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Studies of the ultrastructure and permeability of the blood–brain barrier in the developing corpus callosum in postnatal rat brain using electron dense tracers

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ABSTRACT

The ultrastructure of the capillaries and their permeability to lanthanum ions and ferritin in the corpus callosum was examined in postnatal rat brain. In 1 and 7-d-old rats, numerous pinocytotic vesicles were observed in the endothelial cytoplasm of the callosal capillaries. Tight junctions were present between adjacent endothelial cells which were surrounded by an ill defined layer of basal lamina. The latter was almost devoid of astrocytic association in 1 d rats and partially covered by astrocytic end-feet in 7 d rats. Pericytes were a common feature. Amoeboid microglial cells were in direct contact with some parts of the vascular wall. Large extracellular spaces were present around the capillaries. In 14 d rats, the walls of the callosal capillaries became more well developed and were surrounded by a continuous sheath of astrocytic end-feet. The basal lamina became denser and well defined. The pericapillary spaces had diminished. Immunostaining for GFAP confirmed that, with age, the walls of the callosal capillaries became increasingly covered by the astrocytic end-feet. After perfusion with lanthanum, the tracer was deposited on the luminal surface but not in the abluminal side of the endothelial cells; the passage of the tracer was apparently obstructed in the intercellular space by the tight junctions in both the 1 and 14 d rats. When injected intravenously in 1–7 d rats, ferritin was transported across the endothelial cells by transcytosis and consequently taken up by the pericytes and amoeboid microglial cells contacting the basal lamina. In 14 d rats, the injected ferritin was only found in the endothelial cytoplasm. It was concluded that the difference in the capillary permeability to exogenous material in 1–7 and 14 d rats is due to the difference in the activities of the transendothelial transport. The pericytes and amoeboid microglial cells associated with the capillary probably play a role as phagocytes in maintaining the function of the blood–brain barrier by trapping any serum-derived foreign substances with astrocytes having a regulatory role in the formation of the barrier.

Key words: Brain capillaries; astrocytes; glial fibrillary acidic protein; lanthanum.

INTRODUCTION

The term blood–brain barrier (BBB) has been used to explain the restricted movement of certain materials between the bloodstream and the brain parenchyma. Ultrastructurally, Reese & Karnovsky (1967) demonstrated that after the intravenous injection of horseradish peroxidase (HRP) into mice, the tracer was found in the lumina of the cerebral vessels and in a few vesicles within endothelial cells; none was seen beyond the vascular endothelium. They concluded that the barrier to the passage of peroxidase was due to 2 structural features of the cerebral endothelium: (1) the endothelial tight junctions, which effectively seal the intercellular space, and (2) the scarcity of endothelial vesicles which limits the transendothelial transport from blood to brain.

In the fetus and newborn, the BBB is immature (Saunders, 1977) and some substances penetrate more freely into the nervous tissue than in the adult (Seta et al. 1972). Xu et al. (1993) have recently observed labelled amoeboid microglial cells (AMC) in the postnatal corpus callosum in rats following an intraperitoneal (i.p.) or intravenous (i.v.) injection of the fluorescent tracer, rhodamine isothiocyanate (RhIc). The frequency of RhIc-labelled microglial

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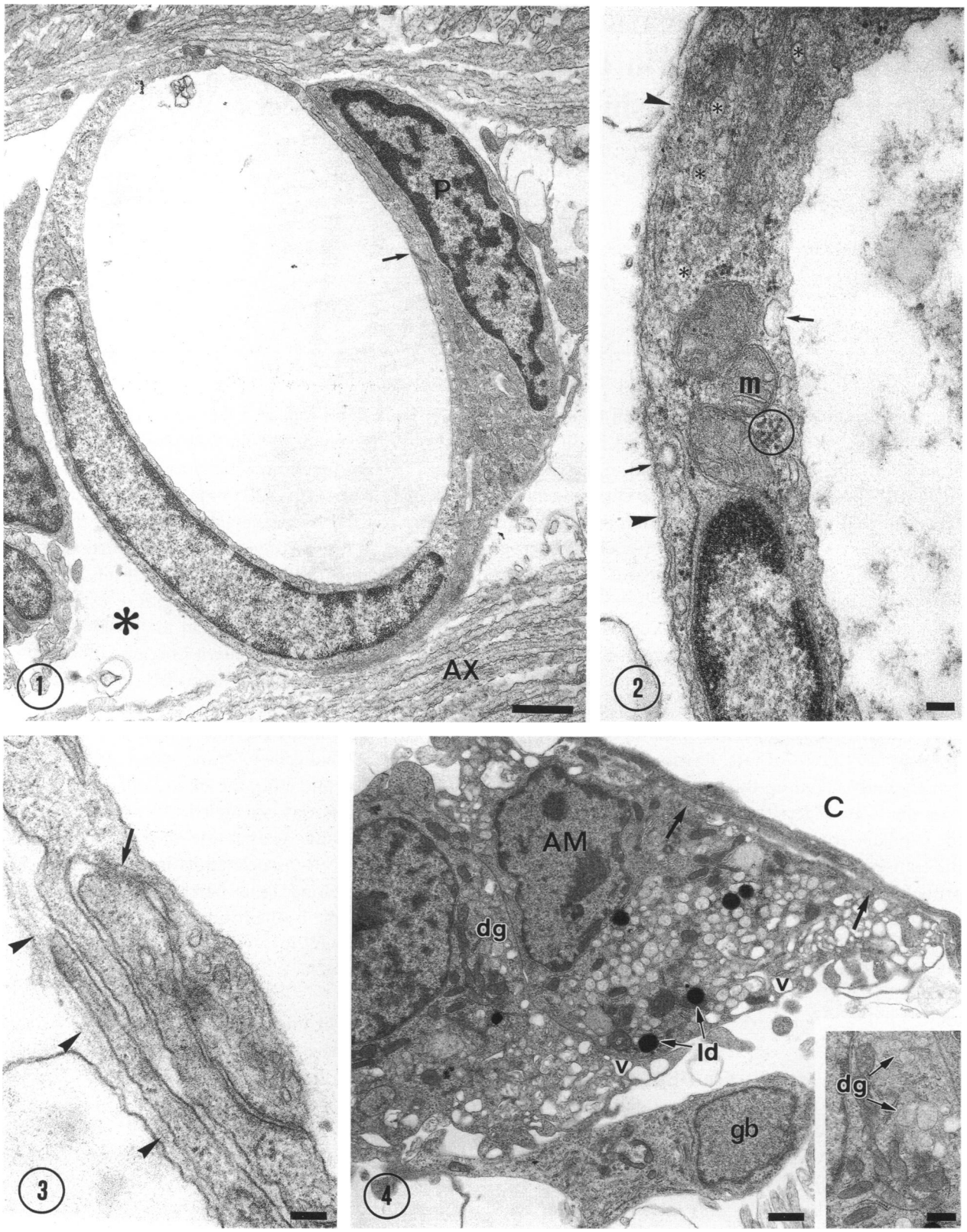


