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INVERTEBRATE NEUROGLIA–JUNCTIONAL STRUCTURE AND DEVELOPMENT

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SUMMARY

The morphological characteristics of the neuroglial cells of invertebrates are reviewed, including the ultrastructural and enzyme cytochemical features of their cell bodies and attenuated cytoplasmic processes, the various ways they ensheath the nerve cells, including the loosely myelinated condition, their modifications due to intraganglionic localization and their interactions with other glial cells in the form of homocellular junctions. The spectrum of heterocellular axo-glial associations that occur in invertebrates is considered with particular reference to the different kinds of intramembranous organization they exhibit as revealed by freeze–fracture. Recent studies on glial cell development in a range of arthropods, during embryonic and pupal stages, reveal the importance of glial cell tight junctions in forming the tracer-excluding blood–brain barrier. These occluding junctions are now shown to be, in some cases, vertebrate-like in their complexity. The stages in their assembly, which may be concurrent with those of gap junction formation, reveal a number of differences from vertebrate glia. During metamorphosis, glial cells dissociate and the dynamics of the concomitant interglial junctional disruption and their intramembranous particle dispersal without apparent internalization, as well as their subsequent reassembly, are examined. The stimuli triggering these glial events and the physiological significance of the various glial modifications are considered.

I. INTRODUCTION

The glial cells in the invertebrates are a varied assemblage, and, unlike those of vertebrates, have thus far defied attempts to categorize them in any rigorous way; there is little that is consistent about their distribution and morphology in different organisms to enable neurobiologists to make definitive classifications. On the other hand, some differences in cytological features and topographical arrangements between different types of neuroglial cell are discernible, and glia have for example, been subdivided into plasmatic, fibrous, perineurial and Schwann-like categories (Radojcic & Pentreath, 1979), although this classification, as with any others put forward, suffers from a number of limitations. Reviews on glial cells in the tissues of invertebrates include specialized reports (Nicaise, 1973) and more generalized accounts (Clayton, 1932; Roots, 1978; Radojcic & Pentreath, 1979; Varon & Somjen, 1979; Treherne, 1980; Lane & Treherne, 1980).

Neuroglia occur in the higher invertebtates, such as Annelids, Arthropods, Molluscs and Aschelmintha, possibly are present in the Platyhelmintha, and seem to be absent

in such phyla as the Porifera and Coelenterata (Roots, 1978; Radojcic & Pentreath, 1979). The glial cells that are found in invertebrate ganglia may be loosely defined as those cells in the nervous system which are non-neural and which ensheath the neurones, ramifying between them. Inevitably, other non-nervous elements are also present, such as those of the blood or connective tissue, and mesenchymal cells or granulocytes (for example, Baskin, 1971*a*) may be difficult to distinguish from true glial cells. In some cases, particularly in early embryonic tissues, any distinction between glial and nerve cells is impossible. Later on in development, the glia become, in comparison with the nerve cells, more irregular in outline and much more attenuated (Fig. 1), and may be rather more electron opaque in thin-sections (Fig. 2). Their initial embryonic origin is not well understood although in annelids and arthropods they could be ectodermal (Roots, 1978); in the latter it is thought that they may arise from the same neuroblast cells that give rise to the nerve cells themselves (Edwards, 1980), although they might also originate from different epidermal cells (Bate, personal communication). No really convincing evidence is as yet forthcoming for either possibility.

The functions of the glial cells in invertebrates appear to be manifold. Clearly, since they encompass the neurones, they, or their intercellular adhesions, provide a degree of mechanical support and are undoutedly also protective; for example, it has been suggested that they dampen the spread of compressional forces (Baskin, 1971*b*). Where they occasionally form extensive myelin-like sheaths they may insulate and presumably speed up impulse conduction by a saltatory mechanism (Heuser & Doggenweiler, 1966; Günther, 1976). In some cases, notably in arthropods, they may form permeability barriers (Lane & Treherne, 1972*a*; Treherne, 1974; Lane, Skaer & Swales, 1977*a*; Lane, Swales & Abbott, 1977*b*; Lane & Skaer, 1980; Lane, 1981*c*). They have always been considered to have a trophic role (Holmgren, 1900; Smith, 1967) with regard to the nerve cells, providing metabolic reserves (Fahrenbach, 1976; Wolfe & Nichols, 1967), and transferring metabolites such as proteins and transmitter enzymes to the axoplasm, although the precise mechanism of this interaction is as yet poorly understood (Gainer, 1978). They may be active in the destruction of nerve cells (Bittner & Mann, 1976; Griffiths, 1979) which could be important during development where cell death of neurones occurs naturally. They may also regulate the ionic composition of the fluid bathing the nerve cells (Treherne & Pichon, 1972), restrict the extracellular space which may serve as a cation reservoir, may sequester and/or release neurotransmitters or transmitter enzymes (Orkand & Kravitz, 1971; Salpeter & Faeder, 1971; Evans, 1974; Houk & Beck, 1977), or indeed, in some cases, may synthesize them. They may also have a tactic role in guiding migrating neurones Lopresti, Macagno & Levinthal, 1973; Lane, 1979*a*; see also Edwards, 1980). The

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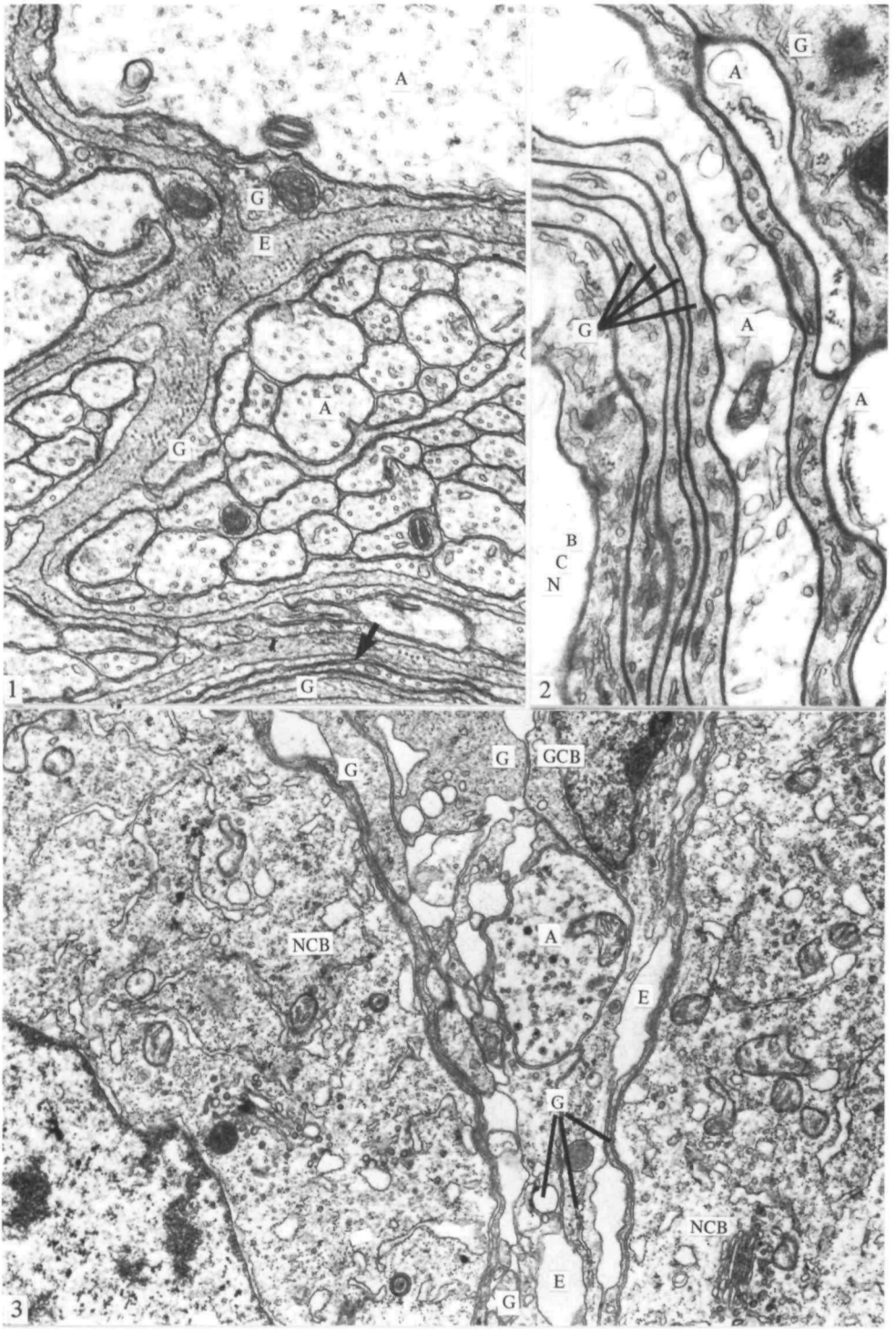
Fig. 1. Thin-section of axons (A) surrounded by attenuated glial processes (G) between which lies extracellular space (E) containing collagen fibrils. Crayfish (*Procambarus clarkii*) central nervous system. Arrow indicates short trans-glial channel. × 33 550.

Fig. 2. Enhanced electron density of the glial cells (G) ensheathing axons (A) and nerve cell bodies (NCB). Locust (*Schistocerca gregaria*) nervous system. × 35 000.

Fig. 3. Nerve cell bodies (NCB) and axon (A) encompassed by attenuated glial cell (G) processes which emanate from the glial cell body (GCB). E, extracellular space. Snail (*Helix aspersa*) ganglion. × 15 600.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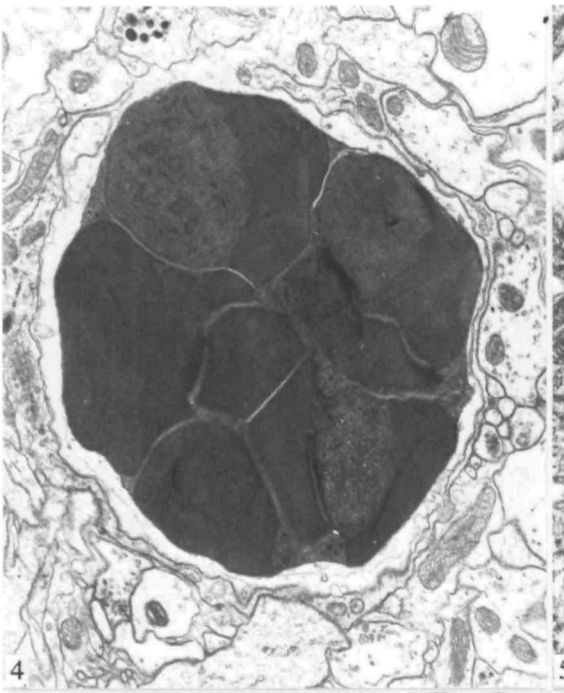
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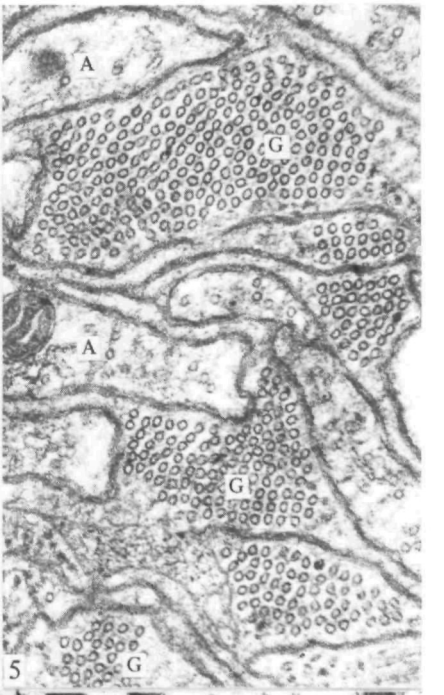
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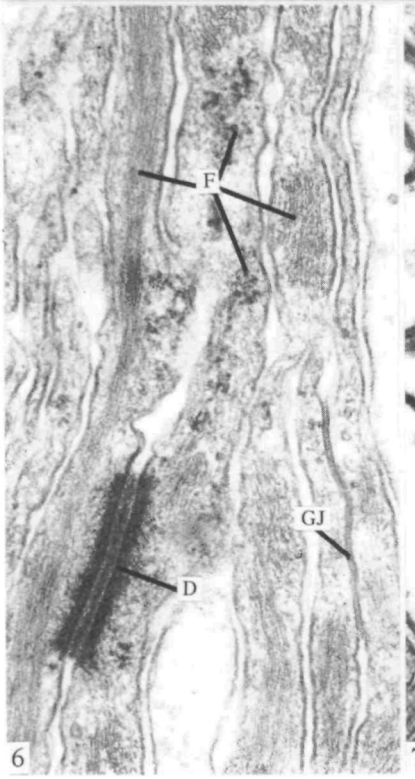
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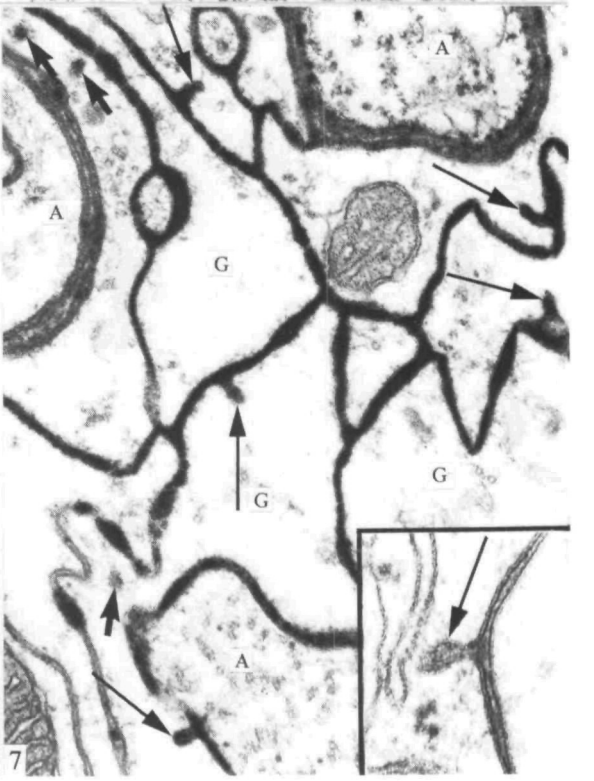
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morphological basis for these various kinds of activities and interactions are not all well established. In this chapter it is hoped to assess what is known about the diverse structures of neuroglia in the invertebrates and the avenues of approach used in the elucidation of their characteristic features.

