THE NUTRITION OF THE CENTRAL NERVOUS SYSTEM IN THE COCKROACH *PERIPLANETA AMERICANA* L.

THE ROLE OF PERINEURIUM AND GLIAL CELLS IN THE MOBILIZATION OF RESERVES

By V. B. WIGGLESWORTH

*Agricultural Research Council Unit of Insect Physiology, Department of Zoology, University of Cambridge*

*(Received 24 March 1960)*

*(With Plate 13)*

The organs and tissues of insects are mostly composed of single layers of cells separated from the circulating blood by thin connective tissue membranes (Wigglesworth, 1956a; Pipa & Cook, 1958). The supply of nutrients to such organs presents no problem. But the central nervous system is an exception. The ganglia are the only solid organs in the insect body. They consist of densely packed peripheral cells surrounding a central neuropile, the whole ganglion being covered by a tough fibrous sheath or neural lamella continuous with the connective tissue membranes elsewhere. The total thickness of the ganglion may be 1 mm. or more.

The need for oxygen is met by the tracheae and tracheoles which pass through the neural lamella and ramify inside. But there is no circulation of haemolymph within the ganglion. All nutrient substances must diffuse through the neural lamella and be passed on to the deeper layers. One would, therefore, expect to find some special provision for the transport and storage of such nutrients.

After passing through the neural lamella the nutrient substances must be taken up by the perineurium cells, transferred to the glial cells, and conveyed by these to the neurones. In an earlier paper (Wigglesworth, 1959b), the probable importance of both perineurium and glial cells in the nutrition of the neurones was emphasized.

The present paper, which deals with histology and histochemistry, is part of a general study of the nutrition of the nervous system in the cockroach. The biochemical observations are published separately by Treherne (1960).

METHODS

Most of the work has been done on the last abdominal ganglion of the adult male cockroach, *Periplaneta americana*, with the cercal nerves behind and the connectives in front. The aim has been to relate the anatomy and histology of the ganglion to the deposition of visible reserves of triglycerides and glycogen under different conditions of nutrition.
For general histology the osmium tetroxide and ethyl gallate method of fixation and staining has been used, horizontal and transverse sections at 0.42–2 μ being cut by the method already described (Wigglesworth, 1959c). This same method reveals triglycerides as blue-black droplets. For glycogen the periodic acid-Schiff test (with saliva-treated controls) has been used after fixation with alcoholic Bouin, Hansen’s iron haematoxylin and light green being employed as counterstains.

ANATOMY AND HISTOLOGY OF THE GANGLION: THE GLIAL LACUNAR SYSTEM

The osmium and ethyl gallate method reveals many new features in the ganglion, but the present description will be limited to those points that are relevant to the problem in hand. The thick laminated neural lamella containing collagen has been described by Baccetti (1955, 1956) and by Hess (1958a, b). The perineurium cells, as in Rhodnius (Wigglesworth, 1959b), contain densely packed masses of filamentous mitochondria (Hess, 1958b) (Text-figs. 1, 2). These cells are described by Hess (1958b) as cubical. Actually they vary in height in different regions of the ganglion. Along the sides they are somewhat flattened, say 3–6 μ (Text-figs. 1, 6D), but at the
anterior and posterior extremities of the ganglion, particularly on the dorsal aspect, they form a columnar epithelium that may be 20–30 µ in height (Text-figs. 2, 6B, C); and all intermediate conditions occur.

Text-fig. 2. Horizontal section (2 µ) through the anterior end of the ganglion just dorsal to the connectives (osmium tetroxide and ethyl gallate). a, neural lamella; b, perineurium cells with clumps of filamentous mitochondria; c, outer glial cells with oval and rod-like mitochondria; d, glial sinus system, partially collapsed; e, inner glial cells bounding the neuropile; f, small neurone.

