

AGE- RELATED HISTOLOGICAL CHANGES IN THE PINEAL GLAND (EPIPHYSIS CEREBRI) OF THE DOG

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ABSTRACT

The histological changes with the progress of age in the pineal gland of dogs were studied. Twenty dogs of both sexes clinically healthy were used. Two dogs from different ages were chosen from 2 days age up to 4 years of age. The size and shape of the pineal gland showed no or little change with the progress of age. The pineal recess of the newly born dogs consisted of arrow cleft remained unchanged up to maturity. The changes were classified into 3 stages. The first one, the parenchyma was highly cellular with either randomly distributed cells or cords-like structure. The main cellular elements were the pinealocytes and few glial cells. The mitotic figure was predominant. From the second stage onward, the number of pinealocytes decreased and the number of both glial and pyknotic cells increased. The glial filaments appeared long and branched at 4 months old dogs. The pigmented cells appeared at 2 days old dogs and became more with the advances of age. In conclusion, the decrease in the number of the pinealocytes in our results could be attributed to either the pinealocytes undergoes differentiation to other cells similar to glial cells, due to change in nuclear morphology and the presence of large number of glial filaments, or by pyknosis in which the pinealocytes were degenerated. The last suggestion augmented by wide separation of the cells from 2 years old dog onward.

INTRODUCTION

The secretory product of the pineal gland considered to be protein in nature (*Przybylska et al.,1991*) in pig (*Abu Easa, 1996*) in goat. *Olcese (1995)* mentioned that melatonin hormone is the main secretion. This hormone is fundamentally involved in many functions in the postnatal life (*Khan, et al., 1997*).

The morphology of the pineal gland is mainly described in different mammals, mainly rodents (*Review of Bhatnagar, 1992*). The gland in large mammal is also studied from its morphology by light microscope (*Calvo et al., 1990*) in dog, (*Taher et al., 1975 and Abbas & Ewes, 1982*) in camel. Ultrastructural features of the pineal gland are studied in adult dog (*Sano & Mashimo,1966 ; Welser et al.,1968 and Calvo et al., 1988*), and in adult cat (*Karasek & Hansen, 1982; Calvo et al., 1991& 1992; Vigh-Teichmann et al.,1991 and Boya et al., 1995*).

Researches dealing with embryonic development are carried out on laboratory animals particularly rats (*Clabough, 1973; Calvo and Boya, 1981 a & b and Saleh et al., 1984*), while in rabbit (*Gabr, et al., 1992*) and *Regodon et al.,(1998)* in ovine.

Postnatal development of the pineal gland is rare (*Calvo, et al., 1990*) in dog; (*Ohshima and Matsuo, 1987*) in goat and Japanese serow and (*Abou-Easa and Abd -El-Gawad, 2002*) in camel and buffalo.

So the aim of the present study is to trace the histological changes with progress of age in the pineal gland of stray dogs in the post-natal life.

Materials and Methods:-

Twenty stray dogs of both sexes, living under natural light and clinically health were used for the present study. The following ages 2,6,10,25,35 days; 5-5.5, 6, 8 months; 1-2 and 3-4 years respectively were used. Groups of two dogs of each age were chosen to carry out the present study. The dogs were sacrificed under chloroform anesthesia ,then the brain was exposed by routine dissection of the head.

The pineal gland associated with block of diencephalon was gently sampled and fixed in Bouin's solution for 24 hours. Routine histological technique was made to obtain 4-6 um sections. These sections were stained by:

- Haematoxylene and Eosin as well as Toluidine blue for general descriptions.
- Van Gieson- Von Kossa for the presence of calcium.
- Finally Phosphotungestic acid heamatoxylene(PTAH)for glial filaments.

All the above mentioned methods were reported by *Bancroft & Stevens (1991) and Woods & Ellis (1994)*.

