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Transformation of monocytes into amoeboid microglia and into microglia in the corpus callosum of postnatal rats, as shown by labelling monocytes by carbon particles

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INTRODUCTION

In an earlier communication (Ling, 1978) circulating monocytes labelled with colloidal carbon particles were shown to enter the brain tissue, in particular the corpus callosum, of 5 days old rats. Sporadic carbon-labelled cells derived from blood elements were observed interstitially between axons in the corpus callosum 1 or 2 days after intravenous carbon injection. This study, however, failed to demonstrate carbon labelling in the so-called amoeboid microglial cells, a prominent group of active macrophages which are constantly present in the loosely structured corpus callosum of neonatal rats (Ling, 1976, 1977). It was inferred from this experiment that some of the brain macrophages were derived from blood elements while others, the amoeboid microglia, arose from subependymal cells in common with astrocytes and oligodendrocytes (Ling, 1976). The preliminary observations did not allow a clear clarification of the relation between infiltrated carbon-labelled monocytes, amoeboid microglia and typical microglia.

Recent findings by Imamoto & Leblond (1978) have cast doubt on this view (Ling, 1976) of the origin of amoeboid microglia in early postnatal rats. They have presented electron microscopic evidence for the presence in the corpus callosum of postnatal rats of cells with monocytic features which could have been attracted by degenerating elements in the corpus callosum, phagocytosing debris and transforming into amoeboid microglia. Radioautographic observations have indicated that such cells involute into microglia as the animal ages. Thus, in the brain of the postnatal rat, as in brain wounds in adult rats (Imamoto & Leblond, 1977), monocytes and macrophages are considered to transform into microglia.

In an attempt to resolve this dilemma as to the origin of microglia, the author's earlier study of postnatal rats has been repeated, but using larger doses of carbon and and longer survival periods. Pilot studies along these lines (Ling, unpublished) have shown that carbon particles ingested by monocytes do remain within the cells and can be identified up to 10 days after injection. The technique, therefore, can be used with confidence to trace the fate of monocytes containing carbon which infiltrate the corpus callosum in recently born rats (Ling, 1978).

MATERIALS AND METHODS

Sixteen albino rats aged 6 days, from two litters, were used. Each rat was given two doses, 24 hours apart, of a 0·04 ml carbon suspension (Günther Wagner India ink of Hanover, Germany; Batch no. C11-1431/a) into the jugular vein. Injections

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were made under ether anaesthesia with the jugular vein exposed. Following each injection the skin was sutured and the wound area sprayed with a plastic wound dressing (Nobecutane). The rats appeared physically healthy after the injection, and the darkened skin was of fairly normal colour again within an hour. The injected rats were then returned to the mother, which had also been anaesthetized earlier with ether, handled gently, and sprayed over its back and limbs with Nobecutane. These procedures were effective in preventing cannibalism.

The rats were killed at 1, 3, 4, 5, 6, 8 and 9 days after the second carbon injection. They were perfused under ether anaesthesia with 4 % glutaraldehyde in phosphate buffer (pH 7·3). The brain was then removed and further fixed in 10 % neutral formalin for approximately 24 hours. Coronal serial sections at 7 μm were prepared and stained with cresyl fast violet.

Electron microscopy was also attempted in order to verify the localization of the various carbon-labelled cells. For this purpose 3 rats, which had been allowed to survive for 3, 4 and 5 days after the second carbon injection, were perfused with a mixed aldehyde solution composed of 2 % paraformaldehyde and 3 % glutaraldehyde in cacodylate buffer (pH 7·3). Blocks of corpus callosum and neighbouring regions were removed and fixed for a further 2 hours in fresh aldehyde solution. After a brief rinsing in sucrose buffer the blocks were post-osmicated in Dalton's fluid. They were then processed for embedding in Araldite. Ultrathin sections were cut, double stained with uranyl acetate and lead citrate, and examined in a Hitachi HS-8 electron microscope.

OBSERVATIONS

Light microscopy

The present observations were primarily based on sections taken at the level of the optic chiasma. The corpus callosum, lateral ventricles, subependyma, and cavum septi pellucidi, were examined carefully for cells containing intracytoplasmic carbon particles. These areas were chosen because it was known from previous work (Ling, 1976) that amoeboid microglia were to be found there. The cerebral cortex and its covering meninges, together with the caudate nucleus were also briefly scanned. Carbon-labelled cells were abundant in the meninges: they were identified as macrophages with the electron microscope (Ling, unpublished observations). A few carbon-labelled cells were observed in association with blood vessel walls in the parenchyma of the cerebral cortex and caudate nucleus. Examination of the so-called epiplexus cells (Carpenter, McCarthy & Borison, 1970) in the lateral ventricles showed that some contained carbon particles in their cytoplasm (Fig. 1). Carbon-labelled cells were most frequent, however, in the corpus callosum.

Corpus callosum

The loosely structured corpus callosum of the postnatal rat shows widely spaced axons, especially in the region over the lateral ventricles. Between the nerve fibres are the usual glial cell types, with immature cells preponderating (Ling & Tan, 1974; Imamoto & Leblond, 1978). Amoeboid microglia constitute approximately 6 % of the glial population in the corpus callosum of 5 and 6 days old rats (Ling & Tan, 1974). Some cells with monocytic features have also been described (Imamoto & Leblond, 1978). Microglia of adult type are not seen in newborn rats, but begin to appear after the fifth day postnatally (Ling & Tan, 1974; Imamoto & Leblond, 1978).