Beneath the perineurium is the glial cell layer (Text-figs. 1, 2). This consists of two ill-defined parts; a peripheral part which invests the ganglion cells where these are present, and a central part which surrounds the neuropile. The glial cells contain oval and short rod-like mitochondria. These are much stouter than the filamentous mitochondria of the perineurium cells. Giant glial cells, the type iii as described in Rhodnius, do not occur in Periplaneta. Tracheae and tracheoles are most abundant in the central part of the glial zone, adjacent to the neuropile (Text-fig. 1).

The glial cell layer is more or less vacuolated. The vacuoles appear to arise within the cytoplasm. But in many ganglia the vacuolation is so extreme that a wide space or sinus separates the two layers of glial cells. This ‘glial lacunar system’ is commonly most evident on the dorsal side of the ganglion, but it also extends
Perineurium and glial cells in the cockroach

laterally and may surround the neuropile on all sides (Pl. 13, figs. 1-3). In its fully developed state the glial nuclei surrounded by scanty cytoplasmin lie within extensive cavities which are traversed in all directions by slender branching filaments or trabeculae (Text-fig. 1). Along with the glial nuclei are the much smaller nuclei of tracheal cells supporting tracheae and tracheoles which are suspended in the sinus cavities by delicate mesenteries.

The contents of these vacuoles or lacunae appear virtually colourless after staining with osmium and ethyl gallate. But the air-containing tracheae show up in the sections as a brighter white than the lacunar spaces, which evidently contain a very tenuous plasma. A vacuolated space of the same type is present in the small abdominal ganglia and in the thoracic ganglia.

The function of the glial lacunar system is uncertain. It might serve as a pool into which nutrients are discharged and from which they can be drawn by those cells which require them. Its development is very variable. In some insects the cavities are collapsed: they are little more than potential spaces among the glial cells (Pl. 13, fig. 4). This condition seems to be characteristic of young and well-nourished insects. In old or in starved insects the spaces are generally large (Pl. 13, fig. 3). This suggests that perhaps the lacunar system is simply a provision for maintaining unchanged the outward form of the ganglion, as defined by the thick fibrous neural lamella, while the cellular structures within the ganglion expand or shrink according to their state of nutrition. This curious structure must often have been observed before; it can be seen, for example, in the terminal ganglion of Periplaneta figured in longitudinal section by Roeder (1948); but I have found no description of it.

THE GLIAL CYTOPLASM; PAS-POSITIVE STRUCTURES

The glial cytoplasm extends everywhere between the ganglion cell bodies, and between the nerve fibres in the neuropile and in the nerves and connectives (as already described in Rhodnius). If the nervous system is fixed with alcoholic Bouin, and glycogen is removed from the sections with saliva before treatment with PAS, the neural lamella and the glial cytoplasm give a positive reaction (Wigglesworth, 1956a).

In the glial cytoplasm the PAS-positive material takes several forms. (i) Irregular deposits, presumably reserve material to be used in the formation of glial membranes, etc. (Text-fig. 3 C). (ii) Delicate homogeneous membranes around the cell bodies and axons, and lining the glial cavities (Text-fig. 3 A, C). Around the giant axons in the connectives these sheaths are multiple (Text-fig. 3 A). (iii) Tapering and branching strands in the cytoplasm of the glial cells (Text-fig. 3 C, D). These may occupy the larger filaments which traverse the spaces in the glial lacunae. They occur as thickenings in the membranes around the ganglion cells and axons. Here they appear in cross-section as purple points around the axon sheaths (Text-fig. 3 A). In surface view of the large axons they appear as a network of fibrils (Text-fig. 3 B). This arrangement was described by Pipa & Cook (1958) in the nerves of the sucking louse.
All these structures are presumably composed of the collagen-like material made up of banded fibrils that has been described in the neural lamella (Hess, 1958a; Smith & Wigglesworth, 1958) and in the glial cytoplasm within the ganglia (Gray, 1959). Besides giving a positive PAS reaction they stain with haematoxylin (slightly) and with light green. As a result they have a purple colour in the sections which, even in the absence of the saliva test, serves to distinguish them from glycogen, which is stained crimson.