Fig. 1. Typical capillary in the supraventricular corpus callosum in a 1 d rat. The apposed endothelial cells are joined by cell junction (arrow). A pericyte (P) caps the capillary on the right. Large pericapillary extracellular spaces (asterisk) occur around the vessel. AX, unmyelinated axons. Bar, 1 μm.

Fig. 2. Portion of a callosal capillary in a 1 d rat. Arrows indicate 2 pit-like invaginations, one on the luminal side and the other on the abluminal side. The cytoplasm shows several pinocytotic vesicles (asterisks), clusters of ribosomes (circle) and mitochondria (m). The basal lamina (arrowheads) is ill defined. Bar, 0.1 μm.

cells in the corpus callosum declined with age. This led to the proposal that the gradual maturation of the BBB in the corpus callosum would lead to a reduction in the transvascular transport of RhIc taken up by AMC.

The present study was undertaken to elucidate the structural and functional differences of the callosal blood vessels with advancing age, first, by examining their ultrastructural changes related to development and, secondly, by investigating the permeability of the blood capillaries using lanthanum and ferritin tracer techniques. These results could help to explain the mode of the labelling of callosal AMC by exogenous substances when the latter are administered by the i.p. or i.v. route into postnatal rats (Kaur et al. 1986; Xu et al. 1993).

MATERIALS AND METHODS

A total of 50 Wistar rats, aged 1–14 d, were used. For routine electron microscopy, 2 rats each from 1, 7 and 14 d of age were perfused for 10–15 min under ether anaesthesia with a mixed aldehyde fixative containing 2% paraformaldehyde and 3% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.3–7.4. After perfusion, the brains were removed, kept in the same fixative for another 4 h before rinsing overnight in 0.1 M cacodylate buffer with 5% sucrose at 4 °C. Coronal sections (300 μm) of the cerebrum taken at the level of the optic chiasma were cut with an Oxford Vibratome and the areas of the corpus callosum above the lateral ventricles (supraventricular corpus callosum) were trimmed under a dissecting microscope. After washing in buffer, the tissue was postfixed in 2% osmium tetroxide and dehydrated in a graded series of ethanol and embedded in Araldite mixture. Ultrathin sections were stained with uranyl acetate and lead citrate and examined in Philips 400T or JEOL 1200EX electron microscope.

For the glial fibrillary acidic protein (GFAP) immunohistochemical study, 2 rats each from ages 1, 7 and 14 d were perfused with 0.2% picric acid in 2% formaldehyde in 0.1 M phosphate buffer at pH 7.4 for 15–20 min after which the brains were removed and postfixed in the same fixative for another 2 h. They were then kept in 0.1 M phosphate buffer containing 10% sucrose overnight at 4 °C. Coronal frozen sections (40 μm) were cut at the level of the optic chiasma and then washed in 3 changes of phosphate buffered saline (PBS) with 0.2% Triton X-100. The sections were incubated overnight at room temperature in mouse monoclonal anti-GFAP diluted 1:100 with PBS containing 1% bovine serum albumin (BSA) and then washed in PBS as above. The sections were subsequently incubated in biotinylated anti-mouse IgG, diluted 1:100, for 1 h, washed, and then placed in the avidin–biotin–peroxidase complex for 1 h at room temperature. The peroxidase was visualised by using nickel-enhanced 3,3′diaminobenzidine (DAB) solution. The sections were then dehydrated in ascending concentration of alcohols and mounted in Permount.

For lanthanum tracer studies, 3 rats each from ages 1 and 14 d were used. After ether anaesthesia and thoracotomy, the right atrium was slit with a pair of fine scissors. The animals were perfused through the left ventricle with the following 3 perfusates according to the method of DePace (1984). The initial perfusate was 10–15 ml of 1% $NaNO_3$ in normal saline. This was immediately followed by a 10 min infusion with 50–100 ml of ionic lanthanum solution composed of 20 mM-$La(NO_3)_3.6H_2O$, 80 mM-NaCl, 3.5 mM-KCl, 1.0 mM-$CaCl_2$, 1.0 mM-$MgCl_2$ and 1.0 mM glucose, pH 7.4. The lanthanum nitrate solution was followed by an additional 10 min perfusion with 50–100 ml of 2% glutaraldehyde and 2% paraformaldehyde made up in a sulphate–salt solution containing 43 mM-Na_2SO_4, 16 mM-$NaHCO_3$, 10 mM sodium acetate, 3.5 mM-KCl, 1.0 $CaCl_2$, 1.0 mM-$MgCl_2$, 1.0 mM glucose, 1.6 mM-Na_2HPO_4, 0.4 mM-NaH_2PO_4 and 33 mM sucrose at pH 7.4. $La(SO_4)_3$ is insoluble and the electron dense precipitates facilitate ultrastructural localisation of the lanthanum. After the perfusion, the brains were cut into coronal 300 μm sections with a vibratome. The supraventricular corpus callosum was dissected out and after several rinses, the tissue blocks were postfixed in 1% aqueous osmium tetroxide for 1 h and processed for electron microscopy as described above.

For the ferritin tracer study, 8 rats each from ages 1, 5, 7 and 14 d were used. Under ether anaesthesia, each rat, between 1 and 7 d of age, was given an i.v.

