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Postnatal development of the dog pineal gland. Light microscopy

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Summary. The light microscopical morphology of the dog pineal gland from the first postnatal day to maturity is described. In the first postnatal week, the pineal parenchyma shows immature cells and many mitotic figures. In this week, pigmented cells are observed for the first time, both in the pineal gland and in extrapineal nodules. Throughout the second week, the pineal parenchyma shows a cordonal pattern that disappears progressively in the following stages. From the 20-30th day onward, it is feasible to discern the cell types characteristic for the adult pineal gland. In the adult animals, the length of the pineal gland axes almost quadruplies that of the pineal gland in neonatal stages. The light microscopical features of the adult dog pineal gland are also described.

Key words: Pineal gland, Dog, Pigmented cells, Postnatal development

Introduction

The structure of the pineal gland of vertebrates shows a wide range of variations. The study of this range of morphological variations of the pineal gland has provided important clues for the knowledge of this gland. Compared to the deep knowledge of rodent pineal gland, the pineal gland of carnivores remains largely unknown. In 1960, Zach described the light microscopic appearance of the pineal gland in adult dogs and cats. Lately, some morphological features of the adult dog pineal gland have been described (Ellsworth et al., 1985). Other light microscope studies on the dog pineal gland dealt with the innervation of the gland (Hartmann, 1957; Matsuura and Sano, 1983; Matsuura et al., 1983). Hitherto, all the studies concerning the dog pineal gland have been performed in adult animals. As a consequence, there is a dearth of knowledge regarding morphological aspects of both the embryonic development and the postnatal evolution of the dog pineal gland.

In the present paper we describe the evolution of the dog pineal gland from birth to the adult stage.

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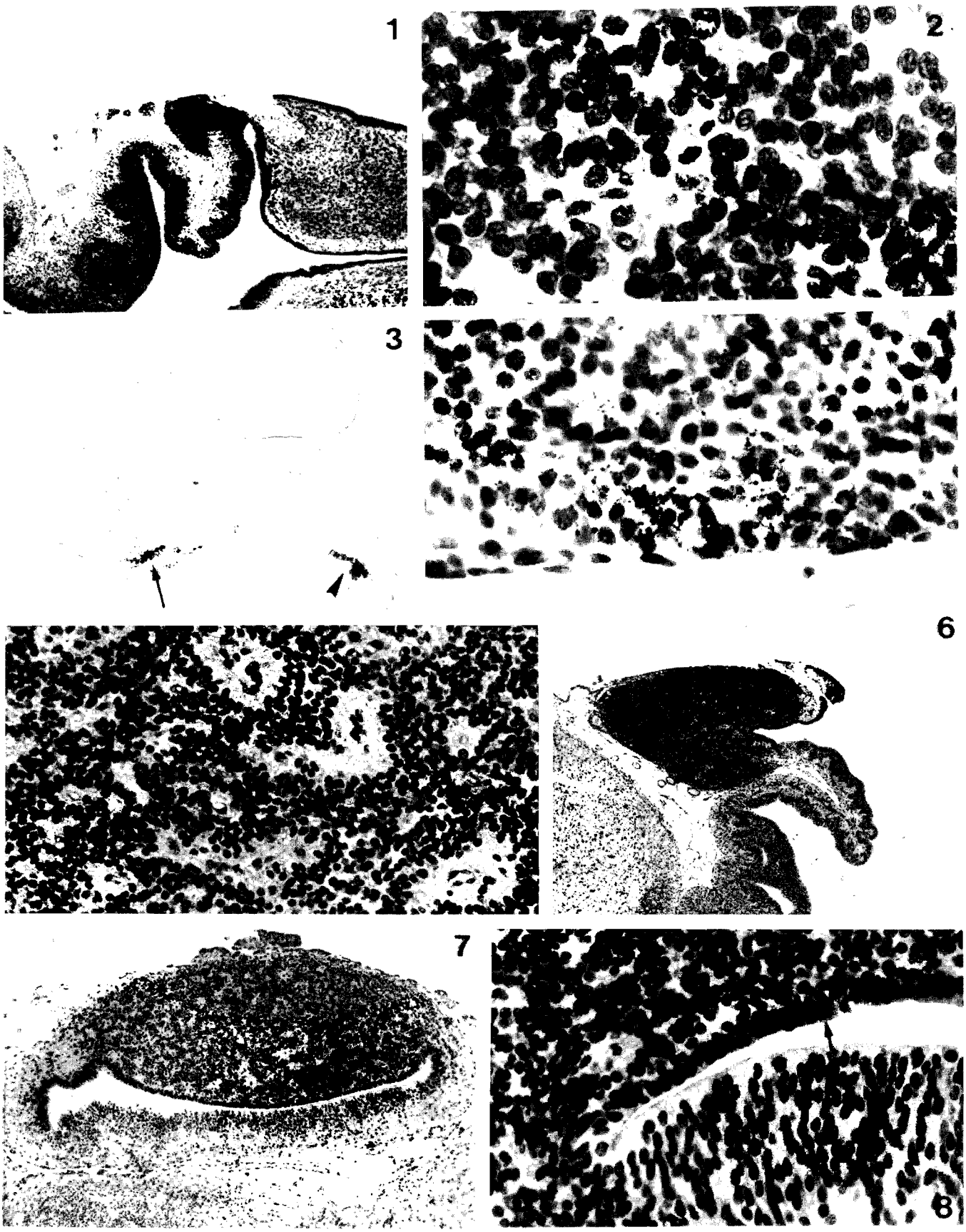
Materials and methods