Text-fig. 3. Sections through the ganglion and connectives (4 μ) (alcoholic Bouin; PAS after saliva) showing PAS-positive structures. A, Transverse section of connective; B, horizontal section of connective showing large axon sheath in surface view; C, lateral margin of ganglion showing neural lamella and PAS-positive deposits, membranes and fibrils in the glial cytoplasm; D, detail of glial cells adjacent to the neuropile, showing branching fibrils in the cytoplasm.

DISTRIBUTION OF GLYCOGEN IN THE GANGLION AND NERVES

Text-fig. 4 shows the general distribution of glycogen in the ganglion of the well-nourished insect. It is most conspicuous in the perineurium cells which are often stuffed with glycogen like fat-body cells. It is scanty in the glial cytoplasm, and it occurs as a fine ‘dust’, barely resolvable under the oil immersion, in the glial membranes which ensheathe the axons in the connectives and cercal nerves, and in the glial component of the neuropile.
The glycogen in the glial cytoplasm is most evident at the base of the cercal nerve and this gives the impression that it is being transmitted from the ganglion to the nerve. In the cercal nerves and in the connectives the cells below the neural lamella combine the functions of perineurium cells and Schwann cells (cf. Hess, 1958a; Wigglesworth, 1959a). They contain glycogen, as in the specialized perineurium cells of the ganglia, and they presumably transmit this glycogen into the deeper parts of the subjacent nerve, where traces are detectable as a fine ‘dust’ in the axon sheaths.

Glycogen is conspicuous in the nerve cells; it lies predominantly on the inner side where the axon is given off. It is well known that in tissues fixed with alcoholic fixatives, glycogen, even more than other cell components, may be swept forwards by the advancing fixative and become concentrated on the opposite side of the cell. The question arises whether the accumulation of glycogen in the axon cone is an artifact of fixation or represents the distribution in the living cell.

There seems little doubt that it is not merely an effect of fixation. (i) The distribution is unchanged if the ganglion is split open so that the fixative can enter the cells from both sides. (ii) In normal ganglia fixed with alcoholic Bouin glycogen is likewise concentrated in the axon cone in those cells in which the axon lies parallel with the surface of the ganglion (Text-fig. 5 A, D). But it will be noted in Text-fig. 5 A that there has been some displacement of glycogen during fixation. (iii) If the ganglion is exposed for 10 min. in buffered osmium tetroxide before transfer to alcoholic Bouin, the cytological fixation is good with no evidence of displacement, but the distribution of glycogen in the ganglion cells is the same; it is concentrated in the axon cone.
GLIAL INVAGINATIONS AND THE TRANSFER OF CARBOHYDRATE TO THE GANGLION CELLS

The cell membrane of the large ganglion cells in insects is invaginated so that the glial cytoplasm penetrates deeply into the body of the cell. This arrangement was termed by Holmgren (1900) the 'Trophospongium'. In *Rhodnius* also it was interpreted as the mechanism by which the glial cells furnish nutrient substances to the ganglion cells (Wigglesworth, 1959b). It was of interest to see whether there is any visible relation between the glial invaginations and the transfer of glycogen.

Text-fig. 7 A shows a longitudinal section through a large ganglion cell fixed and stained with osmium and ethyl gallate. There are small invaginations from all parts of the cell surface, but these are much more conspicuous and numerous in the axon cone. Text-fig. 7 B–F represents a series of transverse 2µ sections cut at intervals of 10–12µ from the equator of a large ganglion cell to the beginning of the axon. It shows the progressive development of glial invaginations as the axon cone narrows to form the axon. These drawings also indicate the thickness of the layer of glial cytoplasm that encloses the ganglion cell at the different levels.

