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Ultrastructural characteristics of blood vessels in the infant and adult human cerebral cortex

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Summary. Blood vessels in frontal and temporal cerebral cortex of adults and two infants aged 5 months and 5 years were studied by electron microscopy. The cells outside the endothelium were classified on their ultrastructural characteristics. Fibroblasts had prominent rough endoplasmic reticulum and few mitochondria in the cytoplasm. They were different from pericytes, which contained a prominent Golgi apparatus but only a few, isolated profiles of rough endoplasmic reticulum. Smooth muscle cells were distinguished from fibroblasts and pericytes by the presence of filaments and caveolae. Perivascular cells were characterised by the presence of lysosomes and granules of different sizes and electron densities, and were present at all ages studied. Plasma cells had abundant rough endoplasmic reticulum in the cytoplasm, and were present only in the 5-month-old infant cortex. Cortical vessel diameter increased with age.

Key words: Capillaries, Pericytes, Perivascular cells, Endothelium

Introduction

Blood vessels in human brain have been studied by electron microscopy, but mostly in foetuses (Dahl, 1963; Pappas and Purpura, 1964; Bauer and Vester, 1970; Hauw et al., 1975; Allsopp and Gamble, 1979) or in aging brain (Stewart et al., 1987). Little is known about the classification or ultrastructural features of cellular components of human cortical blood vessels, although some information is available for subprimate species (Stensaas, 1975; Kida et al., 1993). The present study was therefore carried out to elucidate the ultrastructural features of immature and adult human cortical blood vessels, known to be of importance in maintaining the blood-brain barrier (Cancilla et al., 1972; Dermietzel and Krause, 1991).

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Materials and methods

Specimens and fixation

Three specimens of adult cerebral cortex and two from infants were obtained during neurosurgical operations in which removal of normal tissue was a necessary part of the procedure. The first specimen was obtained from the left frontal cortex (area 8 or 9 of Brodmann, 1909), during removal of a deep frontal lobe tumour in a 60-year-old male. The cortex appeared normal and well vascularised at operation. The second specimen was from a case of left frontal lobe meningioma in a 60-year-old woman and consisted of a block of normal frontal cortex beyond the margin of the tumour. Due to the absence of clear anatomical landmarks, the exact area where this biopsy was taken could not be determined. The third specimen was from the right middle temporal gyrus (area 21), excised during surgery to remove a right temporal lobe glioma in a 55-year-old male. The fourth specimen was obtained from a male infant aged 5 months with a frontoparietal porencephalic cyst. The specimen included the cyst wall and a strip of adjacent normal cortex from areas 9 and 44. The fifth consisted of a block of right area 21, excised during surgery to remove a right temporal lobe glioma in a 5-year-old male. At operation, the cortex appeared normal and without oedema. The specimens were fixed in a solution of 2% paraformaldehyde (PF) and 3% glutaraldehyde (GA) in 0.1M phosphate buffer within seconds of removal and then dissected into smaller blocks. They were divided into even smaller blocks, approximately 1 mm by 2 mm, one hour later. After osmification, they were dehydrated in ethanol, embedded in Araldite and sectioned perpendicularly to the pia.

Sections for electron microscopy

Semithin (1μm) sections were first obtained for general survey. They helped estimate quality of fixation and absence of oedema. Blocks were trimmed such that the entire thickness of cortex was included in a section.

Thin sections were prepared, mounted on copper grids coated with Formvar, and counterstained with uranyl acetate and lead citrate. They were viewed in a Philips 400T or a Jeol 1200EX electron microscope.

Quantitative study

Micrographs were made of cross sections of blood vessels encountered at electron microscopy. We selected vessels for quantitative study if the membranes of their component cells were sharp around the entire circumference of the vessel, and if the processes of the endothelial cells were of fairly uniform thickness around the vessels. A total of 360 micrographs were used for the quantitative study, approximately 120 for each age group (5 months, 5 years and 55-60 years).

The cross-sectional area of the vessel lumen was measured, using an Intergraph Workstation (Interpro 2700), and its diameter calculated according to the formula: $D=2(A/\pi)^{1/2}$ (D=diameter, A=area of profile; Heinsen and Heinsen, 1983). The number of profiles of vessels within a particular range of diameters was expressed as a percentage of the total number of vessels encountered in each age group. A mean value was also calculated for all vessels in each age group. The number of profiles of vessels that contained nuclei of each constituent cell type was counted and expressed as a percentage of the total number of vessels encountered in each age group.

Another 200 micrographs were made of profiles of vessels which showed nuclei and sharp nuclear membranes of the vessel wall cells (approximately 15 micrographs for each type of vessel wall cell). The range of diameter of vessels that contained nuclei of each type of vessel wall cell was tabulated for each age group.

Results

1. General features of blood vessels in infant and adult cortex

Arterioles, venules and capillaries could be distinguished on grounds of size and cellular content.

Capillaries were identified by their single layer of endothelial cells and lack of smooth muscle, and measured from 0.4 to 9.5 μm in diameter. They did, however, have pericytes, perivascular cells and fibroblasts in their walls, and, in the 5-month-old infant, plasma cells. Collagen fibrils were sometimes present outside the endothelial cells. Capillaries with a slit-like lumen were occasionally observed in the infants, but not in the adults.

Arterioles were 9.5-15 μm in diameter. Their tunica intima consisted of a layer of endothelial cells and overlying basal lamina, which was the most obvious feature distinguishing arterioles from venules, that of arterioles consisting of thick electron-dense bands surrounding endothelial and other cells, while that of venules was thinner. The tunica media contained one or two layers of smooth muscle cells, and longitudinal collagen fibres were present in the adventitia. Arteriolar walls also contained perivascular cells. Venules measured 8-20 μm in diameter and contained a discontinuous layer of smooth muscle cells in their tunica media and collagen fibrils in the adventitia. They, too, had perivascular cells.

Perivascular cells were enclosed by basal laminae, and were distinguished from microglia which were outside the vessels and not enclosed by basal laminae. Nerve endings were not observed in the vessel walls. Astrocytic end feet were often found, completely or incompletely surrounding the vessels.

