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The lack of a structured blood–brain barrier in the Onychophoran *Peripatus acacioi*

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Summary

Onychophorans are 'living fossils' frequently purported to have evolved from the same ancestor as the arthropods and annelids. In the CNS of *Peripatus acacioi*, beneath an outer acellular neural lamella, glial cells ensheath the cerebral ganglion and the nerve cords. These glial cells are, however, attenuated and rather few in number and, although they interdigitate with one another, they seem to lack intercellular junctions. Exogenous tracers penetrate between them and into the underlying neuropile, suggesting that there is no structural blood–brain barrier. Throughout the nervous tissue, extracellular spaces occur which contain banded collagen fibrils embedded in a matrix material. Thin glial cell processes, characterized by dense filaments, surround these regions and frequently form hemi-desmosomes with the extracellular matrix. The peripheral nerve cell bodies have a range of diameters; some have the characteristics of neurosecretory neurons. Granules in such neurons are produced by the Golgi saccules and associated fenestrated membranes which also possess many coated vesicles. Comparable granules are also found in axonal tracts, but no distinct peripheral neurohaemal areas have been found. Lysosomes are common in the nerve cell bodies and are frequently in the form of multivesicular bodies or large phagocytic vacuoles. Beneath the outer nerve cells lie many tracheae, arranged as a ring around the central neuropile which consists of glial processes, extracellular matrix, axons and nerve terminals. These nerve terminals occur throughout the central neuropile and are characterized by dense pyramidal presynaptic specializations and postsynaptic subsurface cisternae. The nervous system of *Peripatus* is relatively simple in its organization, in the lack of glial intercellular junctions and in the ready accessibility of substances from the external milieu.

Introduction

The morphology and anatomical arrangement of the nervous system of the group of invertebrates known as onychophorans was rudimentarily described by authors at the end of the last century and the beginning of this century (Sanger, 1869; Balfour, 1883; Gaffron, 1885; Saint-Remy, 1890; Holmgren, 1916; Fedorow, 1926, 1929; Hanström, 1935; Snodgrass, 1938). The ambiguous phylogenetic position of the Onychophora has made them organisms of interest to biologists for many years. More recently, Henry (1948), Bullock & Horridge (1965) and Pflugfelder (1968) reviewed the literature and discussed the nervous system of the onychophorans from a comparative point of view. Gabe (1954), Sanchez (1958) and Campiglia (1969) provided some additional information about the light microscopical distribution of neurosecretory cells within the CNS and about the infracerebral organ.

Pharmacological studies on the nervous system and neuromuscular transmission in onychophorans, although limited, have been carried out by Ewer & van den Berg (1954), Florey & Florey (1965); Gardner *et al.* (1978), Gardner & Robson (1978;); Hoyle & Del Castillo (1979), Hoyle & Williams (1980) and Del Castillo & Hoyle (1982). There has, however, been no exhaustive, fine-structural examination of the onychophoran CNS, although some observations exist on the ultrastructure of the giant fibres in the ventral nerve cord as well as on synapses and nerve–muscle contacts (Schürmann & Sandeman 1976, Lavallard, 1977, Schürmann, 1978a, b; Lavallard & Campiglia, 1979).

The CNS of onychophorans consists of a supra-oesophageal ganglion (brain) and two long, widely separated nerve cords extending from the brain to the posterior end of the body (Federow, 1926, 1929). The brain, which is bilobed, receives the antennal nerves and gives rise laterally to a pair of small optic lobes that subtend the eyes and the infracerebral organs. The nerve cords are connected by nine or ten ventral

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commissures per somite and they give off in each segment a series of dorsal and ventral nerves. Opposite each of the legs the nerve cords are slightly thickened, but we have observed that they have no differentiated ganglia since the neurons appear to be scattered along their length.

Onychophorans belong to a group of animals which does not appear to have changed greatly since the Cambrian period. Since their discovery, the phylogenetic position of onychophorans has been the subject of much discussion (reviewed by Zacher, 1933); it was once considered that they occupied an intermediate position between the annelids and the arthropods. Nowadays, two different opinions prevail: one, led by Manton (1973), incorporates the onychoporans in the phylum Uniramia together with the Myriapoda and Hexapoda; another, held by Zacher (1933), Snodgrass (1938), Cuenot (1949) and supported by recent ultrastructural studies (Lavallard, 1977; Hoyle & Williams, 1980), proposes that onychophorans have to be considered as an independent phylum.

Taking into account the evolutionary importance of the onychophorans and the lack of information on the ultrastructure of their CNS, this paper describes the fine structure of the cerebral ganglion and nerve cord of *Peripatus acacioi* Marcus & Marcus (1955). A preliminary report of this work has been published elsewhere (Lane & Campiglia, 1985).

