

Some Neuron-hypodermis Relationships in the Parasitic Nematode *Trichuris myocastoris* Enigk¹

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Abstract: The association of neurons and hypodermal cells in longitudinal nerve chords and in interchordal positions (perhaps dorso-ventral commissures) in the body wall of *Trichuris myocastoris* Enigk has been examined with the electron microscope. Nerve chords are composed of several groups of neurons separated by processes of hypodermal cells; such groups may be functionally separate. Nerve cell bodies occur within the chords, and appear to be at least bipolar. Neurons emerge from the median chords and enter the interchordal regions, showing progressive invagination in hypodermal tissue with increased distance from the median line. Such invagination results in formation of mesaxons and may be significant in the nutrition of the neuron. Mesaxons may branch and may enclose single or groups of neurons; no spiralling of the mesaxon was seen. Occasionally more than one mesaxon was traced to a single neuron. **Key Words:** Neuron, Hypodermis, *Trichurus myocastoris*, Electron microscopy.

The nervous systems of a few species of nematodes have been examined by light microscopy (1, 2). The central nervous system of *Ascaris* is considered to be a series of ganglia united by the circumesophageal nerve ring, a ventral ganglionated nerve chord, and a rectal ganglion complex. The peripheral nervous system consists of a series of longitudinal somatic nerves lacking ganglia, nerves supplying the various sensory structures of the body, and dorsoventral nerve commissures thought to interconnect and coordinate the motor stimuli in the longitudinal nerves. The nerves lie in close association with the hypodermis of the body wall. This pattern varies considerably among species. Glial cells were described by Goldschmidt in 1910 (as quoted by Chitwood and Chitwood, (2)) in the nerve ring and in association with cephalic papillary nerves of *Ascaris*. The presence of nerves or neurons has been noted in several recent electron microscope studies of nematodes (5, 7, 8, 9) but neuron-hypodermis relationships have not been intensively examined, although Yuen (8) identified "myelin figures" associated with amphidial nerves. This report

describes some ultrastructural aspects of the nerve chords and interchordal neurons of *Trichuris myocastoris*, Enigk.

MATERIALS AND METHODS

Nematodes removed from caecae of freshly killed Nutria (*Myocastor coypu*) were fixed in 1.2% potassium permanganate in 0.9% NaCl for 10 hr at ~4 C or in 10% acrolein in Sorensen's 0.1 M phosphate buffer at room temperature for 15 min and postfixed 1 hr in 1% osmium in veronal acetate buffer at ~4 C. Permanganate-fixed nematodes were embedded in Araldite 502, while acrolein/osmium fixed nematodes were embedded in a 3 : 1 mixture of butyl and methyl methacrylate. Sections were stained with lead citrate.

RESULTS

Highest resolution in this study was achieved in tissues fixed in potassium permanganate. However, only the esophageal portions of the nematodes were available for study due to incomplete penetration of epoxy into the posterior portion of the body. Observations made on these tissues were, however, confirmed and extended to posterior regions of the body in examination of the methacrylate-embedded tissues.

MEDIAN HYPODERMAL CHORDS: The

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dorsal and ventral hypodermal chords in the esophageal region appear similar in structure (Fig. 1), although toward the esophagus base the dorsal hypodermal chord becomes reduced in size as neurons of the dorsal nerves disappear. The chords are composed of two elements, hypodermal cells and neurons (Fig. 2 and 5). The hypodermal cell component is not prominent in the regions examined (Fig. 7). Cell bodies of these cells contain nuclei, mitochondria, extensive granular endoplasmic reticulum, fiber bundles, and complex dense bodies resembling the organelles and inclusions of the interchordal hypodermis (Fig. 8). Toward the cuticle, the hypodermal cell processes extend between muscle cells and are reflected at the cuticle to form the thin layer of interchordal hypodermis which underlies the cuticle. Increased density is evident along the medial cell membranes of these hypodermal cells just before contact is made with the cuticle, suggesting an *adherens*-type of junction between cells (Fig. 2). Cell bodies of neurons contain a nucleus, mitochondria, a few vesicles and scattered granular endoplasmic reticulum membranes with clustered ribosomes in the cytoplasm. A few dense bodies similar to those in hypodermal cells occur. In longitudinal sections, axons can be seen arising from more than one point on these cell bodies (Fig. 5), indicating that some are at least bipolar, or possibly multipolar neurons. The neuron processes lack ribosomes and endoplasmic reticulum but contain occasional mitochondria and vesicles. Cross sections of median chords show that the neurons occur in several irregular groups enclosed by hypodermal cell processes (up to ten groups have been counted). Some of these groups are deep in the chord, while others are peripheral. Those at the periphery are not surrounded by hypodermal cell processes. Thus, no discretely organized longitudinal nerve chord is recognizable.