2. Cells in vessel walls of adults

Vessels in adult cortex were surrounded by well-defined basal laminae. Vacuoles containing degenerating cellular structures were often observed in cells of vessel walls (Fig. 1A). Cytoplasmic interdigitations were rarely observed between endothelial cells and other cellular components. Many collagen fibres were present in the basal lamina.

a) Endothelial cells (Fig. 1A)

Small amounts of heterochromatin were present in the nucleus of endothelial cells. The cytoplasm contained a few free ribosomes, light mitochondria and profiles of rough endoplasmic reticulum with long and stringy or distended cisternae and few attached ribosomes. Large numbers of vesicles, vacuoles, lipid droplets and cellular debris were present in the cytoplasm. «Seamless vessels» with no junction throughout the entire circumference were also observed. Endothelial cells were separated from the other cellular components of the vessel walls by a layer of basal lamina, except in certain places where they were separated by only a narrow (15-17 nm) gap junction.

b) Pericytes (Fig. 1B)

These had round or elongated outlines. The nucleus was regular and contained dense heterochromatin. A few free ribosomes and many light mitochondria were present in the cytoplasm. The rough endoplasmic reticulum formed short, isolated profiles with dilated cisternae, at the periphery of the cells. The Golgi apparatus was prominent and contained many delicate saccules and short membranous cisternae. Centrioles and lipid droplets were frequently observed.

c) Fibroblasts (Fig. 2A)

Fibroblasts were spindle-shaped. The nucleus was regular, except for occasional small indentations, with dense heterochromatin clumps. The cytoplasm was characterised by a few free ribosomes and light mitochondria, and prominent, parallel stacks of rough

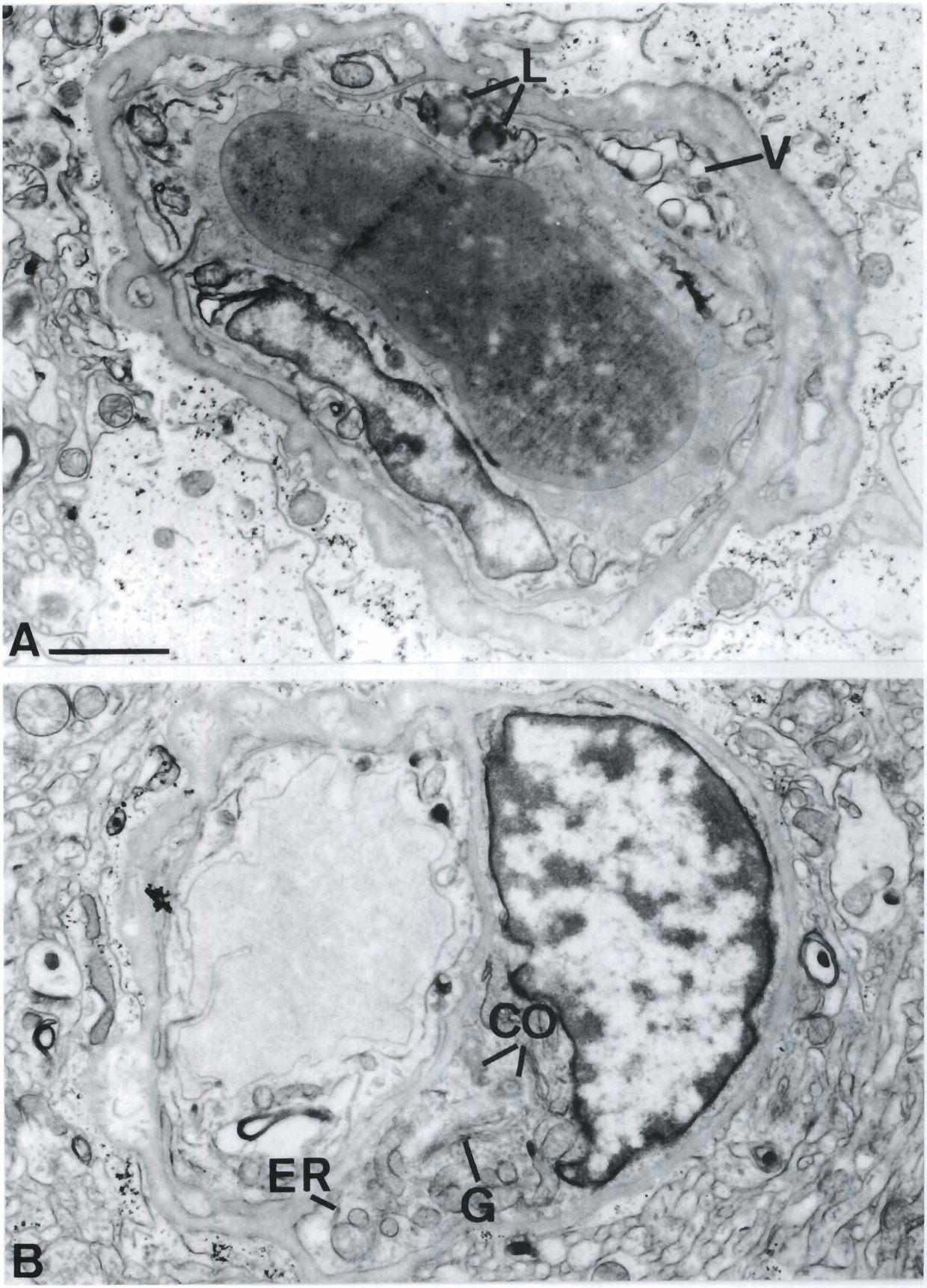


Fig. 1. A. Endothelial cell in a 60-year-old adult. Lipid droplets (L) are observed, and a vacuole (V) containing degenerating cellular structures is present in the wall of the vessel. Scale bar: 1.3 µm. **B.** Pericyte in a 60-year-old adult. It contains a prominent Golgi apparatus (G), and short, isolated profiles of rough endoplasmic reticulum (ER). Centrioles (CO) are frequently observed. Scale bar: 1 µm.

