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Structure and ultrastructure of the pigmented cells in the adult dog pineal gland*

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INTRODUCTION

The presence of pigment in the mammalian pineal gland has been described in man (Bayerova, Bayer & Obrucnik, 1962) as well as in other mammals (bovines: Santamarina & Meyer-Arendt, 1956; rabbit: Romijn, 1973; pocket gopher: Sheridan & Reiter, 1973; chinchilla: Matsushima & Reiter, 1975; bat: Pevet, Kappers & Voute, 1977; horse: Cozzi & Ferrandi, 1984; Cozzi, 1986). Three classes of pigment may be found in the pineal gland: melanin, lipofuscin, and haemosiderin (Quay, 1974). In some of these species, the pineal pigment has been identified as melanin by histochemical methods (Santamarina & Meyer-Arendt, 1956; Cozzi & Ferrandi, 1984). Heretofore, the presence of pigment in the dog pineal gland has only been reported by light microscopy (Zach, 1960). According to this author, about half of the dogs showed pigment granules in the pineal gland. Pigmented cells of the dog pineal gland have not yet been studied with the electron microscope.

In a previous paper (Calvo, Boya & Garcia-Mauriño, 1988), the general ultrastructural features of the adult dog pineal gland have been described. The present paper deals with the light and electron microscopic features of the pigmented cells in the adult dog pineal gland.

MATERIALS AND METHODS

Twelve adult mongrel dogs, living under natural photoperiod (approximately 45° N latitude) and clinically healthy, were used for this study. The animals were killed under sodium pentobarbitone anaesthesia at 11.00 a.m. over a period between March and June. Two dogs, male and female, were studied at each of the following ages: 1 and 2 years for the light microscope study, and 9 months, 1, 2 and 4 years for the electron microscope study.

For light microscopy, the pineal glands were fixed either in Bouin or in 10% formalin. After embedding in paraffin, 7 μm serial sections were obtained. The identification of melanin was carried out by the methods recommended by Pearse (1972): bleaching with hydrogen peroxide and peracetic acid and the Masson Fontana silver method. The Perls method (Pearse, 1972) for demonstration of haemosiderin pigment was also used. Some sections were stained with haematoxylin and eosin for routine light microscope observations.

For the electron microscope study, the pineal glands were fixed by immersion in cold 3% glutaraldehyde in 0·1 M phosphate buffer, pH 7·4. After fixation, small blocks of the proximal region of the pineal gland and the adjacent posterior

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commissure were dissected out and washed in 0·1 M phosphate buffer. After postfixation in 1% osmium tetroxide in the same buffer, the samples were embedded in Vestopal. Semithin sections stained with toluidine blue were used to localise the pigmented cells. Ultrathin sections stained with uranyl acetate and lead citrate were examined in a Philips 201 electron microscope.

RESULTS

At the light microscope level, cells with pigment granules were seen in all the pineal glands examined. These cells were always found at the ventral surface near the base of the gland and beside the posterior commissure (Fig. 1). Pigmented cells usually gathered to form a cluster beneath the capsule of the gland (Fig. 1). Except for the presence of pigment granules, no morphological differences were found with the light microscope between pigmented and non-pigmented pineal cells. No limiting structure was found between the pigmented cell clusters and the surrounding pineal parenchyma.

Variations in the amount of pineal pigment were found among different animals and these were unrelated to the age or sex of the dog. The hybrid genetic character of the dogs used for this study did not allow us to establish any reliable relationship between the amount of pineal pigment and the strain of the animal. In general, those pineal glands that were more deeply pigmented showed more pigmented cells containing a higher number of darker granules.

The pigment granules appeared light to dark brown in colour in unstained paraffin sections and became colourless after bleaching the sections either with 10% hydrogen peroxide for 48 hours or with peracetic acid for 3 hours. The pigment showed a strong argentaffin reaction, as demonstrated by the Masson Fontana silver method (Fig. 2). No staining of pigment was found with the Perls method.

