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Response of intraventricular macrophages to crotoxin-coated microcarrier beads injected into the lateral ventricle of postnatal rats

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INTRODUCTION

Supra-ependymal and epiplexus cells found in association with the ependyma and choroid plexuses of the cerebral ventricles respectively, have been studied by many authors (Carpenter, McCarthy & Borison, 1970; Hosoya & Fujita, 1973; Walsh, Brawer & Lin, 1978; Sturrock, 1979, 1983; Ling, 1981*a*, 1983). It is evident from the above-mentioned studies that these cells are phagocytic. Thus, Ling, Tseng & Wong (1985) demonstrated that these intraventricular macrophages avidly engulfed leaked erythrocytes in the lateral ventricles following maternal administration of an antimetabolite, 6-aminonicotinamide in rats. Recently, Maxwell & McGadey (1988) studied the response of epiplexus and supraependymal cells in the lateral ventricles of adult rats after a penetrant cerebral lesion and found that these cells developed numerous inclusion vesicles and some contained disrupted erythrocytes.

The present study was conducted to find out the response of the supra-ependymal and epiplexus cells to some large foreign particles introduced into the lateral ventricle. For this purpose, Cytodex(R)3 (Pharmacia) microcarrier beads coated with a toxin, a crotoxin complex (phospholipase A^2) were injected into the lateral ventricle because it has been found that these beads attract the aggregation and growth of peritoneal macrophages when implanted into the peritoneal cavity in rats (unpublished observations). Furthermore, it has been shown in our recent study (Ling, Gopalakrishnakone & Tan, 1988) that epiplexus cells in the fourth ventricle in cats were activated when crotoxin complex was introduced into the cisterna magna. This study reports the reactive changes and growth of the intraventricular macrophages on the microcarrier beads introduced into the lateral ventricles in postnatal rats.

MATERIALS AND METHODS

Cytodex(R)3 (Pharmacia) microcarrier beads, 175 μm in diameter, were incubated in crotoxin solution (0·45 μg/ml saline) containing 1% phenol red. Twenty to forty beads were then injected into the lateral ventricles of postnatal albino rats by the following method. The rats, aged 5 days, were anaesthetised with ether and their skull exposed following an incision of the skin in the midline. The beads were then introduced slowly with the aid of a plunger through a 23-gauge needle stabilised vertically at a point approximately 1 mm to the left of the sagittal suture and immediately behind the coronal suture. The tip of the needle was about 2 mm from the surface of the brain. Following the administration of the beads the skin wound was

closed with sutures and the animals were allowed to survive for various time intervals. The rats were killed at 18 hours, 2 days, 1 week, 2 weeks, 3 weeks and 30 days after the injection. 5 animals were killed at each time interval. They were perfused under ether anaesthesia with 50 ml of Ringer's solution followed by a mixed aldehyde solution composed of 2% paraformaldehyde and 3% glutaraldehyde in 0·1 M cacodylate buffer at pH 7·3. The perfusion lasted for 15 minutes after which the brains were removed and fixed for a further 5 hours at 4 °C in a similar fixative. One to two millimetre thick coronal slices of the brain were removed and blocks of the cerebrum containing the lateral ventricles were cut out and postfixed in 2% osmium tetroxide in 0·1 M cacodylate buffer at pH 7·4 for scanning electron microscopy. They were dehydrated in a graded series of ethanol and critical-point-dried in liquid carbon dioxide. They were then mounted on a specimen stub, sputter-coated with gold for 3 minutes and then viewed in a Philips 505 scanning electron microscope operated at 20 kV.

Some of the blocks were later removed from the stubs, immersed in amyl acetate, embedded in Araldite mixture and sectioned for transmission electron microscopy. Ultrathin sections were doubly stained with uranyl acetate and lead citrate and viewed in a Philips 400T electron microscope. By this procedure it was possible to confirm the cell types that had been observed by scanning electron microscopy.

OBSERVATIONS

All rats in the present study survived the injection of crotoxin-coated microcarrier beads into their lateral ventricles. The accuracy of injection was confirmed subsequently when the ventricles were exposed following the perfusion. The phenol-treated beads were visible in the ventricle, even with the naked eye. The beads introduced were lodged in the lateral ventricle on the injected side (Fig. 1). In some rats, the septum pellucidum was disrupted.

