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Postnatal development of cell types in the rat pineal gland

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INTRODUCTION

The adult rat pineal gland contains two main parenchymal cells or pinealocytes which may be identified by their ultrastructural features (Wolfe, 1965; Arstila, 1967). These cells have received different names according to the different authors (see review by Pevet, 1977). Although the ultrastructural features of adult pinealocyte are quite well known, there are few studies on cell type differentiation during pineal gland development. The beginning of this differentiation varies according to different authors. Karasek (1974) describes pinealocyte differentiation in the rat between days 10 and 14 after birth, while Steinberg *et al.* (1981) describe two cell types in rats 2 days old. Bayerova & Malinski (1972) have studied the quantitative postnatal ultrastructural changes, finding few changes between days 5 and 10 after birth and the adult stage. These authors, however, have not described pinealocyte cell types. Moreover, while some studies do exist on changes of the different organelles of the main cell type in the neonatal rat pineal gland (centrioles and cilia: Lin, 1970; junctional complexes: Zimmerman & Tso, 1975; synaptic ribbons: King & Dougherty, 1980), little is known about the development of the other pineal cell type.

The pineal gland of the newborn rat displays undifferentiated cells or pinealoblasts (Calvo & Boya, 1981). In the present study, we describe, using electron microscopy, the differentiation of pineal gland cell types after birth.

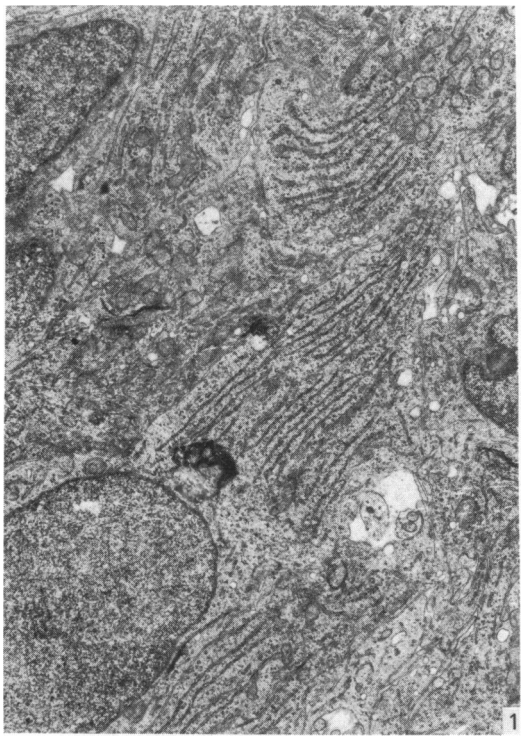
MATERIALS AND METHODS

Forty four Wistar albino rats of both sexes were kept under standard conditions of light (14L:10D) and feeding. The animals were killed by decapitation at the following ages: 1, 3, 5, 7, 10, 15, 20, 25, 30, 45 and 60 days. For each age group, four pineal glands (two of each sex) were taken and fixed by immersion in 0·1 M phosphate-buffered 3 % glutaraldehyde or 2 % glutaraldehyde–2 % paraformaldehyde, pH 7·4. After washing them through the same buffer, they were post-fixed in phosphate-buffered 1 % osmium tetroxide and embedded in Vestopal. Ultrathin sections were obtained in an LKB ultramicrotome, stained with uranyl acetate and lead citrate, and examined in a Philips 201 EM.

