

Central nervous system microangiarchitecture in the human foetus

Riccardo Arisio¹, Mariagrazia Bonissone³, Ettore Piccoli², Giancarlo Panzica³

Summary

It is thought that arterioles penetrating the central nervous system behave as terminal arteries and lack for anastomosis.

The purpose of our study was to define the angiogenesis in the fetal encephalon at different stages of development. To this purpose, we examined 13 fetal and newborn encephalons between the 10th and 33rd week. To label blood vessels, we used an immunohistochemical procedure based on the detection of two antigens located within endothelial cells: CD31 and CD34.

The cerebral vascularization modifies in quantity and in structure during pregnancy, with important topographic differences between cerebral cortex and striatal-limbic areas.

We observed two microarchitectural patterns:

1. Rectangular mesh pattern, characterized by capillaries that join transversally to one or more branches that deepen orthogonally from the surface of the meninges;
2. Hexagonal mesh pattern, which surrounds small groups of neurons and develops with a honeycomb shape. The rectangular mesh pattern is mostly observed from the 13th to 26th week in the white matter, in the hippocampus and in the cortex. The hexagonal mesh pattern is typical of the basal nuclei, and of the cerebral cortex during the 10th-12th week and after the 26th-27th week.

Until the 26th week the vascularization increases mainly in the hippocampus and in the basal nuclei. The cortex shows a vascularization increment, greater than in the limbic system, with a pattern prevalently hexagonal in areas where the neurons' number increases.

Our data demonstrate that, in the human fetus, cerebral capillaries are not of terminal type. On the contrary, they show a rich anastomotic network that has different patterns in white matter (rectangular pattern) or in grey matter (hexagonal pattern). The functional meaning of this difference is unknown, but we can suppose that its role is to warrant availability of nutritional substances within regions where a high number of neurons is present. Recent findings in computational neuroanatomy show that computer simulated axonal symmetric bifurcation can generate a dendritic tree with close similarities with real observed vascular patterns in fetal cortex.

Key words: foetal brain, angiogenesis, vascular microarchitecture.

¹ Servizio di Anatomia e Istologia Patologica e Citodiagnostica – Ospedale Sant'Anna, Corso Spezia 60, 10136 Torino

² Dipartimento di Discipline Ginecologiche e Ostetriche dell' Università, Via Ventimiglia 3, 10136 Torino

³ Rita Levi Montalcini Center for Brain Repair. Dept. Anatomy, Pharmacology, and Forensic Medicine, Lab. Neuroendocrinology, Corso M. D'Azeglio 52, 10126 Torino

Correspondence:

Ettore Piccoli

Dipartimento di Discipline Ginecologiche e
Ostetriche dell'Università
Via Ventimiglia 3
10126 Torino, ITALY
E-mail: ettore.piccoli@unito.it

Introduction

Available data concerning the angioarchitecture of the foetal central nervous system are rare and contrasting. This is partly due to differences in experimental methods (injections of Indian ink, barium sulphate or silicon), but the main problem is represented by the difficulty of a clear identification of arteries and veins. Differences in sectioning methods (paraffin versus cryostate) and in section thickness may contribute to this last problem (1, 2, 3).

Duckett (2) analysed the vascularisation of twenty-five telencephalons from human foetuses between the 5th and the 18th week of life. He divided the development of blood vessels in two separate and successive phases: external and internal.

The external vascularisation depends on the vascular proliferation from the brain surface, the internal one starts with the penetration of blood vessels within the encephalic parenchima; it was, in turn, divided in four different steps. At the end of the 7th week (first stage) the vessels, coming from the brain surface, invade the telencephalic wall, they cross the thick cellular layer named cribrous layer of His and reach the *zona incerta*. During the 8th and 9th week (second stage) these vessels branch in a dycothomic way and the resulting vessels course parallel to the cortical and the ventricular surfaces. Among the 9th and 10th week (third stage) from the pial vessels rise secondary branches, they reach the **mantle layer** where they anastomose with the pre-existing vessels. During the 4th stage (11th-12th gestational week), the blood vessels of the **mantle layer** give off branches in the germinal layer. At this time, being the vascular architecture very close to the final situation, the telencephalic pallium increases in thickness.