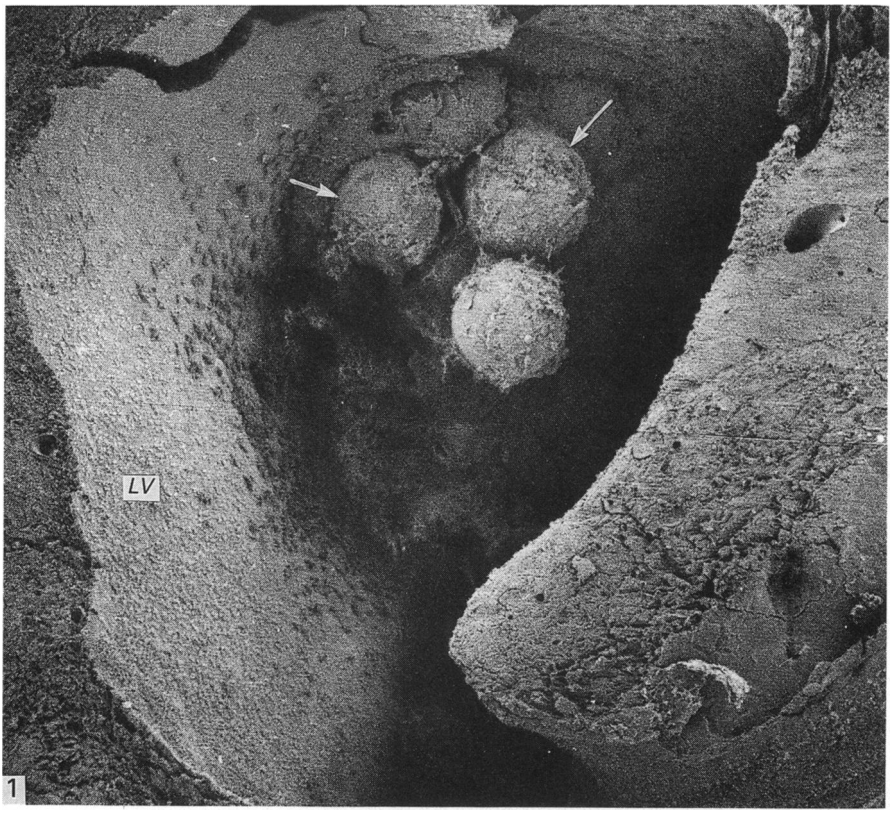
Eighteen hours after the injection, the surfaces of the beads in the lateral ventricle appeared clear except for one or two cells attached to them (Fig. 2). The ependyma of the lateral ventricle appeared normal and its lining cilia did not display any structural alteration. Two days after the injection, a number of cells were seen attached to the surface of the beads (Figs. 3, 4). The cells were spherical with a number of blebs and pseudopodia on their surfaces (Figs. 3, 4). Often, they showed long filamentous filopodia which appeared to anchor the cells to the surface of the beads (Fig. 5). Supra-ependymal and epiplexus cells showing similar features were observed on the ependyma and the choroid plexus of the lateral ventricle (Figs. 3, 4).

Fig. 1. A scanning electron micrograph of the lateral ventricle (*LV*) 2 days after the injection of beads. The lumen of one of the ventricles shows the accumulation of the injected crotoxin-coated microcarrier beads (arrows). × 162.

Fig. 2. Scanning electron micrograph of a microcarrier bead (*B*) in the lateral ventricle 18 hours after the injection of beads. The bead is lying on the ependyma (*E*). A cell (arrow) is attached to the surface of the bead. Note the normal appearance of cilia on the ependyma. × 730.

Fig. 3. Scanning electron micrograph of a portion of the lateral ventricle 2 days after the injection of microcarrier beads. A number of cells (arrows) are attached to the surface of the bead (*B*). Supra-ependymal cells (*S*) bearing similar features can be seen on the ependyma (*E*) of the ventricle. × 1210.

