J. exp. Biol. 132, 59–78 (1987)
Printed in Great Britain © The Company of Biologists Limited 1987

MECHANISMS OF GLIAL REGENERATION IN AN INSECT CENTRAL NERVOUS SYSTEM

BY P. J. S. SMITH, E. A. HOWES AND J. E. TREHERNE

AFRC Unit of Insect Neurophysiology and Pharmacology, Department of Zoology, Downing Street, Cambridge CB2 3EJ, UK

SUMMARY

As in other repairing systems, glial regeneration in insect central nervous connectives, following selective chemical lesioning, involves both exogenous and endogenous elements. Our current evidence, including that obtained with monoclonal antibodies, indicates that the reactive, granule-containing cells are derived from a sub-population of circulating haemocytes which, within 24 h, invade, and are restricted to, the lesion zone. The granule-containing cells are involved in the initial repair of the perineurial region. They also contribute to the first stage in the restoration of the blood–brain barrier and are responsible for recruiting reactive endogenous glia, apparently from the vicinity of the anterior abdominal ganglion. The granule-containing cells transform into or are replaced by functional glia between 3 and 5 days after selective glial disruption, coincident with the appearance in the lesion zone of dividing reactive cells.

INTRODUCTION

A major problem in the study of the mechanisms of recovery from neural damage is the complexity of the ensuing cellular reactions and interactions. These involve a variety of cell types and it is frequently difficult to recognize their relative contributions. Recent research has focused not only on the regeneration of the nerve cells, but also on the role of glia and exogenous cells. Glial cells have, for example, been shown to play a critical role in directing axonal growth in central and peripheral neural grafts (Aguayo, David & Bray, 1981) possibly through their production of extracellular matrix materials and secretory products which appear to be an essential feature of axonal development and regeneration following nerve injury (Bunge & Bunge, 1983; Bunge, 1987). Several studies have also implicated exogenous cells in neural repair, notably the blood monocytes which invade the lesion zone and have the capability of transforming into brain macrophages (Adrian & Schelper, 1981; du Bois, Bowman & Goldstein, 1985). Furthermore, it is also known that blood cells, principally T-lymphocytes, can release glial growth factors (Fontana, Grieder, Arrenbrecht & Grob, 1980; Benveniste *et al.* 1985) and could, conceivably, play an important role in glial regeneration.

Much of the accelerating progress in this field has been derived from *in vitro* studies using cultured neural cells. It is, however, still difficult to relate the findings

Key words: glia, neural repair, CNS, insect.

from such studies to those obtained from *in vivo* investigations, often involving gross lesioning, in the complexity of the vertebrate brain.

In our recent studies, we have used an invertebrate preparation, the abdominal connectives of the cockroach (*Periplaneta americana* L.), to investigate some of the basic features of neural repair. Our preparation is relatively simple and well-characterized, consisting only of axons and two basic glial types with their associated extracellular matrices (Fig. 1A). The perineurial glia form a barrier round the central nervous system, excluding small water-soluble cations, such as potassium, from the axonal surfaces (Fig. 1B: Schofield, Swales & Treherne, 1984; Schofield & Treherne, 1984). The experimental situation is further simplified by the technique of selective glial disruption (Smith, Leech & Treherne, 1984). This results in swift and ordered repair, which contrasts with protracted and complex cellular responses observed following surgical lesioning of the connectives (Treherne, Harrison, Treherne & Lane, 1984). Encouragingly, the sequence of glial repair observed under these circumstances bears a number of similarities with the equivalent processes which occur in the vertebrate central nervous system, especially in the role of blood cells and their interactions with reactive endogenous cells.

SELECTIVE GLIAL DISRUPTION

The abdominal connectives used in our experiments contain only glial nuclei, the neuronal soma being confined to the ganglia. Thus, local application of a DNA-intercalating agent, ethidium bromide (Nelson & Tinoco, 1984), to a mid-portion of the connectives affects only glial nuclei. This compound, which has been previously used to demyelinate mammalian nerve cords (Yajima & Suzuki, 1979; Blakemore, 1982) is taken up from low external concentrations by the cells in cockroach nerve cords (Leech, 1984) and causes extensive glial disruption when applied locally to the connectives *in vivo* (Smith *et al.* 1984). This is seen within 24 h by drastic changes in the ultrastructure of the superficial, perineurial glia: notably, a clumping of nuclear chromatin and extensive cytoplasmic damage (Fig. 2A). During this stage there is a breakdown of the perineurial blood–brain barrier as shown by the rapid decline in

Fig. 1. (A) Semi-diagrammatic drawing of a portion of the cockroach abdominal nerve cord, with a transverse section through the connective between the fourth and fifth abdominal ganglia. The surface of the central nervous system is delimited by the acellular neural lamella, which is leaky to small water-soluble cations and molecules. Beneath the neural lamella is a superficial layer of flattened glia, the perineurium, which (due to junctional complexes at the inner margins of the tortuous intercellular clefts) constitutes the blood–brain barrier. The underlying axons are closely wrapped by sub-perineurial glia which delineate a complex three-dimensional network of extracellular channels containing matrix material. (B) Effects of high-potassium saline on relative spike amplitude and d.c. potential from normal, penultimate abdominal connectives measured using sucrose-gap recordings. Due to the perineurial blood–brain barrier there is no appreciable effect on action potential amplitude (●). The change in d.c. potential (○) represents the depolarization of the outwardly directed perineurial membranes, access to the inwardly directed ones being restricted by the perineurial junctional complexes. The vertical lines through the data points indicate the extent of the standard error of the mean ($N = 3$). (From Smith, Leech & Treherne, 1984.)

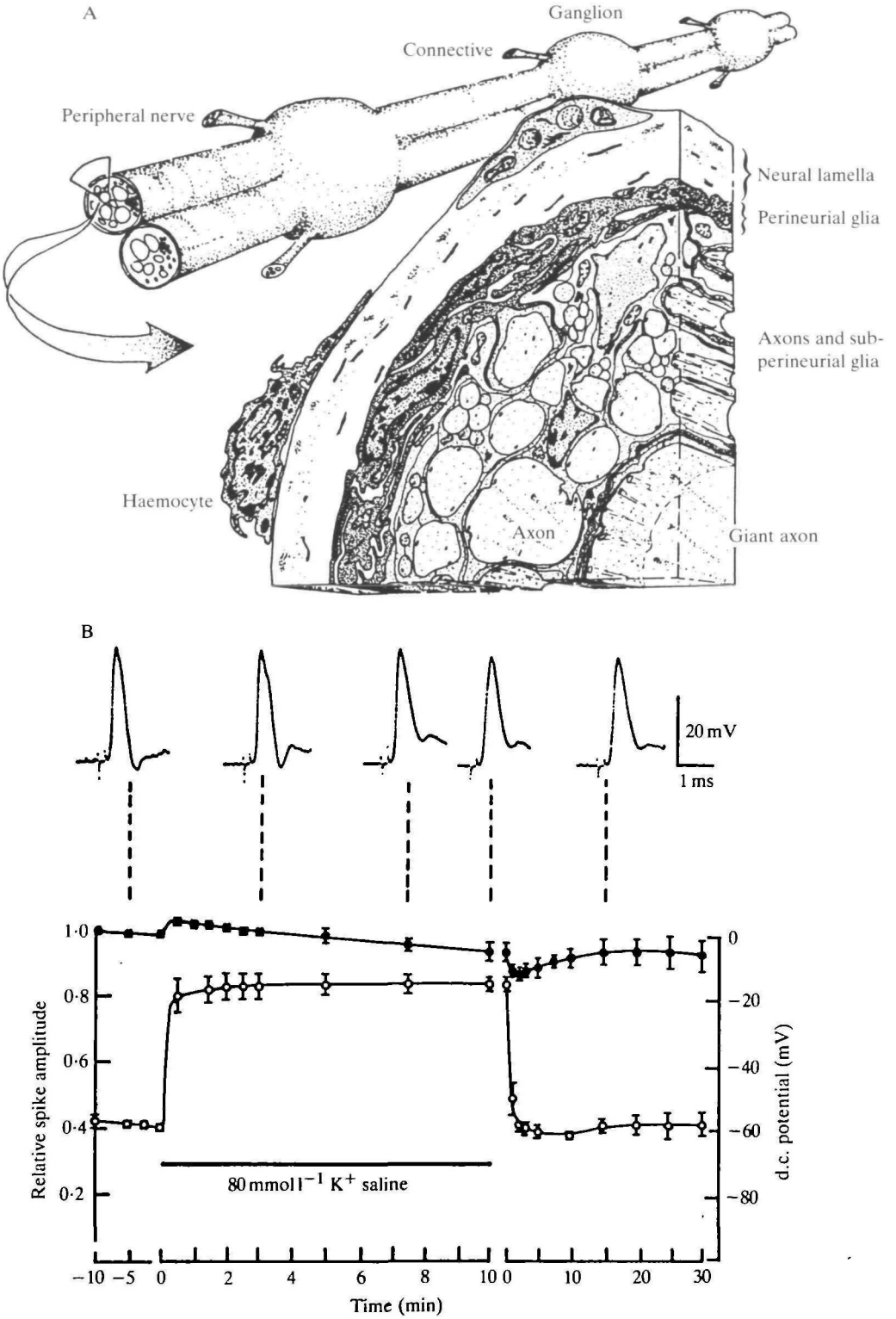

A
Ganglion
Connective
Peripheral nerve
Neural lamella
Perineurial glia
Axons and sub-perineurial glia
Haemocyte
Axon
Giant axon
B
20 mV
1 ms
Relative spike amplitude
1·0
0·8
0·6
0·4
0·2
0
−20
−40
−60
−80
d.c. potential (mV)
80 mmol l^{-1} K$^+$ saline
−10 −5 0 2 4 6 8 10 0 10 20 30
Time (min)

