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Response of intraventricular macrophages after a penetrant cerebral lesion

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INTRODUCTION

The occurrence and form of two varieties of intraventricular macrophages, the so-called epiplexus and supraependymal cells, have been described in a variety of species: cat (Carpenter, McCarthy & Borison, 1970), fetal mouse (Sturrock, 1978), postnatal rat (Ling, 1979) and monkey (Ling, 1981). Other investigators, using the SEM, have concentrated on surface morphology of epiplexus cells in the rat (Hosoya & Fujita, 1973; Chamberlain, 1974; Peters, 1974), dog (Allen, 1975) and monkey (Ling, 1983). Intraventricular macrophages show considerable variation in surface morphology when viewed in the scanning electron microscope but the functional significance of these variations is controversial. They have been thought to reflect different degrees of activation or maturation (Bleier & Albrecht, 1980); different stages in the development of the same cell line (Sturrock, 1979) or to arise in response to foreign material (Ling, Tseng & Wong, 1985).

These last workers induced hydrocephalus in the offspring of pregnant rats by administration of 6-aminonicotinamide (6-AN) and reported morphological changes in intraventricular macrophages of the offspring which they associated with phagocytosis of extravasated erythrocytes. However, 6-AN is known to alter the surface morphology of amoeboid microglia (Tseng, Ling & Wong, 1984) and it is possible that it also alters the surface contours of intraventricular macrophages directly.

We have developed an alternative model, utilising a penetrant lesion to the adult rat cerebrum, that does not penetrate as far as the lumen of the lateral ventricle and allows the study of changes in surface morphology of intraventricular macrophages following bleeding into the ventricular lumen (Maxwell & McGadey, 1987). This approach may show whether surface changes result from the phagocytic activity of these macrophages since the systemic administration of chemicals is avoided. The morphological changes have been followed at intervals up to 16 days after injury in order to elucidate the time scale of activation and quiescence of these cells. Ling *et al.* (1985) examined only late fetal animals. We have investigated the response in adult animals in an attempt to minimise changes related to development and maturation of the intraventricular macrophage population.

MATERIALS AND METHODS

Twenty one adult albino Wistar rats of 6–7 months of age, 250–450 g body weight, were anaesthetised by intraperitoneal injection of sodium pentobarbitone (0·5 ml (60 mg/ml) per kg body weight). The animal's head was held in a stereotactic frame and a parasagittal skin incision made 3 mm to the right of the midline over the frontoparietal bones. A strip of the calvaria was removed with a dental drill to expose

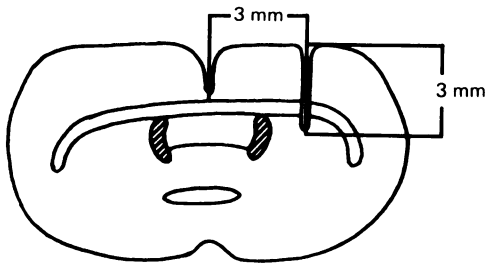

Fig. 1. A diagram showing the position of the lesion used in a schematic coronal section of the rat brain. ▨, Ventricle lumen.

the meninges and the cerebral hemisphere. A Number 11 scalpel blade mounted in a holder on the stereotactic frame was lowered 3 mm into the cerebrum and driven caudally for a distance of 4 mm. The incision did not enter the lateral ventricle (Fig. 1). The skin wound was closed with clips and the animals allowed to recover for 4, 6, 24 and 30 hours, and 4, 8 and 16 days when they were killed by ether inhalation.

Transcardial perfusion of 200 ml of heparinised saline was followed by 300 ml of 2·5% glutaraldehyde in buffered saline (350 m-osmol). The animal was decapitated, the brain removed and fixation by immersion in glutaraldehyde continued for 24 hours. The brain was then sliced coronally by hand in the area of the lesion to expose the lateral ventricles. The slices were osmicated, dehydrated in a graded series of ethanols and critical-point dried from amyl acetate. They were mounted on a specimen stub with silver paint, sputter-coated with gold and viewed in a Jeol T300 scanning electron microscope, operated at 25 or 30 kV. The choroidal and ependymal surfaces of both lateral ventricles were examined by SEM, to compare the cellular responses to the injury on the experimental and normal sides.

When examination was complete by SEM, the lateral ventricles and their contained choroid plexuses were dissected out and dehydrated in several changes of acetone (to remove silver paint), propylene oxide and embedded in Spurr resin with the coated surface orientated parallel to the cutting face. Semithin toluidine blue sections were cut for orientation and light photomicrography; followed by mesas cut from areas of interest. Silver/gold thin sections were cut, mounted on 300-mesh fine grids, stained with uranyl acetate and lead citrate, and examined in a Jeol 100S transmission electron microscope. Material from three unoperated control animals was prepared in similar fashion.

RESULTS

Control animals.

The appearance of the epiplexus cells confirms the earlier descriptions by Ling *et al.* (1979, 1985) and Sturrock (1983). They are randomly distributed over the ventricular surface of the choroidal epithelium. From a flattened and relatively smooth cell body, there arise a number of major cytoplasmic processes each ending in a number of fine filopodia. Some filopodia may arise directly from the cell body. The filopodia are deeply implanted in the microvillous pallisade of the choroidal epithelium (Fig. 2). In thin sections the cells closely resemble monocytes, with a slightly indented nucleus and a moderate amount of cytoplasm. The cytoplasm contains scattered ribosomes, long dispersed strands of rough endoplasmic reticulum, a large Golgi complex, and mitochondria. We confirm the finding by Sturrock (1983) of a relatively smooth type

of cell but are unable to confirm his description of irregular epiplexus cell types. In all our control material the surface of epiplexus cells is smooth (Fig. 2).