Fig. 3. Portion of a capillary in the corpus callosum of a 1 d rat showing a tight junction between 2 lining endothelial cells. Arrow indicates the site of 'fusion' where the intercellular space is obliterated. Elsewhere the intercellular space is evident. The tenuous basal lamina is indicated with arrowheads. Bar, 0.1 μm.

Fig. 4. Two amoeboid microglial cells in a 1 d rat. One (AM) makes direct contact (arrows) with the outer wall of the capillary (C). The cells are characterised by numerous vacuoles (v), dense granules (dg) and lipid droplets (ld). gb, glioblast. Bar, 1 μm. Inset: dense granules (dg). Bar, 1 μm.

injection of 50 μl (10 mg) of ferritin (Sigma, MO) via the left external jugular vein. The 14 d rats were each given 100 μl (20 mg) ferritin injection. The rats were then killed in pairs at 30 min and 1, 3, 6 h after the injection. The subsequent tissue processing followed that described for routine electron microscopy.

OBSERVATIONS

Routine electron microscopy

In 1 and 7 d rats, the unmyelinated axons in the supraventricular corpus callosum were loosely organised. Capillaries of different calibre were observed (Fig. 1). The cytoplasm of the endothelial cells of a typical callosal capillary contained numerous elongated vesicles with an average size of 90 × 125 nm (Fig. 2). Small invaginated pits or caveolae were present on both the luminal and abluminal sides of the plasma membrane (Fig. 2). Mitochondria, cisternae of rough endoplasmic reticulum, Golgi apparatus and ribosomes were also present in the cytoplasm of the endothelial cells (Fig. 2). The lining endothelial cells of the capillaries were held together by tight junction showing one or more 'fusion points' of the apposed membranes (Fig. 3). Exterior to the endothelial cells was a relatively ill defined basal lamina composed of an amorphous substance of varying electron density (Figs 2, 3). While most of the walls along the length of the capillary were made up of a single layer of endothelial cells, other cellular elements also formed an integral component in some segments. Associated with the capillaries were a variable number of pericytes which were invested by a common layer of basal lamina. Astrocytic end-feet characterised by the presence of glycogen and filaments were observed to cover only a limited area of the vascular wall, whereas most areas of the outer capillary wall were devoid of the association of the astrocytic end-feet (Fig. 1). Large interstitial spaces which often extended around the blood vessels as the pericapillary space (Fig. 1) were present throughout the immature corpus callosum. Apart from the astrocytic processes, amoeboid microglial cells were found associated with the walls of the blood vessels (Fig. 4); they appeared to rest directly on the vascular basal lamina in some areas (Fig. 4).

In 14 d rats, the corpus callosum was more compact in texture and contained both myelinated and unmyelinated nerve fibres (Fig. 5). The walls of the callosal capillaries were composed of endothelial cells and pericytes (Fig. 5). The end-feet of astrocytes entirely covered the outer wall of the blood vessels at this stage (Fig. 5). The number of cytoplasmic vesicles in the endothelial cells was drastically reduced in contrast to that in 1 d rats. Tight junctions were clearly evident between the endothelial cells (Fig. 5). The well defined basal lamina was denser and thicker (Fig. 5). The pericytes, which had a paler cytoplasm than the endothelial cells, contained numerous free ribosomes and isolated dilated cisternae of rough endoplasmic reticulum (Fig. 5). The large pericapillary spaces which were present in 1 and 7 d rats had diminished and were filled with closely packed nerve fibres (Fig. 5).

GFAP immunohistochemistry

In 1 d rats, the GFAP-immunostained astrocytes were mostly round with occasional cells bearing a few short processes; some of them contacted the walls of the capillaries. Most areas of the blood vessels, however, were free from astrocytic contacts (Fig. 6).

In 7 d rats, the stained astrocytes assumed a stellate appearance with a variable number of long slender processes (Fig. 7). The terminals of these processes were expanded to form the astrocytic end-feet abutting the outer wall of the capillaries. A large area of the outer walls of the capillaries, however, lacked the covering of astrocytic end-feet (Fig. 7).

In 14 d rats, there was a considerable increase in the number of astrocytic processes compared with that of the younger animals. They formed an extensive network between the capillaries. In the latter, the outer walls were completely invested by the astrocytic end-feet (Fig. 8).

Lanthanum tracer study

Following the infusion of lanthanum solution, the electron dense tracer was deposited on the luminal but not on the abluminal side of the blood vessels in both the 1 and 14 d rats. However, there appeared to be some differences in the pattern of lanthanum deposition between the 2 age groups.

In 1 d rats, the electron dense lanthanum was observed in the lumina of the blood vessels as well as in the intercellular junctions of the endothelial cells. The tracer was frequently observed to have seeped deeply into the endothelial interspace filling almost the entire length of the intercellular junctions (Fig. 9); it was, however, ultimately prevented from reaching the abluminal side of the endothelial cells by the tight junctions (Fig. 9). So far, the tracer has never been observed to traverse beyond the endothelial cells into the capillary basal lamina or the pericapillary spaces.