The techniques available for the study of the structural/functional correlates in these enormously important, yet enigmatic, cells, include electrophysiological approaches and related injection procedures whereby electrodes implanted into the glial cells may fill the cells with fluorescent dyes or with tracers which render them electron opaque in thin sections (fig. 3 in Treherne & Pichon, 1981); the electrically-characterized cells are then identifiable morphologically. Conventional fixation with thin-sectioning and freeze-fracturing has yielded much of the basic information regarding glial distribution, topography, fine structure and membrane modifications. Cytochemical techniques, including immunocytochemistry, have characterized certain aspects of their enzymatic make-up and autoradiographic studies are beginning to elucidate the parameters of glial uptake, storage and glial–neuronal exchanges. Recent developments in the production of cell-type-specific antigenic markers for glial cells may facilitate their classification as well as the study of their features during differentiation.

II. CYTOLOGICAL FEATURES

(A) *Glial cell body*

The cell body of invertebrate glial cells is normally relatively small; from its nuclear region emanate many attenuated cytoplasmic processes (Fig. 3) which may extend for very considerable distances into the surrounding nervous tissue. The glial cell body proper may possess relatively little cytoplasm (Fig. 3), but usually contains endoplasmic reticulum, rough or smooth, free ribosomes, mitochondria, the occasional Golgi complex, microtubules and or filaments. In some cases storage products such as glycogen or lipid may also be found as well as glia grana or lysosomes. The latter may be numerous (Lane, 1968*a*) and of quite a considerable size (Fig. 4) containing lamellae and granules; in such cases they are often referred to as 'gliosomes' (Scharrer, 1939; Pipa, Nishioka & Bern, 1962). Cytochemical studies show, however, that they possess acid phosphatase as well as some thiamine pyrophosphatase (TPPase) and, ATPase (Lane, 1966, 1968*a*), so they do actually seem to be a form of lysosome (Lane,

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Fig. 4. Large gliosome (lysosome) in a glial cell which is lying within the substance of the neuropile. Spider (*Aranaeus*) ganglion. × 12900.

Fig. 5. Axons (A) surrounded by glial cells (G) containing massive, ordered arrays of microtubules as is typical of arthropods. Blood-sucking bug (*Rhodnius prolixus*) nerve. × 81900.

Fig. 6. Glial cells, associated by desmosomes (D) and gap junctions (GJ), exhibiting extensive bundles of intracellular filaments (F), seen cut longitudinally and in cross-section, as is typical of molluscs and annelids. Snail ganglion. × 80000.

Fig. 7. Axons (A) surrounded by glial cells (G) after fixation with colloidal lanthanum. The tracer has leaked past the limited perineurial junctions, has infiltrated the extracellular space and is being endocytosed by the glial cells as indicated by the dense omega profiles (arrows) and vesicles (thick arrows). Spider (*Aranaeus*) peripheral nerve. The insert shows a nerve cell with adjacent glial cell containing omega profile in a locust ganglion. × 57000; Insert × 58000.

1968*b*). On the other hand, the glial granules in certain molluscs are thought to be specialized for the storage of inorganic cations (Nicaise, 1973).

Gliosomes have been shown to increase in number during CNS differentiation and then to decrease after the completion of ganglionic development, in the oligochaete, *Tubifex* (Djaczenko & Cimmino, 1976); this was taken to mean that the gliosomes were important in trophic interactions between glia and neurones during differentiation. They have also been found to increase in number with age or with enhanced phagocytic activity, presumably since they are accumulated rather than extruded (Lane, unpublished data).

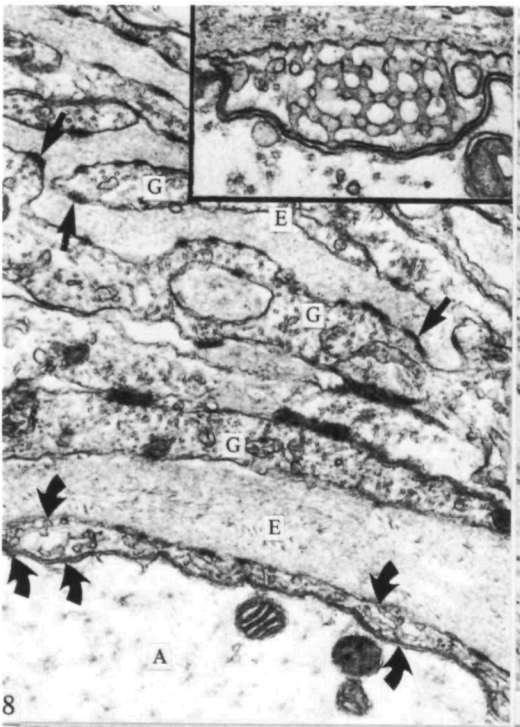
(B) *Attenuated glial cell processes*

The narrow glial projections spiral around axons or nerve cell bodies, and interdigitate with other glial processes, ramifying within the tissues of the nervous system extensively. These processes may be excessively thin (Fig. 3); where they are wider, cytoplasmic organelles are found and, depending on the nutritional state of the organism, glycogen and lipid.

The most striking glial components are the cytoskeletal elements and these seem to be either microtubules or filaments. The first abound in the glia of arthropods (Fig. 5) (Smith, 1968; Lane, 1974) where the latter are rarely to be seen. The reverse is true in molluscs and annelids, where bundles of filaments extend through the glial processes (Fig. 6) (see Nicaise, 1973); here microtubules are rare or non-existent. The physiological significance of this is not clear, although it has been suggested that the microtubules may be required for adequate transport of materials along glial processes given that the arthropods are active organisms with a poor vascular supply to the nervous system (Roots, 1978). This situation contrasts with that in certain vertebrate macroglia, where there is a differential distribution depending on maturity; in astrocytes, for example, filaments abound in differentiated cells but are rare in undifferentiated glia where microtubules are plentiful (Allt, 1980).

The plasma membranes of the glial cell system have the potential to form junctional complexes (see section IV) and of being modified in other ways. They are capable of endocytosing tracer material such as lanthanum (Fig. 7) or ferritin (Reinecke, 1976) by vesicular uptake, rather as horseradish peroxidase is pinocytosed into caveolae by vertebrate Schwann cells (Mugnaini *et al.* 1977); exocytosis also seems possible (Lasek, Gainer & Barker, 1977). Freeze-fracture evidence of such cytotic activity in neuroglial membranes is also available (Fig. 14, insert). In certain situations, well documented only in the crustacea, the glial membrane forms permanent endocytotic vesicles which form trans-glial channels, or a tubular lattice system (Fig. 8) (Holtzman, Freeman & Kashner, 1970; Lane & Abbott, 1975; Nordlander, Masnyi & Singer, 1975; Shivers, 1976; Shivers & Brightman, 1976) which are patent to the entry of tracers (Holtzmann *et al.* 1970; Shivers, 1976; Lane *et al.* 1977*b*). This produces a potential short-circuit route for ions and molecules in their attainment of the axonal surface. The channels also contain cholinesterase and so may be involved in neurotransmitter degradation (Holtzman *et al.* 1970).

Glial cells have been shown to contain certain putative neurotransmitters. For example, in arthropods, acetylcholine and dopamine are preferentially localized in glial processes (Houk & Beck, 1977), while glutamate (Faeder & Salpeter, 1970;



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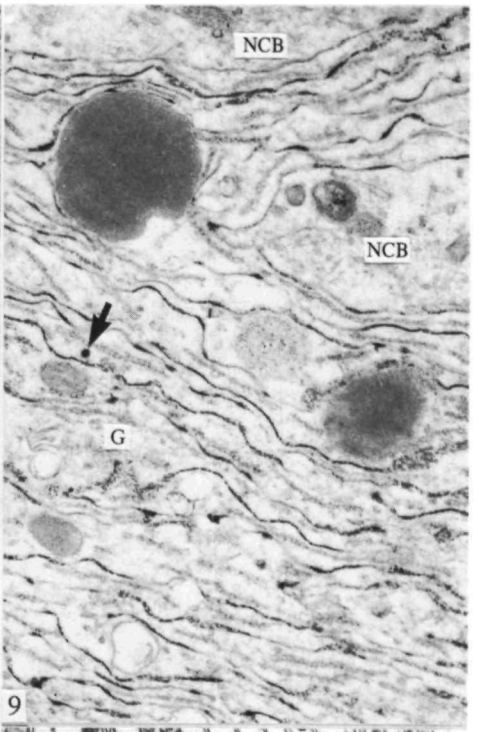

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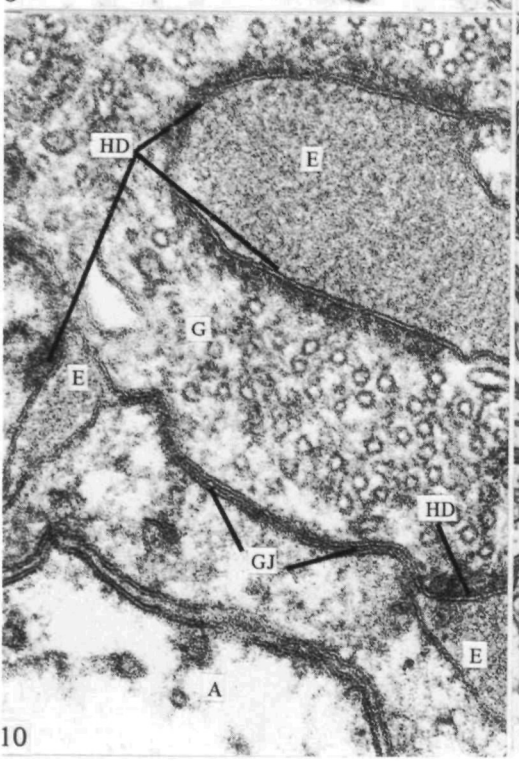

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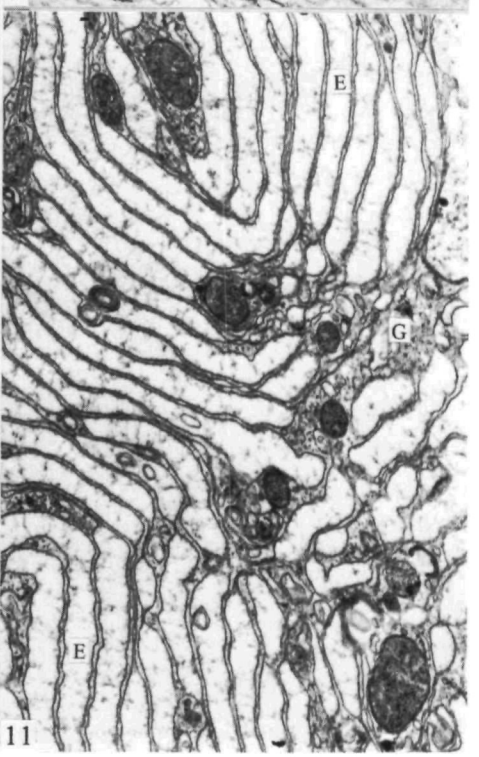

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Evans, 1974) and γ-amino butyric acid (GABA) (Orkand & Kravitz, 1971) are also more concentrated in glia than neuronal cells. This may be due to their selective uptake by glial cells in situations where there is no mechanism for extracellular neurotransmitter inactivation or enzymatic degradation.