Cells with pigment granules were occasionally seen outside the pineal gland lying close to pigmented areas in the gland itself. These extrapineal pigmented cells were mainly localised in the posterior commissure (Fig. 3) or within the adjacent meningeal space (Fig. 1). In both locations, the light microscopic morphology of the extrapineal pigmented cells was very similar to that described for the pineal gland.

The electron microscope study confirms the presence of cells containing electron-dense granules at the sites described above at the light microscope level (Fig. 4). No basal lamina could be seen between the pigmented cells and the adjacent pineal cells nor the myelinated nerve fibres in the posterior commissure. However, meningeal clusters of pigmented cells showed a clearly defined basal lamina.

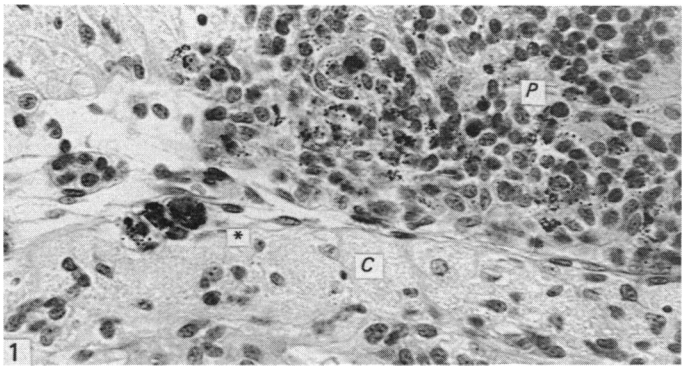
The ultrastructure of the pigmented cells was similar whatever the localisation. The small ovoid nuclei showed a thin peripheral rim of heterochromatin as well as one or two small nucleoli (Fig. 4). The cytoplasm contained free ribosomes and a few short cisterns of rough endoplasmic reticulum (Fig. 5). The Golgi apparatus was well developed and showed numerous associated vesicles. Centrioles and even cilia were frequently found in the Golgi zone. Dense bodies identifiable as lysosomes (Figs. 5, 6, 7) and numerous microtubules were seen throughout the cytoplasm. No microfilament

Fig. 1. Cells showing pigment granules at the ventral surface of the pineal gland. A narrow meningeal sheet enclosing a nodule of pigmented cells (asterisk) is seen between the pineal gland (*P*) and the posterior commissure (*C*) Haematoxylin and eosin. × 400.

Fig. 2. Intense argentaffin reaction of the pineal pigment granules. Masson Fontana silver method. Weak nuclear counterstaining with safranin. × 380.

Fig. 3. Cluster of pigmented cells (asterisk) in the posterior commissure near the pineal gland (*P*). Haematoxylin and eosin. × 100.