RESULTS

1- Dogs at 2-10 Days age:-

The pineal gland of the dog was triangular or lancet-shaped, dorsoventrally flattened and its free end was pointed (fig.1). The gland showed no or little change in its shape with the progress of age. In newly born dog the pineal recess; derived from the third ventricle at the base of the gland was a narrow cleft, which remained unchanged up to maturity (figs.1&8). The pineal gland was covered by thin connective tissue capsule contained the blood vessels of the gland. At the beginning of this stage, the parenchyma of the gland was highly cellular, irregularly arranged or distributed as a cords like structure separated with a space (figs. 2, 3, 4 and 5). The present spaces contained blood vessels associated with fine connective tissue (fig.2, 3,4&5). Few and rarely occurred cyst lined with ependymal cells were also presented(fig.2).

The pinealocytes were densely distributed, had large vesicular, spherical or ovoid nuclei, pale staining with very clear nucleolus. The mitotic division was predominate along this stage (figs.3,5 &6). Few cells with large darkly stained nuclei, contained clumped chromatin were presented, these cells similar to the structure of glial cells (astrocytes) (fig. 4). The pigment cells were located at the periphery of the gland near

its base under the connective tissue capsule opposite to the pigmented area of the brain. The brown pigmented granules were few in number and dispersed (fig. 7).

2-Dogs at 25-35 Days age:-

At the beginning of this stage, the pineal gland was slightly increased in size than the previous stage. There was no change in the pineal recess (fig.8). The cellular parenchyma was distinguished into two parts; under the connective tissue capsule; there was densely packed cells while at the center of the gland, there was loosely arranged cells in cords like structure with spaces between it (fig. 9). The pinealocytes were increased in number and densely packed. At the end of this stage there were randomly distributed cells with darkly stained pyknotic nuclei, which was either that of glial cells or pyknotic cells suggested to be pinealocytes (fig. 10). The pigmented cells had the same location like the previous stage (fig.11), but the pigment granules showed increased number and distribution. The glial cells increased in number and became randomly distributed (fig. 12).

3- Dogs at 5 Months-4 Years age:-

At the beginning of this stage, the parenchyma was highly cellular. The number of pinealocytes decreased than previous stage. The number of cells contained darkly stained pyknotic nuclei increased could not be differentiated from glial cells (fig. 13). The number of glial cells increased in number and became randomly distributed. The first signs of glial filaments appeared at 4 months old dogs by PTAH method. These filaments were long, thick with delicate network of branches (figs. 13 &14).

At the end of this stage (2 years onward),The cellular elements of the gland were widely separated by wide spaces, with the appearance of very delicate processes. The number of pinealocytes with its characteristic nuclei were markedly decreased, on the other hand, the pyknotic cells increased (figs. 15 &16).

LIEGENDS

- Fig. (1):** Sagittal section of the pineal gland of 2 days old dog showing: pineal recess (arrow), pineal gland (P). Stain H&E X 40.
- Fig. (2):** photomicrograph of the pineal gland of 2 days old dog showing: distribution of pinealocytes(P), blood vessel (long arrow) and ependymal cyst (short arrow). Stain H&E X 400.
- Fig. (3):** photomicrograph of the pineal gland of 2 days old dog showing: pinealocytes (P), blood vessel (black arrow), mitotic figure (white arrow). Stain Van Geison-Von Kossa, X400.
- Fig. (4):** photomicrograph of the pineal gland of 2 days old dog showing: pinealocytes (P), glial cells (black arrow), cord like arrangement (white arrow) and space free of cells contained blood vessel (B). Stain H&E X 1000.
- Fig. (5):** photomicrograph of the pineal gland of 2 days old dog showing: pinealocytes (P), mitotic figure (arrow), there was no processes but space free of cells contained blood vessel (S). Stain PTAH, X 1000.
- Fig. (6):** photomicrograph of the pineal gland of 10 days old dog showing: mitotic figure (arrow), pinealocytes (P) and space free of cells contained blood vessel (S). . Stain H&E X 1000.
- Fig. (7):** photomicrograph of the pineal gland of 10 days old dog showing: pigment cells at the ventral aspect of the gland (small arrow) pinealocytes (P) and connective tissue capsule contained blood vessel (thick arrow) . Stain H&E X 400.
- Fig. (8):** Sagittal section of the pineal gland of 25 days old dog showing: pineal gland (P), habenular commissure (H), pineal recess (arrow). Stain H&E X 40.
- Fig. (9):** photomicrograph of the pineal gland of 25 days old dog showing: capsule (arrow), peripheral condensation of cells (P) and loosely arranged cells at the center of the gland(L). Stain H&E X 100.

