FINE-STRUCTURAL CHANGES IN A LEPIDOPTERAN NERVOUS SYSTEM DURING METAMORPHOSIS

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SUMMARY

The fine structure of the metamorphosing abdominal nerve cord of *Manduca sexta* has been studied. In fifth instar larvae, the connectives are ensheathed by a complex, thickened neural lamella. The underlying perineurium at this stage consists of 2 layers. The outer layer consists of interdigitating type I cells which are attached to the overlying neural lamella by hemidesmosomes, and to each other by occasional gap and tight junctions which persist throughout development. They are attached by desmosomes to a thin underlying type II cell layer, which is joined by gap and tight junctions and which has desmosomal attachments with the underlying glial membranes. The larval axons are surrounded by multiple glial wrappings containing bundles of microtubules. During the first week after larval-pupal ecdysis, the neural lamella degenerates and is phagocytosed by invading haemocytes. The underlying perineurial I cells gradually become hypertrophied and vacuolated. At the same time the type II layer, which does not increase in size, appears to be composed of either one or two cells which form a continuous 'bracelet' around each connective. The cellular bracelet is joined at one or two places by extensive gap, tight and septate junctions, and gap junctions are also seen along its perineurial I and glial borders. The underlying axons are embedded in vast amounts of glial cytoplasm containing relatively few microtubules. During the second week after larval-pupal ecdysis, the neural lamella is reformed and the perineurium flattens again. Type I and II cell junctions remain as described in earlier stages. Before adult emergence, the axons are again wrapped by glial cells rich in microtubules.

INTRODUCTION

During metamorphosis the transformation of the larval central nervous system is accomplished by various changes in the disposition of connectives and ganglia along the nerve cord (Newport, 1832, 1834; Brandt, 1879; Pipa, 1963; Ashhurst & Richards, 1964a; Ali, 1973). In the moth *Manduca sexta*, the larval abdominal nerve cord is formed of 8 ganglia (the last two are fused), which are joined by double connectives. By the first 2 days after larval-pupal ecdysis, the nerve cord has shortened and the first and second abdominal ganglia have fused with the last thoracic ganglion. A few days later, the sixth abdominal ganglion fuses with the terminal seventh and eighth ganglia (author's unpublished observations). As metamorphosis proceeds, the neural lamella surrounding the nerve cord degenerates and a connective tissue mass is formed on the dorsal side of the connectives, as described in another lepidopteran (Ashhurst

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& Richards, 1964a). Before adult emergence, the neural lamella has reformed around the connectives and dorsal mass and the nerve cord is suspended in the body cavity by the attachment of its dorsal mass to the ventral diaphragm.

Various studies on adult Manduca nerve cord have suggested that the perineurial cell junctions may restrict the flow of ions and molecules into and away from the immediate extra-axonal environment (Pichon, Sattelle & Lane, 1972; Lane, 1972). These studies also suggest that when the neural lamella is removed by microsurgery, the restriction to ionic movements is destroyed. In the metamorphosing nerve cord of Manduca an event comparable to surgical desheathing occurs, namely neural lamella breakdown. In this study particular attention is given to the perineurial cell junctions and changes in the perineurium which accompany this event.

MATERIALS AND METHODS

Manduca sexta were raised according to the methods of Yamamoto (1969). The larvae used were fully grown fifth instar larvae which were still feeding and whose ocelli were unretracted. To obtain pupae of known ages, fifth instar larvae, which had stopped eating and were ‘wandering’, were allowed to burrow in containers of damp peat and to pupate in the dark under the same temperature and humidity conditions used for rearing larvae. On the fourth day, prepupae were gently removed to individual containers where they were observed for 24-h periods to determine the time of ecdysis. For convenience, age denotes hours after ecdysis for both male and female animals. The period from larval-pupal ecdysis to adult emergence lasts 18–21 days.