RESULTS

Day 1

The pineal parenchyma showed undifferentiated cells or pinealoblasts, whose structure was similar to that described for the last stages of the embryonic period (Calvo & Boya, 1981).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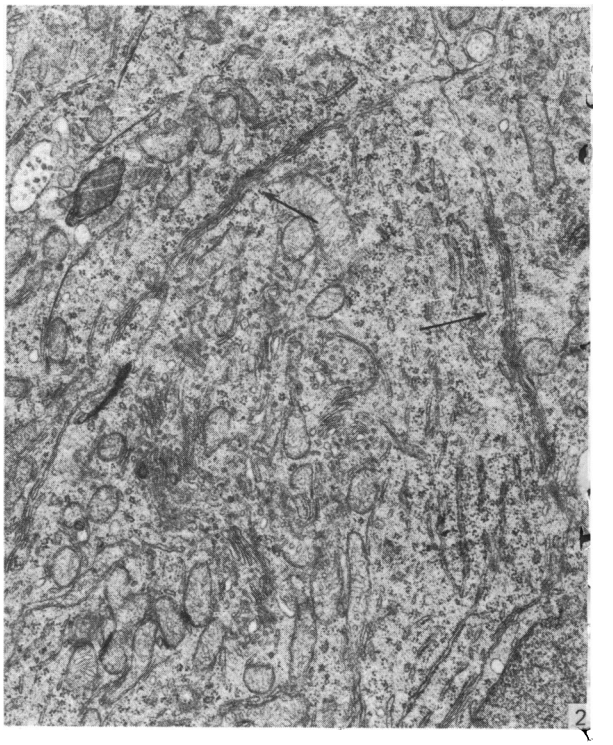
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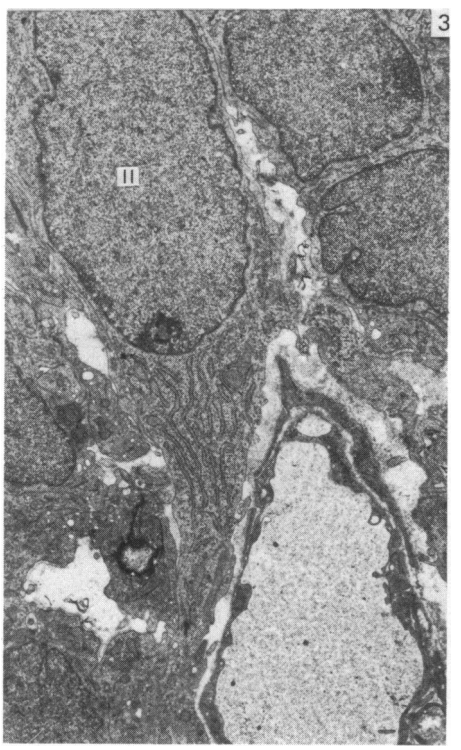

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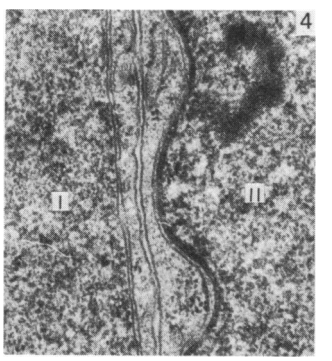

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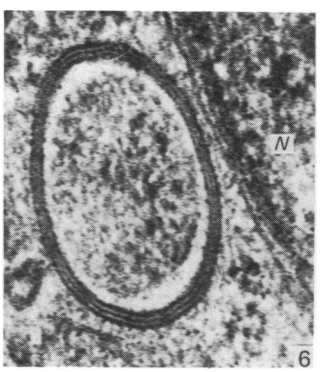

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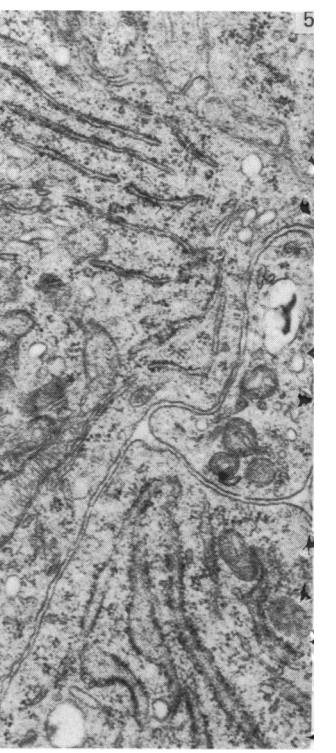
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Day 3

The beginning of pineal cell type differentiation was observed. The main pinealocytes, which will be referred to as Type I according to the terminology proposed by Pevet (1977), were characterised by the appearance of groups of long parallel cisterns of rough endoplasmic reticulum (Fig. 1). Subsurface cisterns were also constantly found in this cell type (Fig. 2). They were found only along the surface of contact between two Type I cells, frequently at the same level in both of them. This cell type frequently displayed centrioles, and cilia were sometimes found.