Fig. (10): photomicrograph of the pineal gland of 25 days old dog showing: increase number of glial cells (arrow) and pinealocytes (P). Stain H&E X 400.

Fig. (11): photomicrograph of the pineal gland of 35 days old dog showing: amount of pigment cells at the periphery (thick arrow) under the connective tissue capsule (thin arrow) and the pinealocytes (P). Stain H&E X 1000.

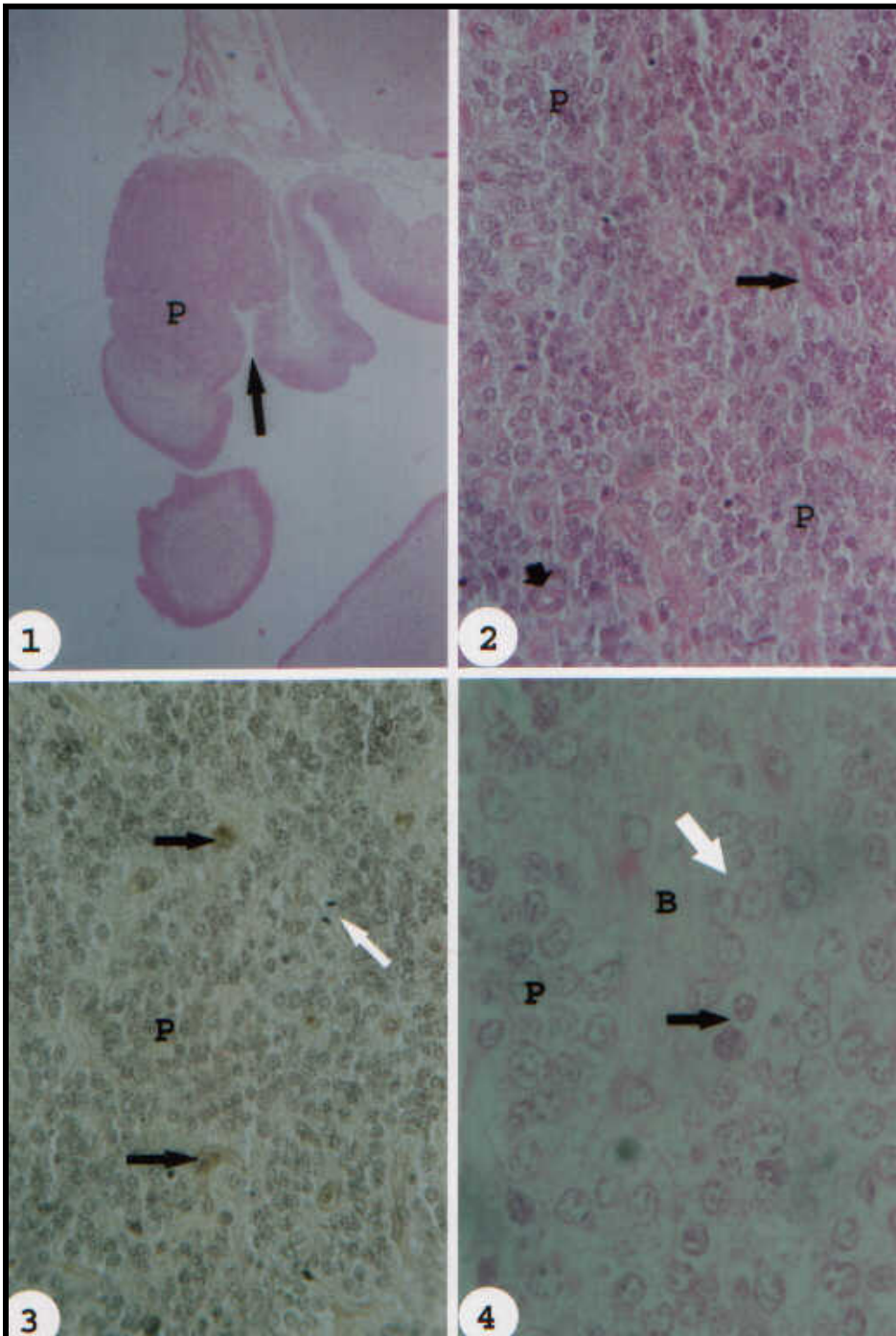
Fig. (12): photomicrograph of the pineal gland of 35 days old dog showing: distribution of pinealocytes (P) and glial cells (arrow) and space free of cells(S). Stain toluidine blue, X 400.