Supraependymal cells are widely spaced over the ependymal surface. They are more rounded up than the epiplexus cells with far fewer filopodia (Fig. 3). Rather, the cell surface is much more irregular showing large numbers of blunt cytoplasmic protrusions or blebs.

Survival for 4, 6, 24 and 30 hours

Four hours after a penetrant cerebral lesion the surface of the choroid plexus appears relatively clean; there are few erythrocytes and epiplexus cells are widely dispersed (Fig. 4). The epiplexus cells have a smooth surface and numerous fine filopodia (Fig. 5). The ependymal surface is clean.

By 6 hours, erythrocytes are more numerous over the choroidal and ependymal surfaces on the experimental side of the brain but they are less numerous on the control side in the same animal. Epiplexus cells on the experimental side now tend to occur in groups, near numbers of erythrocytes (Fig. 6). The epiplexus cells still possess smooth surfaced major cytoplasmic processes terminating in filopodia (Figs 6, 7) but the cell body now shows several short cytoplasmic protrusions or blebs (Fig. 7). In the lateral ventricle of the control side the epiplexus cells are more widely spaced and still possess a smooth cell body and numerous filopodia. Supraependymal cells have an irregular form and their surface has many irregular cytoplasmic protrusions (Fig. 8). Erythrocytes often lie in close relation to these cells (Fig. 8).

After 24 and 30 hours erythrocytes are still more numerous on the experimental side. Epiplexus cells tend to form groups on the choroidal surface; few groups occur on the control side. On the experimental side epiplexus cells form close relations to erythrocytes (Fig. 9), the number of filopodia is reduced and there is great development of irregular surface protrusions or blebs (Fig. 9) which seem to extend towards adjacent erythrocytes (Fig. 9). On the control side the number of filopodia is still high and the cell surface comparatively smooth. The supraependymal cells differ little from those seen after 6 hours. The cell form is irregular and short processes extend from the cell body to closely related erythrocytes. Thin sections show that the cell cytoplasm contains numerous ingested erythrocytes.

Survival for 4, 8 and 16 days

Four days after injury the numbers of erythrocytes appear to be much reduced. On the experimental side erythrocytes still occur in crevices or folds on the choroid plexus. Epiplexus cells situated on the choroidal ridges, with no closely adjacent erythrocytes,

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Fig. 2. An epiplexus cell (*EP*) from a control animal demonstrating the smooth cell body and many fine filopodia (*F*). × 2650.

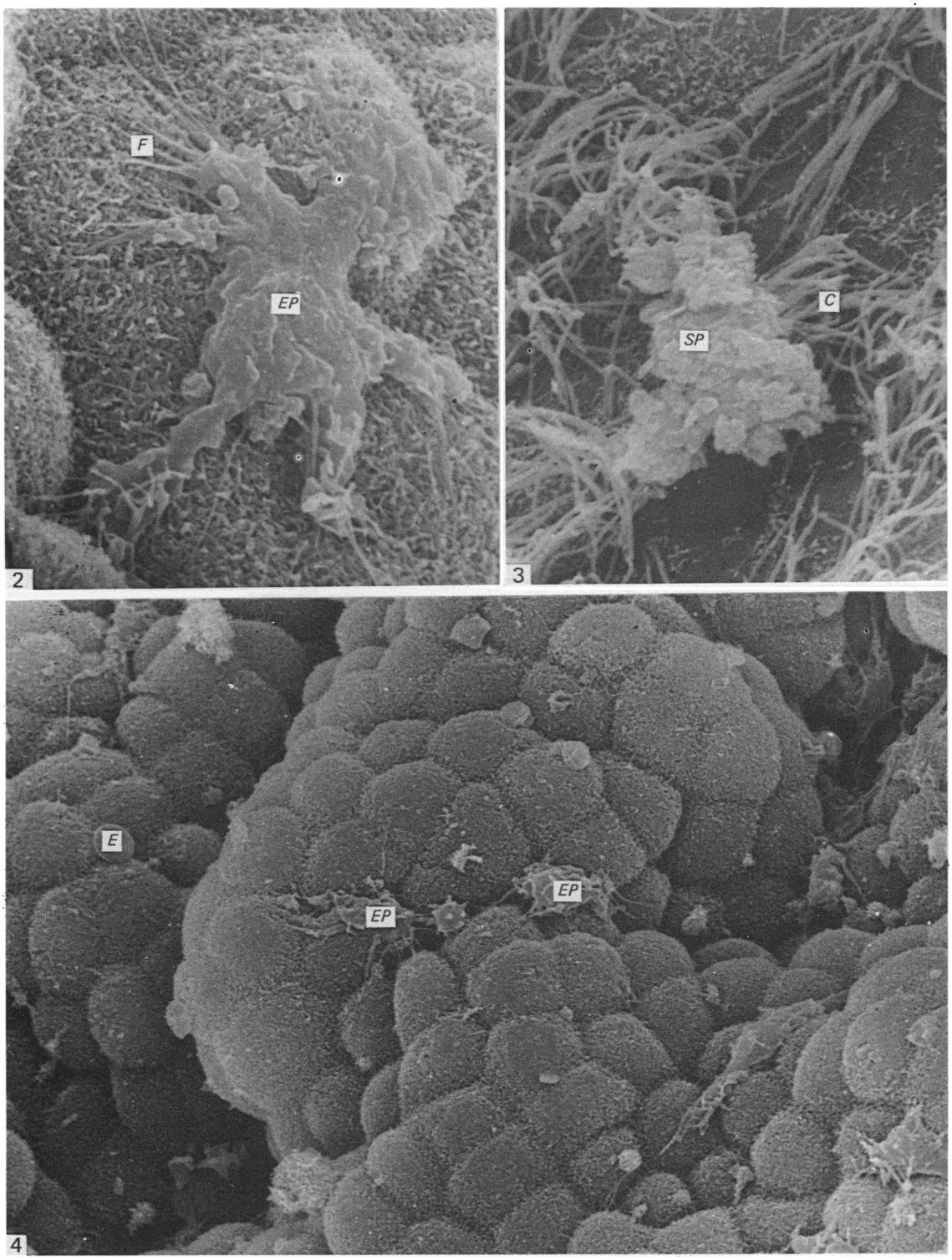
Fig. 3. A supraependymal cell (*SP*) from a control animal among the cilia (*C*) of the ependyma. × 2450.