Fig. 4. Low power magnification of a cluster of pigmented cells in the pineal gland. × 5600.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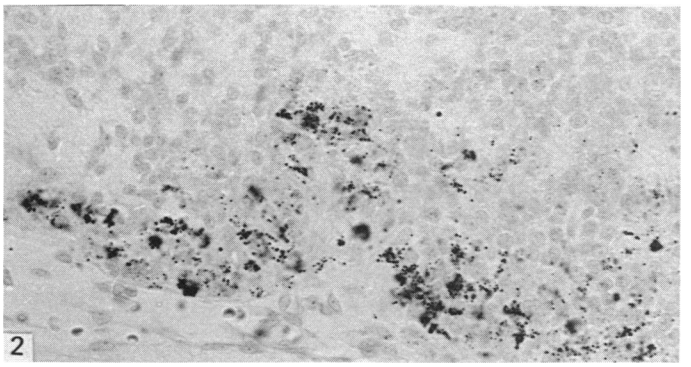
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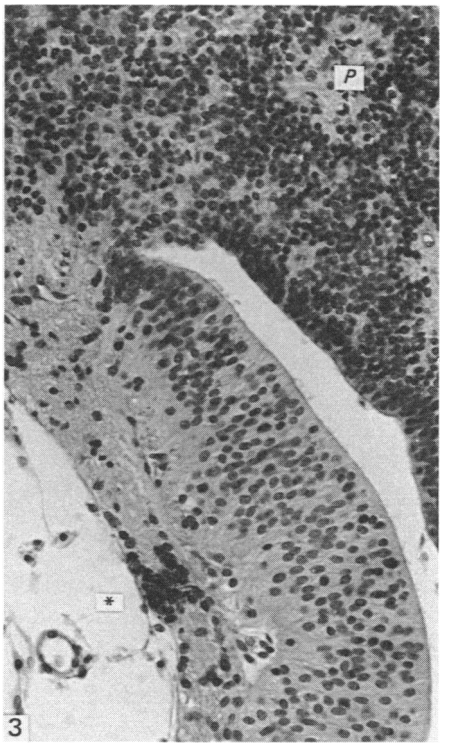
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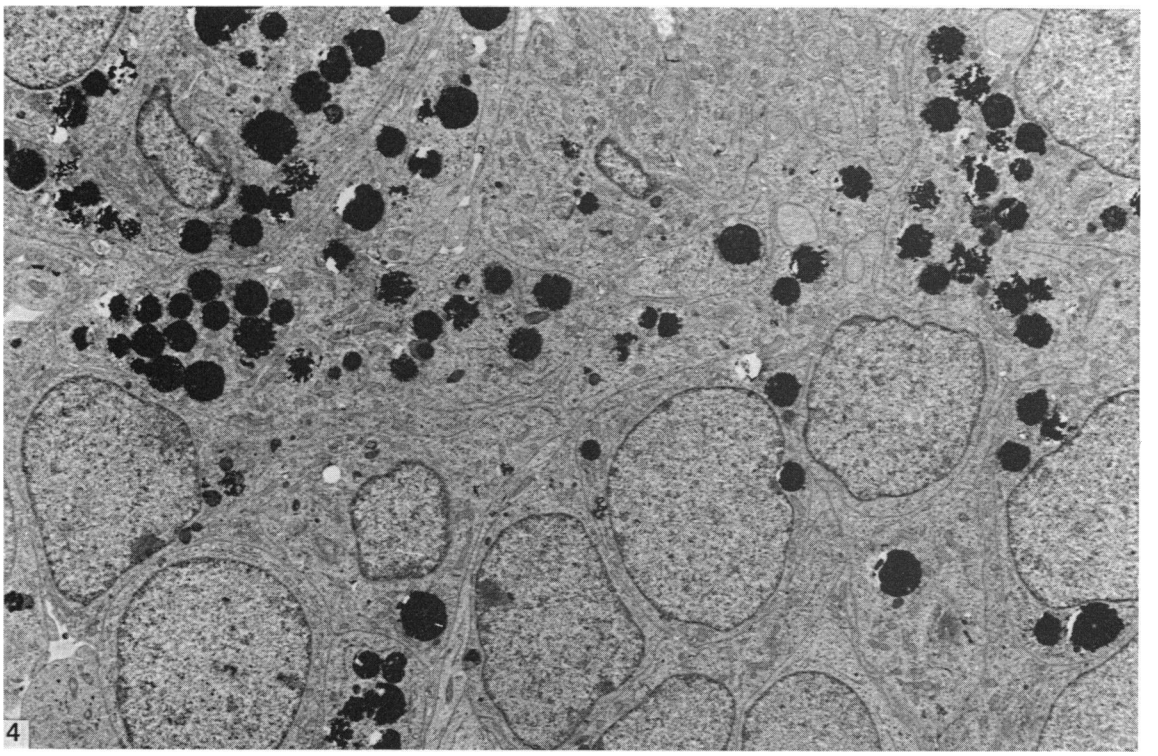
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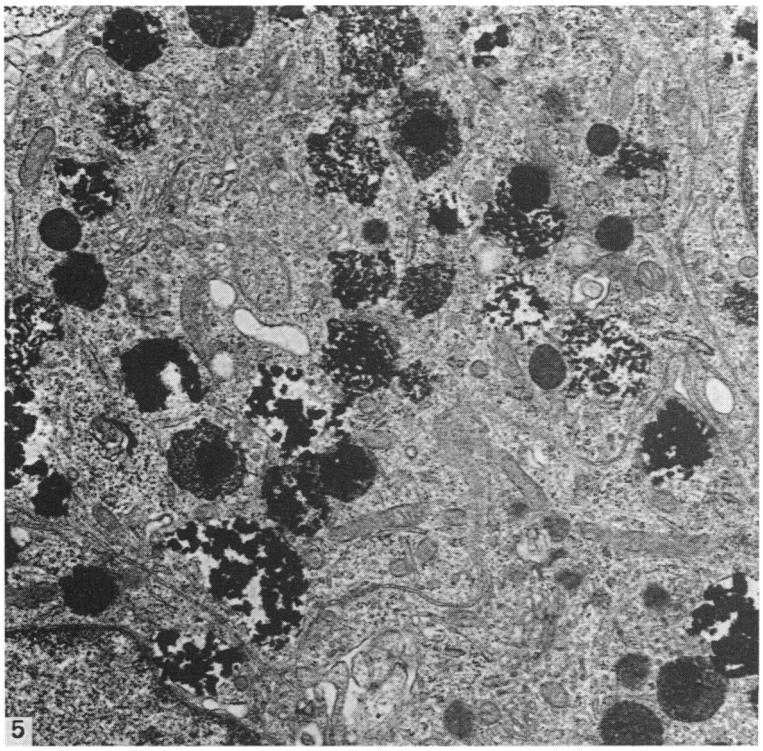

