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The origin of microglial cells

J. BOYA, J. CALVO AND A. PRADO

Department of Histology and General Embryology, Faculty of Medicine, Complutense University, Madrid, Spain

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INTRODUCTION

A mesodermal extracerebral origin for microglia was first suggested by Rio-Hortega (1919, 1921*a*, *b*) in his classical work on the morphology and development of these cells. The cells pass through a series of stages, being round and amoeboid before attaining their typical ramified morphology. Over the years much evidence has accumulated that amoeboid cells are a characteristic feature of perinatal brains (Penfield, 1925, 1932; Rydberg, 1932; Field, 1955; Booz & Felsing, 1973; Schmitt, 1973; Ling & Tan, 1974; Ling, 1976*a*, *b*, 1977, 1978).

The identity of the mesodermal precursor cell and the mechanism of its transformation into a microglial cell has been much debated. A vascular source as proposed by Rio-Hortega (1921*b*) has been accepted by many workers (Dunning & Stevenson, 1934; Field, 1955; Blinzinger & Hager, 1964; Maxwell & Kruger, 1965; Hager, 1969; Baldwin, Wendell-Smith & Blunt, 1969; Mori & Leblond, 1969; Wendell-Smith, Blunt & Baldwin, 1966; Jones, 1970; Baron & Gallego, 1972; Boya, 1975, 1976). However, Stensaas (1975) and Dodson, Tagahira & Wai-Fong Chu (1976) deny a pericyte origin for microglia.

From the first studies of Rio-Hortega (1919, 1920, 1921*a*, *b*), until now, there has been great confusion about the nature and function of amoeboid microglial cells. Rio-Hortega (1921*b*) observed lipid droplets in their cytoplasm and inferred that the cells were phagocytic before they reached the ramified adult state. Recent studies have confirmed this phagocytic function (Booz & Felsing, 1973; Ling & Tan, 1974; Ling, 1976) and pinocytosis has been demonstrated (Stensaas & Reichert, 1971). However, few histochemical studies have been undertaken.

Macrophages in other parts of the body contain a variety of hydrolytic enzymes, including acid phosphatase, peroxidase, arylsulphatase (Dannenberg, Burstone, Walter & Kinsley, 1963; Enomoto & Kitani, 1966; North, 1966*a*; Seeman & Palade, 1967; Van Furth, Hirsch & Fedorko, 1970; Nichols, Bainton & Farquar, 1971; Nichols & Bainton, 1973) and ATPase (North, 1966*b*). These hydrolases have also been studied in amoeboid microglia (Ling, 1976*a*, 1977). Acid phosphatase, arylsulphatase and ATPase activities have been demonstrated, but the reaction for peroxidase was always negative.

In the present paper, we report on the origin and evolution of amoeboid microglia in the rat, beginning with birth, and using techniques for acid phosphatase and peroxidase as well as silver impregnation.

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MATERIALS AND METHODS

Studies were made on white rats (Wistar) 6, 24, and 36 hours and 2, 3, 4, 5, 6, 7, 10, 15, 20, 30, 60 and 100 days old. In all cases the techniques applied were: the Miller & Palade (1964) technique for acid phosphatase, the Fahimi (1969) technique for peroxidase, and the Weil & Davenport (1973) technique for silver impregnation of microglial cells.

RESULTS

At *6 hours* after birth, globular acid phosphatase-positive cells were present in the meninges, particularly in the median meningeal septum and especially in its upper part (Fig. 1).

Similar cells were present in the nervous parenchyma close to the meninges (Fig. 1), but more deeply the cells gradually lost form and became more irregular, with thick cytoplasmic processes. This change was very evident in the corpus callosum, where the acid phosphatase-positive cells 6 hours after birth were elongated and lined up between the nerve fibres.

Silver impregnation confirmed the existence of these globular cells in the superficial layers of the nervous parenchyma close to the meninges, especially near the median septum and in the corpus callosum. In the latter cells short and wide pseudopodia were present. Completely ramified microglial cells of adult type were not found. Very few microglial cells were found more deeply.

