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Postnatal Development of the Dog Pineal Gland: Electron Microscopy

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The ultrastructure of the dog pineal gland from the first postnatal day to the seventh month is described. In the first postnatal stages, pineal parenchyma only shows immature proliferative cells with abundant cytoplasmic glycogen. Nerve fibers are seen in the pineal connective tissue spaces. The differentiation of the dog pineal cell types begins in the first postnatal week. Both pinealocytes and pigmented cells are first seen on the fourth postnatal day. The pineal astrocytes are observed on the tenth day. Immature cells are still found in the pineal gland of 1 mo-old dogs. The differentiation of the dog pineal cell types is completed by the second postnatal month.

Key words: pinealocytes, pineal astrocytes, pimented cells, ultrastructure

INTRODUCTION

The ultrastructure of the postnatal development of mammalian pineal gland has been largely studied in rodents, namely rat [Bayerová and Malinsky, 1972; Steinberg et al., 1981; Calvo and Boya, 1983], mouse [Ito and Matsushima, 1967], hamster [Hewing, 1976; Sheridan and Rollag, 1983], and guinea pig [Banks et al., 1985]. Other mammalian species have been seldom used and dogs are represented by a few studies of the pineal gland in adult animals [Sano and Mashimo, 1966; Welser et al., 1968; Calvo et al., 1988a,b]. Furthermore, no previous reports have been published either on the ultrastructure of the embryonic development or on the postnatal evolution of the dog pineal gland. In a previous report [Calvo et al., 1989, 1990] we described the light microscopy of the postnatal development of the dog pineal. Now, we describe the ultrastructure of the postnatal development of the dog pineal.

MATERIALS AND METHODS

Twenty-six clinically healthy mongrel puppies were used for this study. All the animals were kept under natural light conditions (approximately 40°N latitude). Two dogs (one male and one female) were sacrificed at the following

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age intervals: 1, 2, 4, 7, 10, 15, 20, 30, 40, and 45 d, 2, 3, and 7 mo. The animals were sacrificed under sodium pentobarbital anesthesia at 11:00 A.M. between March and June. The pineal gland was quickly removed, trimmed out, and fixed by immersion in cold 3% glutaraldehyde in 0.1M phosphate buffer. The samples were washed in 0.1M phosphate buffer, postfixed in 1% osmium tetroxide in the same buffer, and embedded in Vestopal. Ultrathin sections stained in uranyl acetate and lead citrate were examined in a Philips EM 201.

RESULTS

First Postnatal Week

The ependymal lining of the pineal recess consisted of cells with electrolucent cytoplasm and nuclei arranged in several layers. These ependymal cells were joined by junctional complexes and showed microvilli and occasional cilia in their luminal surface (Fig. 1). Their cytoplasm showed short cisterns of rough endoplasmic reticulum and small mitochondria. Mitotic figures were frequently observed near the lumen of pineal recess.

In the first 2 postnatal d, the pineal parenchyma showed immature cells (Fig. 2). The identification of pineal cell types is not yet possible. The immature cells had ovoid-shaped nuclei with one to three small nucleoli, a thin peripheral rim of heterochromatin, and some central chromatin clumps (Figs. 2 and 3). Mitotic figures were frequently found in this cell type. Their cytoplasm showed short cisterns of rough endoplasmic reticulum, Golgi apparatus, small mitochondria, and dense bodies. While many of these cells showed a centriole, cilia were seen only occasionally. The most outstanding feature of the cytoplasm of immature cells was the presence of glycogen granules, which were sometimes clumped into large masses (Fig. 3).

The pineal connective tissue spaces contained blood vessels, mostly capillaries, often lined by immature, thick endothelial cells (Fig. 2). The endothelial basal lamina was focally absent or poorly differentiated. The perivascular spaces showed scarce connective tissue fibers and nerve fibers. Nerve fibers either isolated or grouped in small bundles were also found among parenchymal cells.

A layer of thin cell processes lined the parenchyma bordering the connective tissue spaces (Fig. 4). This layer was well developed by the end of the first postnatal week. The cell processes showed small mitochondria, free ribosomes, glycogen granules, occasional small dense bodies, and sometimes coated vesicles and dense-cored vesicles.