Glia plasmalemma in the invertebrates, as in vertebrates (see for example Wood *et al.* 1977*a*), are characterized by cytochemically-demonstrable ATPase (Fig. 9) (Lane, 1968*a*; Houk & Beck, 1975) as well as by alkaline phosphatase (Fernandez, 1966) and also diphosphatases. Energy-requiring regulatory mechanisms may therefore be localized in glia, such as the function of their processes as a 'K^+ sink' (Kuffler & Potter, 1964). In addition, non-specific esterase (Wigglesworth, 1958) and eserine-sensitive cholinesterase have been reported in glial cell folds around nerve perikarya in insect ganglia (Smith & Treherne, 1965) as well as in the glia of molluscs and earthworms (Nicaise, 1973). Moreover, acetylcholine receptors are found in glial membranes of squid nerve fibre (Rawlins & Villegas, 1978) so that the glial cells must be involved in some way in nervous activity.

Perhaps surprisingly, ATPase as well as thiamine pyro(di)phosphatase is also to be found in the endoplasmic reticulum (ER) of some insect glial cells (Lane, 1968*a*). The similarities in enzymatic activity of ER and plasma membrane suggests a functional interrelationship, perhaps relating to transport of substances synthesized in the glia via the extracellular spaces to the neurones (Lane, 1968*a*). Supporting this contention, acid phosphatase-rich smooth ER in glia may show continuity with extracellular sites between glia and axons in fly eyes (Griffiths, 1979); it is thought that the enzyme is exported into axons, particularly those that have been injured. These results argue for a 'destructive' role for glia as has also been proposed in crustaceans (Bittner & Mann, 1976). Moreover, recent enzyme studies on the nervous system of the larval blowfly, *Calliphora*, reveal, in preliminary cytochemical investigations, that adenyl cyclase activity is localized in the plasma membranes of the innermost perineurial cells, that is, the 'bracelet' cells (Lane & Swales, unpublished data). These are the cell borders between which are situated the gap and tight junctions that become disassembled at the onset of metamorphosis (Lane & Swales, 1978*b*, 1980) as the glial cells become separated prior to pupal reorganization. Membrane-bound adenyl cyclase splits ATP to cyclic AMP (cAMP), which is an active phosphorylating intermediate well-known to act as a secondary messenger in many cellular control systems, mediating the activity of a variety of peptide hormones (Bitensky & Gorman, 1973;

Fig. 8. Glial processes (G) with hemi-desmosomes (arrows) abutting onto the collagen-filled extracellular space (E) as is typical of crustaceans. The ad-axonal glia exhibit trans-glial channels (curved arrows and in *insert*), crossing from extracellular space to axonal (A) surface. Crayfish ganglion. × 25000; Insert, × 38000.

Fig. 9. Glial processes (G) surrounding nerve cell bodies (NCB) and exhibiting cytochemically-demonstrable ATPase in their plasmalemma as well as some vesicles (arrows), possibly endocytotic. Grasshopper (*Melanoplus differentialis*) ganglion. × 15500. From Lane (1968*a*).

Fig. 10. Glial cell (G) processes exhibiting hemi-desmosomes (HD) where they abut onto extracellular space (E) which contains a moderately electron opaque matrix, as is typical of insects. An interglial gap junction (GJ) exhibiting the reduced intercellular cleft occurs between the membranes of the glial cells ensheathing the axon (A). Note the projections from some of the glial microtubules. Cockroach (*Periplaneta americana*) abdominal nerve cord. × 102000.

Fig. 11. Thin glial (G) processes separated by electron lucent extracellular spaces possessing only slight fibrous striations. Blood-sucking bug (*Rhodnius prolixus*) ganglion. × 18600.

Berridge, 1979). It therefore seems possible, as suggested earlier (Lane & Swales, 1980), that the late larval hormones of holometabolous insects can induce the glial cell changes and related junctional modifications which occur at the commencement of pupation. If these hormones are steroids, which do not require a second internal signal, they may activate another protein hormone(s) that can then act on the glial cells via an intermediate such as cAMP. Hormonally-induced changes in glial gap junctions have been reported previously for other systems such as those of amphibians (Decker, 1976). Moreover, when isolated glial fractions from the CNS of *Manduca sexta* are treated with serotonin, an increase in cAMP levels is obtained, suggesting the activation of a glial-associated adenyl cyclase (Taylor, Dyer & Newburgh, 1976).

Glial processes are usually separated from the nerve cells or each other by a cleft of only 10–20 nm (Fig. 1) and so they restrict the immediate cellular environment. However, in certain regions of some ganglia the extracellular space may be dilated into extensive spaces or sinuses (Fig. 11) which sometimes contain a fibrous matrix. This matrix may display collagen-like fibrils (Figs. 1 and 8) as in the crustacea, or the spaces may possess deposits of an electron opaque ground substance (Fig. 10) which has been characterized histochemically in insects as hyaluronic acid (Ashhurst & Costin, 1971). In molluscs, and many other systems, the glial cells themselves are thought to elaborate the dense extracellular material (Johnston & Roots, 1972; Prior & Lipton, 1977). This extracellular matrix may be important in furnishing a pool or reservoir for substances, possibly cations, and hence may be of considerable physiological importance (Abbott & Treherne, 1977; Abbott, Pichon & Lane, 1977; Abbott, 1979).

III. TOPOGRAPHICAL AND CELLULAR DISTRIBUTION

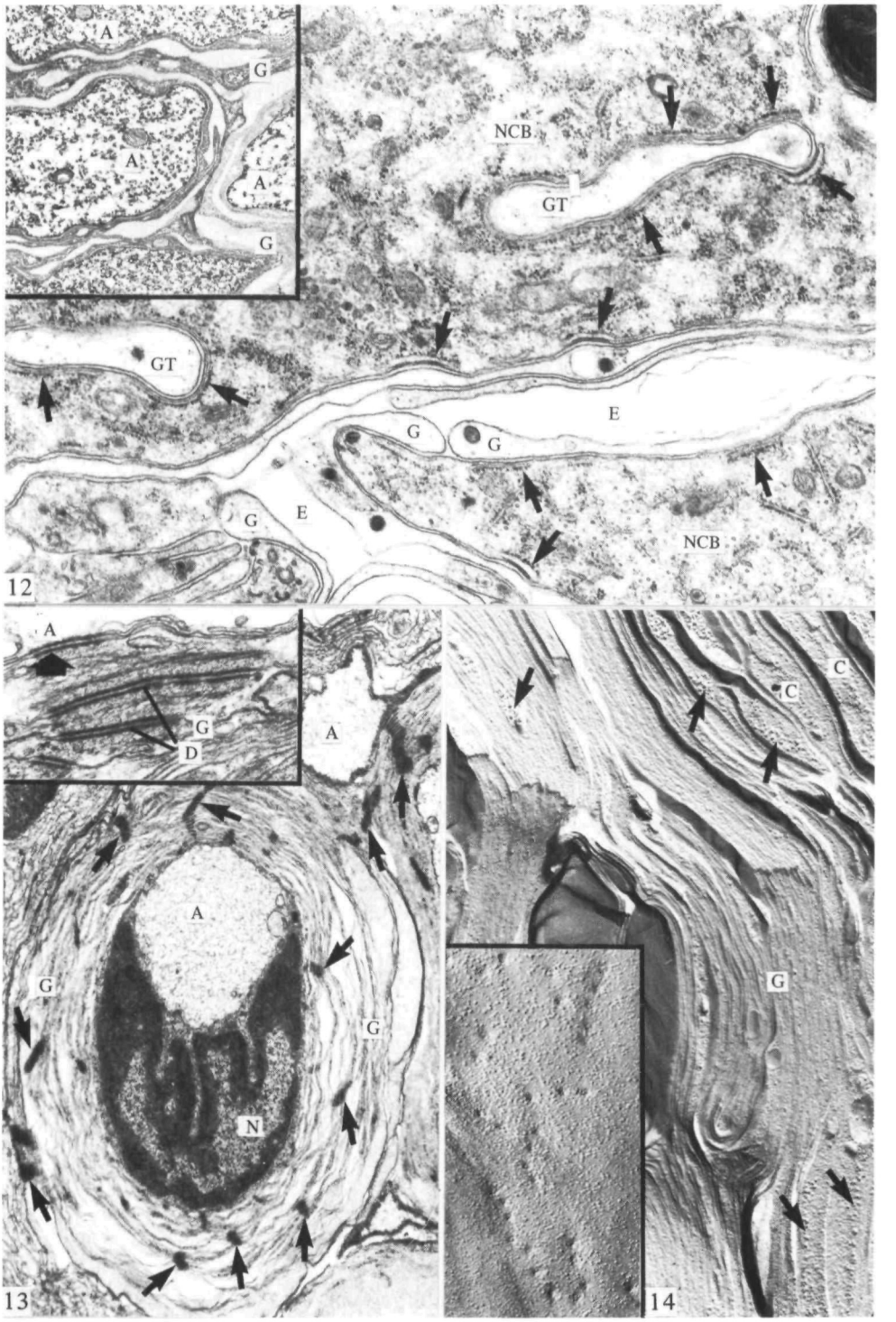
(A) *Trophospongial processes*

The attenuated nature of the neuroglial cells in invertebrate ganglia is such that the glial folds are often almost no more than the width of their two membranes. Around the perikarya of large nerve cell bodies, glial processes often project into the peripheral cytoplasm (Fig. 12), which otherwise is not readily accessible to diffusion from the

Fig. 12. Nerve cell body (NCB) surrounded by attenuated glial cell (G) processes, some of which have invaginated into the perikaryon as trophospongia (GT). Note the sub-surface cisternae (arrows) of endoplasmic reticulum, smooth-surfaced where facing the neurolemma, lying near the glial cells. E, extracellular space. Snail ganglion. ×28500. Insert shows tannic-acid treated glial cells (G) and axons (A) where different glial cells are distinguishable by virtue of their differing electron opacity. Locust ventral nerve cord. ×17300.

Fig. 13. Glial nucleus (N) and 'loose' myelinated glial folds (G) encompassing axons (A); the glia are associated with one another by multiple desmosomal-like radial attachment zones (arrows). Prawn (*Leander serratus*) ventral nerve cord. ×7800. Insert shows giant axon (A) with which the innermost glial layer is joined by a heterocellular desmosome (thick arrow), the other glial folds (G) being held together by homocellular desmosomes (D). Earthworm (*Lumbricus terrestris*) ventral nerve cord. ×36000. From Roots & Lane (1981*a*, *b*).