De Reuck (4) examined six neonatal brains injecting a colloidal solution of barium sulphate. He recognised different patterns of cortical angioarchitecture referred to archi-, paleo-, and neocortex. A simple architecture characterises the archi- and paleocortex, whereas a more complex situation is present in the neocortex. At the surface of cerebral and cerebellar hemispheres the anastomoses are frequently observed, whereas the penetrating branches (running orthogonally into the cortex) give rise to terminal zones within each cortical layer. In the white

matter, terminal zones are located within the arcuate fibres as well as the periventricular zone (5). A developmental model for the rat telencephalic vascularisation has been proposed by Bär (6), on the basis of his results obtained after the injection of 72 rats with Indian ink. According to this study, during the development of neocortex the blood vessels penetrate radially and they grow according to a tridimensional pattern based on stacked hexagonal vascular units.

This model has been confirmed also for the human foetus after the 20th gestation week (Kuban and Gilles, 7) with the use of silicon as injection medium, and it is in agreement with the data reported by Kedzia (8) about foetal brain among 12th and 32nd week. Moreover, this last author reported that the subcortical and paraventricular zones are particularly ipovascularised. This seems to be important to explain the high perinatal incidence of para- and periventricular haemorrhages in these regions (4, 9). In fact, Van Den Bergh (9) hypothesised a vascularisation pattern based on a centripetal organisation: intracerebral arteries start from a peripheral net, penetrate perpendicularly within the parenchima, and converge radially towards the ventricle. Finally, the subependymal arteries originate centrifugal arteries; therefore, the subcortical and paraventricular zones may represent border-zones among the vascularising network of centripetal and centrifugal arteries. The existence of centrifugal arteries has not been confirmed in further studies (10, 11), whereas it has been demonstrated that hypovascularised periventricular regions have high incidence of leucomalacy. More recently, the cortical angiogenesis has been investigated with the use of immunochemical markers. Mito (12), with the use of antibodies against type IV collagen and fibronectin, demonstrated that the density and the diameter of blood vessels rapidly increase starting from the 26th gestation week, reaching a situation similar to that of the adult brain at the age of 35 weeks. Wierzbicka e Lewandowska (13) investigated the vascularisation of cerebral hemispheres in 20 foetuses between the 8th to 17th gestation week and demonstrate the sprouting of endothelial cells from pre-existing vessels in leptomeninges during the formation of a capillary network of the fetal human brain. Following studies (14, 15) detailed the expression of the Vascular Endothelial Growth Factor (VEGF) in the embryonic

brain in comparison to the adult, where the angiogenesis is virtually absent. Conversely, VEGF receptors (fit-1 and flk-1), endothelium-specific tyrosine kinase receptors, are highly expressed during the fetal life, whereas they are at minimal levels in the adult brain.

The aim of this study was to investigate the angiogenesis in the fetal encephalon during the period of the highest differentiation (from 10th to 33rd gestation week) by detecting blood vessels with the aid of immunohistochemical procedures to label two specific antigens of endothelial cells.

Materials and methods

We examined 15 foetal brains (among the 10th to 33rd gestation week) dissected from foetuses during postmortem examination. Autopsy was performed within 24 hours after death. The brains were fixed in 10% buffered formalin for two weeks and then were paraffin embedded; we examined a total of 48 macrosections: after standard stain with haematoxylin-eosin, consecutive sections were immunostained with monoclonal antibodies directed to endothelial antigens, anti CD31 (clone JC/70A, Dako) and anti-CD34 (clone QBEnd/10, Dako) in order to label blood vessels. Quantitative evaluation of vessel tree area was performed using the tools of Adobe Photoshop® software as previously reported (16); briefly, given a total frame area of 327680 pixels, optical density plots of immunostained cells were generated and the number of pixels was used as a measure of the area of the vascular tree. Data were analyzed for descriptive statistics with SPSS for Windows (rel. 8.0.0).