Thirty-four mongrel dogs, living under natural light conditions (approximately 40°N latitude) and clinically healthy were used for this study. Groups of two dogs (male and female) were sacrificed at the following age intervals: 1, 2, 4, 7, 10, 15, 20, 30, 40 and 45 days; 2, 3, 7 and 9 months; 2, 3 and 4 years. The animals were sacrificed under sodium pentobarbitone anesthesia at 11:00 a.m. over a period between March and June. The pineal glands were fixed by immersion in Bouin's solution and embedded in paraffin. Seven micron sections were stained with hematoxylin eosin, phosphotungstic acid hematoxylin and the silver method of Gordon & Sweets (Cook, 1974) for reticulin fibres. The Masson Fontana silver method and the bleaching of sections with hydrogen peroxide and peracetic acid were also used, as recommended by Pearse (1968), for the identification of melanin. The outline of the pineal gland in sagittal and frontal sections was drawn with the aid of a camera lucida. Variations in the size of the pineal gland were studied in these drawings.

Results

No differences could be found between sexes in the process of postnatal evolution of the dog pineal gland. The size of the gland showed the most remarkable changes during the postnatal period, whereas the shape and location essentially remained unchanged (Figs. 1, 6, 12). An increase of 3.5 to 4-fold of the length of the sagittal axis of the gland was measured from the first postnatal day to the adult stage (Figs. 1, 12). The other pineal axes varied accordingly. The dog pineal gland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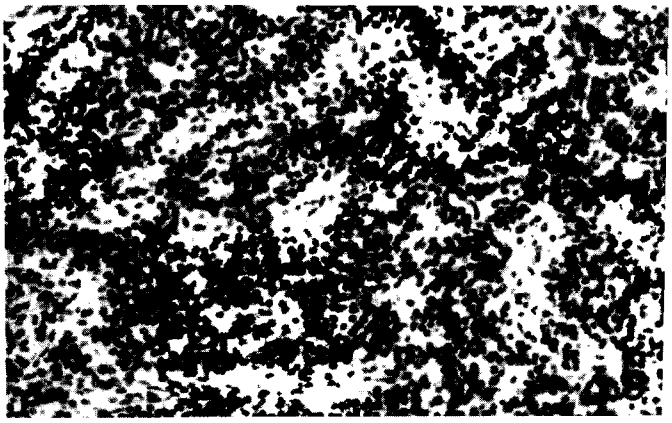