At *24 hours* after birth there were more acid phosphatase-positive cells in the meninges, especially in the thin meningeal septa between the cerebellar folia. At 6 hours very few such cells were present in this latter situation. By 24 hours, however, there were a few phosphatase-positive cells in the interior of the cerebellar folia. Silver impregnation showed them to be globular with vacuolated cytoplasm and few, if any, processes.

At *36 hours* there were still large numbers of phosphatase-positive cells in the meninges, but many had invaded the nervous parenchyma, particularly in the vicinity of the upper part of the median septum (Fig. 2). The number of these cells decreased rapidly as one moved away from the meninges. In the depth of the nervous parenchyma the cells lost their globular form and became more elongated. Some ramified forms were present, but these were always located some distance from the surface of the nervous tissue.

Silver techniques showed many globular and pseudopodic elements in the nervous parenchyma (Fig. 3), particularly in the more superficial layers of the nervous system. Totally ramified microglial cells of adult type were still very rare and were confined to the deeper parts of the brain tissue. At *3 days* there were still more macrophages in the meninges. The most important finding at this time, however, was the first acid phosphatase-positive perivascular cells; they were on the outer surface of the walls of the large blood vessels at the base of the brain (Fig. 4). Cells of this type were not present in the perivascular space or in the adjacent nervous tissue.

At *4 days* there was an even larger population of acid phosphatase-positive cells in the meninges and nervous parenchyma. The large vessels showed a great number of round or ovoid acid phosphatase-positive adventitial cells (Fig. 5). Thus, near the vessels, we found globular cells, but further away the cells gradually lost their globular shape and began to present wide cytoplasmic processes in addition to acid phosphatase positivity.