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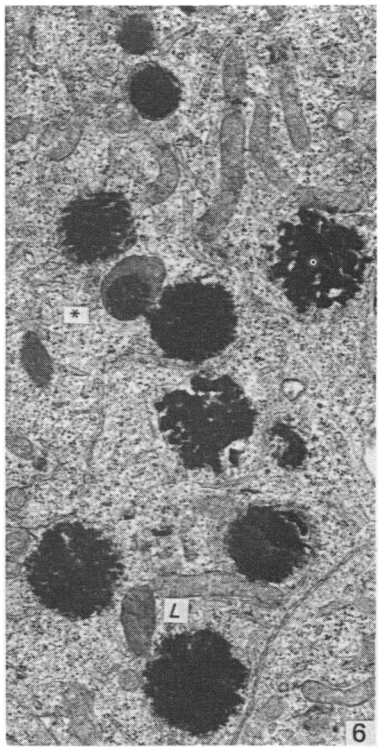

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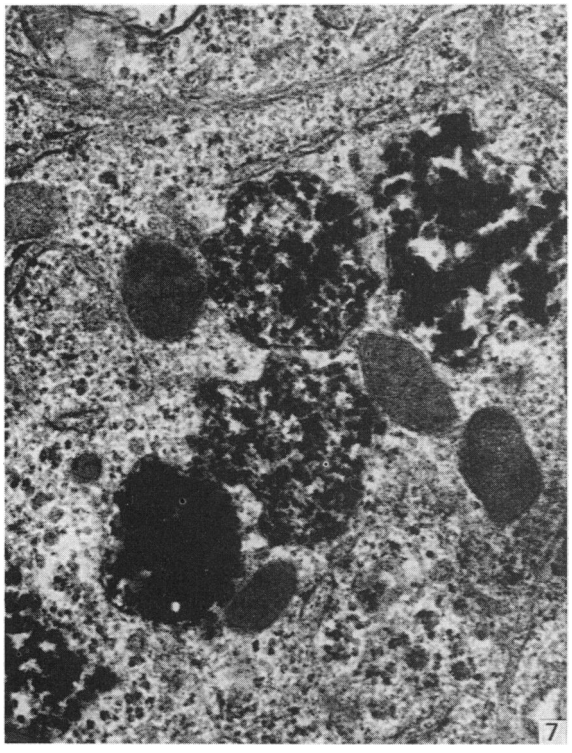