Materials and methods

Both the cerebral ganglion and the two unfused ventral nerve cords were either dissected out in Ringer solution and then transferred to fixative, or flooded *in situ* with fixative before dissection. The fixative used was 1–2.5% glutaraldehyde in 0.05 M or 0.1 M phosphate buffer, pH 7.2, with or without added 6% sucrose. The tissue was left in this fixative at room temperature or 4° C for between 1 and 24 hours. The less hypertonic solutions, around 200 rather than 400 mOsmol or more, gave better preservation. Following primary fixation the material was washed in 0.1 M phosphate buffer with or without 6% sucrose, and treated with 1% OsO_4 in the same buffer. After 1 h at room temperature the tissue was washed in buffer and stained with 2% aqueous uranyl acetate for 30–60 min and then dehydrated through an ascending series of ethanols to propylene oxide. The material was embedded in Araldite and thick and ultrathin sections were cut with a Reichert Ultratome III. These were stained with methylene blue or uranyl acetate and lead citrate, respectively. The thin sections were examined in a Philips EM 420 at 80 kV.

Some preparations were incubated for 1 h at room temperature in cacodylate-buffered 2.5% glutaraldehyde fixative to which 3% colloidal lanthanum had been added, or in Tris-buffered physiological saline (202 mOsmol) with added 10 mM ionic lanthanum. After the latter treatment, the tissue was transferred to 2.5% glutaraldehyde buffered with 0.1 M phosphate buffer, in order to precipitate the lanthanum in an insoluble form. Following fixation, these tissues were treated in the same way as the conventional material described earlier, and were embedded in Araldite, sectioned and examined in a Philips EM 420.

Results

General morphology

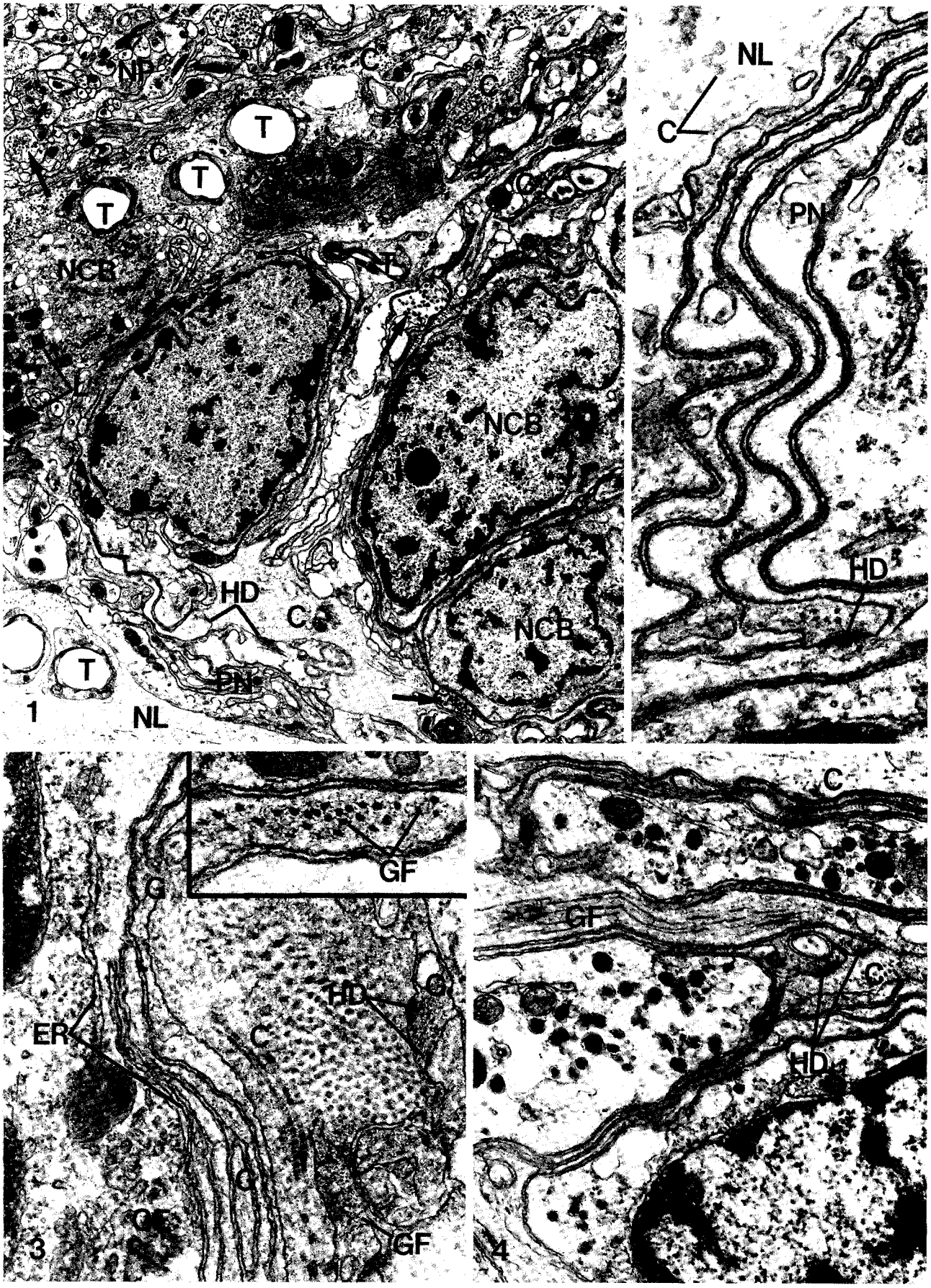
The ventral nerve cord in *Peripatus* is unfused, consisting of two separate cords which project along the length of the body (Federow, 1926, 1929). As mentioned earlier these do not exhibit ganglionic regions; they rather give rise to peripheral nerves along their lengths suggesting that nerve cells are also distributed along the length of the cord. The two nerve cords arise from a single large supra-oesophageal ganglion which forms the 'brain'. The general organization of the cells in the brain and