The best preservation for electron microscopy was obtained by perfusing the animals through the heart with 3% glutaraldehyde (Fisher Scientific Co., Biological Grade, 50%) in either 0.1 M Na₂HPO₄, 2H₂O or 0.094 M sodium cacodylate-HCl, pH 7.4, at 4 °C. After perfusing, the abdomen was opened dorsally, the ventral nerve cord was dissected free, and the connectives between abdominal ganglia IV and V were removed (ganglia included) and fixed for 1.5–3 h in the perfusate at 4 °C. The tissue was then rinsed in either 0.2 M Na₂HPO₄, 2H₂O buffer (if phosphate buffer was used in the fixative) or 0.1 M cacodylate-HCl buffer with 0.2 M sucrose (if cacodylate was used in the perfusate). After rinsing, the tissue was postfixed in 1% OsO₄ in the appropriate buffer (0.1 M Na₂HPO₄, 2H₂O with 0.05 M sucrose; or 0.094 M cacodylate with 0.1 M sucrose) for 1 h at 4 °C. After postfixation the tissue was thoroughly rinsed in either 30% acetone or ethanol followed by distilled water and stained en bloc in 1% aqueous uranyl acetate at pH 4.2 for 15–20 min in the dark at room temperature. The tissue was dehydrated through an ascending series of ethanol, rinsed in 3 changes of propylene oxide, kept overnight in a propylene/Araldite mixture, and flat embedded in Araldite (Ciba, England).

The connectives were sectioned transversely on a diamond knife with an LKB Ultrotome III, placed on coated grids, stained with lead citrate (Reynolds, 1963), and examined in a Philips EM300 electron microscope. For light microscopy, 1–2 μm thick sections were cut with a glass knife and stained with 1% toluidine blue in 1% borax.

RESULTS

Neural lamella

The neural lamella surrounding the interganglionic connectives of the fifth instar larva is a thickened connective tissue sheath containing tracheae which are enclosed by tracheoblast cells (Fig. 3). Extensive septate junctions and occasional desmosomes are seen between the tracheoblasts which border the tracheae and groups of tracheoles (Fig. 4). The outer and inner borders of the lamella are formed by small bundles of longitudinally oriented fibres embedded in an amorphous matrix 1–2 μm wide. The
Entire width of the larval neural lamella varies between 10 μm (in areas where tracheae are collapsed) and 30 μm (tracheae not collapsed).

During the first 3 days after larval-pupal ecdysis (E+1-3), empty spaces, presumably once occupied by some of the larger tracheae, begin to appear in the lamella. The borders of the lamella remain intact and many small groups of tracheoles as well as an occasional collapsed trachea are seen within the substance of the lamella (Fig. 5). The neural lamella of these early pupal stages measures between 6 and 20 μm in width. At E+3 days, several adipohaemocytes (Jones, 1962; Ashhurst & Richards, 1964; Pipa & Woolever, 1964, 1965) containing large inclusion bodies and abundant rough endoplasmic reticulum are seen on the surface of the neural lamella (see Fig. 9). The surface of these blood cells is extended into long cytoplasmic processes which adhere to the outer lamellar border.

At E+4 days the width of the neural lamella remains the same as in E+1-3 days, but many more haemocytes are seen on the surface. Some of these haemocytes have long cytoplasmic processes projecting into the substance of the lamella.

At E+5-6.5 days the disorganized neural lamella is invaded by a number of haemocytes which appear to be actively engaged in phagocytosis of the degenerating connective tissue. These blood cells are engorged with large vacuoles of amorphous material similar to that found in the intact neural lamella (Figs. 6, 9).

By E+7-12 days the neural lamella is absent and a thin basement membrane covers the surface of the perineurium. Although most of the blood cells disappear after.

Fig. 1. Stages of Manduca nerve cord development showing changes in neural lamella and perineurium, invasion by haemocytes, and formation of a dorsal mass. A, fifth instar larval stage; B, E+3 days; C, E+6 days; D, E+8 days; E, E+15 days.
degradation of the neural lamella, a few haemocytes remain around the connectives and in the vicinity of the newly forming dorsal mass.

At E+13-15 days the neural lamella has reformed around the connectives and dorsal mass (Fig. 7). It is 2-4 μm thick and consists of longitudinally and transversely oriented connective tissue fibrils as described for the adult neural lamella of *Manduca sexta* (Lane, 1972).

**Perineurium**

The quality of fixation of the perineurium at various stages of development depended to some extent on the type of buffer used in the fixative. When a phosphate buffer was used, the perineurial cytoplasm appeared more electron-dense than when a cacodylate buffer was used. There were also many more vacuoles between and within perineurial cells perfused with a phosphate-buffered fixative. These differences were most striking at E+1-12 days. In contrast to the perineurium, both axons and glial cells in the connectives were preserved best with a phosphate-buffered fixative, but extracellular spaces tended to be more electron-dense.