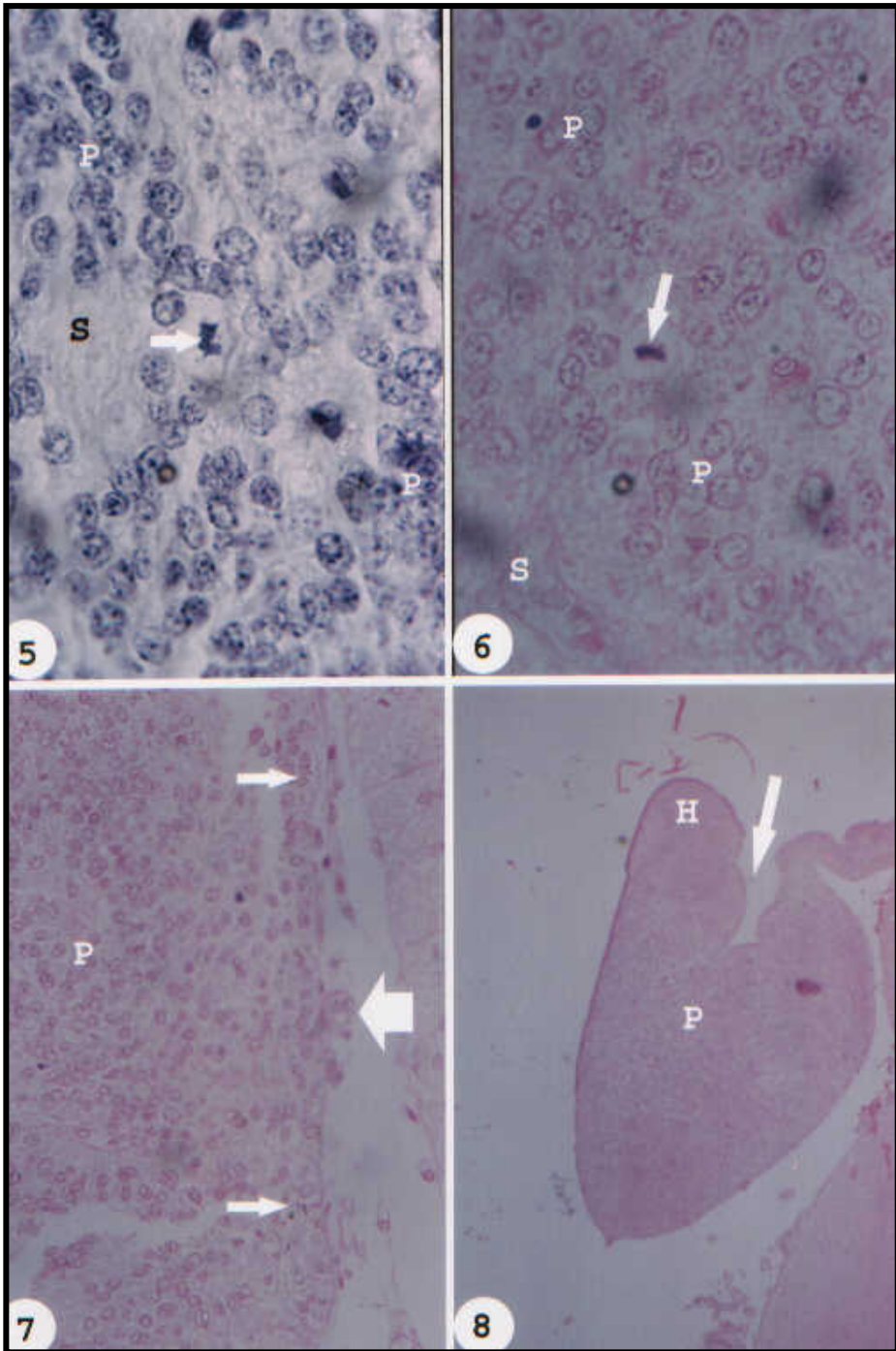
Fig. (13): photomicrograph of the pineal gland of 8 months old dog showing: increase number of glial cells (small arrow) with long branched glial process (long arrow). Stain PTAH X 400.

