AMER. ZOOL., 25:87–97 (1985)

The Stylet Apparatus of Monostiliferous Hoplonemerteans[1]

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SYNOPSIS. This paper compares the ultrastructure, development, and functional morphology of the stylet apparatus in monostiliferous hoplonemerteans (Phylum Nemertea: Class Enopla). The apparatus occurs in the middle region of the worm's eversible proboscis and consists of two main components: 1) a central stylet that is attached to an anchoring device called the basis; and 2) reserve stylet sacs that contain nail-like reserve stylets. The knob-shaped proximal piece of the central stylet is embedded in the anterior end of the basis, and the tapered shaft of the stylet is directed anteriorly. When the proboscis is fully everted, the central stylet is used to stab such prey as polychaete worms and small crustaceans. Wounds inflicted by the stylet allow the introduction of paralytic neurotoxins that are produced by glandular cells in the epithelial lining of the proboscis. Reserve stylets form intracellularly within the reserve stylet sacs and serve to replace the central stylet when it becomes lost or damaged. Fully developed stylets typically measure 50 to 250 μm in length and comprise an organic core surrounded by an inorganic cortex that contains calcium phosphate. In juvenile worms that are four days to several weeks old, reserve stylets are assembled before other components of the stylet apparatus are fully differentiated. Subsequently, a reserve stylet is transferred to the central region of the proboscis and placed on the basis. Stages in the process of stylet attachment are described, and the probable sequence by which the stylet apparatus co-evolved with toxin-producing cells is discussed.

INTRODUCTION

The phylum Nemertea contains approximately 900 species of unsegmented, carnivorous worms that use an eversible proboscis to capture such prey as polychaete worms or small crustaceans (Fig. 1). In the order Hoplonemertea (Table 1), the middle region of the proboscis is armed with a stylet apparatus that contains nail-like calcified stylets. During prey attack, the proboscis is rapidly everted, and the stylet apparatus becomes positioned at the distal tip of the fully everted organ where it can be used to stab the prey (Fig. 2).

The stylet apparatus characteristically comprises: 1) at least one central stylet that is borne on an anchoring device called the basis; and 2) reserve stylet sacs in which reserve stylets are formed. Central stylets are used to inflict wounds into which paralytic neurotoxins can be secreted (Stricker and Cloney, 1983). When a central stylet is lost or damaged, it is replaced by a reserve stylet that is transferred from the reserve stylet sac.

In hoplonemerteans belonging to the suborder Polystilifera, the basis anchors numerous central stylets that are usually less than 15 μm in length. Members of the suborder Monostilifera, however, have a single, relatively large (typically 50 to 250 μm long) central stylet attached to each basis. Polystiliferans are predominantly pelagic and comparatively difficult to collect. Monostiliferous hoplonemerteans, on the other hand, are found in intertidal and subtidal habitats throughout the world, as well as in a few freshwater and terrestrial locations. Consequently, nearly all the information that has been gathered on the nemertean stylet apparatus is derived from studies on monostiliferans.

Nemertean stylets were first described in 1830 by Dugès, who believed that the nail-like structures he observed in the monostiliferan *Prostoma armatum* were located in the worm's intestine. Johnston (1837) and Quatrefages (1846) also thought that the stylet apparatus formed part of the alimentary tract. Oersted (1844), on the other hand, maintained that stylets belonged to an accessory sexual organ that served to arouse the worm's mate. Subsequent to these early studies, several invesigators (*e.g.*, Frey and Leuckart, 1847; Schultze, 1851;

[1] From the Symposium on *Comparative Biology of Nemertines* presented at the Annual Meeting of the American Society of Zoologists, 27–30 December 1983, at Philadelphia, Pennsylvania.

TABLE 1. *Systematics of the phylum Nemertea.**

Phylum Nemertea
Class Anopla (proboscis does not contain calcified stylets)
Order Paleonemertea (4 families; 13 genera)
Order Heteronemertea (5 families; 45 genera)
Class Enopla (proboscis armed with stylets, except in bdellonemerteans)
Order Bdellonemertea (1 family; 1 genus)
Order Hoplonemertea (28 families; 117 genera)
Suborder Polystilifera
Suborder Monostilifera

* Based on the classification of Gibson (1982).

Claparède, 1861; Keferstein, 1862) recognized that the stylet apparatus was part of a prey-capturing organ that was separate from the gut.

It was not until the exhaustive treatise of Otto Bürger was published in 1895, however, that many of the details concerning the stylet apparatus were recorded and correctly interpreted. Bürger (1895) reported that stylet formation occurred intracellularly within a single giant cell that filled the lumen of each reserve stylet sac. He also disputed previously held views (*e.g.*, McIntosh, 1873; von Kennel, 1878; Montgomery, 1894) that the central stylet was formed outside the reserve stylet sacs in the vicinity of the basis.