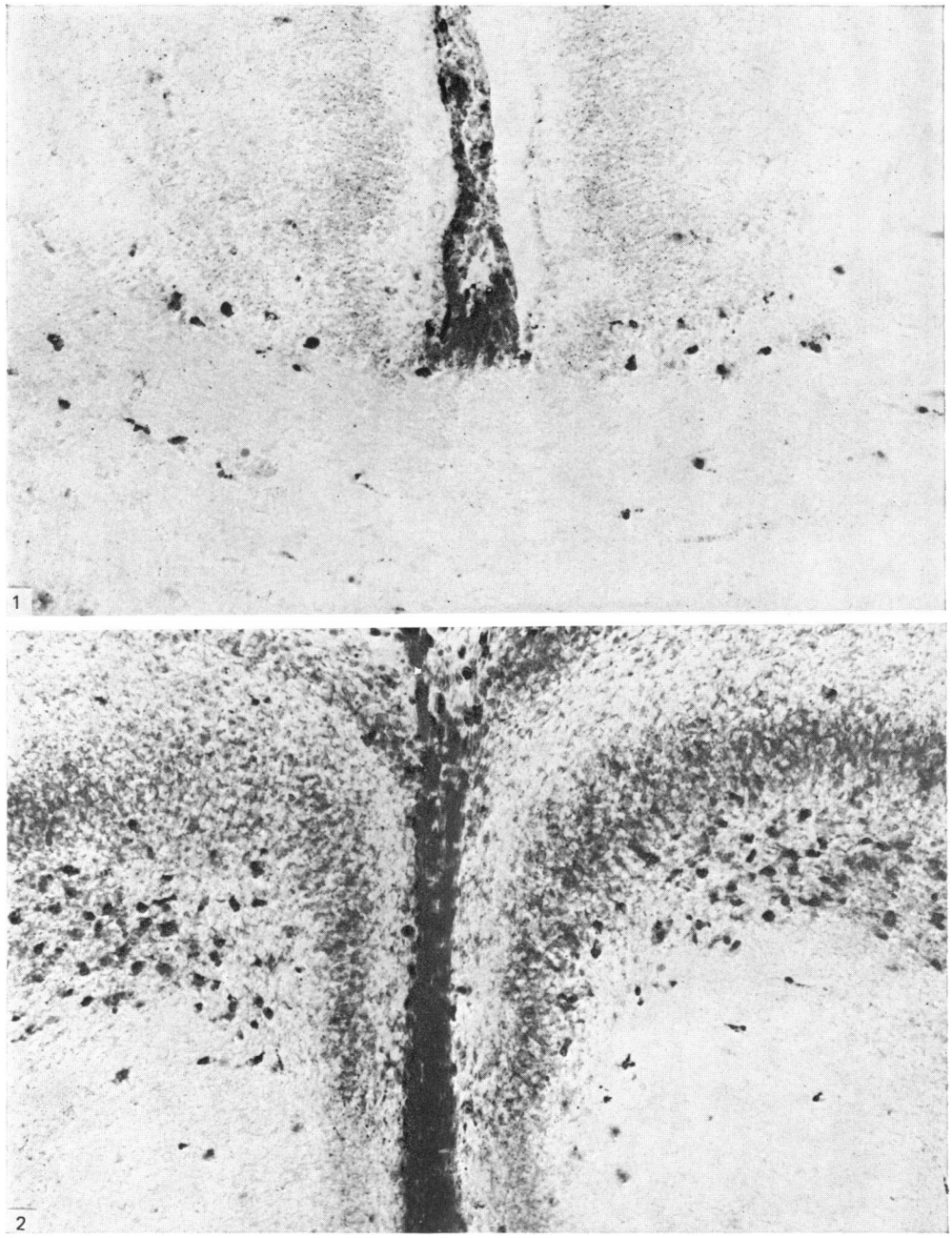
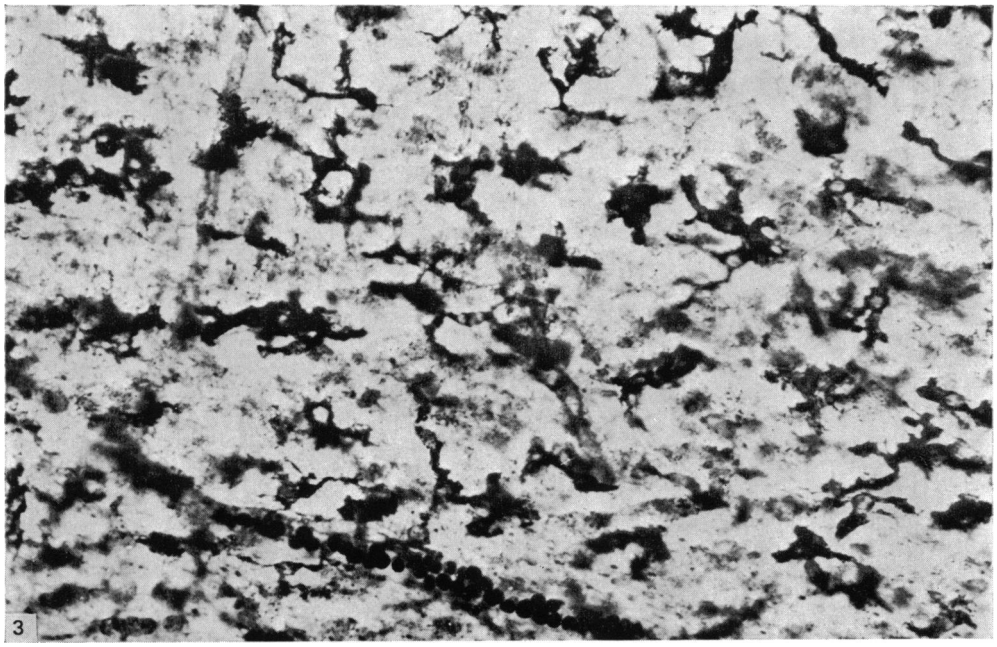


Fig. 1. 6 hours. Area of the medial septum superiorly. Scanty cells with positive acid phosphatase granules are seen in the medial meningeal septum and in the adjacent nervous parenchyma. × 200.

Fig. 2. 36 hours. Medial meningeal septum presenting abundant globular acid phosphatase-positive elements which detach from it and invade the adjacent nervous parenchyma. × 200.