Fig. 4. Scanning electron micrograph of the lateral ventricle 2 days after the injection of microcarrier beads. A bead (*B*) is lying close to the choroid plexus (*CP*). Several cells (arrows) are seen on the surface of the bead. Epiplexus cells (*EP*) can be seen on the choroid plexus. × 740.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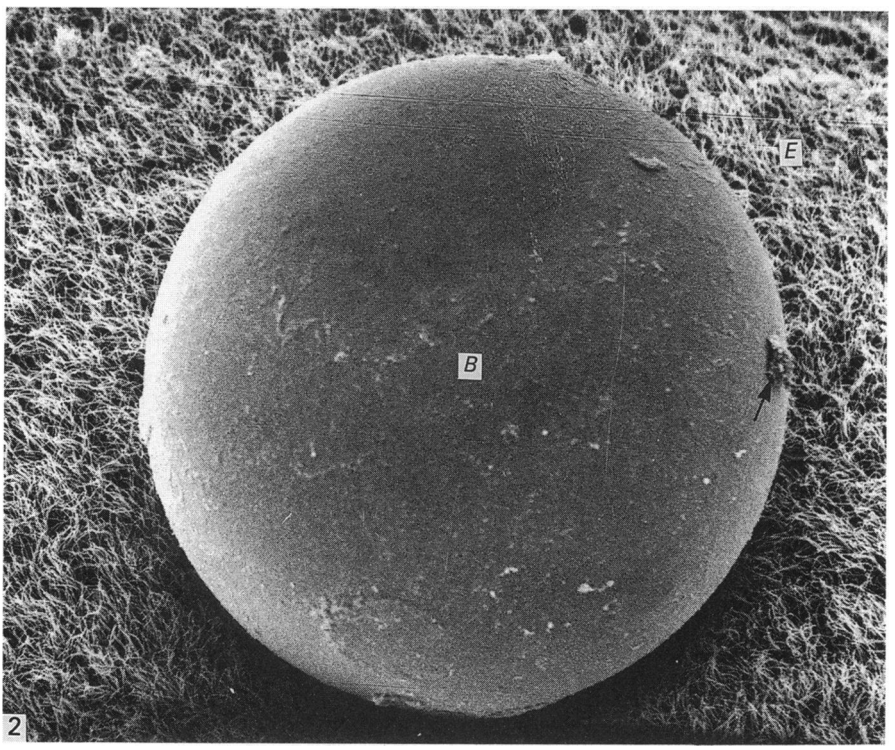
E
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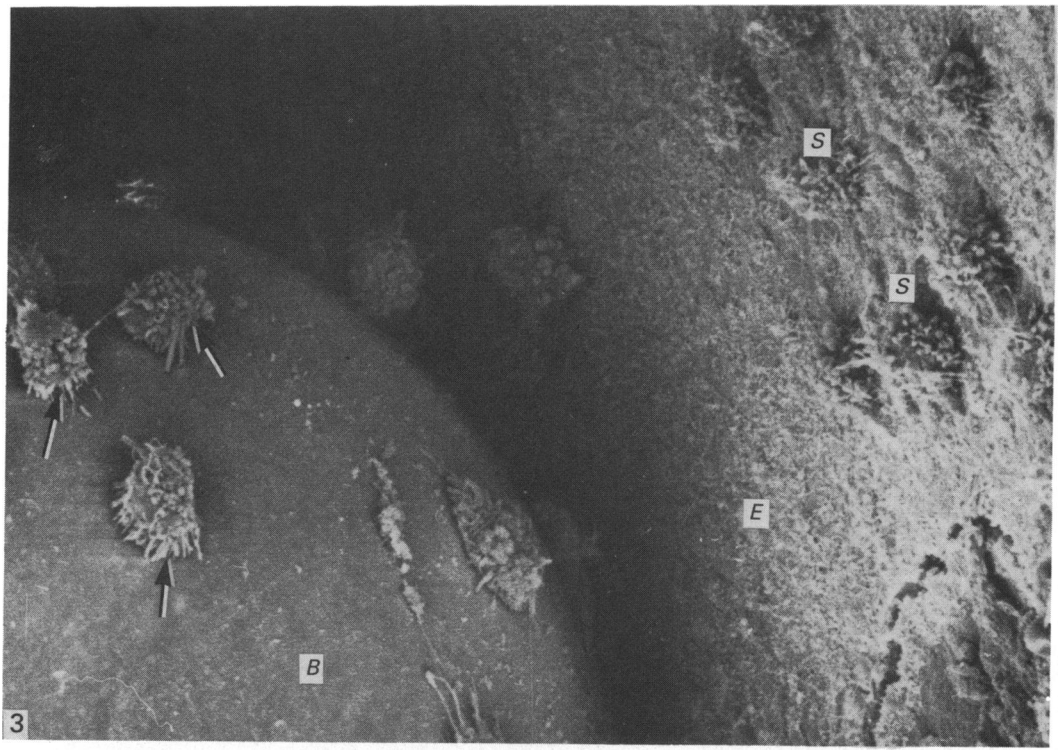

