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The Response of The Cerebral Hemisphere of The Rat To Injury. II. The Neonatal Rat

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THE RESPONSE OF THE CEREBRAL HEMISPHERE OF THE RAT TO INJURY. II. THE NEONATAL RAT

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[Plates 1–7]

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The response to injury of the cerebrum of the neonatal rat was studied in knife wounds by using both light and electron microscopical, and immunohistochemical, techniques. The rats were injured at 2, 4, 8, 12, 16 and 20 days *post natum* and the tissues examined 8 days later.

A mature scar, that is, a layer of fibrous tissue separated from the injured neuropile by a glia limitans, is not formed in the brains of rats lesioned before 8 days *post natum*. Before this time, the neuropile of the severed hemisphere grows together and both the

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glia limitans externa and ventricular lining are repaired. The only evidence of the wound, 20 days after injury, is a subpial and periventricular accumulation of astrocytes and occasional groups of blood vessels; elsewhere glial and neuronal processes traverse the wound obliterating all signs of the original lesion.

After 8 days *post natum*, scar tissue is deposited. The scar first appears in the superficial cortex as fibroblasts and macrophages invade from the meninges. With increasing age at injury, these cells penetrate more deeply and, after 16 days *post natum* at injury, the entire lesion contains these cells. Concomitantly, a glia limitans is formed over the walls of the lesion, firstly in the superficial cortex continuous with the glia limitans externa, and successively in the deeper cortex, white matter and corpus striatum as the meningeal fibroblasts and macrophages invade these regions.

In the developing cerebrum, injured before 8 days *post natum*, the failure to form a scar is unrelated to the maturity of the astrocytes and fibroblasts, because both interact to regenerate the glia limitans externa. The development of a scar, in animals injured after 8 days *post natum*, is correlated with the failure of both axonal and dendritic regeneration. Because there are few oligodendrocytes, and no myelin, it appears that inhibition of axonal and dendritic growth is linked to scar formation, and not to putative inhibitory substrates such as those on the surface of oligodendrocytes. CNS scarring may be initiated by the invasion of fibroblasts and macrophages from the meninges into the injured neuropile. The possible reasons why these mesenchymal cells fail to penetrate before 8 days *post natum* are discussed.

Introduction

Scar tissue develops in lesions of the brains of mature rats by 8 days after injury (Maxwell *et al.* 1990). A glia limitans, continuous with the glia limitans externa, covers the opposing walls of the wound and encloses a central core of mesenchymal tissue derived from the meninges. This glial collagen scar forms a barrier which growing neural processes do not penetrate (Clemente 1964; Windle 1956). In contrast, very immature brains do not develop scar tissue after injury (Berry *et al.* 1983; Clearwaters 1954; De Meyer 1967; Hess 1956; Prendergast & Stelzner 1976*a*, *b*; Maxwell *et al.* 1984; Reinis 1965; Sumi & Hager 1968), and axons and dendrites traverse the lesion by growth *de novo*, or by true regeneration (Kalil 1988; Kalil & Reh 1979, 1982). Thus studies of the injury response of the neonatal brain offer an approach for investigation of both the mechanisms of scarring in the brain and of arrested axonal regrowth after injury. For example, axonal growth fails at a time when the ability to produce scar tissue is acquired by the neonatal brain, and also when myelination begins (Berry *et al.* 1983). Both observations are relevant to hypotheses that implicate scarring (Reier & Houle 1988), myelin breakdown products (Berry 1982) and oligodendrocytes (Caroni & Schwab 1988) in the abortive regenerative response of severed mature axons.

Accordingly, we have analysed the reaction of glial and mesodermal elements in neonatal cerebral wounds and the growth of axons and dendrites across the lesion both electron microscopically and by using antibodies to glial cell proteins.

Materials and methods

(*a*) *Preparation of lesions for microscopy*

Neonatal rats of either sex, selected randomly from standard sized litters (Maxwell *et al.* 1990), were lesioned under Avertin (Tribromoethanol) anaesthesia by a free-hand incision

medial to the superior temporal line through the posterior part of the frontal bone and the anterior part of the parietal bone. This procedure lesions the frontal pole of the cerebral hemisphere. Groups of rats, each containing five animals, were lesioned when 2, 5, 8, 12, 16 or 20 days old and killed 8 days later. None of the animals was a litter-mate.

Animals were anaesthetized and perfused through the left ventricle at atmospheric pressure for 5 min with glutaraldehyde (5% by volume) in 0.1 M phosphate buffer, pH 7.2, with 20 g l^{-1} sucrose added to give an osmolarity of 650 mOsm†. The excised brain was immersed in fixative for 1 h at 4 °C. The lesion was cut coronally into 1.5 mm slices, re-immersed in fixative for 1 h, washed in buffer overnight, post-fixed in osmium tetroxide (1% by volume) in 0.1 M phosphate buffer, pH 7.2 containing 20 g l^{-1} sucrose for 1 h at room temperature, before ethanol dehydration, and embedding in TAAB epoxy resin via propylene oxide.

For light microscopical examination, three 1.5 μm thick coronal serial sections of the whole half cerebrum were cut on 1.0 cm glass knives using a Reichert Ultracut Om U4 ultramicrotome. The sections were floated onto an aqueous ammonia (1% by volume) solution and flattened in an atmosphere saturated with chloroform. They were dried onto slides and stained with alkaline Toluidine Blue.

For electron microscopy, 2.5 mm × 2.5 mm mesas were made to include selected areas of the lesion. Sections were cut on glass or diamond knives, mounted on grids, stained with uranyl acetate and lead citrate, and examined with a Phillips 201, 301, or Jeol 100S electron microscope. Any ultrastructural detail could be located accurately within the semi-thin sections.

(*b*) *Immunohistochemistry*

Animals were anaesthetized with Avertin, and perfused through the left ventricle at atmospheric pressure for 5 min with paraformaldehyde (4% by volume) in phosphate buffered saline (PBS) at pH 7.4, washed overnight in PBS at 4 °C, dehydrated through graded ethanols, infiltrated with polyester wax at 37 °C (Steedman 1957) and stored at 4 °C. Sections, 7 μm thick, were cut on a microtome fitted with a cooled chuck, and the ribbons floated out on a gelatin solution (10 g l^{-1}) on subbed slides and allowed to dry in air.

Sections were dewaxed, rehydrated, washed for 15 min in PBS containing Tween-20 (0.1% by volume), and incubated with rabbit anti-bovine glial fibrillar acid protein (GFAP) (Dakopatt Ltd, High Wycombe, U.K.) at a dilution of 1:200; rabbit anti-mouse sarcoma laminin (Bethesda Research Labs, supplied by Gibco, Paisley, U.K.) at a dilution of 1:100 and rabbit anti-cow S-100 protein (Dakopatt Ltd) at a dilution of 1:200 according to the methods described by Maxwell *et al.* (1990). For controls either the primary, or secondary antibody was omitted; these were consistently negative.

(*c*) *Quantitative analysis*

For each experimental age, one lesion was selected randomly for quantitative analysis. The problems associated with the use of a single animal at each experimental age are discussed in the previous paper (Maxwell *et al.* 1990). The analysis was confined to the mesenchymal cells because it proved impossible to differentiate with certainty between the different types of glia in the brains of neonatal animals (Ling *et al.* 1973; Parnavelas *et al.* 1983). The mesenchymal cells were identified according to the criteria given by Maxwell *et al.* (1990). The cells were

† One osmole contains one mole of osmotically active particles.

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counted and the data were analysed as described in the previous paper (Maxwell *et al.* 1990). The resulting scatterplots show the variation in cell number with distance from the lesion midline at different experimental times (figure 14).

Results

The neonatal brains were lesioned when the rats were either 2, 5, 8, 12, 16 or 20 days old. It was decided to examine all the brains 8 days after injury, because, from a study of lesioned adult rat brains (Maxwell *et al.* 1990), it is known that scar tissue formation is most active at this time.

(*a*) *Microscopical studies of the lesioned areas*

(i) *Animals lesioned when* 2 *and* 5 *days old*

There is no evidence of gliosis or cell swelling within the wounds of these rats. The neuropile at the edges of the lesion has grown together and there is no scar tissue (figures 1–4). Occasional

Abbreviations used on figures

A	astrocyte	ap	astrocyte process
BV	blood vessel	cf	collagen fibrils
E	ependymal cells		
F	fibroblast		
GLE	glia limitans externa		
M	macrophage		
NE	intact neuropile		
V	ventricle		

Description of plate 1

Figure 1. Electron micrograph of the pial surface of a lesion made in a 2-day-old rat. The position of the lesion (arrow) is marked only by the accumulation of astrocytes (A) below the depression in the pial surface. The glia limitans external (GLE) has regenerated and the surface is folded. It is covered by the fibroblasts (F), blood vessels (BV) and matrix containing collagen fibrils (cf) of the pia mater. (Magn. ×3050.)

Figure 2. Electron micrograph to show the expanded lining of the ventricle (V) in a lesion made in a 2-day-old rat. The ependymal lining has not regenerated and the neuropile (NE) of the cortex is separated from the ventricle (V) by astrocytes (A) and macrophages (M). Many enlarged extracellular spaces (arrows) are present in the neuropile. (Magn. ×3050.)

Description of plate 2

Figure 3. Photomicrograph of the cerebral cortex of a lesion made in a 2-day-old rat. The lesion is marked by darkly-stained cells (arrows). There is no scar tissue and the neuropile (NE) bridges the lesion. (Magn. ×135.)

Figure 4. Photomicrograph of the deeper region of a lesion made in a 2-day-old rat to show the ventricle (V). There are many blood vessels (BV) along the site of the lesion (arrows) which is healed. The ventricle is lined by attenuated ependymal cells (E). (Magn. ×135.)

Figure 5. Electron micrograph to show the pial surface away from the lesion made in a rat 5 days old. The glia limitans externa (GLE) has regenerated and it overlies several layers of astrocytes (A) which separate it from the neuropile (NE). Fibroblasts (F) of the meninges cover the surface. (Magn. ×3600.)

Figure 6. Higher magnification electron micrograph of the pial surface of a rat lesioned at 5 days old to show the healed glia limitans externa (GLE). A fibroblast (F) lies on the outer surface which is covered by the basement membrane (arrow heads). There are many layers of astrocyte processes (ap). (Magn. ×10800.)

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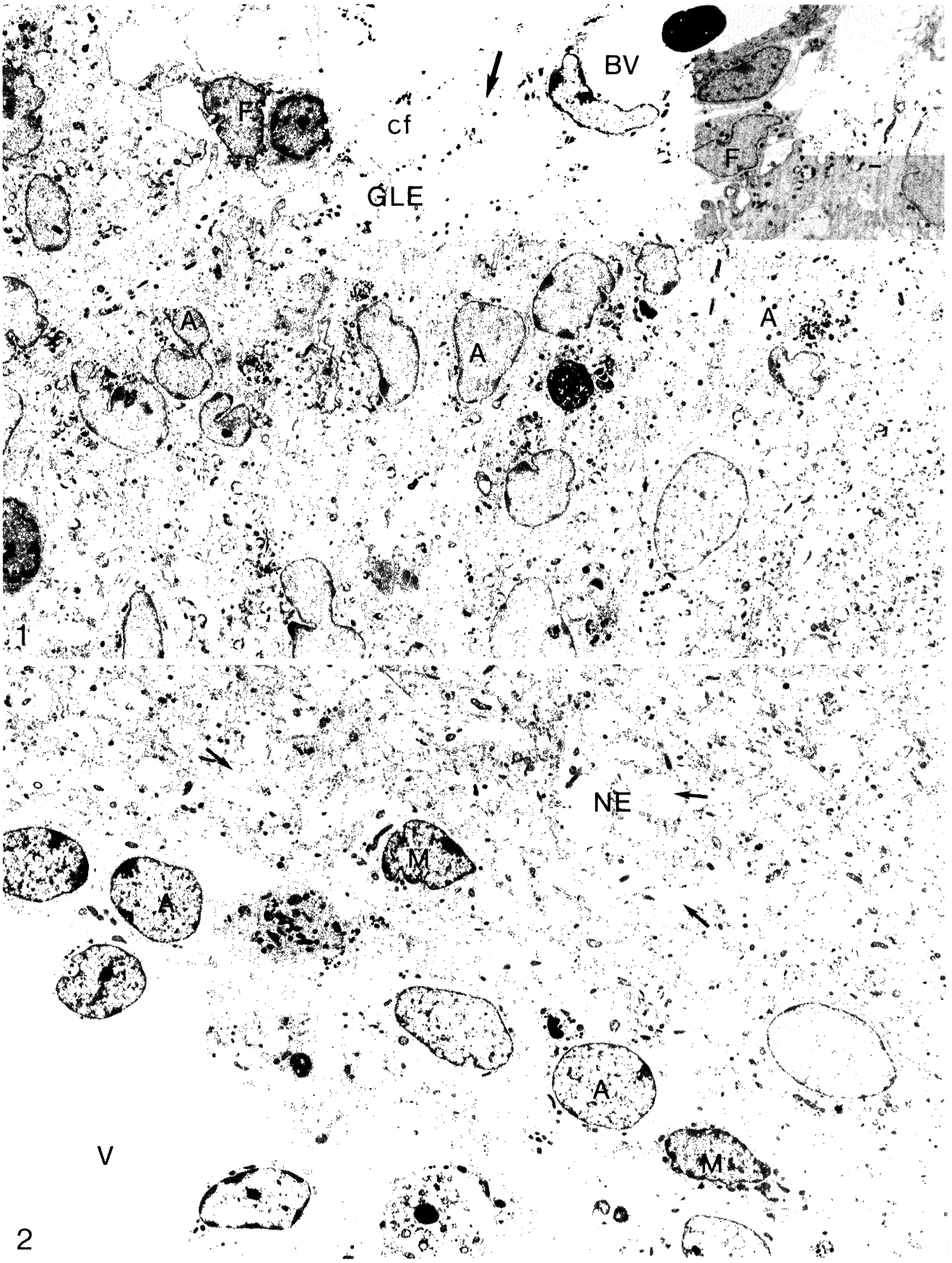

FIGURES 1 AND 2. For description see opposite.