Fig. 1

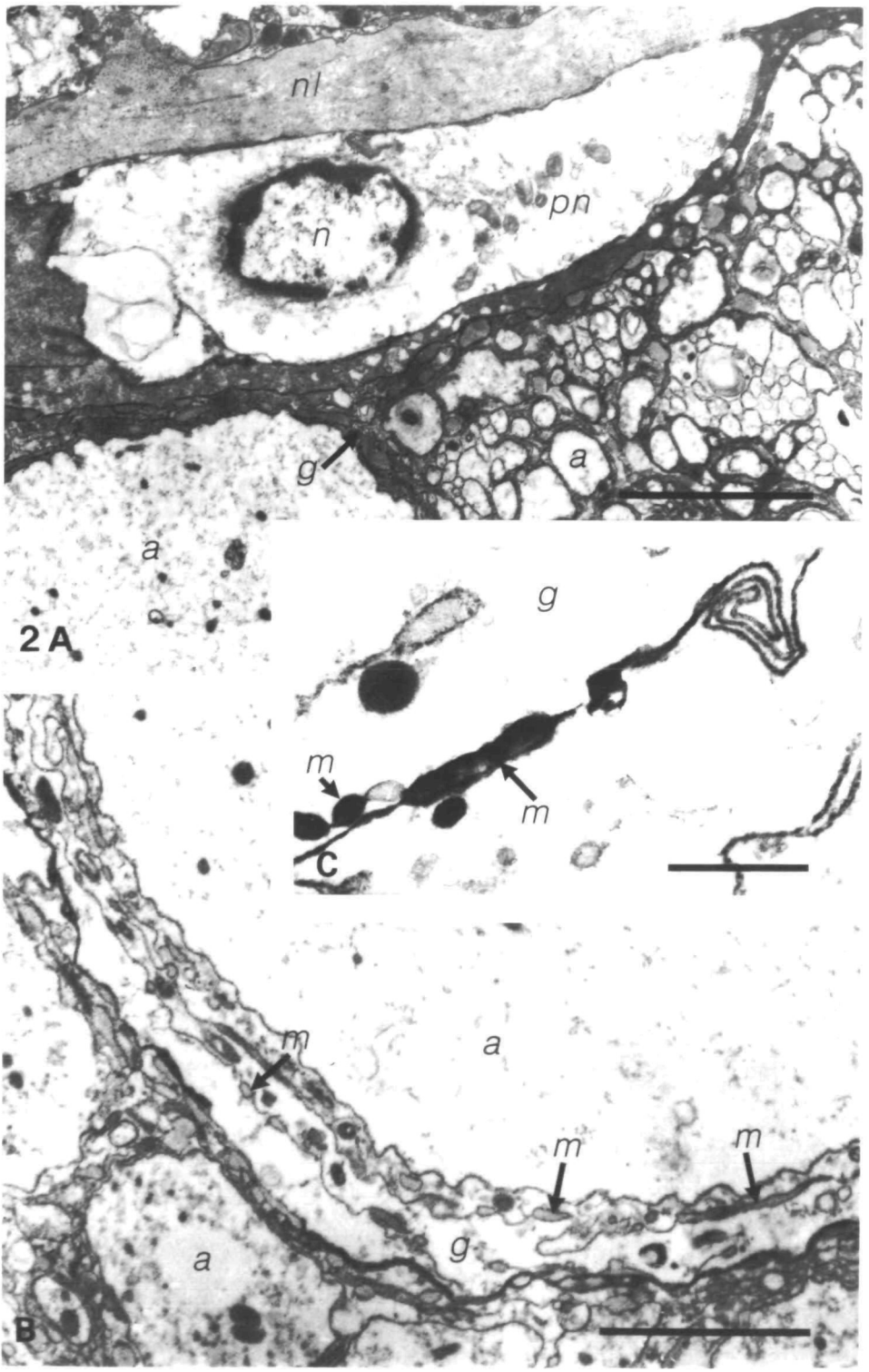


Fig. 2. For legend see p. 64

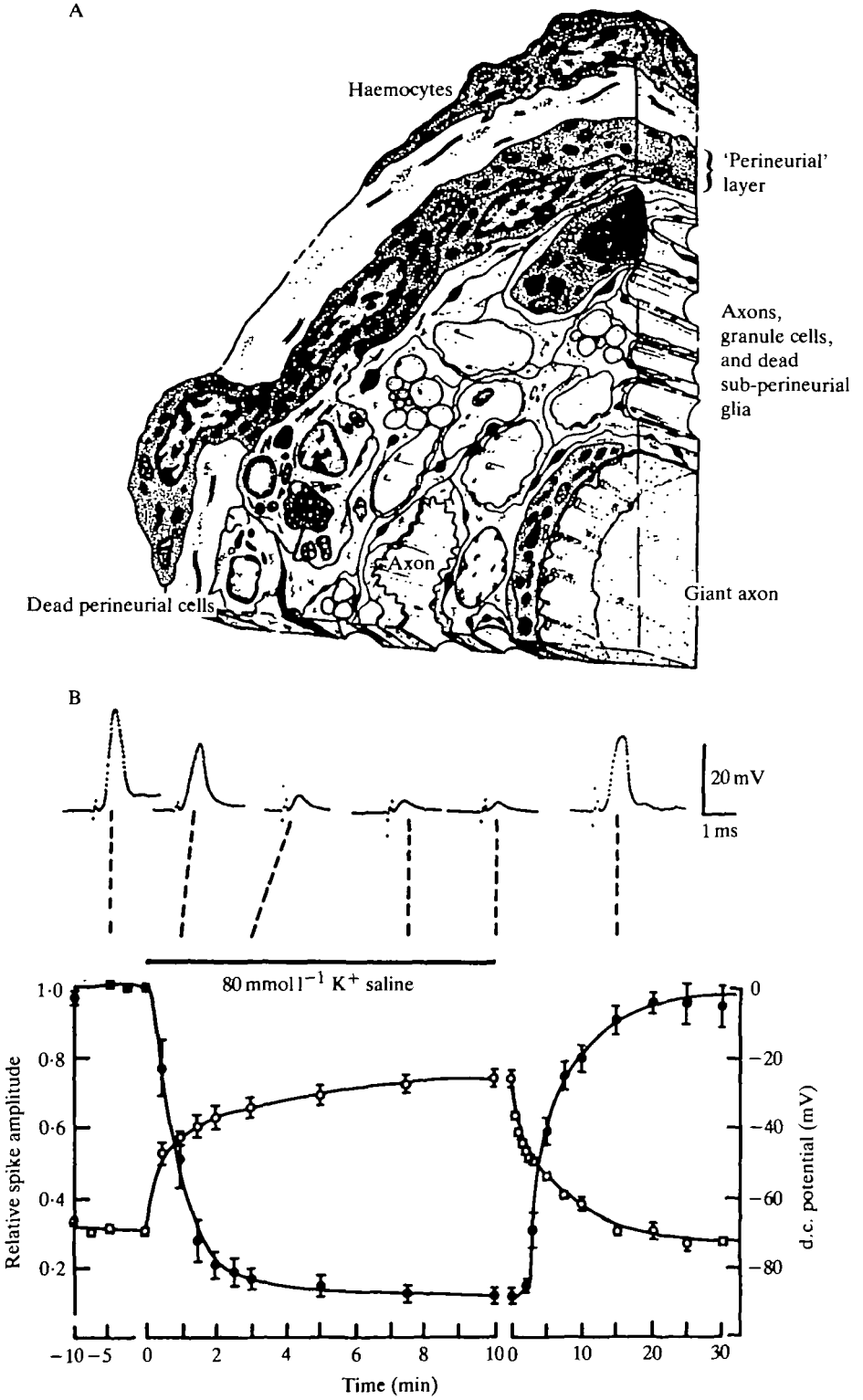


Fig. 3. For legend see p. 64

amplitude of the axonal action potentials, following exposure to high-potassium saline (Fig. 3B), and the penetration of ionic lanthanum into the underlying extracellular system (Fig. 2C). There is also extensive damage to the sub-perineurial and adaxonal glia (Fig. 2B). The ultrastructural appearance of the axons is little modified by the ethidium treatment (Fig. 2B) although, at the light microscope level, axons can appear constricted in diameter and sometimes collapsed. Normal action potentials can, however, be recorded from them at all stages of disruption and repair (Smith *et al.* 1984). Protein synthesis by the glia, including contributions to the axons and acellular neural lamella, is greatly reduced post-lesion (Smith & Howes, 1984).