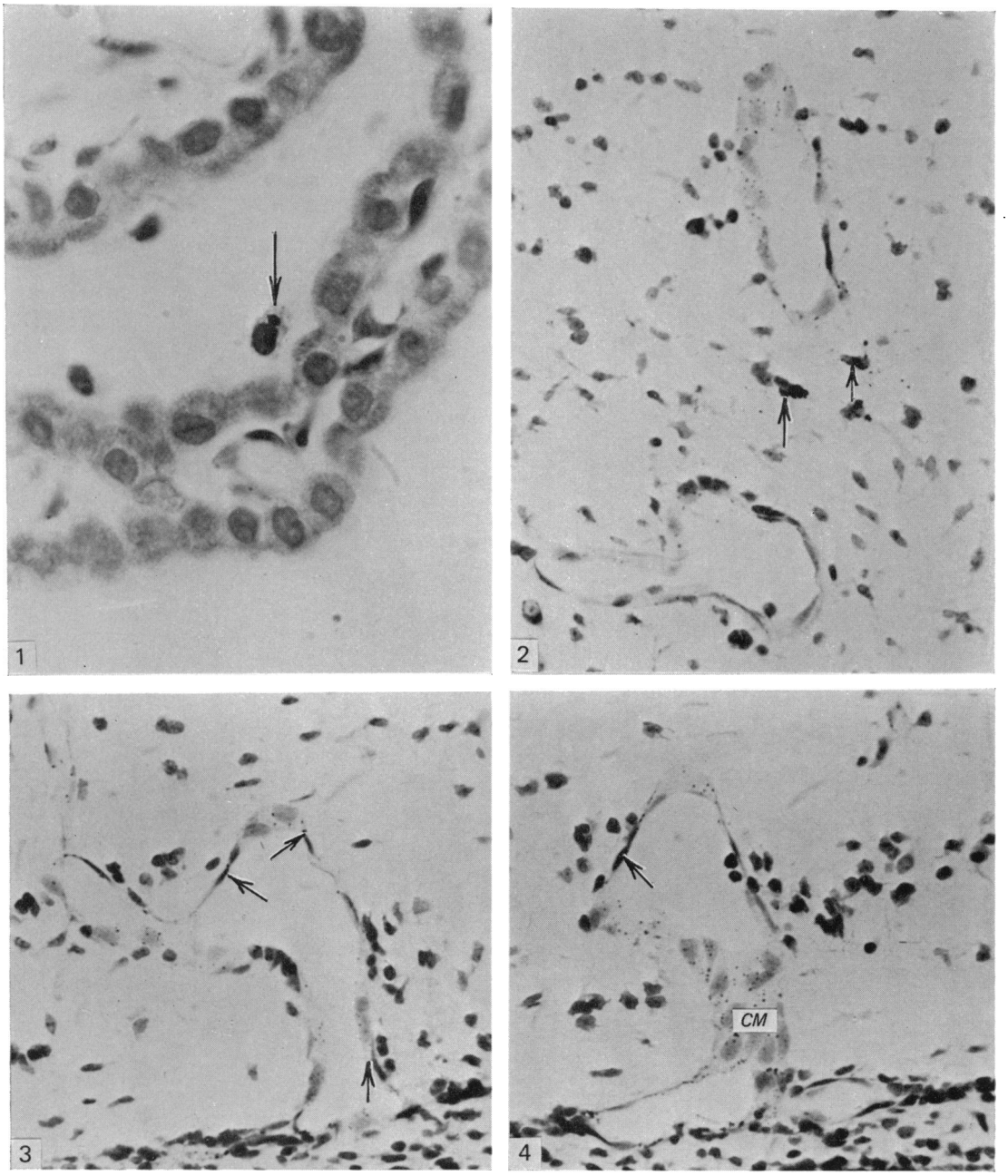
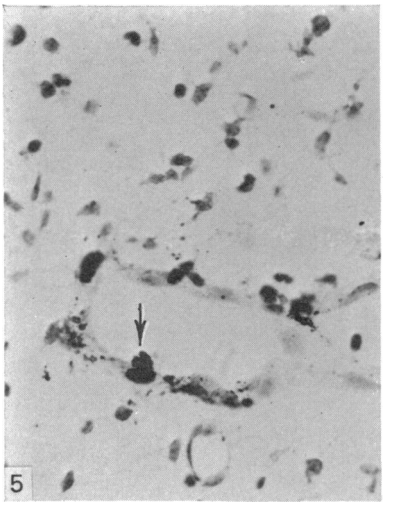


Fig. 1. Carbon-labelled epiplexus cell (arrow) in lateral ventricle of rat, 13 days old, killed 6 days after the second carbon injection. × 1000.