(*Facing p.* 504)

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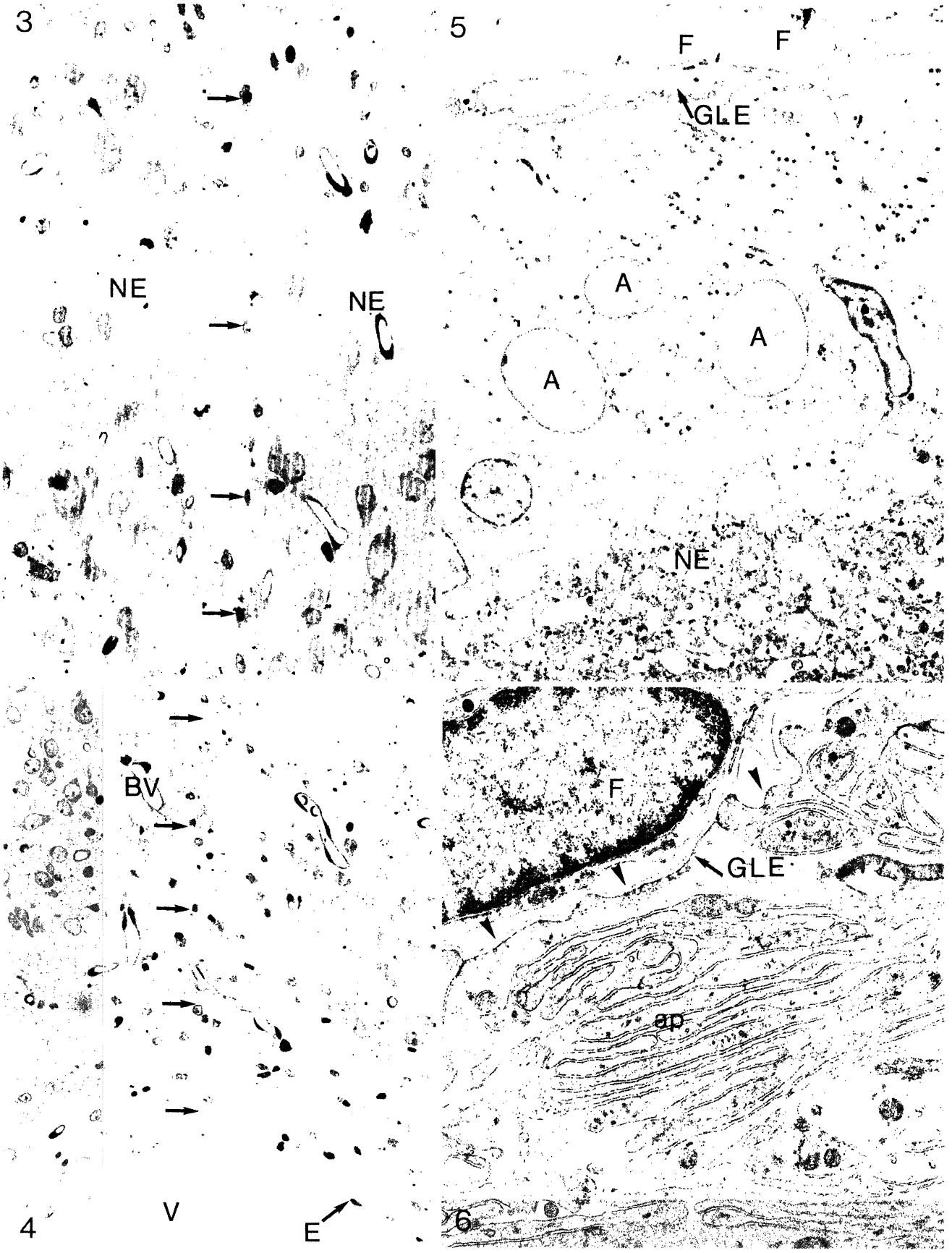

FIGURES 3–6. For description see p. 504.

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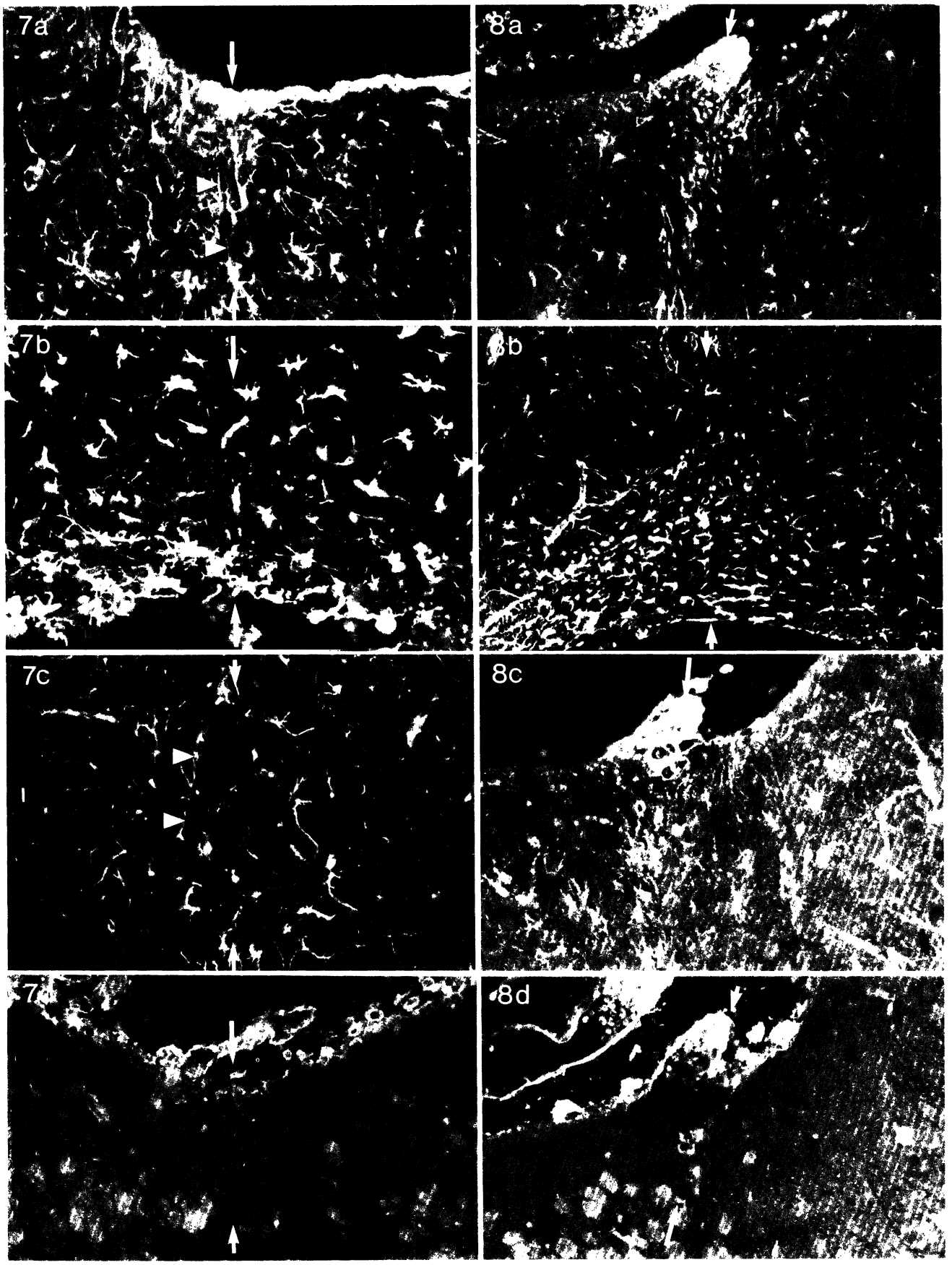

FIGURES 7 AND 8. For description see facing plate 6.

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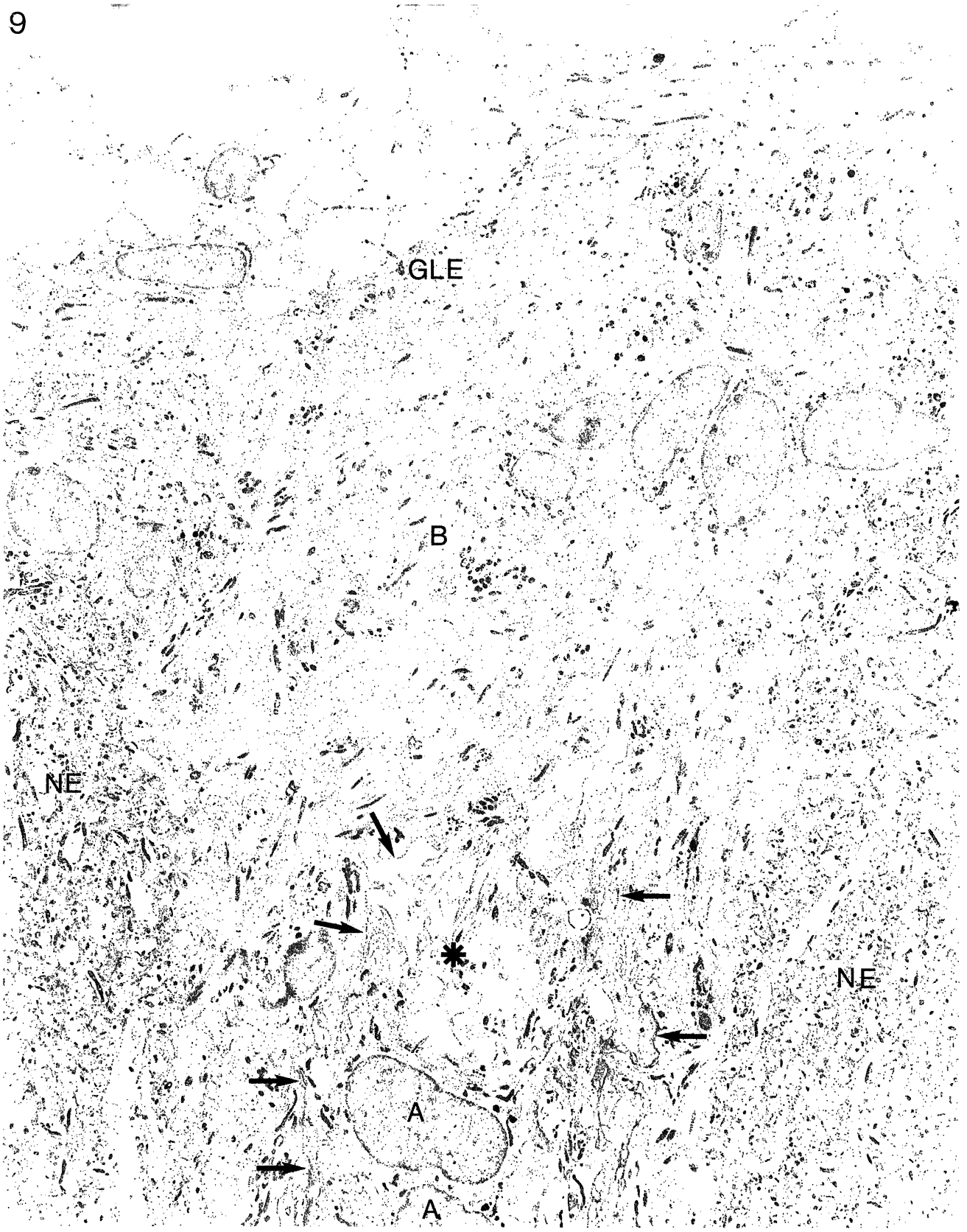

FIGURE 9. For description see facing plate 6.