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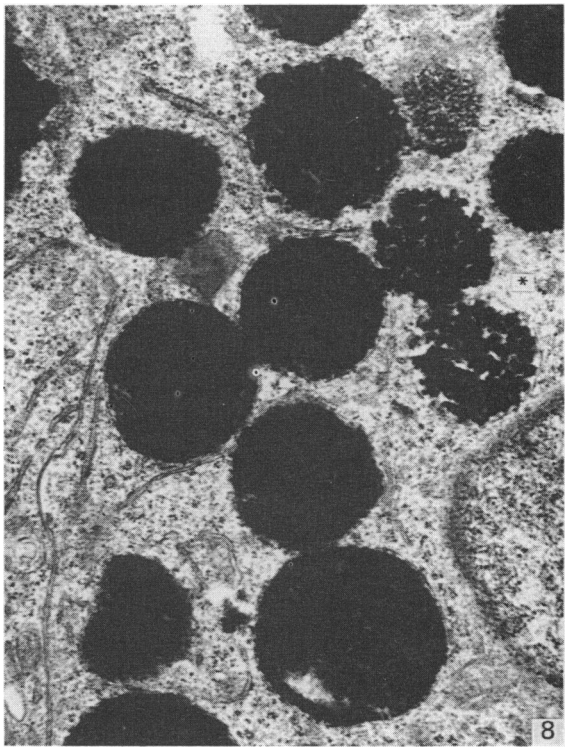

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could be found in pigmented cells. Coated vesicles appeared at the cell surface but subsurface cisterns were lacking. Pigmented cells showed some interdigitating lamellar processes.

The pigmented cells were characterised by the presence in their cytoplasm of large membrane-bound granules with an electron-dense content (Figs. 4–8). Variations in both size and number of pigment granules were found between different animals as well as between cells in the same gland. Based on morphological grounds, the pigment granules may be classified as follows.

(1) Small spherical or ovoid granules (0·4–0·6 μm in diameter), similar to lysosomal dense bodies, but containing a dark central mass (Fig. 6).

(2) Larger granules (0·6–1·1 μm in diameter) containing very electron-dense particles in a clear matrix. These particles sometimes coalesce into progressively larger clumps (Figs. 5, 7).

(3) Spherical granules of 0·8–1·2 μm filled with a very dense homogeneous material. They may represent the final stage in the coalescence of the particles described above (Figs. 4, 6, 8).

All these types of granule were found in the more deeply pigmented glands (Fig. 5). Frequently, a single cell showed granules of the three types described above. The less pigmented glands contained smaller numbers of smaller granules. Moreover, an additional type of pigment granule was seen in these glands, consisting of small granules containing electron-lucent droplets surrounded by a dark matrix.

Cells devoid of pigmented granules but sharing ultrastructural features with pigmented cells were occasionally seen far away from the pigmented area in the pineal gland.

DISCUSSION

According to our results, the pigmented cells are constant elements of the pineal gland in adult dogs. The number of pigmented cells is small in relation to the total number of pineal parenchymal cells. Wide variations could be appreciated between different animals. In the less pigmented pineal glands, only a small number of pigmented cells could be found after serial sectioning of the gland. This may explain the findings reported by Zachs (1960), according to which pigmented cells were only present in about half of the dogs.