Fig. 14. Freeze-fracture replica to show the 'loose' myelin, typical of some oligochaetes and crustacea, wherein multiple stacks of glial membranes (G) exhibit very little cytoplasm (C) between one another. Occasionally the intracellular filaments are seen in cross fracture (arrows). Earthworm ventral nerve cord, giant fibre. ×39000. Insert shows *en face* fracture of a glial membrane displaying many endo- or exocytotic pits. Prawn interganglionic connective. ×45000. From Roots & Lane (1981*a*, *b*).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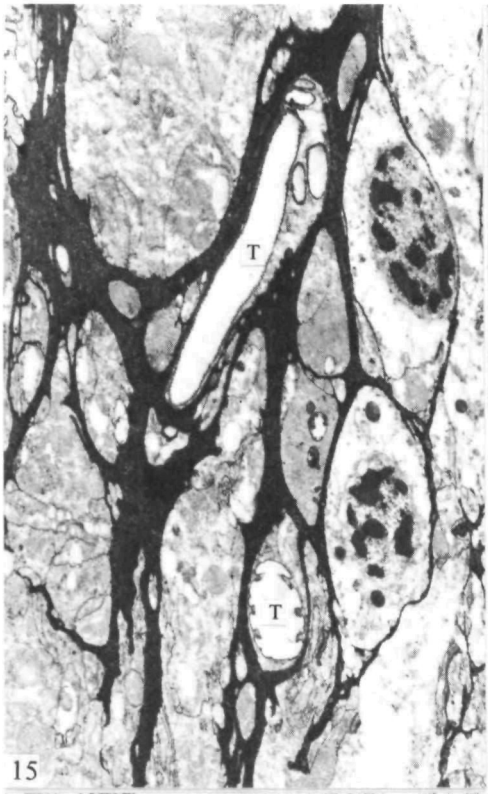
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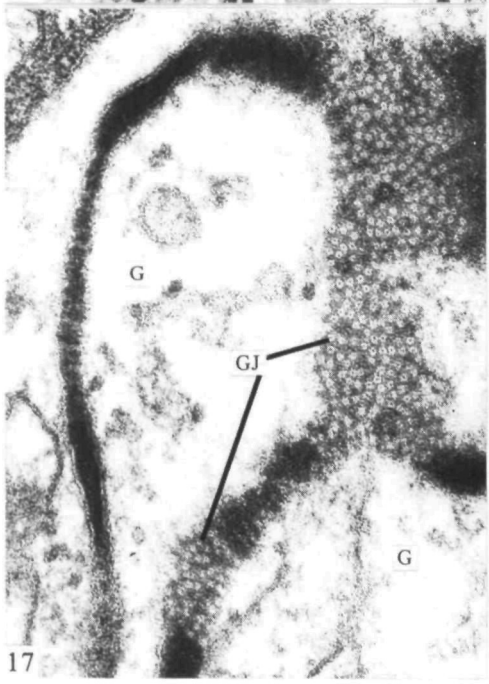
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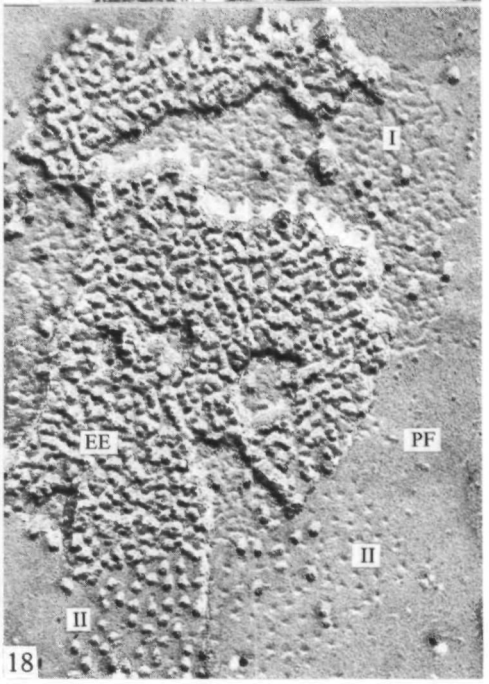
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surrounding glial cells. These structures were originally called trophospongia (Holmgren, 1900) and are also called 'canals of Holmgren', the former name implying, of course, that they are trophic with respect to the nerve cell bodies. This contention is supported by the presence of subsurface cisternae of endoplasmic reticulum which are found lying in close apposition to the trophospongial invaginations (Fig. 12). These have been thought to be the possible sites of specific metabolic exchange (Smith, 1967, 1968) as there is some evidence that directional transfer of glucose from glial to nerve cells may occur during glycogen synthesis in both insects (Wigglesworth, 1960) and the leech (Wolfe & Nicholls, 1967). It is also possible that, as proposed for vertebrate neuronal subsurface cisterns (Henkart, Landis & Reese, 1976), they may couple some intracellular activity to the electrical activity of the plasma membrane.

(B) *Concentric glial wrappings, mesaxons and loose myelin*

The glial cells in invertebrates may send one or more spiral folds around the neurone they are encompassing (Fig. 12, insert); the term tunicated is sometimes applied to this arrangement (Smith & Treherne, 1963). In other cases they form 'mesaxon' folds, which are multiple spiral ensheathments around giant axons; in most cases, however, they do not form myelin as do the vertebrate Schwann cells. The ensheathments of the invertebrate axons tend to be uncompacted so that some glial cytoplasm remains in the spiral wrappings (Fig. 12, insert).

There are a few exceptions to this generalization where a loose or pseudo-myelinated condition occurs; this involves the glial processes wrapping around axons in spiral configurations which may in some cases be so attenuated as to appear to have little or no residual cytoplasm (Fig. 13). This is to be found in the glial wrappings around the giant fibres of the earthworm (Fig. 14) (Hama, 1959; Coggeshall, 1965; Levi, Cowden & Collins, 1966; Günther, 1976; Roots & Lane, 1981*a*) and in the wrappings around most of the axons in the optic nerves and ventral nerve cord of the prawn and shrimp (Fig. 13) (Holmes, 1942; Kusano, 1966; Heuser & Doggenweiler, 1966; Hama, 1966; Doggenweiler & Heuser, 1967; Roots & Lane, 1981*b*) as well as the crab, *Cancer irroratus* (McAlear, Milburn & Chapman, 1958). The number of glial layers or lamellae is variable, from a few to several hundred, and frequently the adjacent glial membranes are associated with one another by contacts which in the

Fig. 15. Desheathed preparation with outer glial layer removed prior to 60 min incubation in 10 mM ionic lanthanum. The CNS is thereby rendered accessible to the dense tracer which has penetrated the matrix of the extracellular spaces of the glial lacunar system lying between glia, nerve cells and tracheoles (T). Cockroach ganglion. × 4000.

Fig. 16. Perineurial cells in the synthetic state actively producing and extruding collagen fibrils (at arrows). Blowfly (*Calliphora*) ganglion. × 31 000. Insert shows a septate junction after incubation in 10 mM ionic lanthanum during which the tracer has penetrated its length. Locust (*Schistocerca gregaria*) ganglion. × 94 000.

Fig. 17. Colloidal lanthanum impregnation into spaces between glial cells (G) in which occur gap junctions (GJ) characterized by loosely clustered particles, each of which contains a stained central channel. Garden spider ganglion. × 117 000.

Fig. 18. Freeze-fracture replica of an arthropod glial–glial gap junction with Eface (EF) particles and Pface (PF) pits. Note that the particles and pits may be closely clustered (as in I) or very loosely aggregated (as at II) within one and the same junction. Locust ganglion. × 85 000.

earthworm appear to be desmosomal (insert in Fig. 13) (Hama, 1959; Coggeshall, 1965; Günther, 1976; Roots & Lane, 1981*a*). In the prawn they are desmosomal-like but clearly exhibit features which are rather atypical (see section IV(A), 1(*a*)) and are often referred to as radial attachment zones (Fig. 13). There have also been reports of whorls of myelin-like glial investments in the CNS of the blowfly *Calliphora* (Lane, 1974) and here the membranes appear to be packed into pentalaminar arrays. In the case of both the earthworm (Günther, 1976) and the prawn (Heuser & Doggenweiler, 1966), glial nodes are present and rapid impulse conduction occurs so that saltatory conduction is presumed to be occurring, albeit less efficiently than in vertebrate myelinated fibres. In freeze-fracture replicas the closely packed membrane folds are very striking (Fig. 14) (Roots & Lane, 1981*b*), although in some cases a little cytoplasm remains and filaments can be seen in cross-fracture (arrows in Fig. 14); the glial membranes in freeze-cleaved preparations display evidence of endo- or exocytotic activity (insert, Fig. 14).

(C) *Specialized glial modifications*

In some cases, the topographical position of glial cells has led to certain physiologically important specializations. For example, the outer peripheral glia have often become extended into a sheath and are termed the perineurial layer (Wigglesworth, 1959), while others, bounding the neuropile, form the glial lacunar system (Wigglesworth, 1960). This latter system is found in insect ganglia and is a series of extracellular spaces or lacunae interwoven with tracheoles and lined by glial cells lying between the outer cortex of nerve cell bodies and the inner medulla of the neuropile (Fig. 15). This system appears to change in volume depending on the nutritional state of the organism (Wigglesworth, 1960), becoming enlarged in starved insects, so that it may be a provision for maintaining unchanged the outward form of the ganglion. If ganglia are desheathed, tracers can gain access to this glial lacunar system (Fig. 15) and lanthanum (La^{3+}) appears to stain its matrix, suggesting the possibility that it could be charged and capable of binding ions.

The term perineurium is now generally used to refer to the outer cellular sheath of many invertebrate ganglia. In the arthropods, the perineurial glial cells are highly modified; they surround the whole of the nervous system both in the large peripheral nerves and in the CNS, forming a complete layer separating the nervous tissue from the circulating body fluids. This tissue has been much studied both electrophysiologically and fine-structurally and appears to form the morphological basis of the arthropod blood–brain barrier (Treherne & Pichon, 1972; Lane, 1972, 1978; Lane & Skaer, 1980). The component cells may possess many gliosomes (Lane, 1968*a*), as well as much lipid and glycogen (Wigglesworth, 1960), thereby being implicated in nutrient storage; they also seem to be specialized in the arthropods for synthesizing the collagen (Fig. 16) and matrix of the neural lamella, particularly in embryonic tissues or early hatchling stages and during metamorphosis (Scharrer, 1939; Ashhurst, 1968; Lane, 1972; Ashhurst & Costin, 1976). They are extensively interdigitated along their lateral borders, which is the location of a variety of intercellular junctions, including tight junctions (Lane *et al.* 1977*a*), and may be ion regulatory, using pumps on the outward facing membranes (Treherne & Schofield, 1978). The perineurial cells

possess amine oxidative activity (Houk & Beck, 1975; 1976) which implicates them in the metabolic regulation of biogenic amines. Relating to this, in molluscs, certain glia also oxidize benzidine and so possess hemoproteins (Schindelmeiser, Kuhlmann & Nolte, 1979); these have been suggested to be associated with gas exchange between the haemolymph and the avascular ganglia, by increasing the respiratory area for the neurones.

Other physiologically interesting glial modifications include such phenomena as the enlarged volume of the metabolically-active 'packet' glial cells in leech ganglia (Coggeshall & Fawcett, 1964) and the membrane specializations that produce glial–glial junctions and axo-glial associations. These latter modifications are considered at greater length in the following section.

IV. INTERACTIONS WITH OTHER CELLS

(A) *Homocellular glial–glial junctions*

(1) *Intercellular junctions*

(*a*) *Desmosomes.* Desmosomal junctions occur between glial cells in the nervous tissues of a whole range of invertebrates (for example, Baskin, 1971*b*; Ribi, 1977; Lane & Skaer, 1980). They usually take the form of *maculae adhaerentes* or junctional plaques rather than belts and exhibit both numerous cytoplasmic filaments, some of which may insert into the membranes, as well as dense intercellular cross-striations (Fig. 6). These junctions are particularly striking in the loosely-myelinated glial wrappings of the earthworm giant fibres where many of these occur lying in stacks, either in register or along diagonals (insert in Fig. 13) (Coggeshall, 1965; Günther, 1976; Roots & Lane, 1981*a*). Similar desmosomal stacking occurs in the prawn and shrimp 'myelin' (Hama, 1959; Heuser & Doggenweiler, 1966) but these desmosomes are rather atypical in structure forming radial attachment zones (Fig. 13) (Roots & Lane, 1981*b*). Frequently hemi-desmosomes are found to occur between glial cells and the collagen or matrix-filled extracellular space (Figs. 8 and 10). Freeze-cleaved replicas of these or of desmosomes may exhibit cytoplasmic cross-fractured filaments but frequently these either do not insert into the membranes, or, if they do, they do not produce a distinguishable intramembranous freeze-fracture profile. The role of the interglial desmosomes seems to be to maintain the structural integrity of the nervous system since they are not found between all glial cells, but only between those apparently subject to strain or motion (Lane, 1972, 1974; Lane & Skaer, 1980).