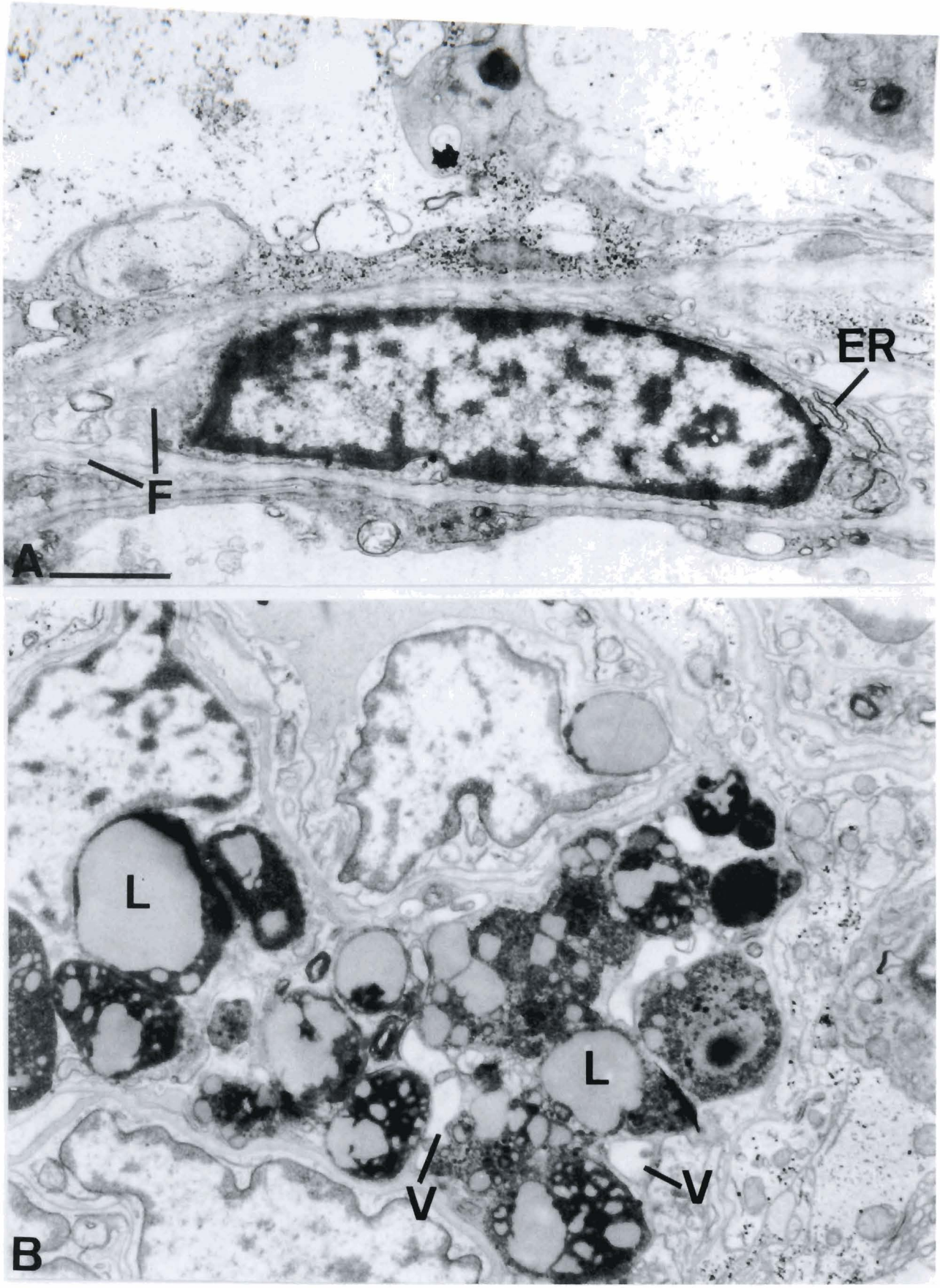


Fig. 2. A. Fibroblast in a 60-year-old adult. It contains prominent profiles of rough endoplasmic reticulum (ER). Collagen-like fibrils (F) are also present near the cell surface. Scale bar: 0.9 µm. **B.** Perivascular cell in a 60-year-old adult. Vacuoles (V), and secondary lysosomes (L), consisting of a thin rim of dense material around a less dense, central zone, are present. Scale bar: 1.6 µm.

Table 1. Proportion of sectioned vessels of various diameter ranges expressed as a percentage of the total number of vessels encountered in each age group.

AGE	DIAMETER RANGE (μm)								
	<1	1-1.99	2-2.99	3-3.99	4-4.99	5-5.99	6-6.99	7-7.99	>8
Adult	0	1	18.1	34.3	19	10.5	9.5	1.9	5.7
5 years	1.9	9.5	30.5	32.4	19	1.9	2.9	0	1.9
5 months	4.1	21.4	29.6	22.4	9.2	8.2	4.1	0	1

Table 2. Proportion of sectioned vessels containing nuclei of various vessel wall cells expressed as a percentage of the total number of vessels encountered in each age group.

AGE	TYPE OF CELL					
	Endothelial cells	Pericytes	Fibroblasts	Smooth muscle cells	Plasma cells	Perivascular cells
Adult	31	7	7	1	0	2
5 years	47	10	20	6	0	14
5 months	63	7	20	1	1	5

endoplasmic reticulum with wide cisternae, and few attached ribosomes. Vesicles, lipid droplets and lysosomes were occasionally present, and collagen fibrils sometimes appeared near the cell surface.

d) Smooth muscle cells

These cells had elongated outlines. The nucleus contained dense heterochromatin clumps and a prominent nucleolus. The cytoplasm contained a few free ribosomes, mitochondria, caveolae and rough endoplasmic reticulum and large numbers of filaments.

e) Perivascular cells (Fig. 2B)

Perivascular cells were irregular. The nucleus contained small amounts of heterochromatin. There were few free ribosomes and light mitochondria in the cytoplasm, and little rough endoplasmic reticulum; however, many vacuoles and secondary lysosomes, consisting of a thin rim of dense material and a less dense central zone, were present.

3. Cells in vessel walls of the 5-year-old infant

Many of the features of the vessel wall cells in the adult were also present in the 5-year-old infant, and only the differences are highlighted below. Endothelial cells (Fig. 3A) contained many more profiles of rough endoplasmic reticulum, light mitochondria, vesicles in actual contact with the cell membrane (plasmalemmal vesicles), glycogen granules and Weibel-Palade bodies (Weibel and Palade, 1964), but only few vacuoles compared with the adult. Pericytes (Fig. 3B) were also different in that fewer heterochromatin clumps were present in the nucleus, while fewer centrioles and lipid droplets, but many more plasmalemmal vesicles were present in the cytoplasm. Fewer filaments and caveolae were present in smooth muscle cells (Fig. 4A) while the perivascular cells (Fig. 4B) contained many more long, stringy profiles of rough endoplasmic reticulum, primary lysosomes, lipid droplets, and Weibel-Palade bodies, but fewer secondary lysosomes and vacuoles than in the adult.