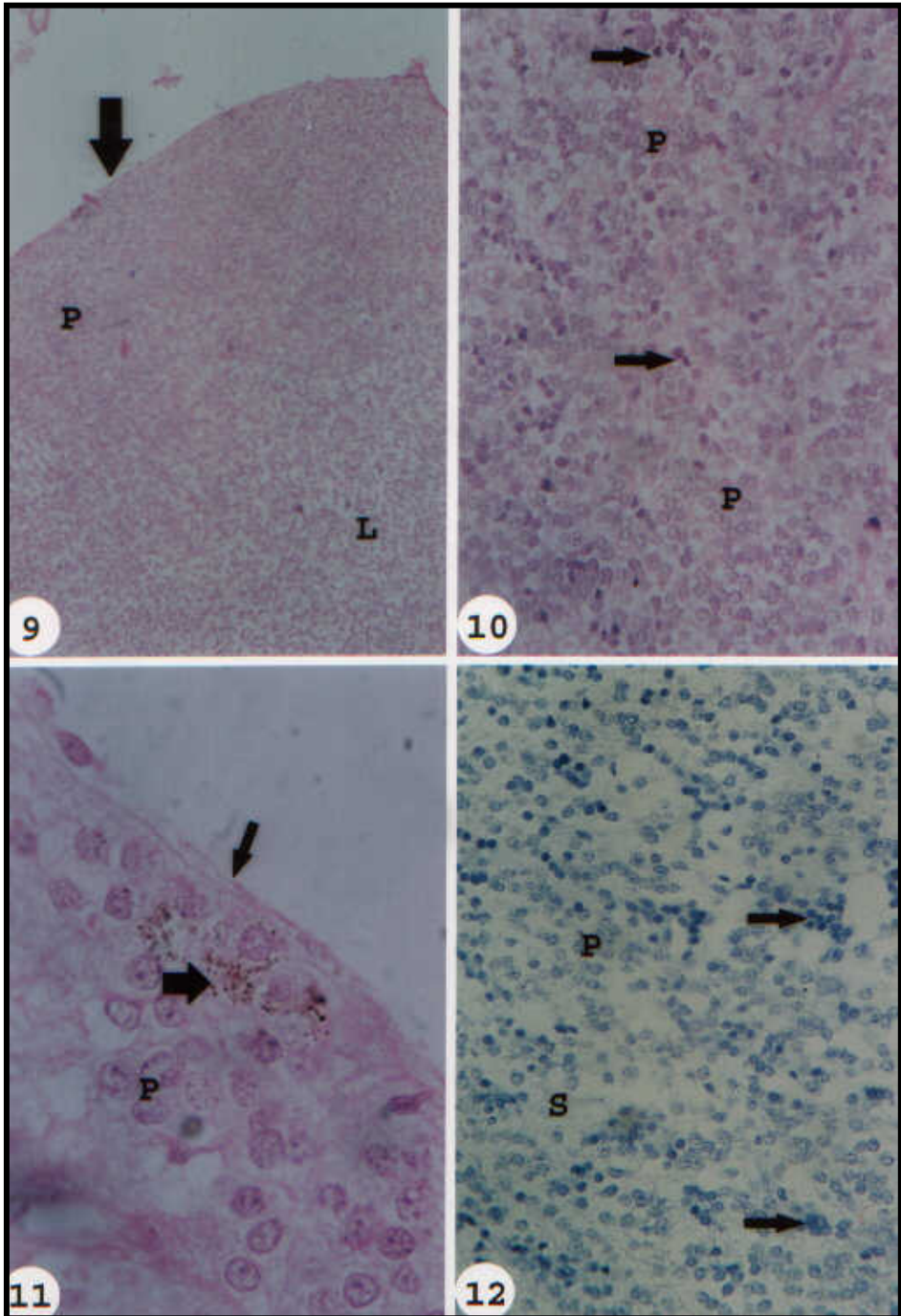
Fig. (14): photomicrograph of the pineal gland of 8 months old dog showing: long branched glial filament (long thin arrow) and glial cells (short thick arrow). Stain PTAH X 1000.