The perineurium of the fifth instar larva is a continuous, flattened, interdigitating layer of cells (0.5-2 μm wide), containing numerous microtubules and large distended rough endoplasmic cisternae filled with electron-opaque material (Figs. 3, 4). Hemidesmosomes are seen on the perineurial cell borders underlying the neural lamella and tight junctions are seen between the more baso-lateral borders. Along the basal perineurial borders the intercellular space narrows to form a uniform 2-3 nm wide ‘gap’, separating the adjacent unit membranes. These gap junctions appear septilaminar in contrast to the pentilaminar appearance of the perineurial tight junctions.

The perineurium is also attached by numerous desmosomes to a thin underlying cell layer (0.15-0.5 μm wide) which resembles the electron-lucent microtubule-filled glial cytoplasm of the connectives. Unlike the glial cells, it does not wrap around axons and therefore will be referred to as a type II perineurial cell in contrast to the overlying type I perineurium. At this developmental stage, the type II layer appears to be formed by one cell which is wrapped around each connective. Its glial border is projected into thin cytoplasmic processes which extend between and around the glial cells. It is attached to the underlying glial cells by numerous desmosomes and its own lateral borders are joined together by gap and tight junctions.

During the first 4 days after larval-pupal ecdysis, perineurial type I cells gradually increase in size (up to 10 μm) and multiple desmosomal attachments are seen between their lateral margins (Fig. 5). Occasional gap and tight junctions occur along their baso-lateral margins and at E + 3-4 days gap junctions as well as desmosomes are seen between type I, II and glial cell borders. The perineurial II cell layer is not changed in appearance or increased appreciably in size at this time.

By 5-7.5 days, the perineurial type I layer is greatly enlarged (up to 20 μm) and there is little interdigitation or overlapping of cell margins (Fig. 6). The type I cells contain many membrane-bound vacuoles in addition to numerous small distended cisternae of rough endoplasmic reticulum, Golgi bodies, and occasional microtubules. Hemidesmosomes are absent from the neural lamellar borders and desmosomes are
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seen rarely between their lateral margins. Other cell junctions remain the same as described previously. In contrast to the hypertrophied perineurial I cells, the type II layer is increased only marginally in size (0.2–1 µm). At this developmental stage, the layer appears to be composed of 2 cells which form a bracelet around each connective (Figs. 10–12). The cellular bracelet is clasped together at 2 different places around the connective; one connexion is formed by multiple interdigitating cytoplasmic processes which are joined by extensive gap, tight and septate junctions (Figs. 13–16) and the other is formed by an overlapping apposition between the 2 cell borders, which may be as long as 4 µm and which is joined either by discontinuous gap junctions.

![Diagram of cell junctions](image)

Fig. 2. A, 1-cell bracelet with interdigitating connexion; B, 2-cell bracelet with both interdigitating and overlapping connexions; C, 1-cell bracelet with overlapping connexion; D, interdigitating connexion; E, overlapping connexion.

in which the intercellular space alternately narrows to a uniform 2–3 nm wide gap and then widens again, or by alternating tight and gap junctional complexes in which the pentilaminar structure of fused adjacent unit membranes alternates with an irregular intercellular space or the septilaminar appearance of interposed gap junctions (Figs. 17, 18). It is possible that many of these overlapping connexions are joined by one continuous gap junction, which is not always oriented in the same plane. In a typical cross-section through the nerve cord, it is common to find only one perineurial II connexion, which suggests that the type II layer consists of alternate bracelets of one or two cells. Desmosomal attachments are no longer as numerous along the perineurial and glial borders of this bracelet layer, but occasional gap junctions are seen between both borders at this time (Fig. 6).

At E + 7.5 days, the perineurial I cells on the dorsal side of the connectives appear to be multilayered as well as hypertrophied. As the dorsal mass develops, these
perineurial I cells remain morphologically indistinguishable from the cells found in the
newly forming dorsal mass.

Between 8 and 15 days after larval-pupal ec dysis, the perineurium gradually decreases
in size and again becomes a flattened interdigitating cell layer (1–3 μm) (Figs. 7, 8). As in the fifth instar larva, the perineurial I cells contain numerous microtubules and
large distended cisternae of rough endoplasmic reticulum filled with electron-opaque
material. Hemidesmosomes are found at their neural lamellar borders with gap and
tight junctions and occasional desmosomes along their lateral margins. The type II
cell layer remains as described, with desmosomal and gap junctions on its perineurial
I and glial borders.

**Interganglionic connectives of the abdominal nerve cord**

The axons of the interganglionic connectives of the fifth instar larva are ensheathed
by glial cells containing massive bundles of microtubules (Fig. 19). Most of the axons
are encased by multiple glial wrappings and numerous desmosomal attachments are
seen between the glial wrappings and between adjoining glial cells.