In 1976, Wourms presented an abstract of some ultrastructural observations that corroborated Bürger's claim regarding the unicellular nature of the stylet sac's central region. In addition, he showed by means of X-ray microanalysis that stylets contain mainly calcium and phosphorus. More recently, I have used light and electron microscopy to compare the structure and function of the stylet apparatus in several species of monostiliferans collected in the vicinity of San Juan Island, Washington (Stricker, 1981, 1982, 1983*a*, *b*, 1984; Stricker and Cloney, 1981, 1982, 1983).

In this paper, I will attempt to summarize our current understanding of the functional morphology of the stylet apparatus in monostiliferous hoplonemerteans. Following a description of the general morphology of the stylet apparatus, I will briefly describe the histology and ultrastructure of its two main components—the central stylet/basis complex; and, the reserve stylet sacs. Next, four major topics are considered: 1) the ontogeny of stylet formation; 2) cytological aspects of styletogenesis in adult worms; 3) mechanisms of stylet replacement; and 4) the role of the stylet apparatus in prey capture. Finally, I will discuss the probable pathway by which the stylet apparatus and toxin-producing cells evolved.

GENERAL MORPHOLOGY OF THE STYLET APPARATUS

The stylet apparatus is situated in the anterior half of the mid-proboscis region, within a muscular septum called the diaphragm. Directly posterior to the apparatus is a dilated ampulla, or stylet bulb, which constitutes the caudal half of the mid-proboscis region. A narrow canal (the ductus ejaculatorius) traverses the diaphragm and thus provides a connection between the anterior and posterior chambers of the proboscis (Fig. 2).

The knob-shaped proximal piece of the central stylet is attached to the anterior end of the basis, and the pointed tip of the stylet shaft is directed toward the anterior end of the worm. Several authors (*e.g.*, McIntosh, 1873; Coe, 1905; Brunberg, 1964) have noted the peculiar case in *Amphiporus pulcher*, where a second central stylet often occurs embedded in the posterior end of the basis. The origin and possible function of this anomolous condition are not known.

The basis and central stylet are usually about equal in length, but in some species the basis is considerably shorter (*e.g.*, *Amphiporus bimaculatus*), or longer (*e.g.*, *Emplectonema gracile*), than the central stylet. The basis is surrounded by a columnar epithelium and a thin underlying layer of connective tissue, which are collectively called the basis sheath. Reconstructions of serial 1-μm Epon sections reveal that the basis sheath of *Paranemertes peregrina* is continuous posteriorly with the lining of the ductus ejaculatorius (Stricker and Cloney, 1981). A similar connection is found in many other monostiliferans (Stricker, 1982, unpublished observations).

Well developed glands (the "diaphragm

glands" of Coe [1905]) surround the basis sheath. Most of the gland cells are filled with acidophilic granules whose morphology and staining properties are similar to those of the granules that constitute the basis. Thus, it seems likely that these glands form at least part of the basis (Hyman, 1951). Toward the anterior half of the diaphragm, however, there are numerous glandular cells that contain small (<1 μm) basophilic granules of unknown function.

The reserve stylet sacs are typically located near the periphery of the proboscis at about the same level as the basis, or slightly anterior to it. In several interstitial species belonging to the genus *Ototyphlonemertes*, however, the reserve stylet sacs occur markedly posterior to the basis (Fig. 3). Most monostiliferans have two to four sacs per proboscis. Adult members of the genus *Carcinonemertes* lack reserve stylet sacs (Fig. 4), and specimens of *Amphiporus formidabilis* typically have about ten reserve stylet sacs.

The Central Stylet and Basis

In most species, the shaft of the central stylet is smooth and straight. Central stylets of *Emplectonema purpuratum, Paranemertes peregrina,* and *P. sanjuanensis,* however, have helically arranged grooves along their shafts, and in *Emplectonema gracile,* the shaft is markedly curved.

Both the proximal piece and stylet shaft contain organic material that is surrounded by an inorganic cortex. The cortex appears to be composed chiefly of calcium phosphate (Wourms, 1976), but it also contains appreciable amounts of barium and strontium (Stricker, 1983*b*). The organic material in the shaft forms a solid core, but in the proximal piece the organic substances comprise diffuse strands that are intermixed with the calcified portion of the stylet. In all species examined, central stylets are solid structures without internal canals, and are essentially identical in ultrastructure to a fully formed reserve stylet (Stricker, 1983*b*).

