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A light microscopic and scanning electron microscopic study of intraventricular macrophages in the brains of aged mice

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INTRODUCTION

Macrophages have been described throughout the cerebral ventricular system. A few free cells may be present but in the main these cells are found lying on the surface of either the choroid plexus (Kolmer, 1921; Carpenter, McCarthy & Borison, 1970; Chamberlain, 1974; Peters, 1974; Sturrock, 1978, 1979; Ling, 1979, 1981) or the ependyma (Clementi & Marini, 1971; Noack, Dumitrescu & Schweichel, 1972; Allen & Low, 1973; Coates, 1973*a*, *b*; Bleier, 1975; Walsh, Brawer & Lin, 1978; Sturrock, 1978, 1979; Bleier & Albrecht, 1980). Most investigations of intraventricular macrophages have been carried out in adult animals, although there have been a few developmental studies (Chamberlain, 1974; Walsh *et al.* 1978; Ling, 1979; Sturrock, 1979; Bleier & Albrecht, 1980). In mature animals, intraventricular macrophages are flattened cells with a variable number of processes (Allen & Low, 1973; Peters, 1974; Scott *et al.* 1975; Leslie, Gwyn & Morrison, 1978; Sturrock, 1979).

So far there has been little interest shown in intraventricular macrophages in the ageing brain. The present study set out to investigate the structure of intraventricular macrophages in the brains of elderly mice as seen in 1 μm sections and the scanning electron microscope. The modal life span of the strain used is 22 months, which means that the animals which were studied, aged 25 to 31 months, were extremely old.

MATERIALS AND METHODS

Brains of ASH/TO strain mice, aged 25, 28 and 31 months, were fixed by vascular perfusion with a solution of 2 % paraformaldehyde and 3 % glutaraldehyde in a 0·165 M phosphate buffer under Sagatal anaesthesia. Three brains were prepared at each age. After completion of perfusion the cadavers were left overnight at 4 °C before the brains were removed. The brains were cut in the sagittal plane. Coronal slices, approximately 1 mm thick, were cut rostral to the caudal boundary of the interventricular foramen. Parasagittal slices were prepared containing the caudal part of the third ventricle, the aqueduct, the fourth ventricle and the medullary and cervical central canal. These slices were post-fixed in 1 % buffered osmium tetroxide and dehydrated in graded alcohols. Some of the coronal slices containing the choroid plexus in the lateral ventricle were embedded in Spurr's resin for 1 μm light microscopy and transmission electron microscopy, whilst the remainder of the blocks were processed for scanning electron microscopy using a method similar to that described for prenatal brains (Sturrock, 1982).