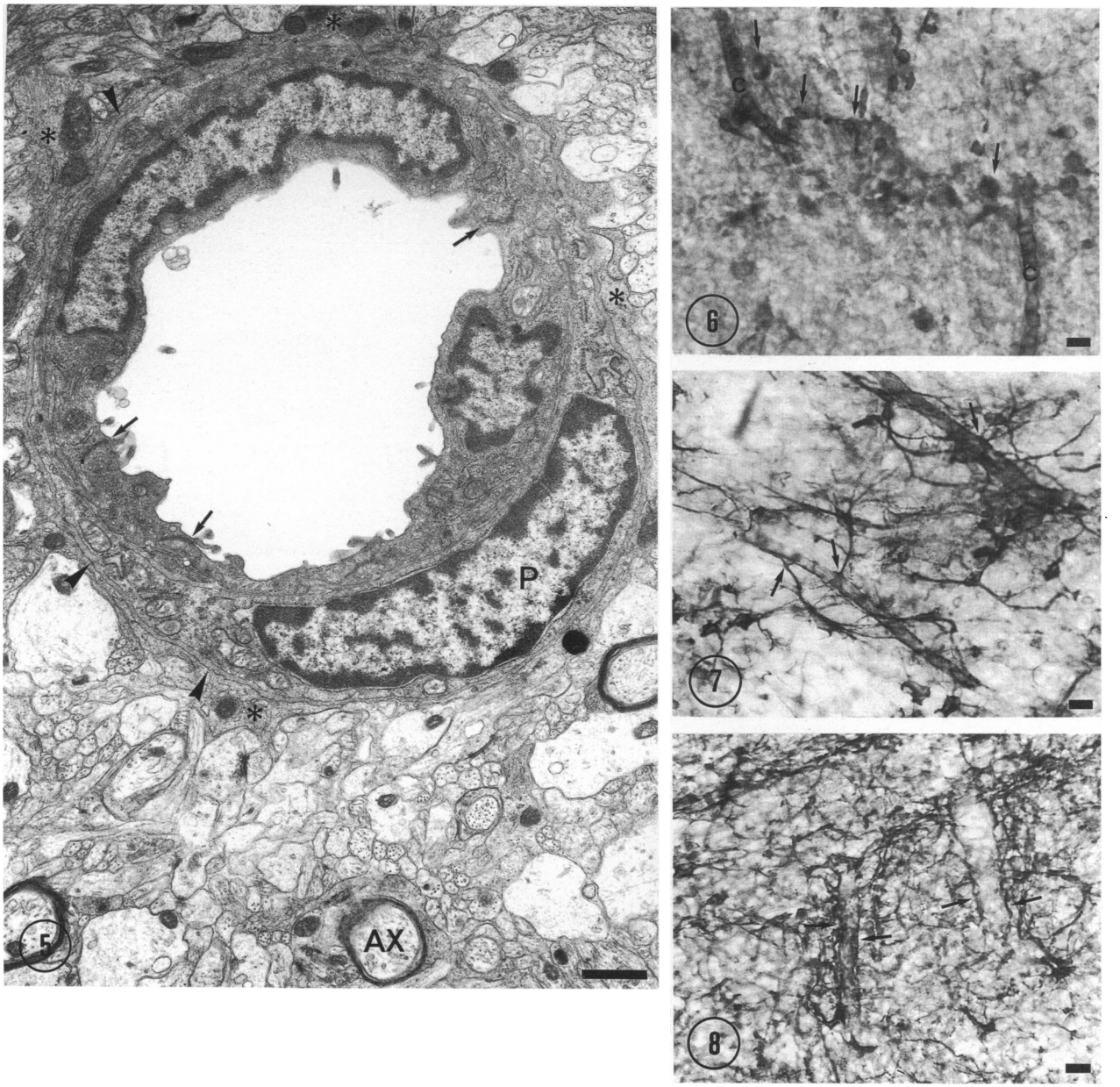


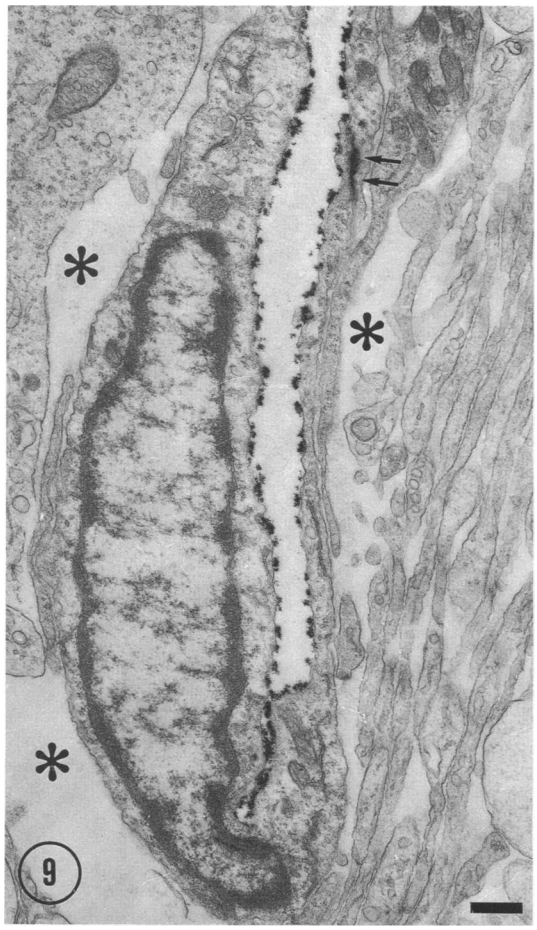
Fig. 5. Transverse section of a capillary with an associated pericyte (P) in the corpus callosum in a 14 d rat. The tissues around the capillary are much more compact than those in the 1 d rat (see Fig. 1). The lining endothelial cells are held together by cell junctions (arrows). Arrowheads indicate its well defined basal lamina which invests the endothelial cells and pericyte. Immediately exterior to the lining basal lamina are the astrocytic end-feet (asterisks) which may be traced to form a complete sheath around the capillary. Numerous unmyelinated and several myelinated axons (AX) are closely packed in the pericapillary region. Note the obliteration of the pericapillary space. Bar, 1 μm.