The second cell type, or pinealocyte Type II (also described as dense, interstitial, glial, etc. by other authors; see Pevet, 1977), appeared as a long cell with rather extensive cytoplasm, generally located near a connective tissue space (Fig. 3). The ovoid nucleus presented little heterochromatin, and had a very clear fibrous lamina in its nuclear envelope (Fig. 4). The cytoplasm contained abundant rough endoplasmic reticulum (Fig. 3). Its cisterns, wider than those of the Type I pinealocyte, contained a visible material (Fig. 5). Finally, Type II pinealocytes presented clear vacuoles in which remnants of organelles were sometimes found enclosed by two fused membranes (Fig. 6). With routine techniques for electron microscopy, the ultrastructural appearance (at high magnifications) of these membranes resembled gap junctions. These vacuoles were specific of this cell type.

At 3 days of age, nerve fibres were first found in the connective tissue spaces of the gland. The axons located near the parenchyma were frequently in contact with Type II pinealocytes, sometimes being partially surrounded by these cells.

Days 5 to 10

Pinealoblasts continued to differentiate into pinealocytes. Thus, the number of pinealoblasts decreased progressively. Although the already differentiated pinealocytes still presented little cytoplasm, they were recognised by the characteristics described in the previous stage.

Days 15 to 20

The pineal parenchyma already showed its typical cord-like appearance (Fig. 7). Type I pinealocytes now had more extensive cytoplasm and clear and dense varieties of this cell type were found (Fig. 7). They differed mostly in the density of the nucleoplasm and the cytoplasmic matrix. The rough endoplasmic reticulum of this cell type was less prominent than in previous stages. Frequently, lipid droplets

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Fig. 1. Day 3. A differentiating Type I pinealocyte shows groups of parellel cisterns of rough endoplasmic reticulum. × 8900.

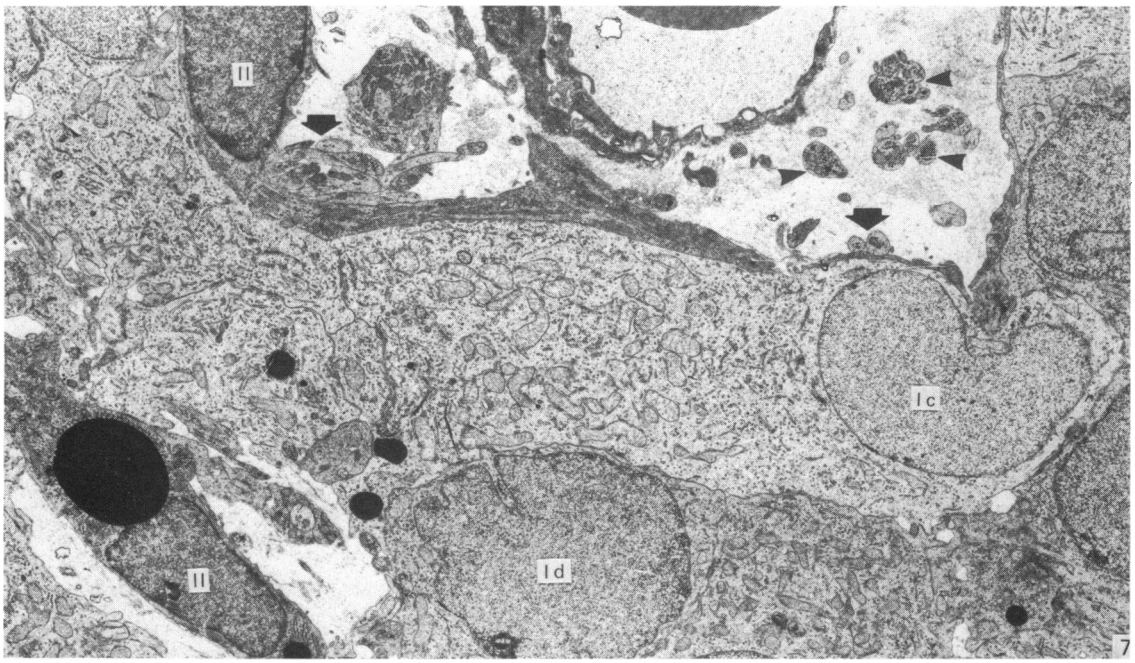
Fig. 2. Day 3. Cytoplasm of Type I pinealocyte exhibits abundant mitochondria and a Golgi complex. Arrows, subsurface cisterns. × 11000.

Fig. 3. Day 3. A differentiating Type II pinealocyte (II) is in contact with a connective tissue space. × 5600.