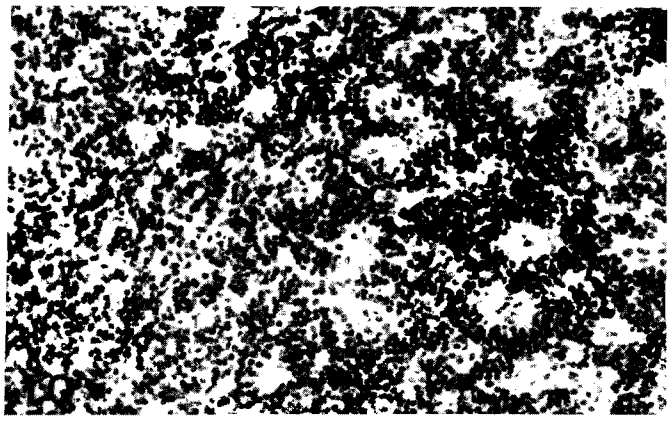

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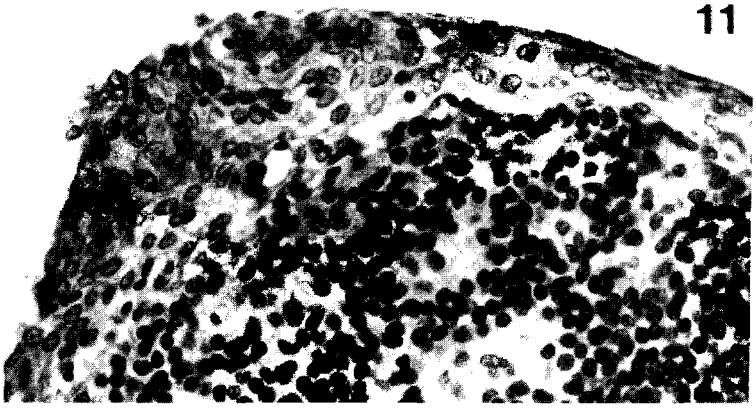

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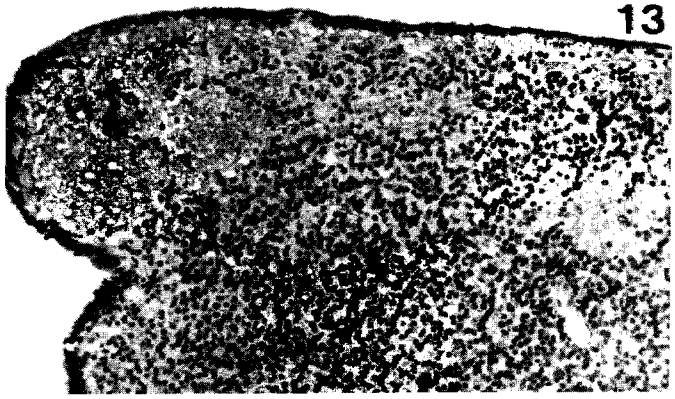

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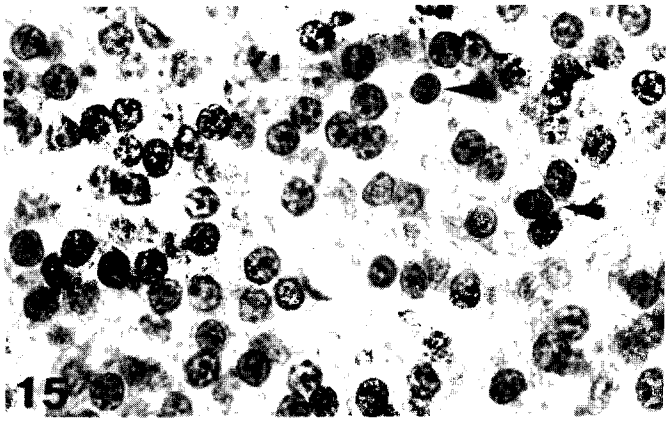