Fig. 4. Choroid plexus on the lesioned side of the brain four hours after injury. Epiplexus cells (*EP*) are widely spaced with fine filopodia. There are only very small numbers of erythrocytes (*E*) present. × 1000.

Fig. 5. Detail of an epiplexus cell four hours after infliction of the lesion. The cell surface is smooth with many long processes (*P*) and filopodia (*F*) extending from the cell body. × 3800.

Fig. 6. At six hours after injury erythrocytes (*E*) are more numerous on the choroid plexus of the experimental side of the brain. Epiplexus cells (*EP*) now tend to occur in groups but still possess many long filopodia (*F*). × 1750.

Fig. 7. Detail of an epiplexus cell (*EP*) six hours after injury in close relation to a number of erythrocytes (*E*). The epiplexus cell surface is no longer smooth but now covered by numerous protoplasmic protrusions or blebs (*B*). × 3800.



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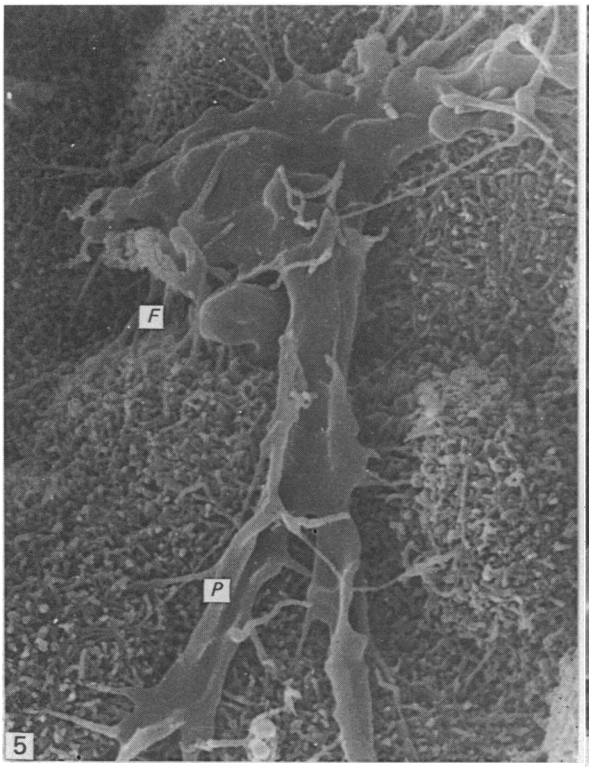

