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Ultrastructural Study on the Origin of Rat Microglia Cells

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Abstract. An ultrastructural study of the origin of microglial cells has been performed in albino rat brains taken from 17-day-old embryos up to 35-day-old rats. Invasion of the nervous parenchyma by macrophagic cells which appear in mesodermal sources is described. Although the two main microglial sources are the meningeal membranes and the vascular adventitia, pericytes may also participate in the formation of microglial cells.

Introduction

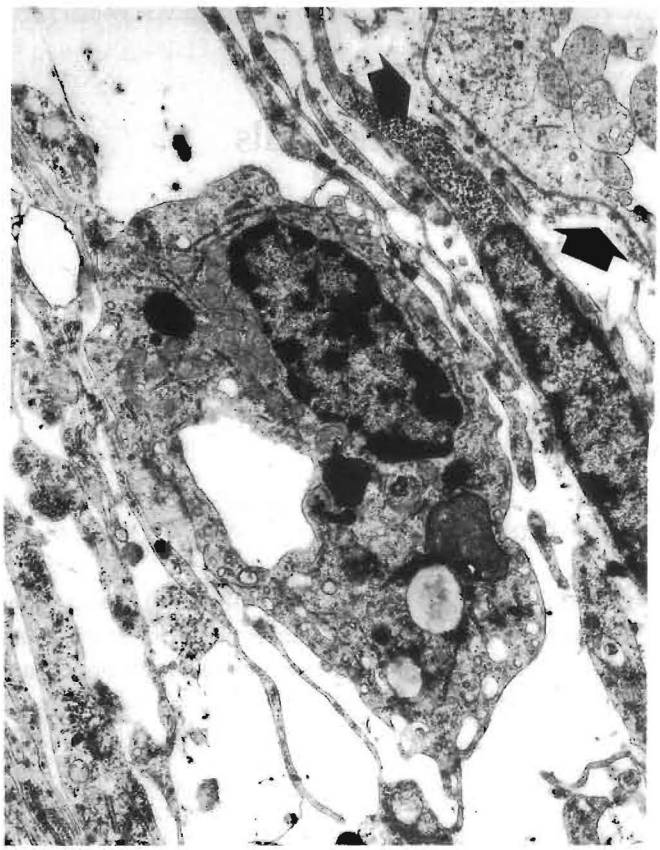
A mesodermal, extracerebral origin for microglial cells was first proposed by Rio-Hortega [1919, 1921a, b, 1932] in his classical studies on the morphology, nature and development of this cell type. According to this author, these cells migrated from the meninges or vascular adventitia into the nervous parenchyma during perinatal stages. This classical theory on the origin of microglia cells is still supported by several modern authors [Cammermeyer, 1970; Kawaguchi, 1978, 1980; Boya et al., 1979]. The presence of amoeboid cells as a typical feature of the perinatal brain has been confirmed by numerous studies [Penfield, 1925, 1932; Rydberg, 1932; Kershman, 1939; Stensaas and Reichert, 1971; Booz and Felsing, 1973; Ling and Tan, 1974; Ling, 1976a, b, 1977, 1978; Sturrock, 1981; Matsumoto and Ikuta, 1985; Lent et al., 1985].

The nature and function of amoeboid microglia described by Rio-Hortega [1919, 1921a, b] has been a matter of further discussion. Based on his findings, Rio-Hortega concluded that these cells were an immature form of microglia which pass through a phagocytic phase before reaching the adult, ramified form. The macrophagic nature of amoeboid cells seems now proven by ultrastructural studies [Stensaas and Reichert, 1971; Booz and Felsing, 1973; Ferrer and Sarmiento, 1980], scanning electron-microscopical obervations [Tseng et al., 1983a], histochemical techniques [Ling, 1977, 1980; Boya et al., 1979; Ferrer and Sarmiento, 1980; Valentino and Jones, 1981; Ling et al., 1982; Tseng et al., 1983b; Kaur et al., 1984] and tissue culture results [Ling et al., 1983].