Fig. 4. Day 3. The nucleus of Type II pinealocyte (II) exhibits a fibrous lamina more developed than that of the nearby Type I pinealocyte nucleus (I). × 21000.

Fig. 5. Day 3. Differences in the rough endoplasmic reticulum between Type I (top) and Type II (bottom) pinealocytes are shown. × 38500.

Fig. 6. Day 3. Type II pinealocyte. A high magnification image of a clear vacuole with a double membrane is shown. *N*, nucleus. × 85000.



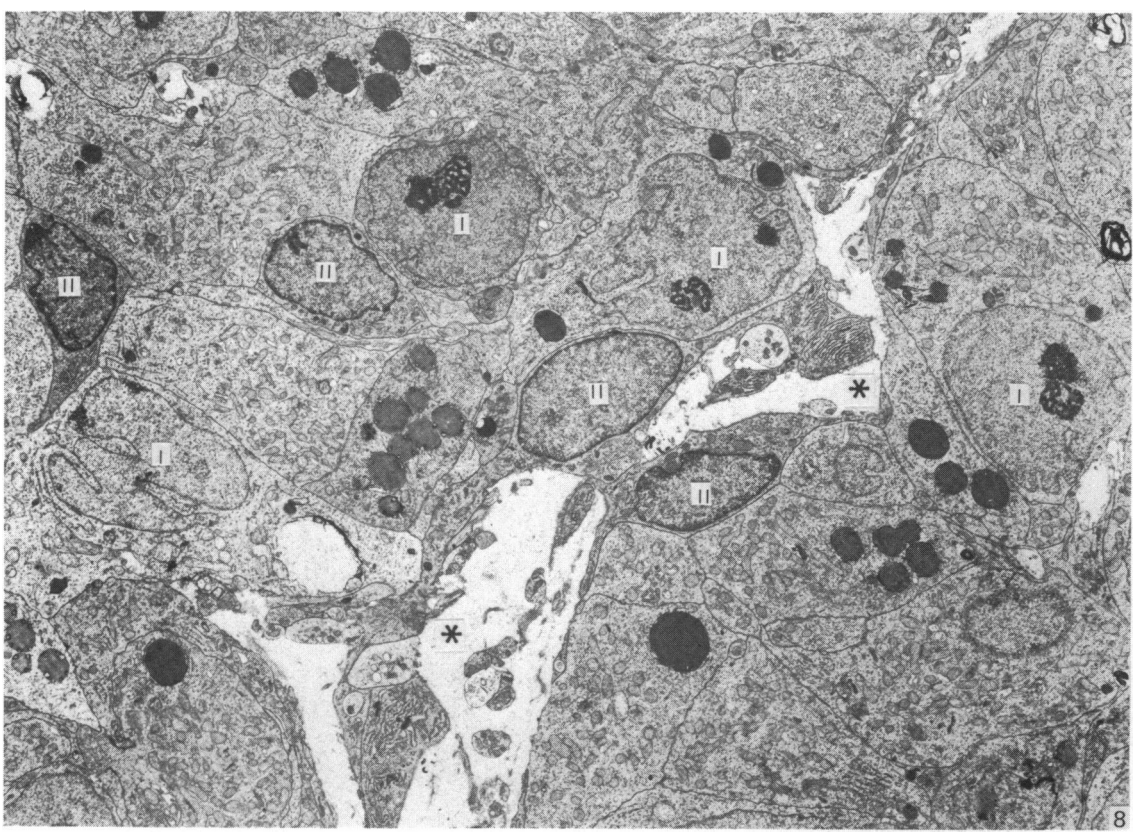


Fig. 7. Day 20. Differentiation of pinealocyte cell types. Two varieties of Type I pinealocyte, clear (Ic) and dense (Id), may be observed. Type II pinealocytes (II) show lamellar processes. Arrowheads, nerve fibres. Arrows, nerve fibres in contact with a Type II pinealocyte. × 5700.

Fig. 8. Day 60. A low magnification electron micrograph shows a connective tissue space (*) and parenchymal cellular cords. I, Type I pinealocytes; II, Type II pinealocytes. × 3800.

(Fig. 7) and small dense bodies (usually in relation to vacuoles) were observed. Centrioles were less frequently found and they sometimes showed an opening in their wall.