Figs. 2–4. Numerous carbon-labelled monocytes adhering to the luminal surface of blood vessels. A cluster of them is seen in Fig. 4 (*CM*). The two labelled cells arrowed in Fig. 2 are in the neuropil. Arrows in Figs. 3 and 4 indicate endothelial cells. Rat aged 8 days, killed 1 day after the second carbon injection. × 1000.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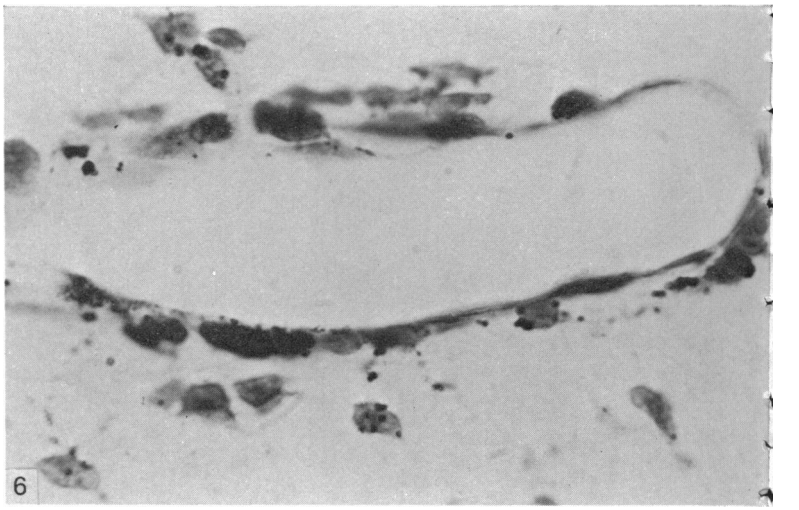
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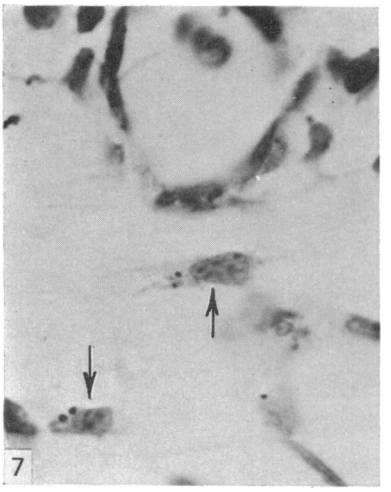
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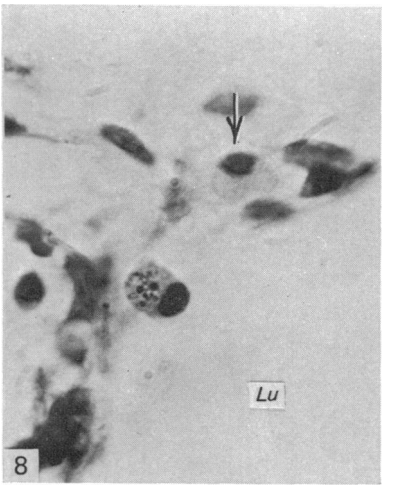

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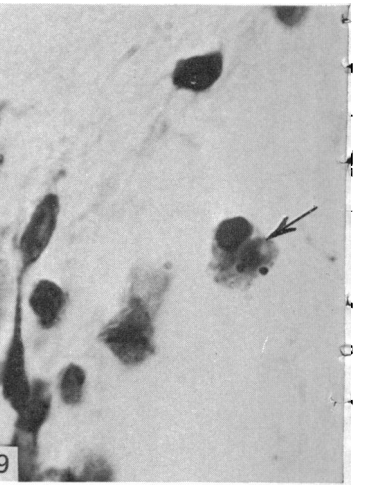
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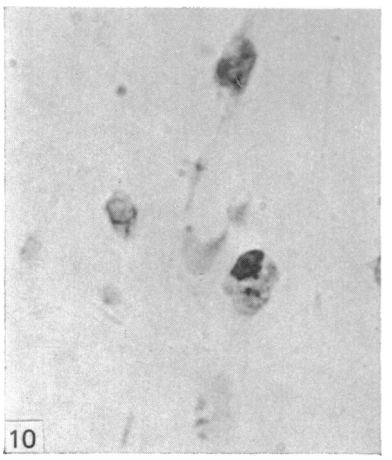
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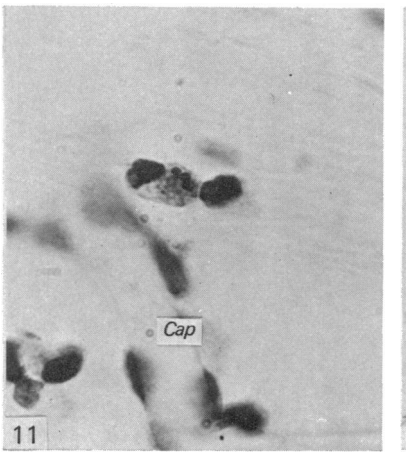

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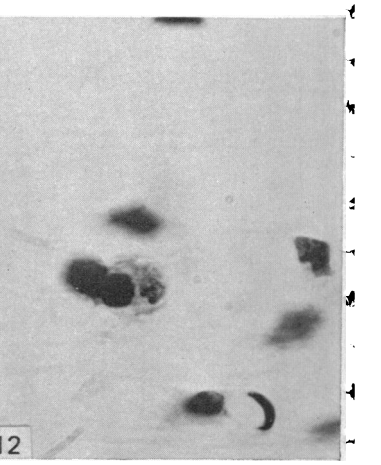
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Carbon-labelled cells

Twenty-four hours after the second carbon injection (rat aged 8 days) a variable number of carbon-labelled monocytes were seen in the lumina of the tortuous blood vessels in the corpus callosum (Figs. 2–4); they were either adherent to the vessel wall or appeared to be penetrating it (Figs. 3, 5). Often, a cluster of carbon-labelled monocytes was seen in the vascular lumina (Fig. 4). The oval nucleus of these cells stained lightly with cresyl fast violet. Outside the wall of a blood vessel an accumulation of labelled cells loaded with carbon particles was frequently seen (Fig. 6). The labelled cells were mostly elongated, with a pale nucleus displaying fine chromatin granules (Fig. 6). These 'perivascular cells' were most likely monocytes which had recently migrated from the vessel. Some labelled cells, however, were seen at some distance from a vessel (Fig. 7).

One day after the second carbon injection the amoeboid microglial cells of the corpus callosum were free of carbon particles. A few amoeboid cells in the cavum septi pellucidi, however, did carry carbon particles (Fig. 8).

In the rats killed 4 and 5 days after the second injection (aged 11 and 12 days respectively), most labelled cells were identified as amoeboid microglia (Figs. 9–12). Such cells were readily distinguished by their round nucleus and coarse chromatin clumps (Fig. 10). Their abundant cytoplasm carried a variable amount of carbon.