The first signs of differentiation of pineal cell types were found on the fourth postnatal day. A few cells showed homogeneous nuclei with dark nucleoplasm and small nucleoli and dense cytoplasm devoid of glycogen granules, so characteristic for immature pineal cells (Fig. 5). Even though poorly differentiated, the developing stages can be identified as developing pinealocytes.

Pigmented cells [Calvo et al., 1988b] were first identified on the fourth postnatal day in the dog. These cells, morphologically similar to immature cells, were characterized by the presence of large osmiophilic pigment granules and the absence of glycogen granules (Fig. 6).

The dog pineal gland was surrounded by a capsule made of a few layers of light cells interspersed with blood vessels and bundles of collagen microfibrils. Short cisterns of rough endoplasmic reticulum and numerous free ribosomas were observed in their cytoplasm.

Second Postnatal Week

Frequent mitoses were seen in the immature cells, which were still the predominant parenchymal cell type. The amount of glycogen in these cells was less than in the first postnatal week, whereas more dense bodies were found in some cells. Degenerated cells with pyknotic nuclei and dark cytoplasm were also seen.

The ongoing differentiation of immature cells yields a wider morphological heterogenity of this cell type. Developing pinealocytes were more numerous (Fig. 7), characterized by a wider cytoplasm rich in organelles. From the tenth day onwards, a new cell type was detected, characterized by a more electron-lucent cytoplasm and nucleus (Fig. 8). Despite the absence of glial filaments at this stage, the development of these cells in further stages allows one to identify them as developing pineal astrocytes [Calvo et al., 1988a].

Third and Fourth Postnatal Week

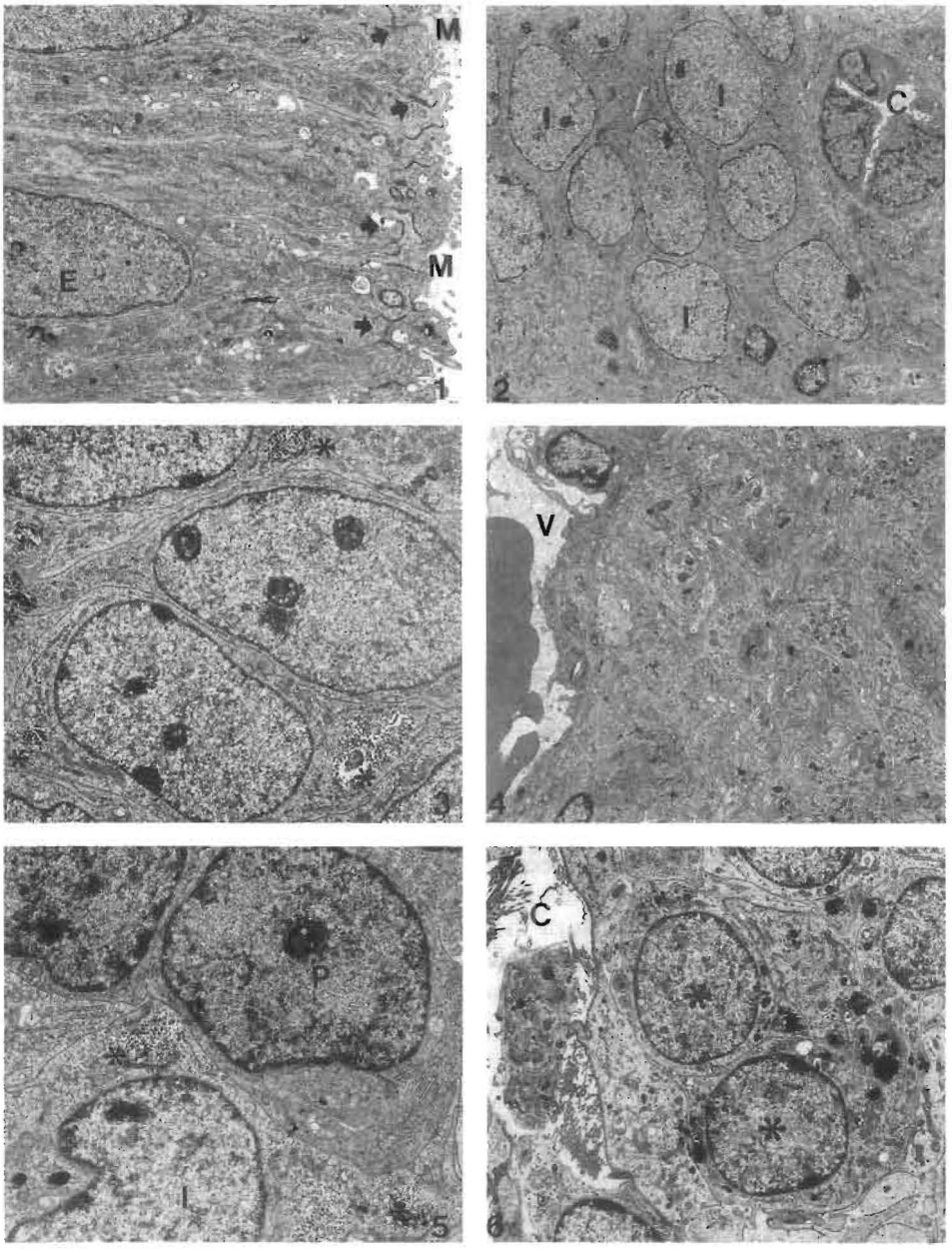
The number of immature cells decreased progressively as these cells differentiated into pineal cell types. However, at the end of the first postnatal month immature cells were still seen, often grouped in small clusters (Fig. 9). Compared to developing cells, immature cells show smaller nuclei with small chromatin clumps and less abundant cytoplasm (Fig. 9). The amount of glycogen granules typical of these cells in former stages was now seldom seen. Mitoses were only occasionally seen.