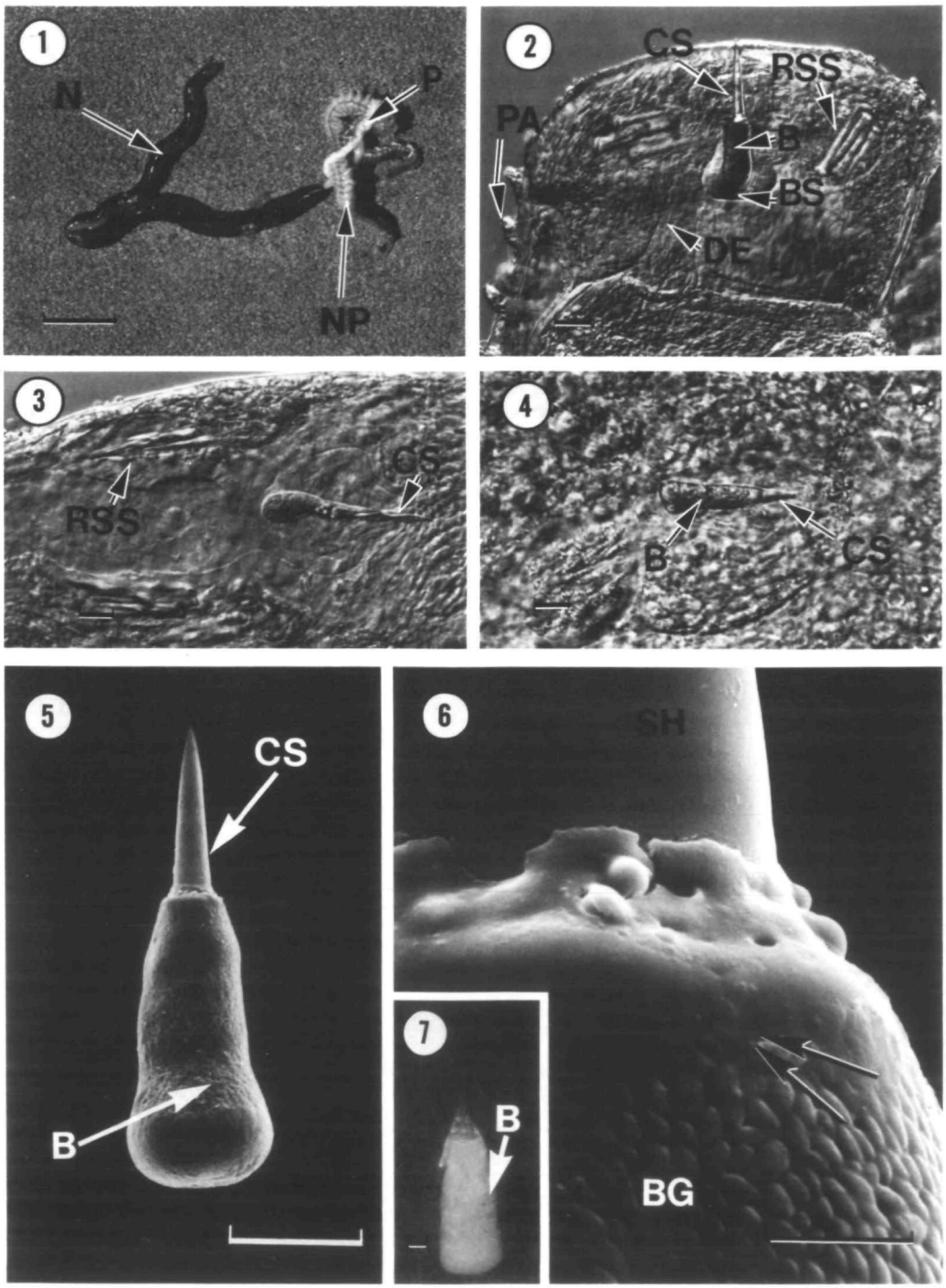
The basis is composed of an extracellular mass of granules that is generally oblong to pyriform in shape (Fig. 5). In many species, there is a short anterolateral diverticulum of the basis directed toward the ductus ejaculatorius. The individual granules that constitute the basis are usually about 1 to 5 μm long. The granules tend to be more or less separate toward the posterior end of the basis, but they are often fused together at the anterior end, especially in the region where the central stylet is attached (Fig. 6).

The basis becomes highly fluorescent under UV illumination (Fig. 7), following incubation of adult worms for one to two weeks in dilute (0.1 to 10 mg/100 ml) sea water solutions of tetracycline (Stricker, 1983*b*). Such findings suggest that the basis is at least partially calcified. This hypothesis is further supported by the following observations (Stricker, 1983*b*): 1) the basis feels hard when touched with a probe or similar instrument; 2) basis granules are birefringent; and 3) the basis becomes more translucent following incubation in E.D.T.A., a chelating agent that dissolves calcified structures. It seems likely that calcification of the basis would aid in supporting the anchoring device. Whether deposits of calcium salts in the basis serve other functions, such as acting as a storage site for divalent cations, is not clear.

The Reserve Stylet Sacs

Reserve stylet sacs are typically ovoid and 100 to 500 μm long in adults of average size. Each sac consists of: 1) squamous cellular processes that constitute the wall of the sac; 2) a single large cell, or styletocyte (Stricker and Cloney, 1981), which fills the lumen of the sac; and 3) a narrow duct that connects the sac with the proboscidial lumen surrounding the central stylet.