Thus the accumulation of glycogen in the ganglion cell goes hand in hand with the development of the glial invaginations. Moreover, longitudinal sections through the beginning of the axon (Text-fig. 5 C, D) commonly show the glycogen...
Perineurium and glial cells in the cockroach

Text-fig. 6. A, Horizontal section of the anterior end of the terminal ganglion just above the connectives, from a cockroach starved for 3 weeks (alcoholic Bouin, PAS to show glycogen). Perineurium cells contain clumps of mitochondria and traces of glycogen. B, The same in starved cockroach 3 hours after feeding on honey and serum. Much glycogen in perineurium cells and traces in glial cells. C, Same insect as B, posterior end of the ganglion showing glycogen in ganglion cells and in glial cytoplasm. D, The same, lateral margin of the ganglion. a, Clumps of mitochondria; b, glycogen.

Text-fig. 7. A, Longitudinal section of large ganglion cell showing invaginations of plasma membrane. B–F, 2 μ sections of a large ganglion cell cut at about 10 μ intervals between the equator and the base of the axon, showing progressive development of invaginations (osmium tetroxide and ethyl gallate).
deposits in elongated strands, strongly suggesting that it is contained within or on the surface of the invaginations. This impression is confirmed by the examination of sections of ganglia fixed for 10 min. in osmium tetroxide before transfer to alcoholic Bouin. These show the glycogen between the glial membranes in some cases, applied to the surface in others.

It might be argued that since the ganglion cells are known to be continuously secreting the axon contents, the deposition of glycogen towards the base of the axon is merely a reflexion of this movement. But glycogen appears in this situation very rapidly after feeding. Young adult male cockroaches have been kept on water alone with no food for 3–4 weeks at 26°C. At the end of this time the fat body is devoid of glycogen and contains only small droplets of fat. Glycogen has practically disappeared from the ganglion; only occasional minute traces are detectable in the perineurium cells (Text-fig. 6 A). But within 3 or 6 hr. after the cockroach has been given a meal of honey the glycogen content of the ganglion has been restored and the deposits in the ganglion cells are again concentrated in the axon cone with no more than a fine ‘dust’ in the remainder of the cytoplasm. In these preparations fixed with alcoholic Bouin and stained with PAS and Light Green, the mitochondria in the perineurium cells appear as green-staining masses. The glycogen is confined to the periphery of the cell and is entirely absent from the clumps of mitochondria (Text-fig. 6 B–D).

DISTRIBUTION OF TRIGLYCERIDE IN THE GANGLION

The ganglia and nerves contain large amounts of lipid in the axon sheaths, in the numerous mitochondria, and in the Golgi bodies; it is this lipid which is mainly responsible for the uptake of osmium and so for the deep coloration with ethyl gallate (Wigglesworth, 1957). The greater part of these lipids are presumably phospholipids which give a grey-brown coloration with osmium and ethyl gallate. Triglycerides appear as blue-black spheres. They are present in much smaller quantity, but they do occur in the cytoplasm of the glial cells in the well-nourished insect (Text-fig. 1). They range from droplets about 2 μ in diameter to fine ‘dust-like’ particles, and occasional minute droplets of fat appear in the cytoplasm of the ganglion cells and perineurium.

As in Rhodnius (Wigglesworth, 1959b) the glial invaginations in the ganglion cells often contain deeply staining lipid material in their inner extremities; and darkly staining inclusions are most evident in the immediate neighbourhood of the invaginations. This localized accumulation of minute lipid droplets over the surface of the glial invaginations persists even in insects starved for 3–4 weeks. But the relation of fat droplets to the invaginations is most evident when the deposition of fat in the ganglion is exaggerated by the injection of large amounts of sugar.

Text-fig. 8 shows sections of a ganglion 20 hr. after the injection of 0.2 ml. of 10% glucose into a normally nourished male cockroach. At the anterior end of the ganglion, just dorsal to the connectives, the high columnar perineurium cells contain no visible deposits of fat, but the underlying glial cells contain abundant
Perineurium and glial cells in the cockroach

small droplets (Text-fig. 8 B). At the margin of the ganglion (Text-fig. 8 A) a few minute droplets of fat are present in the perineurium, but much greater quantities are in the cytoplasm of the glial cells.