The pigmented cells always appear in the same situation in all the glands. Furthermore, extrapineal pigmented cells can be seen in the posterior commissure and the meningeal space, always near the pigmented area of the gland. Ultrastructural features of both extrapineal and pineal pigmented cells are similar. Further studies on the development of the dog pineal gland are required to explain the selective localisation of the pigmented cells both inside and outside the pineal gland.

According to Quay (1974), three types of pigment can be identified in the pineal gland of mammals: melanin, lipofuscin and haemosiderin. The pigment of the dog pineal gland can be identified as melanin on light microscopy by its morphology and staining properties. This pigment is bleached by oxidants such as hydrogen peroxide

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Fig. 5. Cytoplasm of a pigmented cell. Several types of granules coexist in the same cell. × 15000.
Fig. 6. Lysosome-like body (*L*) among different types of pigment granules. One of them (asterisk) is similar to a lysosomal dense body but contains a highly electron-dense material. × 16000.
Fig. 7. Granules of pigment with an irregular outline containing electron-dense particles in a clear matrix. × 40000.
Fig. 8. Rounded granules filled with an electron-dense homogeneous material. Two granules analogous to those of Fig. 7 (asterisk). × 21000.

or peracetic acid and shows a strong argentaffin reaction with the method of Masson Fontana. As stated by Pearse (1972), these characteristics allow one to identify a pigment as melanin with reasonable confidence. On the other hand, the Perls method for the detection of haemosiderin is always negative. Ultrastructurally, the pigment in the dog pineal cells appears very different to lipofuscin.

Ultrastructurally, the pigment granules of the dog pineal gland are similar to those described in other mammals (Sheridan & Reiter, 1973; Cozzi, 1986), and almost identical to those of the rabbit (Romijn, 1973). Compared with other melanin granules, the dog pineal pigment closely resembles substantia nigra neuromelanin (Moses, Ganote, Beaver & Schuffman, 1966) rather than cutaneous melanin as previously described in the horse (Cozzi, 1986). The pigment granules of the dog pineal gland lacked striated fibrils typical of cutaneous melanin granules, showing a coarse granular content similar to that of neuromelanin granules. However, most of the pineal pigment granules were devoid of the lipoid globules described in substantia nigra neuromelanin (Moses *et al.* 1966). Pigment granules lacking both striated fibrils and lipoid globules have also been described in human choroid and pia mater (Moses *et al.* 1966).

The different types of granule observed in our study may correspond to different stages of maturation as previously discussed by Romijn (1973) in the rabbit pineal gland. The ultrastructure of smaller granules suggests that they can be related to lysosomes. Differences in morphology of larger granules can reflect a progressive storage of pigment within them.

Ultrastructurally, the pigmented cells can be classified as a special type of pinealocyte. In a previous study (Calvo *et al.* 1988), two different cell types, pinealocytes and astrocytes, were identified in the adult dog pineal gland. The pigmented cells lack the filaments that are characteristic of astrocytes in the pineal gland. They show instead small nucleoli, microtubules, centrioles and cilia, also present in the dog pinealocytes (Welser, Hinsman & Stromberg, 1968; Calvo *et al.* 1988). However, the presence of pigment granules and other ultrastructural features makes these cells somewhat different from typical dog pinealocytes. Pigmented cells may therefore constitute a special type of pinealocyte. In other mammals, these cells are usually classified as pinealocytes (Romijn, 1973; Pevet *et al.* 1977; Cozzi & Ferrandi, 1984; Cozzi, 1986), yet for some authors they represent a new cell type, different from pinealocytes (Sheridan & Reiter, 1973).

Scattered throughout the pineal gland we have found cells devoid of pigment granules but closely resembling pigmented cells at the ultrastructural level. This may suggest that the second pineal cell type in the dog has a wide distribution in the gland, accumulating pigment only in restricted areas. Nevertheless, the explanation for this behaviour requires further investigations.