The cellular processes that constitute the wall of the sac are anucleate and highly interdigitated. They contain abundant filaments (6–8 nm in diameter) that are apparently capable of contraction (Figs. 8, 9). Although the exact nature of these processes is unclear, it seems likely that they represent basal extensions of epithelial cells whose nuclei occur in the region of the stylet sac duct. A thin sheet of squamous processes can also be seen surrounding the styletocyte of juveniles at the time



FIGS. 1–7. FIG. 1. Photograph of the nemertean *Paranemertes peregrina* (N) wrapping its proboscis (P) around a nereid polychaete prey (NP). Approximately life size. Scale bar = 1 cm. FIG. 2. Photomicrograph of the stylet apparatus in a *Tetrastemma* sp. juvenile, following complete eversion of the proboscis. B = basis; BS = basis sheath; CS = central stylet; DE = ductus ejaculatorius; PA = papilla of the glandular epithelium; RSS = reserve stylet sac. ×522. Scale bar = 10 μm. FIG. 3. Photomicrograph of the stylet apparatus in a proboscis

when the first stylets are assembled (Fig. 10).

The central region of the duct contains cytoplasmic processes that lack filaments and most other organelles (Stricker, unpublished observations). The origin of the intraluminal cytoplasmic processes and their relationship to the rest of the stylet sac remain unknown.

Styletocytes are invariably uninucleate. Depending on the species examined, each stylet-forming cell contains one to a dozen reserve stylets that develop within membrane-bound vacuoles. In addition to the stylets, the styletocyte cytoplasm typically contains smooth endoplasmic reticulum, mitochondria, Golgi bodies, as well as several types of granular or membranous inclusions.

THE ONTOGENY OF STYLET FORMATION

In all species examined (Iwata, 1960; Stricker and Cloney, 1982; Stricker, unpublished observations), the reserve stylets develop before the basis and its surrounding sheath are fully differentiated. In *Emplectonema gracile,* stylets first appear when the juveniles are four days old. In most other species, however, stylet formation begins at about two to three weeks (Stricker and Cloney, 1982; Stricker, unpublished observations).

The first stylets formed are usually about 5 to 10 μm long, and they appear to be miniature versions of the adult stylets. In two and one-half week old specimens of *Paranemertes peregrina,* the first formed stylets display shallow grooves and ridges, such as occur in a more pronounced condition in adult stylets (Fig. 11). In addition, ultrastructural features of juvenile stylets, such as the banding pattern of the organic material in the core of the stylet shaft, resemble their counterparts in adult stylets (Stricker, 1984).

After the basis and basis sheath have differentiated, a reserve stylet is transferred from the styletocyte and attached to the basis. In *Emplectonema gracile,* stylet transfer takes only a few hours, and the process occurs at about the same developmental stage in all healthy individuals. Attachment of the first central stylet in *Tetrastemma* sp. juveniles, however, can take up to two days, and it occurs at markedly different times in individuals developing within the same egg sheath. The mechanisms of stylet attachment are discussed in the section dealing with stylet replacement in adults.

STYLET FORMATION IN ADULT WORMS

When living worms with relatively translucent bodies are examined in a slightly compressed state, most specimens exhibit at least one incompletely formed stylet. Styletogenesis can be seen in starved adults as well as in worms that are allowed to capture prey (Stricker and Cloney, 1982). The stylets produced by adult specimens of *Amphiporus formidabilis, Emplectonema gracile,* and *Tetrastemma* sp. reach full length and have a well formed proximal piece within two to eight weeks (Stricker and Cloney, 1982).

In all specimens that I have examined, stylet formation was observed exclusively in the cytoplasm of the styletocytes. Incom-