One micron coronal sections were stained with toluidine blue and ultrathin

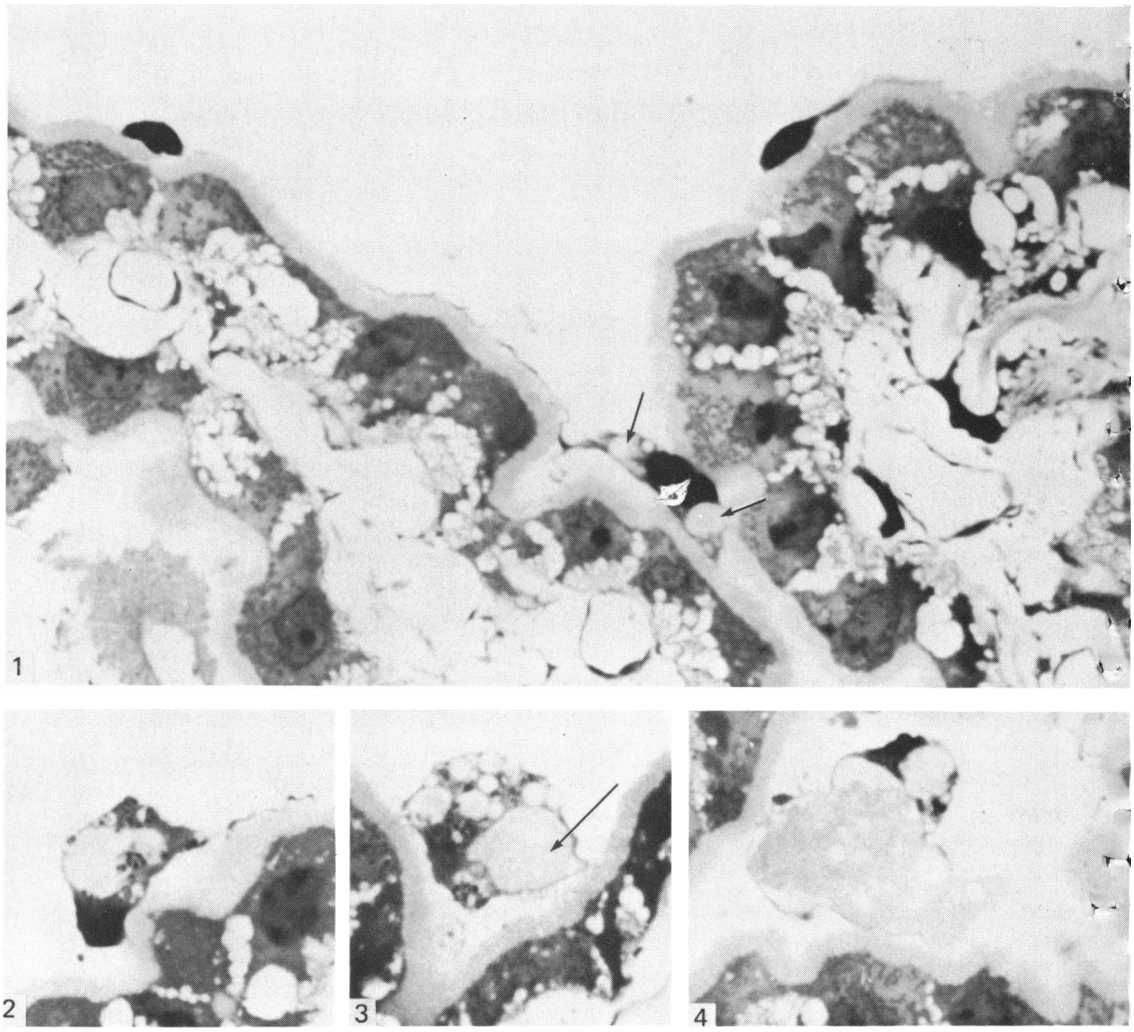


Fig. 1. Three intraventricular macrophages are present in this Figure. Two are of the flattened variety, and a sheet of cytoplasm can be seen extending from the nuclear region of the one on the right. The third macrophage also has a sheet of cytoplasm on one side of the nucleus but the cytoplasm around the nucleus is distended with lipid droplets (arrows). There is a large amount of intercellular space in the choroid plexus. The basal region of the epithelium of the choroid plexus appears to contain numerous vacuoles. 31 months old mouse. Toluidine blue-stained 1 μm section. $\times$ 1250.

Fig. 2. This intraventricular macrophage has distended cytoplasm containing lipid and some dark-staining granules, probably lipofuscin. A sheet of cytoplasm is present on the right side. 31 months old mouse. Toluidine blue-stained 1 μm section. $\times$ 1250.

Fig. 3. The macrophage in this Figure contains numerous small lipid droplets, dark granules and a large foamy mass (arrow). No cytoplasmic sheet is visible. 28 months old mouse. Toluidine blue-stained 1 μm section. $\times$ 1250.

Fig. 4. The main feature of this macrophage is the extensive foamy mass which appears to be extruding through the thin rim of cytoplasm. 31 months old mouse. Toluidine blue-stained 1 μm section. $\times$ 1250.

sections were stained with uranyl acetate and lead citrate and examined in an AEI 801 electron microscope.

RESULTS

In 1 μm sections, almost all the intraventricular macrophages found were lying on the surface of the choroid plexus. About half these were flattened cells with a thin layer of cytoplasm, usually situated on one side of the nucleus (Fig. 1). The remaining cells varied widely in size, shape and cytoplasmic inclusions (Figs. 1–4). Droplets of varying sizes were common (Figs. 1, 3, 4) and were pale yellow in colour in toluidine blue-stained sections. By their appearance in 1 μm sections, they were judged to be lipid. Some cells contained very large amounts of foamy material (Figs. 2–4) which was also pale yellow in colour. Small dark granules, probably lipofuscin, were also occasionally present in the cytoplasm of intraventricular macrophages (Figs. 2–4).

Scanning electron microscopy confirmed that intraventricular macrophages were more numerous on the surface of the choroid plexus than on the ependymal surface. Occasional supra-ependymal macrophages were present, however, throughout the ventricular system, including the aqueduct and central canal. Although no counts of intraventricular macrophages were made one had the impression that they were less numerous, even on the choroid plexus surface, than in young adult mice.