Type II pinealocytes appeared as flattened cells, generally located in the limit between the parenchyma and the stroma, and forming a discontinuous layer (Fig. 7). Sometimes a Type II pinealocyte was located inside a connective tissue space, usually keeping contact with the rest of the parenchyma by its processes.

Up to 15 days of age there were still some cells with sparse cytoplasm similar to those in the younger specimens.

Days 25 to 30

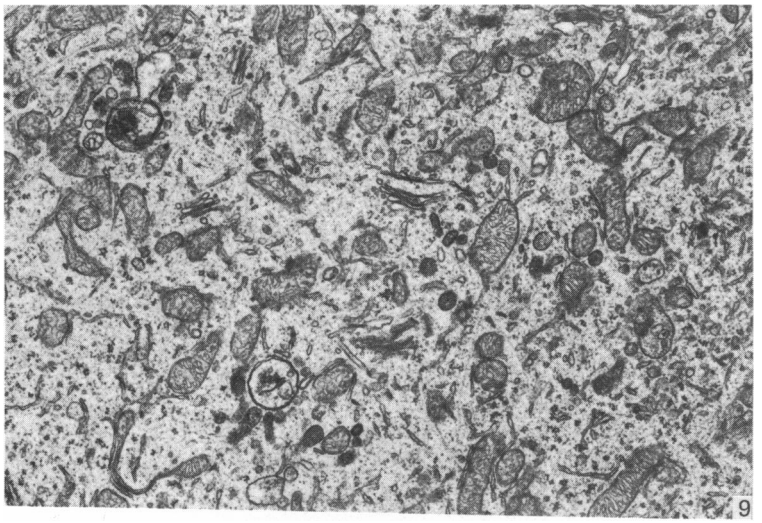
At this stage the process of cell differentiation had already ended. The nucleus of Type I pinealocytes now presented infoldings in its surface and prominent nucleoli. The cytoplasm showed an increase in lipid droplets and dense bodies as well as the appearance of areas rich in both rough and smooth endoplasmic reticulum. Type II pinealocytes presented few changes.

Days 45 to 60

Type I pinealocytes displayed extensive cytoplasm rich in organelles (Fig. 8). Rough and smooth endoplasmic reticulum and free ribosomes were found throughout the cell. However, certain areas near the cell membrane showed more abundant rough and smooth endoplasmic reticulum. In relation to these areas, there were lipid droplets and abundant free polyribosomes. Fewer and shorter subsurface cisterns were still found. Lipid droplets were larger and more abundant, although they were still not found in most cell profiles (Fig. 8). The Golgi complex usually presented small and numerous stacks of cisterns located in a large area near the nucleus. Most Type I pinealocytes now presented dense bodies. These were small, had a dense content, and generally formed groups related to vacuoles and multivesicular bodies (Figs. 9, 10). These vacuoles frequently contained remnants of organelles (Fig. 10). Other findings were microtubules as well as synaptic ribbons which were located both in the soma and in the thin processes near connective tissue spaces (Fig. 10).

From the first day after birth, groups of junctional complexes were found between Type I pinealocytes. The number of these groups was quite low until 15 days, after which they increased progressively. Type I pinealocytes showed a clear polarity toward the group of junctional complexes. The supranuclear cytoplasm decreased progressively towards a narrowed area where it presented junctional complexes (Type zonulae adhaerentes) in the area of union with neighbouring cells (Figs. 11, 12). Microtubules were abundant in this narrowed part. Above the area of junctional complexes there were terminal clubs full of clear round or flattened vesicles (Figs. 11, 12). A few mitochondria, small lipid droplets and multivesicular bodies were also found. The junctional complexes surrounded, often incompletely, a central cavity. Frequently, this cavity opened to a connective tissue space, thus maintaining the location of the junctional complexes at the parenchyma–stroma boundary (Fig. 11). This location explains the frequent finding of terminal clubs in a connective tissue space. The cavities found deep inside the parenchyma often had a minimal diameter thus appearing totally occupied by terminal clubs. In these cases, the clubs were sometimes located in an infolding of one of the nearby cells (Fig. 12).