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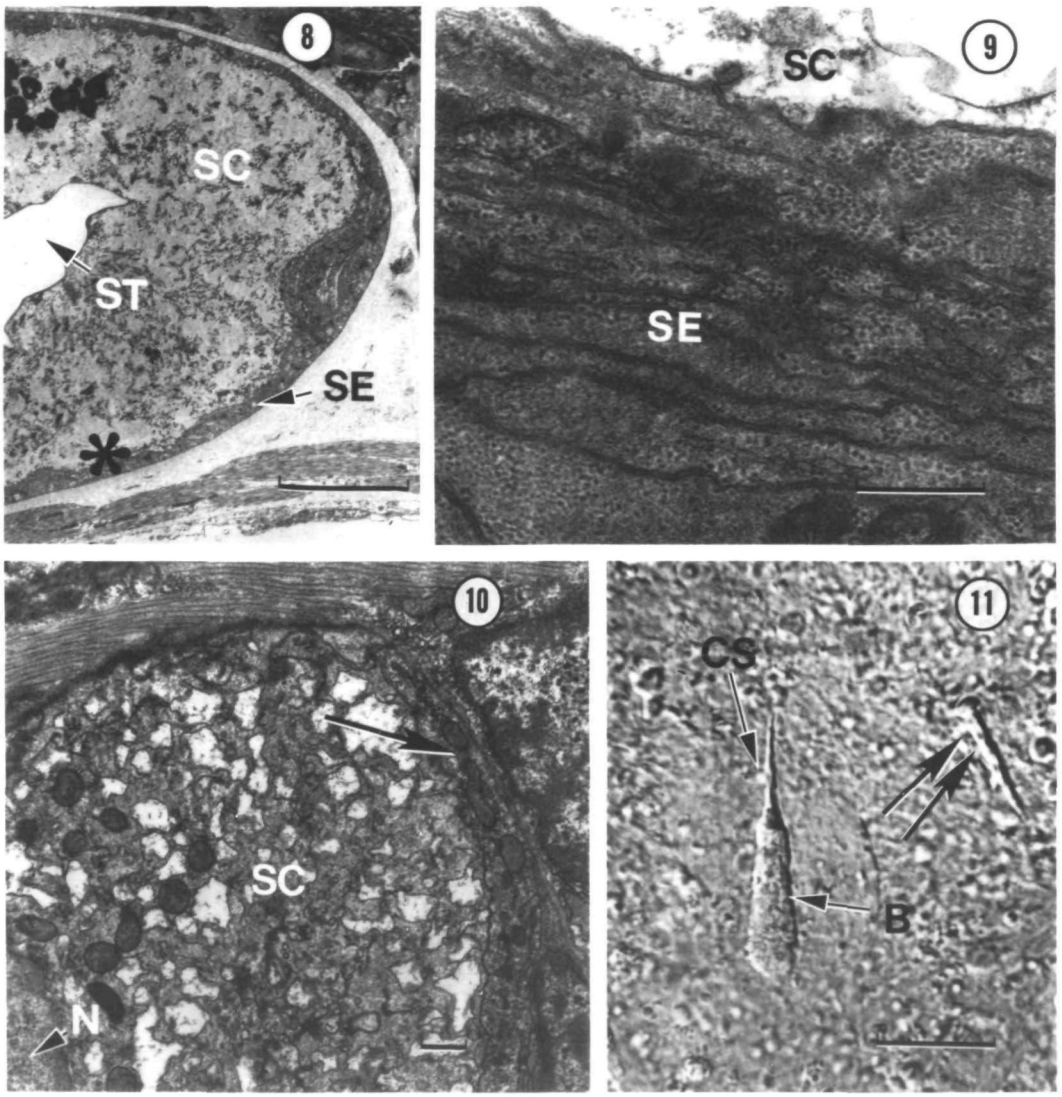
isolated from an adult *Ototyphlonemertes* sp., showing the reserve stylet sacs (RSS) positioned posterior to the basis and central stylet (CS). ×455. Scale bar = 10 μm. FIG. 4. Photomicrograph of the proboscis observed through the body wall of a compressed *Carcinonemertes epialti* adult. Note that a minute central stylet (CS) and basis (B) are present, but reserve stylet sacs are lacking. ×500. Scale bar = 10 μm. FIG. 5. Scanning electron micrograph (SEM) of the central stylet (CS) and basis (B) removed from a relatively small adult *Amphiporus formidabilis.* ×190. Scale bar = 100 μm. FIG. 6. SEM of the attachment site (double arrows) between the central stylet and basis in *Amphiporus formidabilis.* BG = basis granule; SH = shaft of the central stylet. ×2,040. Scale bar = 10 μm. FIG. 7. UV-fluorescence micrograph of the central stylet and basis (B) of a *Paranemertes peregrina* adult that had been incubated for 1 week in a 1 mg/100 ml sea water solution of tetracycline-HCl (pH 7.4). Note that the basis displays intense fluorescence which corresponds to an incorporation of tetracycline, presumably at sites of active calcification. ×285. Scale bar = 10 μm.



FIGS. 8–11. FIG. 8. Transmission electron micrograph (TEM) of part of a reserve stylet sac in *Amphiporus imparispinosus*, showing a stylet (ST) in the cytoplasm of the styletocyte (SC), and squamous cellular processes of the sac epithelium (SE) surrounding the styletocyte. The asterisk marks a site similar to that shown at higher magnification in Figure 9. ×1,710. Scale bar = 10 μμm. FIG. 9. TEM of the filament-containing cellular processes of the sac epithelium (SE) that surrounds the styletocyte (SC) in an adult *Emplectonema gracile*. ×16,850. Scale bar = 1 μm. FIG. 10. TEM of a developing reserve stylet sac in a *Tetrastemma* sp. juvenile, showing the styletocyte (SC) surrounded by the squamous cellular processes (arrow) that will form the wall of the reserve stylet sac. ×5,400. Scale bar = 1 μm. FIG. 11. Photomicrograph of the stylet apparatus viewed through the body wall of a *Paranemertes peregrina* juvenile. The double arrows mark the rudimentary helical grooves that are beginning to form in the stylet shaft. B = basis; CS = central stylet. × 1,600. Scale bar = 10 μm.

pletely formed central stylets were never seen, which argues against the contention of McIntosh (1873) that the central stylet is assembled on the basis.

