chaetoblast and lateral cells. 3700×

Fig. 7. A longitudinal section through a microvillus (*mv*) of the chaetoblast (*c*). Each microvillus contains an axial bundle of 50–70 Å filaments (*f*). These arrays of filaments extend from a macula densa (*md*) at the tip of the microvillus to the basal plasmalemma of the chaetoblast. Each microvillus inserts into the basal end of a rodlet (*r*) of the tuft. 34000×

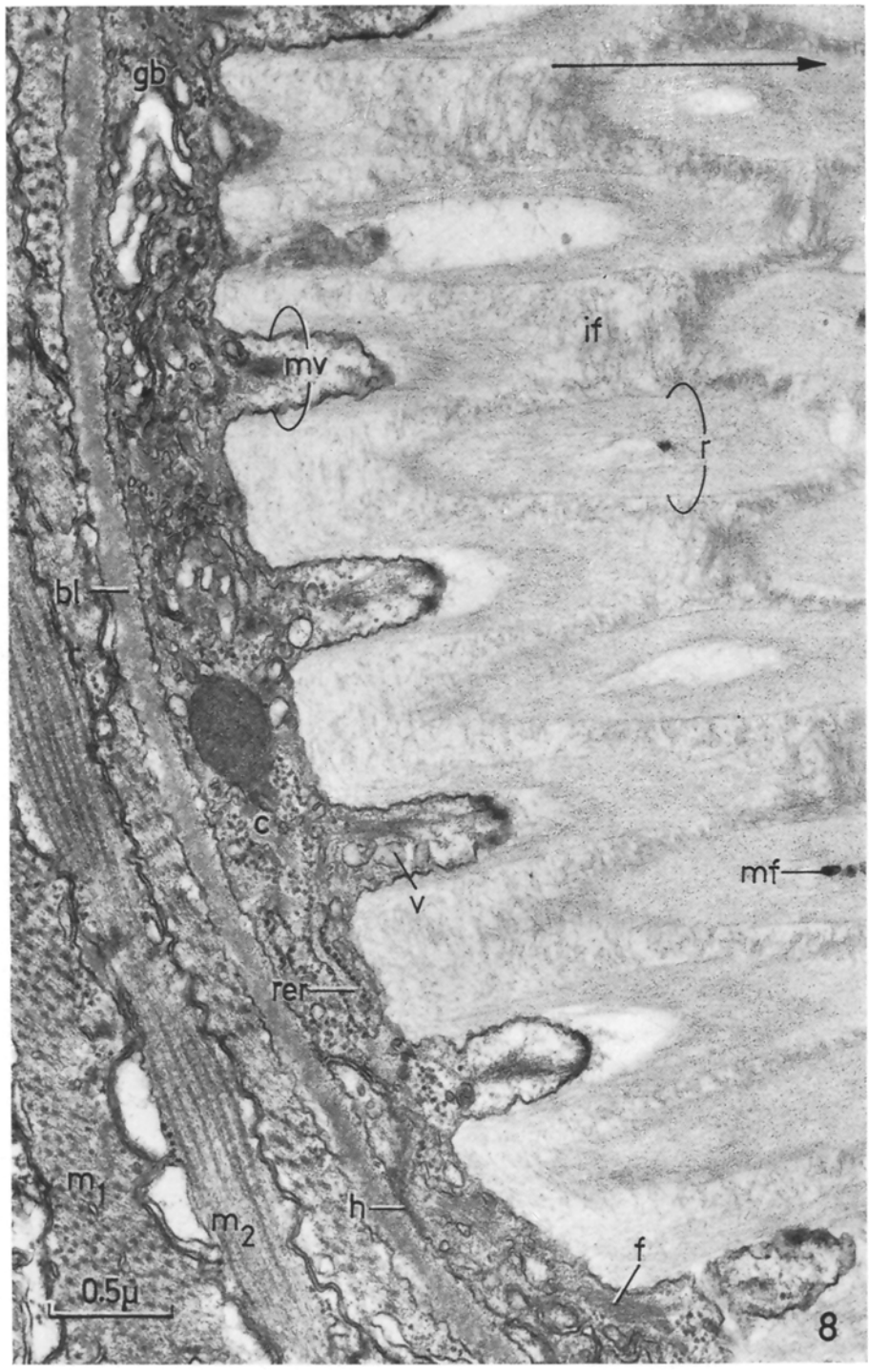


Fig. 8

Discussion

Boletzky (1973) provided the first description of the fine structure of Kölliker's organs in his study of *Octopus vulgaris* and *Eledone moschata*. He has clearly shown that the microvilli of the basal cell insert into the base of each rodlet and that each organ has a complement of dermal muscle fibers that confer motile properties to the tuft. Our observations on *Octopus sp.* are in agreement with those of Boletzky insofar as they overlap but we have extended our analyses to include a comparison of the tufts with setae and to the problem of how the tufts elongate.