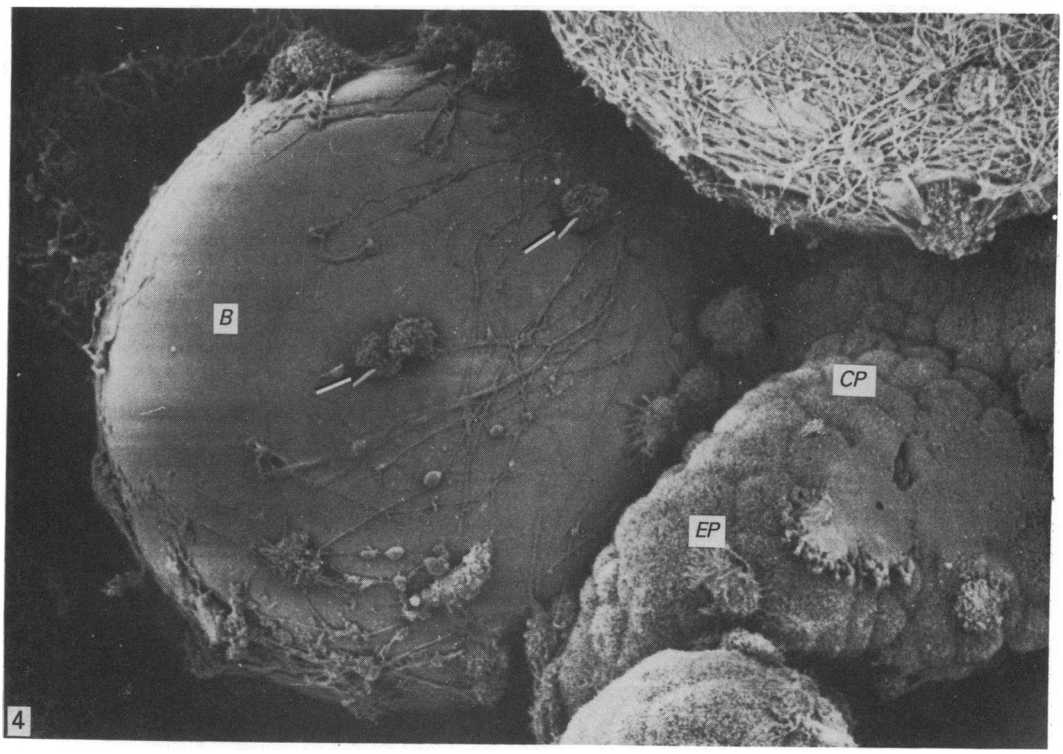

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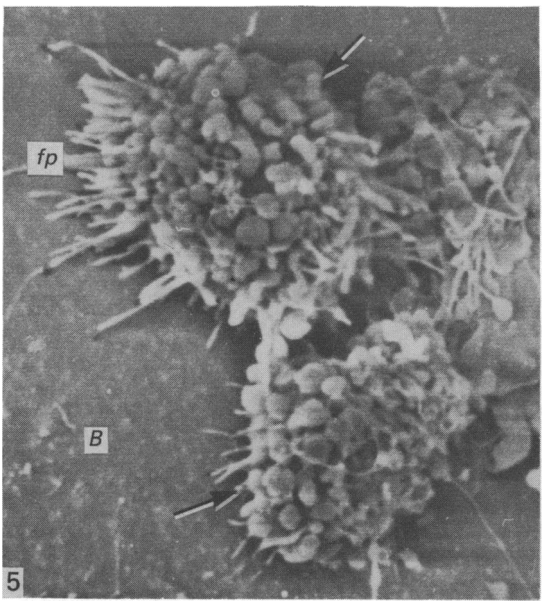
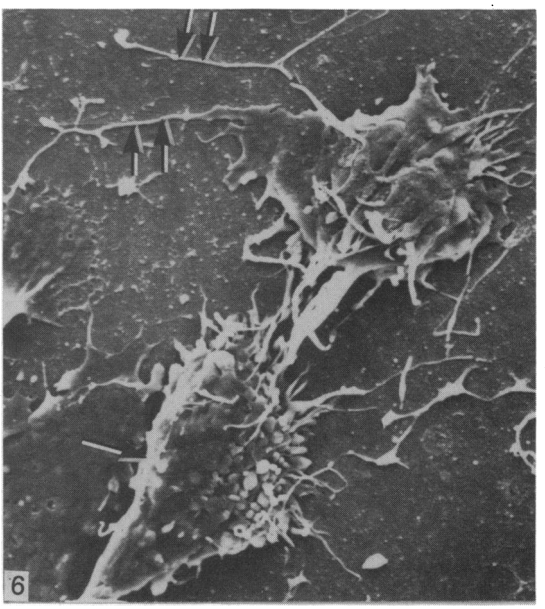