In the rats killed 6 days after the second carbon injection (aged 13 days), occasional amoeboid microglia were labelled. The labelled cells at this age mostly had oval (Fig. 13), angular (Figs. 14–16) or flattened (Figs. 17, 18) nuclei, the last-named resembling those of microglia. Clumping of chromatin in these nuclei was evident. The carbon tended to accumulate at one side of a labelled cell (Figs. 14, 15, 17, 18). It was not uncommon to observe cells with fine cytoplamic processes beaded with carbon particles (Fig. 16).

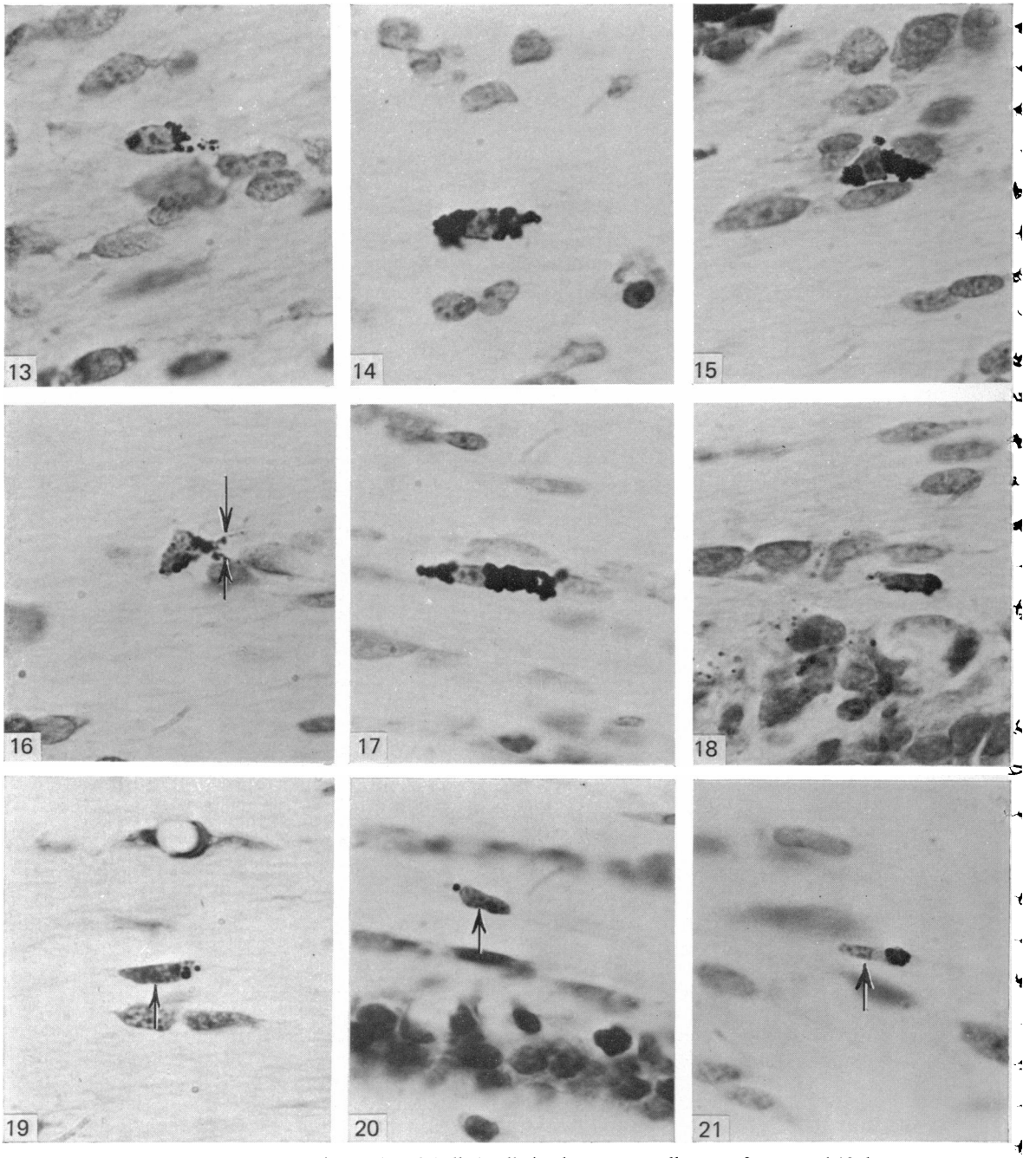
In rats killed 8 and 9 days after the second injection (aged 15 and 16 days respectively) the majority of carbon-labelled cells resembled microglia (Figs. 19–21), with a flattened nucleus containing coarse chromatin clumps, and scanty cytoplasm, often accumulated at one pole, and containing a variable number of carbon particles (Figs. 19–21). The axons of the corpus callosum in these older rats were more closely packed; this might well explain the elongated outline of the labelled microglia-like cells between them. Amoeboid microglia cells were scanty, and none of them showed carbon in their cytoplasm.

Fig. 5. A carbon-labelled cell penetrating the wall of blood vessel (arrow). Rat aged 8 days, killed 1 day after the second carbon injection. ×450.

Figs. 6, 7. A higher magnification of blood vessels in corpus callosum of rats killed 1 day after the second carbon injection. Numerous perivascular labelled cells are present (Fig. 6). Fig. 7 depicts two labelled cells (arrows) which are near a vessel. Note that the nucleus of the labelled cells displays fine chromatin clumps. ×1000.