Fig. 1. Low power view of the periphery of the ventral nerve cord of *Peripatus*, showing the external extracellular neural lamella (NL), the underlying layer of sheath cells (the perineurium, PN) and the nerve cell bodies (NCB), that the perineurium in turn encapsulates. Spaces filled with collagen (C) occur frequently and the glial cells abutting onto them exhibit hemi-desmosomes (HD). A layer of tracheae (T) is interposed between the nerve and glial cell bodies and the neuropile (NP) and many neurosecretory granule-laden axons are found there and elsewhere (arrows). L, lysosomes in NCB. × 6900.

Fig. 2. The outer glial cells, or perineurium (PN), of the ventral nerve cord, encompassed by the acellular neural lamella (NL) which contains collagen (C), lack any apparent intercellular junctions; their clefts are seen to be unmodified except where they abut against collagen-filled extracellular space. There their membranes exhibit hemi-desmosomes (HD). The perineurial cells have fibrils of neural lamellar matrix extending from their outermost membranes. × 59 500.

Fig. 3. Nerve and glial cells in the ventral nerve cord with attenuated glial processes (G) surrounding extracellular spaces filled with collagen (C), against which the cells form hemi-desmosomes (HD). From the glial membranes, fibrils of extracellular matrix may extend. The cisternae of endoplasmic reticulum (ER) in the nerve cell may lie parallel to the plasma membrane and in intimate association with it. Glial processes frequently contain glial fibrils (GF) seen here and in the insert, in cross-section. CE, centriole. × 44 000; Insert, × 70 500.

Fig. 4. Neurosecretory cell bodies and processes in the ventral nerve cord ensheathed by glial cell processes which contain glial fibrils (GF), cut along their longitudinal axis. HD, hemi-desmosomes of the glial cells where they abut onto spaces filled with collagen (C). × 30 000.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those in the nerve cords is essentially similar; in both cases peripheral nerve cell bodies and their encompassing glia underlie an outer cell layer which has an associated connective tissue sheath or neural lamella. The outer rind of nerve cell bodies surrounds a region of tracheae which in turn ensheath the central neuropile of axons and synapses with intermingling glial cell processes. In the nerve cord most nerve cell bodies are located on the ventral–lateral surface, while at the dorsal surface the neuropile is in direct contact with the glia and other connective tissue components.

Glial cells and extracellular matrix

The cells that form the layer surrounding the nervous tissue are relatively undifferentiated; they are flattened cells with thin cytoplasmic processes which may not always form a continuous ensheathment round the CNS. From their position around the nervous tissue it is assumed that they are modified glial cells, or perineurial cells. They are surrounded externally by a neural lamella consisting of collagen fibrils embedded in an amorphous matrix penetrated by occasional tracheae (Fig. 1). Frequently, haemocytes can be seen in this area. The outer glial or perineurial processes partially overlap with one another to form an incomplete encapsulating sheath. No intercellular modifications, such as septate, tight or gap junctions, have yet been found between them (Fig. 2). Perineurial cell processes project down between the underlying nerve cell bodies into the neuropile (Fig. 5). These processes, as well as those of the glial cells underlying them, possess cytoplasmic fibrils which are apparent in cross-section (Fig. 3), longitudinal section (Fig. 4) or tangential section (see Fig. 17) and measure about 16–18 nm in diameter. These fibrils exhibit an irregular centre-to-centre spacing and in some cases appear to be inserted into hemi-desmosomal structures. The hemi-desmosomes are found where glial cells abut against the extracellular matrix (as in Figs 2, 4, 6), and this matrix is found throughout the neuropile in irregularly shaped lacunae of a range of sizes (as shown in Figs 3–6). The glial cells may be involved in the synthesis and elaboration of the extracellular matrix, fibrils of which are frequently seen extending from their membranes (Figs 2, 3). The extracellular matrix has many distinct collagen fibrils embedded within it (Figs 3, 6, 12). These fibrils are roughly circular in cross-section and variable in diameter but exhibit a longitudinal banding periodicity as has been described earlier (Lavallard, 1977). The deeper glial cells send their processes around neurites and axons in the neuropile and intermingle between axonal components with no apparent pattern except that they always line the channels and spaces filled with extracellular matrix

Fig. 5. Neuropile from the medulla of the ventral nerve cord showing spaces filled with collagen (C), and glial processes (G). The remainder consists of axonal and neuritic processes, some of which bear dense neurosecretory granules. Many areas (arrows) are present which contain the paramembranous densities typical of synaptic structures. × 9800.

