The Fine Structure and Morphological Organization of the Peripheral Nerve-fibres and Trunks of the Cockroach (*Periplaneta americana*)

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With three plates (figs. 1 to 3)

Summary

Sections of the peripheral nerve-trunks of the metathoracic leg of the cockroach (*Periplaneta americana*) were studied with the electron microscope. Paraffin sections were also prepared and stained. Protargol succeeds in staining the nerve-fibres. Osmium tetroxide, a modified Weigert procedure, and Luxol fast blue stain the myelin sheaths, as does mercuric bromphenol blue, a protein stain. The axoplasm is relatively free of formed elements; it contains mitochondria. The myelin sheath, when present on the largest and also some smaller fibres, consists of about two or three loose overlapping processes of Schwann cells, covered by their plasma membranes, enclosing lipid-like droplets and having a beaded appearance. Between the nerve-fibres in the nerve-trunk is Schwann-cell cytoplasm, which arises from Schwann cells that surround the whole nerve-trunk. The same fold of Schwann-cell membrane may enter into the formation of the myelin sheath around more than one nerve-fibre. Several small non-myelinated fibres, which may be as small as $0{\cdot}3\,\mu$ in diameter or less, may be enclosed in the same fold of Schwann-cell membrane. Outside of the Schwann-cell layer and surrounding the nerve-trunk is a thin layer of connective tissue, which does not send trabeculae into the interior of the nerve. Tracheae and tracheoles accompany the nerve but are not included within the sheaths surrounding a nerve-trunk, even near the termination of the nerve-fibres in muscle. The structure of the cockroach peripheral nerve is compared with that described by previous investigators, with that of other insects, and with invertebrate and vertebrate nerve.

Introduction

MUCH information has recently been accumulated on the physiological mechanisms of nerve and muscle in insects (Hoyle, 1954, *a*, *b*; 1957). Progress in knowledge of the structure of the nerves of insects has not yet paralleled the advances in physiological information. Pringle (1939) has contributed much information on the anatomy and physiology of the leg muscles and peripheral nerve of the cockroach. In the present investigation, the structure of the peripheral nerve-trunks and fibres of the cockroach has been investigated by staining techniques for the light-microscope and by electron microscopy. Some information has been obtained about the ultrastructure of the nerve-fibres and the relations of the nerve-fibres to each other and their surrounding sheaths.

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Material and Methods

The metathoracic leg of *Periplaneta americana* was used. Whole legs, pieces of leg, and pieces of muscle were fixed in alcohol of varying concentrations, osmium tetroxide, or 50% alcohol with 3 g of chloral hydrate per 100 ml. Cross and longitudinal paraffin sections were prepared. Protargol stains and haematoxylin and eosin were used on the tissues fixed in alcohol. The tissues fixed in alcohol / chloral hydrate were stained by a Cajal block impregnation procedure. Sections were also stained for myelin by a modified Weigert stain (Erhart, 1951) and by Luxol fast blue (Klüver and Barrera, 1953), a sulphonated copper salt of phthalocyanine, generously donated by E. I. du Pont de Nemours & Co. Attempts were also made to impregnate nerve-fibres by formic acid / gold chloride techniques. In addition, the mercuric bromphenol blue stain, a procedure specific for proteins (Mazia and others, 1953), was applied to the paraffin sections. Nerve-fibres were also examined under the polarizing microscope.

For electron microscopy, small pieces of metathoracic leg or of muscle were fixed for 10–30 min in Dalton's fluid (Dalton and Felix, 1955), a solution which contains 1% OsO_4, 1% $K_2Cr_2O_7$ at pH 7·2, and 0·85% NaCl. After dehydration, specimens were embedded in methyl (1 part) and butyl (6 parts) methacrylate and ultrathin cross and longitudinal sections (200–300 Å thick) were cut with the Servall Porter-Blum microtome. The sections were inserted into an RCA–EMU-type electron microscope. Micrographs were taken at a magnification of 2,000 to 6,000 and enlarged to the desired size. The final magnifications are approximate. Thick plastic sections were cut and viewed under the phase-contrast microscope to provide orientation for the subsequent electron microscopy.