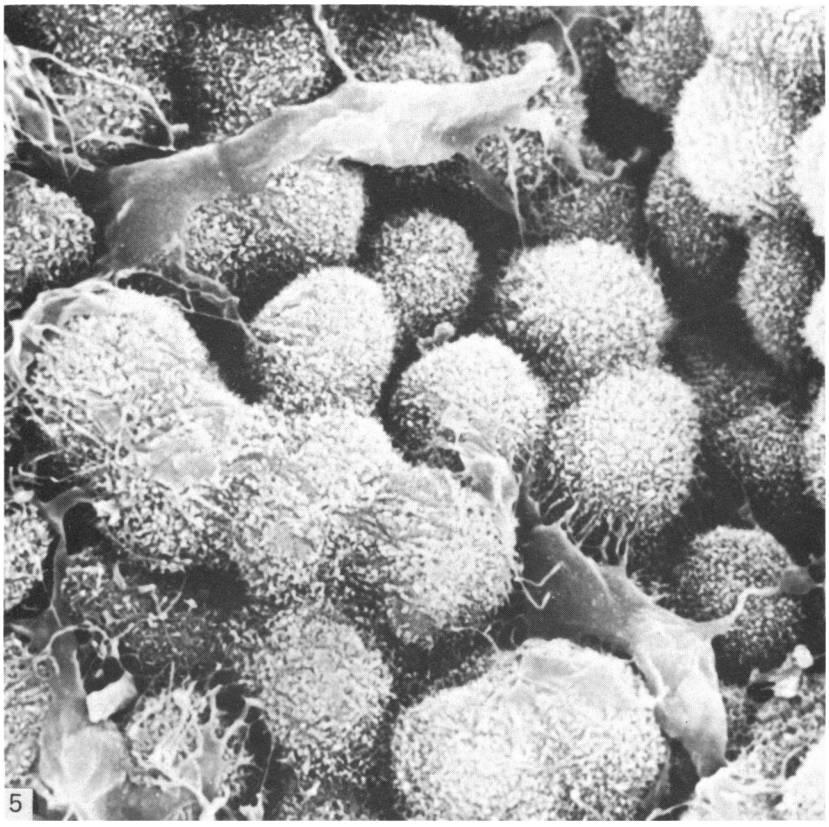
Flattened cells, similar to those seen in young adult mice (Sturrock, 1979), were present and had a number of processes which were usually short and cylindrical (Fig. 5) although some were broader and more ribbon-like (Figs. 5, 6). The cells shown in Figures 5 and 6 are probably similar to the flattened cells shown in Figure 1.

As in 1 μm sections, the non-flattened cells showed a wide variety of form. Some had multi-blebbed masses in one part but retained an area of flattened cytoplasm (Fig. 7) whereas other cells had no areas of flattened cytoplasm (Fig. 8). A feature of many non-flattened cells was the presence of short hair-like processes which were particularly prominent on the cytoplasmic sheet, if present (Figs. 7, 8). Figure 7 shows a macrophage which is probably similar to those shown in Figures 1 and 2 whereas the macrophage in Figure 8 is more probably similar to those shown in Figures 3 and 4.

Because many macrophages contained what appeared to be lipid inclusions, the choroid plexus, ependyma and brain tissue were examined to find a possible source of lipid. Intercellular spaces, varying in size, were observed between choroid plexus epithelial cells at all ages examined (Figs. 1–4, 9). In the basal regions of the choroid plexus epithelium, numerous small spaces were present which resembled leached out lipid droplets (Figs. 1–4). Transmission electron microscopy, however, demonstrated that the spaces were intercellular (Figs. 15, 16).

In some 1 μm specimens, cytoplasmic contents appeared to be herniating out of choroid plexus epithelial cells into the cerebrospinal fluid (Fig. 9) and these extrusions may be the equivalent of the small spherical blebs occasionally seen on the choroid plexus surface in the scanning electron microscope (Figs. 10, 11).

Lipid droplets were very common in ependymal cells (Fig. 12) but these droplets stained much more darkly than those found in intraventricular macrophages, appearing as dark blue–green droplets in contrast to the pale yellow droplets in the macrophages. Unlike intraventricular macrophages microglial cells contained small yellow staining vacuoles but no large lipid droplets (Fig. 13). The vacuoles seen in microglial cells in 1 μm sections may have resulted from loss of the lipid associated with lipofuscin (Fig. 17) during processing. The only foamy masses found in the brain