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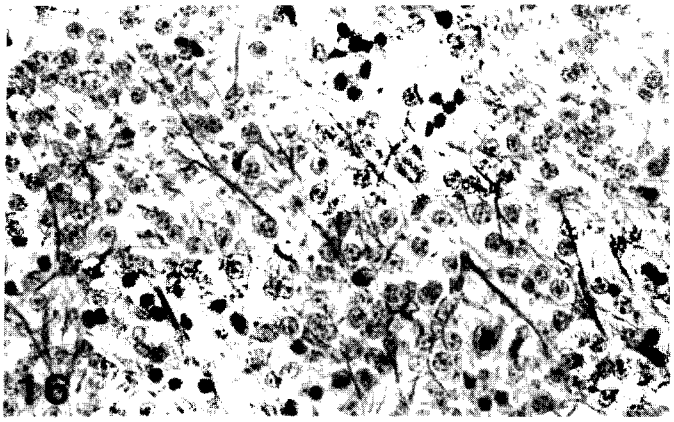

Fig. 1. 1 postnatal day. Sagittal section of a pineal gland seen at low magnification. Compare with figures 6 and 12. Hematoxylin eosin. × 35

Fig. 2. 4 postnatal days. Pineal parenchyma. Immature cells with frequent mitotic figures. Hematoxylin eosin. × 800

Fig. 3. 4 postnatal days. Pigmented cells in the basal aspect of the pineal gland (arrow). Pigmentary nodule (arrowhead) in the basal aspect of the posterior commissure. Unstained thick section. × 120

Fig. 4. 7 postnatal days. Basal aspect of the pineal gland. Pigmented cells. Hematoxylin eosin. × 550

Fig. 5. 10 postnatal days. Cordonal pattern of the pineal parenchyma. Hematoxylin eoxin. × 290

Fig. 6. 30 postnatal days. Low magnification of a sagittal section of the pineal gland. Compare with figures 1 and 12. Hematoxylin eosin. × 35

Fig. 7. 20 postnatal days. Transversal section of the pineal gland. Flat pineal recesss, displaced toward the basal aspect of the gland. Hematoxylin eosin. × 100

Fig. 8. 20 postnatal days. Distal end of the pineal recess. The basal aspect is lined by cells from the subcommissural organ. In the apical surface (arrow), the ependymal cells are in contact with the pineal parenchyma. Hematoxylin eosin. × 300

Fig. 9. 30 postnatal days. Nuclei become apparent in the perivascular area. Compare with figure 5. Hematoxylin eosin. × 200

Fig. 10. 30 postnatal days. Field showing a cordonal pattern (right half) next to an area with diffuse structure (left half); Hematoxylin eosin. eosin. × 150

Fig. 11. 30 postnatal days. Thickening of the capsule at the distal end of the pineal gland. Hematoxylin eosin. × 330

Fig. 12. 2 years. Low magnification of a sagittal section of the pineal gland. Compare with figures 1 and 6. Hematoxylin eosin. × 35

Fig. 13. 2 years. There is no limit between habenular commissure (asterisk) and the pineal gland tissue. Hematoxylin eosin. × 140

Fig. 14. 3 years. Connective tissue stroma of the pineal gland. Silver method for reticulin fibres. × 90

Fig. 15. 4 years. Nuclei of pineal parenchymal cells. Most cells show rounded nuclei with small clumps of chromatin. A second cell type (arrowhead) shows ovoid-shaped nuclei with homogeneous chromatin. Hematoxylin eosin. × 700

Fig. 16. 4 years. Glial fibres in the dog pineal gland. Phosphotungstic acid hematoxylin. × 400

grows specially faster during the first twenty postnatal days, in which the pineal axes showed a two-fold increase.

First postnatal week

In saggital sections, the pineal recess appeared as a short and narrow proximal cleft open to the third ventricle and located between the developing habenular and posterior commisures (Fig. 1).

The pineal parenchyma showed abundant immature cells of small, dark and tightly packed nuclei (Fig. 2). Mitoses were frequently found (Fig. 2). Small blood vessels were observed throughout the gland. The pineal gland was covered by a thin leptomeningeal capsule.