The rough endoplasmic reticulum was still the most developed organelle of



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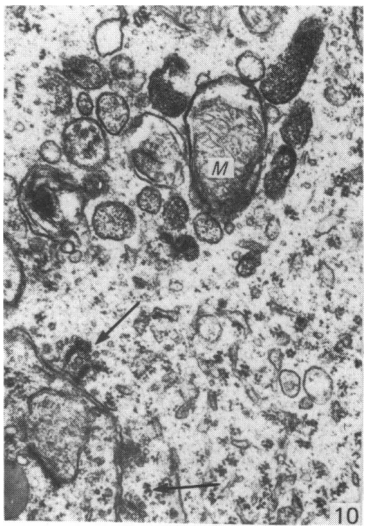

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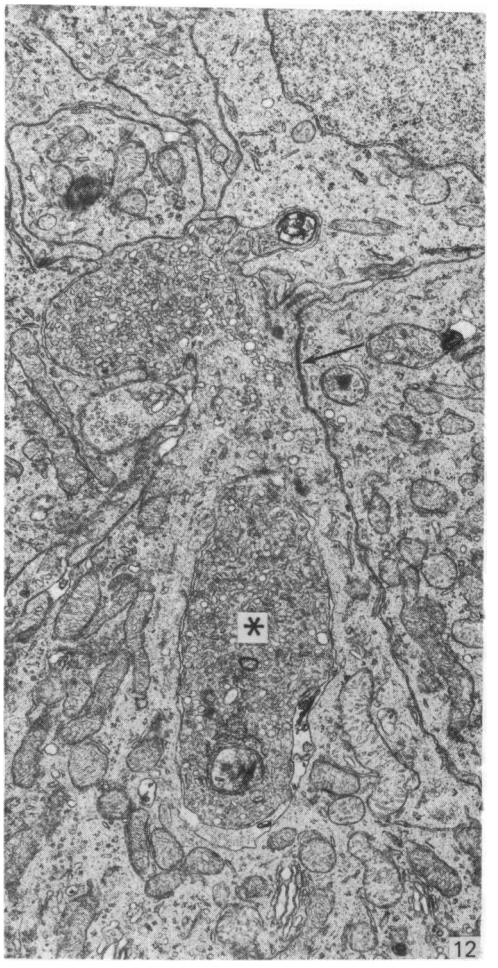

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Type II pinealocytes (Figs. 13–15). Numerous Type II cells showed the vacuoles previously described as typical of this cell type. One cell sometimes contained several vacuoles, and one vacuole sometimes had others inside it (Fig. 14). Junctional mechanisms, whose ultrastructural appearance resembled gap junctions, were observed between Type II pinealocytes. Very rarely, this junction was formed between a wide evagination of one of the cells and an infolding of the other (Fig. 13). Here, the content of the evagination was less dense than the rest of the cytoplasm.

At this stage, connective tissue spaces were relatively large and quite abundant. At the boundary with the parenchyma, Type II pinealocytes were usually found (Figs. 8, 15). The parenchymal basement membrane was fragmented, and was altogether absent in some areas (Fig. 14). Inside connective tissue spaces there were capillaries with fenestrated endothelium, collagen and oxytalan fibres (Calvo & Boya, 1983), a few connective tissue cells and migrating cells coming from the blood (Fig. 14). The nerve fibres close to the parenchyma showed a clear relation to Type II pinealocytes (Fig. 15). Finally terminal clubs of Type I pinealocytes were also observed inside connective tissue spaces (Fig. 16).

DISCUSSION

According to our results, the differentiation of cell types began three days after birth. Karasek (1974) found only undifferentiated cells at 3 days observing cellular differentiation in a later stage (10–14 days). Steinberg *et al.* (1981) described clear and dense cells in rats of 2 days, but their dense cells are apparently identical to the dense pinealoblasts described by us in embryonic development (Calvo & Boya, 1981) and in the first day after birth.

During the first stages of differentiation, the Type I pinealocyte is characterised by the development of its rough endoplasmic reticulum and the frequent appearance of subsurface cisterns. According to Bayerova & Malinski (1972), the rough endoplasmic reticulum is the organelle which increases most during postnatal development, especially from 5 to 10 days of age. Subsurface cisterns have also been described in the adult rat (Wolfe, 1965). Our results showed that these structures appear early and abundantly during the development of Type I pinealocyte.