Text-fig. 8. Sections of ganglion of cockroach 20 hr. after injection of 0.2 ml. of 10% glucose (osmium tetroxide and ethyl gallate). A, Side of ganglion showing a few minute fat droplets in the perineurium cells, numerous droplets in glial cytoplasm (2 μ section). B, Horizontal section of anterior end of ganglion; fat droplets absent from perineurium cells, plentiful in glial cytoplasm. (Mitochondria faintly indicated in the perineurium but omitted from the glial cells.) C, Ganglion cell surrounded by glial cytoplasm. Minute fat droplets in glia and within the plasma membrane invaginations towards the axon. D, Detail of invaginations and fat droplets in part of a ganglion cell (0.85 μ section).

Text-fig. 8 C shows a longitudinal section of a large ganglion cell. As usual, the invaginations from the glial cells are conspicuous in the axon cone and minute blue-black droplets of fat are associated with them and appear to lie between the invaginated cell membranes.

Text-fig. 8 D shows the details of invaginations on the inner aspect of a large ganglion cell. Droplets of fat are plentiful in the glial cytoplasm; they occur in the
base of some of the invaginations into the ganglion cell; and they often occur between the membranes deep in the body of the cell. An occasional droplet may appear to lie free in the cytoplasm; but it is difficult to be sure whether these are droplets which have been liberated from the glial invaginations or whether they are still enclosed within invaginations whose connexion with the cell surface is not visible in that particular section.

The total quantity of visible fat droplets deposited in the ganglion cell is very small in comparison with the glycogen. But the glial invaginations are again clearly concerned in their transfer.

**PERMEABILITY OF THE PERICELLA**

The perilemma is the term used for the total sheath of the ganglion, embracing the inner cellular layer, the perineurium, and the outer fibrous sheath, the neural lamella. It is usually suggested that the neural lamella is responsible for the selective impermeability of the total sheath. But the evidence set out in the earlier paper on the ganglia of *Rhodnius* (Wigglesworth, 1959a) favoured the view that the neural lamella is freely permeable and that it is the cellular perineurium which regulates the passage of solutes into the ganglion.

Observations made during the present work support that conclusion. Trypan blue (0.2 ml. of 0.5% solution in Ringer) was injected. The ganglion was later fixed with Carnoy and the sections stained with Orange G. At 2 days after the injection the neural lamella was coloured a diffuse blue. More dye was concentrated in haemocytes spread out on the surface of the ganglion. At 7 days after the injection there were very small amounts of dye in the perineurium cells and still smaller amounts in the glial cells. There seemed to be no absolute distinction in this respect between the perineurium cells and glial cells (such as was described by Scharrer, 1939); the difference is in degree only. The pericardial cells and dermal glands take up most of the dye; the haemocytes and fat body come next; the perineurium cells take up much less and only traces reach the glial cells.

It would appear that the neural lamella has a sponge-like structure which readily admits even rather large molecules like trypan blue. These results are comparable with those of Röhlich & Weiss (1955) which confirmed in the sciatic nerve of the rat the earlier opinions of Retzius and of Ranvier that the resistance to entry of iron salts is dependent on the cellular layer of the perineurium, the connective tissue sheath being freely permeable (cf. Hoyle, 1953).

**DISCUSSION**

The glial cells in the ganglia of insects have long been regarded as 'trophocytes' with the function of nourishing the neurones. The results here described demonstrate the role of the perineurium cells (which are regarded as a specialized type of glial cell (Wigglesworth, 1959b)) in the storage of glycogen, and the role of the glial cells proper in transferring glycogen and fat (or the precursors of these reserves) to the ganglion cells by way of the plasma membrane invaginations or
Perineurium and glial cells in the cockroach

'trophospongium'. These reserves, particularly the glycogen, are concentrated in the ganglion cell towards the base of the axon. That suggests that they contribute to the secretion from the body of the ganglion cell which is believed to pass continuously into the axon.