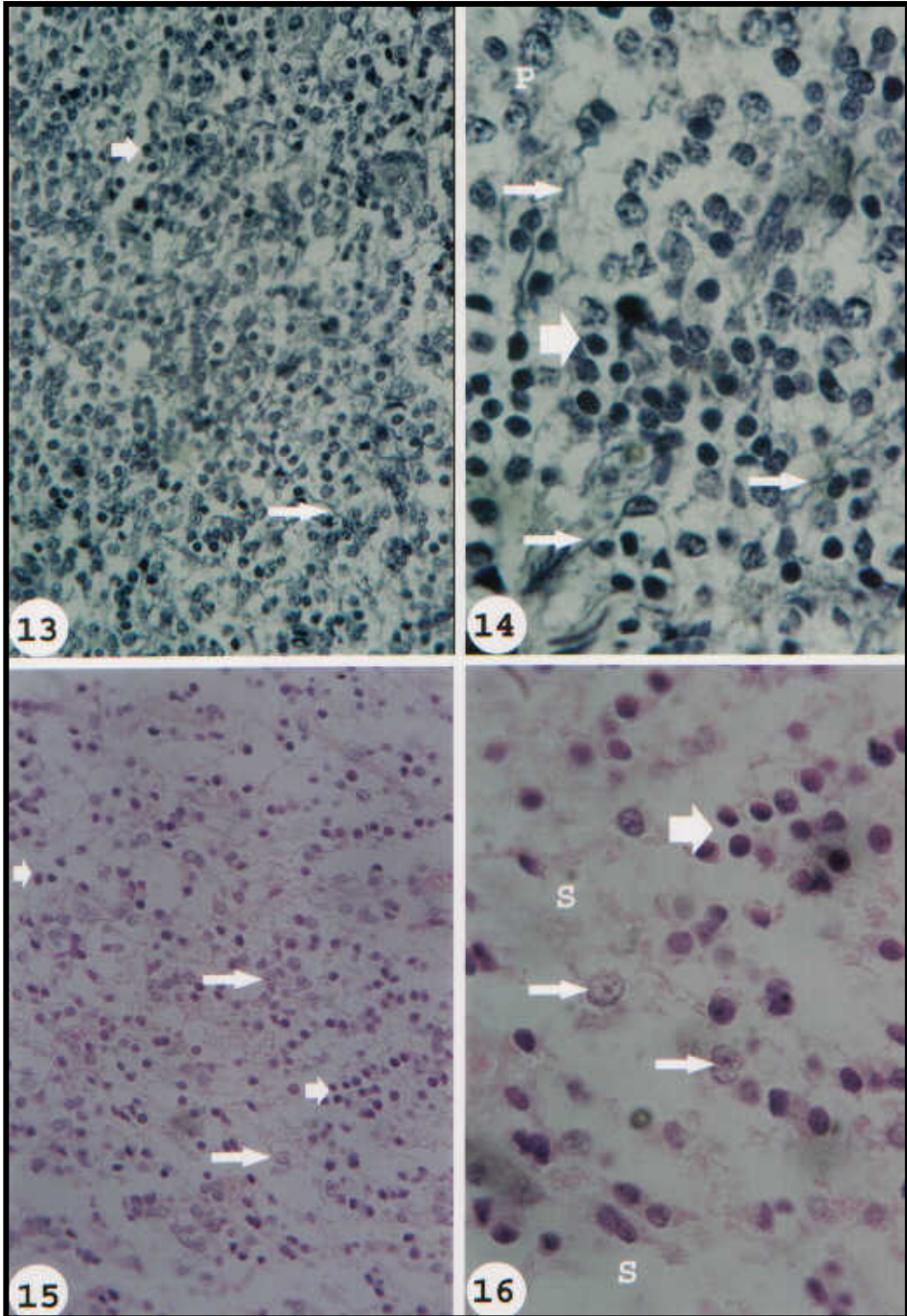
Fig. (15): photomicrograph of the pineal gland of 2 years old dog showing: distribution of pinealocytes (long arrow) and glial cells (short arrow). Stain H&E X400.