Nevertheless, there is no agreement on the source of the mesodermal precursors from which microglia cells are derived. A vascular source, as proposed by Rio-Hortega [1921b] has been supported by many authors [Dunning and Stevenson, 1934; Field, 1955; Blinzinger and Hager, 1964; Maxwell and Kruger, 1965; Hager, 1969; Baldwin et al., 1969; Mori and Leblond, 1969; Mori, 1972; Baron and Gallego, 1972; Brichova, 1972; Boya, 1975, 1976]. Other studies suggest a direct origin from monocytes [Dunning and Furth, 1935; Russell, 1962; Roessman and Friede, 1968; Matthews, 1974; Imamoto and Leblond, 1978; Ling, 1978, 1979, 1980; Ling et al., 1980].

Finally, other authors deny the mesodermal nature of microglia cells, proposing instead a neuroectodermal origin for them [Fujita and Kitamura, 1976; Oehmichen, 1978; Oehmichen et al., 1980; Fujita, 1980; Fujita et al., 1981; Kitamura et al., 1984; Kitamura, 1985].

In the present study we describe the origin and evolution of amoeboid microglia in the rat with the electron microscope.



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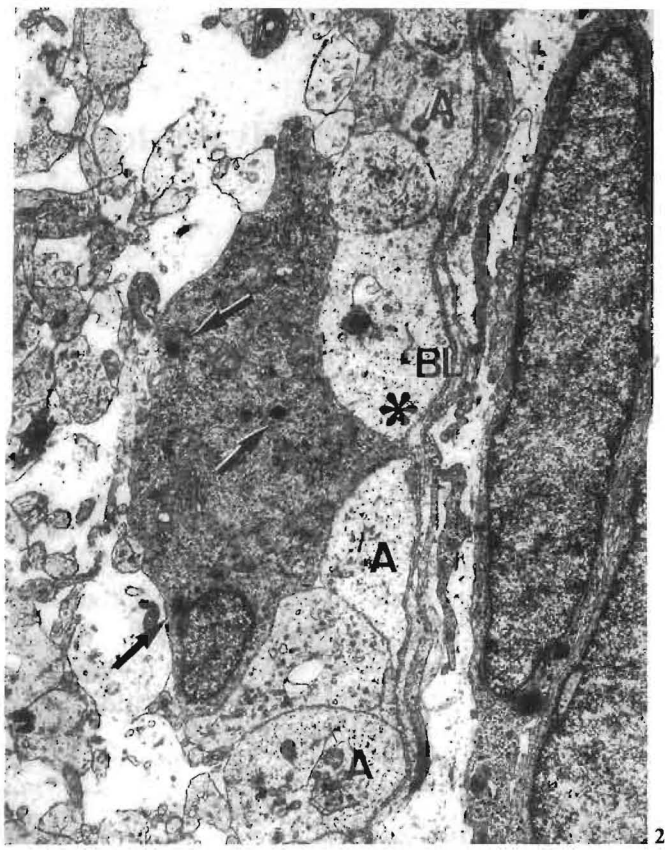

