ORIGINAL ARTICLE

The pinealocytes of the human pineal gland: a light and electron microscopic study

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The pinealocytes of the pineal gland of children and adults were studied at both light and electron microscopic levels. The pinealocytes were classified into light and dark pinealocytes on the basis of their shape, nuclear infolding, cytoplasmic contents and staining density. The light pinealocytes outnumber the dark pinealocytes and both of them have thin processes. The light pinealocytes have round or oval cell bodies and nuclei and have vesicles and ribbons. The dark pinealocytes showed more variations in their shape. The nuclear membrane of the dark cells showed numerous infoldings with deep invagination of parts of the cytoplasm within the nuclear folds, giving the appearance of nuclear pellets. The dark pinealocytes contain pigment in their cytoplasm. In addition to the light and dark pinealocytes a very small cell type with an extremely thin and elongated cell body and nucleus was found. The cells of this type were almost always associated with vacuoles filled with flocculent material and accumulations of presumptive secretion in the extracellular compartment. The findings of this study were discussed in the light of the published data about the pinealocytes of human and non-human species.

Key words: human, pineal gland, pinealocytes

INTRODUCTION

The mammalian pineal gland is mainly composed of pinealocytes. The morphological features of these cells have been studied in detail in non-human species. They have characteristic features such as deep infoldings of the nuclear membrane, vesicles, pigment, cilia and ribbons [1–3, 5, 6, 8, 10, 13, 16–19, 22]. On the basis of their different degrees of staining density the pinealocytes have been classified into light and dark pinealocytes in some previous studies [4, 6, 9, 10, 12].

Human pinealocytes have been studied at both light microscopic level [11, 14, 21] and electron microscopic level [7, 15]. Min et al. [14] and Koshy and Vettivel [11] classified the pinealocytes into light and dark cells on the basis of their different staining densities. Tapp and Huxley [21] demonstrated nuclear

infoldings and pigments in the cytoplasm of the pinealocytes of pineal glands of patients coming to necropsy. The last named study did not classify the pinealocytes into subtypes and therefore it was not clear whether these nuclear infoldings and pigments were present in all human pinealocytes or only in one pinealocyte subtype. The ultrastructure of human pinealocytes was studied in both human foetuses [15] and old patients [7]. Moller [15] demonstrated melanin pigments and vesicles in the human foetal pinealocytes. This study, however, found no ribbons in the pinealocytes of human foetuses. Hasegawa et al. [7], on the other hand, demonstrated ribbons but not melanin pigments or vesicles in the pinealocytes of elderly people. Whether these results represent age-related differences between human foetuses and aged adults was not really clear.

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It was clear that there was a gap related to the ultrastructure of human pinealocytes in children and adults. Study of the pinealocytes of these age groups may shed some light on the possible morphological and functional evolution of the human pineal gland. The objectives of this study were, firstly, to study the morphological features of the pinealocytes of children and adults at both light and electron microscopic levels and, secondly, to compare the pinealocytes of children and adult humans with the pinealocytes already described in human foetuses, in old age and in non-human species.

MATERIAL AND METHODS

This study was based on the examination of the pineal glands of 14 individuals (8 men and 6 women) aged from 5 to 50 years (Table 1) who had died in road accidents. The pineal glands were obtained at autopsy 0.5–2 hours following death and were immediately fixed by immersion in 10% neutral buffered formalin or 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer.

Glands fixed in formalin

Paraffin-embedded sections were cut at 5 μm and stained according to haematoxylin and eosin (HE), double stain (light green and acid fuchsin) as described by Tandler et al. [20], Masson's trichrome and the Papanicolaou method as described by Bio-Optica. The different types of cells and their processes were best stained in the double stain.

Table 1. Age and sex of the 14 individuals used in this study

Individual	Age (years)	Sex
1	5	Female
2	5	Female
3	5	Male
4	7	Female
5	8	Male
6	8	Male
7	15	Male
8	18	Female
9	32	Male
10	36	Male
11	40	Female
12	45	Male
13	45	Female
14	50	Male

Glands fixed in glutaraldehyde

The glands were cut into small pieces (cubes of approximately 1 mm). These small pieces were fixed by immersion in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer for two hours. The pieces were washed with buffer, postfixed in 1% osmium tetroxide in buffer, dehydrated with ethanol and embedded in resin. Ultrathin (70–90 nm thick) sections of the pineal glands were cut and mounted on 200 mesh copper grids and stained with uranyle acetate and lead citrate.