(*b*) *Septate junctions.* In invertebrate ganglia, pleated septate junctions are generally only found arranged as a circumferential belt between the lateral borders of the outer glial cells of the perineurium (Maddrell & Treherne, 1967; Skaer & Lane, 1974). Usually they are not found between unmodified intraganglionic glial cells, although there are exceptions such as in cockroach optic ganglion (Ribi, 1977), dipteran rhabdomeres (Chi, Carlson & Ste Marie, 1979, and Fig. 28) and locust small peripheral nerves or motor nerve terminals (Odhiambo, 1970; Reinecke, 1979). Under certain conditions they have also been found as plaque-like structures in the neuropile (Smith, 1967; Sohol & Sharma, 1973; Lane & Skaer, 1980; Hall *et al.* 1980). Here their precise distribution is not clear and their morphological features are in some respects similar

to, but not identical with, those of pleated septate junctions proper (see Lane & Skaer, 1980). The latter exhibit definite cross-striations or septa in transversely cut thin-sections (insert in Fig. 16), unstained undulating ribbon-like structures in tracer-incubated tangential thin-sections (see Fig. 28), and undulating rows of separated 8 nm intramembranous particles in freeze-fracture replicas (see Fig. 27) (Noirot-Timothée & Noirot, 1980; Lane & Skaer, 1980). These have been considered to form adhesive devices between the outer glial cells of the perineurium, thereby to maintain structural integrity. However, some investigators have also considered them to be occluding, and hence to form the structural basis of the blood–brain barrier. This seems unlikely given their penetration by tracers (see insert, Fig. 16 and Fig. 28), the presence of tight junctional seals in the perineurium and, in addition, the complete absence of perineurial septate junctions in the CNS of certain lepidoptera which have an unequivocal blood–brain barrier (Lane & Swales, 1979; Tolbert & Hildebrand, 1981). Structures considered to be a variant of smooth septate junctions have been found between the marginal glial cells in the first optic neuropile of fly eyes (Chi & Carlson, 1980*b*).

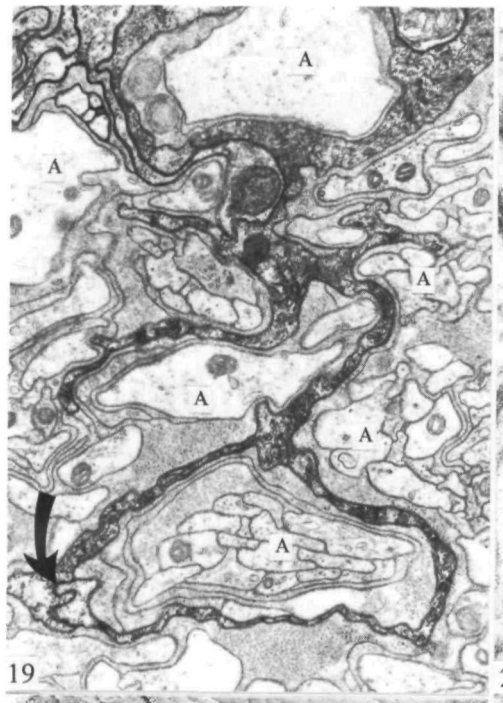
(*c*) *Gap junctions.* Glial cells in nearly all the invertebrate ganglia studied thus far are found to be associated by gap junctions which frequently are present in striking numbers between adjacent glial folds (Fig. 17). They exhibit the typical reduced 2–3 nm intercellular cleft in cross section, and in tangential section, ***en face*** views of the component particles which comprise the junctions are apparent (Fig. 17). These particles may display a stained central channel (Fig. 17) through which ions and molecules are thought to be exchanged when the glial cells thus associated are coupled, thereby functioning as an integrated, communicating unit. In freeze-fracture these junctions are demonstrable as plaques of 13 nm Eface intramembranous particles (Fig. 18); normally the particles are aggregated, but may be found in varying stages of clustering, especially in embryonic tissues even within a single gap junction (Fig. 18). Closely packed junctions may represent ones in the uncoupled state, as suggested for other tissues (Peracchia, 1977; 1978; 1980; Raviola, Goodenough & Raviola, 1980), although frequently those in invertebrate tissues are more loosely packed (Lane & Skaer, 1980) than the hexagonally arrayed nexuses between vertebrate cells.

Fig. 19. Glial cell infiltrated intracellularly with lanthanum to produce enhanced cytoplasmic electron opacity. Note how the cellular distribution can be charted by this density, revealing the complexity of its ramifications around axons (A) and the way it terminates on another of its own processes (at thick arrow). Centipede, *Lithobius* sp., ventral nerve cord. × 16500.

Fig. 20. Inner glial cells (G) encompassing axons (A) in the sub-perineurial region, exhibiting both glial–glial gap junctions (GJ) and tight junctions (arrows). Spider ganglion. × 78000.

Fig. 21. Replica of a freeze-cleaved preparation of outer glial, or perineurial cells showing the complex *zonulae occludentes* present as a network of Pface (PF) ridges and Eface (EF) grooves. EF gap junctional plaques (GJ) co-exist with these tight junctions, Ridges and grooves are seen to be complementary (at arrows) and offset with respect to one another. House spider (*Tegeneria*) ganglion. × 21500. Insert shows a replica of the complex tight junctions from insect compound eye with PF ridges and EFgrooves which are coincident across face transitions (arrows). Blowfly optic retina and lamina. × 38000.

Fig. 22. Glial processes (G) surrounding a nerve cell body (NCB). The glial membranes exhibit gap junctions (GJ) and scalariform junctions (ScJ); beside the latter lie closely-apposed cisternae of smooth ER (SER). The innermost glial membrane at some junctures shows close appositions with the neurolemma which may be a form of neuroglial junction (curved arrow). Locust ganglion. × 41000.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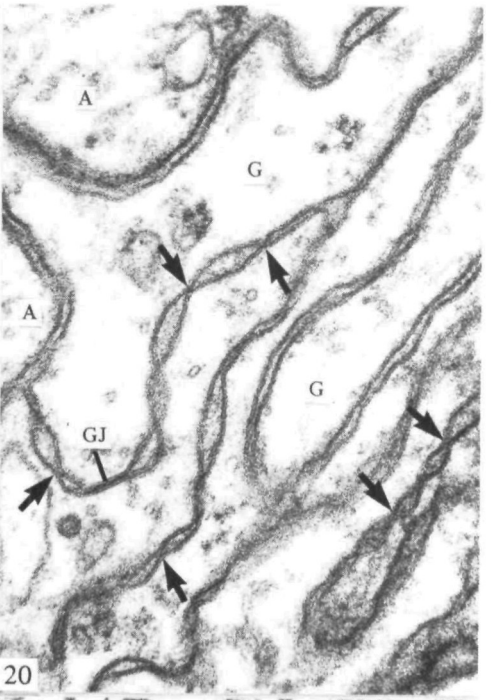
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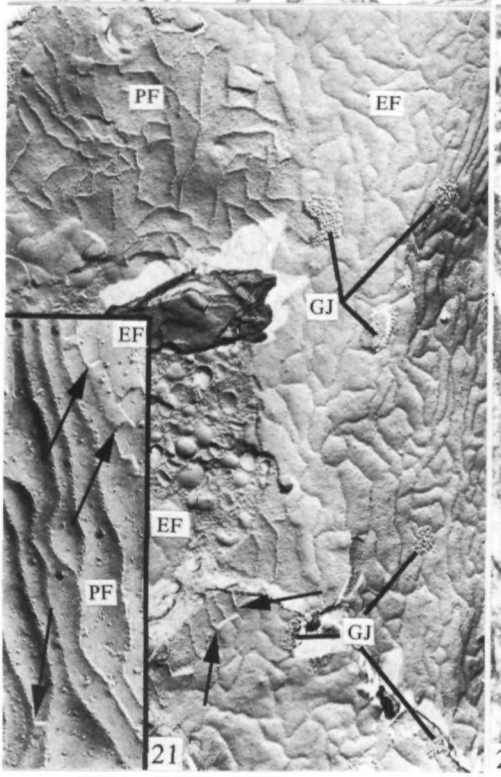
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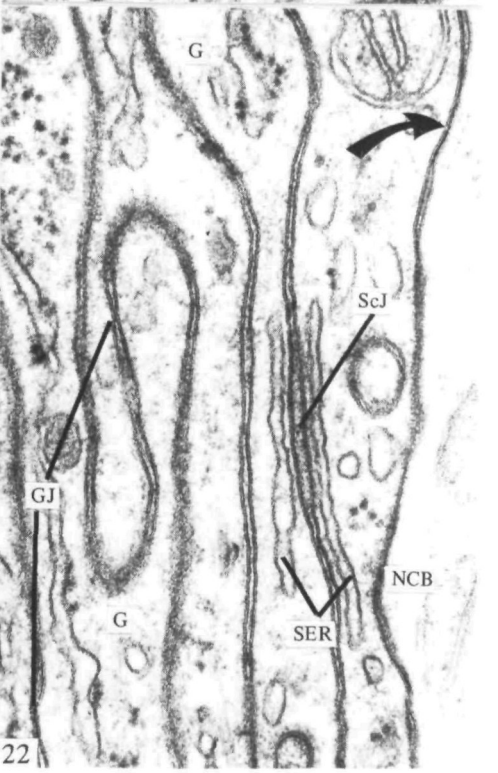
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Recent experiments involving the injection of individual arthropod glial cells with tracers such as horseradish peroxidase (HRP) have made it possible to visualize the injected, and electrically-characterized cell (Swales, Schofield & Treherne, 1981, quoted in Treherne & Schofield, 1981). This high molecular weight tracer (40000 mol.wt.) will not move through the channels within the gap junctional particles, from one glial cell to another, because it is too large a molecule to pass through the restricted diameter of the gap junctional pores (Loewenstein, 1977; Goodenough, Dick & Lyons, 1980). In addition, in some cases, seemingly when the cell membrane is damaged, tracers may enter the glial cytoplasm artefactually (Lane & Treherne, 1969, 1972*a*) and such phenomena may yield information about the spatial distribution of an individual glial cell (Fig. 19). Similarly, when tannic acid is used as a stain, different glial cells may react with different intensities (Fig. 12, insert) and, since they are distinguishable from one another, their separate distributions may be determined. Thus far, attempts to inject electron opaque low molecular weight tracers into neuroglial cells which would then be transported from glial cell to glial cell via the gap junctional channels, have proved technically difficult.

(*d*) *Tight junctions. Zonulae occludentes* occur between both some inner glial cells and the outer modified glial cells of the perineurium in arthropods. There has also been a suggestion that they may exist between glial cells in opisthobranch molluscs where PF particle ridges have been found on glial membranes (Kaczmarek *et al.* 1979; Hall *et al.* 1980). Most reports on other invertebrate groups, however, remark upon the absence of occluding tight junctions in the CNS (see refs. in Lane, 1978; Lane & Skaer, 1980; Lane, 1981*c*).

In the arthropods, tight junctions are most prevalent between the perineurial glial cells; those between the more typical inner glial cells are conventional in thin-section (Fig. 20) but in freeze-fracture replicas are abbreviated and appear to be macular or fasciar, not zonular (Lane & Skaer, 1980; Lane, 1981*c*). The perineurial tight junctions as observed in thin-sections, are all similar, in that they appear to be punctate appositions between adjacent glial membranes (as in Fig. 20). In freeze-cleaved replicas, however, they appear to be either simple or complex in 'strand' morphology (Lane, 1981*c*). The former are found between the outer perineurial cells of insects (Lane *et al.* 1977*a*), and the latter between both certain glial cells wrapping the retinular axons in dipteran compound eyes (Lane, 1981*a*; Fig. 21, insert) and between the perineurial cells of spiders (Lane & Chandler, 1980) and scorpions (Lane, Harrison & Bowerman, 1981). The complex variety, as their name implies, are the more intricate in terms of the number and structural complexity of their composite intramembranous ridges; these are composed of aligned particles which may be fused with one another laterally into ridges or strands (Fig. 21). They form an obvious circumferential tight junctional 'belt' and the component reticulum of Pface (more rarely, E face) ridges are coincident across face transitions with the EF grooves (arrows in Fig. 21) with which they are complementary. These tight junctional networks form seals and therefore maintain permeability gradients; such occlusions provide the morphological basis of the blood–brain barriers observed in arthropods (Lane *et al.* 1977*a*; Lane, 1978, 1981*c*; Lane & Skaer, 1980). The neuroglial cells of the other invertebrate phyla, with the possible exception of some molluscs (Reinecke, 1976; Kaczmarek *et al.* 1979; Hall *et al.* 1980; Abbott, Bundgaard & Cserr, 1981), apparently lack tight junctions

and display no blood–brain diffusion barriers (for example, Pentreath & Cottrell, 1970; Lane & Treherne, 1972*b*; Mirolli & Gorman, 1973).