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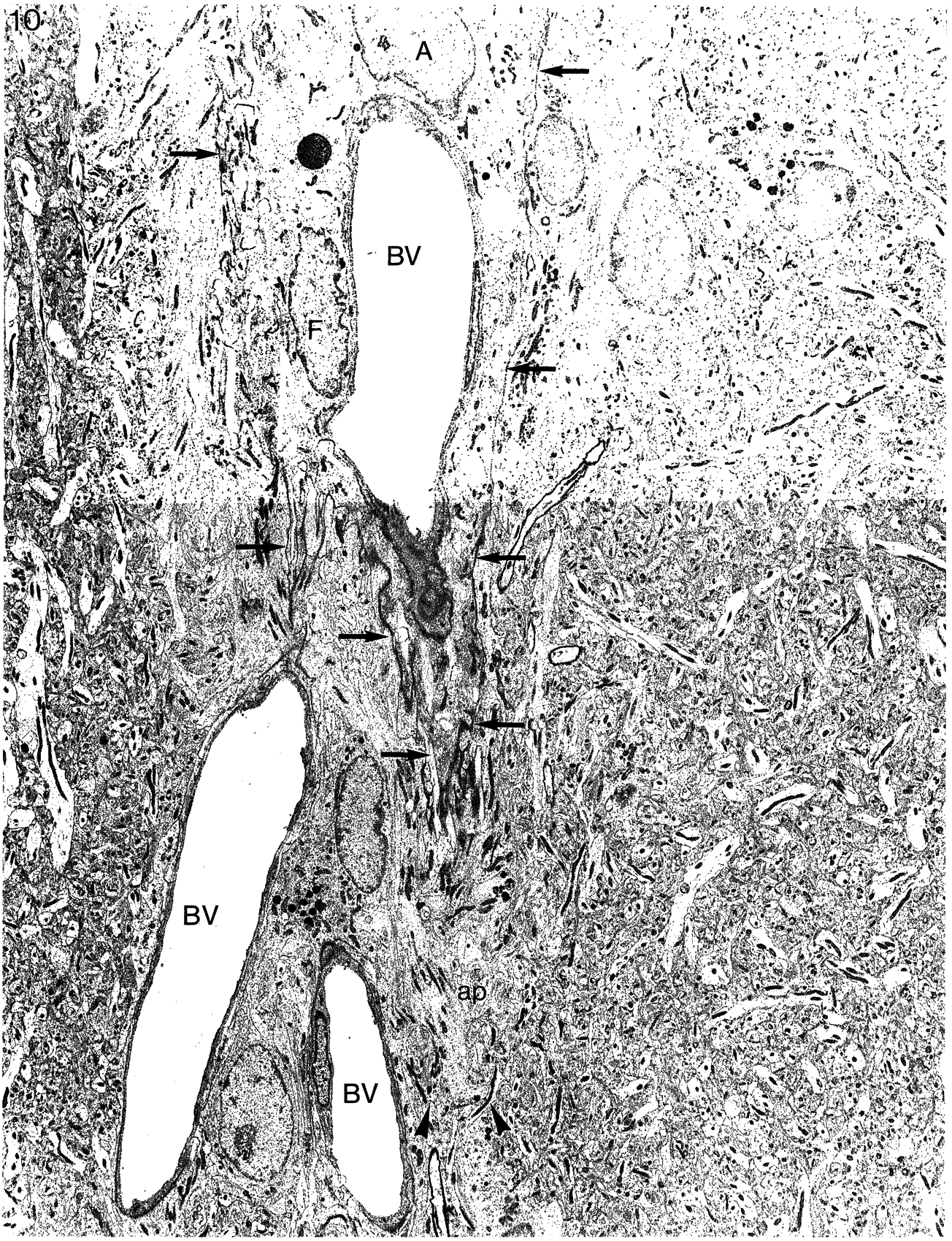

FIGURE 10. For description see p. 505.

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Description of plate 3

Figure 7. Photomicrographs to show the binding of antibodies to GFAP (*a*) and (*b*), S-100 (*c*) and laminin (*d*) by a lesion made in a rat 2 days old. The position of the lesion is indicated by arrows. Astrocytes bind the antibodies in (*a*), (*b*), (*c*); accumulations are seen in the glia limitans externa in (*a*) and lining the ventricle in (*b*). The astrocyte processes are not orientated towards the lesion as they are in the adult lesions (arrowheads). Laminin is restricted to the basement membrane of the glia limitans externa seen in (*d*). (Magn. $\times 240$.)

Figure 8. Photomicrographs to show the binding of antibodies to GFAP (*a*) and (*b*), S-100 (*c*) and laminin (*d*) by a lesion made in a rat 5 days old; the position of the lesion is indicated by arrows. Astrocytes bind the antibodies in (*a*), (*b*) and (*c*). They are grouped at the pial surface to give a region of intense fluorescence in (*a*) and (*c*). There are many astrocytes near the ventricle (V) in (*b*). Laminin is restricted to the basement membrane of the glia limitans externa in (*d*). (Magn. (*a*), (*c*) and (*d*) $\times 240$, (*b*) $\times 120$.)

Description of plate 4

Figure 9. Electron micrograph of the pial surface of a lesion made in a rat 8 days old. The pial surface has healed and the glia limitans externa (GLE) has regenerated to form a bridge (B) across the lesion. The scar tissue extends between the normal neuropile (NE) of the cortex. An area enclosing subarachnoid space (*) is present beneath the bridge of astrocytes; this is presumably continuous with the subarachnoid space of the pia. A glia limitans (arrows) separates the neuropile from an extraparenchymatous astrocyte (A) and the scar tissue. The continuation of this scar is seen in figure 10; the astrocyte nucleus at the bottom of this figure belongs to the same cell as that at the top of figure 10. (Magn. $\times 3050$.)

Description of plate 5

Figure 10. Electron micrograph of the deeper part of the lesion seen in figure 9. The glia limitans (arrows) encloses the scar tissue in which there is an extraparenchymatous astrocyte (A), blood vessels (BV) and a fibroblast (F). The astrocyte processes (ap) of the glia limitans extend below the scar tissue, but then are replaced by neuropile (arrowheads). (Magn. $\times 3050$.)

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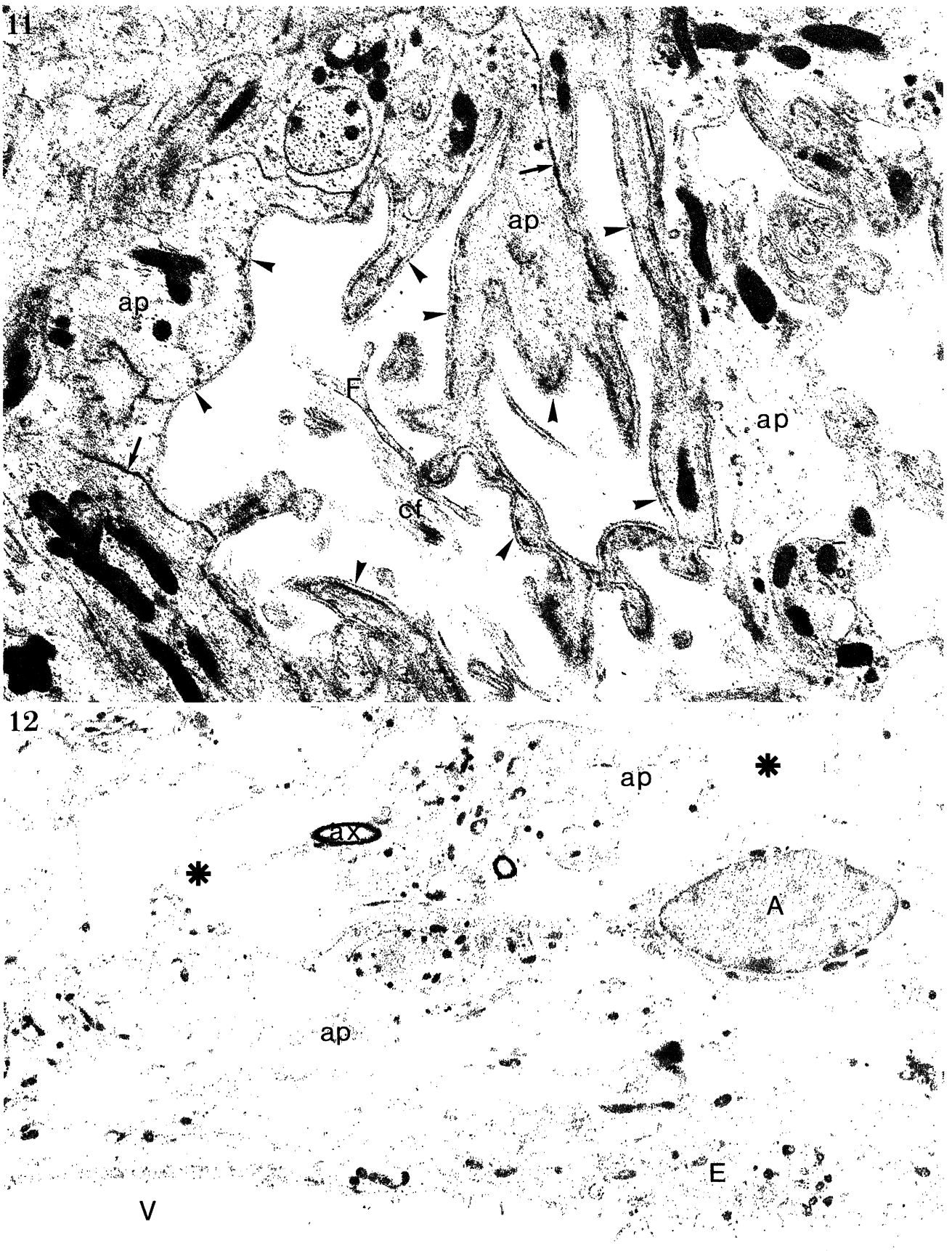

FIGURES 11 AND 12. For description see p. 505.

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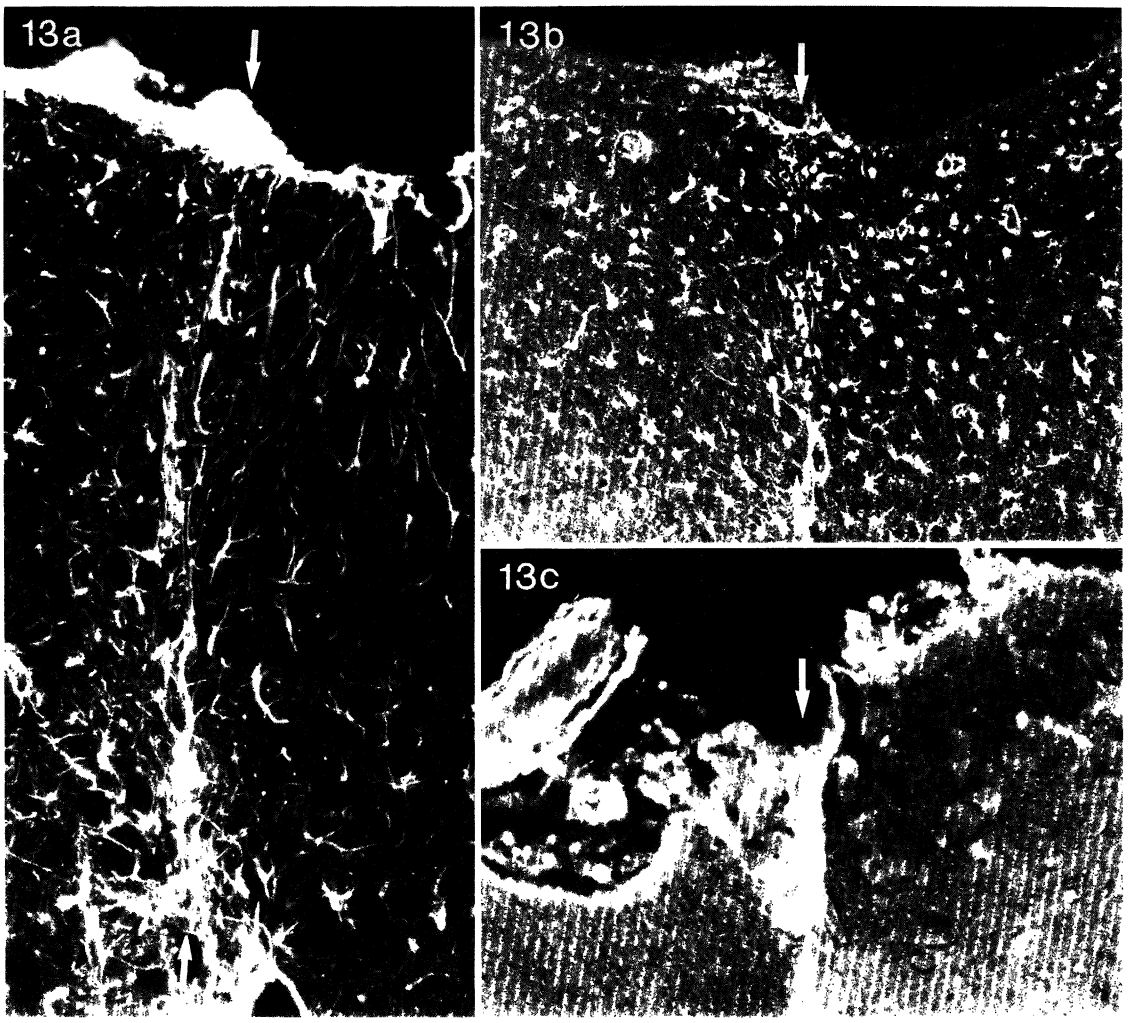

FIGURE 13. For description see opposite.

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macrophages and large blood vessels are found along the site of the lesion in the cortex (figure 3). Few of the astrocytes contain the S-100 protein or are 'reactive', i.e. contain GFAP, in 2-day-old animals (figure 7*a–c*). The astrocytic processes are short, with no preferential orientation. Many astrocytic and neuronal processes traverse the wound site and obliterate all signs of the lesion. Growth cones are found throughout the neuropile and synapses are also seen.

At the pial surface, the glia limitans is reconstituted (figures 1, 5 and 6). There is often a small surface depression at the site of lesion, which is filled by meninges (figure 1). Intensely reactive, subpial astrocytic processes (figures 7*a* and 8*a*, *c*) interlace to form an elaborately folded surface covered with a basement membrane (figure 6) which contains type IV collagen and laminin (figures 7*d* and 8*d*) (Maxwell *et al.* 1984). In 5-day-old rats, the astrocytic reaction at the pial surface is more intense than in younger rats (figure 8*a*). Many reactive astrocytes accumulate in the lesion in the superficial cortex and at the site of transection of the cortical white matter (figure 8*b*).