RESULTS

The results of this study showed that the pinealocytes of human children and adults have characteristic features, including nuclear infoldings, vesicles, pigments and ribbons. These structural features were found in both children and adults. Cilia with a 9 × 2 + 0 microtubular pattern (Fig. 1) were also found. In this study the pinealocytes were classified according to (1) the shape of the cell body and nucleus, (2) nuclear lobulation, (3) the contents of the cytoplasm and (4) staining density. With the use of these criteria two types of pinealocyte (light and dark) were found (Fig. 1). Extremely thin and elongated cells were also found at the electron microscopic level. These cells were clearly different from both the pinealocytes and glia cells seen in this study.

Type I pinealocytes (light pinealocytes)

The light pinealocytes (Figs. 1, 2) represent the majority of pinealocytes in the child and adult human pineal gland. These round or oval cells had a mean diameter ranging from 7 to 11 μm with an average of 9 μm (n = 80). Their round or oval nuclei were large with an average mean diameter of 5.8 μm (n = 80) and had a regular outline with no folds. There was a slight condensation of the chromatin material at the inner surface of the nuclear envelope. These cells contain vesicles in their processes and terminals (Fig. 2). They also had rod-like ribbons in their cytoplasm (Fig. 2).

Type II pinealocytes (dark pinealocytes)