Numerous sections of muscle innervation processes occur around the median chords, yet specialized synaptic junctions have not been clearly identified. Towards the outer regions of the hypodermal chord, however, there frequently are larger nerve profiles which contain numerous vesicles $\sim 600 \text{ \AA}$ in size (Fig. 3). These may be synapses with the somatic muscle innervation processes.

LATERAL HYPODERMAL CHORDS: No extensive accumulations of neurons have been found in the lateral hypodermal chords. However, toward the outer margins of the lateral chord containing the bacillary band, a few neurons have been found between the bases of hypodermal cells (7). One section included a nerve cell body in this region indicating that this may be a chain of neurons. No neurons have been found in the other smaller lateral hypodermal chord which is only 3 cells wide.

INTERCHORDAL NEURONS: Neurons occur in invaginations of the interchordal hypodermis which vary greatly in depth (Fig. 9 to 14). Where neurons, after descending from the nerve groups of the median chords toward the cuticle, first emerge into the interchordal hypodermis, they lie in shallow depressions, so that approximately half of their circumference is adjacent to the hypodermis while the remainder is open to the intercellular space between hypodermis and somatic musculature (Fig. 4 and 6). Farther from the median chord, the depression into the hypodermis is greater so that more of the neuron is enclosed. Ultimately neurons are seen deep within the hypodermis ensheathed by the membrane of the enclosing hypodermal cell; the apposed hypodermal membrane connecting the neuron to the cell surface forms a mesaxon (Fig. 13). The number of neurons enclosed by a single mesaxon may be as many as twelve, but double and single neurons are more common.

Occasionally two mesaxons are connected to a single neuron (Fig. 14), while branching mesaxons also occur in which the terminus of each branch encloses a neuron (Fig. 10). The mesaxon may extend a considerable distance into the hypodermis, often lying close to a large mitochondrion (Fig. 10), but no evidence of spiraling has yet been seen. The intercellular gap between hypodermal and neuronal cell membranes, and also between the apposing membranes of the mesaxon measured in permanganate-fixed tissue is $\sim 60 \text{ \AA}$.

DISCUSSION

The observations reported here on *Trichuris myocastris* reaffirm some of the general concepts of the structure of nematode nervous systems (e.g. the presence of nerve cell bodies within the ventral nerve chord) but further indicate the variability in development of the peripheral nervous system.

Within the median hypodermal chords, the neurons are not extensively enclosed by hypodermal tissue. This differs from *Ascaris* where a single large aggregation of neurons is entirely enclosed by hypodermal cytoplasm except over the areas of neuromuscular junctions (6). Neuromuscular junctions were only tentatively identified since no specialized aggregates of mitochondria were found which might aid in their identification as in *Ascaris*. Although neurons of the median chords were not greatly enclosed by hypodermal cytoplasm, they are subdivided into units by thin processes of the hypodermal cells. Such groups may be expected to be electrically insulated from each other, while individual neurons of each group may interact by the spread of ionic currents from one neuron to another (4). It is suggested, then, that at least within the esophageal region of the median nerves, several functionally distinct nerve tracts may exist. The