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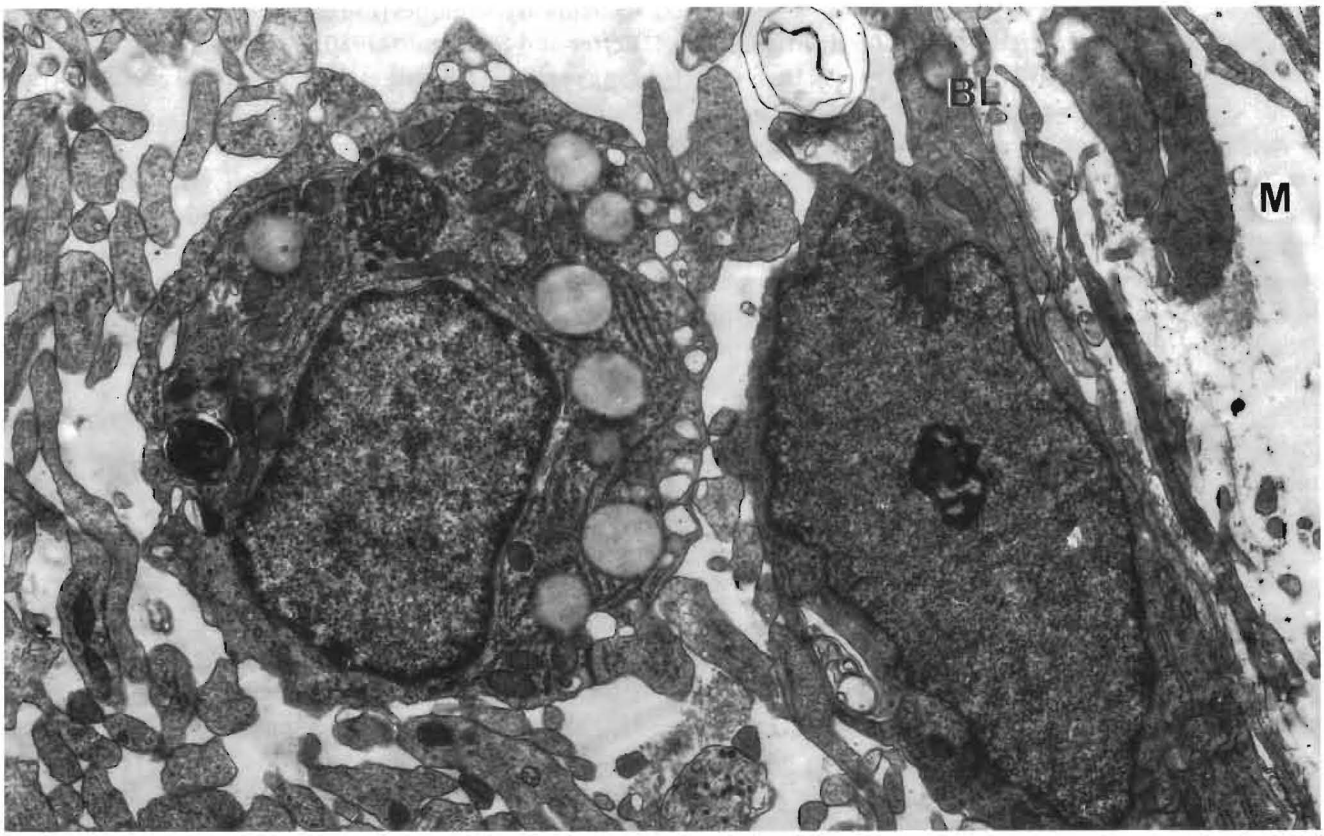

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Materials and Methods

Thirty-two albino Wistar rats were used, ranging between 17-post-conception-day fetuses and 35-postnatal-day juveniles. The brains were fixed by immersion in 0.1 *M* phosphate-buffered 3% glutaraldehyde, pH 7.4. Small blocks of cerebral cortex were excised, especially from the interhemispherical region including the median meningeal septum and also from the basal forebrain regions. These were washed in 0.1 *M* phosphate buffer, postfixed in phosphate-buffered 1% osmium tetroxide and embedded in Vestopal. The techniques of Miller and Palade [1964] and Fahimi [1969] were previously performed in some blocks for the demonstration of acid phosphatase and peroxidase activities, respectively.

Ultrathin sections were cut on an LKB ultramicrotome, stained with uranyl acetate and lead citrate, and examined in a Philips EM 201 electron microscope.