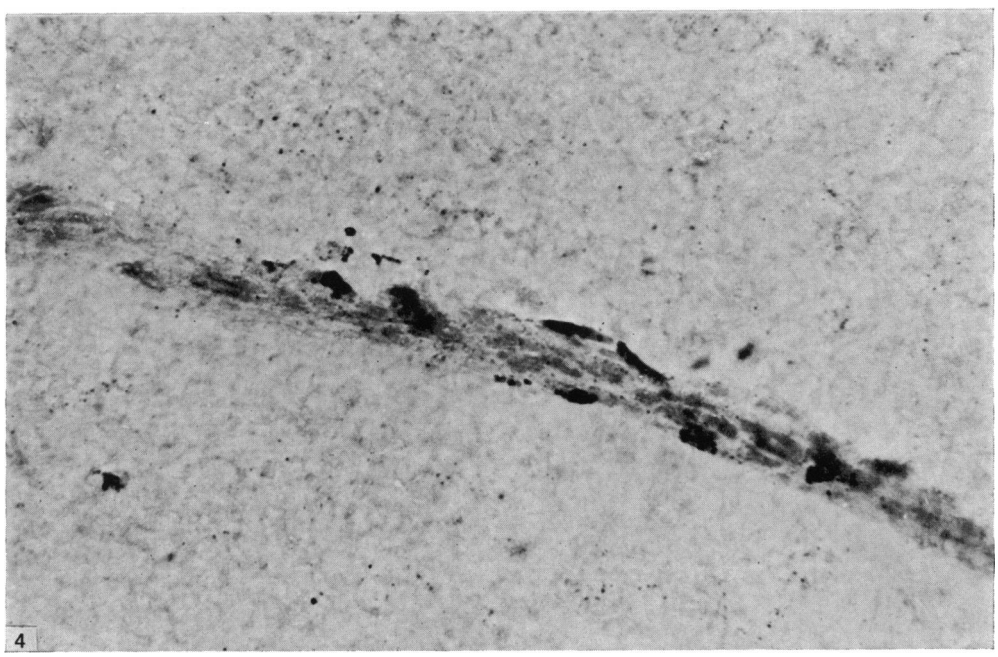
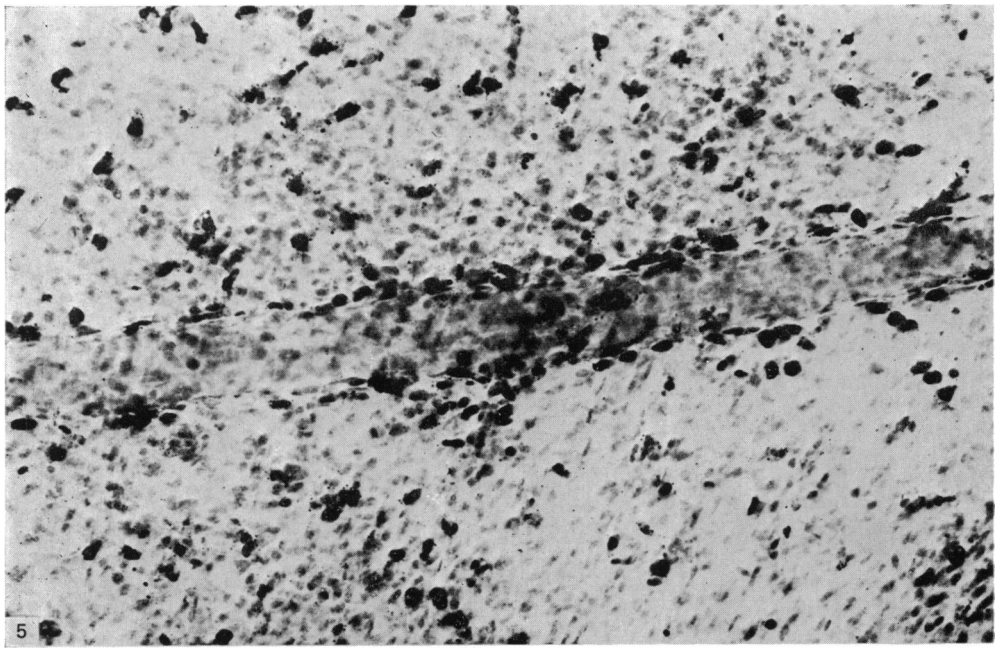


Fig. 3. 36 hours. Pseudopodic microglia in the nervous parenchyma near the meninges. Silver. ×400.

Fig. 4. 3 days old rat. Cells with acid phosphatase-positive granules are present in the wall of a blood vessel in the base of the brain. ×475.