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FIG. 1-4. Neuron-hypodermis fine structure of *Trichuris myocastris*: 1. Cross section through the median chord. The chord is composed mainly of longitudinally oriented neurons, with small intervening areas of hypodermis containing dense heterogeneous inclusions. Hypodermal cell processes can be traced down to the level of the cuticle (c). Sarcoplasmic regions of adjacent muscle cells contain dense, massive deposits of glycogen. KMnO_4 fixation, 18,900 \times ; 2. Adjacent cell membranes of hypodermal cells at the level of the cuticle (c) showing density (arrow) suggestive of "adherens-type" cell-attaching junction. KMnO_4 fixation, 75,200 \times ; 3. Synaptic vesicles within neurons of a median chord. KMnO_4 fixation, 39,700 \times ; 4. Cross section of the chord showing two neurons (arrows), one entering the interchordal area. KMnO_4 fixation, 31,200 \times .

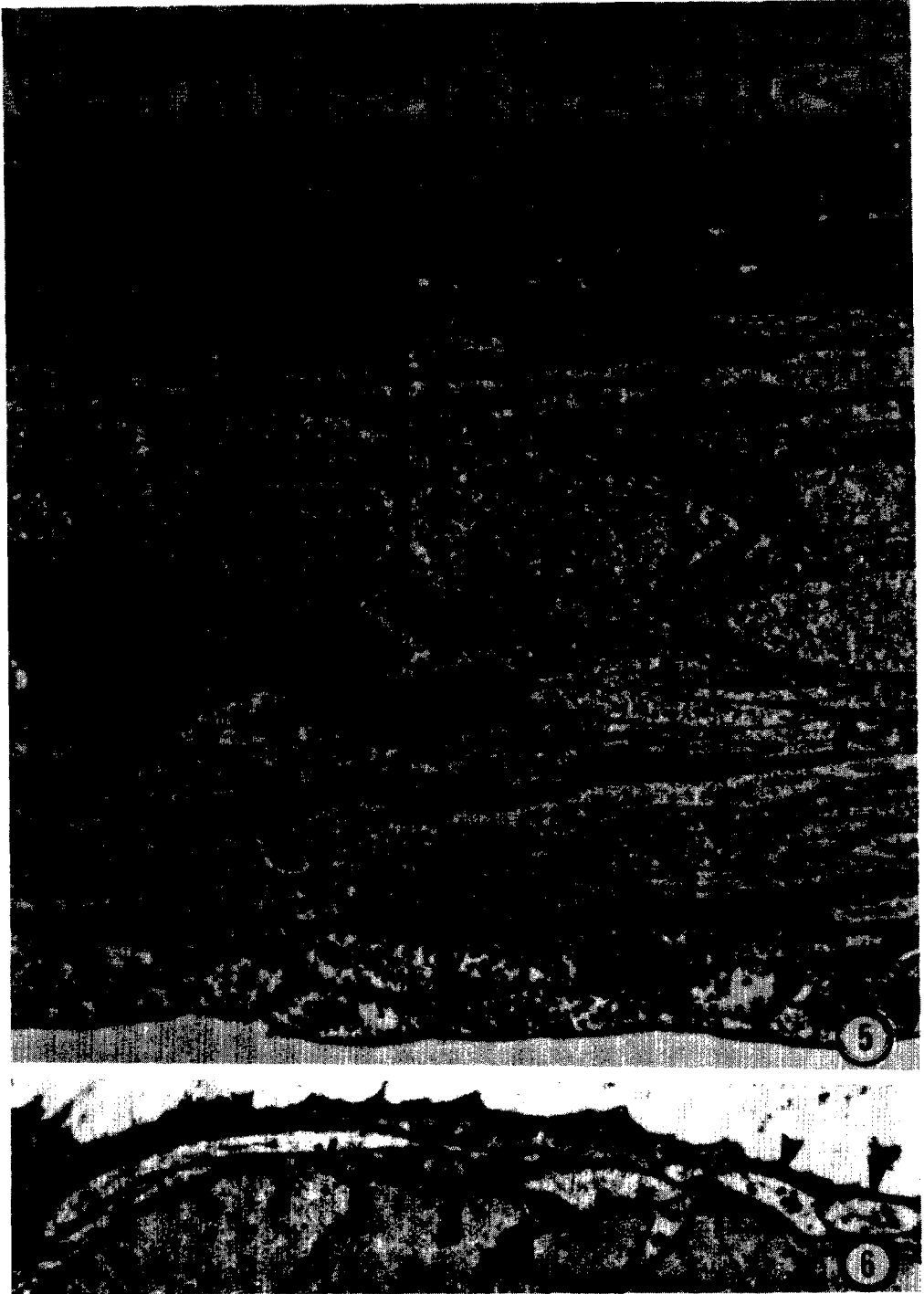
FIG. 5-6. Neuron-hypodermis fine structure of *Trichuris myocastris*: 5. Longitudinal-oblique section of median chord in posterior region of the body showing neuron cell bodies with nuclei in the mid region and sections of axons (a) above and below this. Heavy arrows indicate two axons arising from a single cell body. The contractile regions of muscle cells (CM) lie above the chord and tips of the sarcoplasmic region of muscle cells (SM) lie below. Hypodermal tissue (H) of the chord can be identified by its greater density. Thin arrows at top indicate single interchordal neurons. C = cuticle. Acrolein/osmium fixation, 8,100 \times ; 6. Cross section of the interchordal region adjacent to median chord showing a group of axons (heavy arrows) emerging from the chord. KMnO_4 fixation, 15,400 \times .