(*e*) *Scalariform junctions*. These junctions, first described in transporting epithelia of insects (Fain-Maurel & Cassier, 1972) have also been found between glial cells in arthropod ganglia (Fig. 22) mainly those of insects (Lane, 1968*a*, 1974; Lane & Treherne, 1970, 1980; Lane & Skaer, 1980). They exhibit intercellular columns or striations, not septa, but have no characteristic freeze-fracture profile. Their physiological significance is not yet clear, although the presence of subsurface cisternae alongside them suggest that they may be involved in molecular exchange or the ion pumping associated with glial-mediated homeostasis.

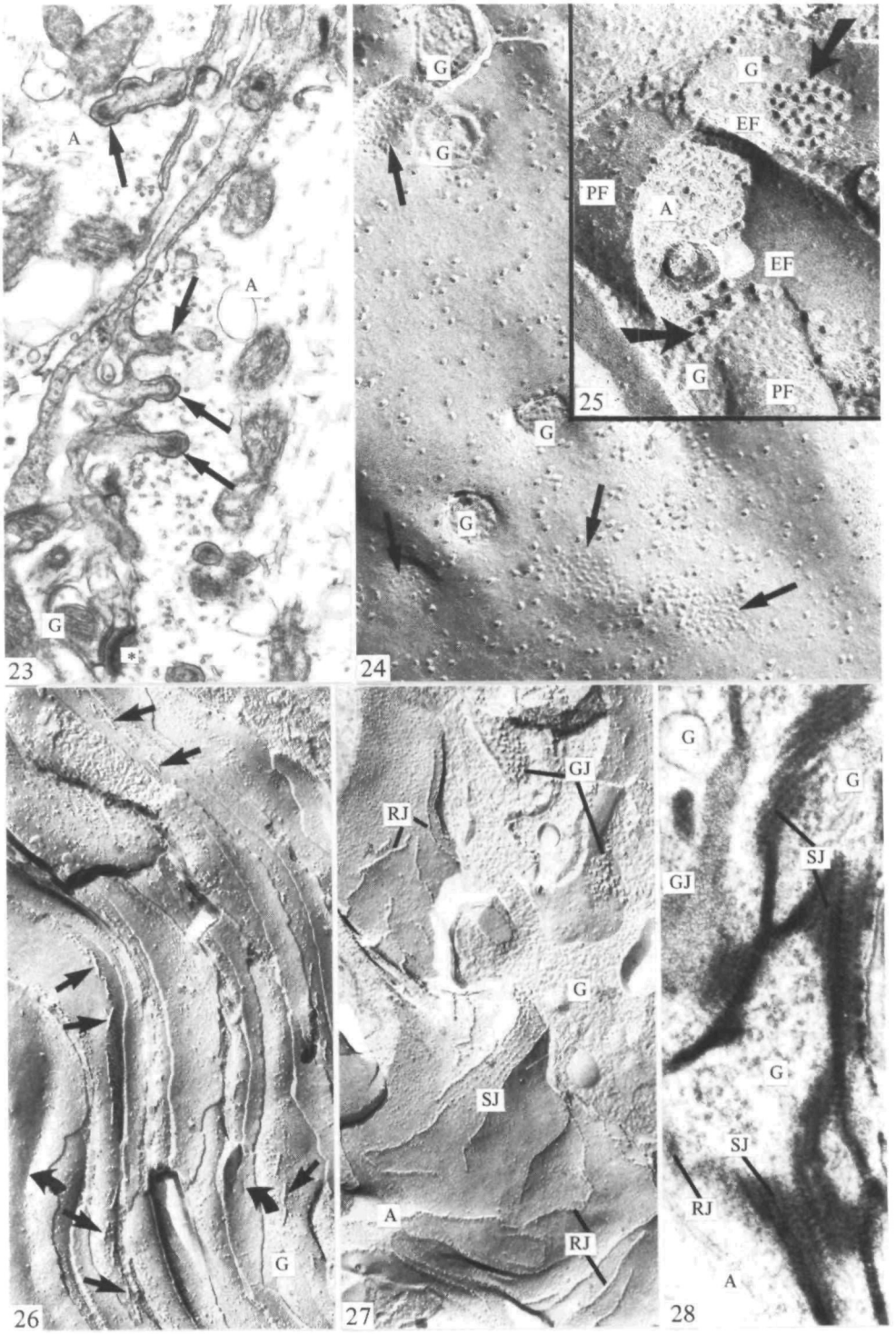
(2) *Co-existence between junctional types*

The apposing lateral borders of glial cells may possess a variety of different junctional types. These primarily include desmosomes, septate junctions, gap junctions, and tight junctions, any of which, particularly the last two, may co-exist side by side (Figs. 20–22) (see Lane & Skaer, 1980). This co-existence suggests a distinct role for each of the junctions, since it is unlikely that two structures with identical functions would have evolved. This poses a problem in cell regulation and co-ordination during the development of these co-existing junctions since different junctional protein particles must be inserted into the gial cell membrane for each junctional type. Little research has gone into problems of this sort except in the case of the outer glial cells in arthropod nervous systems. In arachnids, co-existing gap and tight junctional particles in the outer perineurial membranes can be distinguished in development during their concomitant translateral intramembranous migration due to differences in IMP size and fracturing characteristics (see Fig. 29). This sequence of intramembranous events can therefore be characterized (Lane, 1980, 1981*b*) although the mechanisms and initiating events are as yet obscure.

(B) *Heterocellular axo-glial junctions*

(1) *Trophospongia, subsurface cisternae and axo-glial close appositions*

The trophospongial glial processes that project into large nerve cell bodies (see Fig. 12 and section III(A) are often associated with cisternae of endoplasmic reticulum; these lie immediately beneath the neural plasmalemma with a smooth surface adjacent to it and a ribosome-studded surface facing the cytoplasm (Smith, 1968). These 'subsurface cisternae' (arrows in Fig. 12) form a neuronal–glial association which may have a trophic role involving the exchange of metabolites from glia to nerve cells (Smith, 1967, 1968). Frequently also, there may be close membrane appositions, often five-layered, between invertebrate glial membranes and those of nerve cell bodies (Fig. 22) or their axons (Peracchia, 1974; Lane & Swales, 1978*a*, *b*; Lane, 1978; Lane & Skaer, 1980; Binnington & Lane, 1980; Harrison & Lane, 1981; Keil, personal communication) and these may have associated subsurface cisternae. These junctional structures, seen frequently also in vertebrate tissues in thin-sections (Johnston & Roots, 1976), may be correlated with particle arrays seen by freeze-fracture (Henkart, Landis & Reese, 1976; see section IV(B)3).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Subsurface cisternae are also found to be associated with insect glial scalariform junctions (Fig. 22; Lane, 1968*a*) and with quintuple-layered junctions between retinular axons and glial cells in the ommatidia of the horseshoe crab, *Limulus* (Lasansky, 1967); these latter junctions, which may actually be gap-like junctions, have also been interpreted as devices to promote metabolic or ionic exchanges between retinular and glial cells, the axonal subsurface cisternae serving the purpose of intracellular transport from glial cells to rhabdom.

Structural complexes involving a narrowing to disappearance of the axon–Schwann cell interspace and an axonal undercoating of dense material have also been observed in squid giant axons (Villegas & Villegas, 1976). These complexes were proposed to be the structural correlate of specialized sites for active ion transport since they decrease on treatment with ouabain. A chemical mechanism has been suggested, rather than electrical coupling, due to the localization of acetylcholinesterase and possibly ATPase in the dense axonal undercoating (Villegas, 1978).

(2) *Capitate projections*

Specialized axo-glial associations also exist in the compound eyes of dipteran flies where certain glial cells send 'capitate' projections into the axons that they surround (Carlson & Chi, 1979; Chi & Carlson, 1980*a*; N. J. Lane, unpublished data). These projections may be quite numerous and show an enhanced density of the glial membranes at the point of contact with the axon as well as regions of electron opacity in the axolemma (Fig. 23). By freeze-fracture, it is clear that there is a neuronal intramembranous modification associated with these protuberances, which can be identified by their cross-fractured appearance, in that plaques of intramembranous particles are to be found in these regions as well as with other axolemmal modifications (Fig. 24). The functional significance of these has not been established, but they are morphologically suggestive of release or receptor sites. What appear to be vesicle-laden

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Fig. 23. Axo-glial contacts in the region of the retinular cells where the glial processes (G) invaginate into the retinular axons (A) as 'capitate projections' (arrows). Both glial and axonal membrane shows enhanced density at these areas, and synaptic vesicle-like structures abound in the axoplasm. * = axo-glial synapse-like structure with electron opacity associated with the axolemma. Blowfly compound eye. × 27300.

Fig. 24. Freeze-fracture of capitate projections showing regions where glial processes (G) have been cleaved across and the particle plaques (arrows) present in the axolemma where other axo-glial modifications occur. Blowfly compound eye. × 67000.

Fig. 25. Freeze-cleaved preparation of axons (A) and glia (G) cells in intimate association forming possible axo-glial junctions between axonal EF and glial PF in the form of clusters of EF particles (arrows) and PF pits. (From Binnington & Lane, 1980.) Tick, *Boophilus microplus*, synganglion. × 104000.

Fig. 26. Pface ridges (arrows) arranged along the longitudinal axis of axons and glia (G) with complementary Eface grooves (curved arrows), forming possible axo-glial junctions which may also be involved in cell migration. Blowfly CNS. × 40400.

Fig. 27. Retinular axons (A) and associated glial cells (G) forming axo-glial junctions of both ridge-like retinular (RJ) and septate (SJ) varieties. GJ, interglial gap junctions. Blowfly compound eye. × 40400.

Fig. 28. Axo-glial retinular (RJ) and septate junctions (SJ) as well as glial-glial septate and gap junctions (GJ) after infiltration with lanthanum. A, axon; G, glial cells. Blowfly compound eye. × 62500.

'synaptic' regions of photoreceptor axons with glial cells occur here too (Fig. 23) (Carlson & Chi, 1979; Lane, unpublished data), although the vesicles may be so small as to resemble microtubules; it is therefore possible that these could be regions where glial cells and axons may activate, or interact in some way with, one another. In drone retina, for example, there is evidence for a movement of K^+ from photoreceptor axons to glial cells during stimulation (Coles & Tsacopoulos, 1979).

(3) *Glial/axonal intramembranous particle clusters and ridges*

In addition to the particle plaques described above in the glial capitate projections (Fig. 24) a variety of other intramembranous particle (IMP) modifications and membrane specializations have been reported between apposing glial and axonal membranes. These may in some cases also be recognizable in thin sections as specialized membranous appositions (Fig. 22; Peracchia, 1974; Lane, 1978). One of the most striking examples is in the crayfish ventral nerve cord, where IMP plaques and complementary arrays of pits have been described (Peracchia, 1974) in the regions where the glial cells form undulations into the axonal periphery. It has been proposed that these could represent sites of axo-glial coupling for metabolic exchange, attachment devices or structures involved in excitation. Comparable suggestions have been put forward for similar IMP plaques found in the axonal and glial membranes of insects (Lane, Skaer & Swales, 1977*a*; Lane & Swales, 1978*a*, *b*) centipedes (Reger & Fitzgerald, 1981) and ticks (Binnington & Lane, 1980) (Fig. 25). In insects, glial-axonal intramembranous particle ridges and grooves (Fig. 26) have also been reported (Lane *et al.* 1975, 1977*a*; Wood *et al.* 1977*b*); these may be implicated in cell 'tracking' or contact guidance between axons and glia during development (Lane, 1979*a*). Particulate ridges on both glial and axonal surfaces have also been reported in the gastropod *Aplysia* (Kaczmarek *et al.* 1979) although they have not been termed axo-glial ridges.