4. Cells in vessel walls of the 5-month-old infant

There were many differences in the 5-month-old infant compared with the adult and the older infant. The basal laminae of vessels were different in that they were often ill-defined, of irregular density and thickness, and contained fewer collagen fibres. Endothelial cells (Fig. 5A) were different in that the nucleus was more irregular in outline with denser heterochromatin clumps. The cytoplasm contained many prominent microvilli on the luminal surface of the cells, and many interdigitating processes from overlying cells. Transverse sections of such cytoplasmic interdigitations were often observed in endothelial cells, but rarely in other cellular components. Many more free ribosomes, but only infrequent centrioles, were present in the pericytes (Fig. 5B) compared to those at older ages, while fibroblasts (Fig. 6A) were quite similar. Smooth muscle cells (Fig. 6B) were different from those in older specimens in that few filaments but large numbers of caveolae were observed. Plasma cells (Fig. 7A) were only observed at this age. They were rounded, but sometimes bore projections on the abluminal surface. The nucleus was deeply folded, with dense heterochromatin clumps. The rough endoplasmic reticulum was prominent in the form of long, parallel stacks at the origins of the cytoplasmic projections. Its cisternae were distended and contained a light floccular material. Large numbers of free ribosomes and a few mitochondria were also present. Perivascular cells (Fig. 7B) were different from those at older ages in having many more vesicles, but only a few lysosomes.

Table 3. Relationship between vessel wall cell type and vessel diameter. The range of vessel diameters (in μm) containing nuclei of each cell type is tabulated in each age group.

AGE	TYPE OF CELL				
	Pericytes	Fibroblasts	Smooth muscle cells	Plasma cells	Perivascular cells
Adult	2.4-7.9	3.2-8.7	10.5-14.6	-	3.4-14.8
5 years	1.1-7.4	1.9-7.0	9.5-10.9	-	1.5-10.9
5 months	1.7-7.0	1.0-5.9	10.8-14.6	1.6-2.4	2.9-10.9

Quantitative assessment of blood vessels

The percentages of cross-sectioned vessels having various diameter ranges are shown in Table 1. The mean vessel diameter in the adults was 4.4 μm, and slightly less in the infants (3.1-3.4 μm). The commonest nuclei seen in the walls were those of endothelial cells at all ages (Table 2). Pericytes and fibroblast nuclei were only found in vessels with diameters up to 8.7 μm, that is in capillaries. Smooth muscle cells were mainly found in large vessels (arterioles and venules), while perivascular cell nuclei were seen in all vessel types (Table 3).

Discussion

The present study describes the ultrastructural features of blood vessels in mature and immature human cerebral cortex. Apart from endothelial cells, a variety of other cell types are present.

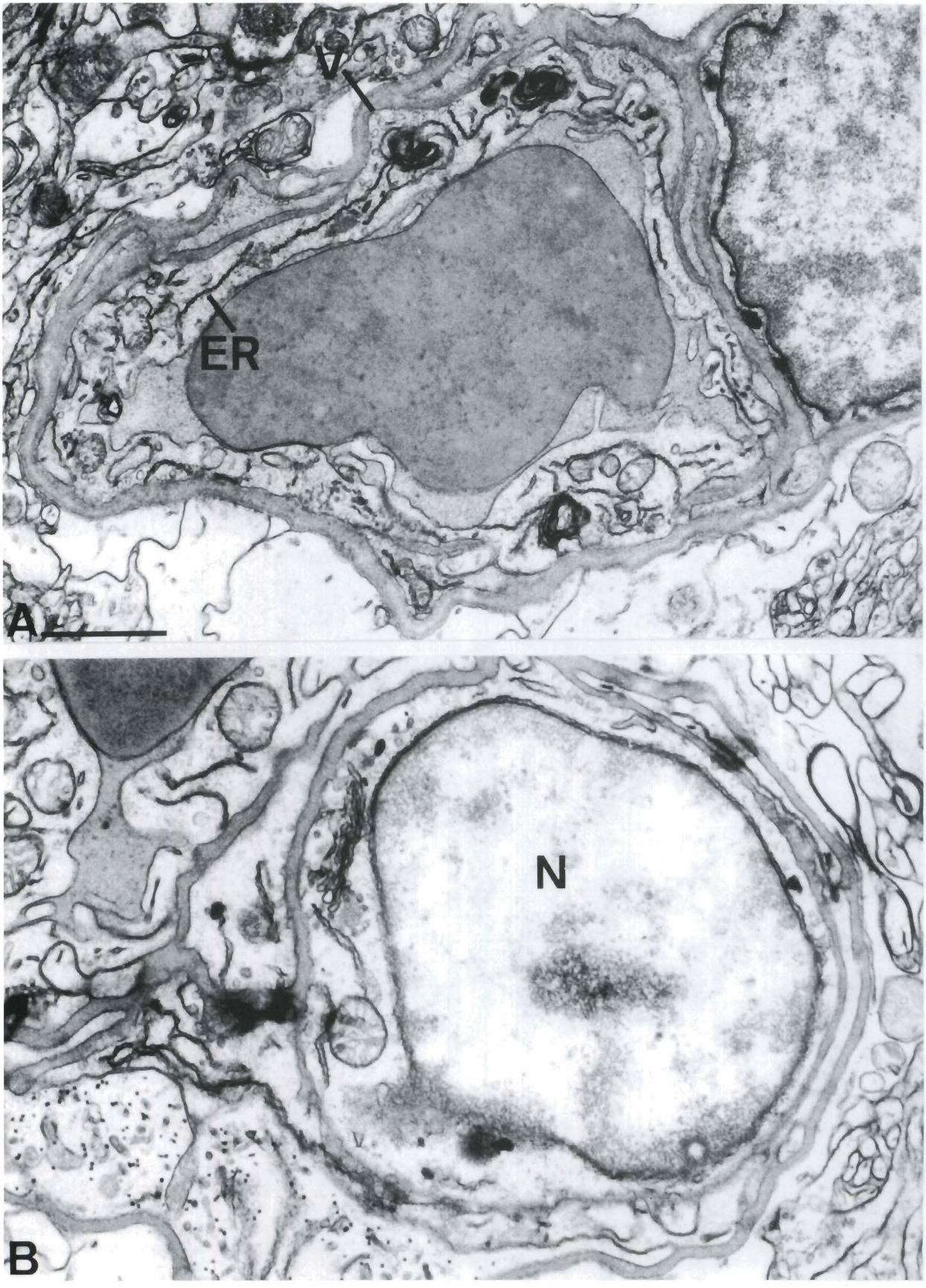
There is inconsistency in the use of the term «pericyte» in the literature (Farrell et al., 1987). Some authors make no distinction between different types of pericytes (Kristensson and Olsson, 1973; Le Beux and Willemot, 1980; Castejon, 1984). Others refer to two types, the first with numerous large, dense granules, which are autofluorescent, acid phosphatase positive, and periodic acid-Schiff positive (Jeynes, 1985), and the second without granules. The pericytes of the present study were characterised by the presence of prominent Golgi apparatus and few dense bodies or profiles of rough endoplasmic reticulum, and were similar to pericytes in rabbit basal forebrain (Stensaas, 1975) and rat cerebrum (Kida et al., 1993). Changes with age in the ultrastructure of pericytes include a reduction in the number of free ribosomes in the cytoplasm, and changes in the morphology of mitochondria and rough endoplasmic reticulum. More plasmalemmal vesicles were observed in the pericytes of the 5-year-old infant and the adult, compared to the 5-month-old infant. The function of brain pericytes is not known. Their ultrastructural features differ from those in other organs (Díaz-Flores et al., 1991) and they are more numerous, forming an almost complete investment of the endothelium in brain, whereas they are rare in other tissues such as muscle (Allsopp and Gamble, 1979). Brain pericytes also express certain proteins, such as a 140 KDa antigen, not present in pericytes of other organs (Krause et al., 1988). It has been suggested that brain pericytes play a part in the maintenance of the blood-brain barrier, since several authors have reported that they account for some of the gamma-glutamyltranspeptidase (GGTP) activity in brain capillaries (Rupnick et al., 1988; Méresse et al., 1989; Risau et al., 1992) and GGTP is a biochemical marker for the blood-brain barrier (Goldstein et al., 1975). Immunocytochemical study of rat, monkey and human cerebral cortex, however, has showed that whilst GGTP is present on the luminal surface of endothelial cells in rat cortical vessels, it is mainly present in astrocytic end-feet and not endothelial cells or pericytes in monkey and human cortex (personal observation).