The ventricle in the injured hemisphere is always enlarged and, if penetrated by the lesion, may have no ependymal lining; in this case, intensely 'reactive' astrocytes line the ventricular surface (figures 2, 4, 7*b* and 8*b*). Long, thin, intensely reactive astrocytic processes (figures 7*b* and 8*b*) enclose large spaces which give the tissue a characteristic honeycomb-like appearance (see figure 2). Where the ventricular surface is re-epithelialized, the ependymal cells are attenuated (figure 4). The neuropile near the hydrocephalic ventricle is more loosely packed than elsewhere. The axons in the periventricular white matter have degenerated and there are many neurons with pyknotic nuclei. The interrupted corpus callosum is starting to myelinate and contains numerous presumptive oligodendrocytes.

(ii) *Animals lesioned when* 8 *and* 12 *days old*

Many mesenchymal cells are now found in the wound. They penetrate more deeply into the lesion from the pial surface in the 12-day-old animals. Collagen fibrils are present in the developing scar in the cortex of 8-day-old animals (figure 11), but, in the 12-day-old animals, fibrils are also found in the corpus callosum. A glia limitans separates the highly vascularized developing scar tissue from the neuropile in the cortex (figures 9 and 10). The glia limitans externa is continuous with the newly formed glia limitans of the lesion as shown by the presence

Description of plate 6

Figure 11. Higher magnification electron micrograph of the enclosed region of subarachnoid space (*, figure 9) to show the glia limitans externa which is made up of astrocyte processes (ap) held together by cell junctions (arrows) and their basement membrane (arrowheads). The cavity encloses cell processes, presumably of fibroblasts (F), collagen fibrils (cf) and the processes of extraparenchymatous astrocytes. (Magn. ×15300.)

Figure 12. Electron micrograph of the surface of the ventricle (V) below a lesion made in a rat 8 days old. The ependyma has regenerated, but the cells (E) are attenuated. The white matter above the ventricle contains a few axons (ax) and many astrocytes (A) and their processes (ap). There are large extracellular spaces (*). (Magn. ×4950.)

Description of plate 7

Figure 13. Photomicrographs to show the binding of antibodies to GFAP (*a*), S-100 (*b*) and laminin (*c*) by a lesion (indicated by arrows) made in a rat 8 days old. There is strong binding of the antibodies to GFAP, but not to S-100 in the astrocytes at the pial surface. In the superficial region of the cortex the astrocyte processes are aligned along the scar tissue (*a*). Laminin is restricted to the basement membrane of the regenerated glia limitans externa and the glia limitans of the scar in the superficial cortex (*c*). (Magn. (*a*) and (*c*) ×240, (*b*) ×120.)

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of laminin in the basement membranes (figure 13*c*). In some regions of the wounds of 8-day-old rats, the glia limitans externa has regenerated to bridge the wound and entraps meningeal tissue including extraparenchymatous astrocytes (figures 9 and 11). Thus the glia limitans of the cortical scar of 8-day-old rats is often discontinuous and elaborately folded. Below the scar tissue, the site of the lesion is marked by an accumulation of large blood vessels (figure 10). In 8-day-old rats, the astrocytic processes of the glia limitans in the cortex are aligned along the plane of the wound (figure 13*a*, *b*). However, in the corpus callosum and the deeper parts of the lesion of the 8-day-old rats, the immunoreactivity of the astrocytes is more intense than in those injured at 5 days old; the astrocytic processes cross the wound and do not form a glia limitans. In such regions neural processes also cross the lesion.

In the 12-day-old animals, the glia limitans of the scar extends into the corpus callosum, where it is discontinuous; fibroblasts and macrophages may be opposed to it. In the corpus striatum, astrocytes, with no basement membrane, are found in the lumen of the lesion which is also traversed by neural processes. Axonal growth is inhibited where a scar is formed, since neuromata are not found in the walls of the scar.

Large empty cysts are frequently present in the deeper parts of the cortical lesion, the walls of which are lined by macrophages and astrocytes juxtaposed to healthy neuropile. These cysts may either communicate with, or be isolated from, the ventricle. The ependyma of the ventricle forms an attenuated epithelium that covers a layer of loosely packed astrocytes and macrophages in which a few myelinated and non-myelinated axons are seen (figure 12).

(iii) *Animals lesioned when* 16 *and* 20 *days old*

By the age of 16 days, the rat has acquired the ability to produce a mature, vascularized scar throughout the lesion; it is identical to that produced by the 30-day-old rat (see Maxwell *et al.* 1990).

(*b*) *Quantitative analysis*

No mesenchymal cells are found in cerebral lesions of 2-day-old animals (figure 14*a*, *b*). A few macrophages (figure 14*a*) are present in the wounds of 5-day-old rats, but higher frequencies are found in the wounds of 8- and 12-day-old rats. The number of macrophages found in the lesions of 16–20-day-old rats reaches normal adult values (Maxwell *et al.* 1990). In 5-day-old rats, the few fibroblasts are confined to the superficial cortex associated with the

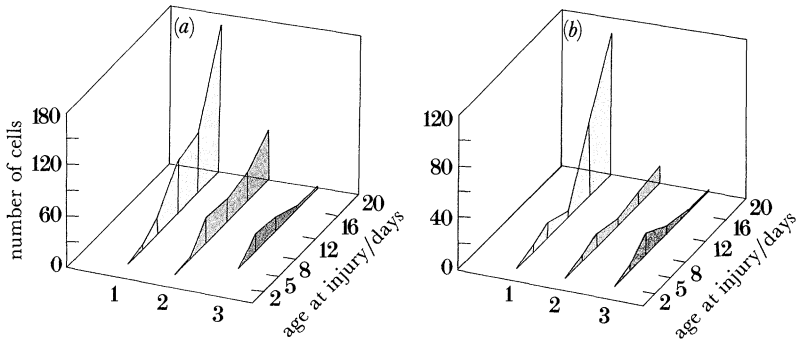

FIGURE 14. Graphs to show the numbers and distribution of the mesenchymal cells associated with the lesion with increasing distance from the midline in animals lesioned at different ages. (*a*) macrophages; (*b*) fibroblasts. Key: 1, cells between midline and 100 μm on either side; 2, cells between 100 and 200 μm on either side; 3, cells between 200 and 300 μm on either side.

meninges (figure 14*b*). Fibroblasts remain restricted to the cortex in 8-day-old rats, but in the 12-day-old animals, fibroblasts have invaded the region of the corpus callosum and in the 16–20-day-old animals, adult numbers are found throughout the lesion.

Discussion

The observations reported here show that in lesions of the cerebral cortex, scar tissue forms only if the rats are 8 or more days old at injury. In younger rats, a glia limitans is formed only in the cortex, but when the animal is 16 days old at injury, a glia limitans is formed throughout the depth of the lesion within 8 days. Before the rat is 8 days old, lesions are bridged by glial cells and neuronal processes. The damaged glia limitans externa repairs, while there is incomplete re-epithelialization of the damaged ependyma of the lateral ventricle.

(*a*) *Repair of the ependymal lining of the lateral ventricle*

Most of the experimental animals developed hydrocephalus and the ventricular lining was repaired by ependymal cells and/or astrocytes derived from the subependymal plate cells which proliferate in response to trauma (Bruni *et al.* 1983; Garfia *et al.* 1980; Lewis 1968; Paterson *et al.* 1973; Weller *et al.* 1978; Willis *et al.* 1976). Collins & Fairman (1990) described ependymal tears in the expanded hydrocephalic lateral ventricles of rats, which were invaded by small round subependymal cells within 48 h. None of these cells differentiate into typical cuboidal, ciliated, ependymal cells, but instead, develop into astrocyte-like cells within 8 days, that is, the same period after which a reactive astrocytic response was observed in the present investigations. Thus the stimulus for the accumulation of the many, GFAP-reactive astrocytes seen in the ventricular lesions is probably compounded of both the original penetrant injury, and the consequent ipsilateral hydrocephalus that separates the margins of the ependymal wound. The latter may augment the mitogenic stimulus to both the subependymal cells and the deeply lying cortical astrocytes.

(*b*) *Repair of the glia limitans externa*

The complete repair of the glia limitans externa observed in animals injured before they are 8 days old, demonstrates the maturity of interactions between glial and mesenchymal cells at the pial surface over this period. The reformation of the neonatal glia limitans externa is dependent upon the juxtaposition of fibroblasts, macrophages and astrocytes at the pial surface (Boya *et al.* 1979). It follows, therefore, that scarring is not initiated because fibroblasts and macrophages fail to invade the lesion in young animals. Lyser (1972) has also shown that in organ cultures, of the chick neural tube, a basal lamina is formed only if mesenchymal tissue is apposed to the developing cord surface.

In penetrant lesions of the mature cerebral hemisphere Cavanagh (1970) observed mitotic astrocytes in all cortical layers. However, Takamiya *et al.* (1988) found dividing astrocytes only in the molecular layer and white matter. A similar differential response of astrocytes to injury, may account for the localization of increased numbers of GFAP-reactive astrocytes to the subpial and periventricular regions of the cerebrum after injury in neonates (see figures 7*a*, *b* and 8*a*, *b*). Candidate molecules for the role of an exogenous astrocytic mitogen are numerous and include platelet derived growth factor (PDGF) and epidermal growth factor (EGF) (Nieto-Sampedro *et al.* 1985; Simpson *et al.* 1982; Takamiya *et al.* 1986; Westmark 1976);

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both these growth factors could be active in the neonate if the astrocytes have acquired the appropriate membrane receptor.

There is evidence that neonatal astrocytes, in both the sub-pial cortex and white matter, may be more mature than elsewhere (Parnavelas *et al.* 1983). Thus, in the molecular layer, the end-feet that form the inner lamina of the glia limitans externa are those of either established radial glia astrocytes, or astrocytes transforming from radial glia (Choi 1981; Choi & Lapman 1978, 1980; Hirano & Goldman 1988; Levitt & Rakic 1980; Rakic 1972, 1981; Schmechel & Rakic 1979). The latter cells are present in relatively high densities in the superficial layers of the neonatal visual cortex (Parnavelas *et al.* 1983). The present observations show that, after injury, the astrocytes of the surface wound are intensively reactive and accumulate to form a sub-pial layer several cells thick, the most superficial layer of which repairs the glia limitans externa.

(*c*) *The wound in the cortex of animals lesioned when less than* 8 *days old*

As discussed above, the failure of scarring in the cortex before the rat is 8 days old could be causally related to the failure of fibroblasts and macrophages to accumulate in the wound (see figure 14*a*, *b*). Fibroblasts probably move into brain lesions in response to the elaboration of fibroblast tropic or trophic factors; one such molecule is fibroblast growth factor (FGF) which is found in high titres in neonatal and adult brains (Gospodarowicz 1988; Logan *et al.* 1985). In the mature brain, elevated levels of FGF and of FGF mRNA are recorded during reactive gliosis and scarring (Finklestein *et al.* 1988; Logan 1988; Nieto-Sampedro *et al.* 1988). FGF is also angiogenic and this property could be responsible for capillary ingrowth into the brain during scarring (Risau 1986). FGF is present in the macrophages that invade a site of injury in the adult brain (Baird *et al.* 1986). Haematogenous macrophages are largely absent from neonatal brain wounds, except in the meninges (Boya *et al.* 1979). Their absence could be a factor in creating the observed differences between the reactions of neonatal and mature rat brains to injury, particularly if the release of brain-derived FGFs into the wound is crucial. One possible reason why few haematogenous macrophages invade neonatal brain wounds is that the cellular debris is rapidly ingested by the large numbers of microglia present (Jordan & Thomas 1988; Ling 1976, 1977; Parnavelas *et al.* 1983; Perry *et al.* 1985). Thus there is no stimulus to which haematogenous macrophages can respond.

In the intact neonatal cortex at the end of the first week of life, GFAP is present in the end-feet of astrocytes associated with the glia limitans externa and the cerebral vasculature (Bignami & Dahl 1973, 1974). The astrocytes in 14-day-old embryonic cerebral cortical transplants, grafted into adult host cortex, appear morphologically mature and synthesize GFAP by 5 days after implantation, which is equivalent to a foetal age of 19 days (Kruger *et al.* 1986). Kruger *et al.* (1986) grafted foetal cortex, aged between 14–18 days, into cavities in the adult cerebral cortex. Irrespective of the orientation of the grafts, complete integration, without scarring, occurred in the depths of the cavity. At the surface of the cavity, the host meninges covered the graft and a glia limitans was formed individually by the tissues of both the host and the graft. This suggests that all cortical astrocytes are competent to form a glia limitans before birth and will do so if juxtaposed to mesenchymal cells (Lyser 1972; Reier *et al.* 1983). If, however, foetal grafts are denuded of meninges and implanted deep into the brain, they fuse directly with the adult host tissue without the formation of an intervening glial scar (Das *et al.* 1980; Jaeger & Lund 1980; McLoon & Lund 1983; Oblinger *et al.* 1980).

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Transitory punctate laminin deposits which are probably secreted by astrocytes, are found along the path of pioneering axons during tract development (Cohen *et al.* 1987; Liesi & Ristelli 1989; Liesi & Silver 1988). The growth of neural processes across the lesion in neonatal rats could be similarly induced by laminin (Silver *et al.* 1982; Silver & Ogawa 1983), but we failed to find punctate laminin deposits using an antibody to mouse sarcoma laminin. By 8 days after injury, any transitory deposits may have disappeared.