Results

Meninges and Border between Meninges and Nervous Parenchyma

In the first stage studied (17-day embryo), globose cells of round globose or sometimes ovoid shape were already found lying in the meninges, locating themselves among the long and thin processes of meningocytes (fig. 1). These cells usually showed an irregular contour with thin short pseudopodical processes. The nucleus was large and ovoid, containing prominent peripheral chromatin clumps and an occasional nucleolus. In the dark-stained cytoplasm large, dense bodies of lysosomal nature (acid phosphatase-positive), lipid droplets and numerous small vacuoles with flocculent material were seen. The cell body also showed mitochondria, free polyribosomes, isolated strands of granular endoplasmic reticulum and a Golgi apparatus. Occasionally, the basal lamina which separates the nervous parenchyma and meninges was missing in some locations, and cells of macrophagic aspect were located in the nervous parenchyma beneath the basal lamina but still in contact with it (fig. 2).

Fig. 1. Twenty-day rat embryo. A globose cell with irregular surface and numerous cytoplasmic dense bodies is located in meningeal tissue. A continuous basal lamina (arrows) separates the meninges from the nervous parenchyma. ×8,500.

Fig. 2. Neonatal rat (16 h after birth). Superficial region of nervous parenchyma close to the meninges. A cell with dark cytoplasm containing some dense bodies (arrows) is interposed between astrocytic processes (A) and in contact (asterisk) with the basal lamina (BL). ×7,200.

Fig. 3. Seventeen-day embryo. Macrophagic cell in superficial region of nervous parenchyma. BL = Basal lamina; M = meningeal space. ×15,700.

The most superficial nervous parenchyma, close to the meninges, showed a loose organization pattern in the earlier stages of embryonic life studied, with large extracellular spaces among the cell processes of neural elements. Macrophagic cells of ultrastructural appearance similar to the ones described above in the meninges were observed in these large spaces (fig. 3). These cells showed round or more elongated shapes, depending on the available space. None of these cells showed peroxidase activity.

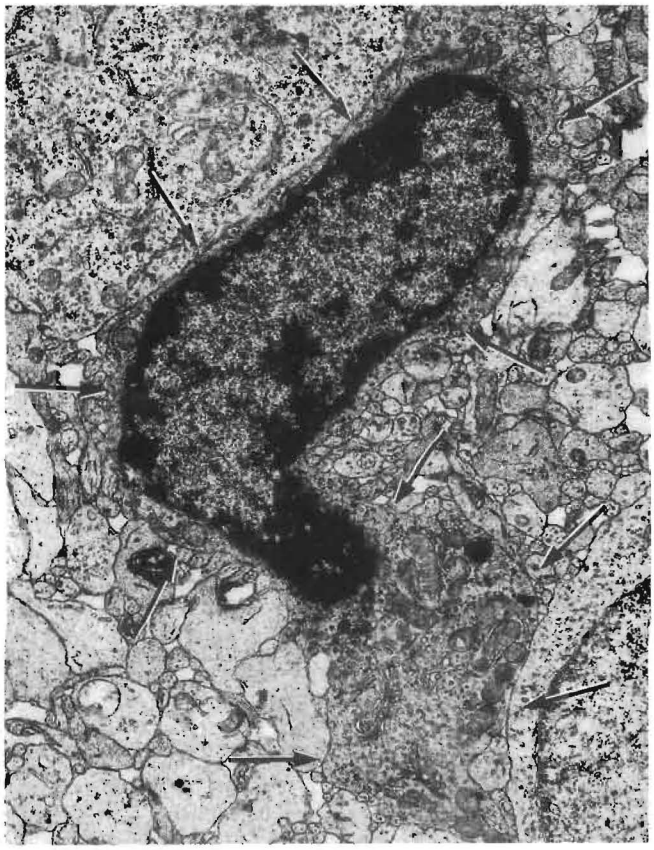
The presence of macrophagic cells in the meninges and superficial regions of the nervous parenchyma, as well as the images suggesting the passage of these cells across the basal lamina, were found from 17-day embryos to 5-postnatal-day rats. The frequency of these findings began to decrease after the 6th postnatal day, and they were scarce from the 10th postnatal day onwards.