The fine structure of Kölliker's tuft resembles that of the bristles generally referred to as "annelid-like" setae, or chaetae, of polychaetes (Bouligand, 1966, 1967; Orrhage, 1971; George and Southward, 1973; Gustus, 1973; Gustus and Cloney, 1973; O'Clair and Cloney, 1974), echiuroids (Orrhage, 1971), pogonophorans (Gupta and Little, 1972; George and Southward, 1973; Orrhage, 1973), and brachiopods (Gustus and Cloney, 1972; Storch and Welsch, 1972; Orrhage, 1973). In all of these groups the bristles are entirely extracellular and are composed of longitudinally oriented filaments organized around longitudinal channels. A bristle is composed either of many laterally associated filamentous tubules or, when the interstices between the tubules are filled with glycoprotein as in the compound setae of *Nereis vexillosa*, of a single rod pierced by longitudinal channels. Each Kölliker's tuft and each seta arises from a follicle of epidermal cells consisting of several lateral cells and a single basal cell. We infer that the morphogenesis of all these bristles is directed by microvilli on the apical surface of the basal cell. The term chaetoblast appears to be appropriate for the basal cell in all taxa that have been examined.

The meshwork of interstitial filaments among the bases of the hexagonally faceted cannular rodlets of Kölliker's tuft may have a counterpart in the base of the larval setae of *N. vexillosa* where dense material separates the longitudinal arrays of filaments organized around the microvilli. The individual cannular rodlets of Kölliker's tuft splay apart during eversion (Boletzky, 1973) and shedding, while there is no evidence that a similar process occurs in other forms, even in those whose setae are composed of laterally associated tubules (e.g., *Terebratalia* (Brachiopoda), Gustus and Cloney, 1973; *Disoma* (Annelida), Orrhage, 1971).

Finally, there may be some differences in the behavior of the distal tips of the microvilli associated with Kölliker's tufts and those associated with annelid-like

Fig. 8. A longitudinal section through the base of a scyphate chaetoblast (*c*) and associated musculature. The concave apical surface of the chaetoblast is covered with short microvilli (*mv*) that serve as templates on which the rodlets are cast. The axial array of filaments (*f*) of the microvillus traverse the chaetoblast and attach by haptosomes (*h*) to the basal plasmalemma. Note the abundant rough surfaced endoplasmic reticulum (*rer*), Golgi bodies (*gb*) and vesicles (*v*) in the cytoplasm of the chaetoblast. We infer that these vesicles fuse with the apical plasmalemma of the chaetoblast and release material that is subsequently incorporated into the tuft. A meshwork of interstitial filaments (*if*) is present between the bases of the obliquely sectioned rodlets (*r*). The musculature of Kölliker's organ consists of orthogonally arranged layers of obliquely striated muscle fibers (m_1, cross section and m_2, longitudinal section) that are attached to the basal lamina (*bl*) of the follicle. The arrow in the upper right indicates the axis of the tuft. 29000×