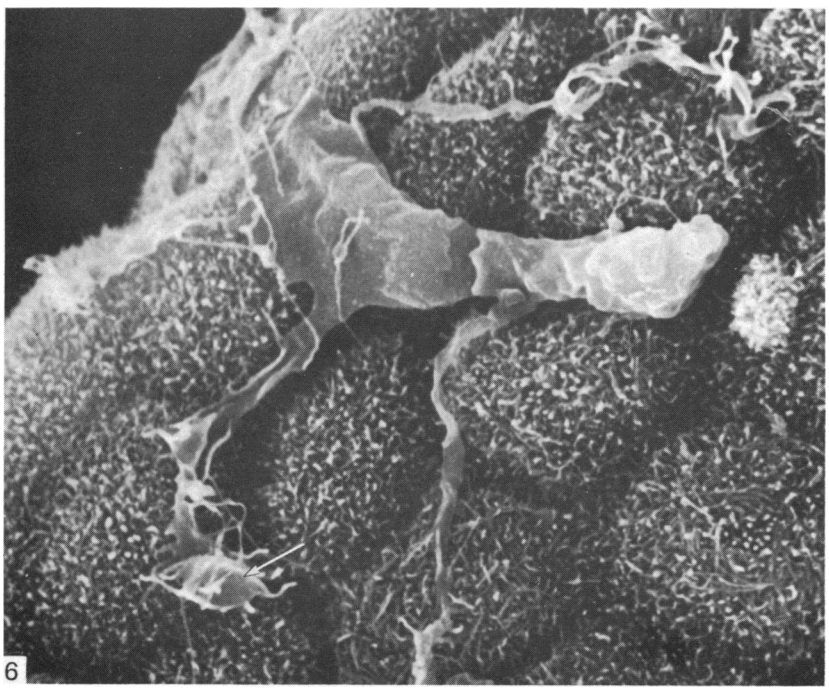
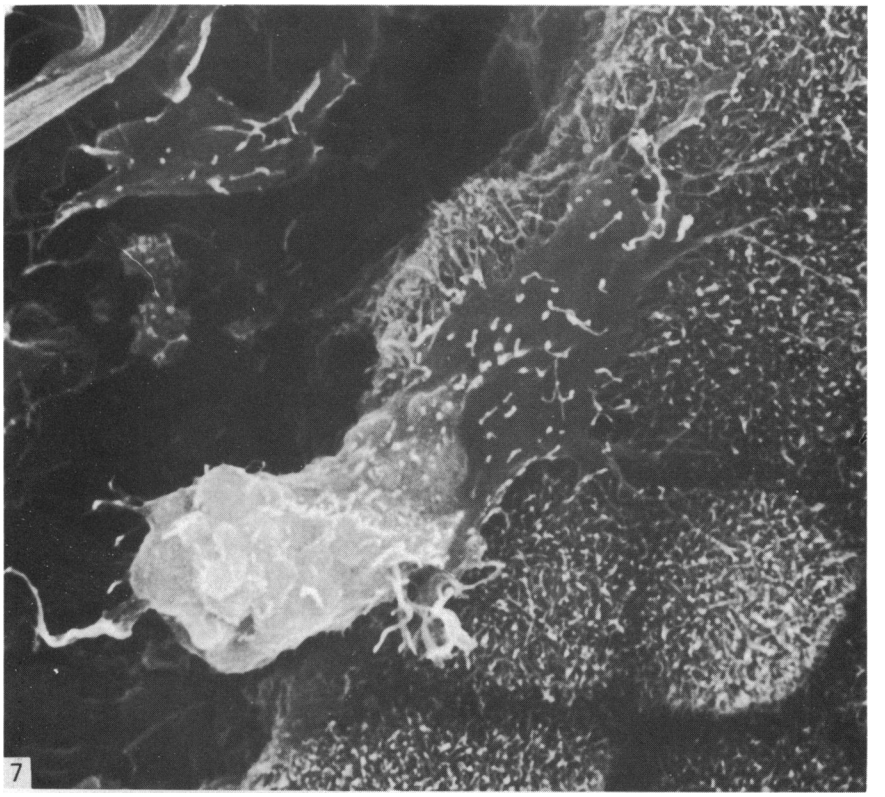


Fig. 5. Three flattened macrophages are visible on the surface of the choroid plexus. Most of the processes are short and cylindrical, but some ribbon-like processes are present. 28 months old mouse. SEM. $\times 2060$.

Fig. 6. This macrophage, lying on the choroid plexus, has a number of ribbon-like processes. One is slightly distended (arrow). There appears to be an artefactual fracture across the body of this cell. 31 months old mouse. SEM. $\times 2950$.



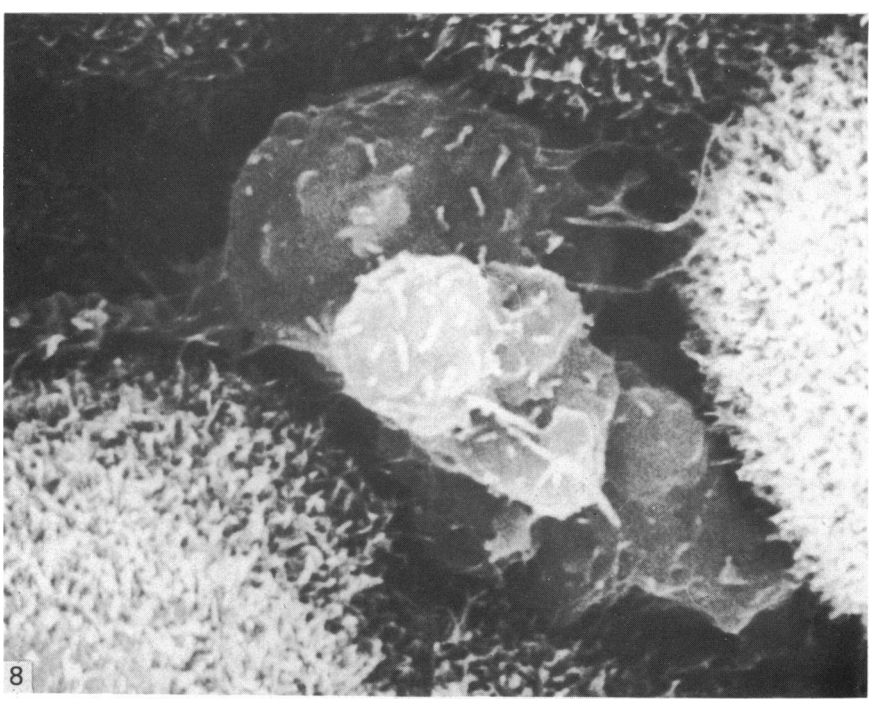
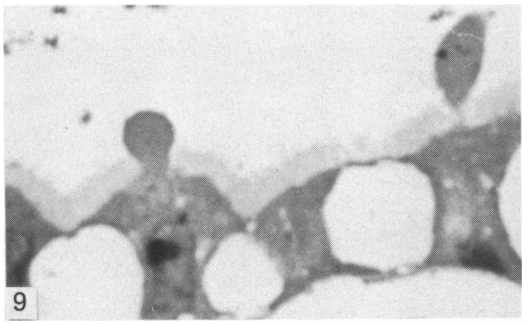