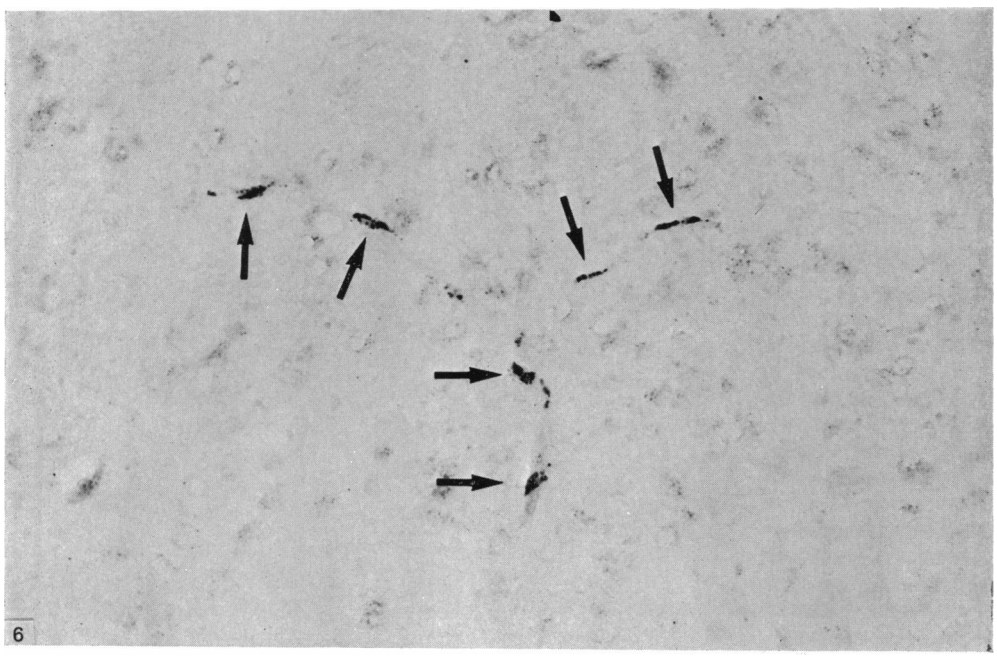
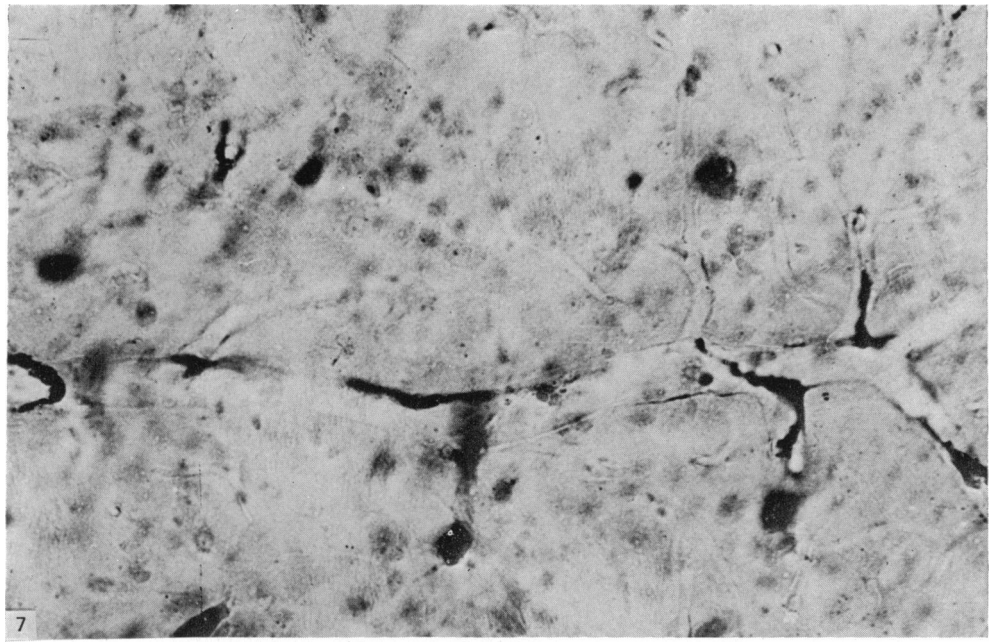


Fig. 5. 4 days old rat. Artery of the base of the brain showing abundant globular cells containing acid phosphatase-positive granules. These cells detach themselves from the vessel and, after occupying the perivascular space invade the nervous parenchyma. × 200.