THE REPAIR SEQUENCE

A consistent feature of the early stages of repair following selective glial disruption is the appearance of cells containing characteristic electron-dense granules within their cytoplasm (Fig. 3A: Smith *et al.* 1984). After 2 days, the granule-containing cells begin to form a mosaic of cells and organized layers beneath the neural lamella. By 4 days, the cells in this region have developed extensive, interdigitating cell processes with septate and tight junctional complexes between the membranes of adjacent processes, but cells carrying the electron-dense primary lysosomes have largely disappeared (Smith *et al.* 1984). At this stage, there is a variable degree of access of potassium ions to the axon surfaces (Fig. 4), although extraneously applied lanthanum is now excluded from the underlying extracellular spaces. Granule-containing cells are also present at deeper levels among the sub-perineurial tissues. Some of these appear to be engaged in the phagocytosis of cellular debris within the reorganizing perineurium and at deeper levels in the lesion (Smith *et al.* 1984).

Fig. 2. (A) Electronmicrograph from the perineurial region of a penultimate abdominal connective 24 h after treatment with ethidium bromide showing a disrupted perineurial cell. Scale bar, 4 μm. (B) Disrupted mesaxonal glia pulling away from an axon surface, 48 h after treatment with ethidium bromide. Scale bar, 3 μm. (C) Extraneously applied lanthanum can now penetrate into the extracellular system after selective glial disruption as shown by precipitated lanthanum phosphate in the matrix of the mesaxonal glia. Scale bar, 0·4 μm. *a*, axon; *g*, mesaxonal glial cell; *m*, matrix; *n*, nucleus; *nl*, neural lamella; *pn*, perineurial glial cell.

Fig. 3. (A) Semi-diagrammatic representation of the ultrastructure of a penultimate abdominal connective at 48 h after selective glial disruption with ethidium bromide. At this stage haemocytes accumulate in relatively large numbers and then penetrate the neural lamella. The perineurial layer now contains numerous granule-containing cells, derived from the invading haemocytes. (B) Effects of high-potassium saline on the electrical responses of the connectives 48 h after exposure to ethidium bromide. Unlike situations in normal connectives (Fig. 1B) there is now a rapid collapse of the action potentials (●), the change in d.c. potential (○) now corresponding to axonal depolarization due to the relatively rapid penetration of potassium ions resulting from the disruption of the perineurial blood–brain barrier. The vertical lines through the data points indicate the extent of twice the standard error of the mean ($N = 5$). (From Smith, Leech & Treherne, 1984.)

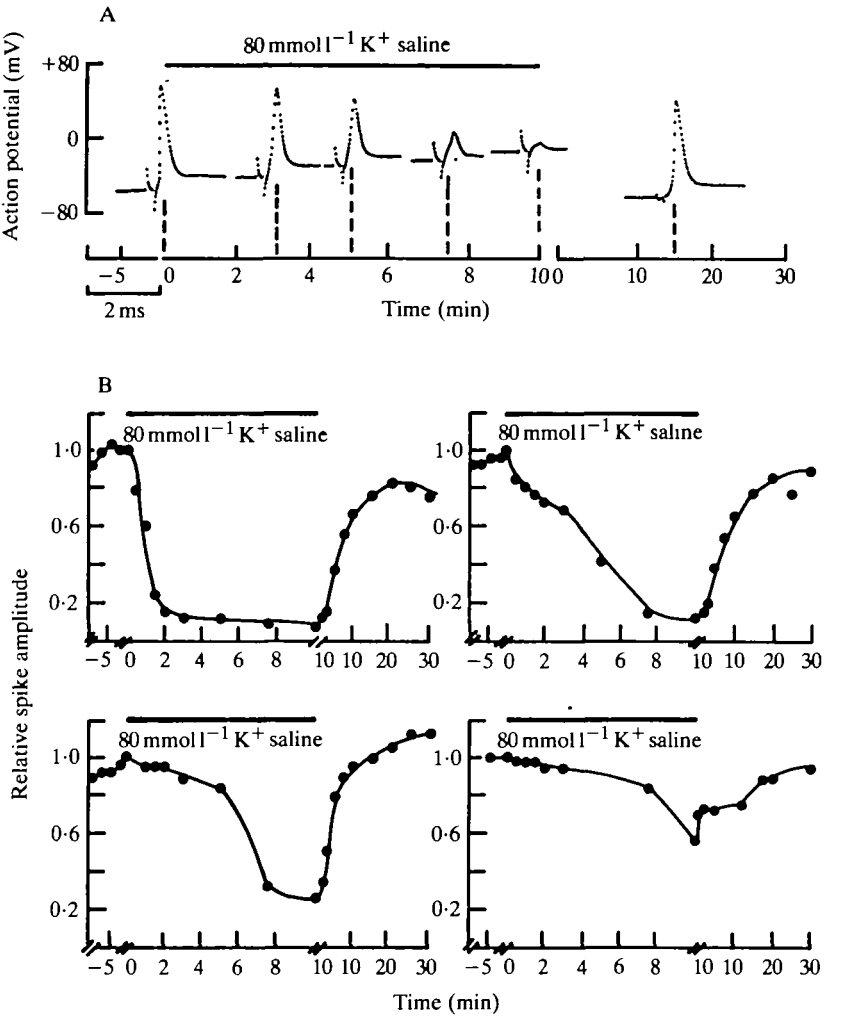


Fig. 4. Electrical responses from ethidium-treated connectives at an intermediate stage of repair. (From Smith, Leech & Treherne, 1984.) (A) Intracellular recording from a giant axon within a connective, 4 days after selective glial disruption, showing maintenance of normal action potentials, but a slower response to high-potassium saline than at 48 h (Fig. 3). (B) At 4 days there is, however, considerable variability in response to elevated potassium, indicating different degrees of barrier repair, as is shown in four sucrose-gap recordings.

At 6 days, the superficial repairing cells begin to acquire the flattened appearance, and narrow processes extending downwards into the underlying tissues, characteristic of normal perineurial cells. By 11 days, a blood–brain barrier has been re-established; the glia are similar to those in control connectives (Fig. 5) and exposure of the repairing lesion to high-potassium saline no longer produces a rapid decline in action potential amplitude. However, the electrical properties of the blood–brain interface are still abnormal, in that the potassium-induced extraneuronal potentials,