Fig. 6. Region of the brain neuropile (NP) indicating the way neuritic processes intermingle with glial processes (G) which lie adjacent to the extracellular spaces containing collagen (C). HD, hemi-desmosomes. × 42 000.

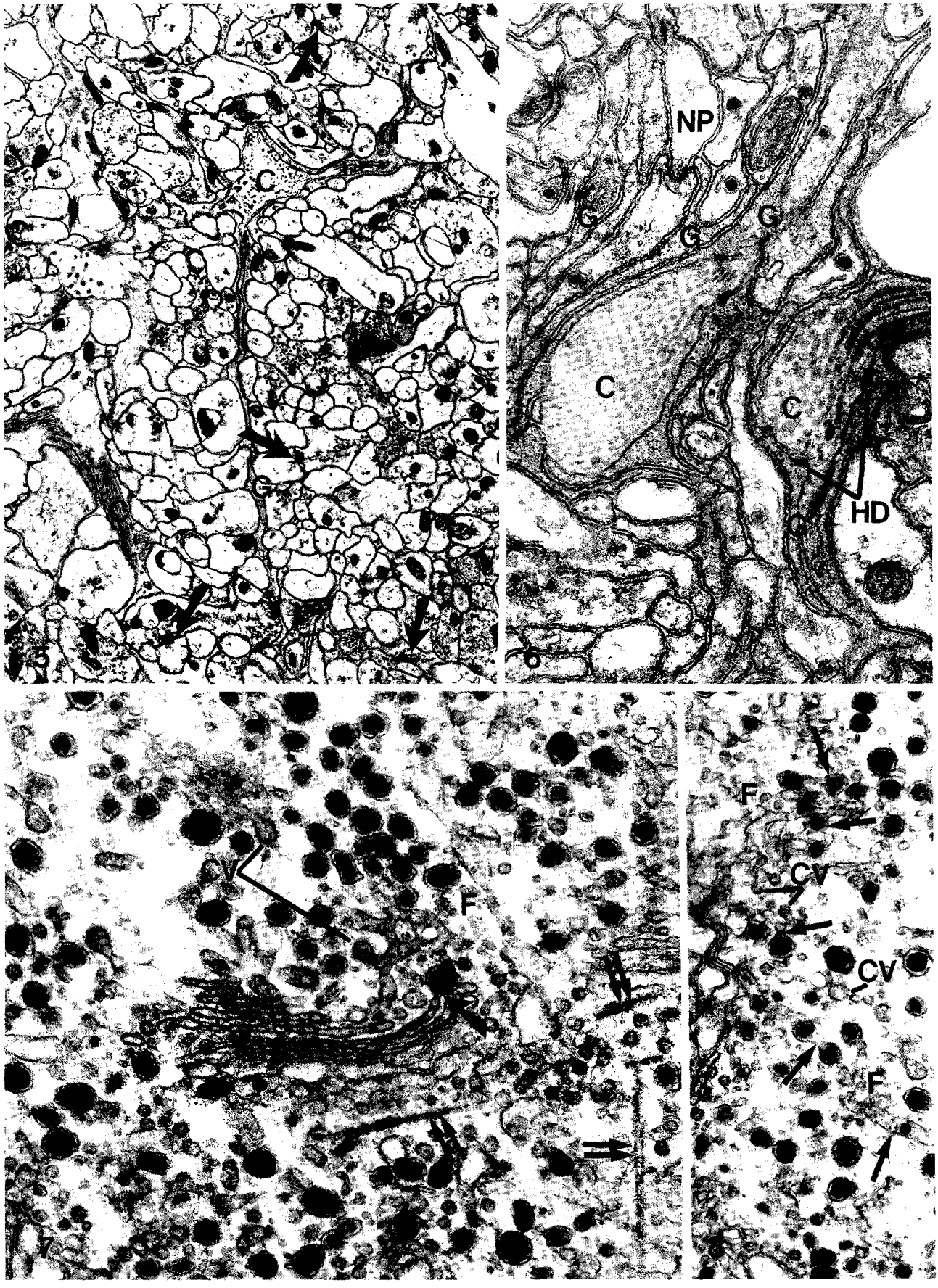
Figs 7, 8. Neurosecretory granule-containing nerve cell bodies from the ventral nerve cord, indicating how the dense granules are produced (arrows) from the smooth membranes of the Golgi, both from the saccules and fenestrated (F) region. Coated vesicles (CV) are also in attendance here. In some cases, striated filaments (double arrows) are prominent in the perikarya. Fig. 7, × 48 000; Fig. 8, × 39 000.