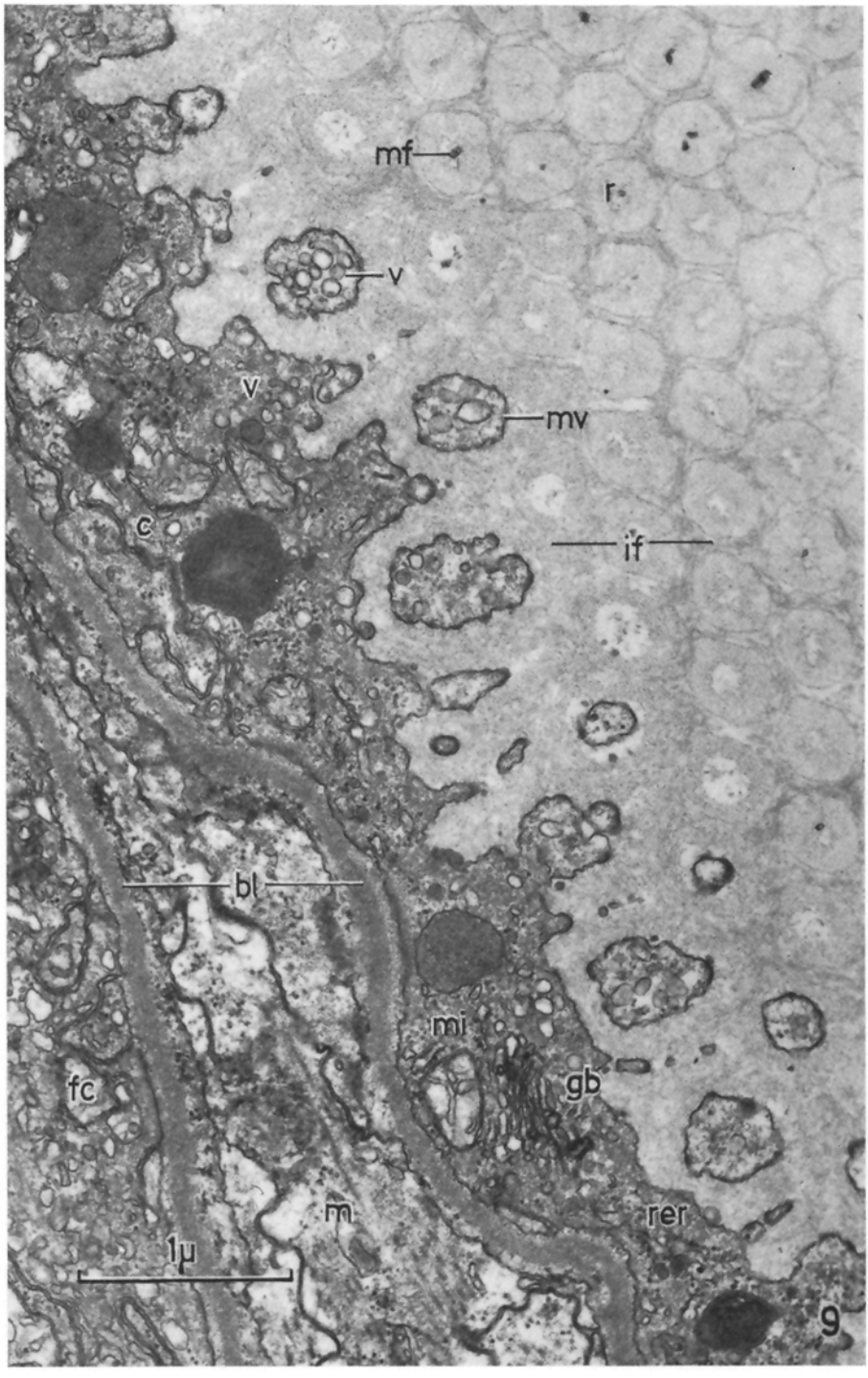


Fig. 9

setae. In the chaetoblasts of *Octopus sp.* the distal tips of the microvilli appear to cleave off periodically during secretion, occluding the hollow cores of the rodlets with irregularly spaced membranous fragments. This phenomenon is probably not an artifact of fixation because the fragments are found along the length of each rodlet and it is unlikely that they could be transported over these distances in narrow channels during or following fixation. The microvilli of most annelid chaetoblasts do not regularly cleave off fragments, although a few possible cases of fragmentation at the tips of cortical microvilli have been described (Bouligand, 1967; Orrhage, 1973). An interesting exception to this is the large medullary microvillus of nereid chaetoblasts. Large membranous sacs periodically separate from the tip of this microvillus and occupy the medullary channel; they probably contribute to the structure of the medullary trabeculae and diaphragms of these setae (O'Clair and Cloney, 1974).