Mature, differentiated oligodendrocytes proliferate in response to trauma within 3 days (Ludwin 1984, 1985; Ludwin & Bakker 1988); adult oligodendrocyte progenitor cells may react in a similar way to injury (Wolswijk & Noble 1989). The newly formed cells do not synthesize myelin proteins (Ludwin 1985). The formation of new oligodendrocytes is not, however, a significant morphological feature in either adult or neonatal wounds (Maxwell *et al.* 1990). Before the tenth postnatal day, there are few mature oligodendrocytes in the cortex (see Imamoto *et al.* 1978; Privat 1975; Parnavelas *et al.* 1983). Immature forms are abundant and highly mitotic during the phase of premyelination gliosis (Schonbach *et al.* 1968) and may originate from radial glial precursors (Hirano & Goldman 1988). Myelination of the corpus callosum of the rat begins between 10 and 14 days *post natum* (Hatotani & Timiras 1967; Jacobson 1963; Seggie & Berry 1972) and myelinated fibres first appear in the cortex at this time also (Parnavelas *et al.* 1983). Thus injury during the period of oligodendrocytosis, or myelination, does not appear to alter significantly the distribution of oligodendrocytes in the hemisphere and immature cells do not react to injury, or contribute to the scar (see also Maxwell *et al.* 1990).

(*d*) *The wound in the cortex of animals lesioned when more than* 8 *days old*

The ability to produce a mature scar in cortical lesions is acquired over the period from 8 to 16 days *post natum* when macrophages and fibroblasts accumulate in the wound (see figure 14*a*, *b*). At first, the glia limitans of the scar develops in continuity with the glia limitans externa, and subsequently invades the deeper parts of the wound progressively. It is unlikely that the haematogenous environment changes over this period and thus the acquisition of scarring by cerebral tissue is attributable to the maturation of mesenchymal–glial interactions. In the first week *post natum*, migratory neuronal movements cease, myelination starts and glia mature and establish their adult numbers (Berry & Rogers 1965; Lund & Mustari 1977; Parnavelas *et al.* 1983; Seggie & Berry 1972). After 8 days *post partum*, lesions in the cortex and deeper white matter interrupt myelinating axons and the ensuing degenerating myelin may be a stimulus for macrophage invasion. Macrophages secrete both FGF, which might induce the entry of fibroblasts, and also astrocytic mitogens which could augment exogenous agents like PDGF, EGF and glial maturation factor (Baird *et al.* 1986; Leibovich & Ross 1975, 1976; Lim 1985; Nieto-Sampedro *et al.* 1985, 1988; Takamiya *et al.* 1986). In turn, fibroblasts, in association with macrophages, might stimulate cortical astrocytes to produce GFAP, organize a glia limitans and secrete type IV collagen and laminin into the basement membrane (Bernstein *et al.* 1985; Liesi *et al.* 1984; Maxwell *et al.* 1984).

The present observations show that newly growing and regenerating nerve processes traverse a lesion in the early neonatal brain before a glia limitans is organized. Later, scar tissue develops at a time when nerve growth is retarded or inhibited, possibly by the development of non-permissive substrates, such as the surface of oligodendrocytes, or by myelin breakdown

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products (Berry 1982, 1983, 1985; Caroni & Schwab 1988). In rats aged between 8 and 10 days, myelination commences in the white matter and accordingly inhibition of axonal growth after lesions is expected to be greatest here (Seggie & Berry 1972). Paradoxically, cicatrization commences subpially and invades the white matter later. Axons grow in the deep regions of the lesion only where there is no glia limitans. By contrast, in the superficial cortex, there is inhibition of both regeneration and growth *de novo* of axons of newly migrated neuroblasts which are establishing the granular and supragranular cortical laminae (Berry *et al.* 1964; Berry & Rogers 1965). Thus axons fail to grow in this superficial neuropile in which there are few oligodendrocytes and no myelin. Indeed, over the period 8–12 days *post natum*, in the deeper parts of the cortex juxtaposed to the white matter where neural processes traverse the lesion, large numbers of oligodendrocytes are present and myelination is underway. These observations corroborate the findings of others (see, for example, Clemente 1964; Reier & Houle 1988; Windle 1956), that there is a strong correlation between the failure of central axonal regeneration and the development of scar tissue. This suggests that a common mechanism may be central to both events.

(*e*) *Conclusions*

A glial-collagen scar is first deposited around 8 days *post natum* in the cerebral wounds of rats. The primary event is the migration of macrophages and fibroblasts into the wound from the meninges. The influence of fibroblasts on astrocytes appears to be essential for the formation of the glia limitans. Before fibroblasts migrate into the lesion, a wound glia limitans is not formed. Macrophages may fail to move into cerebral lesions before 8 days *post natum* because the microglia ingest debris from the wound quickly and thereby remove the stimulus for macrophage invasion. Macrophages secrete fibroblast trophic or tropic factors that attract fibroblasts into the wound.

The present findings also clarify the role of scarring in axonal growth inhibition in relation to the development of putative growth inhibitory substrates. Both axonal regeneration and growth *de novo* are inhibited first in the superficial cortex where there are few oligodendrocytes and no myelin. Inhibition of axonal growth in the cortex is thus correlated only with the development of scar tissue, and is unrelated to myelination, or to the presence of oligodendrocytes.