Results

Axoplasm

The diameters of the nerve-fibres encountered in cockroach nerve vary from about 10 μ to about 0·3 μ or even less. The axons of insect nerve-fibres are usually described as structureless. After the treatment of sections with osmium tetroxide (fig. 1, B) or after gold or silver impregnation, the axon usually remains unstained. At times a granular precipitate can be seen. After staining with mercuric bromphenol blue, the axoplasm is coloured a pale blue (fig. 1, D). Protargol succeeds in impregnating the axons. They are coloured

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Fig. 1 (plate). Sections of cockroach nerve. In A–E (photomicrographs) the scale represents 10 μ; in F–H (electron micrographs) it represents 1 μ. Magnifications are approximate.

A, fixed in Dalton's fixative, embedded in plastic. The sheath around the individual nerve-fibres is seen.

B, fixed in osmium tetroxide solution. The sheath around individual nerve-fibres is blackened.

C, protargol preparation. The axons are impregnated and have a crumpled border.

D, stained with mercuric bromophenol blue. The axoplasm was pale blue. The sheath around individual nerve-fibres was also stained.

E, silver impregnation, to show the nuclei in the nerve.

F–G, mitochondria within the nerve-fibres.

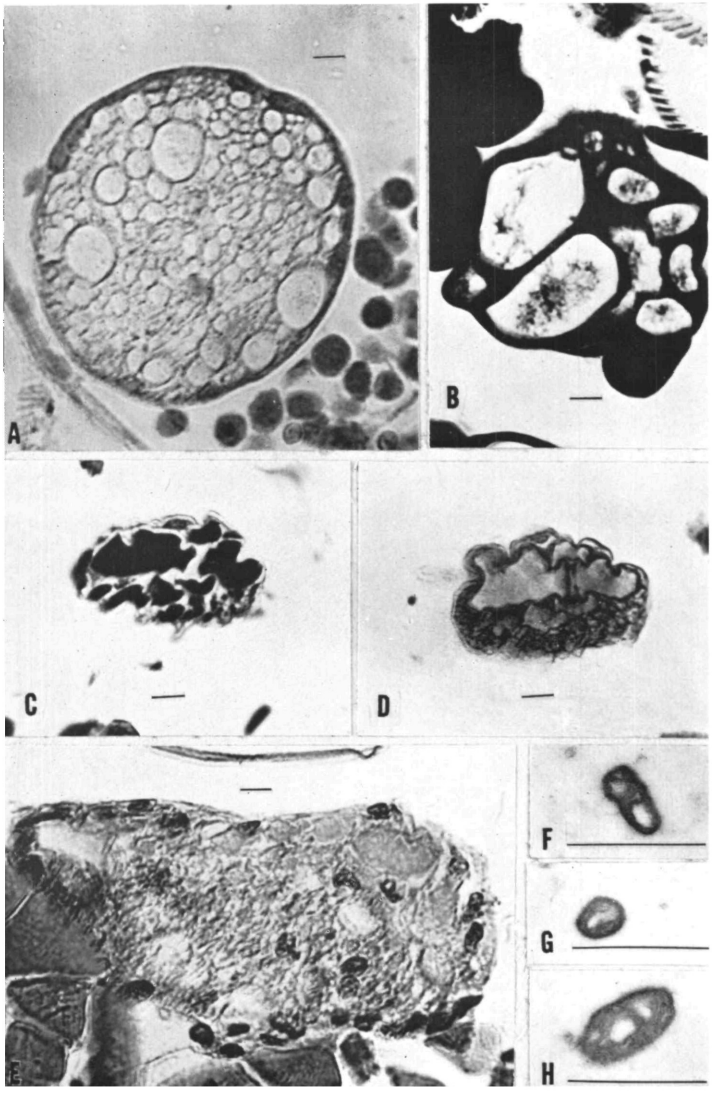

FIG. 1
A. HESS

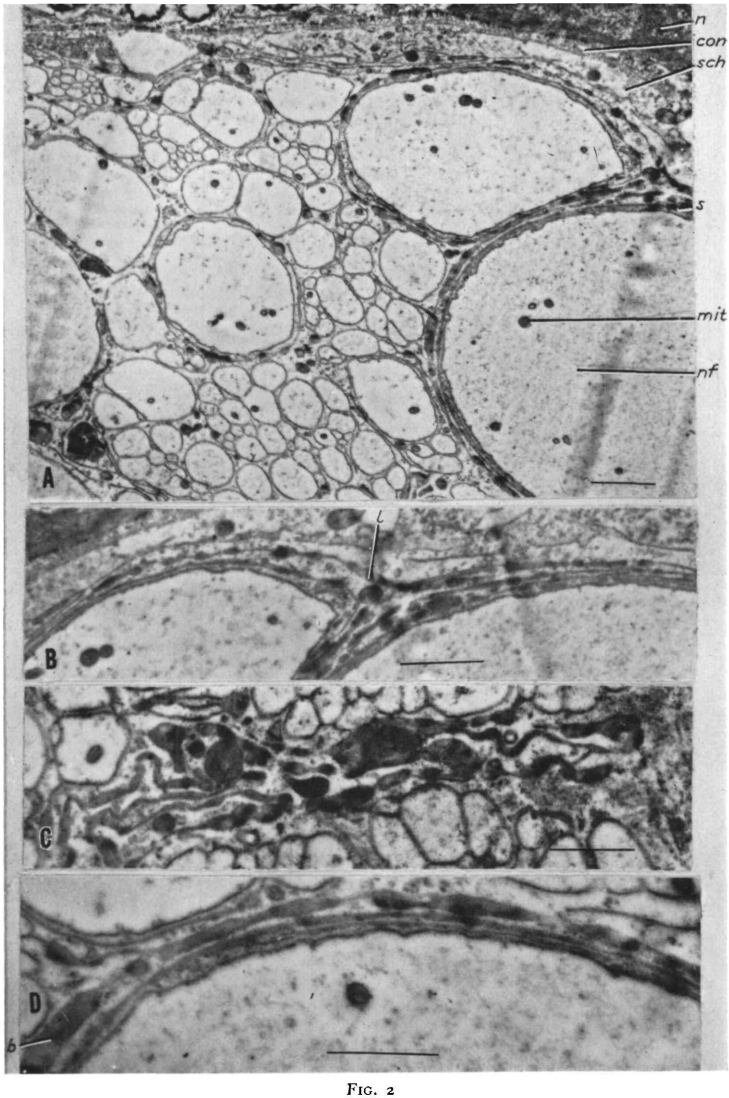

FIG. 2
A. HESS

dark blue or black and frequently appear to lose their round shape and have a crumpled border (fig. 1, C).

Electron micrographs reveal the elements present in axons. The most obvious structures seen are electron-dense bodies (figs. 2, A; 3, A) having an internal structure of folded membranes and frequently having round areas of light density (figs. 1, F, G, H). These dark bodies may be mitochondria. In cross-sections of nerve, they usually appear round, which indicates that they are longitudinally oriented in the axon. The rest of the axoplasm usually presents an amorphous granular appearance (figs. 2, A; 3, A). In cross-sections, some granules have an interior less dense than the periphery, which indicates that they are small tubules longitudinally oriented in the axoplasm. In general, the rather sparse content of organelles and fibrils and light density of the axoplasm are striking, especially when compared with the axons of vertebrates.