The appearance of the connectives at E+1 day is strikingly different. The axons
are embedded in vast expanses of electron-lucent glial cytoplasm, which no longer
contains prominent bundles of microtubules or displays desmosomal attachments
with other glial cells (see Fig. 20). As metamorphosis proceeds, the number of glial
wrappings around axons is diminished and numerous isolated groups of small axons
are evident (Fig. 21). Tight junctions are seen between some of these small axons and
between the glial processes which surround them (Fig. 22).

Before adult emergence, most of the axons are separated from each other by glial
cells containing numerous microtubules (Fig. 24). Some of the axons are surrounded
by glial wrappings and desmosomes and occasional tight junctions are seen again
between the wrappings and between adjacent glial cells.

Throughout metamorphosis various profiles are seen which could represent growing
or degenerating nerve fibres. Some of these are electron-dense and filled with mito-
chondria and/or vesicular accumulations (Fig. 20). Other electron-lucent axons are
found which contain a number of smooth vesicular membranes in addition to the
usual complement of organelles (Fig. 22). In some pupae, irregular electron-dense
profiles (0.5–2 μm) containing membranous whorls and other debris are seen within
the surrounding glial cytoplasm (Fig. 23).

**DISCUSSION**

**Neural lamellar changes during metamorphosis**

The breakdown of the neural lamella during metamorphosis has been documented
in a number of light- and electron-microscopic studies on Lepidoptera (Panov, 1963;
Ashhurst & Richards, 1964a; Pipa & Woolever, 1964, 1965; Heywood, 1965; Nord-
lander & Edwards, 1969; Ali, 1973). In most of these studies the mechanism of neural
lamellar histolysis remains uncertain, but it is generally agreed that the haemocytes
are active in phagocytosing the debris. Pipa & Woolever (1965) found direct evidence
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for this phagocytic activity when neural lamellar fibrils were seen within the adipohaemocytes of *Galleria mellonella*. In the present study haemocytes are seen in various stages of lamellar engulfment, and connective tissue material resembling that of the intact lamella is observed within large vacuoles of the invading blood cells. These observations strongly suggest that the adipohaemocytes of *Manduca sexta* are active also in phagocytosis of the pupal lamella.

The role of the adipohaemocytes in the formation of the neural lamella in *Manduca sexta* is less certain. Blood cells have been implicated in connective tissue formation in several insects (see Jones, 1962). The involvement of haemocytes in neural lamella formation in *Galleria* pupae has been studied by Ashhurst & Richards (1964,a–c; see also Ashhurst, 1968), who found that blood cells were not present when the lamella was formed. They concluded that the underlying sheath cells (perineurium) were involved in formation of the adult lamella. A similar conclusion was reached by Pipa & Woolever (1964, 1965) in light- and electron-microscopic studies on the same animal. Unlike the situation in *Galleria*, the blood cells of *Manduca* do not disappear entirely after neural lamella breakdown but remain in the vicinity of the connectives and newly forming dorsal mass. They are present also during formation of the adult neural lamella. In view of this, the possibility that *Manduca* haemocytes play a role in neural lamella formation remains to be explored.

Perineurial changes

Cytological changes in the perineurial cells during metamorphosis have been described in other moth nervous systems (Ashhurst & Richards, 1964a; Pipa & Woolever, 1964; Ali, 1973). In *Pieris*, Heywood (1965) has reported that the perineurium undergoes autolysis and is reformed by mitosis of the few remaining perineurial cells on the surface of the nerve cord. In a more recent study on the same animal, Ali (1973) reports that although some perineurial cells degenerate, the majority of them remain intact throughout metamorphosis. The perineurial cells of *Manduca* also persist throughout development, but undergo hypertrophy and vacuolation prior to and during neural lamella breakdown (E + 3–7.5 days). At this time the perineurial cells contain large numbers of small dilated rough endoplasmic cisternae, as well as numerous Golgi bodies and membrane-bound vacuoles. As the perineurium flattens and the lamella is reformed (E + 8–15 days), the rough endoplasmic cisternae appear enlarged and their contents are electron-opaque. As has been suggested for *Galleria* (Ashhurst & Richards, 1964a, b; Pipa & Woolever, 1965), the secretory appearance of the perineurial cells in *Manduca* may be related to histolysis and reformation of the neural lamella. It is also possible that some of the perineurial vacuolation is related to artifacts of fixation.