FIG. 7-8. Neuron-hypodermis fine structure of *Trichuris myocastris*: 7. Hypodermal tissue has been cross-hatched in this section to indicate the manner by which neurons are enclosed by the hypodermis. Seven neuron groups are shown. KMnO_4 fixation, 15,900 \times ; 8. Section of interchordal hypodermis from the posterior region of the body showing eight neurons associated with a single mesaxon (heavy arrows). Bundles of filaments are noted by thin arrows. N = nucleus, * = intercellular space. Acrolein/osmium fixation, 38,800 \times .

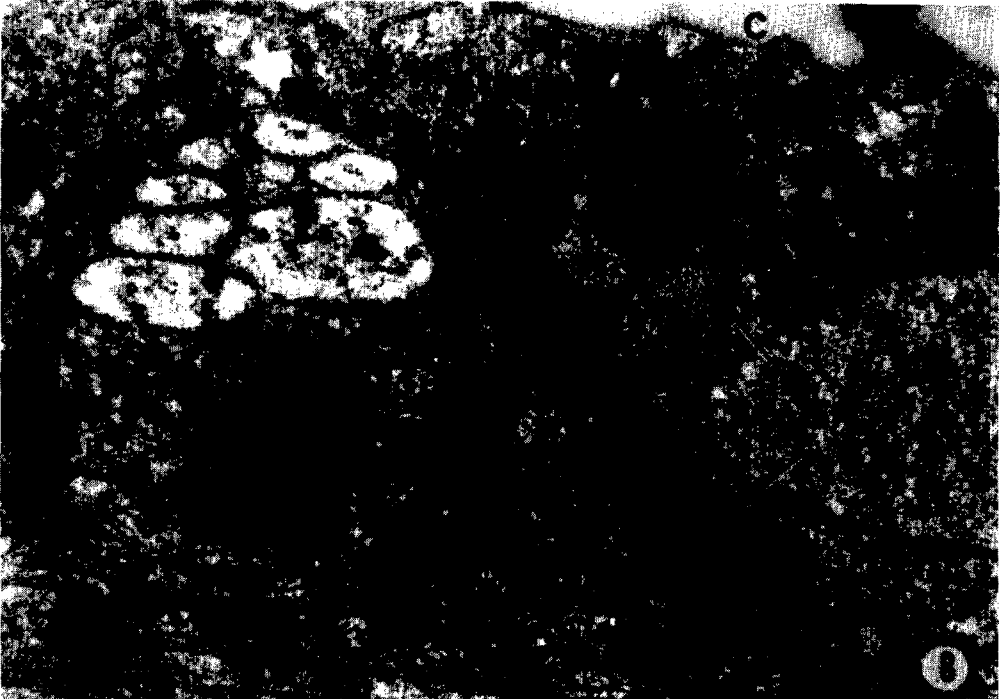
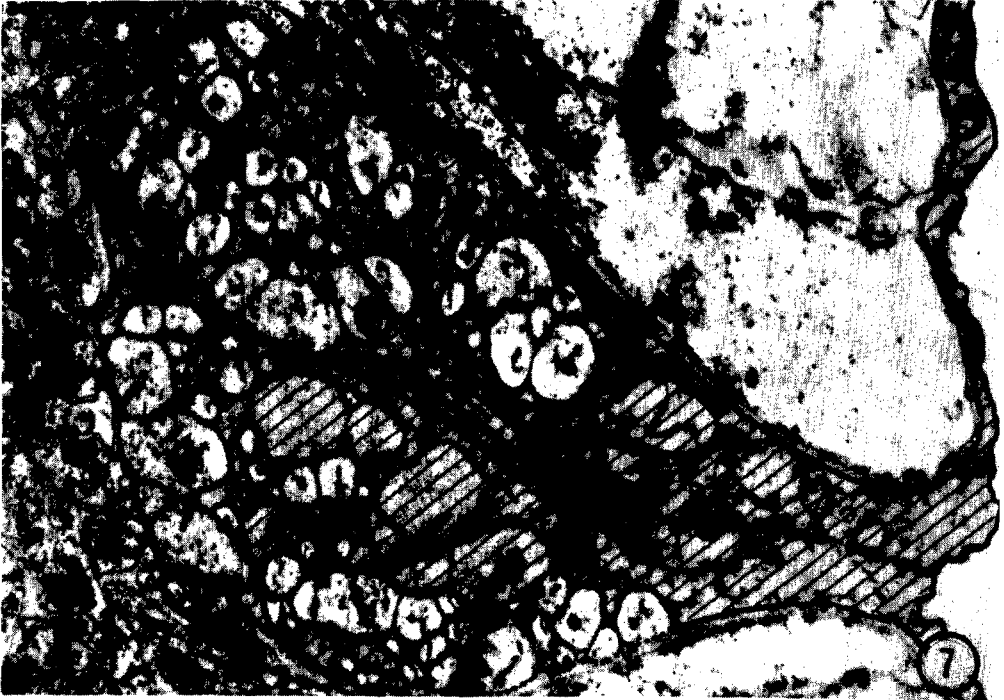
FIG. 9-10. *Trichuris myocastris*: Sections of the interchordal hypodermis of the esophageal region showing neurons within the hypodermis, and associated with mesaxons (heavy arrows). Thin arrows indicate branching mesaxons. * = intercellular space; C = cuticle; N = nucleus; G = glycogen; KMnO_4 fixation; Fig. 9, 29,600 \times . Fig. 10, 47,200 \times .