Sheaths of the nerve-fibres

The nerve-fibres rest in the cytoplasm of Schwann cells which enclose the whole nerve-trunk, a relationship that will be described in more detail below. Processes of these cells, covered by their plasma membranes, twist and turn through the interior of the nerve-trunk. Some entwine themselves around a nerve-fibre so that it may be surrounded by two or three loosely imbricated processes with cytoplasm and its organelles enclosed between the plasma membrane layers (figs. 2, A, B, D). Electron-dense droplets, apparently of a lipid nature, are frequently seen. These are enclosed between the membranes. These droplets thus enter into the formation of a beaded fatty sheath around some nerve-fibres (figs. 2, A, B, D), which may be equivalent to the myelin sheath of other forms. Most commonly, the largest nerve-fibres are surrounded by this beaded fatty sheath. However, this relationship is not invariable as it is not uncommon to see large nerve-fibres without any membrane folds or lipid or to see small nerve-fibres surrounded by membrane folds and beaded droplets. The membranes appear to course at random through the interior of the nerve-trunk. At times lipid droplets can be seen enclosed in

FIG. 2 (plate). Electron micrographs of cockroach nerve. The scales represent 1 μ. Magnifications are approximate.

A, cross-section of cockroach nerve-trunk. The axoplasm has an amorphous granular appearance. The electron-dense bodies in the nerve-fibres are mitochondria. The processes of the cell surrounding the nerve-trunk (Schwann cell) can be seen entwining themselves around the nerve-fibres. Some of the membrane wrappings enclose dense droplets, apparently of a lipid nature. The wall of a trachea is at the top of the photograph.

B, enlargement of A showing the beaded membrane folds, the enclosed cytoplasm, and droplets forming a sheath on one nerve-fibre and passing on to form a sheath on another nerve-fibre.

C, section of a nerve-trunk showing folds of membrane with droplets and not surrounding or forming a sheath on any nerve-fibre.

D, section showing the membrane folds enclosing cytoplasm around a nerve-fibre and the beaded sheath of the nerve-fibre.

b, beaded sheath of the nerve-fibre; *con*, connective tissue layer; *l*, beaded membrane fold passing on from one nerve-fibre to the other; *mit*, mitochondrion; *n*, nucleus of wall of trachea; *nf*, nerve-fibre; *s*, sheath of nerve-fibre; *sch*, cytoplasm of Schwann cell.

the membrane and not in relation to any nerve-fibres (fig. 2, C). In addition, several nerve-fibres can share the folds of membrane and also the fatty sheath. Thus, the outer layer of two or three folds of membrane can be seen to leave the original nerve-fibre and join in forming the membrane folds around another axon (figs. 2, B; 3, E). Nerve-fibres with membrane folds not making complete turns around them or with only one or two droplets in the membrane folds can frequently be seen.

Many fibres are in the cytoplasm without any membrane folds around them. It is common to see bundles of very small nerve-fibres enclosed in the same fold of membrane (fig. 3, D).

That this beaded fatty sheath is equivalent to the myelin sheath can be seen by reference to light- and polarization microscope preparations. A myelin sheath and the sheath around the cockroach nerve-fibres stain with Sudan colouring agents and are birefringent (Richards, 1943, 1944); the latter observation has also been made in the present study. Both myelin and the cockroach nerve-sheath blacken with osmium tetroxide (fig. 1, B) and also are darkened by Dalton's fluid, as is seen in plastic-embedded sections in the phase-contrast microscope (fig. 1, A). Myelin and the cockroach nerve-sheath are also stained by the modified Weigert method employed and by Luxol fast blue. The sheath around the cockroach nerve-fibres also stains with mercuric bromphenol blue, a reagent for protein (fig. 1, D). This probably occurs because of the protein in the sheath contributed by the membranes and cytoplasm intercalated between them. Developmental studies are necessary to determine if the apparently lipid droplets of the membrane folds arise from the cytoplasm and are secondarily included between the membrane folds of the Schwann cells or if the droplets are elaborations of the Schwann-cell plasma membrane itself.

In a bundle of muscle-fibres one can find the large and small motor-fibres that are destined to innervate the muscle-fibres (Pringle, 1939; Hoyle, 1954*a*, 1957) and are near their terminations (fig. 3, C). The complex membranes can be seen twisting, apparently at random, between and around the nerve-fibres.