Results

The observation of the macrosections suggests that the cerebral vascularisation undergoes profound qualitative and quantitative changes during foetal development, with important topographical differences among limbic and cortical regions. We have observed two different microarchitectural patterns: a) a square pattern, characterised by transversally oriented capillaries linking two or three perpendicular branches derived from the pial surface; b) a hexagonal pattern around small groups of neurons, with a

typical honeycomb structure.

The square pattern has been mainly detected within the white matter (Figure 1), the cortex in the early stages (13rd-26th gestation week, Figure 2), and the limbic system (Figure 3). The hexagonal pattern has been detected in the basal ganglia, in the raphe medianus (Figure 4), in the cortex at early stages (10th-12nd gestation week, Figure 5) or at late stages (26th-27th gestation week, Figure 6).

We have identified two main developmental periods for angiogenesis: the first one from the 10th and the 26th gestation week, while the second one correspond to the following weeks.

Starting from the 12th gestation week in the limbic system the mean vascular tree area is 16071±4936 pixels; this value is 1.82 times greater than the one measured within the cortex (8785±2078 pixels). Up to the 24th week, the blood vessels of the white matter (square pattern) are prominent, whereas the number of cortical vessels (square pattern) is scarce. Basal ganglia and limbic system show, on the contrary, a large increase in the vascularisation.

Around the 26th-27th gestation week we have detected high vascularisation of the hippocampus (square pattern) as well as, even if to a lesser extent, in the cerebral cortex. The basal ganglia are characterised by the hexagonal pattern.

During the second period we observed an intense development of cortical vessels. Starting from the 28th week, the increase of the cortical surface is paralleled by an increase in the number, branches, length and size of blood vessels. The increase in cortical volume is even more enhanced from the 30th week. Also the vascularisation is largely increased within the neocortex, this increase is more pronounced than in the limbic system. The cortical pattern of distribution is mainly hexagonal, corresponding to the increase in the number of neurons.

Discussion

It is a general opinion that small arteries, that penetrate orthogonally within the brain, lack of reciprocal anastomosis and they are, for this reason, considered as terminal arteries. Anastomosis are present at the brain surface, but they behave as terminal arteries as soon as they penetrate the cerebral territories.

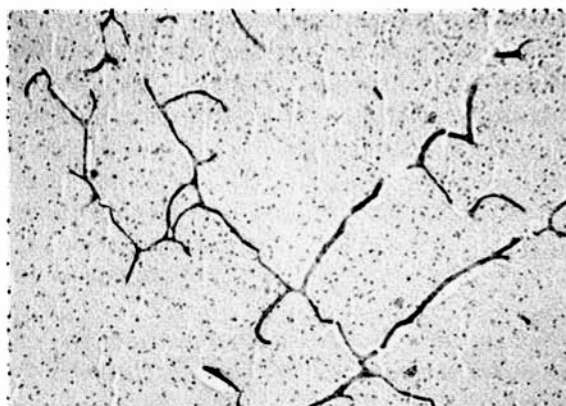


Figure 1.

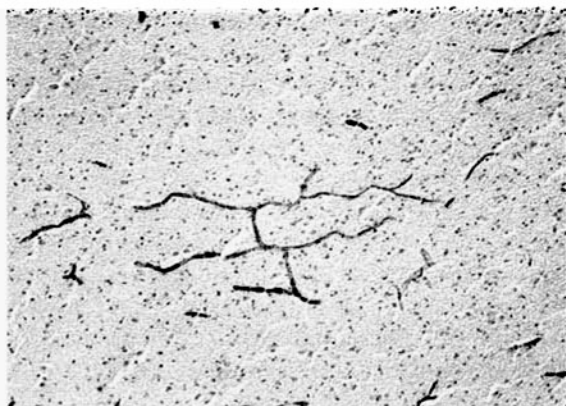


Figure 2.