These round, oval or elongated cells had a mean diameter ranging from 7 to 11.2 μm with an average of 9.4 μm (n = 50). Their nuclei were very irregular and large with a mean diameter equal to 6.4 μm (n = 50). Numerous infoldings of the nuclear membrane were found at both light and electron microscopic levels (Figs. 1, 3) with invagination of parts of the cytoplasm into the nuclear folds, giving the appearance of
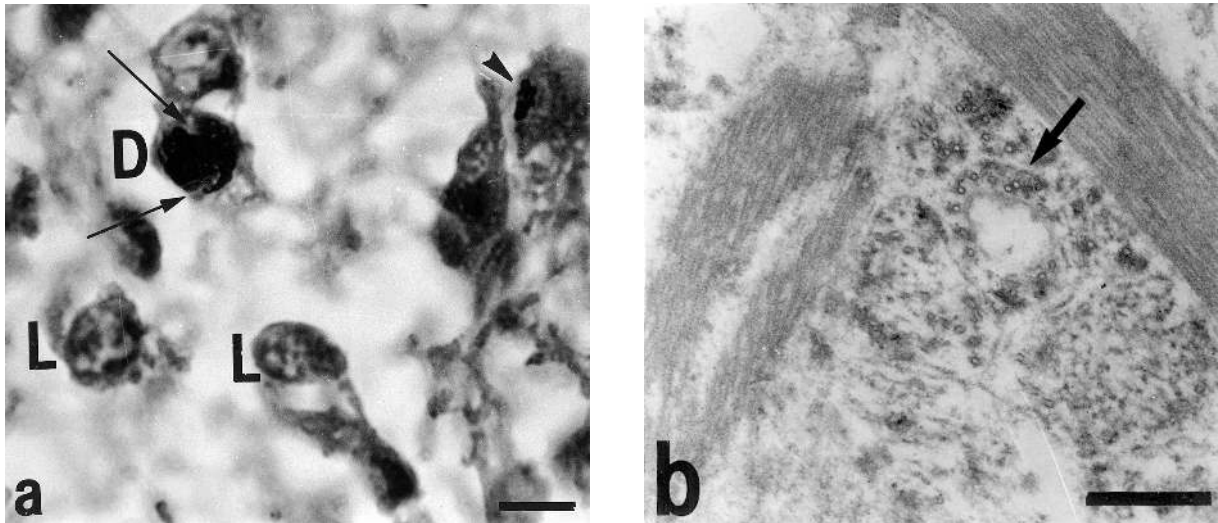
Figure 1. **A.** Light micrograph of two light pinealocytes (L) and one dark pinealocyte (D). Note the nuclear folds (thin arrows) and the sponge-like dark appearance (nuclear pellet) of the nucleus of the dark pinealocyte. A small part of the nuclear pellet of another dark pinealocyte is shown in the upper right part of the figure (arrow head) (Bar = 9 μm). Double stain (light green and acid fuchsin). **B.** Electron micrograph of cilium with a 9 $\times$ 2 + 0 microtubular pattern (arrow) (Bar = 0.4 μm). Note: it was not possible to determine whether the cilia belong to light and/or dark pinealocytes.
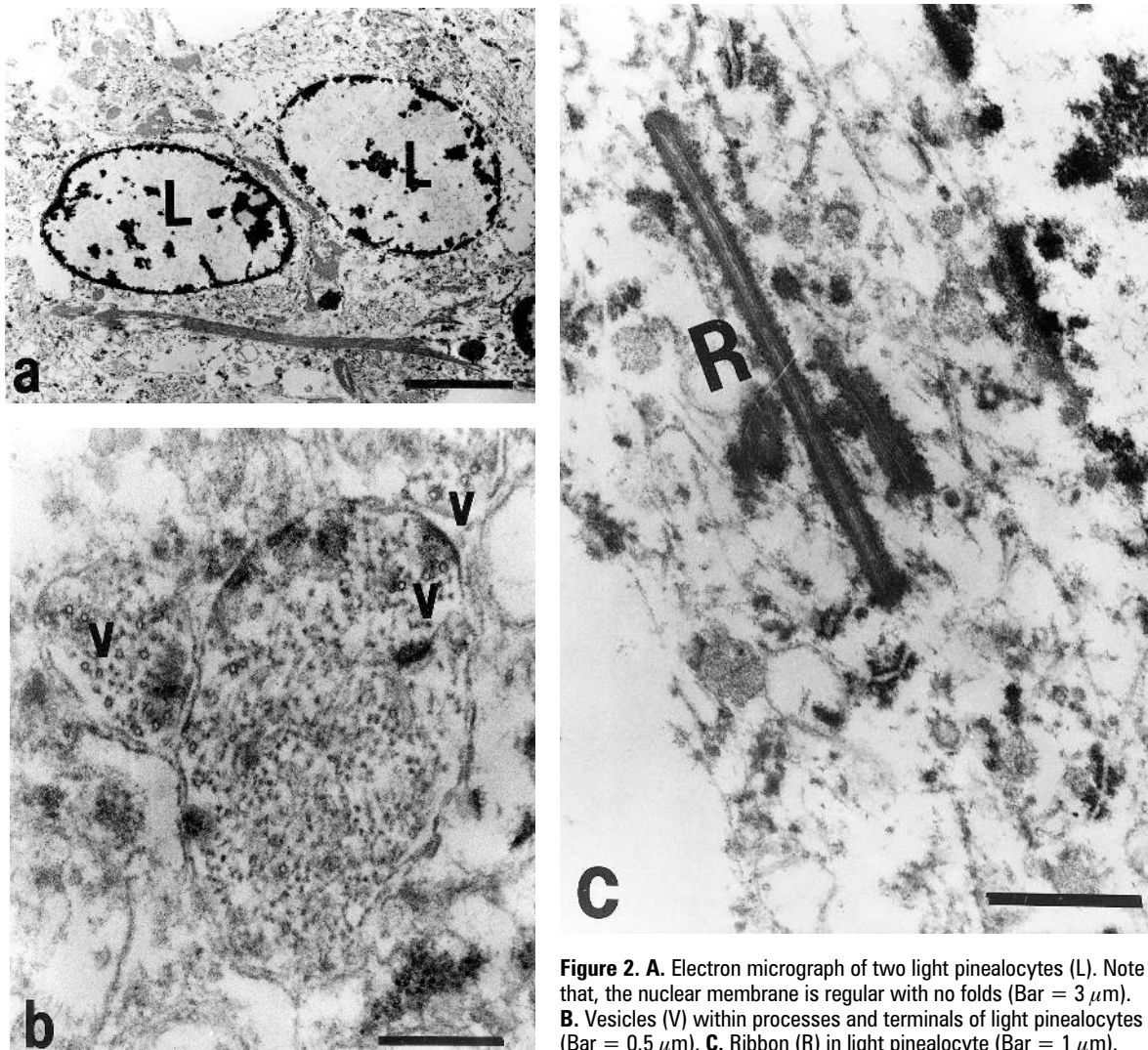