Sheaths surrounding the nerve-trunk

Immediately outside the area occupied by nerve-fibres is cytoplasm containing dense droplets, presumably lipid, large droplets with internal structure comparable to mitochondria, and small tubules (figs. 2, A; 3, A). Extending from the plasma membranes covering this cytoplasm are complexly folded membranes which wind their way into the interior of the nerve (figs. 2, A; 3, A, B). It thus appears that the sheath-cells send cytoplasmic processes into the interior of the nerve-trunk. These cytoplasmic processes surrounded by membranes form the complex sheath system around the nerve-fibres, which was described above.

Nuclei can be found in this cytoplasmic sheath or located either at the periphery or in the interior of the nerve (figs. 1, E; 3, A). In the interior of the

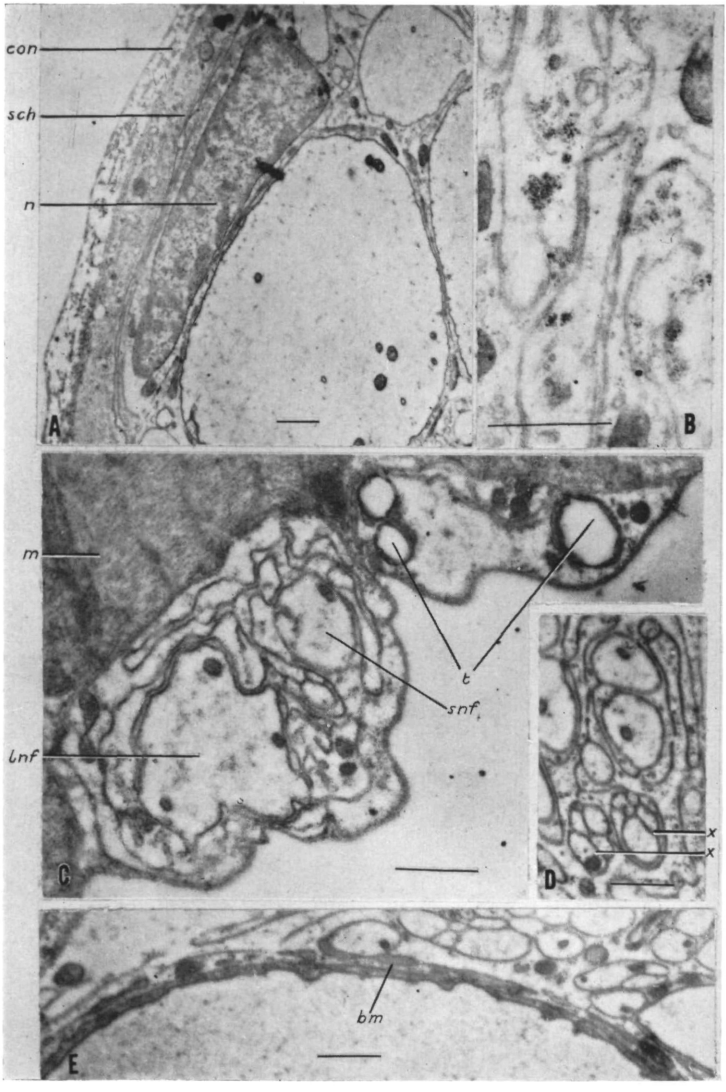

FIG. 3
A. HESS

nerve, the cytoplasm is attenuated and interrupted by the foldings of plasma membrane. However, near the nucleus in the cytoplasm or in an oblique section, the cytoplasm is relatively continuous and can be seen best. Many of these cells occur and contribute to the sheaths around the nerve-fibres. The randomness of the cytoplasmic strands and membranes in the interior of the nerve indicates that the processes of several cells can contribute to the formation of a sheath around a nerve-fibre. The arrangement of these cells and their intimate relationship to the nerve-fibres indicate that these cells are comparable to the cells accompanying nerve-fibres in other animals and are Schwann cells.