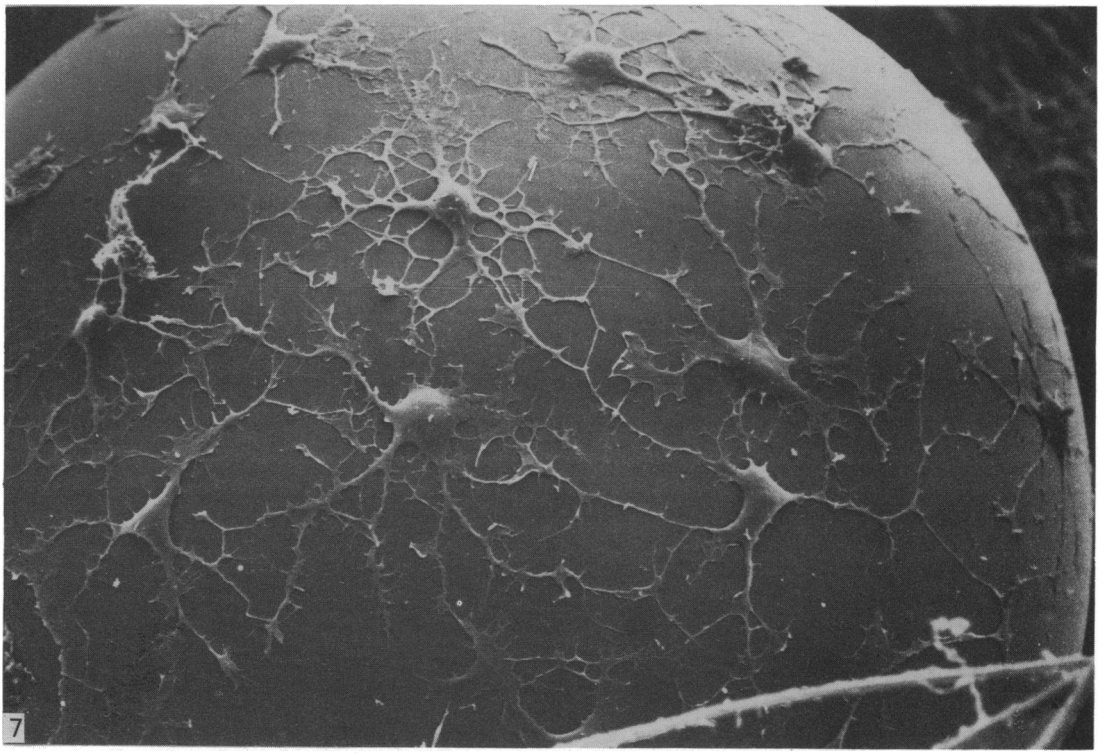
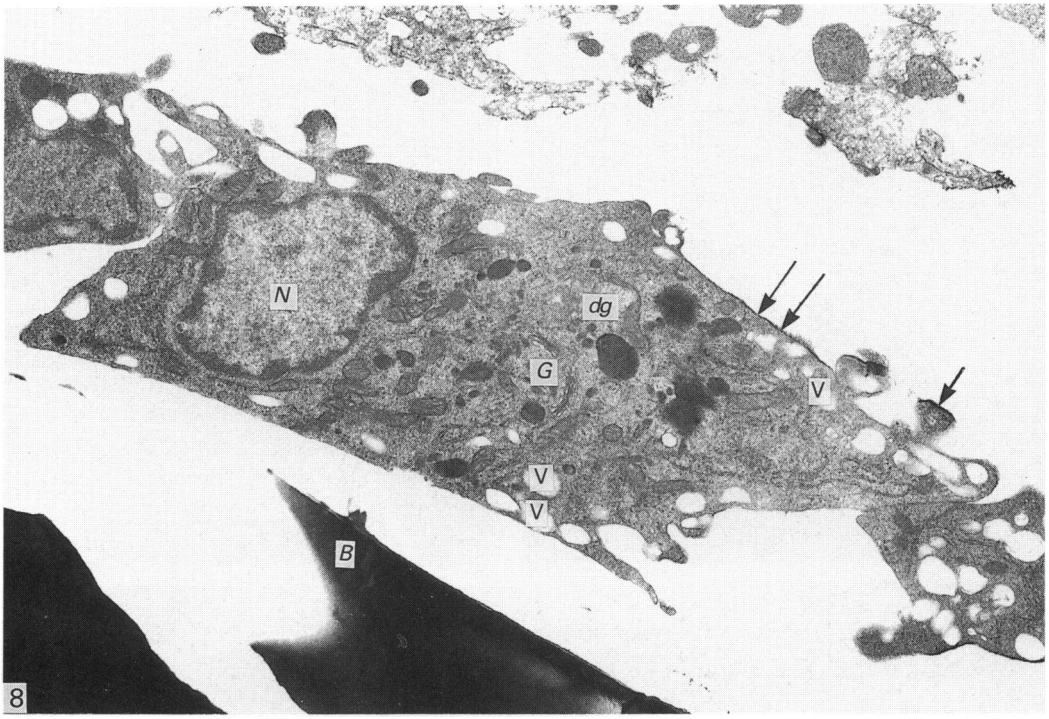