Heretofore no functional role has been assigned to the pineal pigment in mammals. In dogs, the relative scarcity of pigmented cells and wide variations in amount among different animals militated against an outstanding functional role. Quay (1974) could not find any relationship between the amount of pineal pigment with age, sex or the reproductive state in rodents. Nevertheless, the selective localisation of pigmented cells in the dog pineal gland may provide a clue for discerning its physiological role.

SUMMARY

The light and electron microscopic features of pigmented cells in the adult dog pineal gland have been described. The presence of pigmented cells was a constant

characteristic of the dog pineal gland, though wide variations in the amount of pigment could be found among different animals. Conversely, the localisation of pigmented cells was very constant on the basal surface of the proximal region of the pineal gland. Frequently, clusters of pigmented cells were seen in the posterior commissure and the neighbour meningeal spaces, near the pigmented pineal zone. The pineal pigment has been identified as melanin according to its morphological features and histochemical properties. Several types of granules were identified ultrastructurally, apparently corresponding to different stages of a maturation process. The pigmented cells were identified as a special type of pinealocyte according to their ultrastructural features.

REFERENCES

BAYEROVA, G., BAYER, A. & OBRUCNIK, M. (1962). Zur Frage der fluorescenz- und polarisations-mikroskopischen Untersuchungen an der menschlichen Epiphyse. *Acta histochemica* **14**, 276–283.

CALVO, J., BOYA, J. & GARCIA-MAURIÑO, J. E. (1988). Ultrastructure of the pineal gland in the adult dog. *Journal of Pineal Research* (In the Press).

COZZI, B. (1986). Cell types in the pineal gland of the horse: an ultrastructural and immunocytochemical study. *Anatomical Record* **216**, 165–174.

COZZI, B. & FERRANDI, B. (1984). Fine structure and histochemistry of the equine pineal gland, with special reference to the possible functional role of the electrodense intrapinealocyte bodies. *Clinica veterinaria* **107**, 337–346.

MATSUSHIMA, S. & REITER, R. J. (1975). Comparative ultrastructural studies of the pineal gland of rodents. In *Ultrastructure of Endocrine and Reproductive Organs* (ed. M. Hess), pp. 335–356. New York: John Wiley.

MOSES, H. L., GANOTE, C. E., BEAVER, D. L. & SCHUFFMAN, S. S. (1966). Light and electron microscopic studies of pigment in human and Rhesus monkey substantia nigra and locus coeruleus. *Anatomical Record* **155**, 167–184.

PEARSE, A. G. E. (1972). *Histochemistry. Theoretical and Applied.* Edinburgh: Churchill Livingstone.

PEVET, P., KAPPERS, J. A. & VOUTE, A. M. (1977). The pineal gland of nocturnal mammals. I. The pinealocytes of the bat (*Nyctalus noctula*, Schreber). *Journal of Neural Transmission* **40**, 47–68.

QUAY, W. B. (1974). *Pineal Chemistry in Cellular and Physiological Mechanisms.* Springfield: Thomas.

ROMIJN, H. J. (1973). Structure and innervation of the pineal gland of the rabbit, *Oryctolagus cuniculus* (L.). II. An electron microscopic investigation of the pinealocytes. *Zeitschrift für Zellforschung und mikroskopischen Anatomie* **141**, 545–560.

SANTAMARINA, E. & MEYER-ARENDT, J. (1956). Identification of melanin in the bovine pineal gland. *Acta histochemica* **3**, 1–5.

SHERIDAN, M. N. & REITER, R. J. (1973). The fine structure of the pineal gland in the pocket gopher, *Geomys bursarius*. *American Journal of Anatomy* **136**, 363–382.

WELSER, J. R., HINSMAN, E. J. & STROMBERG, M. W. (1968). Fine structure of the canine pinealocytes. *American Journal of Veterinary Research* **29**, 587–599.

ZACH, B. (1960). Topographie und mikroskopisch-anatomischer Feinbau der Epiphysis cerebri von Hund und Katze. *Zentralblatt für Veterinärmedizin* **7**, 273–303.