Figure 2. A. Electron micrograph of two light pinealocytes (L). Note that, the nuclear membrane is regular with no folds (Bar = 3 μm). **B.** Vesicles (V) within processes and terminals of light pinealocytes (Bar = 0.5 μm). **C.** Ribbon (R) in light pinealocyte (Bar = 1 μm).
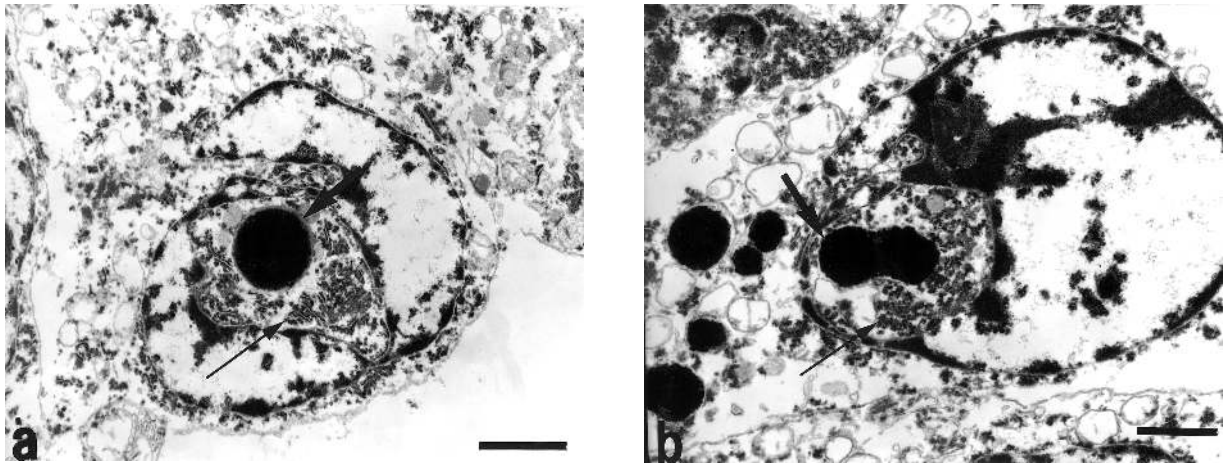
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Figure 3. Electron micrographs of two dark pinealocytes (**A** and **B**). Note the deep infoldings of the nuclear membrane with a rough endoplasmic reticulum (thin arrows) and pigments (thick arrows) in the cytoplasm that invaginate the nuclear folds (Bars = 1.5 μm).

nuclear pellets at the light microscopic level. The nuclear pellets were best seen in the double (light green and acid fuchsin) stain. In this stain the nuclei of the dark cells were formed of sponge-like dark masses that overlap with parts of the cytoplasm (Fig. 1). At the electron microscopic level, it was interesting to note that the parts of the cytoplasm that invaginate into the nuclear folds contain a large amount of rough endoplasmic reticulum (Fig. 3). There was a condensation of the chromatin material on the inner surface of the nuclear envelope. The cytoplasm of these cells contains pigments frequently seen within the nuclear folds (Fig. 3).

Type III cells

In addition to the two types of pinealocytes described above a third unusual type of cell was seen at the electron microscopic level (Fig. 4). Cells of this type have very elongated and thin cell bodies and nuclei. The longest diameter of these cells ranges from 12 μm to 32 μm with an average of 24 μm (n = 12) and their shortest diameter ranges from 0.5 μm to 1.4 μm with an average of 0.8 μm (n = 12). The nuclei almost completely fill these cells and leave only a very thin rim of cytoplasm around them (Fig. 4). The longest diameter of these nuclei ranges from 8 μm to 20 μm with an average of 16 μm (n = 12) and their shortest diameter ranges from 0.4 μm to 1.3 μm with an average of 0.7 μm (n = 12). These cells were almost always associated with vacuoles filled with flocculent material and accumulations of presumptive secretion in the extracellular compartment (Fig. 4). These vacuoles, together with the very small size of these cells and their very elongated shape, differentiate the cells from both pinealocytes and glia cells.