The number of pinealocytes increased progressively to become the predominant cell type on d 30. Both the cytoplasmic volume and the amount of organelles increased. The nuclei became round with one or two small nucleoli and a finely granular chromatin. Features characteristic for adult pinealocytes [Calvo et al., 1988a] such as abundant microtubules and frequent centrioli and cilia became also evident (Fig. 10).

Glial filaments in developing astrocytes were seen for the first time on the 30th postnatal d (Fig. 11). These cells, now more abundant, showed nuclei with a thin peripheral rim of chromatin and peripheral nucleoli. The cytoplasm was relatively more abundant and contained ribosomes and irregular cisterns of rough endoplasmic reticulum. Filaments were still few and sparse. Some microtubules were also seen (Fig. 11). These cells often showed lamellar processes partially surrounding the pinealocytes.

Although the number of pigmented cells varied among animals, towards the end of the first postnatal month groups of pigmented cells similar in size to those seen in adult animals [Calvo et al., 1988b] could be seen. Moreover, pigmented cells began to show larger pigment granules similar to those seen in the adult stage (Fig. 12). However, most cells still only showed small-sized pigmented granules.

The connective tissue spaces showed blood vessels, scarce collagen mi-



Figs. 1–6.

crofibrils, and numerous unmyelinated nerve fibers (Fig. 13), also present in the pineal parenchyma. These nerve fibers showed clear and granular vesicles similar to those found in sympathetic nerve fibers. A thick, immature-looking endothelial lining still could be seen in some blood vessels. Most of the capillaries show a thin continuous endothelial layer. The parenchymal basement membrane was incomplete in many areas. The pineal capsule was formed by flattened clear cells. At the distal tip of the pineal gland, these flat cells were arranged in several layers (Fig. 14).

Second to Seventh Month

The differentiation of the pineal cell types was fairly advanced by the 45th d, to be completed at the second postnatal month when the morphology of the adult pineal gland is achieved. The pineal glands of dogs aged 3 and 7 mo old did not show significant differences compared to those of adult animals. Between the 30th d and the second month of life there is a prominent development of the cell processes of both pinealocytes and astrocytes. Terminal clubs full of clear vesicles were formed at the end of the pineal cell processes (Fig. 15). Finally, from the 40th d onwards myelinated nerve fibers were seen within the pineal parenchyma, particularly near the commissures (Fig. 15), as has been already described in adult animals [Calvo et al., 1988a].

DISCUSSION

The results of the present ultrastructural study in addition to those described in a previous light microscopic study show that the postnatal development of the dog pineal gland follows a course similar to that described for other mammals [reviews: Vollrath, 1981; Gupta and Reiter, 1986].