Fig. 6. 5 days old rat. Cerebral capillaries showing pericytes with acid phosphatase-positive granules (arrows). × 300.



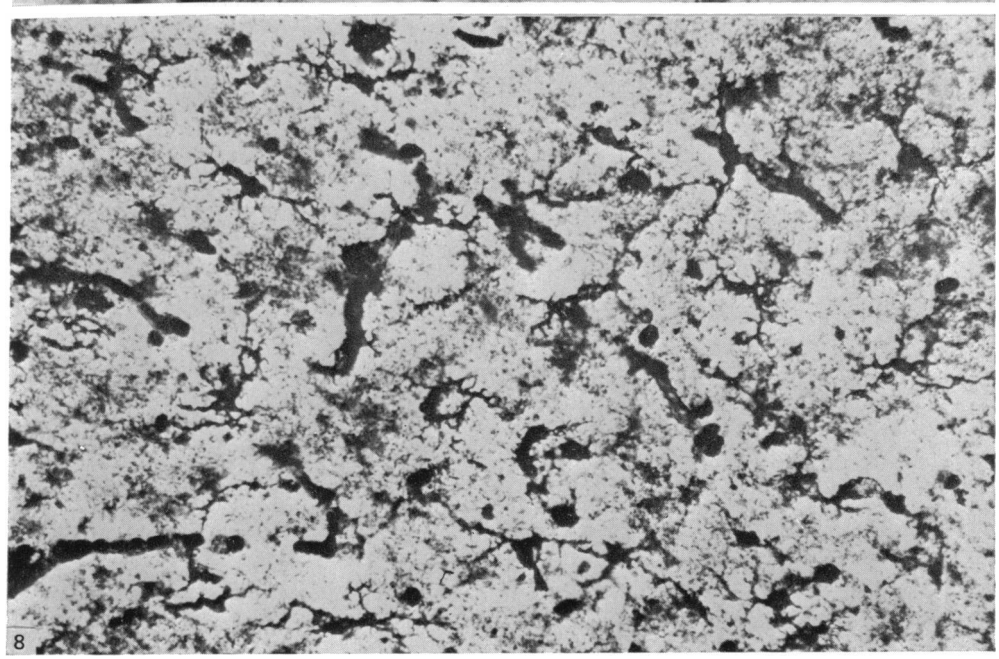


Fig. 7. 10 days old rat. Showing abundant pericytes with acid phosphatase-positive granules. × 500.

Fig. 8. 20 days old rat. Shows abundant ramified microglia in the depths of the nervous parenchyma. Silver. × 200.

The silver techniques showed globular and pseudopodic cells in the perivascular nervous parenchyma. These cells had similar characteristics to those observed in areas close to the meninges.

The most important finding in *5 days* old rats was the positive acid phosphatase reaction in pericytes (Fig. 6). The enzyme was in the processes which encircled or extended along the capillary wall.

Many acid phosphatase-positive cells were still present in the meninges and on the walls of large vessels, from where they appeared to separate off and migrate into the nervous parenchyma.

During *days 6 and 7* there was a slight decrease in the number of meningeal macrophages, but those related to large vessels remained abundant.

By now the number of totally developed microglial cells had increased considerably. Using silver techniques, such cells with thin ramified processes were identified in deep areas. However, superficially near the meninges, and in perivascular areas, globular and pseudopodic forms were still to be found, but no completely ramified microglia. Adult cells were only found at a certain distance from their meningeal and vascular sources.

From *day 10* onwards the number of pericytes giving an acid phosphatase-positive reaction increased (Fig. 7), while their numbers rapidly declined near the meninges. In the interstitial space of the nervous parenchyma there were very few acid phosphatase-positive cells apart from those close to large vessels.

From *day 20* onwards practically all the acid phosphatase-positive cells were in relation to blood vessels. The number of globular elements in the walls of large vessels and in the perivascular space had decreased considerably, while in the adjacent nervous parenchyma they were practically non-existent. However, the acid phosphatase-positive pericytes remained abundant and by *100 days* they were virtually the only acid phosphatase-positive cells to be found.