Nervous Parenchyma

As amoeboid cells penetrate into the depth of the nervous parenchyma, locating themselves in areas with more narrow extracellular space, they lose their globular shape: the cells become more elongated, decreasing the amount of somatic cytoplasm and showing cytoplasmic processes (fig. 4). The same evolution was found with increasing age of the rat, owing to the decrease of extracellular spaces associated with brain maturation. The cytoplasm of these cells contained secondary lysosomes, lamellar bodies and even clusters of lipofuscin-like bodies. These inclusion bodies were progressively less conspicuous with brain maturation. Signs of phagocytosis of cellular debris were also found.

Pericytes and Vascular Adventitia

In the first embryonic stages studied, capillaries with hypertrophic endothelial cells surrounded by pericyte-like cells were found in the loose nervous tissue. In 19-day embryos, some of these pericapillary cells seemed to be detaching from the blood vessel wall, often remaining joint to it only by a small portion of the cell (fig. 5). These cells showed free polyribosomes, scarce dense bodies and short strands of granular endoplasmic reticulum. The nucleus contained peripheral chromatin clumps and dark nucleoplasm. In newborn rats (16 h after birth), some cells were seen sending very thin cytoplasmic processes partially surrounding a capillary while others of their processes were directed towards the nervous tissue (fig. 6). Their cytoplasm showed lamellar bodies, secondary lysosomes and lipid droplets. Some of these cells were surrounded by a basal lamina-like material not always tightly attached to their surface.



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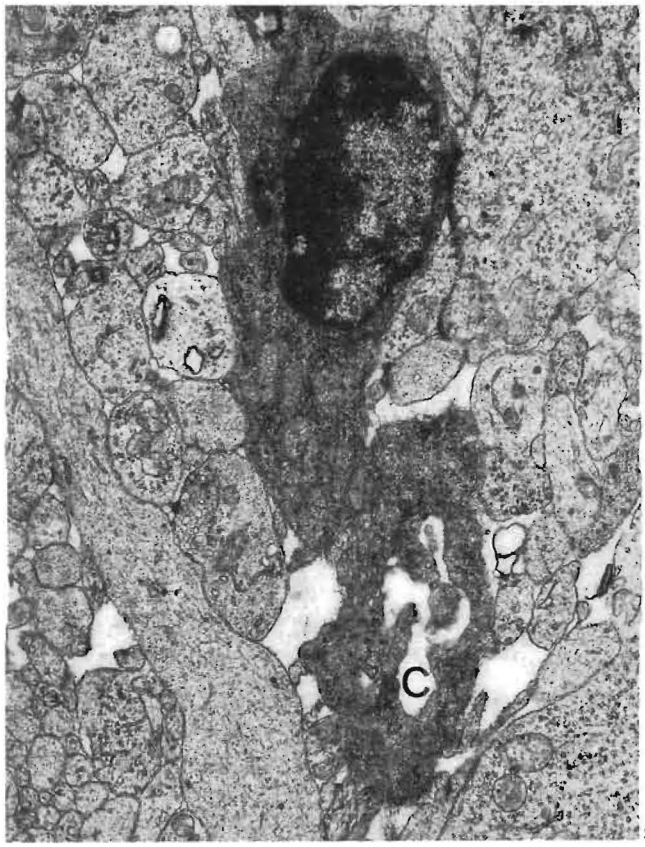