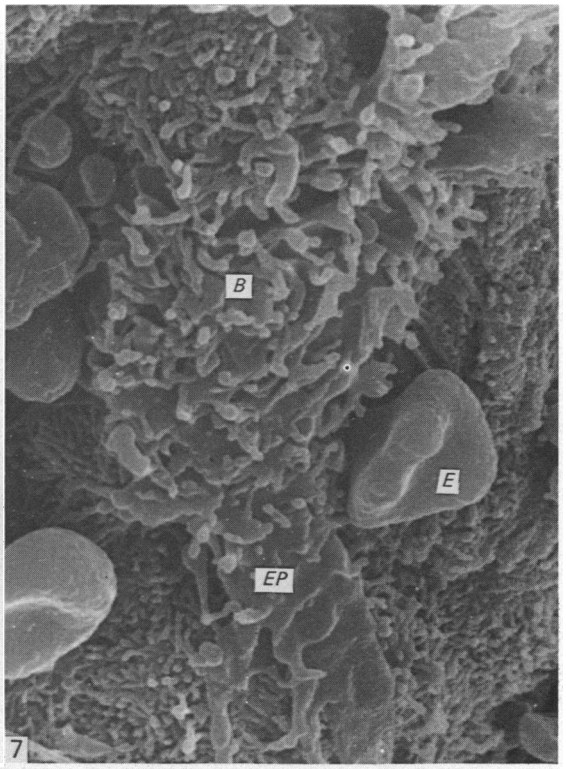

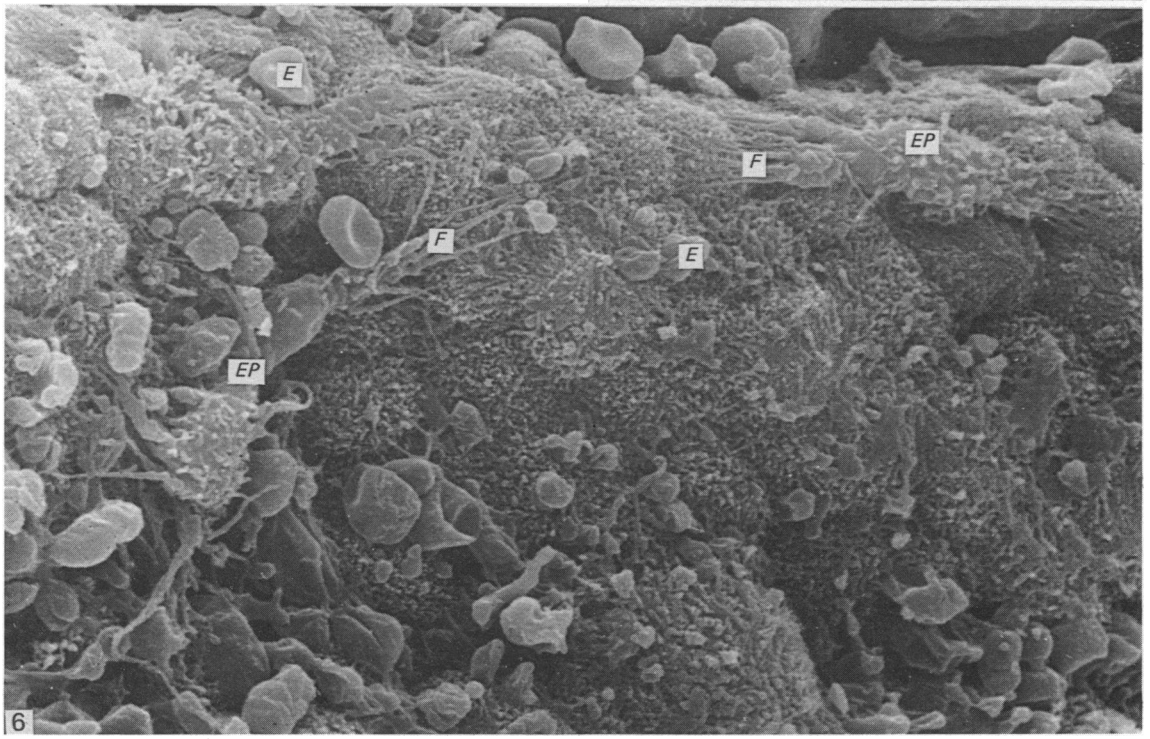

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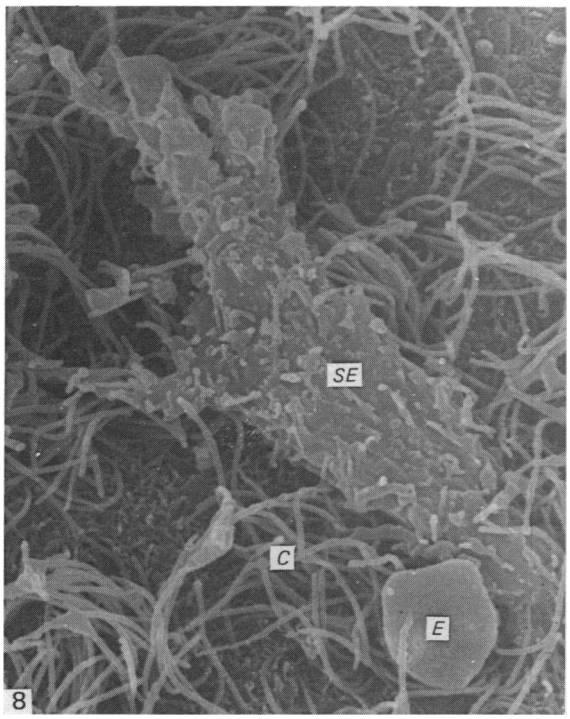