In light-microscope preparations, as, for instance, after protargol staining, the nerve-trunk appears surrounded by a refractile sheath that is unstained (apart from the nuclei) (fig. 1, C). This sheath stains with connective tissue dyes and is stained by eosin in haematoxylin and eosin preparations. In electron micrographs, outside the Schwann-cell cytoplasm, short fibrils occur with indications of a periodicity and an appearance reminiscent of connective tissue in other animals (figs. 2, A; 3, A). This layer does not send trabeculae into the interior of the nerve. The nuclei in and on this connective tissue-layer may well belong to cells analogous to fibroblasts. At the interface of the connective tissue and Schwann-cell layers, a thick membrane occurs and is made up of the outer limiting membrane of the Schwann cells and the basement membrane of the surrounding connective tissue (fig. 3, A).

The above are the sheaths belonging to the nerve-trunk. Outside the thin connective tissue layer is the haemolymph with its haemocytes. Very frequently, nerve-trunks lie beside tracheae and tracheoles. In such instances the portion of the nerve near the trachea and the wall of the trachea are in very intimate relation (fig. 2, A). However, the trachea is never included within the connective tissue-sheath of the nerve (fig. 2, A). The same relation holds near the terminations of nerve-fibres. Tracheoles accompany the nerve, but are not included within the sheaths surrounding the nerve-fibres (fig. 3, C).

FIG. 3 (plate). Electron micrographs of cockroach nerve. The scales represent 1 μ. Magnifications are approximate.

A, cross-section showing the nerve-fibres, showing a Schwann-cell nucleus in the nerve, the Schwann-cell layer, and the connective tissue layer.

B, section through Schwann-cell cytoplasm around the nerve-trunk, showing the complex membrane folds and other organelles.

C, section through muscle, showing the complex foldings of Schwann-cell membrane between and around the large and small motor-nerve fibres in their sheath. The tracheoles are enclosed in their own cell.

D, section of two bundles of small nerve-fibres. Each bundle is ensheathed by a fold of Schwann-cell membrane.

E, section through a nerve-fibre and its sheath of folded membranes and enclosed cytoplasm. The beaded membrane fold of the large nerve-fibre leaves it to encircle partially the small nerve-fibre.

bm, beaded membrane fold passing from a large nerve-fibre to a small one; *con*, connective tissue layer; *lnf*, large motor-nerve fibre; *m*, muscle; *n*, nucleus of Schwann cell; *sch*, Schwann-cell cytoplasm; *snf*, small motor-nerve fibre; *t*, tracheoles; *x*, *x*, two bundles of small nerve-fibres, each bundle ensheathed by a membrane fold.

Discussion

The description of the structure of cockroach nerve in electron micrographs conforms very well to that of previous investigators who employed light- and polarization microscopy. The structure of the sheath around the individual nerve-fibres described in the present investigation may well be responsible for the optical properties of this sheath, as described by Richards (1944). That the protein of the individual nerve-sheaths of insects may be collagenous (Richards, 1944) seems to be incorrect.

The sheaths around the nerve-trunk also require comment. The nerve-trunk is usually described as ensheathed by an outer homogeneous layer called the 'neural lamella' and an inner cellular sheath denoted as the 'perilemma' (Hoyle, 1952). The 'perilemma' is equivalent to the Schwann-cell layer and the 'neural lamella' appears to be the connective tissue-sheath. Richards (1944) suggests that the neural lamella does not seem to be collagen 'since it does not swell, dissolve or even lose its birefringence in dilute acetic acid (3 days) and since immersion experiments give different results for the neural lamella and the presumably collagenous sheaths around individual nerves'. However, as was mentioned above, the sheaths around individual nerve-fibres are probably not collagenous; the neural lamella appears to be so. The descriptions of the nerve-sheaths of insects by Scharrer (1939) and Twarog and Roeder (1956) are restricted to the ganglia. Contrary to general belief, the composition of the sheaths on the ganglia and those around the nerves differ (Hess, 1958); hence discussion of the sheaths around the ganglia will be reserved.