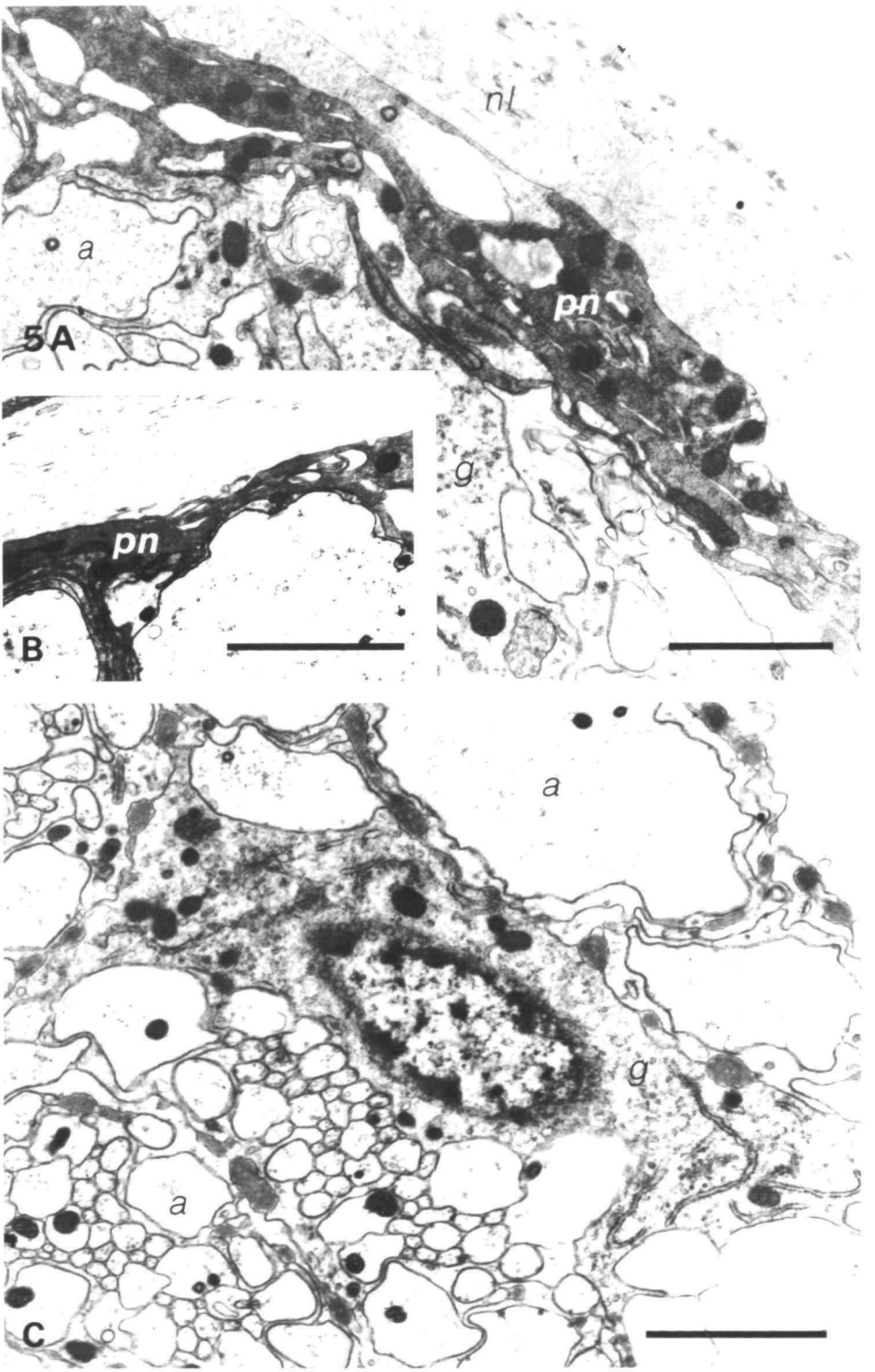


Fig. 5. For legend see p. 68

corresponding to the depolarization of the outwardly directed perineurial membranes, are much smaller than in normal connectives (Fig. 6).

After 28 days the ultrastructural appearance of the perineurial and sub-perineurial glia is indistinguishable from that of untreated connectives. There has also been an increase in the extent of the potassium-induced extraneuronal potentials although

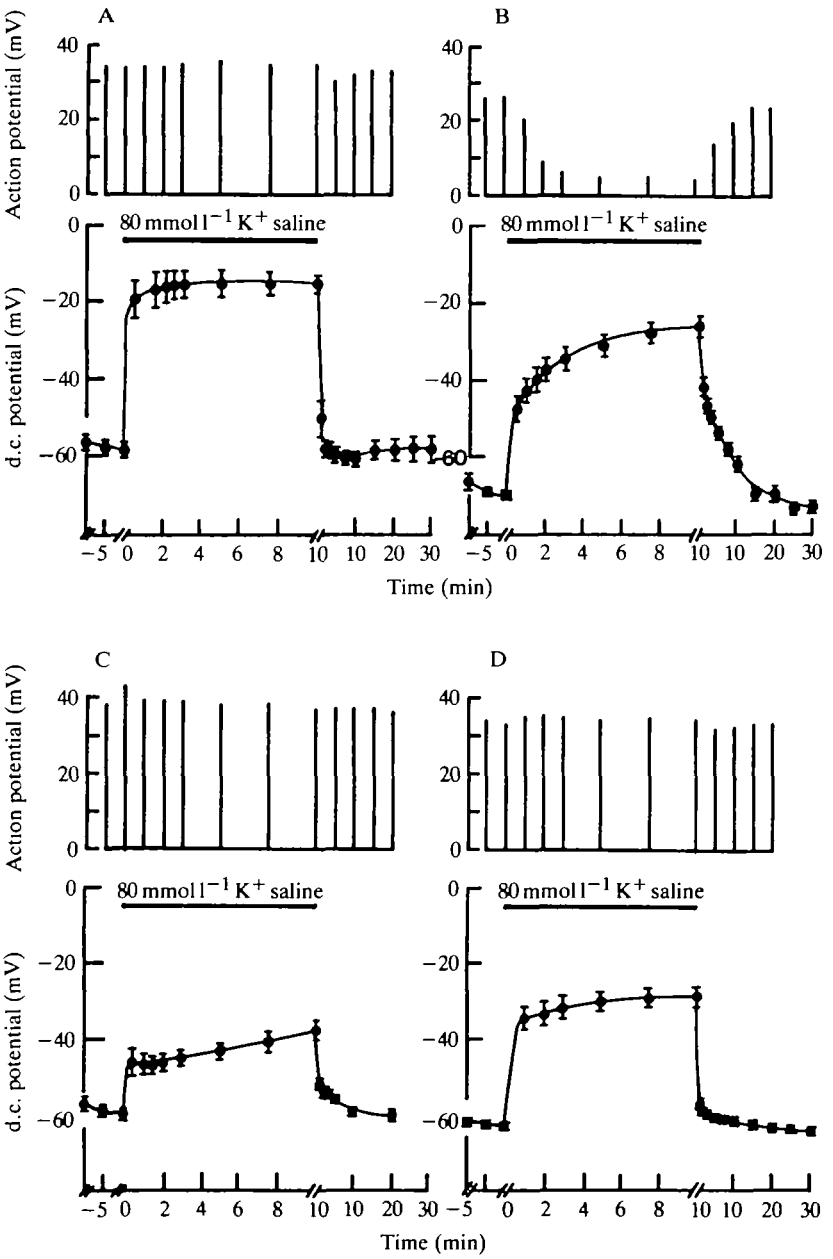


Fig. 6. For legend see p. 68

these are still smaller than those recorded in control connectives. Thus, it seems that, despite the swift re-establishment of the perineurial blood–brain barrier, there is a relatively slow restoration of the original electrophysiological characteristics of the blood–brain interface.

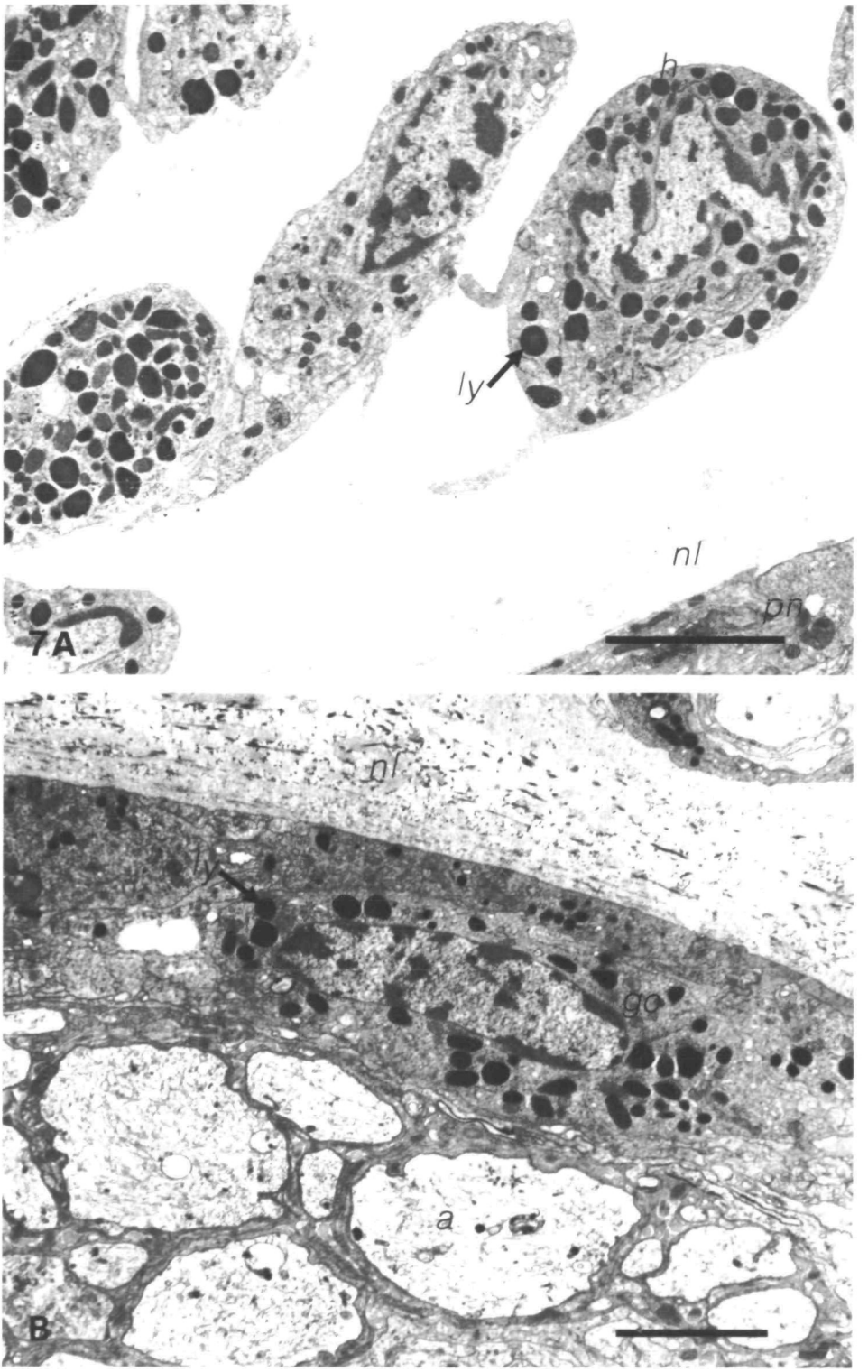
IDENTITY OF THE REACTIVE CELLS

The first question which arises is the origin of the granule-containing cells that are so prominently involved in the initial stages of glial repair. They are unlikely to be endogenous, for no cells containing such primary lysosomes are seen in normal nervous connectives. The granule-containing cells are, however, strikingly similar to the circulating blood granulocytes (Figs 3, 7: Smith *et al.* 1984), that are only very occasionally seen adhering to the surface of undamaged connectives (Treherne *et al.* 1984), but which swiftly accumulate on, and penetrate, the neural lamella following chemical lesioning of the underlying glia (Smith *et al.* 1984; Treherne, Smith & Edwards, 1987*b*). These and other observations suggest that the granule-containing cells may be of exogenous origin, being derived from circulating haemocytes (Smith, Howes, Leech & Treherne, 1986), a supposition which is confirmed by recent experiments using antibodies raised against haemocytes (E. A. Howes, B. M. Chain, P. J. S. Smith & J. E. Treherne, unpublished observations). In these experiments,