(4) *Axo-glial desmosomes, retinular and septate junctions*

Structures very like homocellular desmosomes or maculae adhaerentes have been found in a few cases between neurones and glial cells. Examples occur in the leech ganglion (Coggeshall & Fawcett, 1964) and in the earthworm, *Lumbricus terrestris*. In the latter, desmosomal densities have been found to occur between the axolemma of the giant fibres and the initial glial membrane to form distinct axo-glial contacts (insert, Fig. 13) (Coggeshall, 1965; Günther, 1976; Roots & Lane, 1981*a*). Here the axonal filaments may be seen to insert into the axolemma at the point of contact; rather similar axonal modifications have been observed in the giant axons of the estuarine sabellid, *Mercierella* (Skaer *et al.* 1978) but as no comparable glial structures occur, they are thought to be hemi-desmosomal axonal structures, and not axo-glial ones.

The retinular axons of the photoreceptor cells in compound eyes of dipteran flies are associated by retinular or R junctions to each other and to the encompassing glial cells (Chi *et al.* 1979; Lane, 1979*b*; Lane & Skaer, 1980; Chi & Carlson, 1981). These are modifications which consist of a constant intercellular cleft of 15–20 nm across

which appear to exist columns or striations. After freeze-fracture it is apparent that they contain intramembranous ridges and grooves (Fig. 27) arranged at arbitrary angles to one another. These junctions, near the point of entry of the rhabdomeres into the basal lamina area of the retina, become transformed into septate junctions which are typical of the pleated variety as judged by both freeze-fracture (Fig. 27) and thin-section (Fig. 28) criteria. The adjacent glial cells are also joined by septate and gap junctions (Figs. 27 and 28). The functions of such axo-glial junctions is as yet uncertain, but it has been suggested that the retinular junctions may serve to maintain the local concentration gradients in the intercellular clefts (Chi *et al.* 1979). They may also have a cohesive role (Chi *et al.* 1979) but this seems more likely to be served by the axo-glial septate junctions which develop at the rhabdomere base (as in Figs. 27 and 28), since here the axons begin to insert into a different glial layer and a number of complicated structural modifications occur. Septate-like junctions between axons and Schwann cells occur in the paranodal region of vertebrate myelinated axons (Schnapp & Mugniani, 1975; Wiley & Ellisman, 1980) and these have also been considered to play some role in adhesion as well as possibly being implicated in the restriction of permeability or ion exchange.

V. DEVELOPMENT OF GLIAL CELL JUNCTIONS

(A) *Embryology*

(1) *Insect embryos*

The outer glial or perineurial cells in the developing CNS of the locust, *Schistocerca*, have been studied throughout embryonic life until hatching (Swales, Lane & Schofield, 1981). The glial cells and axonal processes are indecipherable in early stages, but subsequently become distinguishable as the inner glia grow more electron opaque and come to ensheath the axons. The outer glia that form part of the perineurial sheath do so by migrating to an outer region where a lengthy wisp of matrix material has been laid down around the presumptive ganglion. Larger perineurial cells form extensive overlaps with one another in the manner described as 'bracelet cell' arrays (McLaughlin, 1974; Swales *et al.* 1981; Lane, 1981*c*) and ultimately tracers are excluded, seemingly when the tight junctional ridges develop between the last region of bracelet cell overlap. In other insects, tight junctional formation between glial cells may take place more extensively and the precursor IMPs and short junctional ridges in the process of assembling are found scattered over much of the basal perineurial Pface (Lane & Swales, 1978*a*, 1979). Septate junctions and gap junctions also co-exist with tight junctions along the perineurial borders of the locust and the blowfly, *Calliphora*, (Lane & Swales, 1978*a*), but no septate associations occur in the perineurium of the *Manduca* embryo (Lane & Swales, 1979).

The differentiation of septate junctions has been studied in the developing CNS of both the locust (Lane & Swales, 1981; Lane, 1981*d*) and the blowfly (Lane & Swales, 1978*a*) and the stages in their development are very similar to those described in a regenerating system, that of *Hydra* (Wood & Kuda, 1980) where junctional reassembly has been investigated. The junctional particles appear to become inserted into the glial membranes and arrayed in linear alignments over the presumptive junctional

areas; these in turn become arranged in parallel rows to form the mature structur that is characterized by stacks of these particle rows, lying on the Pface, with complementary rows of EF pits in the glial membranes (Skaer & Lane, 1974; Lane *et al.* 1977*a*). The intercellular septa insert into the membranes, possibly in association with the junctional particles. These clearly act to maintain the structural integrity of the system and may also aid in inhibiting the free inward diffusion of certain molecules (Wood & Kuda, 1980; Lane & Skaer, 1980; Noirot Timothée & Noirot, 1980).

The gap junctions that characterize the lateral borders of the outer perineurial layer as well as the associated membranes of inner glial cells have been studied during formation in embryonic life in such insects as the blowfly, *Calliphora* (Lane & Swales, 1978*a*), the moth *Manduca* (Lane & Swales, 1979) and the locust (Swales *et al.* 1981). In all cases the 13 nm gap junctional particles which are at first scattered at random over the Eface of the glial membranes, undergo translateral migration into linear arrays, then into irregular, loose clusters and finally into more closely-packed aggregates (Fig. 18). As mentioned earlier, these are believed to be the basis of coupling between adjacent glial cells and hence may be the sites of exchange of ions, informational molecules or metabolites. Electrophysiological evidence for low resistance coupling is available for the giant packet glial cells in the leech CNS (Kuffler & Potter, 1964) but not as yet for those of arthropods, where the glial cytoplasm is much more attenuated and difficult to impale with electrodes.

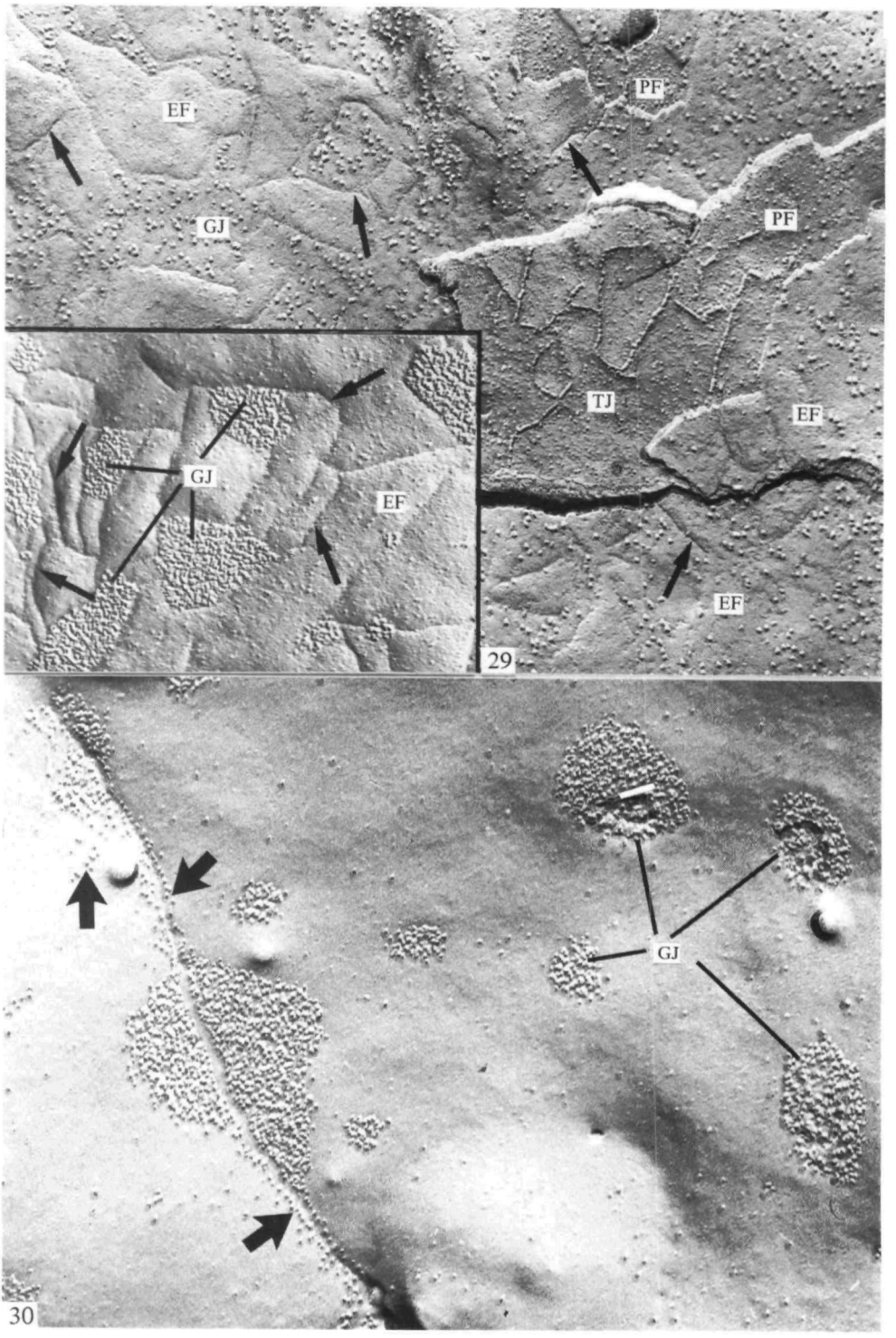
One other feature of the glial cells in embryonic invertebrate ganglia is that they appear to synthesize collagen precursors, since mature, polymerizing collagen can sometimes be seen emanating from glial membranes (Fig. 16) (Ashhurst, 1968; Lane, 1972). Whether or not this occurs in precisely the same way as in vertebrates is not clear but the synthesized collagen appears to be biochemically like that of the vertebrate protein (Ashhurst & Bailey, 1980).

(2) *Arachnid embryos and hatchlings*

The glial cells in the CNS of arachnids may also be followed during the period when they become associated with one another to produce both the outer perineurial layer around the nervous system and the ensheathment of the inner glial cells. The final stages of these processes in spiders occur just after hatching and relate to the assembly of the intercellular junctions by which these glial cells become attached to one another (Lane, 1980, 1981*b*).

Both outer and inner glial cells are coupled by gap junctions and, at least in the spiders and scorpions, the outer glial or perineurial cells are also sealed together by tight junctions; in the arachnid systems thus far studied, including the horseshoe crab (Harrison & Lane, 1981), the tick (Binnington & Lane, 1980), scorpions (Lane *et al.* 1981) and the spider (Lane & Chandler, 1980), no septate junctions are to be found between the glial cells in this position. This contrasts with the perineurium of most insects (Lane & Skaer, 1980) but is also true of some crustacean perineurial cells (Lane & Abbott, 1975) and those of certain moths (Lane & Swales, 1979, 1980).

The interglial tight junctions appear to begin development just before the gap junctions, but both types tend to differentiate concurrently (Lane, 1980, 1981*b*, *c*). Since they co-exist on the same membrane faces, investigations of either junctional type tend to reveal stages in the assembly of both (Fig. 29). The development of thes


EF
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particular structures can be clearly distinguished as involving two separate categories of intramembranous particle (IMP), since each of these junctional types involves IMPs of different sizes and fracturing characteristics (Fig. 29). In both cases, junctional precursor particles migrate translaterally within the presumptive junction-bearing membrane, and from free particles assemble into short linear arrays; these, which in the case of tight junctions, are PF and 8–10 nm in diameter, become extended into lengthy ridges which may fuse together laterally (as in Fig. 21). The precursor IMPs for gap junctions are EF and 13 nm in diameter (Fig. 29) and become aggregated into loose clusters, then more closely packed maculae (Fig. 29, insert; Fig. 18). Clearly these junctions do not arise from the same precursor particles, as could be the case for vertebrate tissues (Decker, 1976) where both junctions possess 8–10 nm PF particles. The processes involved in these events in arachnid tissues exhibit certain differences from the stages that occur in similar situations in vertebrates (see Lane, 1981*b*, *c*) which include such features as larger 'precursor' particles, special formation plaque areas and the addition to the final tight junctional ridges of 'coating' material (Montesano *et al.* 1975; Elias & Friend, 1976; Porvaznik, Johnson & Sheridan, 1979). When the junctions in spiders are fully formed, the tight junctions perform an occluding role to produce circumferential glial permeability barriers (Lane & Chandler, 1980), while the gap junctions are thought to permit the exchange of ions and small molecular weight substances. These junctions appear to be maintained throughout the various instars, as the organisms are heterometabolous. In holometabolous insects, they only remain through larval life, for, with the onset of metamorphosis, the glial junctions undergo disruption followed by reassembly (Lane & Swales, 1978*b*, 1980).