Fig. 9. Neuronal perikarya from the ventral nerve cord showing nucleus (N), endoplasmic reticulum (ER), massed mitochondria (M), Golgi saccules (GS) and lysosomes (L), some of which may be multivesicular bodies (MVB). The latter may have membranous pits dipping into the periphery (arrows). The attenuated glial processes (G) around the nerve cells exhibit their usual fibrils (GF). × 31 500.

Figs 10–12. The neuropile of both brain (Fig. 11) and ventral nerve cord (Figs 10, 12) possesses neurosecretory granule-laden axons; normal synaptic vesicles (asterisk) frequently coexist with them and these mixed terminals make synaptic contacts (small arrows). The glial processes here tend to be restricted to the regions around the extracellular spaces (still laden with collagen, C), even in the central regions. The dense granules may be aligned in parallel with both the axonal membranes and their internal microtubules (arrow in Fig. 11), as if axonal transport might be occurring. Fig. 10, × 36 500; Fig. 11, × 63 000; Fig. 12, × 22 000.

Figs 13, 14. Presynaptic regions in both brain and nerve cord are characterized by pyramidal paramembranous densities (arrows) around which the synaptic vesicles cluster. The postsynaptic region may exhibit a density and subsurface cisternae (SSC). A distinct synaptic cleft is present, containing some dense striations. Fig. 13, × 82 000; Fig. 14, × 70 000.

Figs 15–17. Tissues from the CNS, both brain (Fig. 17) and ventral nerve cord (Figs 15, 16), incubated with lanthanum; this tracer (at arrows) has free access into the clefts between the axons (A) and glial (G) cells as well as into dilations of the extracellular space (ECS) and the clefts by synapses (S). The tracer is in the form of loose crystals in the extracellular matrix but becomes compacted into denser aggregates in the clefts. The glial cells (G) contain fibrils, here cut tangentially (Fig. 17). Fig. 15, × 50 000; Fig. 16, × 80 000; Fig. 17, × 48 000; Insert, × 52 500.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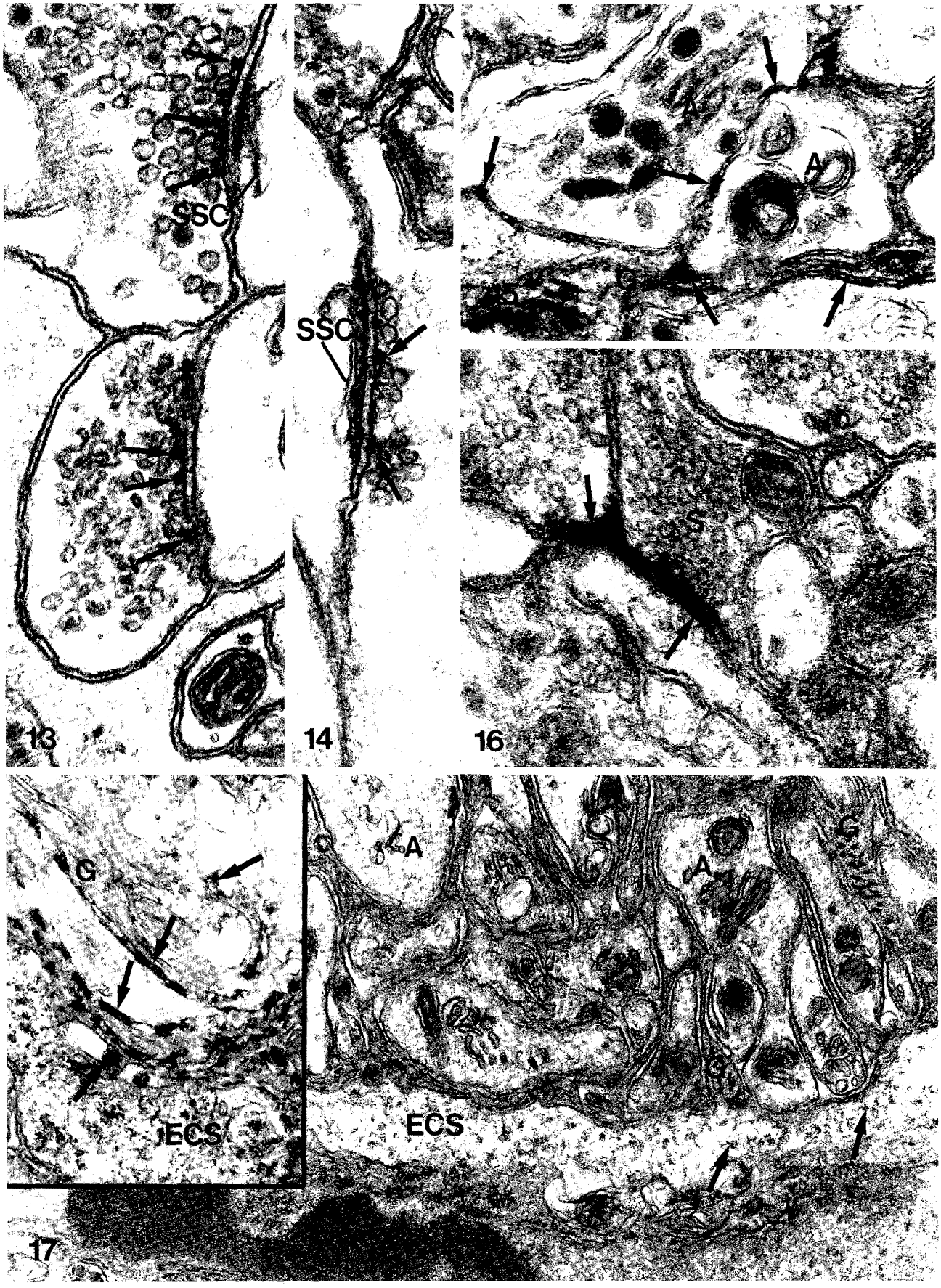
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(Figs 5, 6). No vertebrate-like myelination occurs nor are clear-cut spiral, mesaxon-like wrappings of axons or axon bundles apparent. It seems, therefore, that only two types of glial cell occur: the outer perineurial glial cells forming a sheath and the inner glial cells that surround the axons and line the extracellular spaces.