Fig. 5. A higher magnification of the cells (arrows) attached to the bead (*B*) 2 days after the injection of beads into the ventricle. Note the prominent blebs and their long filopodia (*fp*) perching on the surface of bead. × 2300.

Fig. 6. Scanning electron micrograph of two cells on the surface of a bead 1 week after the injection of beads into the lateral ventricle. One of them (arrow) is elongated and shows blebs on one side whereas the other side appears smoother. The other cell appears to have spread out with long extending processes (double arrows). × 1420.

Fig. 7. Scanning electron micrograph of a bead in the lateral ventricle 2 weeks after the injection of beads. About a dozen cells are seen with long branching processes on the surface of the bead. × 625.



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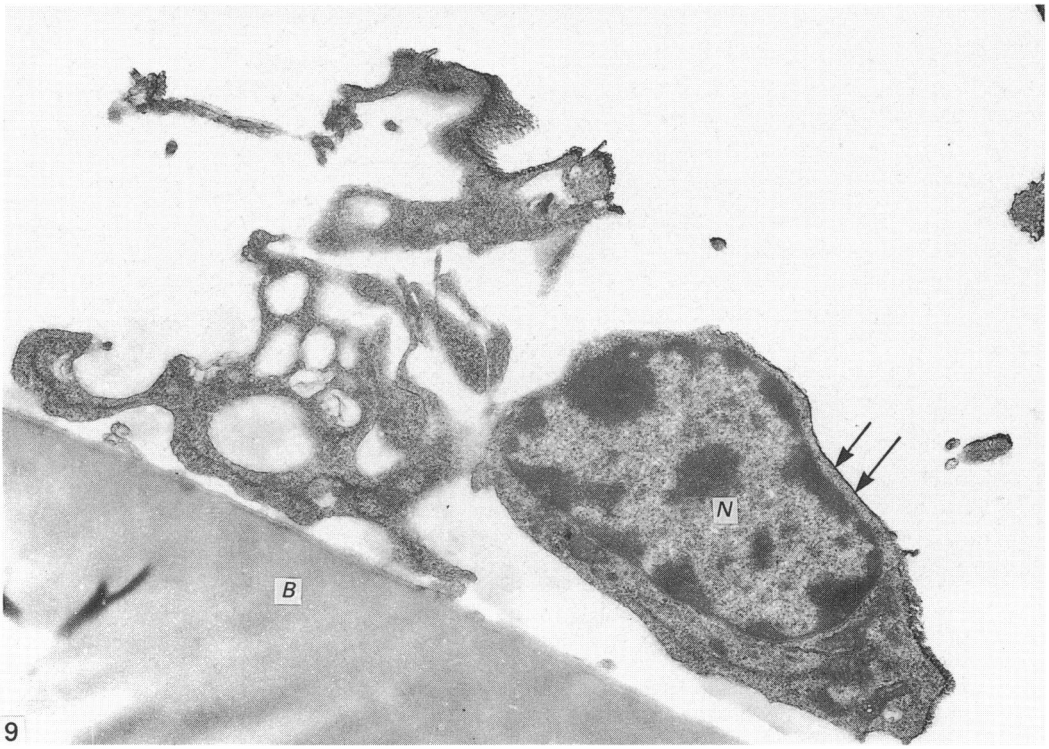

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One week after the injection, the spherical cells on the surface of the beads appeared to spread out slightly and had fewer blebs at the cell surface (Fig. 6). Some cells became oval or elongated although they were still anchored to the beads with their long filopodia.