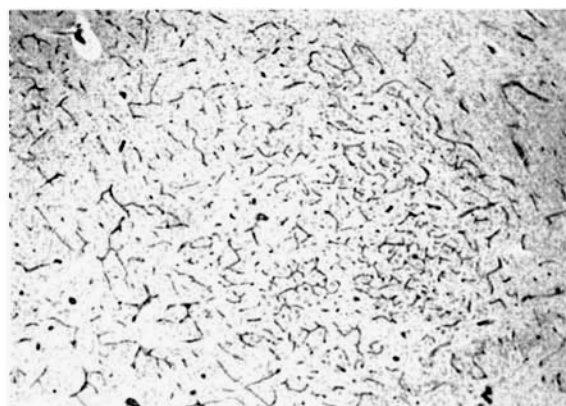


Figure 3.

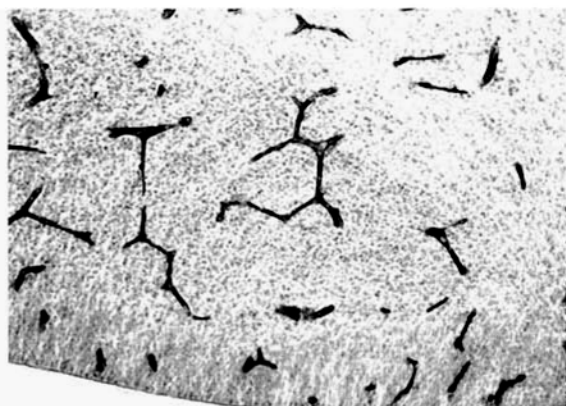


Figure 4.

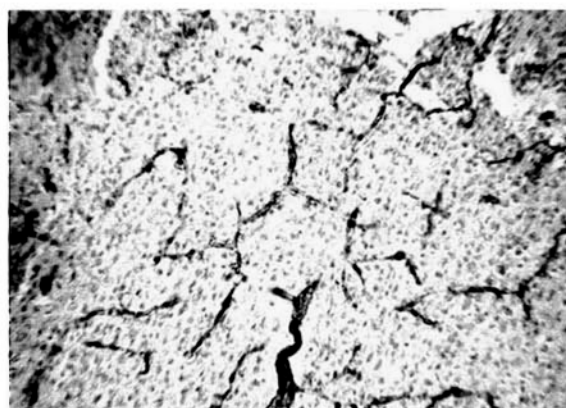


Figure 5.

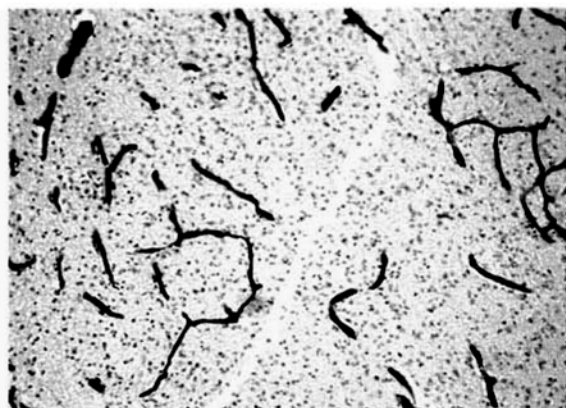


Figure 6.

Present results indicate that the cerebral capillaries of the human foetus are not of the terminal type, on the contrary, they merge in a wide anastomotic net. This net shows different patterns of organisation within the white (square pattern) or the grey matter (hexagonal pattern).

In the white matter, the blood vessels are mainly distributed along the fibres' trajectories, whereas in the regions characterised by a more active neuronal development the capillaries are organised in a more complex pattern similar to a honeycomb. The functional significance of these dif-

ferent patterns of distribution is not clear at this time. However, it is conceivable that the hexagonal pattern of distribution could guarantee a larger availability of nutritional substances in those regions where a greater number of nervous cells is present. A rectangular pattern implies more heterogeneous neurone-vessel distances and it may probably be insufficient to satisfy the nutritional requirements of delicate cells as the neurones.

Due to the lack of data on the distribution of blood vessels' markers in the adult brain it is not possible to know if anastomosis are present or not after birth. However, available data, reviewed in the introduction, strongly suggest the lack of them in the adult brain. If these data will be confirmed by the use of immunohistochemical markers, this would imply that after birth, a profound rearrangement of blood vessels' organisation should take place.