Fig. 7. The macrophage lying on the choroid plexus has an extensive area of flattened cytoplasm from which small hair-like processes protrude. The left half of the cell is distended and the surface is blebbed. These blebs may be due to lipid droplets within the cytoplasm. This cell may be similar to those shown in Figures 1 and 2. 31 months old mouse. SEM. × 2800.

Fig. 8. No flattened area of cytoplasm appears to be associated with this macrophage which is markedly distended. Numerous short processes extend from the cytoplasm. This cell may be similar to those shown in Figures 3 and 4. 28 months old mouse. SEM. × 5400.



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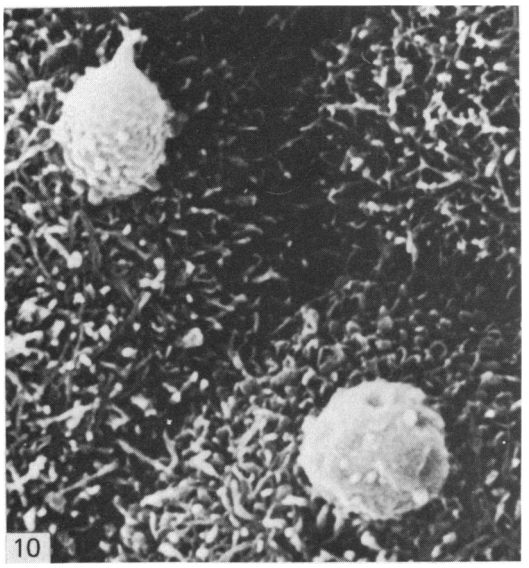

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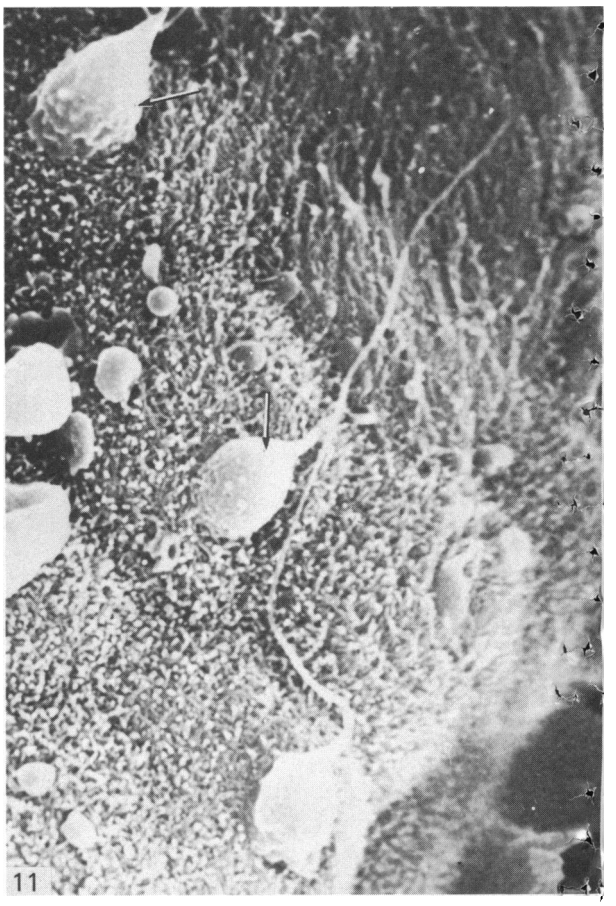

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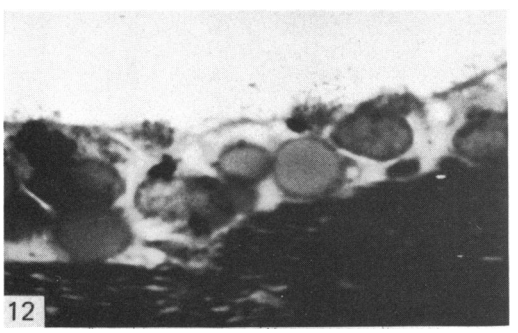