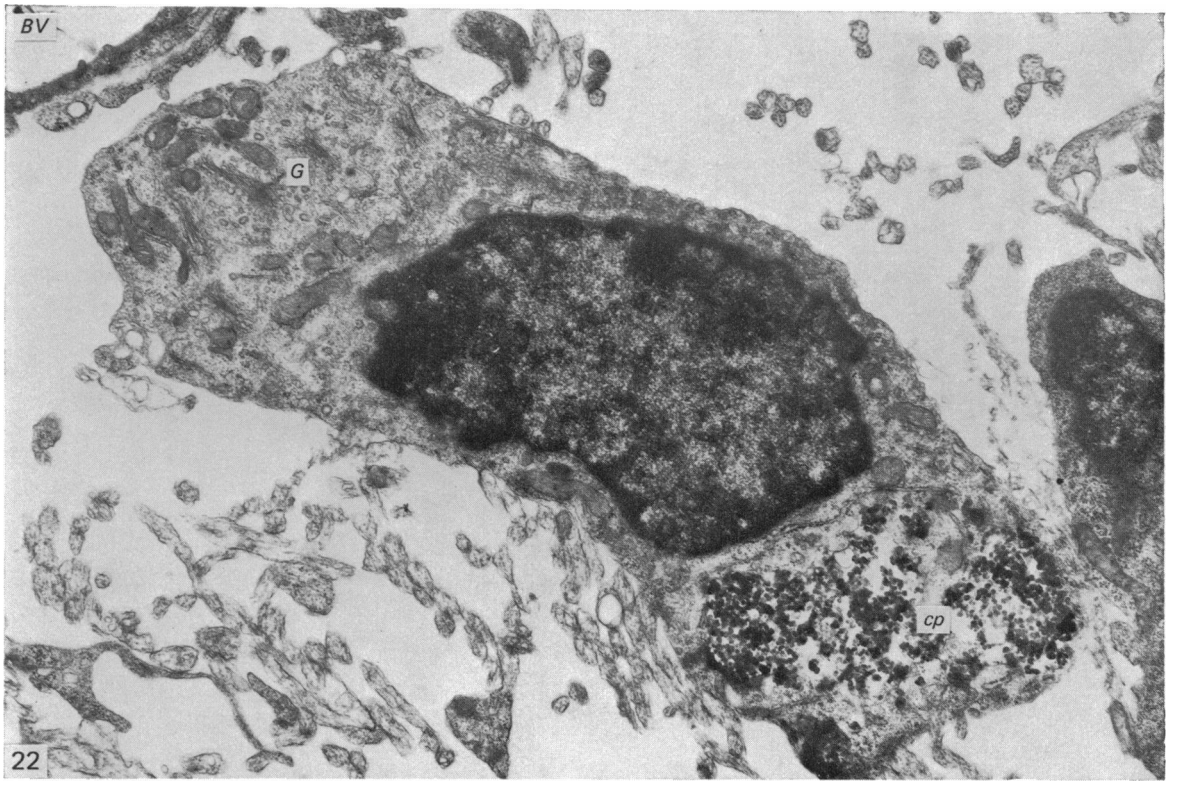
Fig. 8. A carbon-labelled amoeboid microglial cell on the wall of the cavum septi pellucidi. Another is unlabelled (arrow). *Lu*, lumen of cavum septum pellucidum. Rat killed 1 day after the second carbon injection. ×1000.

Figs. 9–12. Representative carbon-labelled amoeboid microglial cells in the corpus callosum of rats 5 days after the second carbon injection. Marginal chromatin masses are evident in Fig. 10. The cell depicted in Fig. 9 also includes a large dense body (arrow). *Cap*, capillary. Age of rats at death, 12 days. ×1000.



Figs. 13–18. Representative carbon-labelled cells in the corpus callosum of rats aged 13 days, 6 days after the second carbon injection. × 1000. The labelled cells show either oval (Fig. 13), angular (Figs. 14–16) or flattened (Figs. 17, 18) nuclei. The amount of carbon varies considerably from cell to cell. Fig. 16 depicts a labelled cell with two fine cytoplasmic processes with carbon particles (arrows). Figs. 17 and 18 show cells which are considered microglia-like and which are common in rats killed after longer survival times (compare with Figs. 19–21).

Figs. 19–21. Representative carbon-labelled microglia-like cells (arrow) in the corpus callosum of rats aged 15 days, 8 days after the second carbon injection, × 1000. All labelled cells have a flattened nucleus. The cells are elongated with scanty cytoplasm at one pole.