In the first postnatal days, the pineal parenchyma is formed by masses of mitotically active indifferentiated cells. In this first stage two features characterize the developing dog pineal gland. First, the presence of glycogen granules appear to be a reliable cytological marker for immature dog pineal cells. Glycogen granules have been reported in developing pineal cells of other mammals,

Fig. 1. Wall of the pineal recess. Ependymal cells (E) showing junctional complexes (arrows) and microvilli (M) oriented toward the recess lumen; first postnatal day. ×9,500.

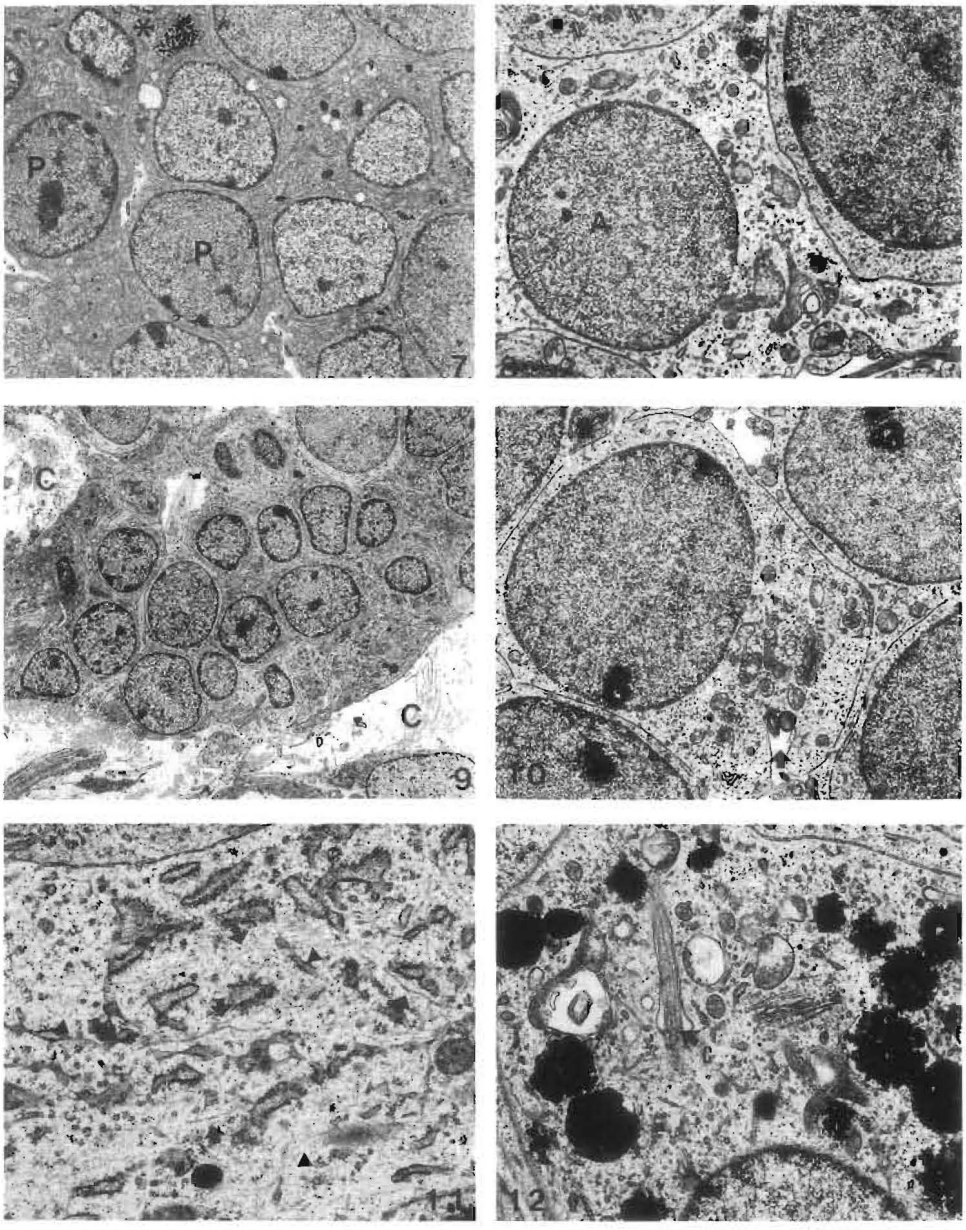
Fig. 2. Immature cells (I) of the pineal parenchyma. The endothelial cells of the capillary (C) also show signs of immaturity; first postnatal day. ×3,700.