Fibroblasts were characterised by the presence of prominent rough endoplasmic reticulum and few mitochondria. They often contained collagen fibrils in both adult and immature cortex. Fibroblasts may be responsible for the synthesis of basal lamina, extracellular fibrils and ground substance (Tilton, 1991).

Cells with filaments and caveolae, typical of smooth muscle, were observed in cortical vessels at all ages, but the number of filaments increased with age. Although Toda et al. (1980) reported that the rounded outline of smooth muscle cells became irregular with age, such was not observed in this study. The factors which result in contraction or relaxation of smooth muscle in cortical arterioles and venules are unclear. Nerve endings were not observed in the walls of vessels in the present study, but it is possible that axons terminating near the vessels play a role. Large numbers of nicotinamide adenine dinucleotide phosphate diaphorase-containing axons form a perivascular plexus in the human cortex (DeFelipe, 1993; Yan et al., 1996) and may cause contraction or relaxation of vascular smooth muscle by releasing nitric oxide.

Plasma cells were characterised by the presence of large amounts of rough endoplasmic reticulum, similar to those described in the perivascular compartment of central nervous tissue in multiple sclerosis (Prineas and Wright, 1978). These cells were only observed in the cortex of the 5-month-old infant. The reason for this is

Fig. 3. A. Endothelial cell in a 5-year-old infant. More rough endoplasmic reticulum (ER) and plasmalemmal vesicles (V) are present in the cytoplasm compared with the adult. Scale bar: 0.7 μm. **B.** Pericyte in a 5-year-old infant. The nucleus (N) contains fewer heterochromatin clumps than in the adult. Scale bar: 0.6 μm.