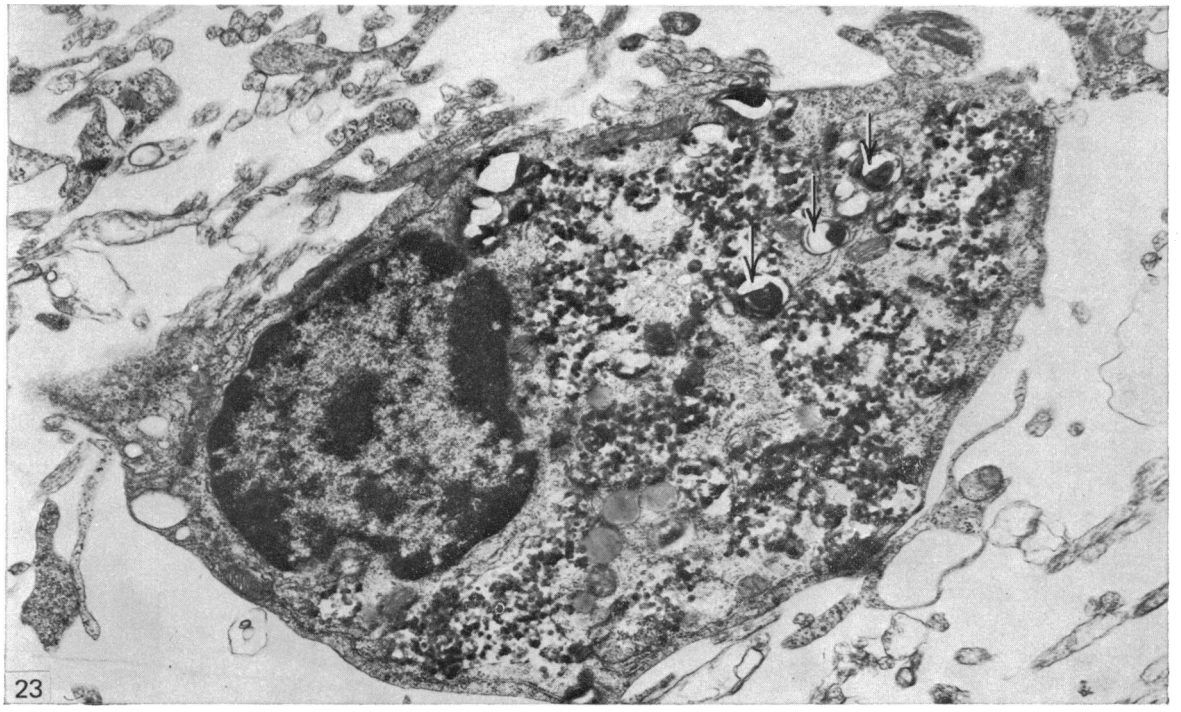
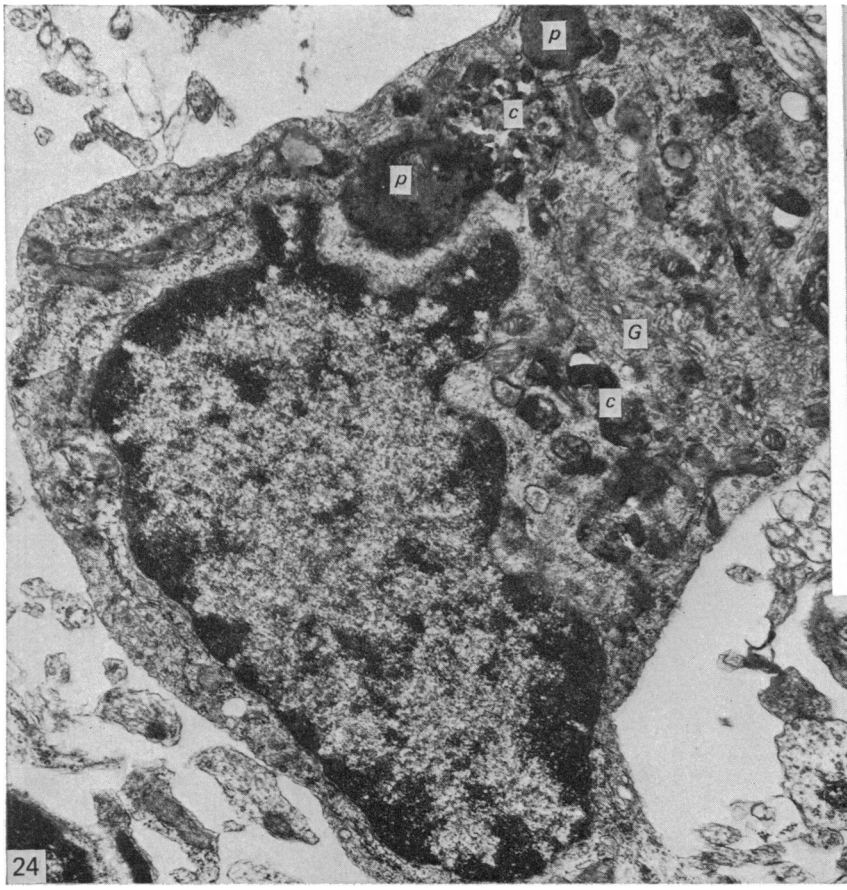


Fig. 22. Electron micrograph showing a perivascular carbon-labelled cell of monocytic type near a blood vessel (*BV*). The cytoplasm is devoid of phagosomes but contains a large mass of carbon particles (*cp*). Golgi body forms a complex (*G*). × 15000.