Only a very few acid phosphatase-positive globular cells were seen in the meninges after day 20.

Using silver techniques we found that from day 20 onwards the predominant microglial element was the intensely ramified mature cell with thin processes, now present throughout the nervous parenchyma (Fig. 8).

Tests for peroxidase were negative at all the stages studied.

DISCUSSION

These results confirm the mesodermal origin of microglial cells first proposed by Rio-Hortega (1919, 1921*b*). In none of our observations did we find any relation between them and the amoeboid ependymal cells described by Ling (1976, 1977). The invasion of the nervous parenchyma by globular acid phosphatase-positive cells from the meninges seems to begin before birth, because a few such cells were already present in the nervous parenchyma 6 hours after birth. During the first days after birth the number of these cells increased, first in the meninges and later in the superficial layers of the nervous tissue. Silver impregnation demonstrated that the microglial population was scanty and there were very few mature microglial cells.

At 3 days the adventitia of the large vessels became a second important source of microglial cells. From there they entered the perivascular space, and invaded the nervous parenchyma. In the following days globular and pseudopodic cells

accumulated both in the superficial layers of the nervous tissue and in the vicinity of the vessels. As was once described by Rio-Hortega (1921*b*), as these globular amoeboid mesodermal cells invaded the nervous tissue they lost their globular shape and adapted themselves to the interstitial spaces of the nervous parenchyma. Thus globular cells were confined to those areas of the nervous parenchyma which, because of their immaturity, possessed a loose structure with large spaces between their elements. However, as microglial cells invaded nervous tissue of a denser structure and greater maturity, they adapted their shape to their surroundings, sending out to begin with wide and irregular processes which were detectable with histochemical techniques because of their content of acid phosphatase-positive granules (lysosomes). In the more mature, greatly ramified microglial elements the cell processes were much narrower and any lysosomes they might contain were not detectable at light microscopic level. Thus totally ramified adult microglial cells were not visible using histochemical techniques for acid phosphatase and silver impregnation was necessary to study them. So, from day 20 onwards, cells with acid phosphatase-positive particles were very rare, although silver impregnation demonstrated a large population of adult microglial cells.

At 5 days after birth acid phosphatase-positive pericytes began to appear. These progressively increased in numbers and were common from then onwards. However, we do not consider pericytes to be an important source of microglia perinatally because we never found globular cells containing acid phosphatase-positive granules in the nervous parenchyma adjacent to the capillaries as they were in relation to the meninges and large vessels. Stensaas (1975) and Dodson, Tagashira & Wai-Fong Chu (1976) deny even the possibility of a pericyte microglial origin. On the contrary, we admit the possibility of the transformation of pericytes into microglial cells or macrophages (Boya, 1975, 1976), but regard this as restricted to the maintenance of the microglial population throughout life or to the increase in this population in special pathological circumstances.

Matthews (1974) and Ling (1978) describe the passage of monocytes into the nervous parenchyma and their transformation there into microglial elements. Peroxidase has been detected in the monocytes of several animals (Van Furth *et al.* 1970; Nichols *et al.* 1971; Caxton-Martins & Daimon, 1976) and in the rat this has been confirmed by Daems, Wisse, Brederoo & Emeis (1975), Daems, Koerten & Soranzo (1976) and Bentfeld, Nichols & Bainton (1977). However, in none of our observations did we find any positive peroxidase reaction in amoeboid cells. Ling (1977), moreover, states that the amoeboid cells of the corpus callosum in the rat are peroxidase-negative, a finding that contradicts his view that they are of monocytic origin.

SUMMARY

The rat brain has been studied between 6 hours after birth and 100 days, using histochemical techniques for acid phosphatase and peroxidase, and silver impregnation for microglial cells.

The results indicate that microglia come initially from acid phosphatase-positive cells of the meninges. These invade the nervous parenchyma and transform into ramified microglia. At 3 days of age similar cells are present on the outer surface of the large blood vessels, from which site they migrate into the nervous parenchyma. In 100 days old rats the acid phosphatase-positive cells are practically all pericytes.

None of the microglial cells or their precursors give a positive reaction for peroxidase.

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