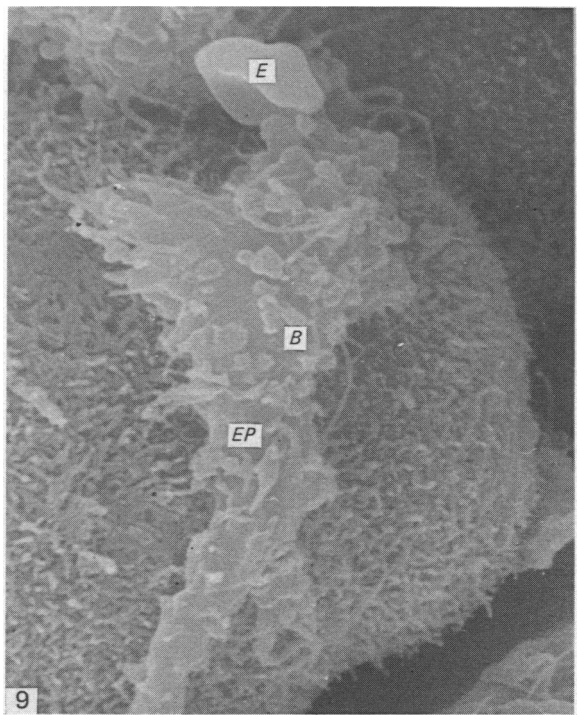

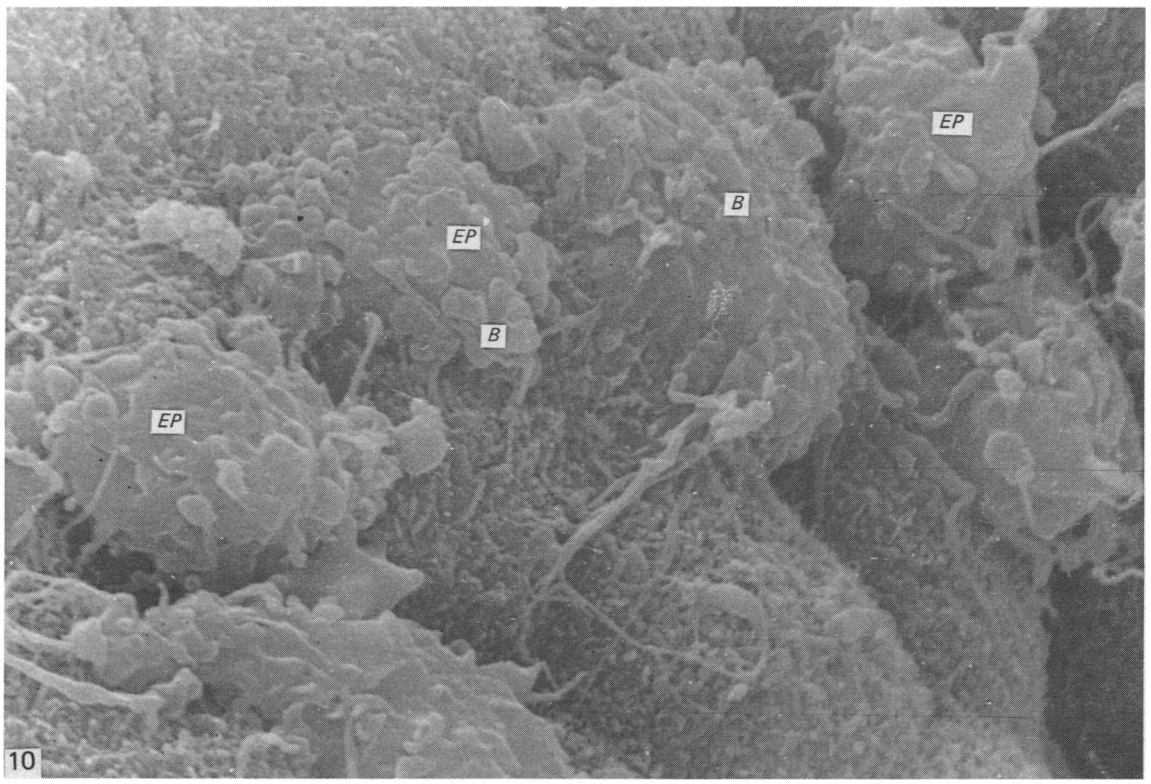

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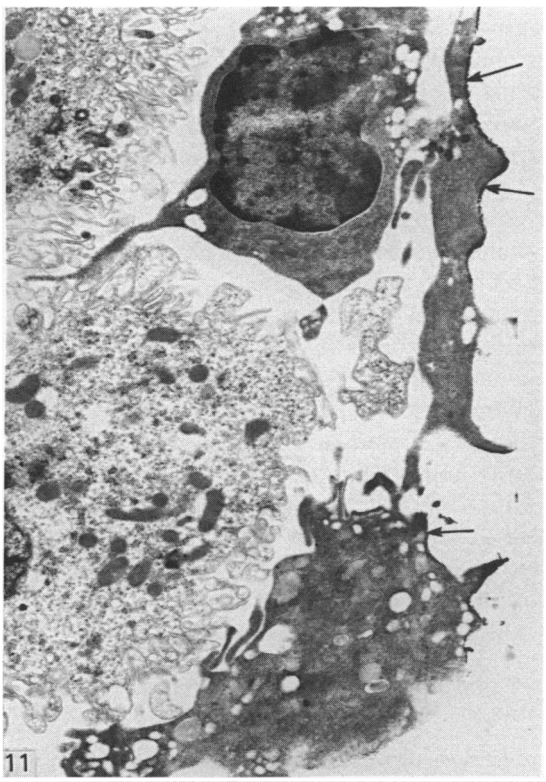

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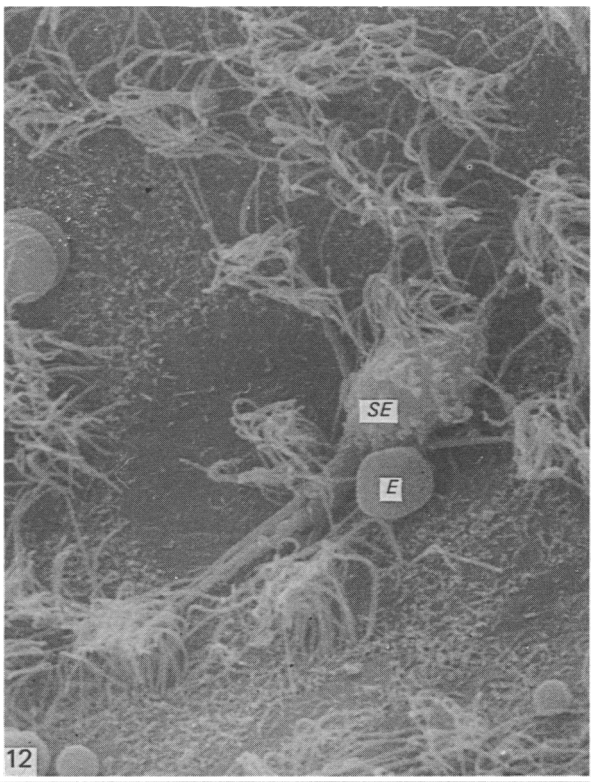