Nerve cells

As in other invertebrate nervous systems, glial-ensheathed nerve cell bodies comprise a rind around the neuropile in the cerebral ganglia and nerve cords (Fig. 1). Neural and glial cell processes make up the neuropile itself. Numerous tracheae are found immediately beneath the neuronal perikarya (Fig. 1). These are somewhat different to those of insects, particularly with regard to the distribution and number of their orifices, as has been described in detail elsewhere (Pflugfelder, 1955; Lavallard, 1977). The majority of nerve cell bodies themselves are relatively small. Hence, they usually appear to lack the extensive glial trophosphongial invaginations (Holmgren, 1916; Hoyle *et al.*, 1986) which are characteristic of large invertebrate perikarya, particularly in arthropods (Lane, 1985); possibly smaller cell bodies do not require the trophic support these processes are considered to supply. Giant cell bodies can be found in certain parts of the brain as in the nerve cord, but their number is relatively small. Some of the neurons appear to be neurosecretory insofar as the presence of dense granules is characteristic of such cells. There appear to be three categories of these neurons with regard to their size and shape: small unipolar cells, bipolar cells which lie against the outer neural lamella, and multipolar neurosecretory cells. These are all found lying between apparently non-neurosecretory neurons. The neurons possess cisternae of endoplasmic reticulum, of which the outermost ones may lie close against the plasma membrane (see Fig. 3), stacked Golgi saccules (Fig. 7) and some lysosomes (Fig. 9). The Golgi complex is frequently associated with coated vesicles (Fig. 7) and, in the neurosecretory cells, dense neurosecretory granules are apparently produced both from its saccules (Fig. 7) and its fenestrated regions (Fig. 8). The lysosomes may be in the form of undifferentiated dense bodies (Fig. 1), multivesicular bodies (Fig. 9) or secondary lysosomes which contain undigested remnants (Fig. 9). The perikarya sometimes contain striated filaments (Fig. 7) but these do not appear in the axons. The nerve cell bodies become prolonged into axonal processes (Fig. 11) and dense granules are often found in these throughout the central region of the nervous tissues (Fig. 10). Frequently they can be found in rows (Fig. 11) lying between neurotubules; no distinct neurofilaments are to be found. Some of these neurosecretory granule-laden processes, together with other axonal processes, are found terminating in neuropilar areas; frequently the terminals are 'mixed' with both normal synaptic vesicles and neurosecretory vesicles in the same terminal (Fig. 12). The terminals are characterized by synaptic vesicles and presynaptic paramembranous densities (Fig. 13) which often take the form of dense pyramidal structures. The vesicles vary in size and outline which may be round or flat (Fig. 13). A distinct, moderately electron-opaque, synaptic cleft underlies these and postsynaptic densities are found, sometimes with associated sub-synaptic cisternae or membranous structures (Fig. 14).

Lanthanum infiltration

It is evident that the nerve cells in both cerebral ganglia and nerve cord are accessible to some of the substances present in the circulating haemolymph. When either the brain or nerve cord is incubated in the presence of an exogenous tracer such as lanthanum, the tracer penetrates into the interior of the tissue (Figs 15–17) and into the clefts between glial cells (Fig. 15 and insert, Fig. 17), axons and synaptic terminals (Fig. 16).