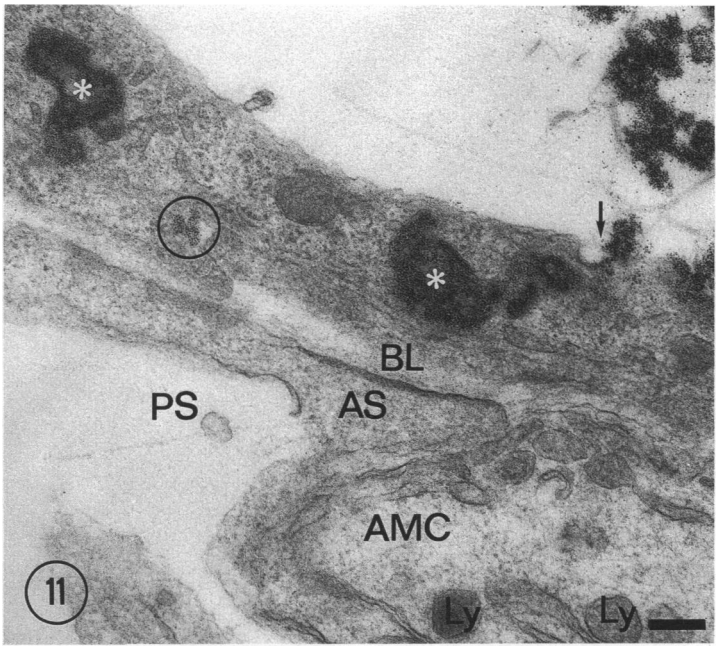
Figs 6–8. Photomicrographs showing the corpus callosum in different age groups stained with GFAP. The immunoreactive astrocytes (arrows) are round with a few processes in 1 d rats (Fig. 6). The outer walls of the 2 capillaries shown are devoid of astrocytic contact. In the 7 d rat (Fig. 7), the immunoreactive astrocytes assume a stellate form. Their long extending processes are anchored to the outer walls of the capillaries (arrows). In some areas, the walls of the capillaries lack the covering of the astrocytic end-feet. In the 14 d rat (Fig. 8), the outer walls of the capillaries are completely covered by the extensive processes of astrocytes (arrows). Bar, 10 μm.

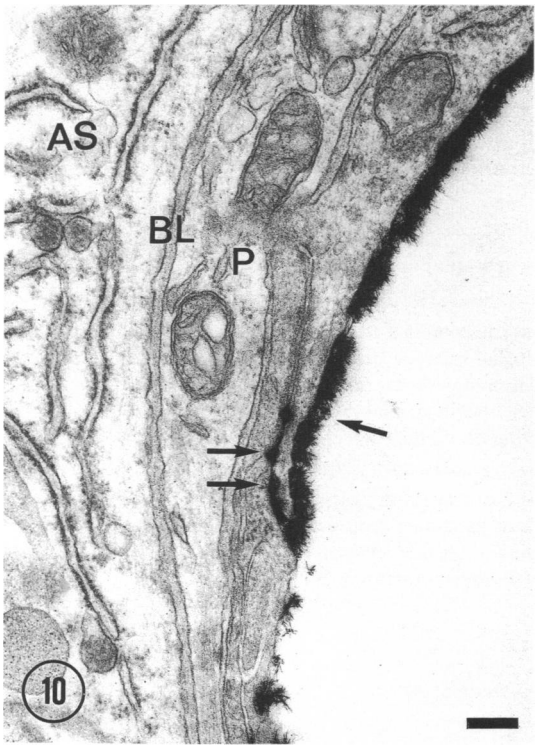
In 14 d rats, the lanthanum deposit was located on the surface of the blood vessels and was arrested by the tight junction at the apical region between adjacent endothelial cells (Fig. 10). The tracer usually did not extend beyond the luminal half of the intercellular cleft where it was impeded by the membrane fusion (Fig. 10).

Ferritin tracer study

Between 30 min and 6 h after injection into 1–7 d rats, ferritin particles were transported across the endothelial cells of the callosal capillaries. The tracer particles were found on the luminal plasma membrane and its invaginations (Fig. 11). Numerous multi-







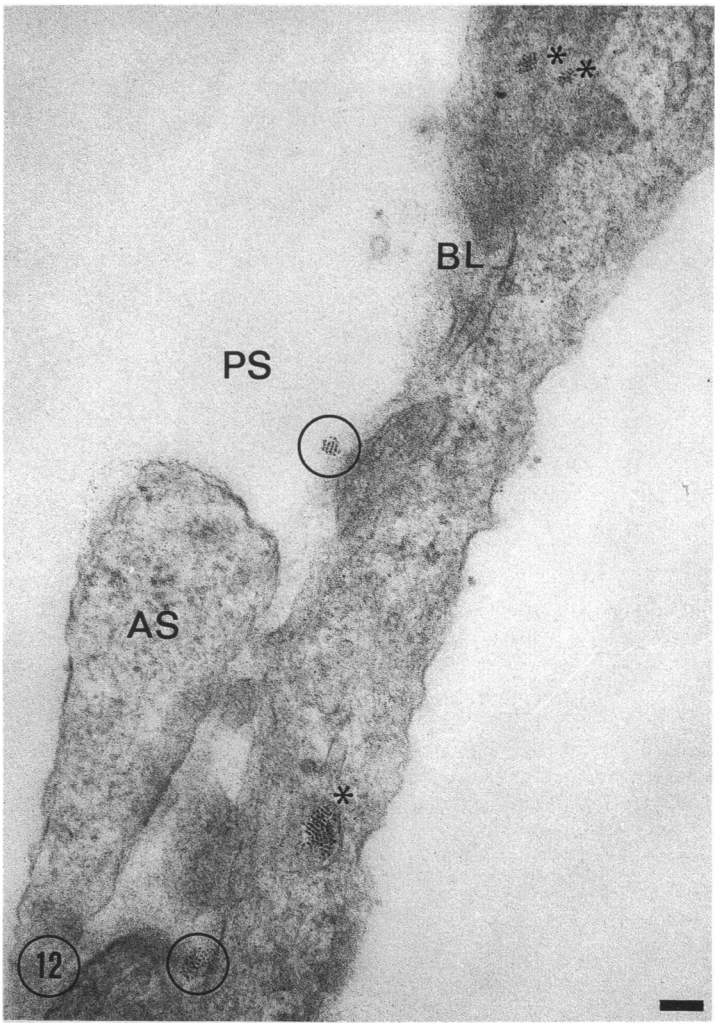


Fig. 9. Longitudinal section of a callosal capillary in a 1 d rat after perfusion with lanthanum. The electron-dense tracer is deposited on the luminal surface and penetrates deeply into the intercellular space (arrow). The abluminal side of the endothelium and the large pericapillary extracellular spaces (asterisks) are devoid of the tracer. Bar, 0.5 μm.