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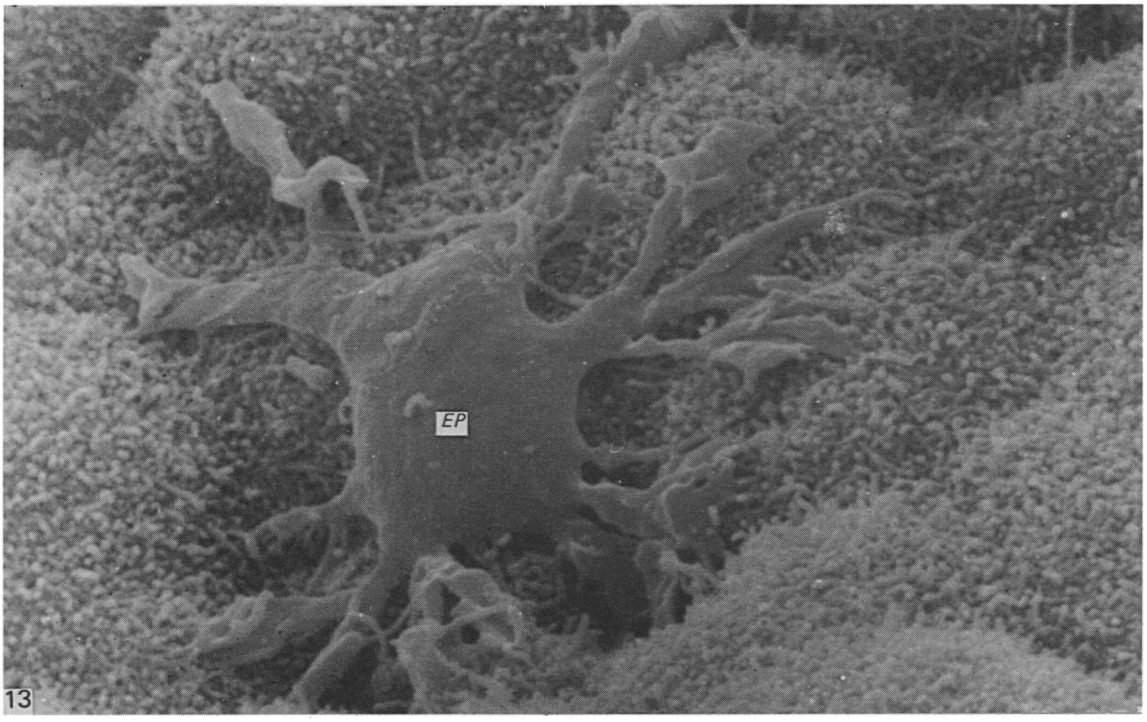

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demonstrate numbers of filopodia but there are still some blebs on the cell body. Epiplexus cells in choroidal crevices still form groups (Fig. 10) and there are numerous blebs on their cell bodies. These cells possess relatively few filopodia (Fig. 10). Thin sections show numbers of possibly lipid-filled inclusions in their cytoplasm (Fig. 11). On the unlesioned side, the epiplexus cells are widely spaced and demonstrate a smooth cell body surface. Large protoplasmic processes with terminal filopodia extend away from the cell body.

On the experimental side a small number of erythrocytes are still present on the ependymal surface. Amongst these occur a small number of supraependymal cells (Fig. 12) possessing only a few filopodia and blebs on the cell body.

There is little difference between the 8 and 16 days survival animals. Epiplexus cells are widely spaced in each lateral ventricle and the choroidal surface has only a very small number of erythrocytes at 8 days and has none by 16 days. The epiplexus cell body is flattened against the choroid epithelium (Fig. 13) and the cell surface is smooth. A large number of processes extend from the cell body to end in fine filopodia. The cytoplasm contains very few inclusion vesicles and is now markedly less electron-dense. The ependymal surface is 'clean', with only a very few, widely spaced supraependymal cells. The cell body may still lie in relation to small numbers of erythrocytes and possesses bleb-like cytoplasmic processes. Alternatively, thin sections show that the cell body is relatively smooth with a small number of blebs visible. The cell cytoplasm contains a few empty vesicles which may be lipid inclusions.

DISCUSSION

The precise mechanism of the extravasation of erythrocytes into the lateral ventricle is uncertain. Within one minute after brain injury there is a transient but significant elevation in cerebral blood flow and lowered cerebrovascular resistance, followed by a hypotensive phase (DeWitt *et al.* 1986). In the present study, 4 hours after a penetrant lesion, few erythrocytes are seen on the choroidal surface but there is a dramatic increase by 6 hours. This suggests that blood leakage does not occur at the time of injury but follows it. We have not seen ruptured blood vessels within the choroid plexus or the margins of the ventricles, either in material processed for SEM or in semithin sections. Although the precise mechanism of bleeding is obscure, it is only after the bleeding occurs that any change is seen in the surface morphology of the intraventricular macrophages.

The pleomorphism of epiplexus cells is well documented, in a variety of mammalian species. In the SEM, they normally have a smooth surface, with a centrally located cell body and several long protoplasmic extensions (Hosoya & Fujita, 1973; Peters, 1974;

Fig. 8. A supraependymal cell (*SE*) from the ependyma of the experimental side, six hours after injury, lying upon the ependymal cilia (*C*). A single erythrocyte (*E*) lies near the supraependymal cell. × 3010.

Fig. 9. Detail of an epiplexus cell (*EP*) thirty hours after injury in close relation to an erythrocyte (*E*). The epiplexus cell possesses numerous blebs (*B*) but very few filopodia. × 3650.

Fig. 10. Groups of epiplexus cells (*EP*) within a choroidal crevice on the lesioned side four days after injury. These cells still possess numerous blebs (*B*) on their cell bodies but few filopodia. × 2450.

Fig. 11. A thin section of a similar group of epiplexus cells to that shown in Figure 10, illustrating their irregular profile with numbers of electron-lucent inclusions within their cytoplasm. The cytoplasm is still electron-dense. Arrows indicate the gold coating for SEM observation. × 7980.

Fig. 12. A supraependymal cell (*SE*) in close relation to an erythrocyte (*E*) on the ependymal surface of the experimental side four days after injury. × 1650.

Fig. 13. An epiplexus cell (*EP*) on the experimental side sixteen days after injury. The cell body is smooth with a number of flattened, protoplasmic protrusions from which arise filopodia. × 3560.