(C) *Insect metamorphosis – junctional disruption and reassembly*

With the advent of pupation, the glial cells in the insect larval CNS rapidly undergo disassociation and the elements separate, to become redistributed ultimately into the CNS characteristic of the adult (Lane & Swales, 1978*b*, 1980). This is true both for the outer perineurial and the inner glial cells and appears to be stimulated by the hormonal changes associated with the initiation of metamorphosis (Lane & Swales, 1978*b*, 1980); with the disruption of the perineural tight junctions, the blood–brain barrier breaks down and tracers are able to penetrate into the CNS. The suspected presence of adenyl cyclase in the perineurial bracelet cell membranes (Lane & Swales, unpublished data) makes it possible that cAMP acts as a second messenger in these cells to trigger off a reaction which leads to changes in distribution of the intramembranous junctional particles, thereby uncoupling and unsealing the glial cells

Fig. 29. Replica from perineurium during development, showing stages in the assembly of tight junctions (TJ) and gap junctions (GJ) wherein the latter are present as loose 13 nm Eface (EF) particles, in the process of aggregating to form mature plaques (GJ in the insert). The tight junctional PF ridges gradually fuse together to form a network of PF ridges or EF grooves (arrows). Spider ganglion. × 59200. Insert, × 42000.

Fig. 30. Freeze-cleaved replica from glial cells in a ganglion that has just entered metamorphosis. The macular EF gap junctional plaques (GJ) characteristic of the larval tissues, become dispersed in a gradual process which initially involves the gap junctional particles streaming out from the formed maculae (at large arrows). Moth, *Manduca sexta*, ganglion; first day of pupation. × 50000.

(Lane & Swales, 1980). Although it is initially surprising that the cells should utilize cAMP, given the steroid nature of the ecdysone thought to be implicated in the onset of pupation (Nijhout & Williams, 1974), it is entirely possible that there is a second hormone, possibly a peptide, activated itself by ecdysone, which has the effect of stimulating or enhancing the glial adenyl cyclase.

These changes in glial cells during pupation involve redistribution of the particles that comprise the gap junctions, which lose their macular, larval configurations (as in Figs. 18 and insert in 29) and become dispersed over the glial membrane faces (Fig. 30). Towards the end of insect metamorphosis, it has been established both in moths (Lane & Swales, 1980) and flies (Lane & Swales, 1978*b*), that when the nervous system has become reorganized into the adult configuration, the glial junctions reassemble by translateral migration of intramembranous junctional particles; this process is not dissimilar to that observed in vertebrate glial junctions (Decker, 1976). These IMPs may be in some part those which originally made up the larval junctions, perhaps being reutilized to form the adult ones (Lane & Swales, 1978*b*, 1980); this economical policy might be a modification of the hemi-conservative junctional assembly process (Dermietzel *et al.* 1977), described for vertebrates, wherein remnants of tight junctions from the separated cells are reused in the assembly of the new adult junctional elements (see also Polak-Charcon & Ben-Shaul, 1979).

By the time of emergence of the adult insect from pupation, the new mature glial–glial and axo-glial associations have been established, and the junctions so formed seem capable of carrying out their various roles in an effective way. Thus far no information is available on the initiation or mode of assembly of the axo–glial junctions.

VI. PHYSIOLOGICAL SIGNIFICANCE OF GLIAL MODIFICATIONS

The specializations of the glial cells with which we have been chiefly concerned are the homocellular interglial junctions and the heterocellular axo–glial associations. The latter clearly could fulfill a number of roles, including mechanical adhesion or, more interesting physiologically, coupling via intramembranous channels. It is possible that trophic molecules are sent to axons from the glial cells, and if these molecules were sufficiently small, they could move via channels in intramembranous particles such as occurs via the gap junctional particle pores (Loewenstein, Kanno & Socolar, 1978). Crayfish axo–glial particle clusters (Perachhia, 1974) provide an example for which this is a possibility, but in other systems, such as the leech, there is no electrical evidence for glia–neuronal coupling (Kuffler & Potter, 1964). Other mechanisms for exchange would include exocytosis from glial cells and pinocytosis by nerve cells, or transport of trophic substances directly across both plasma membranes (Johnston & Roots, 1976; Lasek *et al.* 1977).

Intramembranous particle assemblies (Landis & Reese, 1974) also occur in glial membranes adjacent to neurones and to cerebrospinal fluid (CSF) and blood compartments in vertebrate tissues and are thought to be involved in an active exchange of ions or metabolites between the CSF and haemolymph (Landis & Reese, 1981; Cullen & Gulley, 1980). Similar assemblies have been reported in invertebrate nerve cells (Gemne, 1969; Quick & Johnson, 1977; Lane, 1979*a*) but their function is as

let obscure although it has been suggested that they too could permit metabolic exchange between cells and environment.

In addition to these intramembranous gap junctional-like axonal and axo-glial particle clusters there are also the extensive ridges shared between axons and glia to be considered (Lane *et al.* 1977*a*; Lane & Swales, 1978*a*, *b*; Lane & Skaer, 1980). These could act in axonal guidance, particularly during embryonic and pupal development (Lane, 1979*a*), since they are always aligned parallel to the longitudinal axis of the axonal processes and hence to the direction of cell migration.

In the case of the subsurface cisternal structures, the proximity of the glial membranes to the neurolemma and underlying neuronal cisternae of endoplasmic reticulum (as in Fig. 12), suggests the possibility of transport of trophic substances via these modifications in some way. Since the subsurface cisternae are often associated with the glial trophospongia, exchange is expected to be between the glial elements and the nerve cell body. Use of the mechanism of exocytosis from, or endocytosis into, the glial cells seems a possibility as glial membranes possess many coated pits and omega profiles, some of which can be seen to actively engulf tracer substances (Fig. 7), while typical freeze-fracture images of vesicle release or uptake are also common (insert, Fig. 14). However, the mode of neuronal uptake of any material that is released by the glia into the extracellular space is far from clear. In some cases, as with the glial capitate projections into the photoreceptor axonal substance (as in Figs. 23 and 24), the glia, which are known to take up labelled neurotransmitters, are thought to be delivering some substance, possibly GABA, which is the neurotransmitter for the retinular axons (Campos-Ortega, 1974), to the axons. Here intramembranous particle clusters again appear at the site of intimate physical contact (Fig. 24) (Carlson & Chi, 1979), and although they could merely be receptor loci, they might represent the actual site of exchange. Interestingly, in rat embryonic neocortex, the glial cells, arranged in ephemeral axo-glial synapses, are thought to accumulate and release GABA, hence playing a part in the early stages of synaptogenesis (Wolff, Rickmanor & Chronwall, 1979). In both the vertebrate and insect tissues the axons in these 'synaptic' areas contain many vesicles which have the appearance of synaptic vesicles. In the rat, the glial cells may transitorily promote synaptogenesis, but in insects, they are likely to be more permanent structures and it may be that the axons might also give information to the glial cells by conventional vesicle fusion and transmitter release.

The axo-glial desmosomes, retinular junctions and septate junctions, seem unlikely, due to their relatively wide 15–20 nm intercellular space, to be involved in anything other than maintaining spatial associations and structural integrity between the two cell types. However, the possibility of such junctions contributing to a restriction in permeability has also been proposed for the latter two (Chi *et al.* 1979). Axo-glial septate-like associations have been reported in vertebrate Schwann cells (Schnapp & Mugniani, 1975) where they may also restrict intercellular permeability and/or act as adhesive devices. During development, the Schwann cells have the capacity to recognize the nodal sites in axons where sodium exchange may occur (Wiley & Ellisman, 1979, 1980), but comparable events have not yet been demonstrated for invertebrate glial cells.

The homocellular glia-to-glia cell interactions are, as we have seen, of many types. Clearly the desmosomes function primarily as adhesive devices. The tight junctional elements between glial cells serve to produce a seal which is highly effective if the junctions are circumferential in distribution (see Lane & Chandler, 1980; Lane, 1981*c*) and, if fasciar, are rather more focal in restricting permeability. The formation of these junctions between the outer glial cells ensheathing the CNS in arthropods, in embryonic life (Lane & Swales, 1978*a*, 1979) and towards the end of pupation (Lane & Swales, 1978*b*), can be correlated with the restriction of the inward diffusion of tracers as well as with the advent of, in embryos, excitability and a functional CNS (Goodman & Spitzer, 1980; Swales, Lane & Schofield, 1981). Their breakdown in early insect metamorphosis can be correlated with the inward leakage of tracers and the opening and disassembly of glial contacts prior to CNS reorganization and reformation of the blood–brain barrier (Lane & Swales, 1978*b*).

The septate and scarlariform junctions have a more equivocal role. The former certainly aid in maintaining the structural integrity of outer glial sheaths, but they may also be involved in regulating the interglial diffusion of ions and molecules. Some tracer studies (for example, see Fig. 16, insert) indicate that the septate junctions do not prevent the entry of substances, since ionic lanthanum is found to permeate along the junctional path. However, other reports consider that these junctions may be important in creating permeability barriers (Noirot-Timothée & Noirot, 1980; Thurm & Küppers, 1980); if both septate and tight junctions are present however, the latter may be the occluding structures but they would not always, until very recently, have been recognized to be present. Hence their role would have been unappreciated.

The scalariform junctions present an even more elusive problem; when found between other cell types (Berridge & Gupta, 1967; Lane, 1979*c*) they appear to be involved in ion transport. Their presence between glia, especially between attenuated glial processes near nerve cell bodies (see Fig. 22) suggests some role in ion exchange or pumping as related to the extracellular environment.

The extracellular space surrounding the glial cells, containing mucopolysaccharide produced by the glia, is physiologically significant (Abbott & Treherne, 1977) in that it may have a filtering or buffering effect. Glia in different systems are surrounded by either essentially negligible space or by dilatations which vary in size and density and so the glia to some extent determine the amount and kind of extracellular matrix present. The matrix also contributes indirectly to any permeability barriers as does the restricted extracellular space that the glial cells maintain (Abbott *et al.* 1977). These matrix-filled spaces may be relatively electron lucent (as in Figs. 3, 11 and 12) or moderately dense (as in Fig. 10). When electron dense, it may be homogeneous (as in Fig. 10) or full of fibrils of collagen-like nature (as in Figs. 1, 8 and 16). In certain cases the matrix has been shown to contain hyaluronic acid (Ashhurst & Costin, 1971) which suggests that it could serve as a cation reservoir for the glial cells (see Treherne & Schofield, 1981; Abbott *et al.* 1977; Abbott & Treherne, 1977; Abbott, 1979). X-ray microanalysis of frozen hydrated sections of leech glial cells indicate that the extracellular space contains Na^+ and Cl^-, while the neuroglia themselves have high levels of phosphate and Cl^- (Sauberman & Riley, 1980). If the extracellular matrix were to bind ions it could be of enormous physiological importance in maintaining excitability. In addition, Kuffler (1967) has proposed that the concentration in th

lefts of K^+, released by the nerve cells, could determine the level of glial metabolism, thereby providing a signalling mechanism between the two cell types.

The gap junctions which occur with striking frequency between glial cells (see Figs. 6, 10, 17 and 18) are considered in many systems to represent the sites of coupling between cells. By this, ions and small molecules may be exchanged via the intraparticulate pores (Fig. 17) that occur in the intramembranous gap junctional particles (Fig. 18). The molecules thus exchanged, or transported, one way or another, could be regulatory, to ensure concerted action in synchrony by the glial cells, although there is, alas, no evidence as yet for this; the substances exchanged could also be physiologically important ions.

It is thus clear that the precise functional significance of many of the structural modifications of invertebrate glial cells are not yet clear, but can only be the subject of speculation. Further refinements of available techniques such as microinjection into glial cells of electron opaque, low-molecular-weight compounds, improved resolution with elemental microanalysis of frozen hydrated sections of the CNS, rapid freezing of glial tissue without fixation or cryoprotection, and isolation and purification of glial elements together with specific glial antibody production, may ultimately lead to our greater understanding of some of the current enigmas.

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