A large part of the nutrients entering the ganglion cell will be small molecules, glucose or trehalose and the intermediary metabolites derived from these (Treherne, 1960). Such substances cannot be located by the procedures described in this paper; but the movements of glycogen and fat which follow massive ingestion or injection of sugars may perhaps serve to indicate the route they must follow in reaching the neurones.

The perineurium cells and the glial cytoplasm of Rhodnius are very rich in enzymes: ‘esterase’ of various types (Wigglesworth, 1958), succinic dehydrogenase (Wigglesworth, 1956b); and since these properties are common to the perineurium cells of the ganglion and of the peripheral nerves, a nutritional function was assumed to be common to both (Wigglesworth, 1959,a, b). F. O. Schmitt (1958) emphasized the high metabolic and enzymic activity of the satellite cells (glial cells, Schwann cells) in the peripheral nerves of mammals. He attributes to these cells a ‘nutritional function’, to provide for local metabolic energy and perhaps also the type of energy necessary for ion pumping.

The present work demonstrates the role of the glial cells in the massive transfer of carbohydrates and fats to the neurones in the central nervous system. There is some visible transfer also in the large nerves and connectives. It seems probable that this nutritive function is general throughout the nervous system.

SUMMARY

1. The histology of the last abdominal ganglion and the cercal nerves and connectives of the cockroach are briefly described. Attention is called to the large cavities, termed the ‘glial lacunar system’, that are present in the glial cell layer of the ganglion; and to the branching filaments of collagen-like material which are laid down within the glial membranes and trabeculae of the ganglia and nerves.

2. Glycogen is stored in large amounts in the perineurium cells, and in small amounts in the interaxonal glial membranes in the neuropile and nerves. Invaginations of the plasma membrane of the large ganglion cells (the ‘trophospongium’) are apparently concerned in the transfer of glycogen. Invaginations and glycogen deposits increase progressively towards the base of the axon.

3. Very small amounts of triglycerides are stored in the ganglion. There are traces only in the perineurium cells; rather more in the glial cells. The invaginations of the glial cells into the large ganglion cells seem to be concerned also in the transfer of lipids to the neurones.
REFERENCES


WIGGLESWORTH, V. B. (1959c). A simple method for cutting sections in the 0.5 to 1 μ range, and for sections of chitin. *Quart. J. Micr. Sci.* 100, 315-20.

EXPLANATION OF PLATE

All sections fixed and stained with osmium tetroxide and ethyl gallate, and cut at 2 μ.

Fig. 1. Horizontal section of terminal ganglion slightly dorsal to the cercal nerves and connectives, showing, from without inwards, the layer of ganglion cells, the clear 'glial lacunar system', and the darkly staining neuropile.

Fig. 2. The same as fig. 1, cut at a more dorsal level: glial lacunae very extensive.

Fig. 3. Transverse section of terminal ganglion from an old and starved cockroach: glial lacunar system well developed.

Fig. 4. The same as fig. 3, from a young and well-nourished cockroach: glial lacunae largely collapsed.

Fig. 5. Detail of the glial lacunae from the ganglion represented in figs. 1 and 2 above, showing glial cells connected by branching trabeculae.

Fig. 6. Large ganglion cells invested by glial cells, and with their axons crossing the glial lacunae. The outer limit of the neuropile is seen to the left below.
WIGGLESWORTH—THE NUTRITION OF THE CENTRAL NERVOUS SYSTEM IN THE COCKROACH PERIPLANETA AMERICANA L. THE ROLE OF PERINEURIUM AND GLIAL CELLS IN THE MOBILIZATION OF RESERVES

(Facing p. 512)