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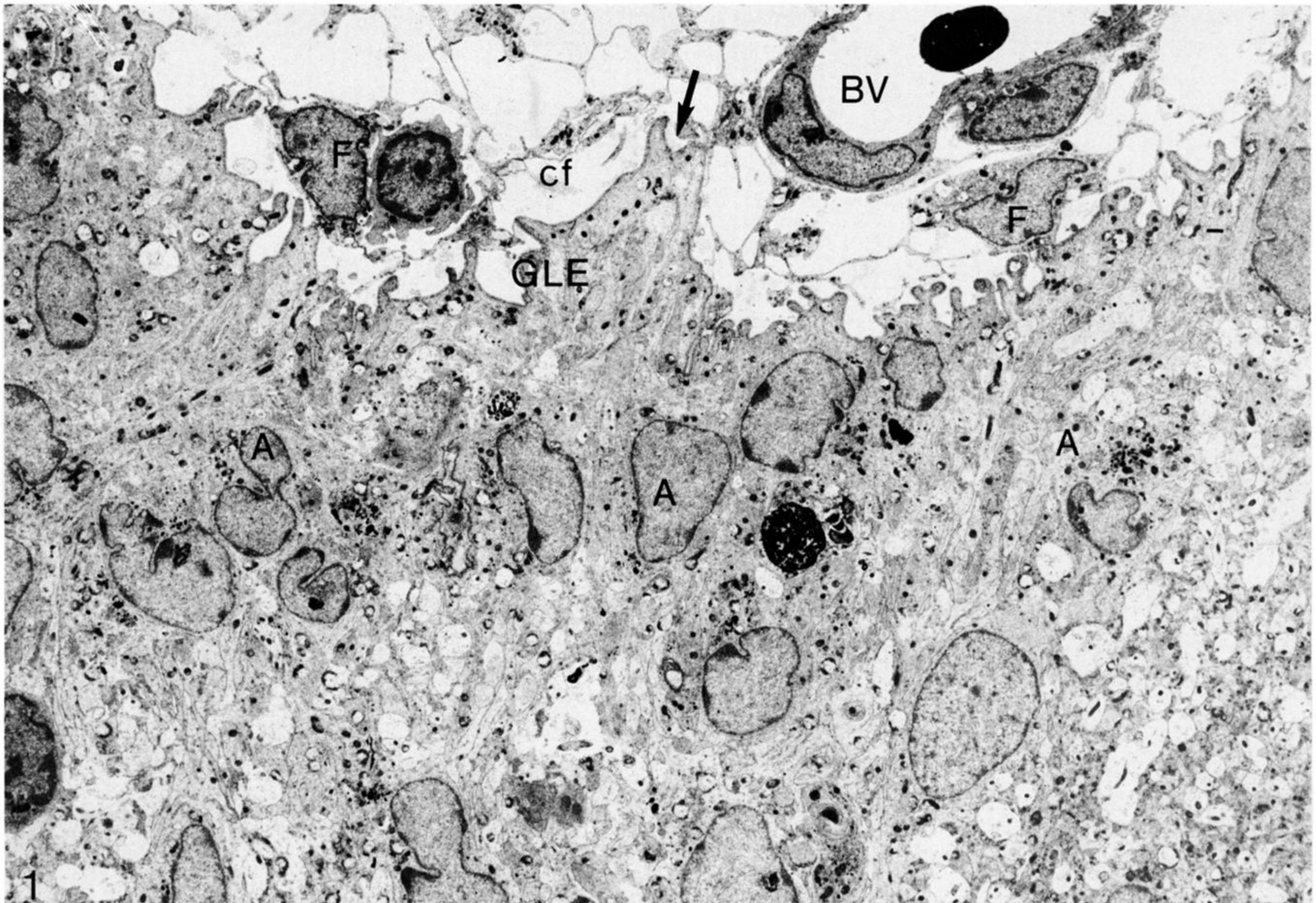
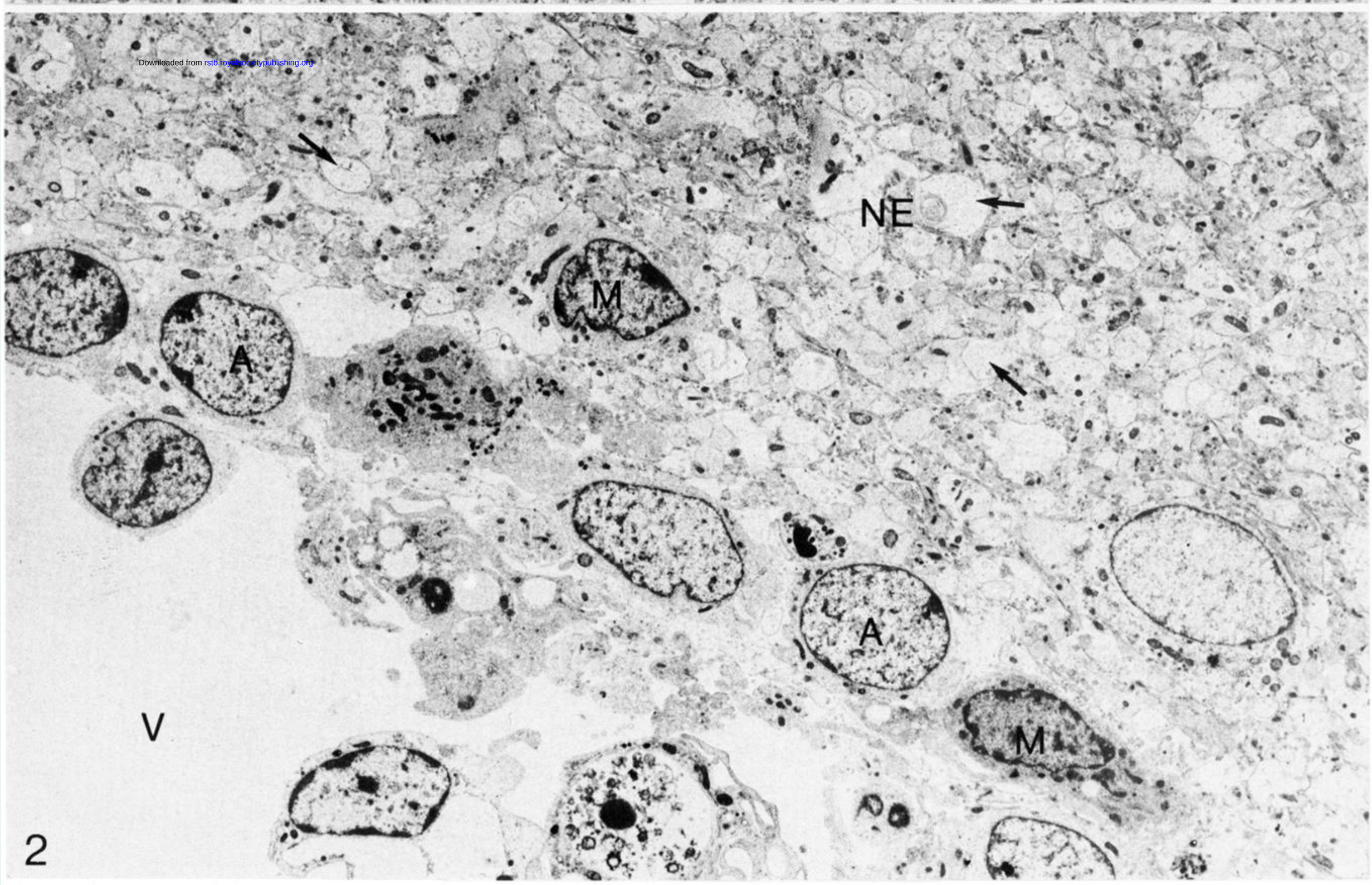
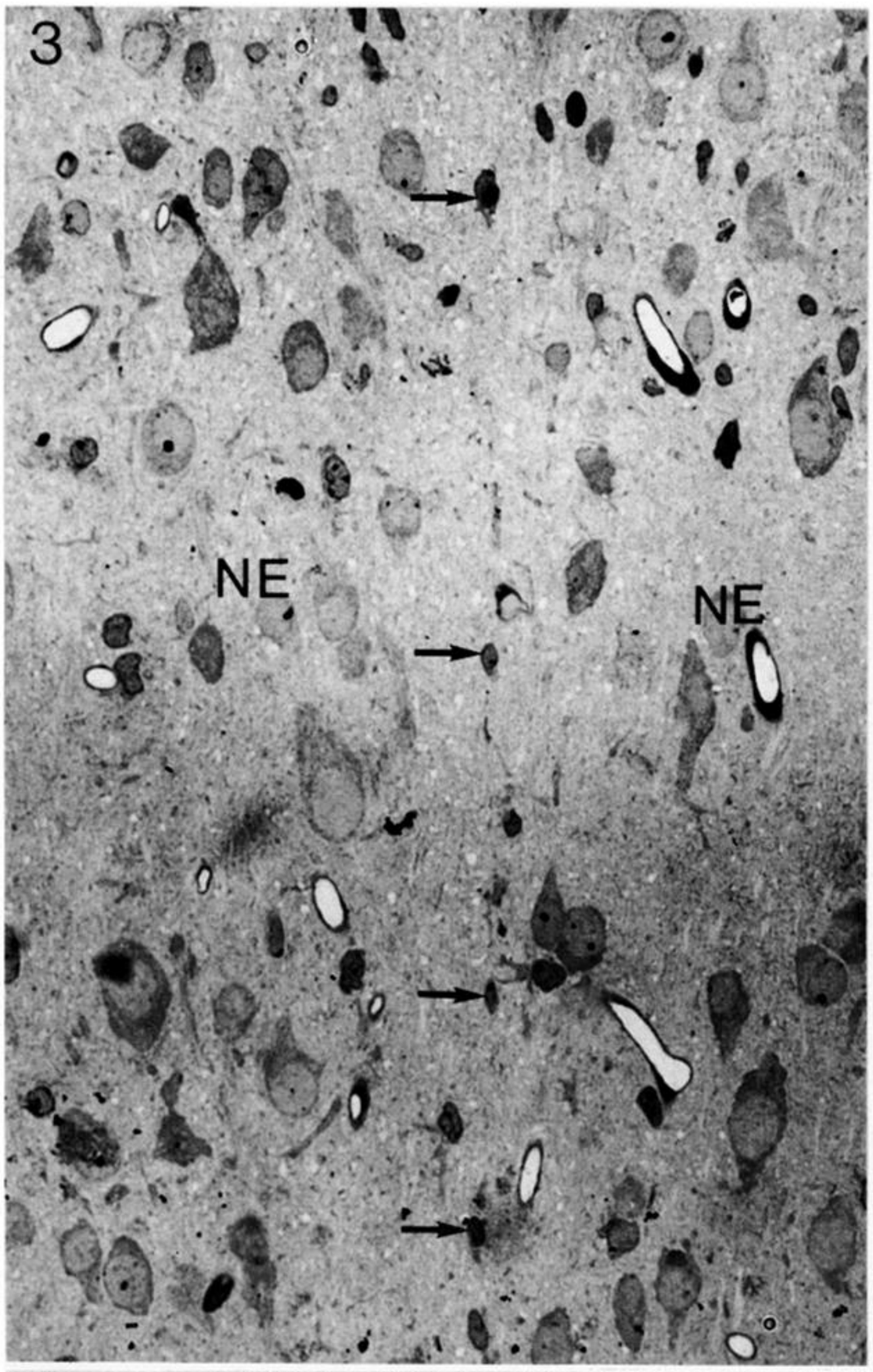
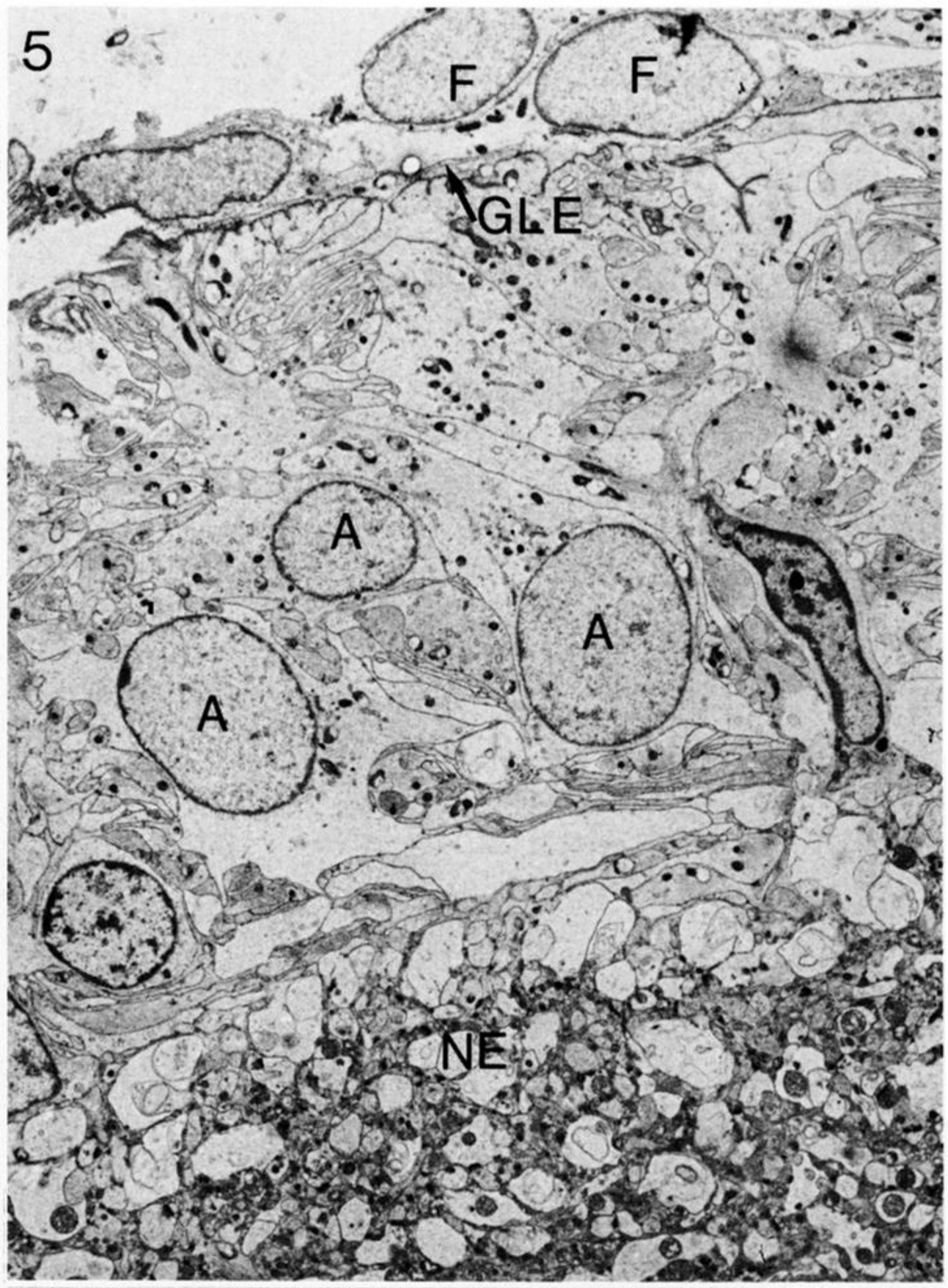
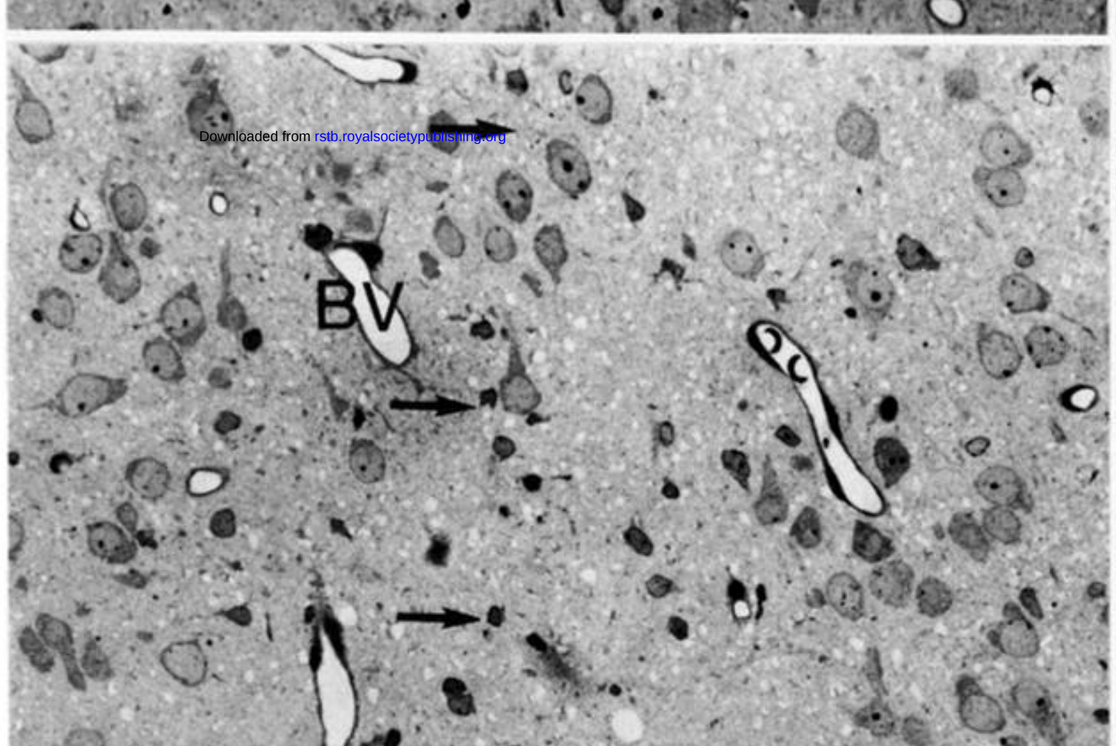


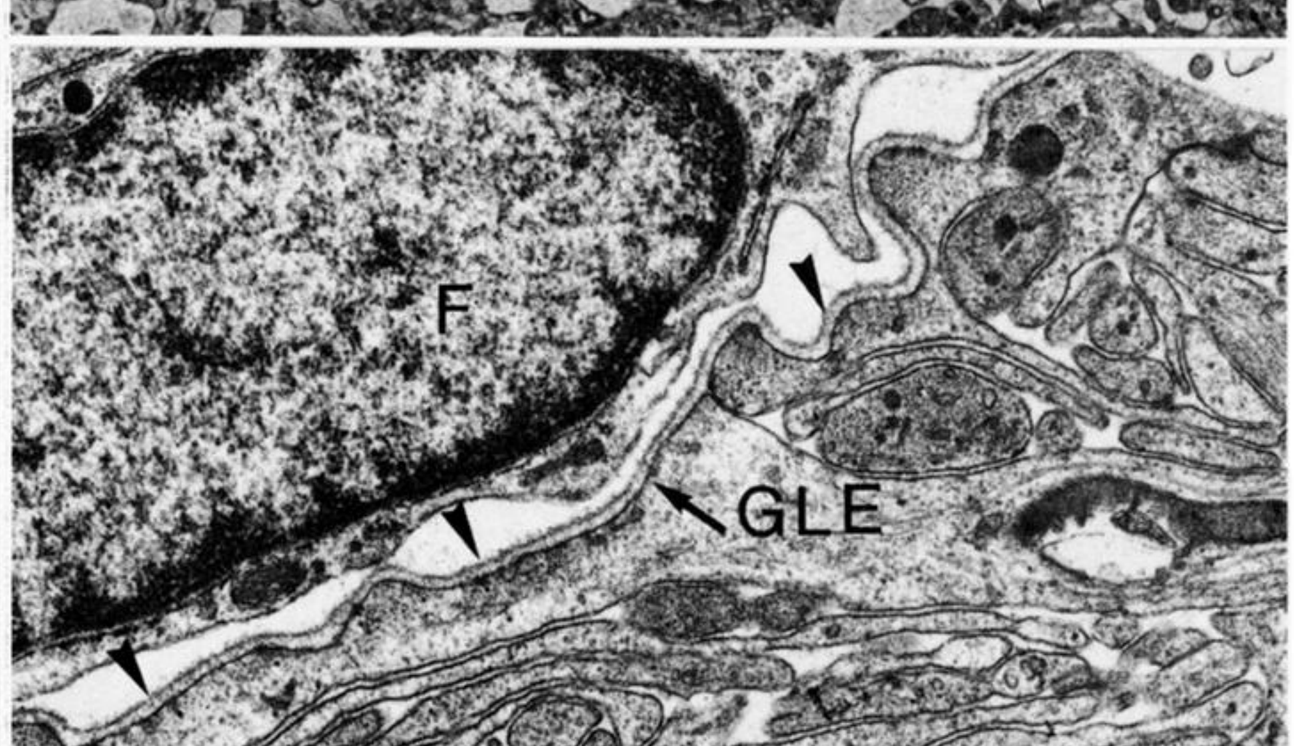


FIGURES 1 AND 2. For description see opposite.