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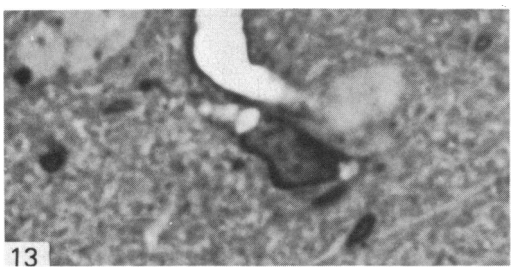

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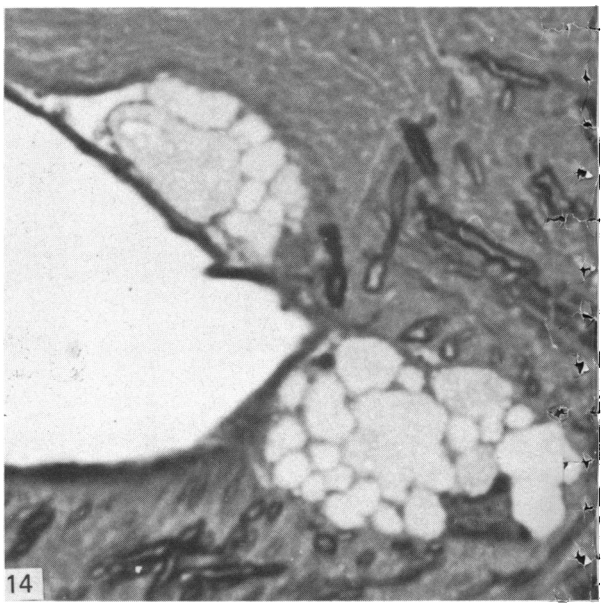

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substance were those associated with the specialised type of pericytes named neurolipomastocytoid cells by Ibrahim, Al-Wirr & Bahuth (1979). The foamy masses found in intraventricular macrophages differed from the foamy cytoplasms of neurolipomastocytoid cells in that the latter were compartmentalised by very fine bands of cytoplasm (Figs. 14, 18). The foamy masses in neurolipomastocytoid cells appeared pale yellow in toluidine blue-stained sections.

DISCUSSION

Some intraventricular macrophages in the ageing brain resemble the flattened type of cell commonly seen in young adult animals (Allen & Low, 1973; Peters, 1974; Scott *et al.* 1975; Leslie *et al.* 1978; Sturrock, 1979) whilst others contain a variable amount of cytoplasmic inclusions, varying from a few lipid droplets and lipofuscin granules to a vast quantity of foamy material grossly distending the cytoplasm. These structural changes may be a primary response of macrophages to ageing but a more likely explanation is that they represent different stages of phagocytotic activity, since all stages between the flattened macrophage and the grossly distended macrophage were observed. This phagocytic activity may be either phagocytosis of lipid or of cellular debris as discussed below. One piece of evidence against the macrophage population simply being an ageing population was the presence of a mitotic macrophage which suggests that the macrophage population is undergoing a slow turnover. Also if the changes were simply the effects of age on the intraventricular macrophages one might expect to find similar changes in microglia, which probably share a common origin and development with intraventricular macrophages (Sturrock, 1978, 1979, 1981; Ling, 1979, 1981). There is, however, relatively little change in the structure of microglial cells with age, apart from the accumulation of lipofuscin.

The most obvious feature shown by many intraventricular macrophages in the ageing brain is the accumulation of what seems to be lipid, both in the form of droplets and in foamy aggregations. It is not clear what the source of this lipid is.

Fig. 9. Choroid plexus from a 31 months old mouse showing very large spaces between adjacent cells and protrusions of cytoplasm through the microvilli. The nucleus in one of the cells with such a protrusion appears normal. Toluidine blue-stained section. × 1500.

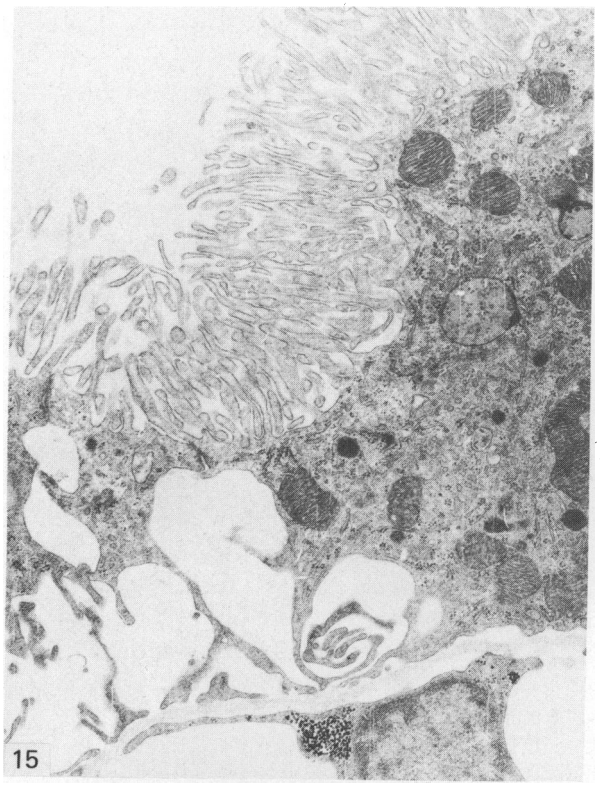
Fig. 10. Surface of the choroid plexus showing the presence of two small spherical structures which may be cytoplasmic protrusions. 31 months old mouse. SEM. × 6050.