Fig. 5. (A) Repair of the perineurial layer, 11 days after treatment with ethidium bromide, showing superficial glia with typical interdigitating cell processes and reduced extracellular volume. Scale bar, 3 μm. (B) Control section, illustrating the normal perineurial structure from an untreated connective. Scale bar, 3 μm. (C) A repaired mesaxonal glial cell, 11 days after treatment with ethidium bromide, showing recovery of normal cell organelles including free ribosomes and rough endoplasmic reticulum. The surrounding tissue is now of normal appearance. Scale bar, 2 μm. *a*, axon; *g*, mesaxonal glial cell; *nl*, neural lamella; *pn*, perineurial glial cell.

Fig. 6. Electrophysiological responses to pulses of high-potassium saline of the penultimate abdominal connectives at various stages of repair following selective glial disruption. (A) Control connectives showing persistent action potentials during application of high-potassium saline. The d.c. potential changes result largely from depolarization of the outwardly directed perineurial membranes ($N = 3$). (B) At 24 h post-lesion, the disruption of the perineurial glial cells results in a rapid access of potassium ions to the axon surfaces and a swift decline in the action potential amplitude. At this stage the recorded d.c. potential change results largely from axonal depolarization ($N = 4$). (C) At 11 days post-lesion, there is now a restricted access of potassium ions to the axon surfaces as shown by the maintenance of action potential amplitude. The d.c. potential changes, corresponding to perineurial depolarization, are however much smaller than in control preparations ($N = 3$). (D) At 28 days post-lesion, the potassium-induced d.c. potential changes have increased but are still smaller than in control preparations ($N = 3$). Sucrose-gap recordings. The vertical lines through the data points indicate the extent of the standard error of the mean.

Fig. 7. (A) Circulating haemocytes in the vicinity of the neural lamella of a lesioned connective. Scale bar, 4 μm. (B) Granule-containing cells, in the perineurial region of a connective 48 h after selective glial disruption. Like the haemocytes, the cells are characterized by the presence of electron-dense primary lysosomes. (From Smith, Leech & Treherne, 1984.) Scale bar, 4 μm. *a*, axon; *gc*, granule-containing cell; *h*, haemocyte; *nl*, neural lamella; *ly*, primary lysosome; *pn*, perineurium.


h
ly
nl
pn
7A
nl
ly
go
a
B

no cell within normal abdominal connectives binds monoclonal antibodies raised against cockroach haemocytes. Monoclonally labelled cells do, however, appear in the disrupted perineurial and sub-perineurial tissues within 24 h of selective glial disruption (Fig. 8). As with the granule-containing cells they increase in number over the first 1–3 days post-lesion and then decline.

Clearly, therefore, this category of reactive cell is of exogenous origin, invading the lesion zone in the early stages of repair to play an important role, not only in phagocytosis, but also in structural replacement of disrupted glia.

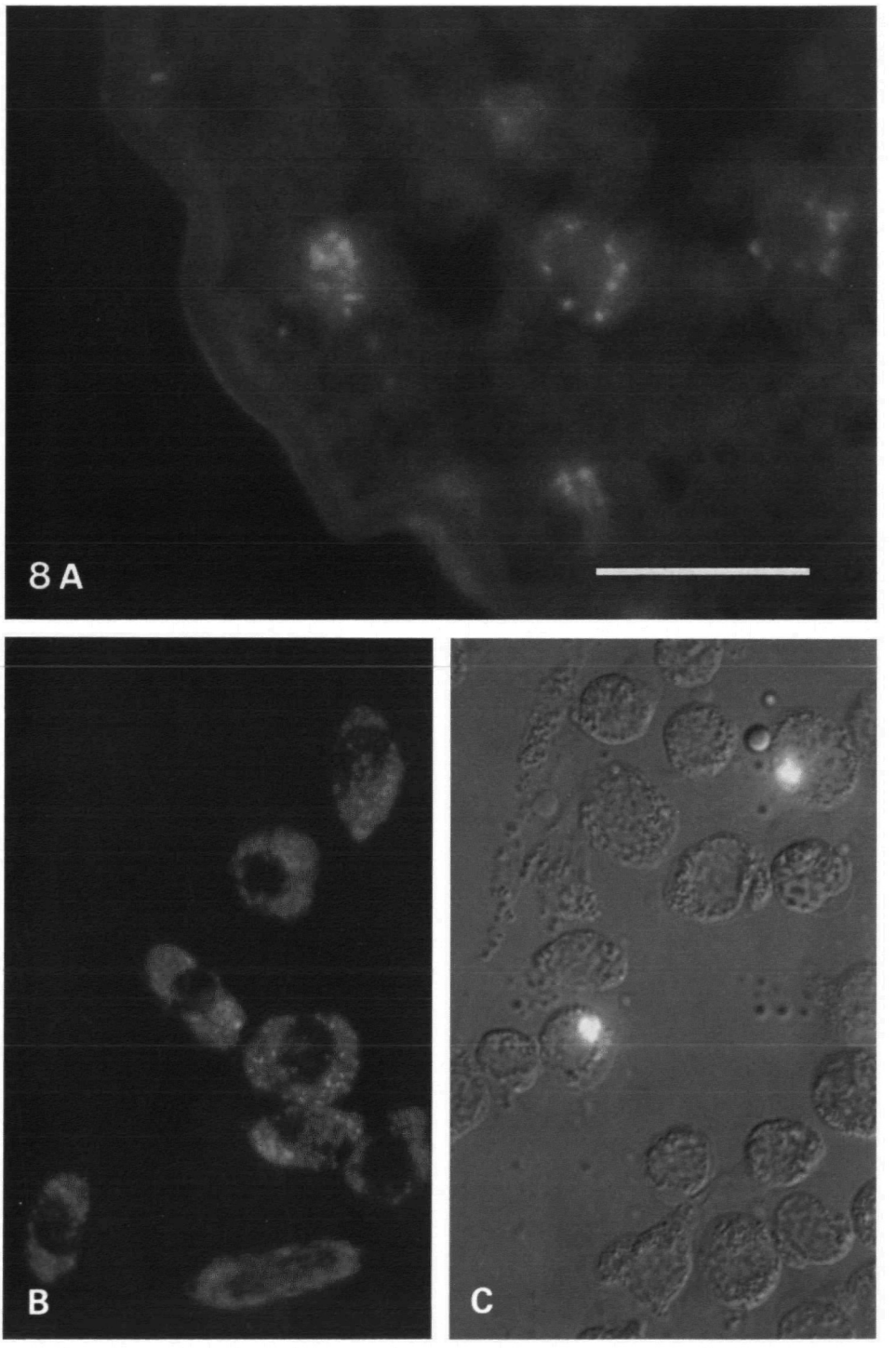
The exogenous, haemocytotic cells appear to work in concert with, and perhaps orchestrate, endogenous reactive cells. The involvement of such endogenous cells is indicated by several lines of evidence. For example, we have shown that glial cells are capable of division and repair following selective disruption in cultured nerve cords, in which there can be no haemocyte involvement and, consequently, in which granule-containing cells are never seen in the lesioned tissues (Howes, Smith & Treherne, 1987*a*,*b*). Glial repair in such *in vitro* preparations is slower and less organized than *in vivo*. Nevertheless, restructuring does occur. Initially, patches of viable cells appear among the disrupted glia eventually extending to form a continuous covering over the repairing lesion; aberrant repair of the sub-perineurial glia also occurs. Essentially similar sequences of repair occur when the appearance of granule-containing cells in the lesion is reduced by the DNA-scission drug bleomycin (Treherne, Howes, Leech & Smith, 1986) or by injection into the blood of physiologically inert particles (fluorescent microspheres), which are taken up by a relatively small proportion of the haemocytes (approx. 10 %) (Fig. 8C) and thus prevented from entering the lesion area following selective glial disruption (Smith *et al.* 1986).

The participation of endogenous reactive cells is also indicated by the substantial increase in cell numbers which occurs in undamaged tissues adjacent to the lesion zone following selective glial disruption (Treherne *et al.* 1987*b*). This increase is unlikely to have resulted from invading haemocytes, for granule-containing cells are rarely seen adjacent to the lesion zone, either in electron microscope sections or in monoclonal antibody-binding studies (E. A. Howes, B. M. Chain, P. J. S. Smith & J. E. Treherne, unpublished observations).