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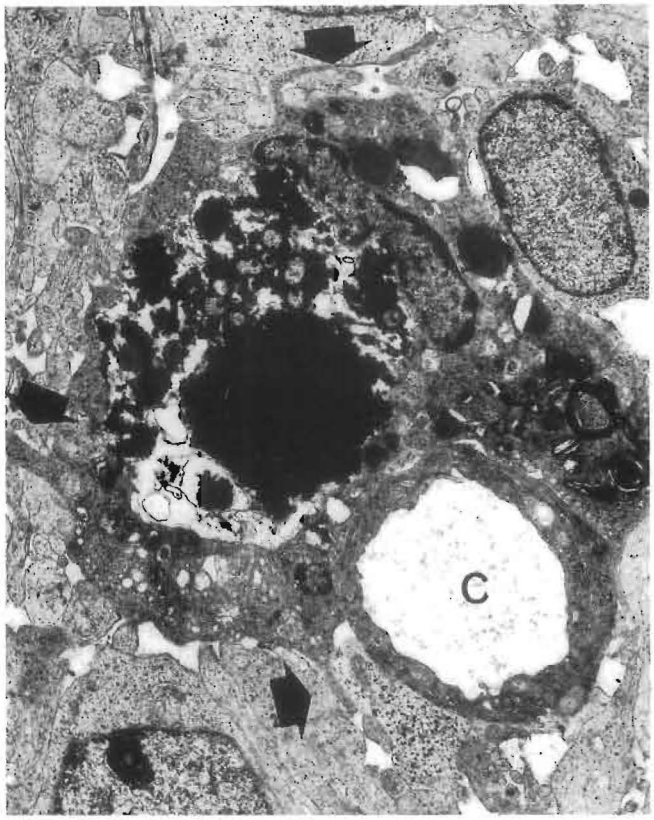

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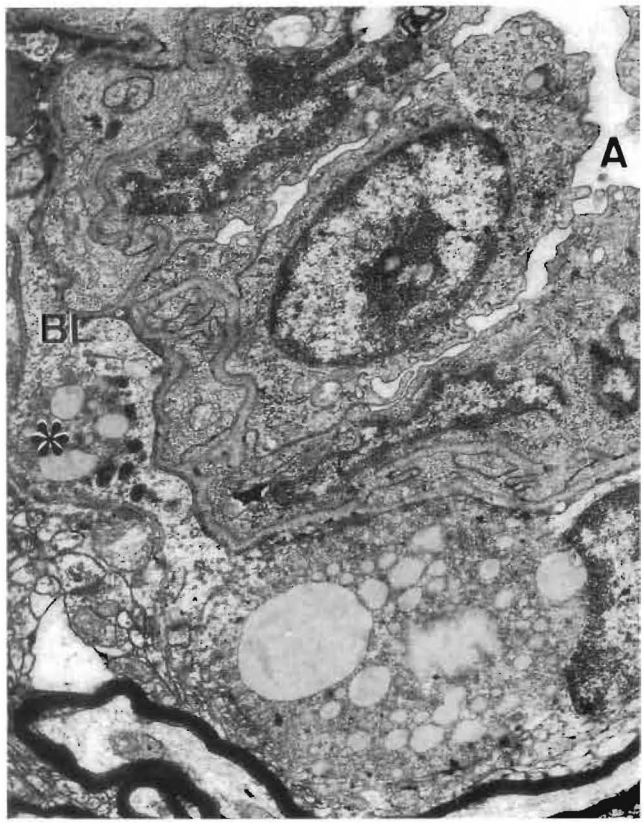

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The number of lysosomal dense bodies in the cytoplasm of these cells increased at 4–5 postnatal days, and large lipid droplets also appeared occasionally. Cells with similar features, namely, abundant lysosomes and presence of lipid droplets, were seen in the adventitia of large blood vessels. Discontinuities or rarefactions of basal lamina were found associated with these cells (fig. 7).

Activated adventitial cells were observed in all the stages studied. After the 5th postnatal day, however, pericytes covered by and in close contact with a basal lamina were already found. A layer of astrocyte-vascular end feet can be seen outside the capillary basal lamina.

The appearance of macrophagic cells isolated in the nervous parenchyma but located near blood vessels was a constant finding in all the stages studied. Peroxidase-positive cells were never found in the stages studied.