V
ER
A
N
B

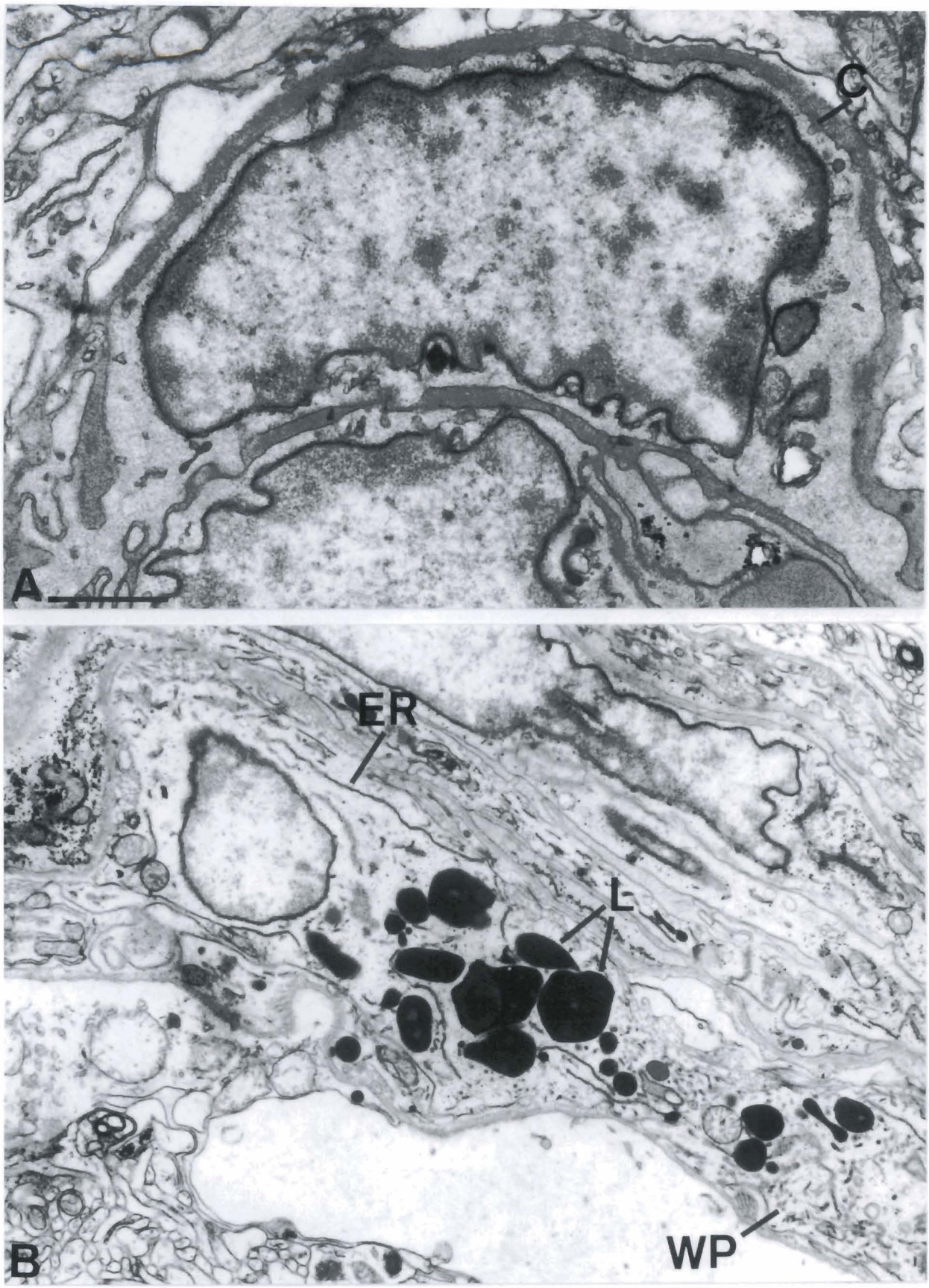


Fig. 4. A. Smooth muscle cell in a 5-year-old infant. In contrast to the adult, the cytoplasm contains fewer filaments and caveolae (C). Scale bar: 0.8 µm. **B.** Perivascular cell in a 5-year-old infant. Many more long, stringy profiles of rough endoplasmic reticulum (ER), lipid droplets (L) and Weibel-Palade bodies (WP) are observed, compared with the adult. Scale bar: 1.4 µm.

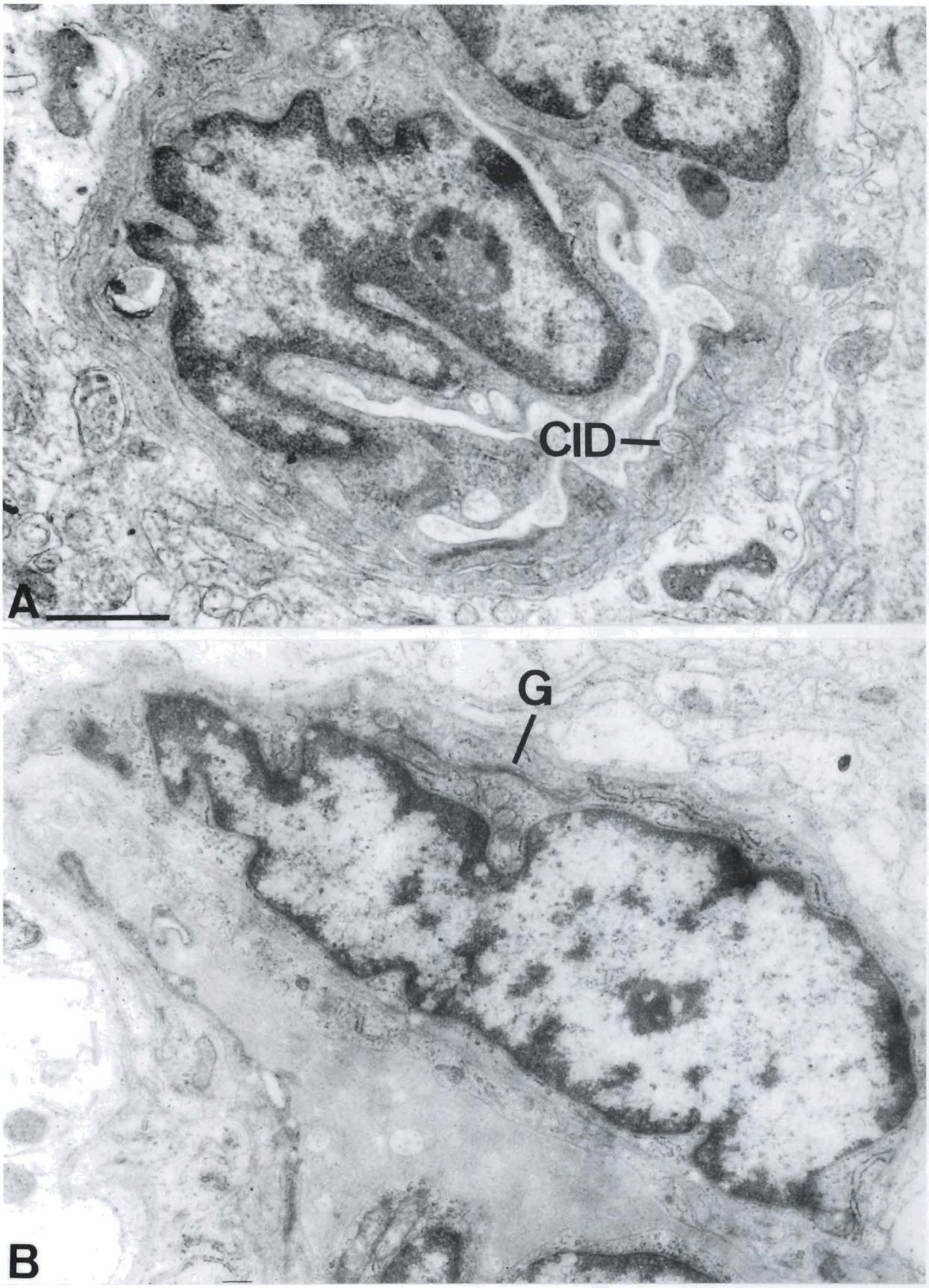


Fig. 5. A. Endothelial cell in a 5-month-old infant. There is a cytoplasmic interdigitation (CID). Prominent microvilli are present on the luminal surface. Scale bar: 0.7 µm. **B.** Pericyte in a 5-month-old infant. Many more free ribosomes are visible than at older ages. Note the prominent Golgi apparatus (G). Scale bar: 1.1 µm.