Fig. 3. Glycogen deposits (asterisks) in the cytoplasm of immature cells; second postnatal day. ×8,500.

Fig. 4. Layer of thin cells processes around a blood vessel (V); seventh postnatal day. ×6,000.

Fig. 5. Developing pinealocytes (P) with dark nuclei and cytoplasm, lacking the glycogen granules (asterisks) characteristic of immature cells (I); fourth postnatal day. ×9,000.

Fig. 6. Pigmented cells (asterisk) close to the pineal capsule (C); fourth postnatal day. ×6,000.



Figs. 7–12.

including human fetal pinealocytes [Moller, 1974] and postnatal mouse pinealocytes [Kachi et al., 1975]. According to our results, glycogen disappears from the differentiating pineal cells in later stages. Glycogen granules have not been described in the cell types of the adult dog pineal gland [Welser et al., 1968; Calvo et al., 1988a,b]. Since routine fixation methods have been used in all of these studies, the presence of glycogen cannot be completely ruled out given the lability of this cell component to the fixation procedures. Notwithstanding, our results in routine fixed material demonstrate the coexistence of glycogen-rich immature cells and developing glycogen-free cells in the same pineal gland. In the nervous tissue, glycogen is considered characteristic for developing and mature astrocytes [Sturrock, 1974; Peters et al., 1976]. This might lead to the conclusion that all cells containing glycogen may be precursors for pineal astrocytes; however, the presence of glycogen in most if not all the immature cells argues against this hypothesis. Immature cells bearing cytoplasmic glycogen appear to be the only cell type present in the first postnatal days. Consequently, such cells probably are the precursors of both pinealocytes and pineal astrocytes.

The second peculiarity of the developing dog pineal gland is the absence of the small cavities or follicles lined by junctional complexes, which are described in the development of the rat pineal gland [Zimmermann and Tso, 1975; Calvo and Boya, 1981, 1983]. Such cavities have not been described in adult dog pineal [Welser et al., 1968; Calvo et al., 1988a].

The ultrastructural differentiation of the pineal cell types in the dog becomes apparent in the first postnatal week. The first pinealocytes begin to differentiate on the fourth postnatal day, while the first astrocytes are observed on the tenth postnatal day. In other mammals, the pineal cell types also appear first during development [rat: Zimmermann and Tso, 1975; Steinberg et al., 1981; Calvo and Boya, 1983; hamster: Clabough, 1973]. According to our results, the differentiation of the pineal cell types both in dogs and rats [Calvo and Boya, 1983] takes place at the time nerve fibers are seen in the pineal gland. To test for synchronicity between the appearance of light stimuli in the postnatal life and the development of the pineal cell types in mammals may be interesting.

Fig. 7. Developing pinealocytes (P) among immature cells. The immature cells show nuclei of clear nucleoplasm and cytoplasm with glycogen deposits (asterisks) and dense bodies; tenth postnatal day. ×5,100.

Fig. 8. Developing astrocyte (A) with an electron-lucent cytoplasm; tenth postnatal day. ×11,000.

Fig. 9. A small cluster of immature cells. C, connective tissue space; thirtieth postnatal day. ×3,300.

Fig. 10. Developing pinealocyte showing an increase in organelles. Arrow, cilium. ×8,700.

Fig. 11. Astrocyte cytoplasm with abundant filaments (arrowheads) and few microtubules (arrows); 30th postnatal d. ×21,200.

Fig. 12. Large dark granules in a pigmented cell. C, cilium; 30th postnatal d. ×16,800.