Fig. (16): photomicrograph of the pineal gland of 2 years old dog showing: decrease number of pinealocytes (long arrow) and increase glial cells and pyknotic cells (short arrow) with the increase spaces between the cells (S). Stain H&E X1000.









DISCUSSION

Our results showed that, the pineal gland of dog was triangular or lancet- shaped, dorsoventrally flattened and its free end was pointed. The results of *Boya et al., (1995) and Abou-Easa, (1997)* support our finding in that the dog pineal gland attached directly without a stalk by its base to habenular and caudal commissure, in the other hand, *Evans, (1993)* described the gland as wedge-shaped, small excrescence on the dorsal midline surface of the diencephalons.

According to our results, the dog pineal gland showed no or little changes in its shape with the progress of age, this was confirmed by *Calvo et al., (1990)*. In contrast the developing gland in rodents was lengthens progressively (*Kappers,1960 and Calvo & Boya, 1984*) due to the distal end of the pineal is firmly attached to the vault through the sinuses of the dura mater. They added that, during the development, the commissural region sets itself apart from the vault, thus determining the elongation of the pineal gland. In our result, the absence of tight bonds between the distal end of the gland and the dura mater, avoids the stretching of the gland.

Our study clarified that, the pineal recess of the gland of newborn dog consists of narrow cleft, remain unchanged up to maturity. The same results was observed in other animals (*Kappers, 1960; Clabough, 1973; Sheridan & Rollang, 1983; Calvo & Boya, 1984 and Calvo et al., 1990*). However in rodents, the pineal recess becomes almost completely closed with the elongation of the gland (*Kappers, 1960; Clabough, 1973; Sheridan & Rollang, 1983; Calvo & Boya, 1984*).

In the dogs (2-10 days age), the parenchyma of the pineal gland appeared highly cellular with randomly distributed cells. Some cells were arranged in a cords-like structure separated by spaces contained blood vessels, which disappeared at the end of the second stage (15 – 35 days). *Garcia-Maurino and Boya, (1992)* along the postnatal period in rabbit observed rosette - like arrangement of pinealocytes which no longer seen

beyond 30 postnatal day. *Abou-Easa, (1996)* described an irregularly distribution of pinealocytes and rarely follicle arrangement of cells in puppies after vision. On the other hand, *Zach, (1960)* described the pineal parenchyma as lobulated and showing follicles at the periphery of the gland.

The presence of many mitotic divisions in the first stage was agreed with that of *Calvo et al., (1990)* who stated that the dog pineal parenchyma showed immature cells with many mitotic division in the first postnatal week, he added that it is feasible to discern the cell types characteristic for the adult pineal gland from 20 – 30 days onward, the same result were obtained by *Abou- Easa, (1996) and Ellsworth et al., (1985)*.

The cord-like structure that observed in our study at the first stage, which disappeared at the second stage was noticed by *Calvo et al., (1990)* which attributed this finding to, firstly the wide separation among the cells probably due to the hypertrophy of individual cell, secondary the thick layer surrounding the blood vessels is invaded by cells thus blurring the limits of the adjacent cell cords. *Ohshima and Matsuo, (1987)* observed large intercellular spaces having extremely low cell density in the parenchyma but not observe cords-like structure.

We observed that, from the second stage, the pineal parenchyma distinguished into densely packed cells at the periphery and loosely arranged cell at the center while *Boya et al., (1995)* described no difference in its distribution.