Fig. 23. Electron micrograph showing a carbon-labelled amoeboid microglial cell. The abundant cytoplasm is filled with carbon masses. A few phagosomes (arrows) are also present. × 15000.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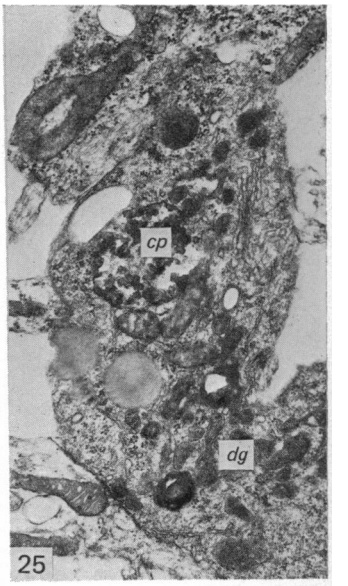
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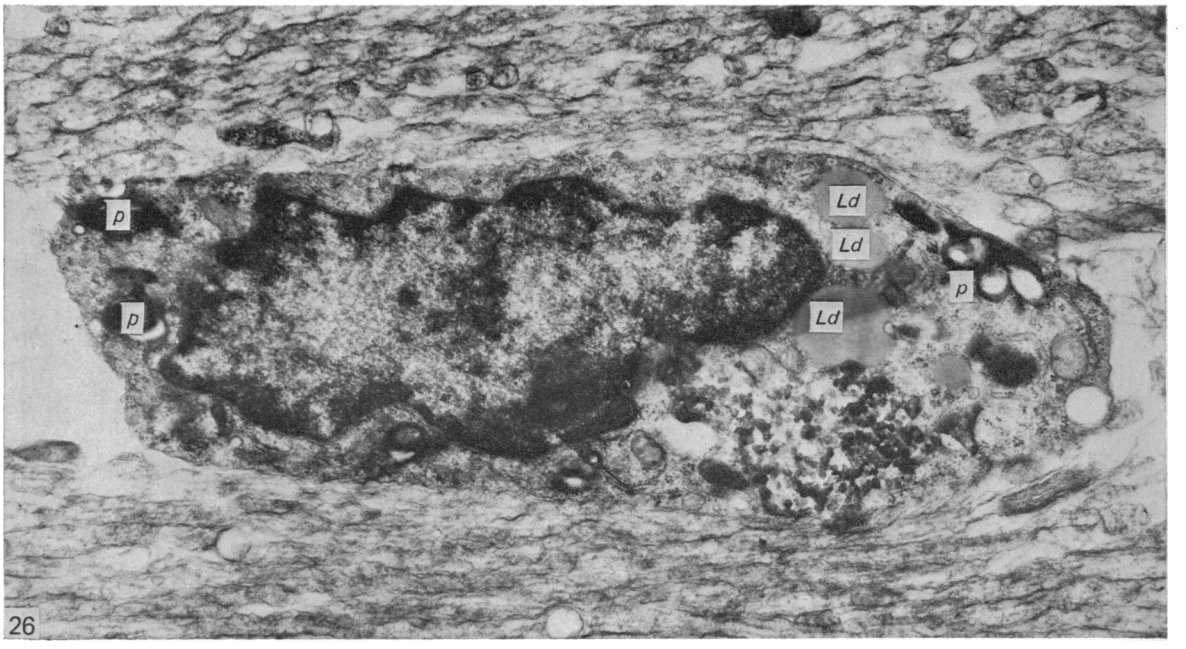
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Electron microscopy

Electron microscope studies of the tissues taken from rats killed at 3, 4 and 5 days after the second carbon injection have thus far confirmed the presence of the various types of carbon-labelled cells in corpus callosum. Perivascular carbon-labelled cells, believed to be recently emigrated monocytes, are shown in Figure 22. Their cytoplasm was generally devoid of phagosomes apart from the carbon particle masses. Carbon-labelled amoeboid microglia containing pleomorphic phagosomes were seen (Figs. 23–25). Carbon-labelled microglia-like cells were often observed between axons (Fig. 26). The morphological features of amoeboid microglia and microglia have been detailed elsewhere (Ling, 1976).

DISCUSSION

Microglia

Microglia are absent at birth in the rat's corpus callosum but make their appearance about 5 days postnatally (Ling & Tan, 1974; Imamoto & Leblond, 1978). At weaning they form 6–7 % of the total glial population in the corpus callosum (Ling & Tan, 1974) and that proportion remains fairly constant, at least up to the age of 5 months (Ling & Leblond, 1973). These cells are not labelled one or two hours after a systemic [^{3}H]-thymidine injection, indicating that they do not undergo mitosis (Mori & Leblond, 1969; Imamoto & Leblond, 1977). As microglia appear between the fifth and nineteenth postnatal day (Imamoto & Leblond, 1978) they must clearly be derived from some precursor cell type.

In the search for a precursor cell from which microglia could have arisen by transformation or differentiation, attention has been drawn to the following cell types: (1) amoeboid microglia, or cells of a similar nature but given a different name: this view was initiated by Rio-Hortega (1919, 1932) and has been widely supported subsequently (Penfield, 1925, 1932; Cammermeyer, 1970; Imamoto & Leblond, 1978); (2) subependymal cells adjacent to the lateral ventricle (Lewis, 1968; Vaughn & Peters, 1968; Vaughn, Hinds & Skoff, 1970; Fujita & Kitamura, 1975); (3) monocytes (Imamoto & Leblond, 1977, 1978); and (4) pericytes, cells located on the capillary wall but invested by a layer of basement membrane: it has been suggested that these cells become detached, giving rise to microglia (Mori & Leblond, 1969; Baron & Gallego, 1972).

Amoeboid microglia

At birth, amoeboid microglia are abundant in the corpus callosum but their numbers decrease with age and very few are present on the tenth postnatal day

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Fig. 24. Electron micrograph showing a carbon-labelled amoeboid microglial cell. The nucleus displays coarse chromatin masses peripherally. The cytoplasm contains phagosomes (*p*) and clusters of carbon particles (*c*). *G*, Golgi complex. × 20000.

Fig. 25. Portion of a carbon-labelled amoeboid microglial cell. A cluster of carbon particles (*cp*) is seen near to a group of electron-dense granules (*dg*), the latter being one of the characteristic features of the cell type (Ling, 1976, 1978). × 15000.

Fig. 26 Electron micrograph of a carbon-labelled microglia-like cell lying between axons. The cell shows a little cytoplasm with prominent carbon masses. *Ld*, lipid droplets; *p*, phagosomes. × 21000.

(Ling & Tan, 1974; Imamoto & Leblond, 1978). These cells are known to be active macrophages (Stensaas & Reichert, 1971; Booz & Felsing, 1973; Ling, 1977; Imamoto & Leblond, 1978).

The origin of amoeboid microglial cells is uncertain: they were thought to be derived from subependymal cells (Rydberg, 1932; Ling, 1976), but this contradicts Rio-Hortega's (1919) hypothesis that amoeboid microglia are cells of mesodermal origin which have migrated in from the brain surface. The hypothesis that amoeboid microglia are of mesodermal origin has been strongly supported by Imamoto & Leblond (1978) who take the view that they are derived from monocytes which have infiltrated the corpus callosum.