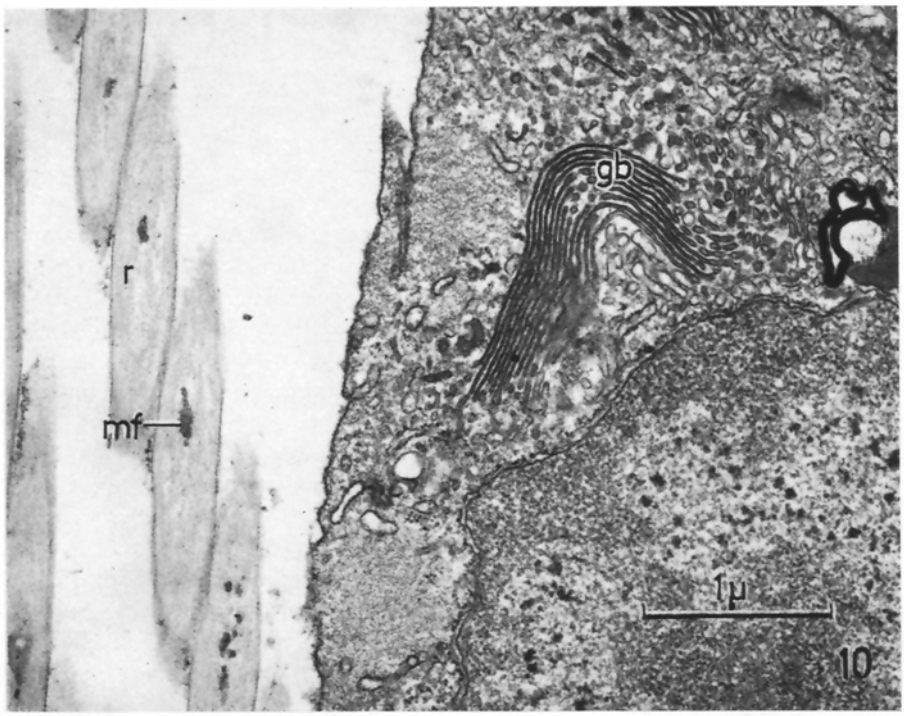
In addition to the biochemical aspects of secretion a model of the formation of Kölliker's tuft must account for 1) the cellular origin of the secreted material, 2) the presence of membranous fragments in the cores of the rodlets, 3) the presence of layers of interstitial filaments between the basal parts of the rodlets, 4) the tapered cannular configuration of the rodlets, 5) the mechanism of elongation of the tuft, and 6) the parallel arrangement of the filaments of the rodlets.

The principal feature of our hypothesis is that the tuft elongates by basal appositional growth and that the precursor materials, secreted by the chaetoblast, and perhaps by the lateral cells, are organized into positively birefringent longitudinally oriented arrays of filaments on the external apical surface of the chaetoblast.

The chaetoblast and lateral cells have the structural characteristics of secretory cells; they contain abundant rough surfaced endoplasmic reticulum and numerous Golgi bodies. The presence of many vesicles in the microvilli and the lack of a terminal web are unusual features of the chaetoblast. Vesicles are rare in the microvilli of most vertebrate and invertebrate epithelial cells and are absent from the microvilli of polychaete chaetoblasts. It appears probable from the micrographs we have examined that many of these vesicles fuse with the plasmalemma of the apical surface of the chaetoblast and release the precursors of the tuft by exocytosis.

A problem immediately arises when exocytosis is considered because continual fusion of vesicles with the apical plasmalemma would increase its surface area. This extra membrane could be disposed of by endocytosis or by cleaving off parts of the membrane (apocrine secretion). Since the core of each rodlet contains many small membranous fragments, we favor the view that these fragments arise as a result of an apocrine process. The surface of the microvilli would be constantly

Fig. 9. A cross section through a part of a Kölliker's organ (along line a′–a″ of Fig. 6) showing rodlets (*r*) of Kölliker's tuft and accompanying chaetoblast (*c*). The core of each rodlet becomes narrow distally (toward the upper right) and contains membranous fragments (*mf*) from the microvilli of the chaetoblast. The rodlets are composed of highly oriented filaments and are surrounded by a meshwork of interstitial filaments (*if*). The chaetoblast contains many mitochondria (*mi*), rough surfaced endoplasmic reticulum (*rer*), Golgi bodies (*gb*) and vesicles (*v*). The vesicles are particularly abundant within the microvilli (*mv*). 30000×