Fig. 10. Portion of a callosal capillary in a 14 d rat perfused with lanthanum. The tracer is localised on the luminal surface of the endothelial cells as well as between the intercellular spaces (arrows). Note that the passage of the tracer is blocked half way down the intercellular

vesicular bodies laden with ferritin were observed in the cytoplasm of the endothelial cells (Fig. 11). The internalised ferritin was also sequestered in small vesicles and tubular structures which appeared to be membrane bound. On the abluminal side of the endothelium, free ferritin particles in small clusters were observed in the basal lamina, pericapillary space (Figs 11, 12), pericytes (Fig. 13) and amoeboid microglial cells (Figs 14–16). Ferritin particles were not observed in the intercellular junctions between endothelial cells nor in the astrocytic end-feet.

In 14 d rats, only a limited amount of ferritin was observed in the endothelial cells of the callosal capillaries. The ferritin particles ingested were present in membrane-bound multivesicular bodies (Fig. 17). Free ferritin was not observed on the abluminal side of the endothelium.

DISCUSSION

Many studies have shown that the BBB of immature animals is more permeable than that of adults (Seta et al. 1972; Kristensson & Olsson, 1973; Saunders, 1977; Betz & Goldstein, 1981). In the adult, it is generally agreed that the brain capillary endothelial cell is the anatomical site of the BBB (Reese & Karnovsky, 1967; Van Deurs, 1980). The structural basis for the increased permeability in immature animals, however, remained to be explored. Our previous study (Xu et al. 1993) had demonstrated the extravasation of the fluorescent tracer, rhodamine isothiocyanate, into the corpus callosum in early postnatal rats but not in the rats older than 13 d. This led us to postulate that the full function of the BBB in the brain tissue was established from the 13th postnatal day onwards.

In the present study, the ultrastructure of the walls of the callosal capillaries in 1 and 7 d rats was simple. In most of the segments, the wall consisted of only 1 layer of endothelium so that the outer wall was exposed directly to the large pericapillary spaces. The cytoplasm of the endothelial cells contained numerous pinocytotic vesicles. The basal lamina was ill defined and the vessels lacked the characteristic investment by astrocytic end-feet. In 14 d rats, the cytoplasm of the endothelial cell of the callosal capillaries contained significantly fewer pinocytotic vesicles than that of 1 and 7 d rats. The basal lamina of the callosal capillaries had increased considerably in its thickness and density. The large pericapillary spaces had diminished and these were replaced by astrocytic end-feet resting directly on the basal lamina. In 1, 7 and 14 d rats, tight junctions were evident between adjacent endothelial cells. Pericytes were a persistent feature. GFAP immunostaining showed that, with age, the callosal capillaries became increasingly covered on their outer surface by the astrocytic end-feet. Our observations of the fine structural changes of the callosal capillaries in the developing rats are in agreement with those observed in the cerebral cortex of the developing rats described by Donahue & Pappas (1961) and Caley & Maxwell (1970).

The fact that astrocytic end-feet form a continuous sheath around the cerebral capillaries suggests an involvement of astrocytes in the maintenance of the BBB. Some studies, however, have indicated that the astrocytic end-feet could not halt the diffusion of the HRP since they are connected by gap junctions rather than tight junctions (Brightman & Reese, 1969). This observation would exclude the astrocytic sheath as a restrictive barrier. On the other hand, astrocytes might act to induce and maintain the development of the characteristics of the BBB in the endothelial cells (Stewart & Coomber, 1986). Transplantation studies in quail-chick chimeras have shown that when fragments of unvascularised embryonic quail brain were transplanted into the coelomic cavity of chick hosts, the mesenteric vessels that grew into the graft developed structural, functional and histochemical characteristics of typical barrier vessels. Conversely, when fragments of unvascularised somite were transplanted into the brain of host chicks, the brain vessels that grew into the somitic tissue failed to develop barrier features (Stewart & Wiley, 1981). These results indicated that endothelial blood–brain barrier characteristics formed as a result of an inductive influence from the surrounding neural tissue. In this connection, astrocytes have been considered to play such a role

space by an intercellular tight junction. Lanthanum is not seen beyond the vascular endothelium. AS, astrocyte; BL, basal lamina; P, pericyte. Bar, 0.2 μm.

Fig. 11. Portion of a callosal capillary in a 1 d rat, 3 h following an intravenous injection of ferritin. The tracer is localised in the lumen and on the luminal plasma membrane and its invagination (arrow). A large amount of ferritin is sequestered in 2 large irregular dense bodies (asterisks). Note that a cluster of ferritin particles (circle) appears on the abluminal side of the endothelial cell. A portion of an amoeboid microglia cell (AMC) abuts on the basal lamina (BL). AS, astrocytic end-feet; Ly, lysosomes; PS, pericapillary space. Bar, 0.2 μm.

Fig. 12. Portion of a callosal capillary in 1 d rat, 3 h after ferritin injection. The injected ferritin is localised in the endothelial cell (asterisks) and on the abluminal side and pericapillary space (circles). AS, astrocytic end-feet; BL, basal lamina; PS, pericapillary space. Bar, 0.1 μm.