DISCUSSION

The human pinealocytes of children and adults were studied and compared with their counterparts in non-human species and in human foetuses and elderly patients. The results of this study showed that the pinealocytes of children and adults have characteristic features including nuclear infoldings, pigments, vesicles, ribbons and cilia. In this study vesicles and ribbons were found in light pinealocytes, whereas pigments and nuclear folds were found in dark pinealocytes (Table 2). Vesicles and melanin pigment were observed in human foetal pinealocytes [15], while nuclear folds and ribbons were demonstrated in elderly patients [7]. These results, shown in Table 2, may suggest that some of these morphological features such as ribbons and nuclear folds were developed after birth, while other morphological features such as pigments and vesicles developed in foetuses, continue in children and adults and disappear in old age.

Min et al. [14] showed that the dark pinealocytes were the predominant cell type at birth. However, the number of light pinealocytes gradually increased with age, and by the age of one year only scattered dark pinealocytes were still present in the human pineal gland. The presence of a good number of dark pinealocytes in human children and adults in this study suggests that the loss of the dark pinealocytes during the first year of life does not continue with age and even after thirty or forty years the human pineal gland still has a good number of dark pinealocytes [14].

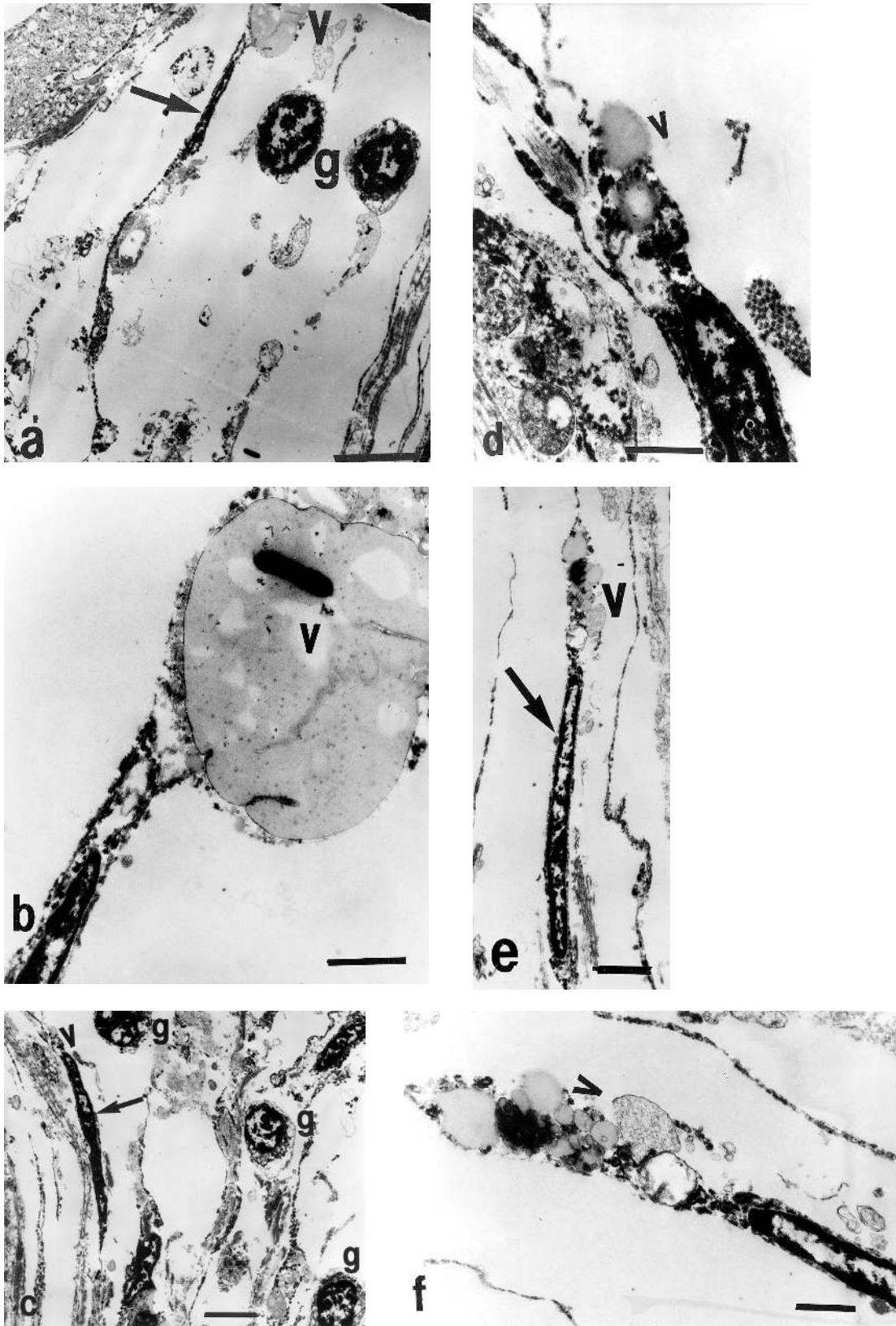The presence of pigment (most probably melanin pigment) in dark but not light pinealocytes as

