Some Observations on the Ultrastructure of Developing Rat Cerebral Capillaries

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Summary. Developing blood vessels in rat cerebral cortex were studied at a number of stages between 3 and 28 days postnatal, in an attempt to obtain data on the mechanisms by which the lumen is established within cords of mesodermal cells. A combination of techniques was utilized in an attempt to elucidate these mechanisms. These were: (a) aldehyde fixation and block staining with phosphotungstic acid; (b) aldehyde perfusion followed by perfusion of a lead solution and post-fixation in osmium tetroxide; (c) conventional preparation of tissue with aldehyde and osmium fixation.

Support for interendothelial lumen formation was readily forthcoming, including vessels with junctions between two or more endothelial cells cut transversely. There was some support for intraendothelial lumen formation, in the form of “seamless” endothelial cells. Other features noted included the presence of free ribosomes and vacuoles in the endothelial cells, endothelial flaps, sprouts and tendrils, intraluminal debris, endothelial degeneration and a junction with a nonendothelial cell.

Large numbers of endothelial vacuoles were noted, many of them occurring at the abluminal edge of the cells. These vacuoles may be involved in the formation of intraendothelial lumina and also in the enlargement of both types of lumina. This study provides evidence that besides the well-established interendothelial lumen formation, intraendothelial mechanisms may also be operative in rat cerebral cortex. The techniques employed in this study offer the potential for clarifying these and related issues.

Key words: Capillaries — Cerebral cortex — Endothelial cells — Intraendothelial lumen — Interendothelial lumen — Rat.

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Introduction

Growth of capillaries in the cerebral cortex takes place by the extension of a cord of mesodermal cells from existing functioning vessels (Klosovskii, 1963), while a period of intensive vascularization occurs in the rat brain shortly after birth (Caley and Maxwell, 1970; Hannah and Nathaniel, 1974). The patent lumina detectable by light microscopy have enabled a three fold increase in vessel numbers per area of tissue to be recorded between 9 and 15 days postnatal. The timing of this vascularization acquires significance on account of its temporal overlap with two other important developmental events, namely, the maturation of cortical electrical activity (Deza and Eidelberg, 1967) and the metabolic transition of the cortex from a glycolytic, ketone body utilizing organ to one dependent on oxidative metabolism (Mourek et al., 1968).

A number of ultrastructural changes have been recorded as brain (and spinal cord) capillaries develop. These include enlargement of the lumen, attenuation of the endothelial cytoplasm and a decrease in its electron-opacity, alterations in the quantity and distribution of the endoplasmic reticulum and ribosomes (Donahue and Pappas, 1961; Hannah and Nathaniel, 1974) and the establishment of a glial cell investment (Caley and Maxwell, 1970; Hannah and Nathaniel, 1974). In addition, the terminal bars of the endothelial cells decrease in prominence (Donahue and Pappas, 1961), while the nuclei of these cells undergo ultrastructural changes (Hannah and Nathaniel, 1974) and the basement membrane acquires a continuous form (Maynard et al., 1957; Donahue and Pappas, 1961; Caley and Maxwell, 1970; Bär and Wolff, 1972). In spite of the availability of such data, two important questions remain unanswered. These concern first, the stimulus which initiates capillary formation and opening and second, the mechanism by which the lumen is established within the cord of mesodermal cells.

Studies in non-neural tissues have led to the suggestion that lumen formation within a developing capillary sprout occurs by the enclosure of space between adjacent endothelial cells (Cliff, 1963). Subsequent perforation of a tissue septum may constitute the means of communication of the space with an already functioning lumen (Cliff, 1965).

The establishment of patency in pre-existing endothelial cords has also been suggested as a means of establishing the cerebral circulation (Caley and Maxwell, 1970), although the mechanism has not been elucidated. It seems likely that an interendothelial mechanism similar to that seen in other tissues may be occurring. Nevertheless, such a process will not account for the formation of lumina in the "seamless" endothelia that have been shown to exist in the rat (Wolff and Bär, 1972). These may be categorized as intraendothelial lumina.

In the present study we have considered the respective contributions of these two methods of capillary lumen formation in rat brain, as part of a more extensive re-examination of a number of the ultrastructural features of cerebral capillaries.

Materials and Methods

Cerebral cortical tissue from male rats was studied at days 3, 4, 5, 7, 8, 15, 20 and 28 days postnatal as well as from adult tissue. The animals were anaesthetized by intrahepatic injection of 60 mg/kg of Nembutal and fixed by perfusion through the left cardiac ventricle. The perfusion pressure was