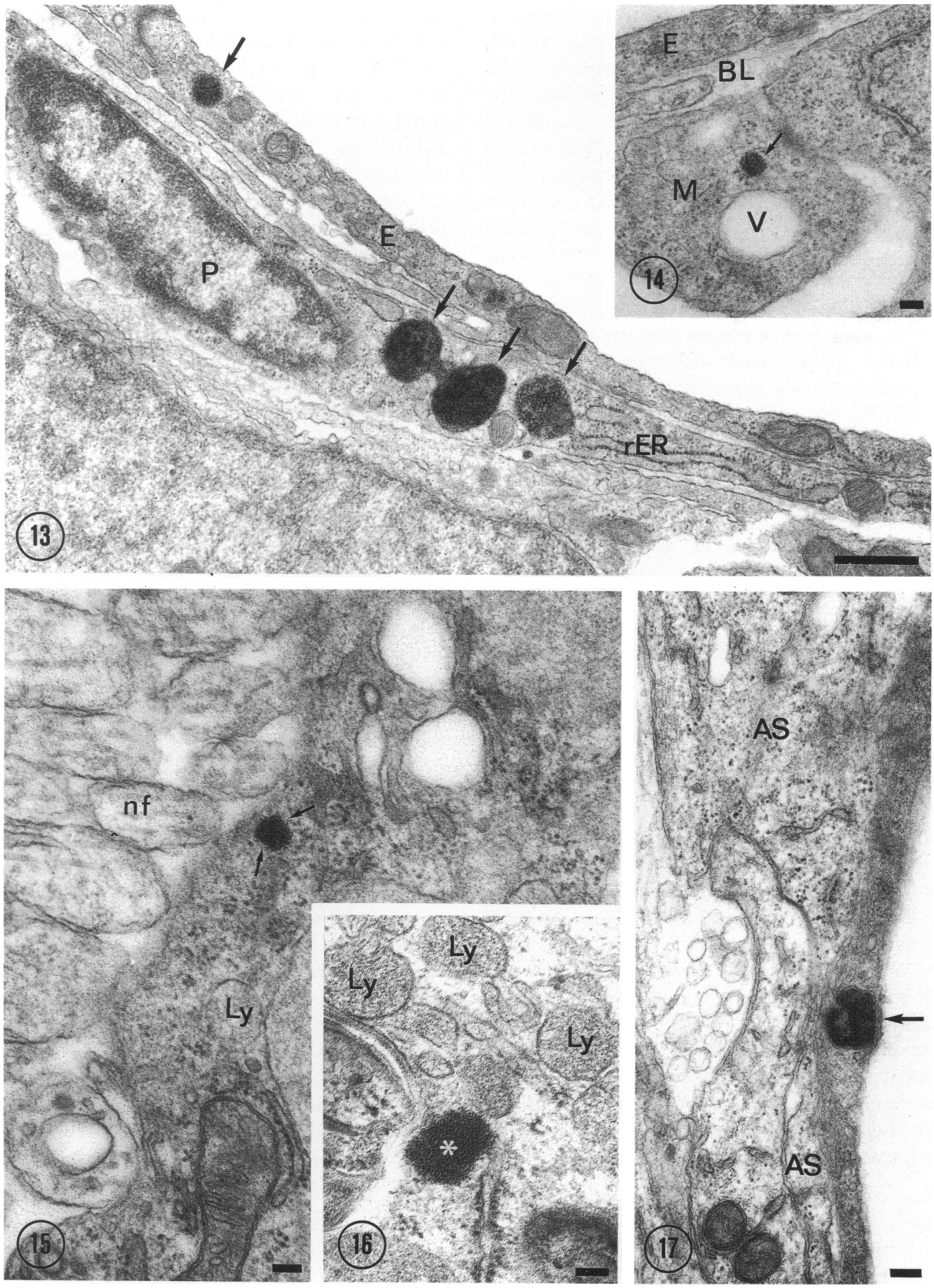


Fig. 13. Portion of a callosal capillary in a 1 d rat, 6 h after ferritin injection. The injected ferritin (arrows) appears both in the endothelial cell (E) and pericyte (P). In the latter, 3 large dense bodies laden with ferritin particles can be seen. rER, rough endoplasmic reticulum. Bar, 0.5 μm.

Fig. 14. Portion of an amoeboid microglial cell (M) characterised by its vacuole (V) abuts against the basal lamina (BL) investing the endothelial cell (E). The cell contains endocytosed ferritin particles (arrow). 1 d rat, 6 h following ferritin injection. Bar, 0.1 μm.

because of their close anatomical relationship with the capillaries (Goldstein & Betz, 1986; Risau & Wolburg, 1990). In fact, Janzer & Raff (1987) provided direct evidence that purified type I astrocytes were capable of inducing BBB properties in nonneural endothelial cells in vivo. The present study showed that in 1 and 7 d rats most of the outer surface areas of the callosal capillaries lacked the investment of astrocytic end-feet and the injected ferritin in these age groups could readily pass through the endothelial cells. In 14 d rats, on the other hand, the blood vessels were entirely covered by astrocytic end-feet and the ferritin injected in this age group failed to reach the abluminal side of the endothelial cells, although it was found in their cytoplasm. It would appear, therefore, that the time of the formation of a complete astrocytic sheath around the callosal capillaries coincided with the time of the full function of the BBB during the course of development. This corroborates the view that astrocytes have a regulatory role in inducing the development of the BBB.

Reese & Karnovsky (1967) attributed the impediment to the passage of HRP across the cerebral parenchyma following its injection into the blood stream of the adult mouse to 2 characteristic features of the endothelium of cerebral capillaries. The first was the presence of endothelial tight junctions which prevented the extravasation of HRP via the intercellular spaces. The second was the presence of few micropinocytotic vesicles which did not appear to be involved in transendothelial transport from blood to brain. This view was confirmed by Brightman & Reese (1969) who showed that endothelial and epithelial tight junctions occluded the spaces between blood and brain parenchyma or cerebral ventricles, thereby constituting a structural basis for the blood–brain and blood–cerebrospinal fluid barriers.

It is therefore justifiable to assume that the 'leaky' BBB in the fetus or newborn may be due to poorly formed or incomplete tight junctions. Recently, ionic lanthanum has been used successfully in a number of investigations as a vascular probe to study endothelial blood–brain and blood–nerve barriers based on its high permeability, small size (atomic size = 0.114 nm) and nontoxic properties (Bouldin & Krigman, 1975; Bundgaard, 1982; Shaklai & Tavassoli, 1982; Mackenzie et al. 1987; Chau et al. 1991; Chien et al. 1991). Using lanthanum as an ultrastructural tracer, tight junctions were divided into 2 types, 'leaky' and 'tight', depending on their permeability to La^{3+} (Machen et al. 1972; Bouldin & Krigman, 1975). In the present study, the lanthanum did not penetrate the junctions of the endothelial cells of the callosal capillaries either in 1 or 14 d rats. The failure of lanthanum to penetrate these intercellular spaces was clearly due to the tight junctions. Although lanthanum precipitate permeated the intercellular clefts and sometimes filled almost the entire length of the intercellular junctions in 1 d rats, it was never observed in the abluminal side of the endothelial cells. The deeper penetration of lanthanum into the intercellular spaces in 1 d rats when compared with the 14 d animals suggests that at least in some segments along the capillaries, the tight junctions were not as fully developed as in the older rats. This would imply that the apposed plasma membranes of the endothelial cells had not formed a tight seal. On the other hand, since the lanthanum infused was never observed in the abluminal side of the endothelial cells in 1 d rats, it seems likely that some parts of the tight junctions had fully developed locally being capable of preventing any extravasation of tracer via the intercellular spaces. This finding is consistent with the observations of Delorme et al. (1970) and Møllgard & Saunders (1975) who showed that the tight junctions of the cerebral endothelial cells began to differentiate and became tighter during embryonic development. Results from the present study therefore exclude the possibility of the tight junction as a barrier factor to account for the difference in permeability to exogenous materials in 1 and 14 d rats (Xu et al. 1993).