Our results showed that with the advances of age, the number of pinealocytes were decreased, the number of glial cells were increased and the pyknotic cells also. *Calvo et al (1990)* described pyknotic cells from 45 days onward in dogs, while *Lalitha and Seshadri, (1992)* in buffalo and *Humbert and Pevet, (1995)* in rat stated that, The degeneration was apparent in the parenchymal cells. *Calvo et al., (1990)* were distinguished subtle difference in the appearance of nuclei,

suggesting this finding to be a leading signs of cell type differentiation. The depletion of pinealocytes is associated with the lost of blood vessels in buffalo and camel (*Abou-Easa and Abd-El- Gawad, 2002*) who added that, it is a signs of involution because of the pinealocytes are crowded at the age of 1 – 6 month in buffalo but become loosely arranged at the age of 1 – 10 years, while in camel severe depletion occurred at the age of 3 – 10 years. The losing of blood vessels in buffalo is associated with calcification in its wall but in camel it is occurred by hyalinization of its wall. These finding not observed in the present study either by Von Kossa stain for the presence of calcification or general stains for the hyalinization. In this respect changes in the structure of the pinealocytes in our results could be attributed to either the pinealocytes undergoes differentiation to other cells specially glial cells, due to change in nuclear morphology and the presence of large number of glial filaments, or by pyknosis in which the pinealocytes were degenerated. The last suggestion augmented by wide separation of the cells from 2 years old dog onward.

The pigmented cells were localized at the periphery of the gland (ventral aspect) and near the blood vessels opposite to pigmented area in the brain. These cells were appeared at 2 days age with few pigment granules. The density of the granules increased with the progress of the age. These results were agreed with *Calvo et al., (1990)* and *Abou-Easa (1996)* in dogs, and *Ohashima and Matsuo, (1987)* in goat, but neither that of *Zach, (1960)* who described pigment cells as it is present in halve of the dogs she studied, nor *Calvo et al., (1988)* who described it in adult dogs only. They added that, the localization of pigmented cell always appear in the same situation in all glands, extra pineal pigmented cells can be seen in meningeal space near pigmented area of the glands (*Calvo et al, 1988*). *Abou-Easa ad Abd El-Gawad (2002)* in camel and buffalo described melanin pigment is scattered allover the cellular elements of the pineal tissue; some pinealocytes, glial cells and even fibroblasts in camel, they added that no pigment granules were observed in buffalo.

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التغيرات الهستولوجية فى الغدة الصنوبرية مع تقدم العمر فى الكلاب

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أجريت هذه الدراسة لمعرفة التغيرات الهستولوجية فى الغدة الصنوبرية فى الكلاب مع تقدم العمر عشرون كلباً من كلا الجنسين استخدمت فى هذه الدراسة تحت الظروف الضوئية العادية.

أوضحت هذه الدراسة أن الشكل الخارجى للغدة الصنوبرية لا يتغير فى الشكل بعد الولادة مع تقدم العمر وكذلك الانغماد الصنوبرى.

قسمت النتائج إلى ثلاث مراحل على أساس التغيرات. المرحلة الأولى تحتوى على عدد كبير من

الخلايا الصنوبرية وعدد قليل من الخلايا الدبقية. وكانت هذه المرحلة تحتوى على عديد من الإنقسام

الميتوزى فى الخلايا الصنوبرية. من المرحلة الثانية وما بعدها وجد أن الخلايا الصنوبرية تقل فى العدد مع زيادة عدد الخلايا الدبقية والخلايا ذات الأنوية الداكنة . وبدأ ظهور الزوائد السيئوبلازمية للخلايا الدبقية من الشهر الرابع وتفرعت إلى أفرع كثيرة.الخلايا التى تحتوى على صبغة الميلانين ظهرت من عمر يومان وكانت قليلة فى العدد وتحتوى على كمية صبغة قليلة ولكن مع تقدم العمر زادت هذه الخلايا وزادت كمية الصبغة داخلها.

من هذه الدراسة يتضح أن :-

- 1- الخلايا الصنوبرية تقل مع زيادة العمر وربما تتحول إلى الخلايا الدبقية وهذا الافتراض يعضد بالتغيرات المورفولوجية فى شكل النواه وزيادة عدد الزوائد السيئوبلازمية.
- 2- الخلايا الصنوبرية تقل مع زيادة الخلايا التى تحتوى على نواه داكنة وهذه علامة من تحلل الخلايا وهذه تعضد بزيادة المساحة البينية بين الخلايا فى العمر الكبير من سنتين إلى ما بعدها.