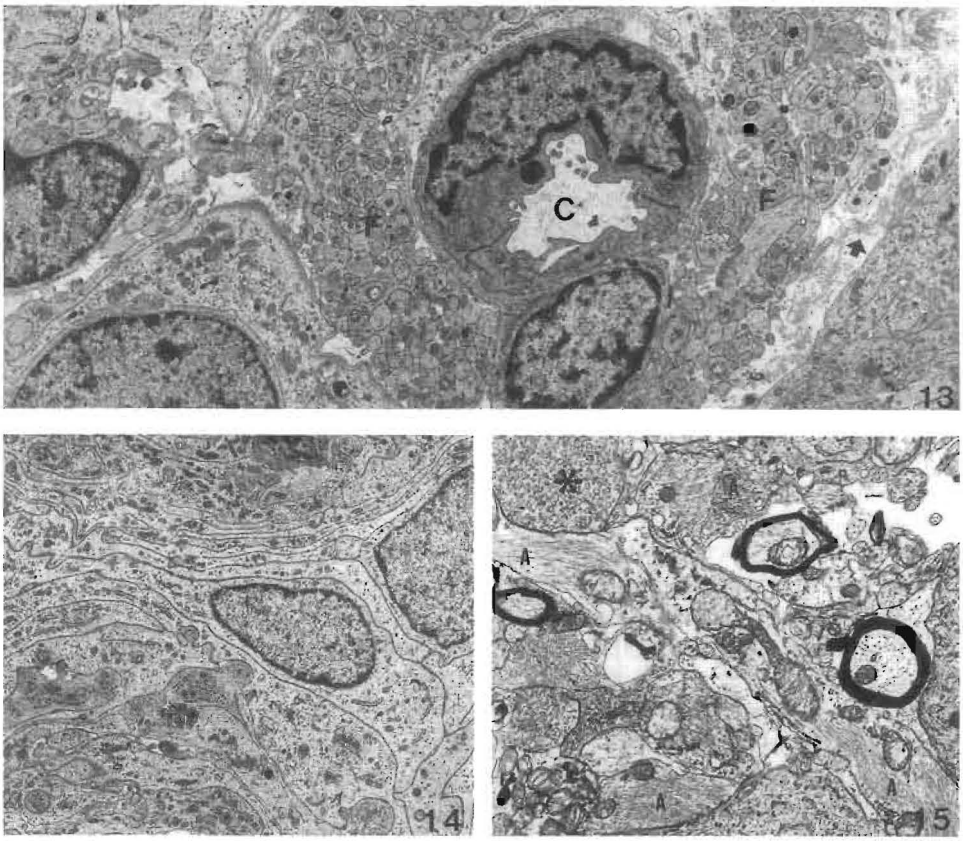


Fig. 13. Unmyelinated nerve fibers in a connective tissue space. C, capillary. Arrow, collagen microfibrils. × 10,000.

Fig. 14. Distal tip of the pineal gland. Pineal capsule with several layers of flat cells; 30th postnatal d. × 6,500.

Fig. 15. Myelinated nerve fibers among parenchymal cell processes. Asterisk, pinealocyte terminal club. A, astrocyte process; 45th postnatal d. × 14,500.

The adult dog pineal gland contains a special cell type, the pigmented cell [Zach, 1960; Calvo et al., 1988b]. The results of the present study corroborate our light microscopic observations [Calvo et al., 1990]. In both these studies, we have demonstrated the presence of pigmented pineal cells in dogs from the very first postnatal day, sharing common ultrastructure and localization with those of the adult dog pineal gland.

With the light microscope, and principally in the first 2 postnatal wk, the pineal blood vessels appear surrounded by a eosinophilic layer [Calvo et al., 1990]. The present results demonstrate that such a layer corresponds to immature cell processes. The discontinuity of the parenchymal basement membrane lining the connective tissue spaces also has been described in the rat [Calvo and Boya, 1983, 1984]. Frequently, nerve fibers can be seen passing into the pineal

parenchyma through these discontinuities. Flattened clear cells probably of leptomeningeal nature surround the pineal gland. In the distal end of the gland these cells are multilayered. These characteristics confirm our previous light microscopic data [Calvo et al., 1990]. Ultrastructurally these flat cells are identical to cells surrounding the pineal stalk in the albino rat [Calvo and Boya, 1985].

According to our results, the blood vessels in the developing dog pineal gland show a continuous endothelial lining. Capillaries of continuous type have been described in the adult dog [Sano and Mashimo, 1966; Welser et al., 1968; Calvo et al., 1988a] and other mammals [see review in Vollrath, 1981]. Undoubtedly, the presence of a continuous or fenestrated endothelium must be closely related to differences in the capillary permeability. However, it is very difficult to hypothesize about this question given the scarcity of studies dealing with such functional aspects in species other than rodents.

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