Fig. 11. Choroid plexus showing a region with numerous surface blebs which vary in size and shape. Some of these may be artefactual, but at least two (arrows) do appear to be cytoplasmic protrusions. One object has a fine process extending a considerable distance from it. These blebs are too small to be cells (contrast with Figures 6 and 7 which are of a similar magnification). 31 months old mouse. SEM. × 3000.

Fig. 12. Ependymal layer of a 31 months old mouse containing a number of large medium-staining lipid droplets. These differ in size and staining intensity from the droplets found in intraventricular macrophages. Toluidine blue-stained 1 μm section. × 1500.

Fig. 13. Microglial cell in the neostriatum of a 31 months old mouse. The small vacuoles are probably due to lipid/lipofuscin complexes similar to those depicted in Fig. 17. Toluidine blue-stained 1 μm section. × 1500.

Fig. 14. Two neurolipomastocytoid cells (Ibrahim *et al.* 1979) are present in this micrograph. The cytoplasm of both cells is grossly distended with foamy material. The foamy material is compartmentalised by bands of cytoplasm. 31 months old mouse. Toluidine blue-stained 1 μm section. × 1250.



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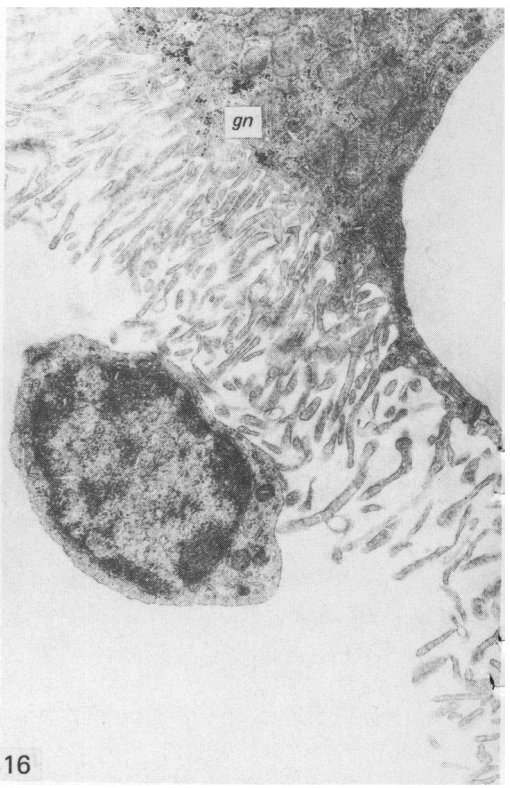

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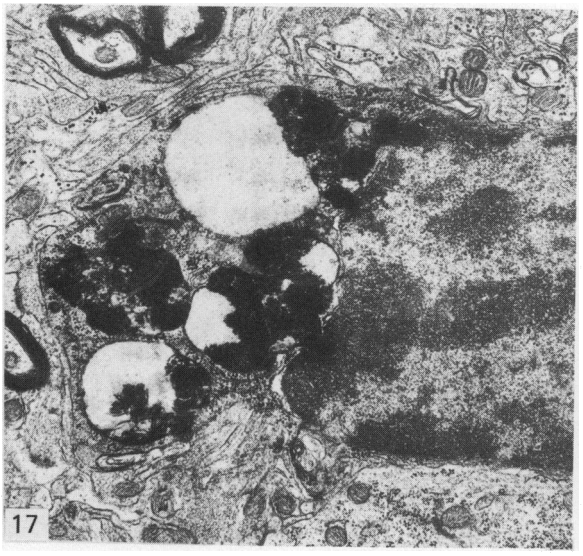