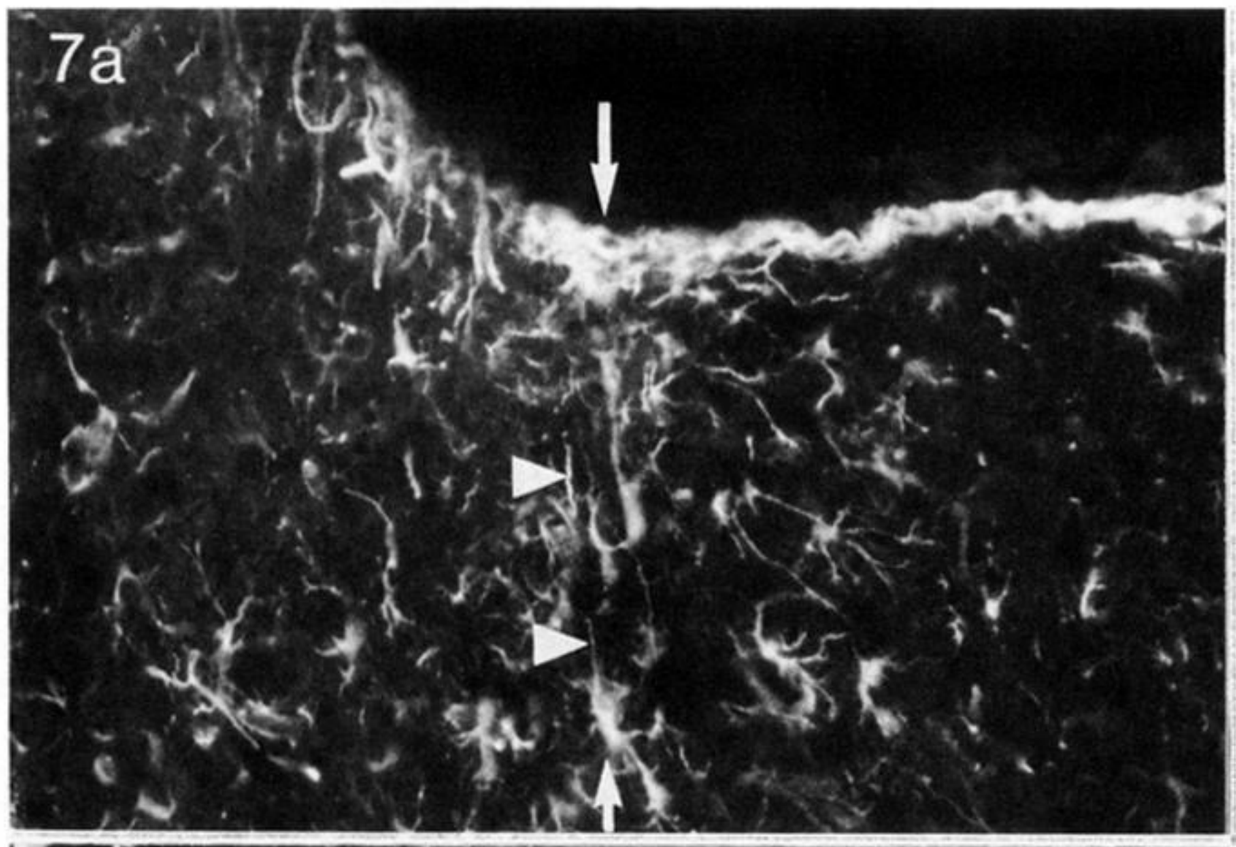


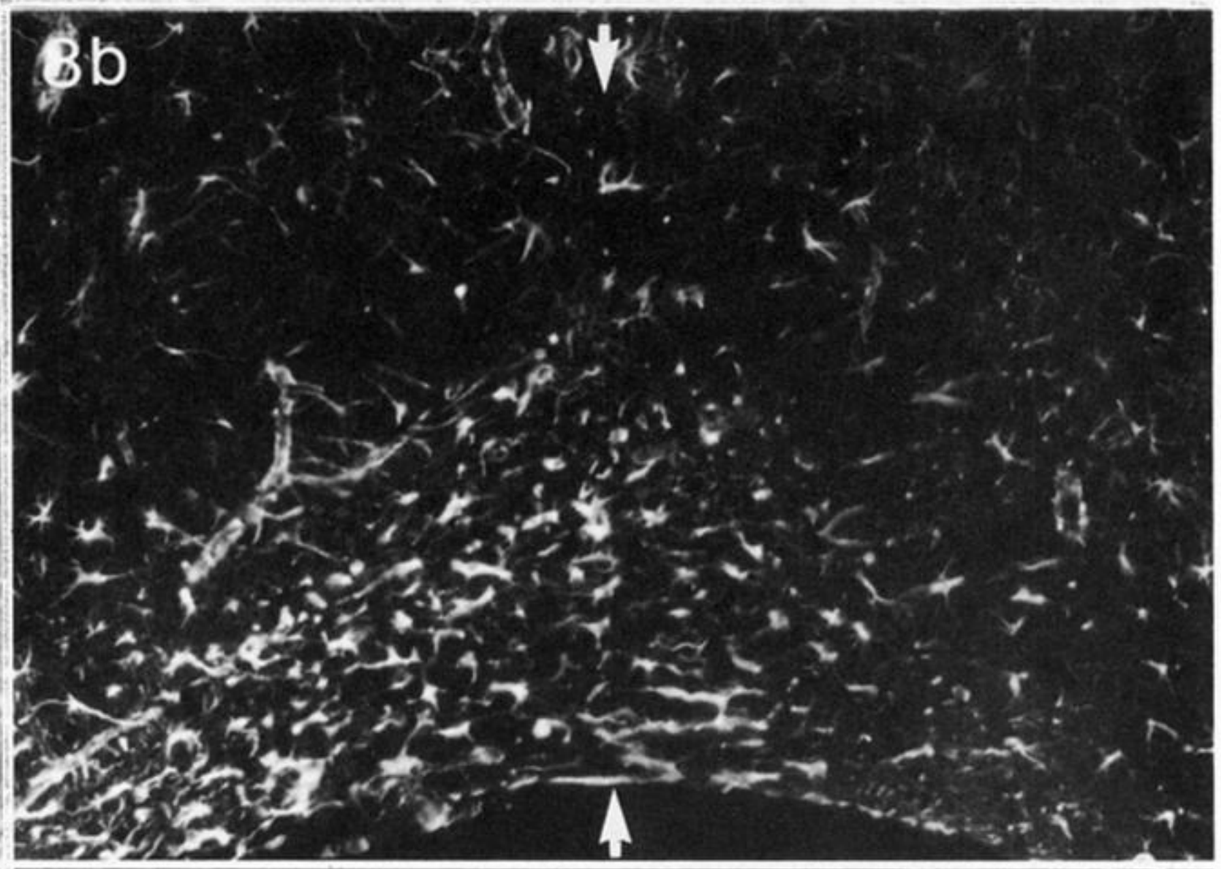


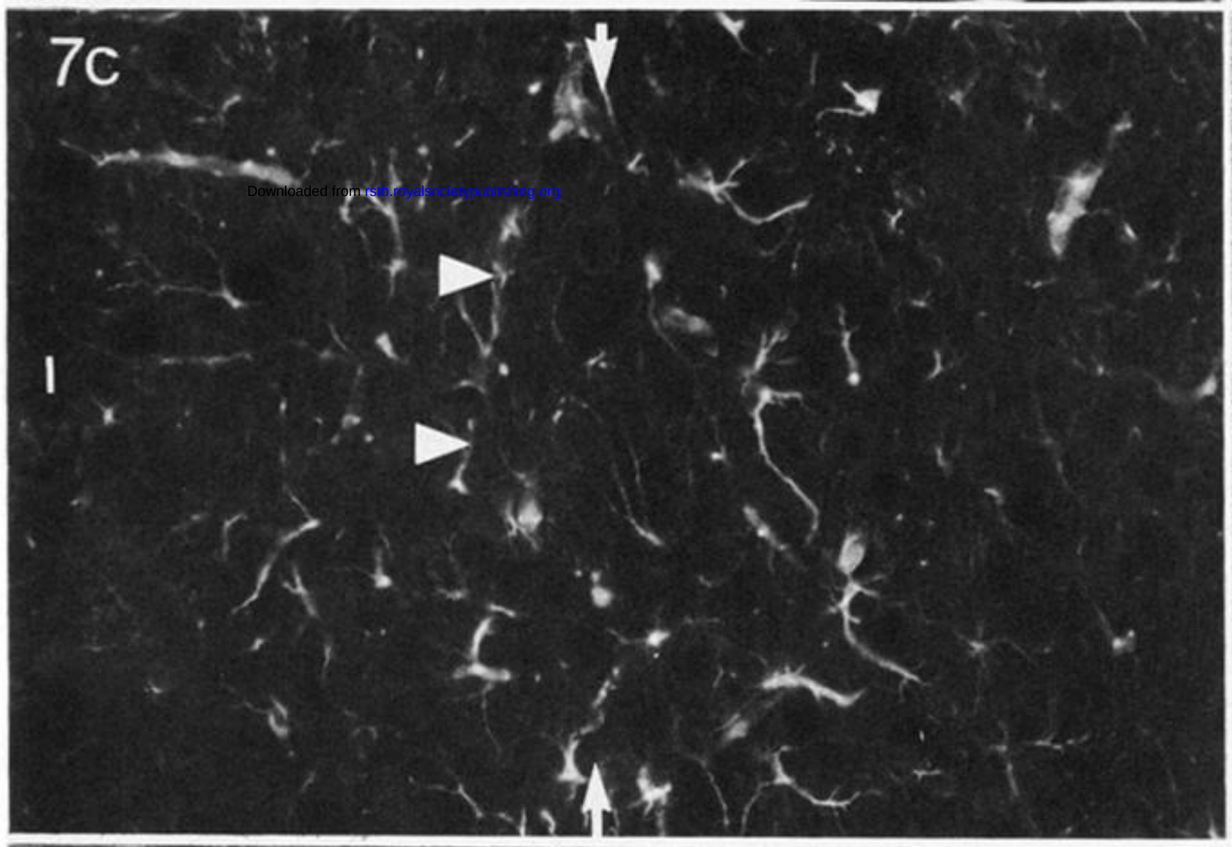


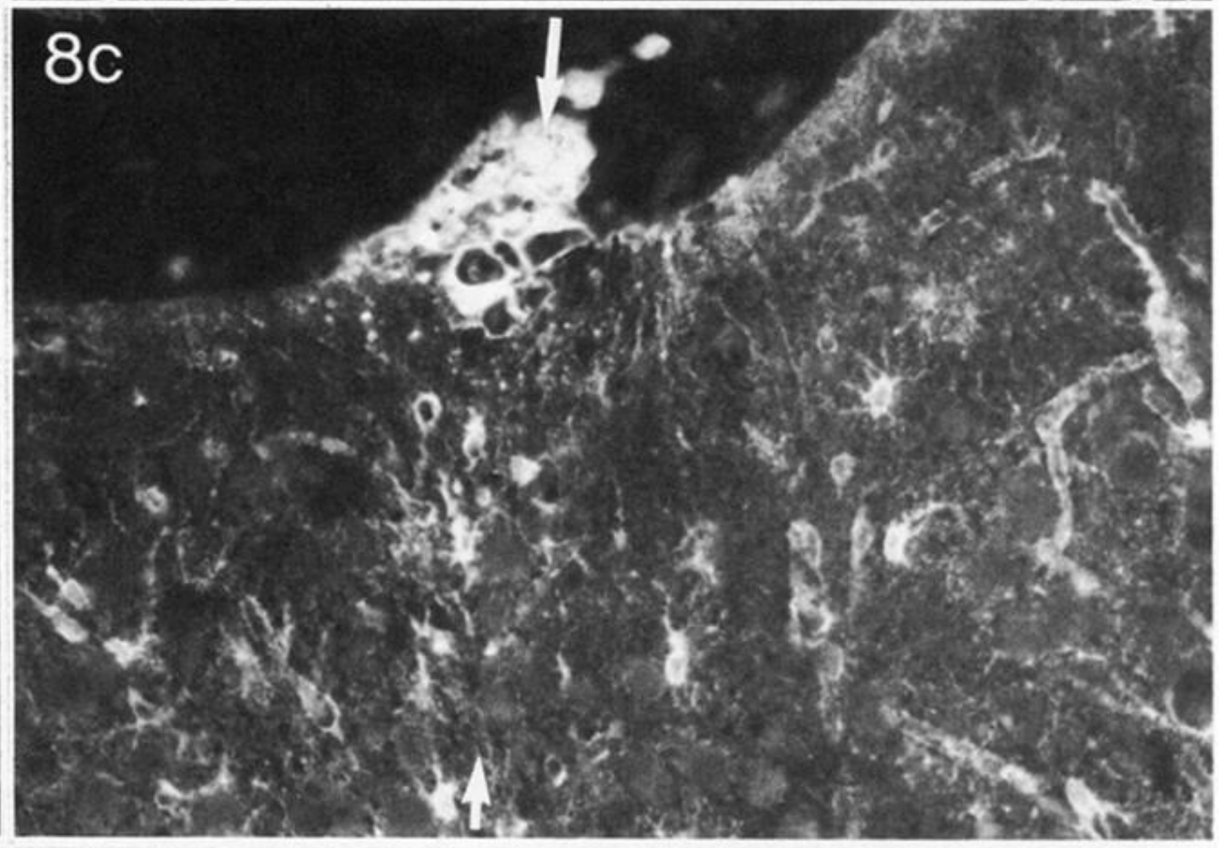


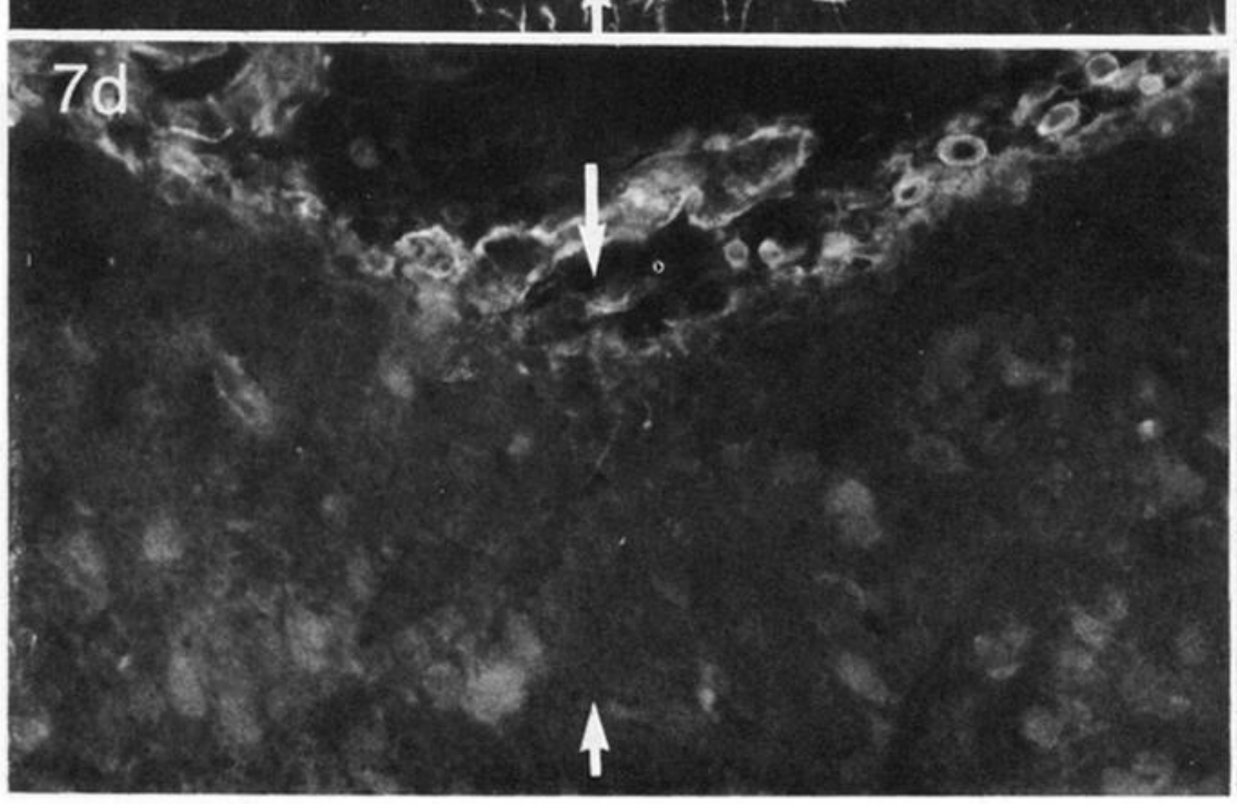
FIGURES 3–6. For description see p. 504.

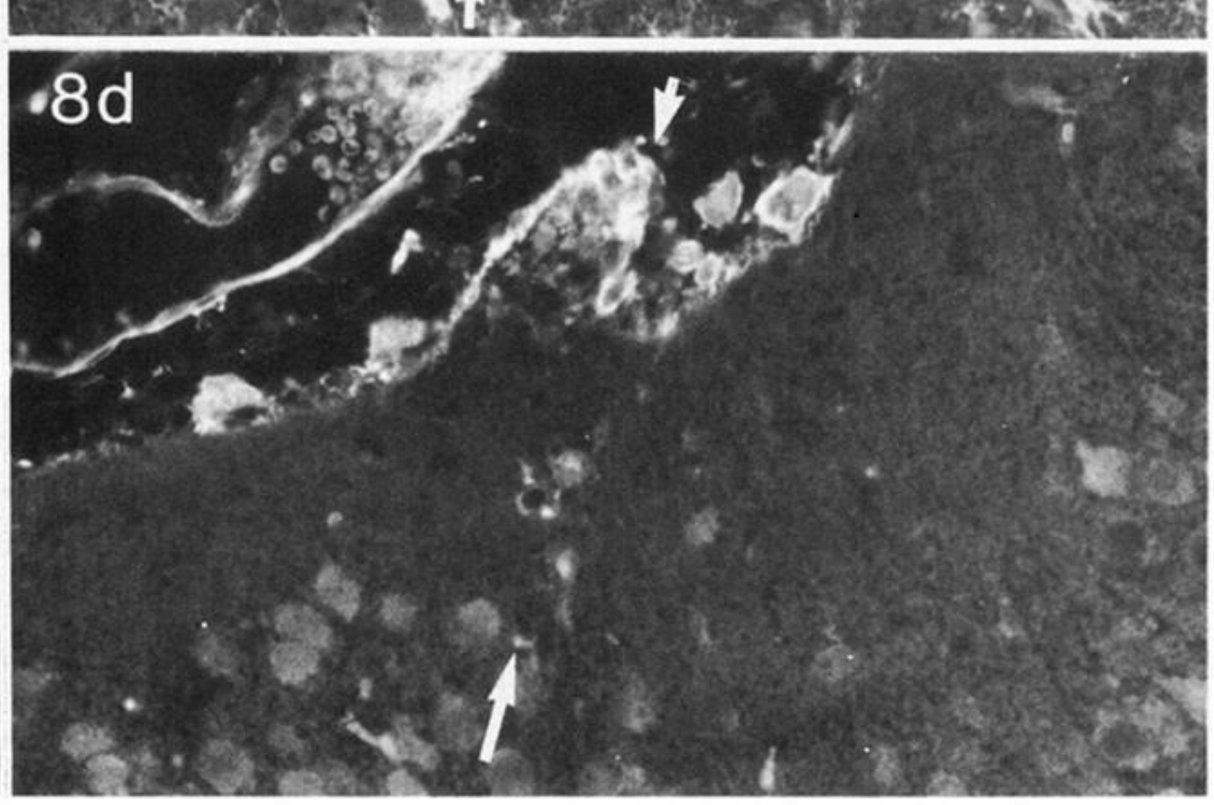












PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

FIGURES 7 AND 8. For description see facing plate 6.

FIGURE 9. For description see facing plate 6.

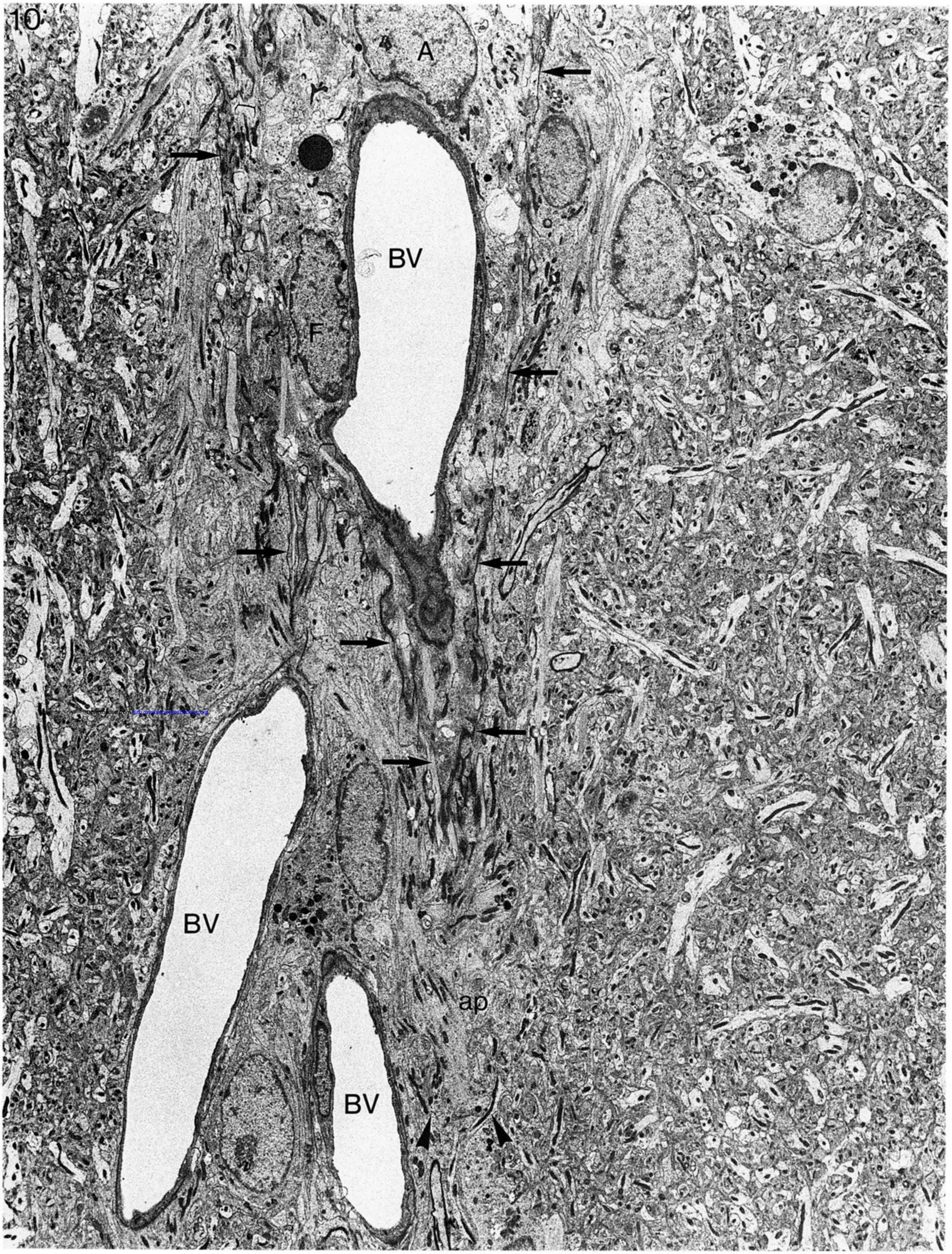


FIGURE 10. For description see p. 505.

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PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