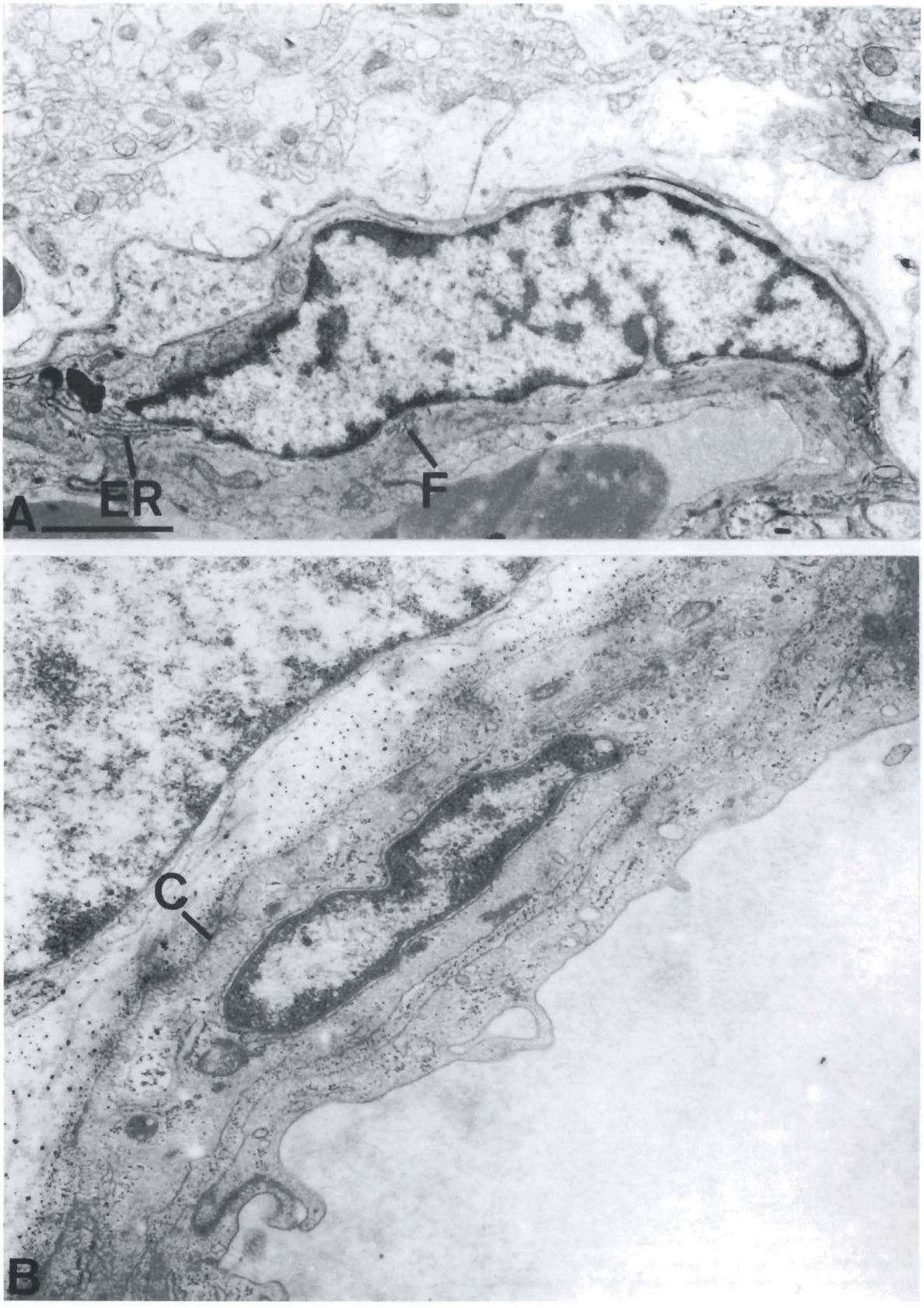


Fig. 6. A. Fibroblast in a 5-month-old infant. It resembles those at other ages in that the cytoplasm contains parallel stacks of rough endoplasmic reticulum (ER). Collagen-like fibrils (F) are also present near the surface of the cell. Scale bar: 1.6 µm.
B. Smooth muscle cell in a 5-month-old infant. Many caveolae (C) are present on the abluminal surface of the cell. Scale bar: 1 µm.