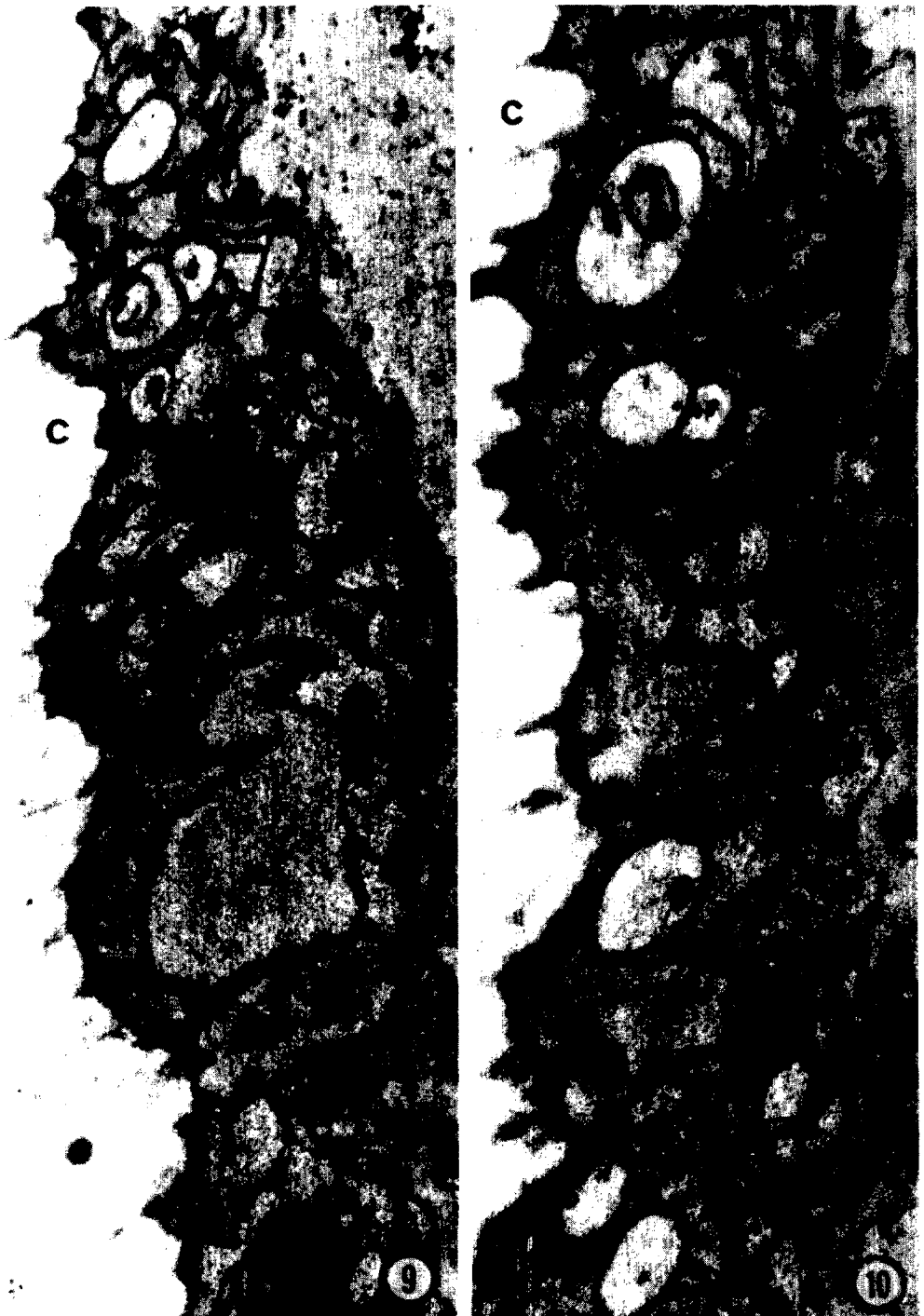
FIGURES 1-4.



FIGURES 5, 6.



FIGURES 7, 8.



FIGURES 9, 10.