Allen, 1975; Coates, 1975, 1977; Persky, 1978; Sturrock, 1979; Peters & Swan, 1979; Ling, 1983). However, rounded epiplexus cells with microvilli or cytoplasmic laminar folds have also been described (Sturrock, 1979). The surface morphology of epiplexus cells suggests that they are capable of moving actively on the choroidal surface and of altering their shape (Allen, 1975; Coates, 1975), so that they show great intraspecific and interspecific variation (Persky, 1978). The functional significance of this is controversial. Interpretations range from stages of development of the same cell line (Sturrock, 1979, 1983), through a reflection of different degrees of activation or states of maturation (Bleier & Albrecht, 1980), to a representation of phagocytic activity (Ling *et al.* 1985). Since the last-named authors used late fetal rats it is possible that the changes that they described in cell morphology reflect both development of the epiplexus cell line and the results of phagocytic activity. Since their material was examined at only one time-interval their data give no information on possible temporal changes following the phagocytic activity of intraventricular macrophages. Two other studies have noted changes in the surface morphology of epiplexus cells under experimental conditions. Nielson & Gauger (1974) reported the change to a verrucous cell body with many long cell processes in animals surviving for long periods after induction of hydrocephalus in hamsters by intraperitoneal injection of Land strain reovirus Type I. Merchant (1979) reported changes from a smooth cell surface to a surface with abundant microappendages, blebs, ruffles and microvilli, three days after intracisternal injection of Bacillus Calmette–Guérin (BCG) but his study did not include longer survival times.

There is thus only circumstantial evidence that surface changes in epiplexus cells reflect their phagocytic activity. In the present study we have shown a temporal sequence of development of surface changes in epiplexus cells after bleeding into the ventricular lumen, following a penetrant cerebral lesion. Comparison of control and experimental sides has shown that epiplexus cells aggregate near areas where erythrocytes occur on the choroid surface; this aggregation is less marked on the control side of the brain. A number of workers have reported an increase in number of supraependymal cells after injury (Nielson & Gauger, 1974; Ling, Tseng & Wong, 1985), while Merchant (1979) found an approximate tenfold increase in epiplexus cells three days after intrathecal injection of BCG. Ling *et al.* (1985), however, found no obvious change in the number of epiplexus cells. Therefore the apparent numerical increase of epiplexus cells may only be a reflection of their aggregation in areas where damage has occurred. We have shown their grouping in areas of the choroid plexus where erythrocytes have leaked on to the choroidal surface. In thin sections an occasional macrophage was found in the pericapillary spaces of the choroid plexus but no evidence was obtained for passage of monocytes through either the choroidal or ependymal epithelium. In the absence of evidence for this migration we feel that the changes in distribution of epiplexus cells are the result of their movement to areas of damage.

Our long term analysis suggests strongly a sequence of activation and senescence of intraventricular macrophages, particularly in the epiplexus cells. A cell with a smooth cell body and a number of long, cytoplasmic processes and filopodia develops blebs or short, blunt cytoplasmic protrusions which appear to be directed towards extravasated erythrocytes. The cell surface becomes very irregular and numbers of inclusion vesicles occur in the cytoplasm. Erythrocytes are surrounded by cytoplasmic processes and ingested. As this process continues filopodia are lost. Possibly these changes provide either for redistribution of plasmalemma and cytoplasm, as suggested by Ling *et al.* (1985), or for increased ingestion of cerebrospinal fluid and analysis of

its composition (Malloy & Low, 1976; Merchant & Low, 1977*a*, *b*). Once the ingested material has been destroyed the epiplexus cells resume a sessile form with the loss of surface blebs and the reformation of protoplasmic processes and filopodia. In our study the cells are activated soon after the liberation of blood into the ventricular lumen, 6 hours after cerebral injury, and are still active 24 hours later. By 4 days some epiplexus cells in 'clean' areas of the choroid plexus are becoming quiescent but, where large numbers of erythrocytes are still present, epiplexus cells remain rounded and irregular in form. The epiplexus cell quiescence is more advanced by 8 days and is uniform by 16 days, when the cells are again widely distributed over the choroid plexus and possess a smooth cell body with numerous cytoplasmic processes and filopodia.

The response of the supraependymal cells is much less marked. There is some reported variation in form of supraependymal cells; some with a triangular profile (Clementi & Marini, 1972) are located in the lateral recesses of the floor of the third ventricle; others are more rounded with many branching processes and occur over the non-ciliated areas of the preoptic and infundibular recesses (Coates, 1973*a*, *b*); others again are rounded cells and covered with many finger-like microvilli, but without long cytoplasmic extensions (Jennes, Sikora, Simonsberger & Adam, 1977). The supraependymal cells of the lateral ventricle in this present study fall into the last group. Although they actively ingest erythrocytes there is no marked change in their surface morphology and distribution throughout the experimental period. This lesser response may be reflected in the slower rate of removal of erythrocytes from the ependymal surface.

SUMMARY

The response of epiplexus and supraependymal cells to extravasated blood after a penetrant cerebral lesion was investigated. Epiplexus cells respond more actively than supraependymal cells. The epiplexus cells tend to aggregate near areas of extravasation of erythrocytes, this being most marked 6 hours after injury. Epiplexus cells lose their smooth surface appearance, retract their filopodia and adopt a more spherical form, with short microvilli or blebs. Numerous inclusion vesicles develop; some contain disrupted erythrocytes 6–12 hours after injury and these are still present 24–30 hours after injury. By 8–16 days after injury epiplexus cells resume a smooth surface appearance and the number of inclusion vesicles is much reduced. This suggests reversion to a quiescent state, from an earlier active state.

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REFERENCES

ALLEN, D. J. (1975). Scanning electron microscopy of epiplexus macrophages (Kolmer cells) in the dog. *Journal of Comparative Neurology* **161**, 197–214.

BLEIER, R. & ALBRECHT, R. (1980). Supraependymal macrophages of third ventricle of hamster: morphological, functional and histochemical characterisation *in situ* and in culture. *Journal of Comparative Neurology* **192**, 489–504.