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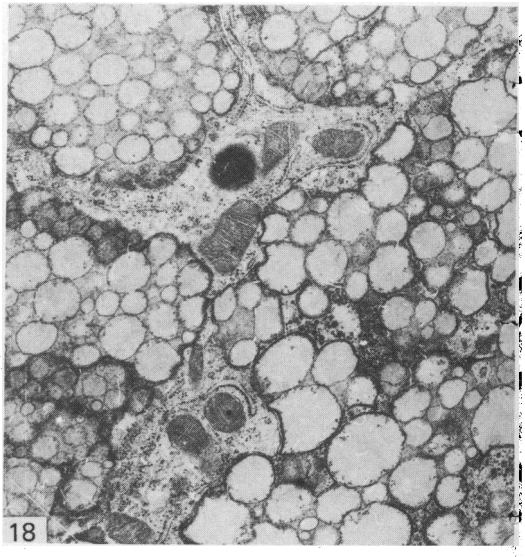

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There was no evidence in the present study for the presence of lipid in any quantity in the choroid plexus epithelium. Large lipid droplets are present in the ependyma but these are more darkly staining and probably contain more unsaturated fatty acids than the lipid droplets in the intraventricular macrophages. It is, nevertheless, possible that ependymal cells secrete lipid into the cerebrospinal fluid and that this lipid is subsequently taken up by the intraventricular macrophages.

The lipid might also be a by-product of phagocytosis (Chu-Wang & Oppenheim, 1978; Sturrock, 1981). The preferential distribution of intraventricular macrophages in the choroid plexus surface may be due to the epithelium of the choroid plexus undergoing gradual degeneration. The large amount of intercellular space, particularly at the basal surface, might be an indication of this. A similar increase in extracellular space has been noted between tanycytes of the third ventricle in aged rats (Scott & Sladek, 1981). Great care, however, must be taken in interpreting extracellular space and its significance (Sturrock & Smart, 1980). As well as an increase in intercellular space, a number of epithelial cells of the choroid plexus could be seen apparently extruding part of their cytoplasm through the surface layer of microvilli. In these cells, however, the nucleus was intact and showed no signs of pyknosis. The extrusions may form the blebs on the surface of the choroid plexus in the SEM. It is possible that phagocytosis of these blebs gives rise to the lipid in the intraventricular macrophages. The epithelial cells of the choroid plexus with extruding cytoplasm were found mainly in the upper part of the lateral ventricle. These extrusions could be artefactual because this part of the plexus is furthest from the root of the plexus and so, perhaps, most likely to suffer from hypoxia during the initial stages of fixation.

One cannot rule out the possibility that the apparent preferential distribution of intraventricular macrophages on the choroid plexus is due to loss of macrophages from the surface of the ependyma during processing, perhaps because they are less firmly adherent to the ependymal surface. It seems more likely, however, that the presence of intraventricular macrophages on the choroid plexus surface is a result of the more obvious structural changes with age found in the choroid plexus epithelium. The possibility cannot be ruled out that the increase in the epithelial intercellular space of the choroid plexus could lead to a more rapid passage of monocytes from the choroidal capillaries to the plexus surface and lead, in turn, to an increase in the macrophage population. There was, however, no evidence of such a migration, either within the choroid plexus or in the spaces between adjacent epithelial cells of the choroid plexus.

Although it was stated in the results that about half the intraventricular macrophages are large, lipid-containing cells, this figure should be treated with some caution. Despite occasional microscope fields of the plexus containing more than

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Fig. 15. This micrograph demonstrates that the spaces between adjacent epithelial cells of the choroid plexus do not contain lipid. The vacuolar appearance in semithin sections is due to numerous narrow processes. The microvilli are narrower than those usually present in young animals. 31 months old mouse. Transmission electron micrograph. × 12600.

Fig. 16. The epithelial cell of the choroid plexus contains small amounts of glycogen (*gn*) which is not present in Fig. 15. An intraventricular macrophage is present. 31 months old mouse. Transmission electron micrograph. × 12600.

Fig. 17. Microglial cell containing lipofuscin and lipid/lipofuscin complexes. 28 months old mouse. Transmission electron micrograph. × 12600.

Fig. 18. Neurolipomastocytoid cell showing the foamy masses separated by strands of cytoplasm. 28 months old mouse. Transmission electron micrograph. × 12600.

one macrophage lying on the surface, intraventricular macrophages are relatively sparsely scattered and the results were obtained from counts of only about 40–50 cells per age group. Because no account was taken of differences in size (and the lipid-containing cells can be very large, in comparison to the flattened variety) the lipid-containing cells probably make up a smaller proportion of the population than appears at first sight.

SUMMARY

Intraventricular macrophages in the aged mouse brain (25–31 months) are found mainly on the surface of the choroid plexus. About half the population are flattened cells similar to those described by a number of authors in adult animals. The remainder are partially or completely distended due to the presence of varying amounts of lipid, either in the form of droplets or of foamy masses. These variations in shape are visible in the scanning electron microscope, as well as in semithin sections.

The source of the lipid is unclear. It may result from passage of lipid into the cerebrospinal fluid from the ependyma, because many ependymal cells contain large lipid droplets. Alternatively, the lipid may be the result of phagocytosis of degeneration products of epithelial cells of the choroid plexus, either of complete cells, or of parts of cells extruded into the cerebrospinal fluid.

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