The pigmented pineal cells, previously described in adult animals (Zach, 1960; Calvo et al., 1988), were found from the second postnatal day onwards (Fig. 3). As described for the adult dogs, pigmented cells appeared in the parenchyma beneath the basal surface of the gland, close to the posterior commissure (Fig. 3). The dark to brown granules of the pigmented cells were often fewer and smaller in the pigmented cells of the postnatal pineal gland than in the pigmented pineal cells of adult animals (Fig. 4). Extrapineal nodules of pigmented cells present in the posterior commissure or within the adjacent meningeal space in adult dogs (Calvo et al., 1988) have been seen also in some dogs from the first postnatal week onwards (Fig. 3). In contrast to the pigmented pineal cells, extrapineal nodules of pigmented cells were an inconstant feature present occasionally in some dogs. The pigment granules became colourless after bleaching of the sections with hydrogen peroxide or peracetic acid. With the Masson Fontana method, these granules showed a strong argentaffin reaction too.

Second postnatal week

The pineal blood vessels were surrounded by a thick, un-nucleated eosinophilic layer (Fig. 5). Parenchymal cells were arranged in thick, irregular cords among the vessels (Fig. 5). The nuclei of the parenchymal cells, less dense and somewhat more separated between each other, showed at this stage subtle differences, yet not enough to make feasible the separation into definite cell types. Mitoses are still abundant. Occasionally, deeply basophilic globules, interpreted as pyknotic nuclei of degenerated cells, were seen isolated or forming groups of two to three cells. All the animals studied showed pigmented cells, although the amount of pigment granules was variable among them and even within the same gland.

15-30 postnatal days

In the ongoing evolution, the pineal gland had increased notably in size with respect to the first postnatal days (Fig. 6). In the transversal sections, the pineal recess appeared as a flat cavity, displaced toward the basal surface of the gland (Fig. 7). The basal or inferior wall of the pineal recess, immediately above the posterior commissure, was outlined by tall columnar cells of the subcommissural organ. The dorsal aspects showed shorter ependymal cells in direct contact with the pineal parenchyma (Fig. 8). The transition between the posterior commissure and the pineal gland took place at the distal end of the pineal recess.

Throughout this period, we could observe a progressive disappearance of the cordonal organization described in former stages. This was due in part to the wider separation among the nuclei, probably secondary to the hypertrophy of individual cells. Secondly, the thick eosinophilic layer surrounding blood vessels was progressively invaded by nuclei thus blurring the limits of adjacent cell cords (Fig. 9). Because the transformation was not completed

at 30 days, areas of diffuse structure as well as thick cords of small cells could be seen scattered in the same gland (Fig. 10). The nuclear types, characteristic in the adult phase, could be recognized from the 20-30 days onwards. These cell types were still mixed with smaller, denser nuclei, similar to those described in previous stages. The amount of mitotic figures decreased mainly at the phase of 30 days and was very scarce at 45 days. Cells with pyknotic nuclei were also frequently seen. The pineal capsule was formed by several layers of flat cells with ovoid nuclei. Toward the distal end of the gland, the pineal capsule thickened to form a cap around that end (Fig. 11).

Adult pineal gland

From 45 days onward the dog pineal gland evolved very slowly toward the definitive structure. In sagittal sections, the gland appeared pear-shaped, with a wide proximal region and a narrow distal part (Fig. 12). The length of the sagittal axis of the gland was about 2 mm. The ependymal lining of the dorsal aspect of the recess was formed by a single layer of cuboid, cilliated cells closely apposed to the pineal parenchyma. No boundaries were found between the pineal tissue and the bundles of commissural fibres, mainly in the habenular commissure (Fig. 13).

During the postnatal life and throughout the adult phase, the amount of connective tissue stroma of the dog pineal gland was very scarce, limited to a thin sheet with connective tissue fibres, essentially reticulin fibres, surrounding large blood vessels and a narrow capsule surrounding the gland (Fig. 14).

The nuclei of the parenchymal cells were widely spread. Any remnant of the cordonal pattern characteristic of the first stages was no longer seen (Fig. 13). Most of the parenchymal cells showed a rounded nucleus with peripheral clumps of heterochromatin and one or two central nucleoli (Fig. 15). A second cell type was characterized by rounded or ovoid nuclei with homogeneous chromatin (Fig. 15). Neither cavities, follicles nor signs of cell polarization were observed in the parenchymatous cells. Using phosphotungstic acid hematoxylin, glial fibres were stained throughout the pineal parenchyma, being somewhat abundant at the periphery of the gland (Fig. 16).