FIGURES 11 AND 12. For description see p. 505.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

FIGURE 13. For description see opposite.

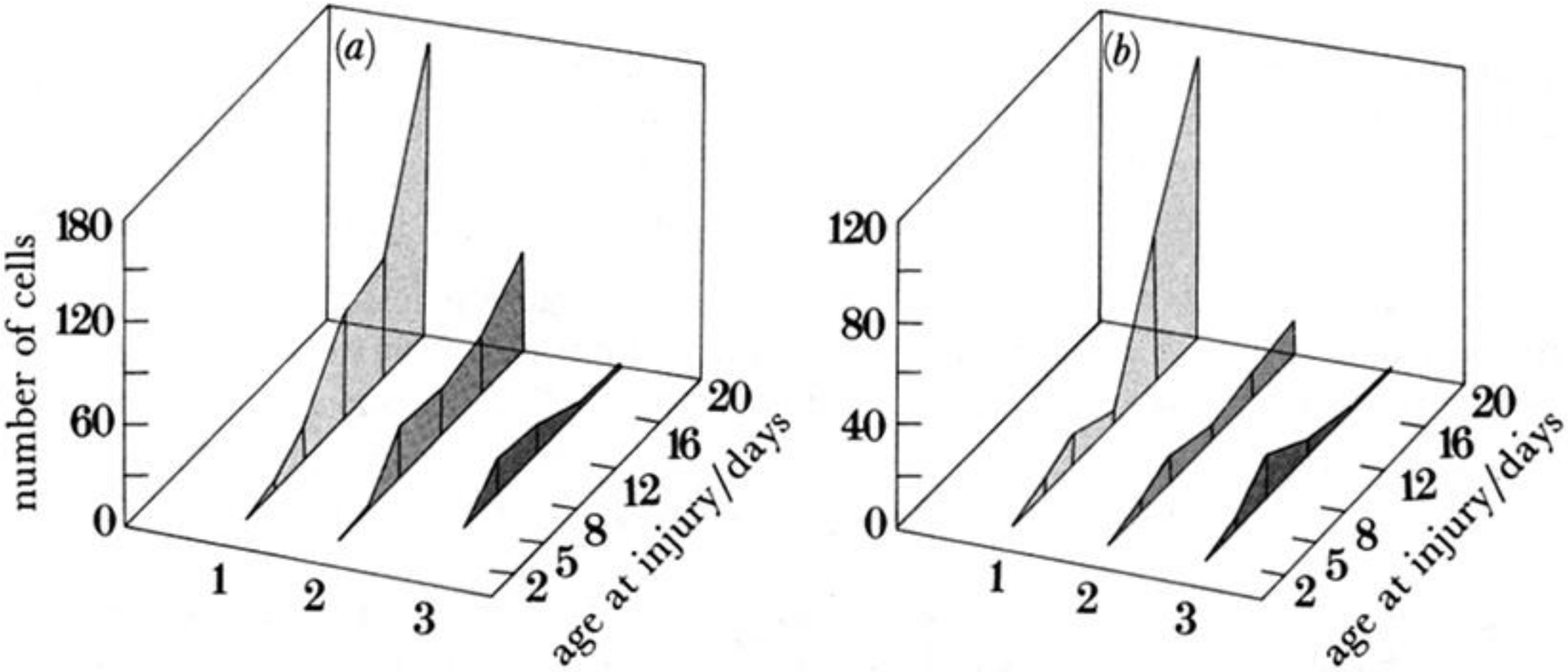

FIGURE 14. Graphs to show the numbers and distribution of the mesenchymal cells associated with the lesion with increasing distance from the midline in animals lesioned at different ages. (*a*) macrophages; (*b*) fibroblasts. Key: 1, cells between midline and 100 μm on either side; 2, cells between 100 and 200 μm on either side; 3, cells between 200 and 300 μm on either side.