The basic process of styletogenesis occurs as follows: 1) an oblong, membrane-bound vacuole develops in the styletocyte cytoplasm; 2) the organic core of the stylet shaft

differentiates on the inner surface of the vacuolar membrane at one pole of the stylet vacuole; 3) calcification of the shaft occurs around the organic core; and 4) after the shaft is well developed, a knob-shaped proximal piece is assembled on the broadened base of the shaft.

Ultrastructural studies of stylet formation (Stricker, 1981, 1983*a*, *b*, 1984) indicate that the contents of the stylet vacuole are derived mainly from the smooth endoplasmic reticulum. Well developed Golgi bodies also appear to participate in the formation of the vacuole. The organic material of the stylet shaft displays a species-specific banding pattern in decalcified thin sections. The shape of the organic core corresponds to that of the surrounding inorganic cortex, which suggests that the organic material serves as a template during styletogenesis. Following formation of the core of the shaft, mitochondria become aggregated around the stylet vacuole and presumably aid in accumulating the calcium salts used in the production of the stylet. The organic material of the proximal piece is non-banded, and there is a peripheral rim of electron-dense substances in this region of the stylet that is not visible in the shaft.

Stylet Replacement

Replacement of the central stylet occurs periodically during growth of the worm, as larger stylets are substituted for previously formed smaller versions (Coe, 1943). In fully grown adults, the central stylet is also replaced by a reserve stylet following loss or damage incurred during prey attack. In addition, stylet replacement can occasionally be observed in the absence of prey attack (Stricker and Cloney, 1982). Specimens of *Prostoma rubrum* apparently replace their central stylet after nearly every feeding episode (Reisinger, 1926). Observations of other species, however, suggest that stylet replacement occurs relatively rarely, at least in laboratory experiments where several species of amphipods are offered as prey (Stricker and Cloney, 1982).

The complete sequence of stylet replacement has not been observed in any one adult specimen, apparently because the process is halted by relaxation in $MgCl_2$ and compression of the body wall, treatments which are necessary to observe the intact adult stylet apparatus with a compound microscope (Reisinger, 1926; Stricker and Cloney, 1982). Reconstructions of stages observed in different adult worms, as well as observations made of the attachment of the central stylet in juveniles, indicate that the process occurs in the following manner (Stricker and Cloney, 1982). One to several reserve stylets are ejected from the reserve stylet sac, apparently by contractions of the surrounding muscles and/or the squamous epithelial cell processes of the sac wall. The stylets travel through the duct of the stylet sac and enter the lumen of the proboscis near the region where the anterior end of the basis is located. Once in the lumen, the stylets are actively moved about by contractions of the proboscis and the musculature of the body wall. One stylet is thereby oriented with its proximal piece toward the basis, and contractions of the longitudinal muscle in the wall of the proboscis draw the stylet toward the basis. The stylet in proximity to the basis becomes attached, presumably owing to adhesive properties of the basis granules or some other substances in the basis. The basis may attach more readily to the thin peripheral layer of organic material in the proximal piece, and thus facilitate orientation of the stylet during stylet replacement (Stricker, 1984).

The Stylet Apparatus and Prey Capture

During a typical feeding episode, the central stylet stabs the prey several times, and the prey becomes rapidly immobilized owing to the introduction of venom into the stylet-inflicted wounds. Histological and ultrastructural studies of the stylet apparatus of *Paranemertes peregrina* (Stricker and Cloney, 1981, 1983) suggest that the venom is introduced in a twofold manner. First, any toxin occurring in the stylet bulb of the mid-proboscis or in the lumen of the posterior chamber may be squeezed through the ductus ejaculatorius by means of muscular contractions. Since the lining of the ductus is continuous with the basis

sheath, material emananting from this canal will most likely pass through the narrow space between the basis sheath and basis, and thereby exit near the proximal piece end of the central stylet. Passage of material may be facilitated by the contractions of radially-arranged muscles that are inserted in the anterior part of the basis sheath (Stricker and Cloney, 1981). The extruded contents of the ductus ejaculatorius would then presumably travel along the surface of the stylet. In species with grooved stylets, the surface ornamentation may aid the introduction of the venom discharged by the posterior parts of the proboscis.

The second mode of envenomation probably entails a rubbing in of venomous mucus as the proboscis is wrapped around the prey. The anterior chamber contains over 95% of the toxin that can be extracted from proboscidial tissues (Kem, 1971), and it seems likely that toxin secreted by cells in the everted anterior chamber can gain entry into the prey when the proboscis is wrapped around the prey (Stricker and Cloney, 1981, 1983). Such toxins would thus re-enforce any toxin that had been previously transferred to storage sites in the more posterior chambers of the proboscis and ejected through the ductus ejaculatorius.