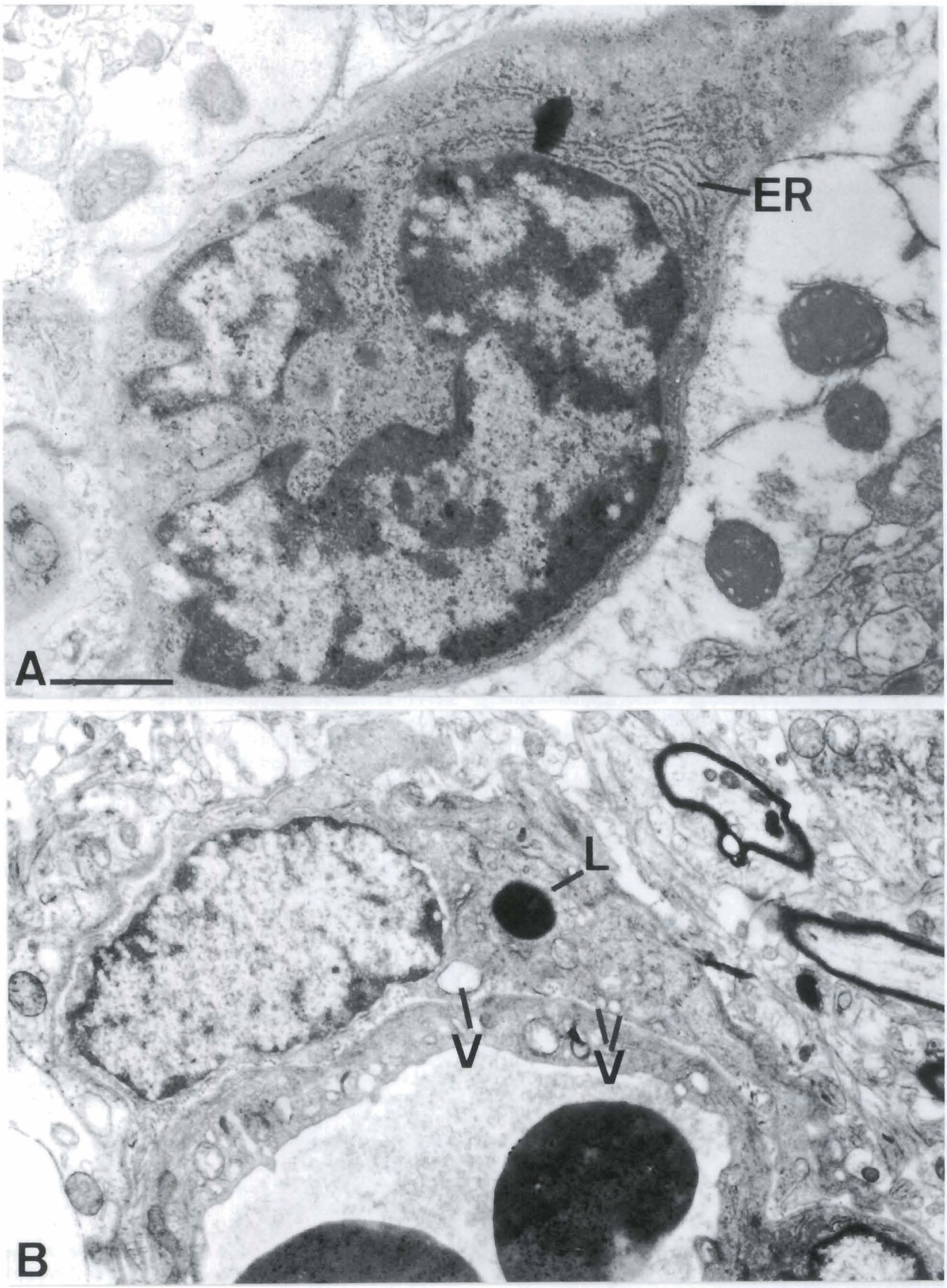


Fig. 7. A. Plasma cell in a 5-month-old infant. The nucleus is rounded. The rough endoplasmic reticulum (ER) is prominent, and forms long, parallel stacks at the origins of the cytoplasmic processes. Scale bar: 1 µm. **B.** Perivascular cell in a 5-month-old infant. Many vesicles (V) and a lysosome (L) are present in the cytoplasm. Scale bar: 1.1 µm.

unknown, but they may pass more easily from bloodstream to brain in the early postnatal period and play some role in the immune response of the neonatal brain.

Perivascular cells were characterised by the presence of lysosomes and granules of varying electron densities and sizes, similar to those previously described in the rat (Kida et al., 1993) and human (Graeber et al., 1992; Ong et al., 1995) cerebral cortex. They were also similar to «granular cells» described by Tagami et al. (1990). Developmental changes in the structure of perivascular cells included a decrease in the amount of heterochromatin, and a change in morphology of rough endoplasmic reticulum from short, dilated cisternae to long, stringy ones. More vacuoles and secondary lysosomes were also observed with advancing age. These findings are consistent with previous observations of larger numbers of vacuoles, and a greater variability in size and stainability of intracellular granules in perivascular cells in older individuals (Mato and Ookawara, 1981). Perivascular cells can be identified by immunocytochemistry, using antibodies to MHC class II antigens (Graeber et al., 1992; Kida et al., 1993; Ong et al., 1995). These cells are capable of presenting antigens to T lymphocytes during immunopathological processes in the central nervous system, such as experimental allergic encephalomyelitis, an animal model of multiple sclerosis (Hickey and Kimura, 1988), and are thought to transform into brain macrophages under experimental conditions (Raedler and Raedler, 1984).

Seamless vessels, with no junction throughout their entire circumference, were observed at all ages in human cerebral cortex. They may represent tangential sections through the tips of capillary loops, or may represent endothelial cells perforated by the lumen. They were also observed in developing and adult rat cortex, but were especially common at 9-20 postnatal days (Wolff and Bär, 1972). Since this age coincides with a period of intensive vascularisation, the presence of seamless vessels is thought to be an indication of increasing arborisation in the capillary network (Wolff and Bär, 1972).

Cellular debris in vacuoles was common in the walls of cortical vessels in the adults, as has been demonstrated in the walls of human retinal vessels (Hogan and Feeney, 1963; Spitznas, 1974). The significance of these vacuoles is unclear. Cytoplasmic interdigitations, described in immature capillaries of human granulation tissue (Wakui et al., 1989), were commoner in endothelial cells than in other vessel cells in the human cortex.

There was no significant difference between the mean luminal diameters of vessels of the 5-month-old and 5-year-old infants ($p>0.05$), although they were significantly larger in the adults ($p<0.01$), in agreement with Hunziker et al. (1978).

Endothelial cells were the commonest cell type in the vessel wall, and their nuclei were present in 31-63% of cross-sectioned profiles of vessels. The next most frequent cell type was the fibroblast. The percentage of vessel profiles containing nuclei of fibroblasts in the adults (7%) was significantly less ($p<0.01$) than that in the infants (20%). The nuclei of pericytes were present in 7-10% of vessel profiles, comparable to previous reports in human brain (Stewart et al., 1994). No significant difference was noted in the percentage of vessel profiles containing nuclei of pericytes, between the different age groups ($p>0.05$).

The developmental changes of endothelial cells included a decrease in the amount of heterochromatin in the nucleus, and fewer free ribosomes in the cytoplasm. The mitochondria contained closely packed cristae and a dense matrix in the 5-month-old infant, but a light matrix in the 5-year-old infant and the adult. The cisternae of rough endoplasmic reticulum were widely distended in the 5-month-old infant, but were sometimes long and stringy in the 5-year-old infant and the adult. Fewer free ribosomes were present on the rough endoplasmic reticulum, and more plasmalemmal vesicles and Weibel-Palade bodies (Weibel and Palade, 1964) that have been linked to endothelial thrombocytoplastic and clotting activity (Burri and Weibel, 1968) were observed in the 5-year-old infant and the adult, compared to the 5-month-old infant.

This study is the first to provide detailed ultrastructural analysis of the various cell types forming walls of arterioles, venules and capillaries in infant and adult cerebral cortex and to correlate this with luminal diameters of the vessels. The data should be useful in providing a baseline for analysis of pathological conditions affecting cerebral cortical vessels.

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