It is unequivocal from this study that ferritin injected intravenously readily passed through the endothelial cells by transendothelial transport in 1 to 7 d rats. Such a process, however, was clearly impeded in 14 d rats. In 1 to 7 d rats, the injected ferritin was localised in the invaginations of the luminal plasma membrane, pinocytotic vesicles, multivesicular bodies and on the abluminal sides of the endothelium, either in the basal lamina, pericapillary spaces, pericytes or amoeboid microglial cells. This indicates that ferritin given intravascularly is transported across the endothelial cells by transcytosis. It is suggested that the ferritin which binds to the luminal plasma membrane

Fig. 15. Portion of an amoeboid microglial cell some distance from a capillary in a 1 d rat, 6 h after ferritin injection. A cluster of ferritin particles taken up by the cell is present near the cell surface (arrow). nf, nerve fibres; Ly, lysosomes. Bar, 0.1 μm.

Fig. 16. Portion of an amoeboid microglial cell in the corpus callosum in a 5 d rat, 30 min after ferritin injection. The ferritin (asterisk) is deeply internalised among the lysosomes (Ly). Bar, 0.1 μm.

Fig. 17. Portion of a callosal capillary in a 14 d rat, 3 h following ferritin injection. The injected ferritin is seen in the cytoplasm of the endothelial cell (arrow) but not in the abluminal side. AS, astrocytic end-feet. Bar, 0.2 μm.

is internalised and sequestered in either the pinocytotic vesicles or multivesicular bodies. These would then move across the endothelial cytoplasm where they will be exocytosed by the abluminal cytoplasmic membrane. In 14 d rats, only a trace amount of ferritin was found in the endothelial cells of the callosal capillaries and none on the abluminal side of the endothelium. It is therefore speculated that the endothelial cells in the 14 d rats became functionally tightened thereby preventing the entry of exogenous substances into nervous tissue. It is significant that the greater number of pinocytotic vesicles in the endothelial cells in 1 and 7 d rats when compared with that in 14 d rats, since the number of endothelial vesicles has been shown to be correlated with vessel permeability (Reese & Karnovsky, 1967; Brightman, 1977; Oldendorf, 1977). Considering the fact that the tight junctions in the callosal capillaries of 1 and 7 d rats have developed fully in function, the difference in permeability to exogenous materials in rats of different ages could be attributed to the different activities of transcytosis in the endothelium. In other words, the endocytosis of the endothelial cells of the callosal capillaries in 1 and 7 d rats is active as shown by the number of pinocytotic vesicles. It becomes attenuated with reduced transendothelial transport of exogenous substances in 14 d or older rats. This view is compatible with the observations by Lossinsky et al. (1986) who demonstrated that massive leakage of injected HRP across microvessels to the brain neuropil could be observed in 1 and 4–7 d mice but appeared to diminish between 12 and 14 d after birth. Vorbrodt et al. (1986) showed that alkaline phosphatase activity was detectable in the luminal plasma membrane of the endothelial cells of brain capillaries between the 12th and 24th day of life in the mouse, which coincided with the loss of permeability of the blood vessels to i.v. injected HRP. The authors therefore believed that the development of BBB function is accompanied by the formation of an enzymatic barrier in the endothelium of blood vessels. Zanetta et al. (1985), using 2 specific antibodies of 2 membrane antigens characteristic of mature endothelial cells, showed that the maturation of endothelial cells in the rat cerebellum was at the 13th to 18th postnatal days. The results of the present study indicate that a gradual maturation of the BBB at the level of endothelium in the corpus callosum led to the reduction of the transvascular transport of ferritin in the course of development from 1 to 14 d rats.

The present study has also shown that pericytes are capable of endocytosing ferritin across the endothelial cells. Following the uptake of the tracer, the phagocytic pericytes retained their intimate spatial association with the vessel walls. It is suggested that pericytes which function as phagocytes also constitute a functional component of the BBB. This view is in line with the studies by Cancilla et al. (1972) and Van Deurs (1976) in mouse and rat brain respectively that pericytes actively endocytose HRP extravasated following the disruption of the BBB. The fate of the endocytosed ferritin by pericytes in our present study is uncertain. One possibility is that it could be digested by the lysosomal enzymes in the pericytes.

The present investigation has also shown the close association of the phagocytic amoeboid microglial cells (Ling, 1981; Ling & Wong, 1993) with the outer walls of the callosal blood vessels in 1 and 7 d rats. These cells were capable of picking up ferritin introduced in the blood circulation. This, taken together with our earlier studies (Leong et al. 1983; Kaur et al. 1986), therefore indicates that amoeboid microglial cells serve as an effective barrier system to remove any serum-derived foreign substances which may have entered the pericapillary space by transendothelial transport at a time when the BBB is not well established. With the full development of the BBB in advancing age, the phagocytic function of the amoeboid microglial cells is no longer necessary and they therefore regress to become the inactive form, namely resting or ramified microglia (Kaur et al. 1984). The fact that the functional maturation of the BBB coincides with the transformation of amoeboid microglial cells into resting or ramified microglial cells between 13 and 15 d of age in the rat brain (Wu et al. 1993) strongly supports this view. The fate of the ferritin ingested by the amoeboid microglial cells is unclear, although it is likely that the ferritin will be incorporated into the abundant lysosomes present in the cells.

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