There appear to be many differences in structure between the peripheral nerve of locusts, as described by Hoyle (1954*a*), and that of cockroaches, as described here. The locust nerve apparently has some kind of connective tissue in its interior, the cockroach has Schwann-cell cytoplasm between its nerve-fibres. The locust nerve-trunk is surrounded by a tracheolated membrane and a fatty envelope, that of the cockroach is not. Hoyle (1957) includes a trachea within the outer sheath of locust nerve near a motor end-plate. Although I have not yet studied neuromuscular endings, the tracheoles near nerve terminations are not included within the outer sheaths of the nerve. Whether these differences in structure between locust and cockroach nerve are real or whether they will break down upon more detailed study remains to be seen.

In lobster and squid nerve-fibres, osmiophil dense-edged layers, similar to the membrane foldings of the Schwann cell described here in insect nerve, occur at the axon interface and in the cytoplasm of the Schwann cell (Geren and Schmitt, 1955). However, the insect nerve usually has in addition lipid-like droplets enclosed or intercalated between the membrane folds of Schwann cell that surround the nerve-fibres; thus the insect myelin sheath is commonly beaded in appearance.

The differences in the relation between cockroach nerve-fibres and Schwann

cells and between vertebrate axons and Schwann cells are interesting. In vertebrate nerves, each myelinated nerve-fibre is enclosed by a Schwann cell, while several non-myelinated fibres share a Schwann cell (Gasser, 1955; Hess, 1956). In the cockroach, Schwann cells surround the whole nerve-trunk. Each vertebrate non-myelinated fibre is suspended in the cytoplasm of the same Schwann cell by a mesentery of Schwann-cell membrane called a 'mesaxon' (Gasser, 1955; Hess, 1956). The 'mesaxon' of insect nerve-fibres can surround several small non-myelinated fibres, which are included as a bundle in the same Schwann-cell membrane. The myelin sheath of mammalian nerve-fibres consists of concentric lamellae wrapped around the axon (Hess and Lansing, 1953). It has been suggested that these lamellae are formed by wrappings of the Schwann-cell membrane (Geren, 1954). The insect fatty sheath is also apparently formed by wrappings of the Schwann-cell membrane, which form lamellae on the nerve-fibre. The insect lamellae are not so tightly packed and cytoplasm intervenes between them. In this respect, the insect fatty sheath bears a striking resemblance to a developing immature vertebrate myelin sheath. The fatty sheath of insects includes droplets apparently of a lipid nature in addition to the wrappings of Schwann-cell membrane and is beaded in appearance. The lamellae of the vertebrate myelin sheath are of even thickness and consist only of wrappings of the Schwann-cell membrane. The wrappings of the Schwann-cell membrane in vertebrates are restricted to individual nerve-fibres so that most commonly two myelinated axons do not share a myelin sheath and are not included within the same Schwann cell, although rarely the latter has been seen to occur (Hess, 1956). In insects, several myelinated and non-myelinated fibres share the same Schwann cells and the wrappings of the same Schwann-cell membrane can frequently be seen to leave one nerve-fibre and join in the formation of the beaded fatty sheath of other fibres, so that several nerve-fibres can be seen to share in the wrappings of the same Schwann-cell membrane and fatty sheath. The apparent randomness of the foldings of the Schwann-cell membrane in insect nerve is striking. Perhaps a study of the development of insect peripheral nerve-trunks will reveal that the sheath system of their constituent nerve-fibres is not as random as it appears in mature forms.

The sheaths of the cockroach nerve-trunk and those of vertebrate nerve are comparable. The connective tissue layer surrounding the whole nerve-trunk of the cockroach nerve is analogous to that of the epineurium of vertebrate nerves. However, this layer does not send trabeculae into the interior of the nerve and hence, at least for cockroach nerve, there is no peri- or endoneurium. The thick membrane at the interface of Schwann-cell and connective tissue layers and composed of the outer limiting membrane of the Schwann cells and the basement membrane of the connective tissue is reminiscent of the neurilemma or Schwann sheath of vertebrate nerve, which is made up of similar membranous layers (Hess, 1956). There are also close functional parallels in the sheaths investing insect and vertebrate nerve tissue (Hoyle, 1953; Twarog and Roeder, 1956).

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