Figure 4. A. Electron micrographs of one type III cell (arrow) and two glia cells (g). Note the branch (b) of the type III cell that arises from one end of the cell and the large vacuole with flocculent material (V) at the other end of the cell (Bar = 6 μm). This end of the cell with the vacuole is shown at a higher magnification in **B** (Bar = 1 μm). **C.** Electron micrograph of one type III cell (arrow) with vacuoles (V) at one end (Bar = 6 μm). This end of the cell with vacuoles (V) is shown at a higher magnification in **D** (Bar = 1 μm). **E.** Electron micrograph of one type III cell (arrow) with vacuoles (V) at one end (Bar = 2 μm). This end of the cell with vacuoles (V) is shown at a higher magnification in **F** (Bar = 1 μm). Note that the nuclei of type III cells occupy most of the cell bodies, leaving only very thin rim of cytoplasm around them.

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Table 2. Ultrastructural features of human pinealocytes of different ages

Ultrastructural feature	Foetus [15]	Children (this study)	Adult (this study)	Old [7]
Nuclear folds	–	+ (Dark pinealocytes)	+ (Dark pinealocytes)	+
Pigments	+	+ (Dark pinealocytes)	+ (Dark pinealocytes)	–
Vesicles	+	+ (Light pinealocytes)	+ (Light pinealocytes)	–
Ribbons	–	+ (Light pinealocytes)	+ (Light pinealocytes)	+
Cilia	+	+	+	+

reported in this study comes in agreement with a similar finding reported in human infants [14].

The cilia found in this study were similar to the cilia described in the human foetal pineal gland [15] and those found in old age [7]. They contained nine pairs of microtubules but did not show a pair in the centre. Hasegawa et al. [7] suggested that the cilia they found in the pineal gland of elderly humans belonged to photoreceptor cells. The presence of cilia with a $9 \times 2 + 0$ microtubular pattern in foetuses, children, and adults and in old age suggests that the human pineal gland may maintain photoreception function at all ages.

The nuclear folds with rough endoplasmic reticulum and pigments invaginating them seem to represent the anatomical substrate of the sponge-like dark nuclear pellet of the dark pinealocytes seen under the light microscope.

Previous human and non-human studies classified pinealocytes into light and dark cells mainly on the basis of different staining densities [9–12, 14]. In this study the human pinealocytes were classified into two distinct subtypes on the basis of their shape, nuclear lobulation, cytoplasmic contents and staining densities. The absence of classification of the pinealocytes into subtypes in some studies [7, 15, 21] may be caused by limitations of the techniques used in these studies. For example, Tapp and Huxley [21] applied the routine haematoxylin and eosin (HE) stain, which also failed to distinguish between the light and dark pinealocytes in this study. At the light microscopic level, only the double stain developed by Tandler et al. [20] and applied in this study clearly distinguished between the light and dark pinealocytes with nuclear pellets for the dark pinealocytes.

The very small type III cells described in this study had very thin and elongated cell bodies and nuclei. They were almost always associated with vacuoles filled with flocculent material and accumulations

of presumptive secretion in the extracellular compartment, suggesting a high level of secretory activity. These features made these cells different from any cell type previously described in the pineal gland. The very thin and elongated shape of these cells and their small number may explain why these cells were not demonstrated in any of the previous studies. The presence of this, it is hoped, new cell type may suggest species differences between human and non-human species and may form part of the continuous process of morphological and functional evolution of the pineal gland.

ACKNOWLEDGEMENT

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