The classification of the perineurium of *Manduca sexta* into type I and II cells is similar to that used for the perineurium in *Carausius* and *Periplaneta* (Maddrell & Treherne, 1967). The perineurial II cells of *Manduca* and these other insects are similar in that they display long cytoplasmic flanges along their gial borders. They differ in that the perineurial II cells of the cockroach and stick insect form a multicellular interdigitating layer with the perineurial I cells, whereas the *Manduca* type II
layer is composed of only one or two cells which form a thin cellular bracelet underlying perineurium I.

The perineurial cell junctions of *Manduca* do not change appreciably during metamorphosis. The occasional gap and tight junctions observed between perineurial I cell margins persist throughout larval and pupal stages. Desmosomal attachments between the type I cells show more variability. They are not seen in the last instar larvae, but are numerous in the early pupal stages when the perineurium is increasing in size. As the hypertrophied perineurium flattens, desmosomes are less numerous. In contrast, the elaborate junctional complexes between the type II cells are a consistent feature at each developmental stage. In the larva, numerous desmosomes are seen along the perineurial I and glial borders of the type II layer. Later in development, gap junctions are observed along both borders and desmosomal attachments are less numerous.

Because the nervous system of *Manduca sexta* is bathed directly by the haemolymph, any ionic or molecular exchange between the axons and the blood must occur via the interposed structures. Electrophysiological studies on the ventral nerve cord of adult *Manduca* and other phytophagous insects indicate a peripheral barrier to the movements of sodium and potassium and a much higher sodium concentration in the immediate extra-axonal fluid than that of the haemolymph (Pichon *et al.* 1972; see also Treherne & Pichon, 1972). Further studies on *Manduca* as well as other insect species suggest a form of ‘blood-brain barrier’ at the level of the perineurial tight junctions (Lane & Treherne, 1969, 1970, 1971; Treherne, Lane, Moreton & Pichon, 1970; Pichon & Treherne, 1971; Pichon, Moreton & Treherne, 1971; Pichon *et al.* 1972; Lane, 1972). It is possible that the perineurial junctions which persist throughout metamorphosis also function to restrict ions and molecules in the developing nervous system of *Manduca*. Of these perineurial junctions, the more consistent tight junctions between the type II cell(s) are the likely candidates. The additional appearance of gap junctions between perineurial I, II and glial cell borders at E + 3-7-5 days is of interest in view of the recent suggestion that an intercellular low-resistance pathway linking the perineurium with the glial membranes would serve to regulate the high extra-axonal sodium concentration by providing an intracellular route for the movement of sodium ions to the glial cells surrounding the axons (Treherne & Pichon, 1972). It has also been suggested that the perineurial tight junctions would serve to maintain the extra-axonal sodium concentration by reducing the escape of sodium ions from the extracellular environment (Treherne & Pichon, 1972).

**Axons and glia**

The apparent increase in glial cytoplasm and diminished glial wrapping around axons during metamorphosis agrees with the fine-structural changes described in *Galleria* developing nerve cord (Pipa & Woolever, 1965; Tung & Pipa, 1972). The electron-dense axonal profiles containing accumulations of organelles and vesicles which are encountered at various pupal stages are similar to the degenerating axons reported by Tung & Pipa (1971) in transected connectives of the wax moth. It is possible that the dense irregular autophagic profiles found within the surrounding
glial cells of *Manduca* may also be related to the degenerative processes of metamorphosis. Other characteristics of the developing nervous system, such as the predominance of axons containing smooth vesicular membranes, may reflect growing nerve fibres. Vesicular membranes have been described in axons and their growing tips in a number of studies dealing with developing vertebrate nervous systems and cultured neurons (Bodian, 1966; del Cerro & Snider, 1968; Grainger & James, 1970; Tennyson, 1970; Kawana, Sandri & Akert, 1971; Bunge, 1973).

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**ABBREVIATIONS ON PLATES**

\[\begin{align*}
ax & \text{axon} & \text{olb} & \text{outer lamellar border} \\
g & \text{glial cell} & \text{pn} & \text{perineurium} \\
h & \text{haemocyte} & s & \text{septate junction} \\
tc & \text{intercellular space} & t & \text{tight junction} \\
ilb & \text{inner lamellar border} & tr & \text{tracheole} \\
nl & \text{neural lamella} & tra & \text{trachea} \\
\end{align*}\]
Figs. 3 and 4. For legend see p. 380.
Fig. 3. Thickened neural lamella of the fifth instar larval connective consisting of tracheae (tra), tracheoblast cells (trb) and inner and outer lamellar borders (arrows). The perineurium (pn) is interposed between the neural lamella and the underlying axons (ax) and glial cells (g). Cacodylate buffer. × 6480.