Recent data derived from computational neuroanatomy (17) provided models of neuronal growth (in particular of the dendritic architecture) (18). The algorithms used for the tridimensional reconstruction of the dendritic growth are based on stochastic rules and on microenvironmental constrictions that point on the effects of the local availability of metabolic resources, as well as on the presence of neurotrophic gradients. Among these models, the bifurcation angle of the axonal growth may vary from $90^{\circ} \pm 20^{\circ}$ for motoneurons, $142^{\circ} \pm 40^{\circ}$ for the Purkinje cells. In the published illustrations a clear hexagonal pattern is present. Therefore, we hypothesise that in the foetal cortex should act an angiogenetic factor addressing the development of the vascular net in a parallel way to the growth factor determining the development of the dendritic arborisation.

References

- 1) Padgett DH. The cranial venous system in man in reference to development of adult configuration and relation to arteries. *Am. J. Anat.* 1956;98: 317-330.
- 2) Duckett S. The establishment of internal vascularization in the human telencephalon. *Acta Anat.* 1971;80:107-113.
- 3) De Reuck J. The cortico-subcortical arterial angioarchitecture in the human brain. *Acta Neurol. Belg.* 1972;72:323-329.
- 4) De Reuck J. The significance of the arterial angioarchitecture in perinatal cerebral damage. *Acta Neurol. Belg.* 1977;77:65-94.
- 5) Mayer PL, Kier EL. The controversy of the periventricular white matter circulation: a review of the anatomic literature. *A.J.N.R.* 1991;12:223-238.
- 6) Bär T. The vascular system of the cerebral cortex. *Adv. Anat. Embryol. Cell Biol.* 1980;59:1-60.
- 7) Kuban KC, Gilles FH. Human telencephalic angiogenesis. *Ann. Neurol.* 1985;17:539-548.
- 8) Kedzia A. Evaluation of human telencephalon microangioarchitecture in fetal period. *Folia Neuropathol.* 1995;33,3:181-186.
- 9) Van Den Bergh R. Centrifugal elements in the vascular pattern of the deep intracerebral blood supply. *Angiology* 1969;20:88-93.
- 10) Takashima S, Mito T, Houdou S, Ando Y. Relationship between periventricular hemorrhage, leukomalacia and brainstem lesions in prematurely born infants *Brain Dev* 1989;11(2):121-4.
- 11) Nakamura Y, Okudera T, Hashimoto T. Vascular architecture in white matter of neonates: its relationship to periventricular leukomalacia. *J Neuropathol Exp Neurol* 1994 Nov;53(6):582-9.
- 12) Mito T, Konomi H, Houdou S, Takashima S. Immunohistochemical study of the vasculature in the developing brain. *Pediatr. Neurol.* 1991;7(1): 18-22.
- 13) Wierzba-Bobrowicz T, Lewandowska E. Morphological study of endothelial cells in the human fetus during early period of gestation. *Folia Neuropathol.* 1995; 33(4): 241-245.
- 14) Risau W. Development and differentiation of endothelium. *Kidney Int. Suppl.* 1998;67: 53-56.
- 15) Samoto K, Ikezaki K, Ono M, Shono T, Kohno K, Kuwano M, Fukui M. Expression of vascular endothelial growth factor and its possible relation with neovascularization in human brain tumors. *Cancer Res.* 1995;55(5): 1189-1193.
- 16) Lehr HA, Mankoff DA, Corwin D, Santeusanio G, Gown AM. Application of photoshop-based image analysis to quantification of hormone receptor expression in breast cancer. *J Histochem Cytochem* 1997 Nov;45(11):1559-65.
- 17) Ascoli GA, Krichmar L, Scorcioni R, Nasuto SJ, Senft SL. Computer generation and quantitative morphometric analysis of virtual neurons. *Anat Embryol* 2001;204:283-301.
- 18) <http://www.krasnow.gmu.edu/L-Neuron>