CELL RECRUITMENT AND INTERACTIONS DURING GLIAL REPAIR

An increase in cell numbers within repairing connectives is first seen in the perineurial region 2 days after selective glial disruption, and is confined to the lesion

Fig. 8. (A) Cells from within the lesion zone of a cockroach connective staining with antibody 3A7/F9, 3 days after selective glial disruption with ethidium bromide. (B) A fluorescein-labelled monoclonal antibody (3A7/F9) binding to cockroach haemocytes, for which it is specific. (C) Haemocytes containing fluorescent microspheres 24 h after their injection into the haemocoel. Approximately 10 % of the blood cells take up the microspheres. Scale throughout as in A. Scale bar, 20 μm.


8 A
B
C

zone (Fig. 9). This stage corresponds to the phase of haemocyte invasion into the lesioned tissues.

The increase in perineurial cell numbers in the undamaged tissues starts 3 days after selective lesioning (Fig. 9). It is then seen as a separate peak, anterior to the lesion and close to the fourth abdominal ganglia. This secondary peak is still evident after 5 days, with the appearance of having moved towards the lesion zone. By 7 days, the peaks appear to have fused and are now confined to the site of original lesion (Fig. 9). This increase in cell numbers is maintained in the lesion zone over the next 1–2 months, with further multiplication in the adjacent areas of the connectives (Treherne *et al.* 1987*b*).

An increase in cell numbers is also a feature of sub-perineurial repair following selective disruption, although the anterior polarity seen in the perineurium is less evident. As with perineurial repair, the increased numbers are maintained 1 month after selective lesioning (Treherne, Smith & Edwards, 1987*b*).

The above results are consistent with the notion that glial repair in penultimate cockroach connectives involves exogenous and endogenous elements: the haemocytes, which invade the lesioned tissues, and the reactive glia, that originate anterior to the damaged area, perhaps in the vicinity of the fourth ganglion, and migrate into the lesion.

We also have evidence that haemocytes play a critical role in the recruitment of the reactive glia. Thus, if haemocytes are excluded from the lesion (by prior injection and uptake of fluorescent microspheres, Fig. 8C) then there is no activation of the reactive glia equivalent to that seen in control lesion experiments (Fig. 10A,B). Similarly, in experiments with cultured cords (Howes *et al.* 1987*a*) and in the presence of bleomycin *in vivo* (Treherne *et al.* 1987*b*), there is no equivalent activation of endogenous reactive cells (Fig. 11A,B). Significantly, in the experiments with bleomycin (a drug which perturbs cell function and division: Kuo, 1981) there is a clear *reduction* of cells in the lesion area following selective glial disruption. It is unlikely that the activation of the reactive glia results from direct contact stimulus, as when, for example, growing neurites initiate division of cultured Schwann cells (Bunge & Bunge, 1983), for our studies show that the monoclonally labelled haemocytes appear to be confined to the damaged tissues. A remaining possibility is that the activation of the endogenous reactive cells is triggered by the release of diffusible growth factors from the invading haemocytes within the repairing connective.

We are, at present, still ignorant of the identity of the reactive glia that, in our preparation, appear to originate within, or in the vicinity of, the anterior ganglion and which impose an anterior polarity on the initial cellular responses to selective glial disruption. They could be uncommitted progenitor cells, such as have been shown to exist in the vertebrate CNS (Raff, Miller & Noble, 1983) or, alternatively, be derived from existing glia, perhaps after dedifferentiation as has been postulated for the regenerating amphibian limb (Brockes, 1984).

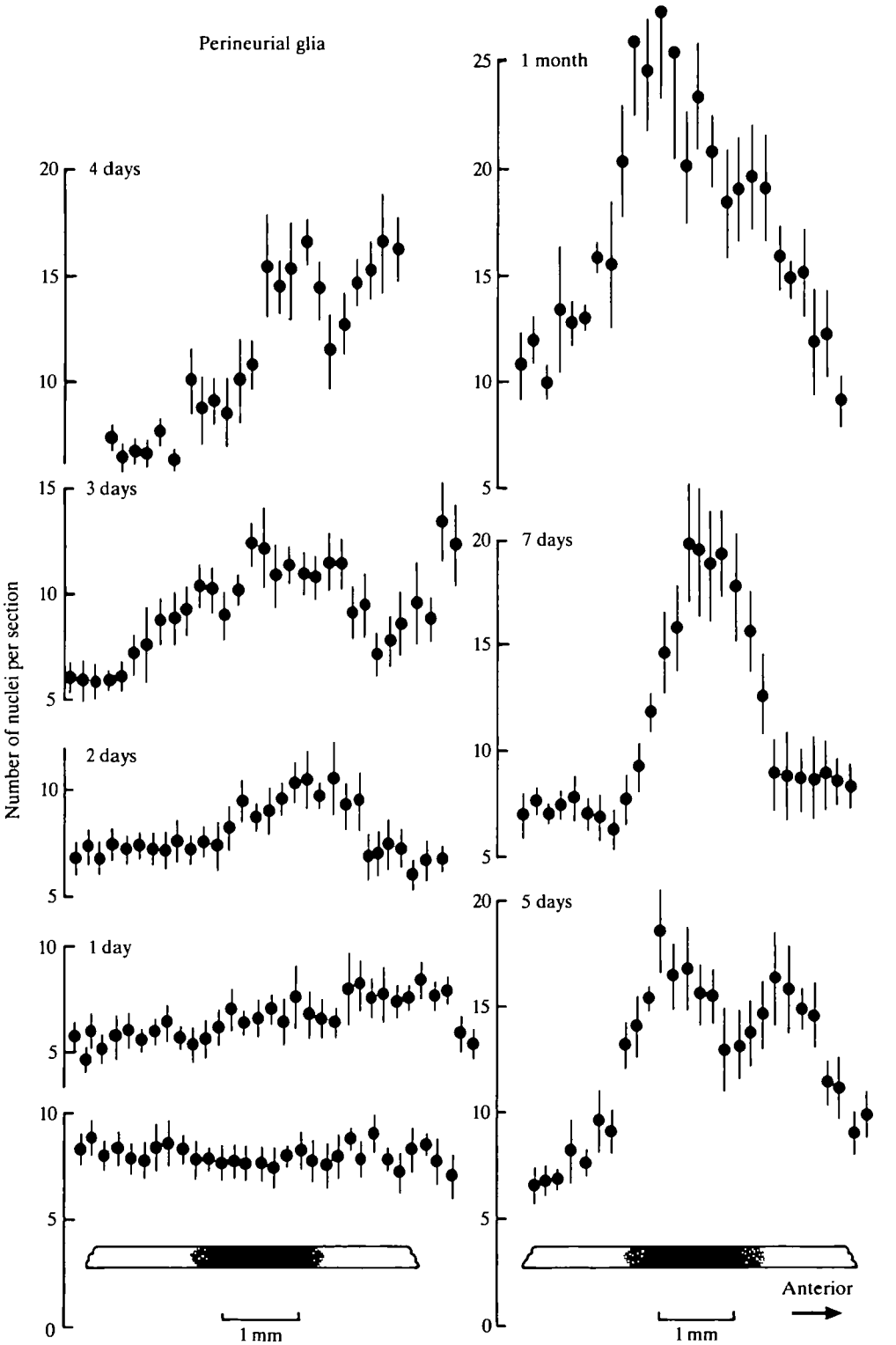

Perineurial glia
4 days
3 days
2 days
1 day
1 month
7 days
5 days
Number of nuclei per section
1 mm
1 mm
Anterior

Fig. 9

THE FATE OF THE REACTIVE CELLS

The invading haemocytes, which so swiftly enter the lesioned tissues following selective glial disruption, clearly serve an important role in the early stages of repair, not only in initiating the recruitment of endogenous reactive cells, but also in the initial structural replacement of damaged glia. This is most clearly seen in their role in restructuring the superficial glia which constitute the perineurial blood–brain barrier. However, this process involves marked changes in the morphology of the repairing cells. There is also a progressive loss of cells containing characteristic cytoplasmic granules 2–4 days after selective glial disruption (Fig. 12). Furthermore, these changes are accompanied by an onset of cell division in the repairing perineurium, as indicated by the increasing number of nuclei labelled with tritiated thymidine (Fig. 12: Smith & Howes, 1987).

There are two possible explanations for these events: the granule-containing cells could be transformed or they could be replaced by cells of glial origin (Howes *et al.* 1987*b*; Treherne *et al.* 1987*a*). Both possibilities would account for the increase in nuclear thymidine labelling, for transformation could involve the division of granule-containing cells, while replacement by reactive glia is also likely to be achieved by division.

LONG-TERM CHANGES IN GLIAL REPAIR

Despite the apparently swift initial repair of selectively lesioned connectives, the cellular reorganization is, in fact, a protracted business. This is seen in the relatively slow restoration of the normal potassium permeability of the blood–brain interface. Thus, even after 28 days the potassium-induced extraneuronal potentials do not attain their original amplitude, though the blood–brain barrier is re-established within 14 days (Smith *et al.* 1984).