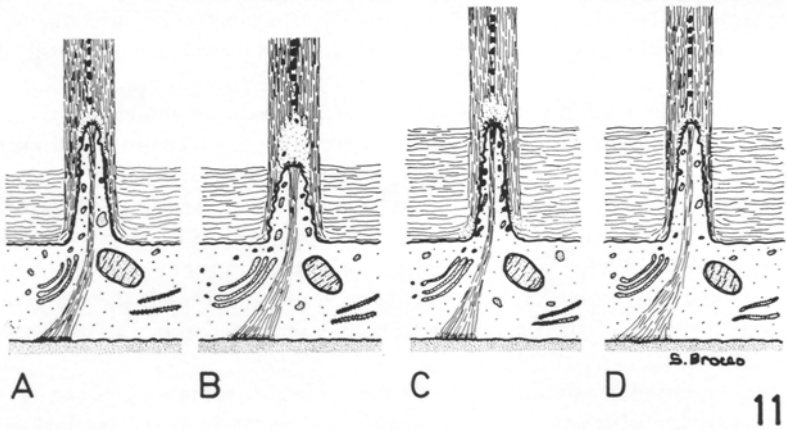


Fig. 10. Section of a lateral follicular cell with well developed Golgi bodies (*gb*). The obliquely sectioned rodlets (*r*) of Kölliker's tuft, on the left, contain membranous fragments (*mf*) of the microvilli. 25000×

Fig. 11. Diagrammatic model illustrating elongation of a rodlet. (A) The microvillus has extended within an enlarged fluid filled basal concavity of the rodlet. Secretory material exteriorized during the previous cycle has been formed into filaments and deposited on the basal part and inner walls of the rodlet. (B) The array of 50–70 Å filaments contracts and shortens the microvillus. Membranous fragments that have separated from the surface of the microvillus by apocrine secretion are present in the concavity of the rodlet. (C) The array of filaments has relaxed and the microvillus has extended by hydrostatic pressure. The microvillus has pushed the rodlet upward, exposing new surfaces for the addition of more filaments to the rodlet. Secretory vesicles have moved from the cytoplasm to the apical plasmalemma of the chaetoblast where they have released additional precursors of the tuft by exocytosis. (D) The formation of new filaments has been completed. The rodlet has been lengthened and its basal wall thickened. The layer of interstitial filaments has increased in depth

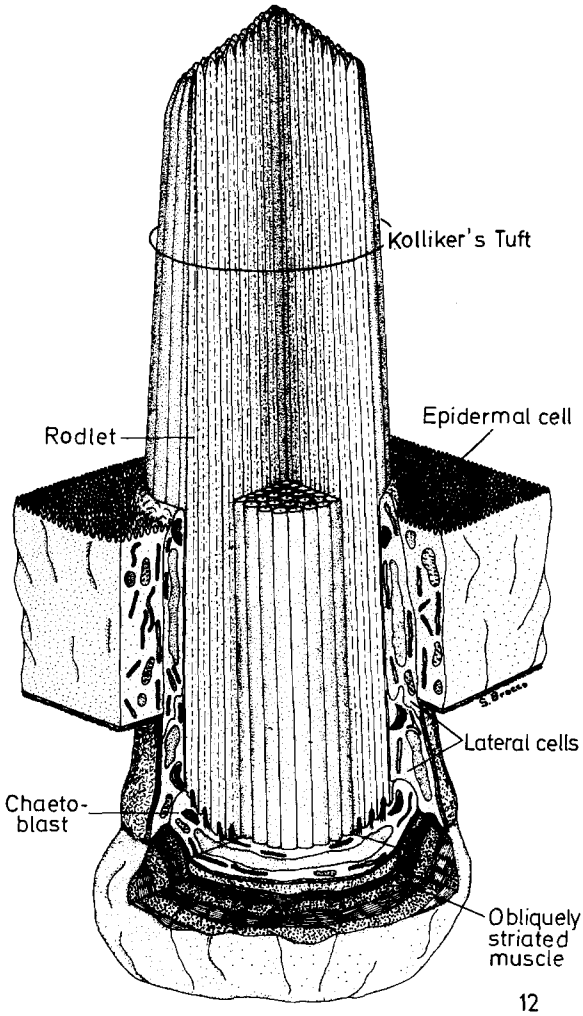


Fig. 12. Diagrammatic drawing of a Kölliker's organ with an erupted tuft. The organ consists of approximately 1500 rodlets contained within an epidermal follicle composed of lateral cells and a basal chaetoblast. The cytoplasm of the lateral cells and the chaetoblast contains abundant rough surfaced endoplasmic reticulum and Golgi bodies. The scyphate chaetoblast has numerous microvilli on its apical surface. Each microvillus inserts into the base of a corresponding rodlet and serves as a template around which the rodlet is formed. The configuration and dynamic activity of the apical surface of the chaetoblast determine the morphology of the tuft. Basally the chaetoblast is separated from the underlying orthogonally arranged layers of obliquely striated muscle fibers by a thick basal lamina. The interstitial filaments have been deleted for clarity