Discussion

Certain aspects of the ultrastructure of the onychophoran nervous system suggest that it is less complex than that of some so-called 'higher' arthropods. For example, there is no a blood–brain barrier insofar as can be judged by the free entry of exogenous tracers. This is apparently due to peripheral perineurial glial cells not being connected by the junctional complexes that are typical of insects and arachnids; these are the interglial tight junctions that appear to form the basis of the blood–brain barrier in these two arthropod classes (Lane, 1981a, 1984). However, like the onychophorans, certain other arthropods, such as the crustacea, ticks and the marine horseshoe crab, *Limulus*, offer little or no restriction to the entry of substances into the nervous system (Lane & Abbott, 1975; Binnington & Lane, 1980; Harrison & Lane, 1981). Like *Peripatus*, these groups lack occluding peripheral, glial tight junctions.

Onychophorans such as *Peripatus* also appear to lack gap junctions. This is in contrast to all other arthropod groups thus far examined, where gap junctions are present between both the outer glial cells that form the blood–brain barrier and between the processes of underlying glial cells that ensheath the axons and nerve cell bodies, within both interganglionic connectives and ganglia as well as peripheral nerves (Lane, 1981b). In many annelid species this also seems to be the case (Radojcic & Pentreath, 1979). Onychophorans, therefore, are

presumably rather primitive in their apparent lack of interglial gap junctions in the CNS and, accordingly, communication between their component glial cells. Interestingly, it was never possible to observe any gap junctions in lanthanum-treated or freeze-fractured midguts of *Peripatus* either (Dallai & Giusti, 1979). Recently Lavallard (1986) has demonstrated, using thin sections, that structures with a reduced intercellular cleft do appear to occur in the intestinal tract, but these are not typical gap junctions. They are found in association with scalariform junctions which are characterized by fine cross-striations that are less obvious than the septa in septate junctions. These latter junctions are found in the transporting epithelia of onychophorans (Lavallard, 1981) while septate junctions are also found in many epithelial tissues (Dallai & Giusti, 1979; Lavallard, 1977), as they are in numerous other invertebrates. The membranes of *Peripatus* may also become modified to form desmosomes and hemi-desmosomes (Lavallard, 1977) which are also common in annelids and arthropods (Radojcic & Pentreath, 1979; Lane, 1981b). As in many insects, such as the lepidopterans (Lane, 1972), and crustaceans (Lane & Abbott, 1975), hemi-desmosomes are particularly prevalent where glial cells abut onto the extracellular matrix in the CNS.

The glial cells of *Peripatus* possess intracellular fibrils, as do annelid glial cells (Radojcic & Pentreath, 1979; Lane, 1981b). Arthropod glia, on the other hand, do not exhibit comparable gliofilaments but possess microtubules (Roots, 1978). In this respect, then, *Peripatus* resembles the annelids more than the arthropods, although its glial fibrils are about 16–18 nm in diameter and distinctly separated from one another, while those of the annelid are closely packed in irregular bundles and are reported to be 5 nm in diamter (Radojcic & Pentreath, 1979). In annelids the gliofilaments are considered to counteract the stresses and shearing forces to which the nervous system is subjected; perhaps this is also the case in *Peripatus*. There is always the possibility that these fibril-laden glial processes could be derived from or be part of the musculature of the animal since the muscles of *Peripatus* are characterized by thick myofibrils, 18–20 nm in diameter (Lavallard, 1977).

No glial (Schwann) cell myelination of axons occurs in *Peripatus* as it does in vertebrates. Myelination proper is not found in annelids or arthropods either, except for the 'pseudomyelination' of the loose glial wrappings around the giant axons of the earthworm ventral nerve cord (Roots & Lane, 1983) and around some axons in the CNS of certain crustaceans (Heuser & Doggenweiler, 1966; Lane 1981b). The glial cells in *Peripatus* ensheath the nerve cell bodies and axons with only one or a few folds as is common in the CNS of other invertebrates.

Tracheae in the *Peripatus* CNS are particularly prominent in the region just under the nerve cell bodies but outside the central neuropile; this distribution is very like that of the tracheae in the similarly located 'glial lacunar system' of insects (Wigglesworth, 1960; Lane, 1981b). Clearly, such a position must be an efficient one for ensuring adequate oxygen supplies to the nerve terminals. The tracheae themselves differ from those of insects in the distribution of their orifices and in the involvement of the basal lamina in the formation of the mestracheon (see Lavallard, 1977); they do not even penetrate the muscle fibres (Hoyle & Williams, 1980), while annelids lack such structures entirely.