The early increase in cell numbers is also continued in the apparently fully repaired lesion. This is most clearly seen in the sub-perineurial glia. Here, the cell numbers at 28 days are more than twice those at 7 days. Furthermore, the increase in cell numbers now tends to spread beyond the limits of the original lesion (J. E. Treherne, P. J. S. Smith & E. A. Howes, in preparation). A similar long-term increase in glial cell numbers has been observed in cultured leech central nervous connectives after lesioning by crushing (Morgese, Elliott & Muller, 1983). A substantial increase in cell numbers also occurred, after 1 week, in regions adjacent to the lesion in the leech preparation.

Unlike the situation following surgical lesioning, the glial proliferation in the chemically lesioned cockroach connectives occurs in an existing compartment defined by the extracellular matrices and axons, so that substantially more cells are now deployed into what is effectively the same, limited, domain. That they

Fig. 9. Cell fluxes in repairing cockroach connectives at various times after selective glial lesioning. The lesion zone is indicated by the shaded areas on the schematic drawings of the connectives. The cell numbers were estimated from nuclear counts made on stained 12 μm light microscope sections. The vertical lines through the data points indicate the extent of twice the standard error of the mean. (From Treherne, Smith & Howes, 1987*a*.)

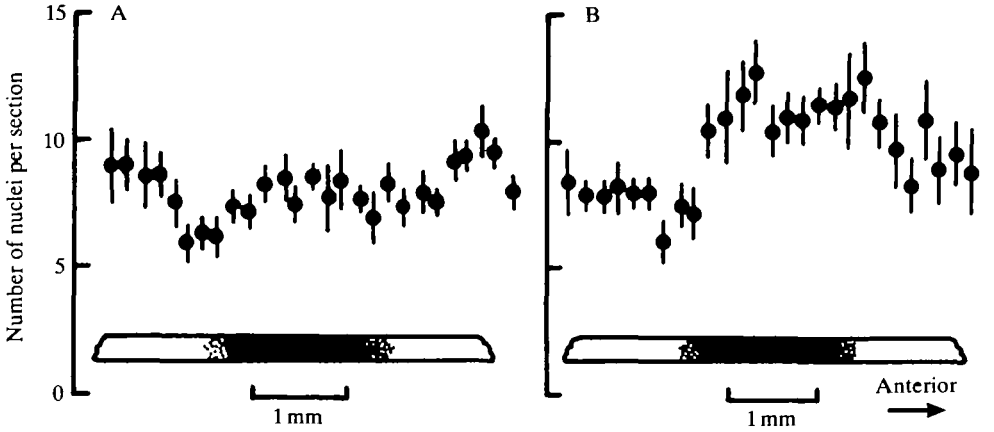


Fig. 10. Effects of haemocyte blockage, caused by uptake of suspended inert microspheres (see Fig. 8C), on perineurial cell fluxes in chemically lesioned cockroach connectives. At 4 days (A) there is no anterior recruitment of repairing cells as is seen in normal, lesioned preparations (Fig. 9); at 7 days (B) there is only a modest increase, apparently centred on the lesion zone. Data obtained from nuclear counts in 12 μm light microscope sections. The vertical lines through the data points indicate the extent of twice the standard error of the mean. (J. E. Treherne, P. J. S. Smith & E. A. Howes, unpublished observations.)

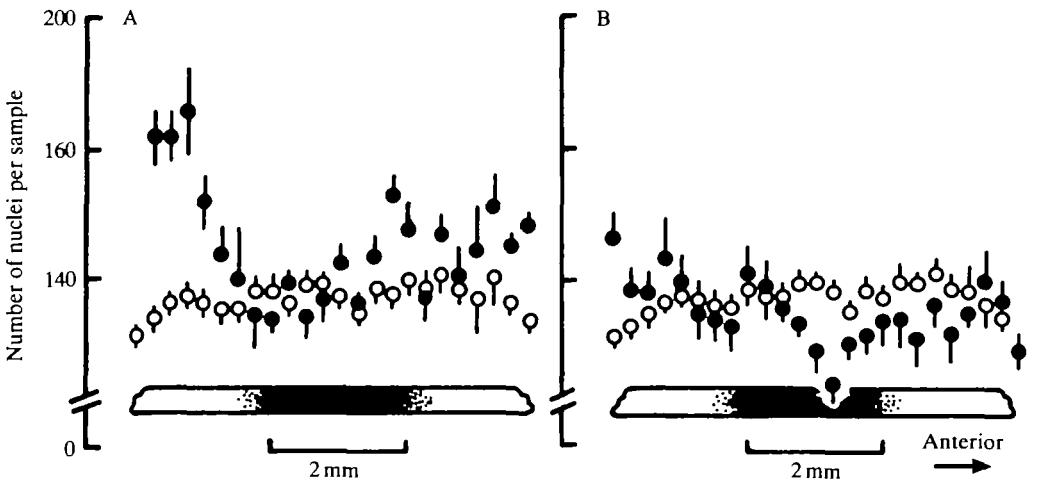


Fig. 11. Perturbation of cell fluxes in repairing connectives 7 days after ethidium bromide lesioning in (A) cultured nerve cords and (B), *in vivo*, in the presence of injected bleomycin; treatments which preclude or substantially reduce the appearance of the haemocytically derived granule-containing cells in the lesioned tissues. In the cultured connectives (closed circles) there is aberrant repair with no appreciable increase in cell numbers in the lesion zone and now an apparently posteriorly oriented recruitment of cells: *in vivo*, with bleomycin (closed circles), there is again no anterior recruitment of cells and significantly lower numbers in the lesion zone as compared with normal, untreated connectives (open circles). In these experiments the cell numbers were estimated from nuclear counts made on flattened, stretched connectives and include both perineurial and sub-perineurial cells. The vertical lines through the data points indicate the extent of twice the standard error of the mean. (From Treherne, Smith & Edwards, 1987*b*.)

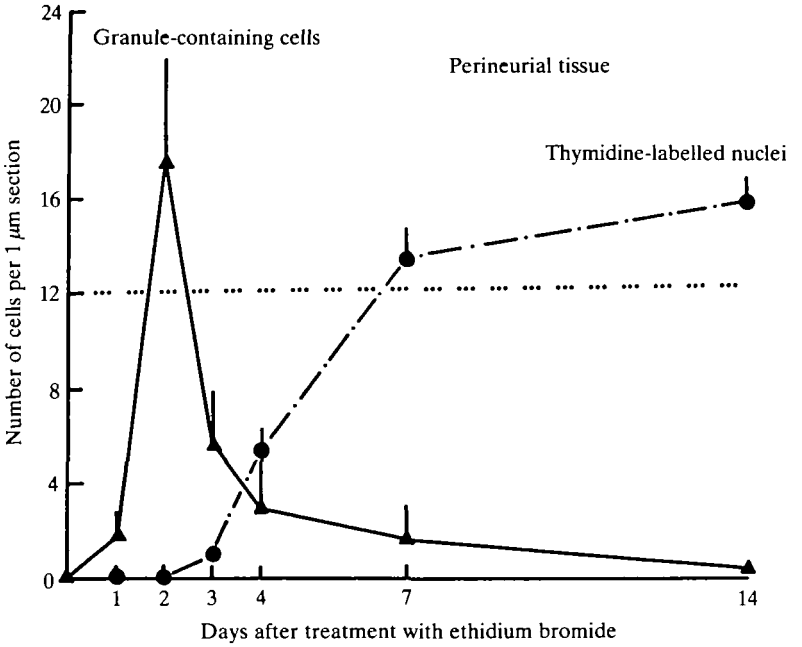


Fig. 12. Numbers of granule-containing cells plotted with the number of nuclei labelled by tritiated thymidine counted from 1-μm sections through the lesion zone of the connective, at various stages of repair. Labelled thymidine was continuously present during the experimental periods. The dotted, broken line is approximately the number of nuclei present in control sections from undamaged connectives. Standard errors are plotted on either side of mean values. (From Smith & Howes, 1987; Howes, Smith & Treherne, 1987*b*.)

proliferate to this extent implies the existence of powerful and sustained mitogenic signals within the repairing system which are capable of activating cell division in existing G-phase cell populations. If the mitogenic signals are powerful enough then they could override the contact-inhibition of cell division which, we presume, must arise as the available space is filled. Under these circumstances, proliferation in a restricted environment could result in an increased population of smaller cells. Alternatively, the increase in the number of glial cells in the repairing lesion could be merely a consequence of the recruitment of a population of progenitor cells which are smaller than those of the original neuroglia.