Two weeks after the injection, there were very few cells showing blebs. Most of the cells on the surface of the beads had a smooth oval or angular cell body with branching processes arising from the cell body (Fig. 7). These long processes appeared to give rise to secondary and tertiary processes, perpendicular to each other, forming a network on the entire surface of the beads (Fig. 7).

In longer survival animals, i.e. 3 weeks and 30 days after the injection, the cells on the surface of the beads did not show further changes when compared with those observed 2 weeks after the injection.

Transmission electron microscopy of the re-embedded materials showed that 2 days after the injection of beads, most of the cells on the surface of the beads had a nucleus showing dense masses of chromatin (Fig. 8). The cytoplasm contained a small Golgi apparatus, dense granules, vacuoles (Fig. 8) and some phagocytosed materials. The surface of these cells showed blebs and pseudopodia. With longer survival times, the cells showed progressive structural changes. The majority of cells adherent to the surface of the beads were oval (Fig. 9) or elongated. Their elongated nucleus showed dense chromatin masses. The amount of cytoplasm was greatly reduced with a concomitant diminution of dense granules and rough endoplasmic reticulum. The cytoplasmic vacuoles which were abundant in the cells observed in the early time intervals were either absent or were greatly diminished.

DISCUSSION

Following the injection of crotoxin-coated microcarrier beads into the lateral ventricles of the postnatal rats, numerous cells showing blebs and filopodia were seen to be associated with their surface by scanning electron microscopy. These surface features, i.e. blebs and filopodia, are identical to those described in monocytes and their derivative macrophages, including the subarachnoid macrophages (Warfel & Elberg, 1970; Cloyd & Low, 1974; Parakkal, Pinto & Hanifin, 1974; Allen & Low, 1975; Polliack & Gordon, 1975; Malloy & Low, 1976; Deimann & Fahimi, 1979; Polliack, 1981) and amoeboid microglia *in vitro* (Ling, Tseng, Voon & Wong, 1983; Giulian & Baker, 1986). The supra-ependymal and the epiplexus cells in the ventricular cavities of the brain also show similar features (Coates, 1973; Hosoya & Fujita, 1973; Peters, 1974; Sturrock, 1979; Ling, 1981*a*). It is therefore suggested that the cells adherent to the beads are intraventricular macrophages. Indeed, with the transmission electron microscope, the pleomorphic cells observed in the scanning electron microscopy displayed features of macrophages: cytoplasmic vacuoles and lysosome-like dense granules. Again, these are ultrastructural features common to neural macrophages (Imamoto, Fujiwara, Nagai & Maeda, 1982; Ling & Tan, 1974; Ling, 1976, 1981*b*; Kaur, Ling & Wong, 1985, 1986).

Fig. 8. Transmission electron micrograph of the re-embedded material 2 days after the injection of the beads. The nucleus (*N*) of the cell associated with the bead (*B*) displays dense chromatin masses at its periphery. The cytoplasm contains vacuoles (*V*), dense granules (*dg*) and a Golgi apparatus (*G*). The surface of the cell shows the gold coating (arrows). × 12000.

Fig. 9. Transmission electron micrograph of the re-embedded bead shown in Figure 7. The cell lying on the surface of the bead (*B*) shows a nucleus (*N*) with dense chromatin masses. The scanty cytoplasm shows some dense granules. The surface of the cell shows gold coating (arrows). × 13000.