Re-appraisal of subependymal cells–amoeboid microglia–microglia hypothesis

The findings in the present study have certainly cast some doubt concerning the present author's previous belief in the 'unitary origin' of astrocytes, oligodendrocytes and microglia (Ling & Tan, 1974; Ling, 1976). The view that multipotential subependymal cells may also give rise to microglia must be re-evaluated.

The present study used colloidal carbon particles as an intracellular marker, readily detected with the light microscope. A previous study (Ling, 1978) has shown that, following intravenous injection of colloidal carbon, numerous monocytes and polymorphonuclear leucocytes become labelled. Likewise, in the present experiment, numerous carbon-labelled monocytes were seen adhering to the walls of blood vessels 1 or 2 days after the injection of carbon. Carbon-labelled polymorphonuclear leucocytes are rare, as most of them are washed out during perfusion. Shortly after carbon administration some labelled cells could be seen penetrating the vascular wall, and there was often an accumulation of such cells ('pervascular monocytes') in the neuropil surrounding the blood vessels, strongly suggesting that they had migrated from the blood stream. The presence of carbon-labelled cells in neural tissue following a single carbon injection has been reported in a previous study (Ling, 1978), but aggregation of carbon-labelled cells in the perivascular region was not observed in that study. It now seems obvious that the single dose of carbon suspension used earlier did not label enough cells to demonstrate migration of carbon-labelled monocytes from the vessel lumina into the neuropil.

In addition to the perivascular carbon-labelled cells, a number of labelled cells were found at a distance from the vessel walls shortly after carbon injection: how they got there is not clear.

No carbon-labelled amoeboid microglia were found in the corpus callosum 1 or 2 days after injection, although some labelled cells identified as amoeboid microglia were seen in the cavum septi pellucidi beneath the corpus callosum. This suggests that some monocytes may already have transformed into amoeboid microglia as early as 24 hours after injection.

Four or five days after carbon injection there were very few perivascular, carbon-labelled monocytes: instead, amoeboid microglia in the same region were now labelled. This progressive diminution of carbon-labelled, perivascular monocytes, with a concomitant increase in labelled amoeboid cells, taken together with the fact that neither degenerating carbon-labelled monocytes nor free carbon particles were seen in the area, strongly suggests that transformation of the former into the latter cell type is taking place.

The cytoplasm of some of the labelled amoeboid microglia showed clear spaces which probably represent the pale vacuoles seen with the electron microscope

(Ling, 1976). Other cells had relatively homogeneous cytoplasm or else contained dense inclusions mingled with black carbon deposits.

By 8 or 9 days after the second carbon injection (age, 15 days), amoeboid microglial cells containing carbon had disappeared: they could have emigrated into other regions, they could have degenerated *in situ*, or they could have transformed into another cell type. The first suggestion can be ruled out because in 15 days old rats there are extremely few amoeboid microglia anywhere, though an occasional unlabelled one was present in the cavum septi pellucidi. The second possibility cannot be ruled out because degenerating amoeboid cells have been observed in the developing corpus callosum (Ling & Tan, 1974, 1976), but not in numbers sufficient to account for the disappearance of all the amoeboid cells labelled initially. It is therefore justifiable to assume that they have transformed into cells which are no longer recognizable as amoeboid microglia. The presence of heavily labelled cells with the morphological features of microglia (i.e. elongated cells with flattened nucleus, coarse chromatin and cytoplasm mainly at one pole) in rats killed after longer time intervals, plus the fact that microglia are not labelled initially, is most easily and obviously explained by amoeboid microglia becoming converted into microglia while retaining their carbon particles. That amoeboid microglia become microglia was a conclusion reached by many workers (Rio-Hortega, 1919; Penfield, 1932; Imamoto & Leblond, 1978) including the author (Ling, 1976). However, when a single dose of a carbon suspension is given to 5 days old rats and they are killed one day after injection, a few labelled cells already appeared microglia-like (Ling, 1978), raising the possibility that infiltrating monocytes can sometimes give rise to microglial cells directly without passing through an amoeboid microglial stage.

Finally, the presence of carbon-labelled epiplexus cells, even though they were seen only in rats killed after the longer time intervals, suggest that these may also arise from blood cells, but the whole question of the mode of formation of epiplexus cells and their relation to microglia remains to be investigated.

SUMMARY

Two successive intravenous doses of carbon suspension were given at 24 hourly intervals into six days old rats. These animals were killed at intervals ranging from 1 to 9 days after the second injection. The corpus callosum and neighbouring structures were examined for cells containing ingested colloidal carbon particles in their cytoplasm.

Twenty four hours after the second injection, a variable number of carbon-labelled monocytes were adherent to the luminal wall of blood vessels in the corpus callosum. Numerous carbon-labelled cells appeared to have left the lumen and entered the brain tissue surrounding the vessels. These perivascular carbon-labelled monocytes in the neuropil displayed a large pale nucleus with fine chromatin granules. The phagocytic amoeboid microglia in the corpus callosum were unlabelled at first, although a few cells of a similar nature in the cavum septi pellucidi did show carbon particles in their cytoplasm.

Four or five days after the second carbon injection perivascular carbon-labelled monocytes were rare, but carbon particles were now present in the amoeboid microglia.

At 8 days amoeboid microglia were virtually absent from the corpus callosum but

carbon particles now appeared in cells which closely resembled microglia (flattened nucleus, coarse chromatin, scanty cytoplasm at one pole).

The sequential appearance of carbon particles in monocytes, amoeboid microglia, and microglia, suggests that monocytes transform into microglia by way of an amoeboid microglial stage.

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