CONCLUSION

Our results show that glial repair in the insect CNS can be divided into three separate phases. The first involves an initial invasion of the lesion by circulating haemocytes. These accumulate on the surface of the nervous system within 24 h of selective glial disruption, penetrate the neural lamella and then transform into a novel cell class, the granule-containing cells, which are never seen in undamaged connectives. The granule-containing cells have three major roles: a limited involvement in phagocytosis, structural replacement of damaged glia and activation of

endogenous reactive cells. The second phase involves a reduction in the number of granule-containing cells, proliferation of reactive glia from the vicinity of the anterior abdominal ganglion and restoration of the perineurial blood–brain barrier. The final phase is characterized by a progressive, and then maintained, increase in the numbers of perineurial and sub-perineurial glia within the lesion site and, subsequently, in the adjacent undamaged tissues.

This sequence bears a number of striking similarities to the equivalent events which occur in the repair of the vertebrate central nervous system. A notable similarity is the initial invasion of the lesion by haemocytes which parallels the entry of blood monocytes into vertebrate brain tissues following surgical damage (Adrian & Schelper, 1981). The timing of these events can be closely similar. As with the insect haemocytes, the vertebrate blood monocytes arrive within 2 days of lesioning and by 5 days transform to macrophage cells (du Bois *et al.* 1985): a timing which corresponds to the transformation/replacement of the insect granule-containing cells. Cell division of the astrocytes commences at 2 days and continues for 6–7 days, which coincides with the onset of thymidine labelling of the perineurial glia in the insect preparation.

The role of the haemocytes in triggering glial cell recruitment again parallels events in vertebrate systems, where it is known that blood cells, principally T-lymphocytes, can release glial growth factors (Fontana *et al.* 1980; Benveniste *et al.* 1985). The presence of glial mitogenic and morphogenic factors in the vertebrate central nervous system (Giulian & Baker, 1985; Giulian & Young, 1986; Giulian, Tomozawa, Hindman & Allen, 1985; Giulian, Allen, Baker & Tomozawa, 1986) also indicates the possibility of endogenous activation of glial proliferation. Equivalent activators in the insect CNS could provide the stimulus for the prolonged glial proliferation and might themselves be initially triggered by the arrival of the exogenous reactive cells, the haemocytes. This would be analogous to the situation in vertebrates. Mammalian microglia, which are probably derived during development from monocytic stem cells (see Rio-Hortega, 1932; Perry, Hume & Gordon, 1985), also produce growth factors that act selectively on populations of macroglia (Giulian & Baker, 1985).

This work was partly supported by the Leverhulme Trust.

REFERENCES

ADRIAN, E. K., JR & SCHELPER, R. L. (1981). Microglia monocytes and macrophages. In *Glial and Neuronal Cell Biology*, 11th International Congress of Anatomy, Part A (ed. E. A. Vidrio & A. Federoff), pp. 113–124. New York: A. R. Liss Inc.

AGUAYO, A. J., DAVID, S. & BRAY, G. M. (1981). Influence of the glial environment on the elongation of axons after injury: transplantation studies in adult rats. *J. exp. Biol.* **95**, 231–240.

BENVENISTE, E. N., MERRILL, J. E., KAUFMAN, S. E., GOLDE, D. W. & GASSON, J. C. (1985). Purification and characterization of a human T-lymphocyte-derived glial growth factor. *Proc natn. Acad. Sci. U.S.A.* **82**, 3930–3934.

BLAKEMORE, W. F. (1982). Ethidium bromide induced demyelination in the spinal cord of the cat. *Neuropath. appl. Neurobiol.* **8**, 365–375.

BROCKES, J. P. (1984). Mitogenic growth factors and nerve dependence of limb regeneration. *Science* **225**, 1280–1287.

BUNGE, R. P. (1987). Tissue culture observations relevant to the study of axon–Schwann cell interactions during peripheral nerve development and repair. *J. exp. Biol.* **132**, 21–34.

BUNGE, R. P. & BUNGE, M. B. (1983). Interrelationship between Schwann cell function and extracellular matrix production. *Trends Neurosci.* **6**, 499–505.

DU BOIS, M., BOWMAN, P. D. & GOLDSTEIN, G. W. (1985). Cell proliferation after ischemic injury in gerbil brain. An immunocytochemical and autoradiographic study. *Cell Tissue Res.* **242**, 17–23.

FONTANA, A., GRIEDER, A., ARRENBRECHT, ST. & GROB, P. (1980). *In vitro* stimulation of glial cells by a lymphocyte-produced factor. *J. neurolog. Sci.* **45**, 55–62.

GIULIAN, D., ALLEN, R. L., BAKER, T. J. & TOMOZAWA, Y. (1986). Brain peptides and glial growth. I. Glial promoting factors as regulators of gliogenesis in the developing and injured central nervous system. *J. Cell Biol.* **102**, 803–811.

GIULIAN, D. & BAKER, T. J. (1985). Peptides released by ameboid microglia regulate astroglial proliferation. *J. Cell Biol.* **101**, 2411–2415.

GIULIAN, D., TOMOZAWA, Y., HINDMAN, H. & ALLEN, R. L. (1985). Peptides from regenerating central nervous system promote specific populations of microglia. *Proc. natn. Acad. Sci. U.S.A.* **82**, 4287–4290.

GIULIAN, D. & YOUNG, D. G. (1986). Brain peptides and glial growth. II. Identification of cells that secrete glial promoting factors. *J. Cell Biol.* **102**, 812–820.

HOWES, E. A., SMITH, P. J. S. & TREHERNE, J. E. (1987*a*). Glial repair in the cultured central nervous system of an insect. *Cell Tissue Res.* **247**, 111–120.

HOWES, E. A., SMITH, P. J. S. & TREHERNE, J. E. (1987*b*). Reactive cells and their role in glial regeneration in an insect CNS. *Am. Zool.* (in press).

KUO, M. T. (1981). Preferential damage of active chromatin by bleomycin. *Cancer Res.* **41**, 2439–2443.

LEECH, C. A. (1984). Effects of ethidium bromide on cells of the cockroach central nervous system. *J. Cell Sci.* **70**, 17–24.

MORGESE, V. J., ELLIOTT, E. J. & MULLER, K. J. (1983). Microglial movements to sites of nerve lesion in the leech CNS. *Brain Res.* **272**, 166–170.

NELSON, J. W. & TINOCO, I., JR (1984). Intercalation of ethidium ion into DNA and RNA oligonucleotides. *Biopolymers* **23**, 213–233.

PERRY, V. H., HUME, D. A. & GORDON, S. (1985). Immunohistochemical localization of macrophages and microglia in the adult and developing mouse brain. *Neuroscience* **15**, 313–326.

RAFF, M. C., MILLER, R. H. & NOBLE, M. (1983). A glial progenitor cell that develops *in vitro* into astrocytes or an oligodendrocyte depending on culture medium. *Nature, Lond.* **303**, 390–396.

RIO-HORTEGA, P. DEL (1932). Microglia. In *Cytology and Cellular Pathology of the Nervous System*, vol. 2 (ed. W. Penfield), pp. 481–584. New York: Paul B. Hocker.

SCHOFIELD, P. K., SWALES, L. S. & TREHERNE, J. E. (1984). Potentials associated with the blood–brain barrier of an insect: recording from identified neuroglia. *J. exp. Biol.* **109**, 307–318.

SCHOFIELD, P. K. & TREHERNE, J. E. (1984). Localization of the blood–brain barrier in an insect: electrical model and analysis. *J. exp. Biol.* **109**, 319–331.

SMITH, P. J. S. & HOWES, E. A. (1984). Glial toxin effect on protein synthesis in an insect connective. *J. Cell Sci.* **70**, 83–92.

SMITH, P. J. S. & HOWES, E. A. (1987). Neural repair in an insect central nervous system: cell kinetics and proliferation after selective glial disruption. *Cell Tissue Res.* **247**, 129–135.

SMITH, P. J. S., HOWES, E. A., LEECH, C. A. & TREHERNE, J. E. (1986). Haemocyte involvement of the insect central nervous system after selective glial disruption. *Cell Tissue Res.* **243**, 367–374.

SMITH, P. J. S., LEECH, C. A. & TREHERNE, J. E. (1984). Glial repair in an insect central nervous system: effects of selective glial disruption. *J. Neurosci.* **4**, 2698–2711.

TREHERNE, J. E., HARRISON, J. B., TREHERNE, J. M. & LANE, N. J. (1984). Glial repair in an insect central nervous system: effects of surgical lesioning. *J. Neurosci.* **4**, 2689–2697.

TREHERNE, J. E., HOWES, E. A., LEECH, C. A. & SMITH, P. J. S. (1986). The effects of an anti-mytotic drug bleomycin on glial repair in an insect central nervous system. *Cell Tissue Res.* **243**, 375–384.

TREHERNE, J. E., HOWES, E. A. & SMITH, P. J. S. (1987*a*). Glial repair in an insect. *J. Physiol., Paris* (in press).

TREHERNE, J. E., SMITH, P. J. S. & EDWARDS, H. (1987*b*). Neural repair in an insect: cell recruitment and deployment following selective glial disruption. *Cell Tissue Res.* **247**, 121–128.

YAJIMA, K. & SUZUKI, K. (1979). Ultrastructural changes of oligodendroglia and myelin sheath induced by ethidium bromide. *Neuropath. appl. Neurobiol.* **5**, 49–62.