Discussion

Our results seem to confirm the mesodermal origin of microglia proposed by Rio-Hortega [1919, 1921b]. We have found macrophagic cells of similar appearance in both the meninges and the superficial nervous parenchyma, some of which were attached to the basal lamina which separates these tissues (mesoderm and neuroectoderm). Both findings strongly suggest that amoeboid microglia may originate by an immigration of macrophagic mesodermal cells from the meninges. Invasion of the nervous parenchyma by these globose cells undoubtedly happens before birth, since they were already found in the first stages studied (17-day embryos). Similar findings were reported by Tseng et al. [1983b].

Exactly as Rio-Hortega [1921b] described with light microscopy, when amoeboid cells penetrate deeply into nervous tissue they lose their globular shape. Due to its lack of maturity, the nervous parenchyma is loosely organised in this first stages, showing large extracellular spaces. With increasing maturity, amoeboid microglia must adapt their cell shape to a progressively decreasing interstitial space, thus sending out cytoplasmic processes which make the cell contour very irregular.

In a previous light-microscopical study [Boya et al., 1979], the origin of microglia in postnatal rats was investigated with histochemical and silver impregnation techniques. Acid phosphatase-positive globose cells were seen migrating from the meninges into the nervous parenchyma in which they were already found 6 h after birth. The silver method for microglia initially showed few impregnated cells which, however, later increased in number at the same time as acid phosphatase-positive cells became more scarce. These findings can be explained by the present ultrastructural results. In the present study we find a remarkable decrease in the size and number of lysosomes associated with maturation and branching of microglia cells. A similar evolution has recently been described by Kaur et al. [1985].

With regard to the possibility that pericytes could be a source of microglia, we have found images at early stages (19-day embryos) that strongly suggest a detachment of pericytes from capillary walls. Although pericytes were not previously seen with histochemical techniques until 5 days after birth [Boya et al., 1979], the small size and number of pericyte lysosomes in early stages could make them indetectable with the light microscope. Although several authors [Stensaas, 1975; Dodson et al., 1976; Fujita and Kitamura, 1976] deny a pericytal origin for microglia cells, we admit a possible transformation of pericytes into microglia or brain phagocytes [Boya, 1975, 1976; Boya et al., 1979]. This would maintain the microglial population throughout life or increase it in pathological situations.

As to vascular adventitia, we found cells loaded with dense bodies in 4- to 5-day-old rats. The basal lamina which separates them from the nervous parenchyma was frequently blurred, and macrophagic cells were often seen near these blood vessels. In the previous light-microscopical study [Boya et al., 1979] we reported acid phosphatase-positive round cells located in the vascular adventitia and the nearby nervous parenchyma of rats of the same age. These findings support the classical view of Rio-Hortega [1921b] that the vascular adventitia is an additional source of microglia.

In this study we did not find peroxidase activity in the cells described, thus confirming previous results [Boya et al., 1979]. Contradictory results have been reported on this enzyme. Thus, Ling, who did not initially observe this activity [Ling, 1977], found it later [Ling, 1980], but again

Fig. 4. Four-day-old rat. Microgliocyte located in neuropil beginning emission of cell processes (arrows). × 18,600.

Fig. 5. Nineteen-day rat embryo. Cell in apparent process of detachment from an embryonic capillary (C) to which it remains joined by a small portion. × 14,300.

Fig. 6. Neonatal rat (16 h after birth). Macrophagic cell with numerous dense bodies attached to a capillary (C). Thin processes from this cell (arrows) penetrate into the adjacent nervous parenchyma. × 7,900.

Fig. 7. Five-day-old rat. Perivascular space around an arteriole (A) occupied by macrophagic cells with abundant lipid droplets and dense bodies. The basal lamina (BL) which separates them from the nervous parenchyma shows a discontinuity (asterisk). × 6,600.

describes the absence of peroxidase activity in cultured microglia cells [Ling et al., 1983]. Peroxidase is known to be present in rat monocytes [Daems et al., 1976, 1977; Bentfeld et al., 1977], and thus its absence can be interpreted as evidence against a monocytic origin for microglia.

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