Discussion

The results of the present report indicate that the evolution of the dog pineal gland during the postnatal period follows a pattern similar to that described for other mammals (Kappers, 1960; Ito and Matsushima, 1967; Clabough, 1973; Calvo and Boya, 1984). The postnatal growth of the dog pineal gland followed two steps that partially overlaped in time. In the first postnatal 15 days, mitotic figures were very frequent in the pineal parenchyma. Afterwards, this number declined progressively, although at 30 days it was still high. From the second postnatal month onwards, mitoses were no longer seen. Therefore, the period of proliferation was prolonged in the dog, though this may be related to the longer life span of this species compared to other studied. From the second postnatal week, the nuclei of the parenchymatous cells were progressively set apart due to the increasing cellular volume. These two processes, namely proliferation and cell hypertrophy, have been previously described in the postnatal evolution of the rat pineal gland (Quay, 1974; Calvo and Boya, 1984).

According to our results, the shape of the dog pineal gland shows only slight variations throughout the postnatal period. This contrasts with the descriptions for other mammals, particularly rodents, in which the developing gland lenghthens progressively (Kappers, 1960; Calvo and Boya, 1984). In rodents, the distal end of the pineal gland is firmly attached to the vault through the venous sinuses of the duramater. During the development, the commissural region sets itself appart from the vault, thus determining the elongation of the pineal gland. In the dog, the absence of tight bonds between the distal end of the pineal gland and the duramater, avoids the stretching of the gland during the developmental period.

As stated in other animals (Kappers, 1960; Clabough, 1973; Sheridan and Rollang, 1983; Calvo and Boya, 1984), the pineal recess of newborn dogs consisted of a narrow cleft in the proximal region. The pineal recess remained unchanged up to maturity. Conversely, in rodents the pineal recess becomes almost completely closed with the elongation of the gland (Kappers, 1960; Clabough, 1973; Sheridan and Rollag, 1983; Calvo and Boya, 1984). According to Zach (1960), the dog pineal recess is pear-shaped. However, in our material, transversal sections have demonstrated that it corresponded to a flat transversal cleft.

An unexpected finding was the early appearance of pigmented cells in the dog pineal gland. According to Zach (1960), pigmented cells were only seen in older adult dogs. Both the pattern of distribution and the morphology of these cells, hitherto only described in adult animals (Zach, 1960; Calvo et al., 1988), were similar in newborn dogs and adult dogs. Further studies on the embryonic development of the pineal gland in this species, not yet performed, should contribute to explain the characteristic localization of these cells. The function of the pigmented cells is largely unknown. However, their presence in neonatal stages, when the pineal parenchyma only shows proliferative immature cells, suggests that the pigmented cells are neither related to the maturation of the gland nor to the sexual development.

Mainly during the second postnatal week, the pineal parenchyma showed a cordonal pattern, due to the development of a perivascular eosinophilic layer, probably formed by cell processes. In the next stages, the eosinophilic layer became progressively less prominent until it disappeared. In the adult dog, the parenchymal cells were not polarized, showing rather a diffuse arrangement throughout the gland. Studies with the electron microscope do not describe any particular polarization of the parenchymal cells in adult animals (Sano and Mashimo, 1966; Welser et al., 1968; Calvo et

al., 1988). The cavities or cysts described as characteristic for the pineal gland surface in the adult dog (Zach, 1960), were not found in our study. Neither are they referred to in ultrastructural studies on the adult dog pineal gland (Sano and Mashimo, 1966; Welser et al., 1968; Calvo et al., 1988).

From the second postnatal week onward, subtle differences could be appreciated in the appearance of nuclei, suggested to be a leading sign of cell type differentiation. Such specific cell types were better defined from the 20-30th postnatal days. Nevertheless, further studies, essentially with the electron microscope, are required to establish with a certain degree of security the sequence of maturation of the different pineal cell types. In the adult dog, according to the nuclear structure, two cell types can be separated that correlate well with those cell types formerly described light microscopically (Zach, 1960; Ellswoth et al., 1985) and ultrastructurally (Welser et al., 1968; Calvo et al., 1988).

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