The Evolution of Toxin-Producing Cells and the Stylet Apparatus

Most authors (*e.g.*, Hyman, 1951; Gibson, 1970, 1972; Halstead, 1978) have maintained that toxins used in prey capture are produced caudal to the stylet apparatus in the posterior parts of the proboscis, because such a location would be advantageous for pumping venom into the prey. Biochemical studies of the toxin produced by *Paranemertes peregrina* (Kem, 1971, 1973) have shown, however, that nearly all of the proboscidial toxin is found in extracts of the anterior chamber. Recently, the glandular epithelium of the proboscis in *P. peregrina* has been studied by ultrastructural methods, and a cell type in the anterior chamber has been identified as a putative toxin-producing cell (Stricker and Cloney, 1983).

In this last section, I will attempt to delineate the basic evolutionary stages that may have given rise to the monostiliferan condition in which a stylet apparatus is present in the middle region of the proboscis and toxin-producing cells occur predominantly in the anterior chamber. The sequence is diagrammed in Figure 12 and discussed in greater detail by Stricker (1983*b*).

According to most phylogenies, the ancestral nemerteans, or "Urnemerteans," arose from proboscidiate turbellarians that resembled modern day kalyptorhynchid neorhabdocoels (Hyman, 1951) or haplopharyngid macrostomids (Karling, 1965). Based on the widespread occurrence of various toxins in the body wall of extant nemerteans, it seems likely that Urnemerteans, or some of their close descendents, possessed integumentary toxins that served to deter predators.

In extant monostiliferans, the glandular epithelium of the proboscis develops from an invagination of the ectoderm that forms the epidermis (Friedrich, 1979). During development of Urnemerteans, the invaginated proboscidial rudiment probably included differentiating cells with toxin-producing capabilities. Thus, some of the primitive venomous nemerteans may have also acquired toxin-producing cells in their proboscides (Fig. 12A). It is unlikely, however, that these proboscidial toxins were used in prey capture without some means of wounding the prey.

The next step that can be envisioned is that selected species developed puncturing devices, such as nematocyst-like pseudocnidae (Martin, 1914). Pseudocnidae, or other non-calcified structures that may represent stylet analogues, occur in the glandular epithelium of the proboscis in extant paleonemerteans (Jennings and Gibson, 1969), heteronemerteans (Müller, 1852; Iwata, 1967; Anadón, 1971), and perhaps in some polystiliferans (Gibson, 1983). In primitive nemerteans, these puncturing devices probably arose as antipredatory structures in the body wall and subsequently became distributed through-