Collagen fibrils lie in the outer acellular neural lamella and, together with the extracellular matrix, in the spaces within the nervous system itself; they exhibit a range of cross-sectioned diameters with banding periodicities like those described by Lavallard (1977). These are similar to those of some insects and annelids (Smith & Treherne, 1963; Baccetti, 1967; Ashhurst & Bailey, 1980). However, in onychophorans the fibrils are circular in cross-section, while those of insects are floret-shaped (Lane, 1981b). The presence of masses of collagen fibrils within extracellular spaces inside the CNS is similar to that reported for crustacea such as the crayfish (Lane & Abbott, 1975), but this is not the case for insects or arachnids (Lane, 1981b). In annelids (Coggeshall & Fawcett, 1964), like the insects, collagen is restricted to the external neural lamella or basal lamina and the inner spaces contain only mucopolysaccharides or glycoproteins (Ashhurst & Costin, 1971). Whether the collagen fibrils in *Peripatus* are involved in forming a cation reservoir to help maintain a constant internal milieu for the nervous system, as has been suggested for certain crustacea (Abbott, 1979), has yet to be established.

The structures suggested to be neurosecretory nerve cells in the periphery of the *Peripatus* nerve cord are characterized by the presence of dense granules surrounded by non-dense haloes. Such cells have been reported earlier (Sanchez, 1958; Rosenberg & Seifert, 1978). The granules appear to be elaborated or condensed within the fenestrated membranes (GERL) and saccules of the Golgi complex; they are also in intimate association with lysosomes, especially multivesicular bodies, as is similar to the situation described in other invertebrates (for example, see Lane, 1985). Moreover, the spatial relationship of the neurosecretory granules with microtubules in the axonal processes where the granules tend to lie in rows parallel to the longitudinal axis of the axon, suggests that neurosecretory products may be transferred along the axon to the terminal via these tubules, perhaps mediated by microtubule-associated proteins or kinesin (Vale *et al.*, 1985). Since *Peripatus* has no clear-cut neurofilaments in its axons, it is very

like the arthropods whose axons possess only microtubules; in contrast, neurofilaments are a characteristic feature of annelid axons (Mellon *et al.*, 1980).

In the onychophoran neuropile, 'mixed' terminals containing both dense-cored granules and synaptic vesicles are found, suggesting that neurohormones may be released there. Specialized neurohaemal sites for the release of this neurosecretory material, however, have not yet been observed in the onychophoran nervous system (Campiglia, 1969). Presumably, release of the neurosecretory material may occur via intercellular clefts and extracellular matrix-bearing lacunae, directly into the circulating haemolymph, since no blood–brain barrier is present to restrict leakage from the site. Mixed terminals are also a common occurrence in the crustacea and insecta (Lane, 1985). The presynaptic pyramidal densities bear some resemblance to the presynaptic modifications found in vertebrates and certain insects (Wood *et al.*, 1977; Lane, 1985). Previous investigations (Hoyle & Williams, 1980) into the fine structure of the presynaptic terminals of *Peripatus* at neuromuscular junctions revealed that they contained accumulations of round, clear vesicles, 48–70 nm in diameter, accompanied by dense-core vesicles. The clear vesicles were clustered around presynaptic dense bodies. The synaptic specializations described here are clearly very similar to these neuromuscular junctions.

The synaptic cleft in the neuromuscular junctions, as described by Hoyle & Williams (1980), measured about 100 nm in width and contained dense material like that of the synapses described here, while the postsynaptic site in the muscle was characterized by multiple, complexly branching invaginations. In the neuropile, the postsynaptic subsurface cisternae seen in this study are like those described earlier in *Peripatus* (Lavallard & Campiglia, 1979). In contrast, although some neuromuscular junctions in arthropods have subsurface cisternae, they possess a cleft which is less wide than that of *Peripatus* (~25 nm) with no density or postsynaptic folding (Atwood & Morin, 1970; Osborne, 1975), while annelids exhibit two types of cleft, either 15 nm or 90 nm wide, with only the wider one distinguished by complex subsynaptic foldings (Rosenbluth, 1972; Schürmann, 1978b).

On the basis of their neuromuscular physiology (Hoyle & del Castillo, 1979) and the characteristic ultrastructural features of their muscle fibres (Lavallard, 1977) and neuromuscular junctions (Hoyle & Williams, 1980), onychophorans should therefore be treated as a unique phylum, unrelated to the annelids or the arthropods. This is at variance with the conclusions of Schürmann (1978) who considers that the fine structure of the onychophoran nerve–muscle junction indicates a close relationship with the annelids. Further, although onychophoran cuticle was thought originally to be arthropod-like (Robson, 1964; Hackman & Goldberg, 1975), it is now thought to have unique features (Lavallard, 1977). The onychophoran photoreceptor structure is, however, considered to resemble the nereid eye in some features and the insect ommatidium in others (Eakin & Westfall, 1964), suggesting an indeterminate phylogenetic relationship.

In conclusion, the structure of the CNS of *Peripatus* has some features in common with the annelids, and others that are similar to certain of the arthropods. As they lack glial intercellular junctions and a blood–brain barrier, the nerve cells apparently do not require isolation from the haemolymph to function adequately. They may, like other invertebrate groups such as the molluscs (Abbot, 1979), use the components of the extracellular matrix, which they have in abundance throughout the CNS, to establish the cationic reservoirs essential to nerve cell function.

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