changing under these conditions but would maintain a state of dynamic equilibrium if the rates of exocytosis and apocrine secretion were in balance. These membranous fragments may be analogous to the epicuticular particles of oligochaete cuticle (Hess and Menzel, 1967; Krall, 1968; Potswald, 1971).

We have not seen any examples of membranous fragments among the interstitial filaments. In order to account for this we infer that apocrine secretion occurs only on the microvilli, however this does not preclude the possibility that exocytosis occurs between the bases of the microvilli.

The interstitial filaments are disposed in layers orthogonal to the axis of the rodlets and more or less parallel to the plasmalemma between the bases of the microvilli. We envision that the entire apical surface of the chaetoblast is involved in the organization of filaments and that the filaments are laid down more or less parallel to the plasmalemma wherever they are formed. The orientation of the layers of interstitial filaments according to this hypothesis can be accounted for by considering only the plane of the plasmalemma between the microvilli.

The conical basal region of the core of each rodlet is occupied by a tapered microvillus and a substance of low electron density, perhaps a watery fluid. We infer that during the initial phase of rodlet formation filamentous glycoproteins are organized on the sides of each conical microvillus and form the tapered tip of each rodlet. We envision that additional glycoproteins are added appositionally to the inner surface and to the basal end of the previously formed filaments. Glycoprotein secreted at the base of the microvillus would become organized onto the basal part of the rodlet forming the outermost layer; glycoprotein secreted higher on the microvillus would add to the inner wall of the rodlet and concomitantly increase the thickness of the walls and reduce the diameter of the cores of the rodlet. The axis of the core remains essentially hollow because there is less surface area for addition of material and the tips of the microvilli are continually cleaved off.

If the mechanism of elongation simply involved secretory pressure from the base of the rodlet, it would be difficult to explain how the high degree of organization of the tuft arises.

Thuneberg and Rostgaard (1969) have demonstrated pulsatory movements of microvilli in living preparations of mammalian gut and kidney. Most of the microvilli of the chaetoblast have a ruffled plasmalemma and appear to be in a partially retracted state within the proximally enlarged basal region of the cores of the rodlets.

With these facts in mind we postulate that the microvilli reciprocate within the fluid filled basal concavity of each rodlet by contraction and relaxation of the arrays of 50–70 Å diameter axial filaments within the microvilli. The addition of filaments of glycoprotein to the basal ends of the rodlets could be effected by a continuous secretion of subunits if it were coupled with oscillations of the microvilli that push the rodlets out far enough so that new material could be added at their basal ends. The sequence of events involved in elongation of a rodlet is represented diagrammatically in Fig. 11.

It will not be possible to explain how the filaments within each rodlet become so tightly packed and organized in parallel arrays without more information about the chemistry of the tuft. At this point we can only suggest that as the filaments are assembled on the surfaces of the microvilli they become crosslinked with one another. The environment on the surface of the microvilli may be different from that of the plasmalemma between the microvilli because the interstitial filaments remain loosely packed and do not form parallel arrays.

Some features of this model of the formation of the tuft could be tested by labeling precursors of the rodlets and analyzing the pattern of their incorporation during secretion autoradiographically.

Boletzky (1966) and Mangold *et al.* (1971) have proposed that Kölliker's tufts act as catch mechanisms preventing the octopus from slipping back into the egg case during hatching. The association of muscles with the tuft suggests that the tufts may be actively flexed or rotated against the chorion aiding the animal to release itself from the egg case during hatching.

Boletzky (1973) has observed rapid cyclic eversions and retractions of the tufts in living specimens of *Eledone moschata*. In this species the rodlets of the tuft spread distally as they are everted. He has suggested that the tufts when everted may slow the rate of sinking of planktonic larvae (juveniles) when they are not actively swimming. The configurations of Kölliker's tufts that we have observed by scanning electron microscopy (Figs. 3, 4, and 5) may represent stages in the eversion-retraction cycle described by Boletzky.

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