Fig. 4. Higher magnification of perineurial I and II cells. The inner lamellar border (ilb) separates the perineurium from the overlying tracheoblasts (trb) which are joined by septate junctions (arrows). Fifth instar larva. Cacodylate buffer. × 42900. Inset: tight junction (arrow) between type I cells. × 141200.

Fig. 5. E + 3 days connective showing degenerating neural lamella with empty spaces, small groups of tracheoles (tr) and inner (ilb) and outer lamellar borders (olb). The type I perineurial cells are enlarged and the underlying type II cell layer is unchanged. Several cytoplasmic flanges (arrows) are seen along the glial border of the type II cell layer. Cacodylate buffer. × 5800. Inset: desmosomal attachments between type I cells. × 51300.

Fig. 6. E + 6 days. The neural lamella is absent (arrow) and the type I cells are hypertrophied and vacuolated. The underlying type II cell layer is not increased in size. A haemocyte (h) is seen on the surface of the perineurium. Cacodylate buffer. × 4800. Inset: gap junction (arrows) between perineurial I and II cell layers. × 99800.
Figs. 7, 8. E + 18 days. The neural lamella (nl) is reformed and the type I cells are decreased in size and have numerous distended cisternae of rough endoplasmic reticulum filled with electron-opaque material. The type II layer remains as in previous stages. Cacodylate buffer. × 9200 and × 7700, respectively.

Fig. 9. Haemocyte (h) engulfing neural lamellar material (nl). E + 5 days. Phosphate buffer. × 4700.

Figs. 10–12. E + 7 days. The perineurial II cell bracelet underlying the hypertrophied perineurial I cells. The cell bracelet is joined by an interdigitating connexion (arrow). × 4500.
Fig. 13. An interdigitating connexion between the type II cell(s). The connexion begins at the perineurial I and II interface (arrow) and ends at the glial (g) interface (arrow). E + 7.5 days. Cacodylate buffer. \( \times 14200 \).

Fig. 14. Higher magnification of part of the interdigitating connexion in Fig. 13 showing septate (s) and tight (t) junctions. \( \times 103000 \).

Fig. 15. Another interdigitating connexion between the type II cell(s). The ends of the connexion are indicated by arrows. E + 7 days. Cacodylate buffer. \( \times 11700 \).

Fig. 16. Higher magnification of some of the connexion in Fig. 15. Most of the junctions are lengthy gap junctions interrupted by occasional non-junctional intercellular spaces (ic). \( \times 90600 \).

Fig. 17. An overlapping connexion between the type II cell(s). E + 7 days. Cacodylate buffer. \( \times 27300 \).

Fig. 18. Higher magnification of an overlapping connexion between the perineural II cell(s) showing a lengthy gap junction (arrows) at the glial end (g) of the connexion. E + 14 days. Cacodylate buffer. \( \times 77000 \).
Fig. 19. Fifth instar larval connective showing multiple glial wrappings (g) around axons (ax). The glial cells contain numerous microtubules and desmosomes (arrows) are seen between the wrappings. Phosphate buffer. × 39,900.

Fig. 20. An electron-dense axon containing numerous mitochondria and vesicular accumulations is seen among other electron-lucent axonal profiles (ax) and surrounding glial cytoplasm (g). E + 7 days. Phosphate buffer. × 11,400.

Fig. 21. Axons (ax) and glial cells (g) in the E + 11.5 days connective. The glial cytoplasm is electron-lucent and contains relatively few microtubules. A group of small axons is adjacent to the glial-wrapped larger axons. Phosphate buffer. × 14,400.

Fig. 22. Higher magnification of a group of small axons which are joined to each other by tight junctions (arrows). The axons contain microtubules in addition to smooth vesicular membrane. E + 12 days. Phosphate buffer. × 57,900.

Fig. 23. An electron-dense profile containing membranous whorls and other debris within a glial cell (g). E + 12 days. Phosphate buffer. × 15,100.

Fig. 24. Glial wrappings (g) around axons (ax) in the E + 18 days connective. Numerous microtubules are seen within the glial cytoplasm and desmosomes (arrow) are found between the wrappings. Cacodylate buffer. × 25,100.
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ax, g