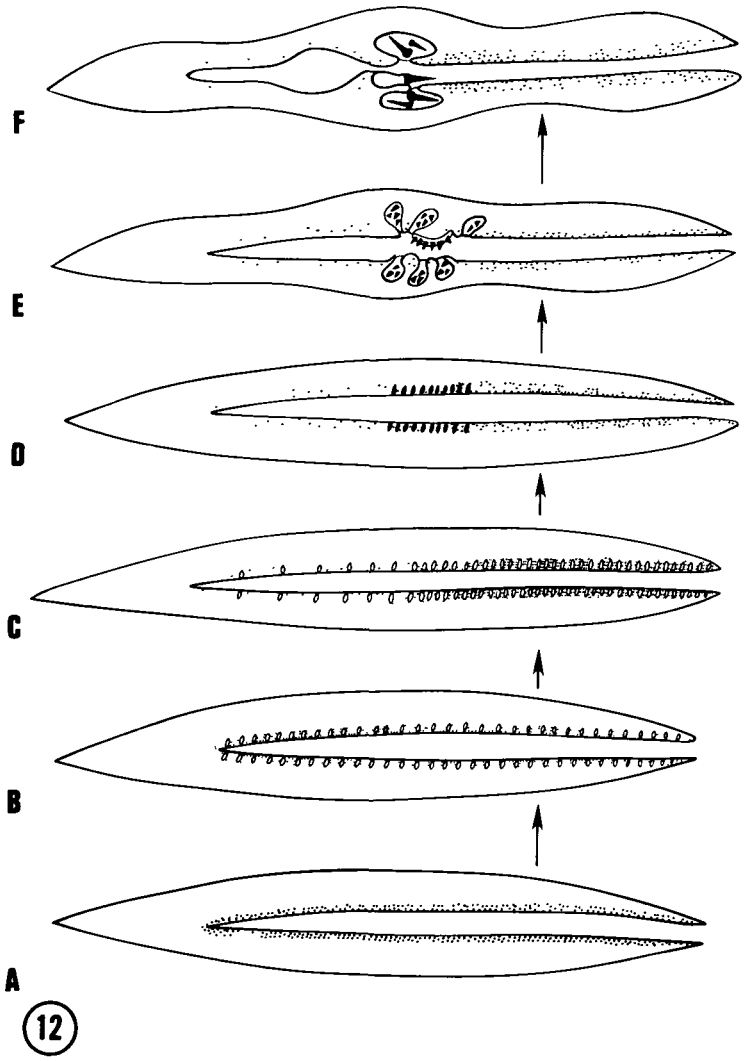


FIG. 12. Diagram of a hypothetical series of nemertean proboscides depicting various stages in the co-evolution of the stylet apparatus and toxin-producing cells. Anterior is to the right; the proboscis sheath, retractor muscle, and proboscis insertion are not illustrated. Legend: stippling = proboscidial toxin; clear ovate structures = non-calcified puncturing devices such as pseudocnidae; black ovate structures = calcified puncturing devices; black triangular or nail-shaped structures = calcified stylets. A. The proboscis of a hypothetical Urnemertean with toxin-producing cells distributed throughout the length of its glandular lining epithelium. B. A proboscis with toxin-producing cells and non-calcified puncturing devices throughout the glandular epithelium. C. A proboscis with toxin-producing cells and non-calcified puncturing devices concentrated in the glandular epithelium of the anterior chamber. D. A proboscis with calcified puncturing devices concentrated in the mid-proboscis region and toxin-producing cells mainly in the anterior chamber. E. A polystiliferan-like proboscis with numerous stylet-producing cells, and several minute stylets attached to a large basis in the mid-proboscis region. Toxin-producing cells are concentrated in the anterior chamber. F. A monostiliferan-like stylet apparatus. A single stylet is attached to the basis, and only a few reserve stylet sacs are present. Toxin-producing cells occur mainly in the anterior chamber.

out the glandular epithelium of the proboscis (Fig. 12B).

In addition to deterring predators, proboscidial pseudocnidae could have enabled the worm to use its proboscidial toxins in prey capture, by providing wounds into which the toxins could enter. Subsequently, the pseudocnidae probably became concentrated in the anterior chamber of the proboscis (Fig. 12C), since this region is the first part of the everted organ to be wrapped around the prey.

A further modification that may have arisen is a trend toward calcification of the puncturing devices. Such a process presumably made these structures more efficient weapons and thus enabled the worms to capture prey that were previously unobtainable. The longer assembly times that were probably associated with producing calcified puncturing devices, coupled with the greater metabolic output that may have been required, could have necessitated a reduction in the number of calcified structures produced by each worm. These few calcified structures probably became restricted to the middle region of the proboscis (Fig. 12D), since calcified devices at this site would be in the optimum position to stab the prey, following an initial immobilization effected by a wrapping around of the everted anterior chamber.

The next advance probably entailed the formation of an anchor that would have served to minimize the loss of puncturing devices. Attachment of calcified structures to the anchoring body, or basis, most likely brought about structural changes in the devices such that they acquired a broadened base for holding them in place and a tapered shaft for stabbing the prey. These attached structures would have thus resembled extant stylets.

Based on a wide variety of morphological characters that indicate polystiliferans are more primitive than monostiliferous hoplonemerteans (Stiasny-Wijnhoff, 1923), it seems likely that the original version of the stylet apparatus consisted of a number of stylet-forming cells in the glandular epithelium of the proboscis and numerous small stylets attached to a relatively large basis (Fig. 12E). Subsequently, worms with larger stylets may have gained a selective advantage in that they could capture larger prey, or prey with a relatively hard body wall. The increase in the size of the stylets probably precluded attachment of more than one stylet per basis, and thereby brought about a reduction in the number of styletocytes in each proboscis (Fig. 12F). This form of the stylet apparatus is similar to that found in extant monostiliferans, the largest and presumably the most highly evolved group of living nemerteans (Stiasny-Wijnhoff, 1923; Gibson, 1982).

The evolutionary scheme presented here accounts for the seemingly anomolous localization of toxin-producing cells anterior to the stylet apparatus. Toxin-producing cells in the anterior chamber of a monostiliferan proboscis may thus represent an "evolutionary holdover" from an ancestral condition in which a large number of anteriorly located toxin-producing cells were associated with non-calcified puncturing devices such as pseudocnidae. Moreover, it can be argued that maintenance of this distribution is adaptive in that it allows a greater number of toxin-producing cells to develop, than could form in the relatively short posterior chamber. Accordingly, a monostiliferan with anteriorly located toxin-producing cells would have the ability both to store toxin in the posterior parts of the proboscis and to rub venom into stylet-inflicted wounds as the everted anterior chamber is wrapped around the prey.

ACKNOWLEDGMENTS

I thank R. Cloney for his collaboration and advice over the past several years. I also appreciate the many helpful discussions I had with M. Cavey, R. Fernald, J. Norenburg, C. Reed, P. Roe, T. Schroeder, G. Shinn, W. Wilson, and J. Wourms. I am especially grateful to J. Norenburg for supplying specimens of *Ototyphlonemertes*. Studies reviewed in this paper were conducted at Friday Harbor Laboratories of the University of Washington and were supported by a Marine/Freshwater biomedical core grant #67-0671, an N.I.H. grant #GM 07270, and an Alberta Heritage biomedical research post-doctoral fellowship.

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