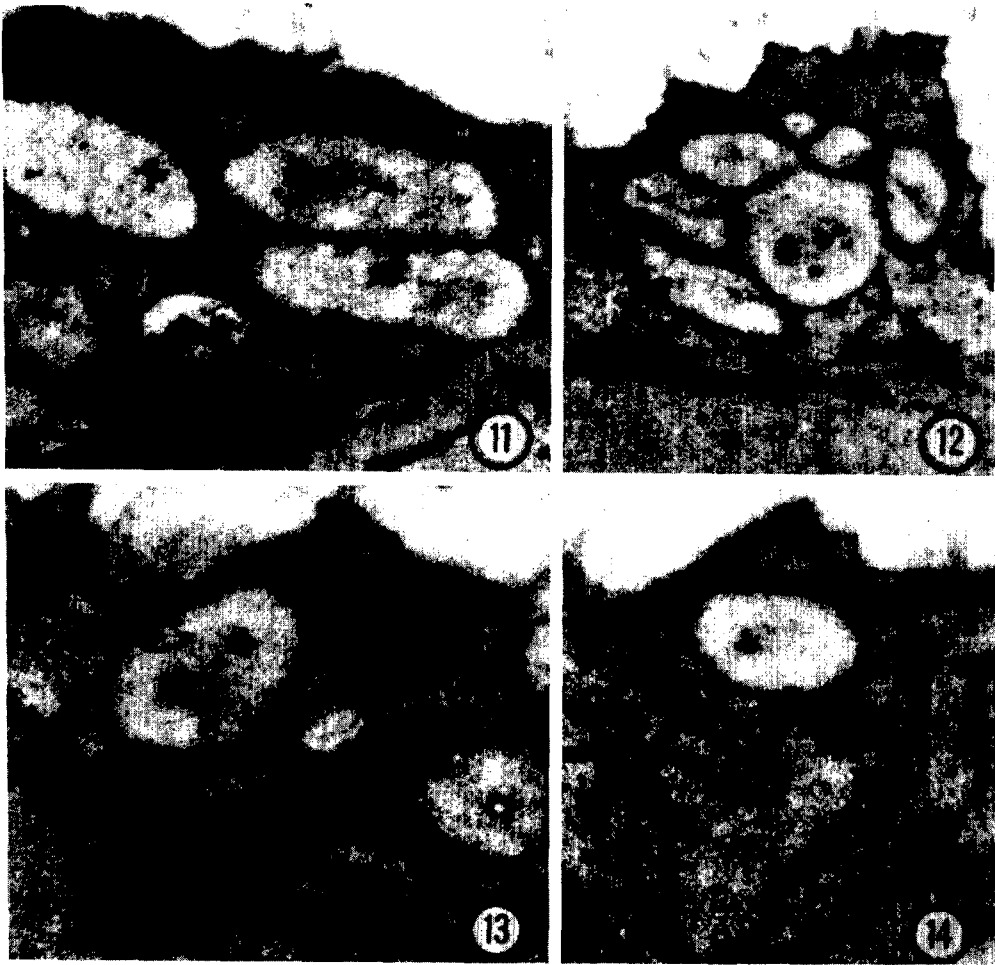


FIG. 11-14. *Trichuris myocastoris*: Various degrees of invagination of neurons into hypodermis. No mesaxon has formed in figure 11 (arrow), while two mesaxons occur in figure 14 (arrows). * = intercellular space. KMnO_4 fixation; Fig. 11, 42,500 \times ; Fig. 12, 32,200 \times ; Fig. 13, 53,800 \times ; Fig. 14, 53,800 \times .

nerve tracts are insulated from contiguous muscle cell processes only by the basement membrane which occurs between them and which is also present in the regions of neuromuscular junctions (6).

The association between hypodermis and interchordal neurons shows a progressive change from neurons lying in only a shallow depression close to the median chord to neurons lying deep within the hypodermis

but connected to extracellular space by a long mesaxon. This suggests that as neurons (perhaps dorsoventral commissures) progress farther from the median chords they become progressively more enclosed by the surrounding hypodermis. Similarly it may be suggested that although commissures emerge from the median chords in groups they become gradually separated, so that farther from the median line predominantly

single neurons occur, each within a mesaxon. Remaining groups of neurons enclosed by mesaxons may be somatic nerves. The reason for progressive invagination of neurons into mesaxons is not clear, but may be related to the nutrition of the neuron (3). Thus, by increasing the area of hypodermis in contact with neurons, more efficient uptake of nutrients from the hypodermis (which serves in nematodes as glial tissue as well as in its other roles) may be possible.

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