CARPENTER, S. J., MCCARTHY, L. E. & BORISON, H. L. (1970). Electron microscopic study on the epiplexus (Kolmer) cells of the cat choroid plexus. *Zeitschrift für Zellforschung und mikroskopische Anatomie* **110**, 471–486.

CHAMBERLAIN, J. G. (1974). Scanning electron microscopy of epiplexus cells (macrophages) in the fetal rat brain. *American Journal of Anatomy* **139**, 443–447.

CLEMENTI, F. & MARINI, D. (1972). The surface fine structure of the walls of cerebral ventricles and of choroid plexus in cat. *Zeitschrift für Zellforschung und mikroskopische Anatomie* **123**, 82–85.

COATES, P. W. (1973*a*). Supraependymal cells in recesses of the monkey third ventricle. *American Journal of Anatomy* **136**, 533–539.

COATES, P. W. (1973*b*). Supraependymal cells: Light and transmission microscopy extends scanning electron microscopic demonstration. *Brain Research* **57**, 502–507.

COATES, P. W. (1975). Scanning electron microscopy of a second type of supraependymal cell in the monkey third ventricle. *Anatomical Record* **182**, 275–287.

COATES, P. W. (1977). The third ventricle of monkeys. Scanning electron microscopy of surface features in mature males and females. *Cell and Tissue Research* **177**, 307–316.

DEWITT, D. S., JENKINS, L. W., WEI, E. P., LUTZ, H., BECKER, D. P. & KONTOS, H. A. (1986). Effects of fluid-percussion brain injury on regional cerebral blood flow and pial arteriolar diameter. *Journal of Neurosurgery* **64**, 787–794.

HOSOYA, Y. & FUJITA, T. (1973). Scanning electron microscopic observation of intraventricular macrophages (Kolmer cells) in the rat brain. *Archivum histologicum japonicum* **35**, 133–140.

JENNES, L., SIKORA, K., SIMONSBERGER, P. & ADAM, H. (1977). Ventrikuläre Topographie des diencephalen Ependyms bei *Rattus rattus* (L.) – eine rasterelektonenmikroskopische Untersuchung. *Journal für Hirnforschung* **18**, 501–520.

LING, E. A. (1979). Ultrastructure and origin of epiplexus cells in the telencephalic choroid plexus of postnatal rats studied by intravenous injections of carbon particles. *Journal of Anatomy* **129**, 479–492.

LING, E. A. (1981). Ultrastructure and mode of formation of epiplexus cells in the choroid plexus in the lateral ventricles of the monkey (*Macaca fascicularis*). *Journal of Anatomy* **133**, 555–569.

LING, E. A. (1983). Scanning electron microscopic study of epiplexus cells in the lateral ventricles of the monkey (*Macaca fascicularis*). *Journal of Anatomy* **137**, 645–652.

LING, E. A., TSENG, C. Y. & WONG, W. C. (1985). An electron microscopical study of epiplexus and supraependymal cells in the prenatal rat brain following a maternal injection of 6-aminonicotinamide. *Journal of Anatomy* **140**, 119–129.

MALLOY, J. J. & LOW, F. N. (1976). Scanning electron microscopy of the subarachnoid space in the dog. IV. Subarachnoid macrophages. *Journal of Comparative Neurology* **167**, 257–283.

MAXWELL, W. L. & MCGADEY, J. (1987). SEM studies of the surface morphology of intraventricular macrophages after a penetrant lesion of the adult rat cerebral cortex. *Journal of Anatomy* **152**, 254.

MERCHANT, R. E. & LOW, F. N. (1977*a*). Identification of challenged subarachnoid free cells. *American Journal of Anatomy* **148**, 143–148.

MERCHANT, R. E. & LOW, F. N. (1977*b*). Scanning electron microscopy of the subarachnoid space in the dog. V. Macrophages challenged by Bacillus Calmette–Guérin. *Journal of Comparative Neurology* **172**, 381–408.

MERCHANT, R. E. (1979). Scanning electron microscopy of epiplexus macrophages responding to challenge by Bacillus Calmette–Guérin. *Acta Neuropathologica* **47**, 183–188.

NIELSEN, S. L. & GAUGER, G. E. (1974). Experimental hydrocephalus: surface alterations of the lateral ventricle. Scanning electron microscopic studies. *Laboratory Investigation* **30**, 618–625.

PERSKY, B. (1978). Scanning electron microscopy of the epiplexus cells of the dog, gerbil, and guinea pig. *Anatomical Record* **190**, 508.

PETERS, A. (1974). The surface fine structure of the choroid plexus and ependymal lining of rat lateral ventricle. *Journal of Neurocytology* **3**, 99–108.

PETERS, A. & SWAN, R. C. (1979). The choroid plexus of the mature and aging rat: the choroidal epithelium. *Anatomical Record* **194**, 325–354.

STURROCK, R. R. (1978). A developmental study of epiplexus cells and supraependymal cells and their possible relationship to microglia. *Neuropathology and Applied Neurobiology* **4**, 307–322.

STURROCK, R. R. (1979). A semithin light microscopic, transmission electron microscopic and scanning electron microscopic study of the macrophages in the lateral ventricles of mice from embryonic to adult life. *Journal of Anatomy* **129**, 31–44.

STURROCK, R. R. (1983). A light microscopic and scanning electron microscopic study of intraventricular macrophages in the brains of aged mice. *Journal of Anatomy* **136**, 761–771.

TSENG, C. Y., LING, E. A. & WONG, W. C. (1984). A scanning and transmission electron microscopic study of amoeboid microglial cells in the prenatal rat brain following a maternal injection of 6-aminonicotinamide. *Journal of Anatomy* **138**, 733–743.