The macrophages observed on the surface of the beads may be derived from two sources: (1) the supra-ependymal or the epiplexus cells found normally in the ventricular cavities, and (2) macrophages derived from the direct transformation of the monocytes. In the first instance, the supra-ependymal and epiplexus cells may be attracted to the crotoxin-coated beads and attach themselves to the beads by means of their long filopodia. How the cells reached the surface of the beads initially remains speculative. It would seem that the beads introduced intraventricularly in this study became closely adherent to the ventricular surface (see for example Fig. 4). This then elicited the migration of macrophages along the ependymal surface to accumulate at the site of contact. The latter would then provide a further pathway for the migration of the cells to reach the entire surface of the beads. However, since it is known that free floating macrophages in the lateral ventricles are not uncommon (Ling, 1979), it is conceivable that some of them might have settled onto the surface of the crotoxin-coated beads directly. Since supra-ependymal and epiplexus cells are known to be active phagocytes (Carpenter *et al.* 1970; Ling *et al.* 1985; Maxwell & McGadey, 1988), their response to the crotoxin-coated beads administered intraventricularly may be an attempt to phagocytose them. In the present investigation large numbers of supra-ependymal cells were observed in the vicinity of the injected beads. They could have been attracted from other regions of the ventricle. The increase in number of supra-ependymal cells in response to injury has been reported in other environmental alterations (Nielson & Grauger, 1974; Ling *et al.* 1985). An increase in the number of epiplexus cells has also been reported three days after an intrathecal injection BCG (Merchant, 1979).

The possibility that some of the cells on the surface of the beads are derived from monocytes cannot be ruled out since the cerebral vessels would be expected to be traumatised during injection. The transformation of monocytes into neural macrophages has been shown in one of our previous studies where a stab wound was made in the cerebrum (Kaur, Ling & Wong, 1987).

The present study has shown that the microcarrier beads introduced intraventricularly form a useful substratum for the growth of intraventricular macrophages. Their subsequent growth and functional activity, therefore, may be followed closely with time by scanning election microscopy. Indeed, the cells associated with the beads initially display numerous blebs and pseudopodia and these features reflect the active phagocytic activity of macrophages (Tseng, Ling & Wong, 1983). However, with time, they underwent morphological changes, becoming oval, angular and consequently ramified, with long processes. This is probably a regressive process since a comparable phenomenon of transformation has also been observed in *in vitro* culture in which the monocyte-derived amoeboid microglia were transformed into ramified microglia (Ling *et al.* 1983; Giulian & Baker, 1986; Bocchini *et al.* 1988). The changes in macrophages closely associated with the microcarrier beads in the cerebral ventricle therefore closely simulate the developmental events *in vitro*. It may be deduced from this study that the morphological forms of intraventricular macrophages are dependent upon their micro-environment.

Transmission electron microscopy in the present study has confirmed the transformation of macrophagic cells into elongated cells resembling the microglial cells described in various parts of the brain (Mori & Leblond, 1969; Ling, 1981*b*; Kaur *et al.* 1985). The microglia-like cells generally possess few lysosome-like granules and little cytoplasm. Furthermore, cytoplasmic vacuoles, which are common in active macrophages, are absent in these cells. It is therefore postulated that they are the less active form or quiescent type. A similar developmental process has been described in

the normal postnatal brain where the active macrophagic amoeboid microglial cells subsequently regress into ramified microglial cells with increasing age (Imamoto *et al.* 1982; Ling, 1981*b*; Kaur *et al.* 1985). Another example is the transformation of neural macrophages into microglial cells in the healing wound of the cerebrum (Kaur *et al.* 1987).

SUMMARY

Crotoxin-coated microcarrier beads were injected into the lateral ventricles of 5 days old postnatal rats. The morphology of the cells attached to the beads at various time intervals was studied by scanning and transmission electron microscopy. Scanning electron microscopy showed that very few cells were associated with the surface of the beads 18 hours after the injection. After 2 days a large number of spherical cells showing blebs and filopodia were attached to the surface of the beads. One week after the injection, these cells became oval and, in longer survival periods between 2 weeks and 30 days after the injection, the cells developed a flattened or angular cell body bearing a number of radiating slender processes. Transmission electron microscopy of the re-embedded materials from animals killed 2 days after the injection showed many cells with an eccentric nucleus containing dense chromatin masses. Their abundant cytoplasm was endowed with a variable number of lysosome-like dense granules and vacuoles. In longer surviving animals, the cells became elongated with scanty cytoplasm showing relatively fewer dense granules and cytoplasmic vacuoles. It is postulated from this study that the cells attached to the crotoxin-coated beads are derived from the intraventricular macrophages. These are functionally active initially in response to the beads injected. With time, however, they undergo morphological alteration and regress into quiescent cells which are microglia-like.

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