Type II pinealocytes also appeared at 3 days. We have not been able definitely to identify a precursor cell for this cell type in one day rats or during embryonic development (Calvo & Boya, 1981). From the beginning, Type II pinealocytes showed similar features and location to those found in the already differentiated cell. In later stages, there were changes mainly in shape and electron density of these cells. The

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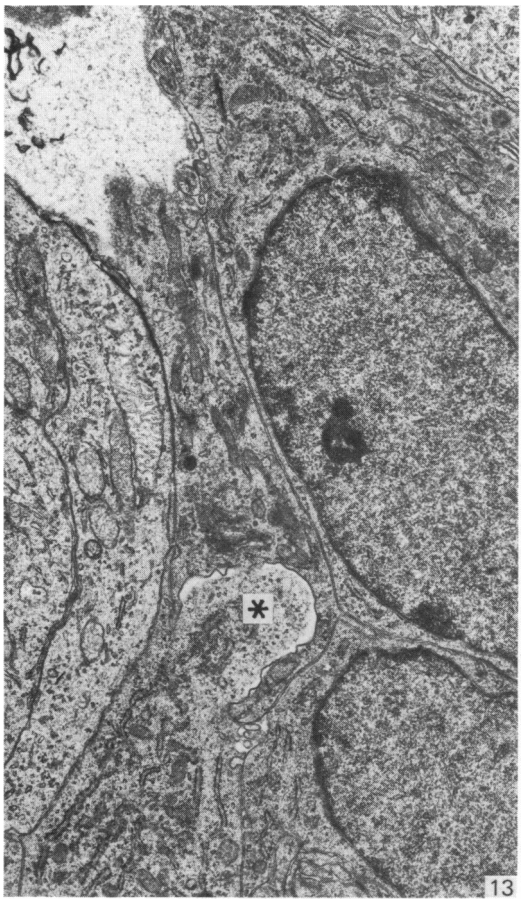
Fig. 9. Day 60. Type I pinealocyte. Groups of dense bodies are related to vacuoles. × 12500.

Fig. 10. Day 60. Type I pinealocyte. A group of dense bodies is in relation to vacuoles, one of which contains a mitochondrion (*M*). Synaptic ribbons (arrows) are at the cell surface. × 22500.

Fig. 11. Day 60. Type I pinealocytes with junctional complexes (arrowheads) limit a C-shaped surface in contact with a connective tissue space. In the upper part there are no junctional complexes, and Type II pinealocytes may be observed as usual in the parenchyma–stroma boundary × 4800. Inset. Higher magnification of the terminal club framed in the micrograph. × 9800.

Fig. 12. Day 60. A Type I pinealocyte exhibits terminal clubs located in infoldings of nearby cells. In transverse section (*), these clubs are apparently isolated in the interior of the cell. Arrow, junctional complex. × 13500.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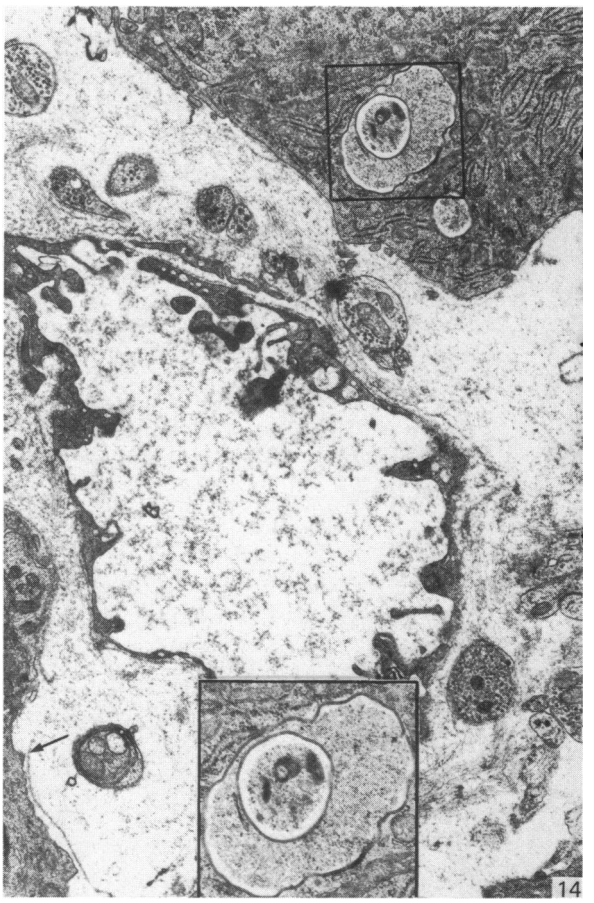
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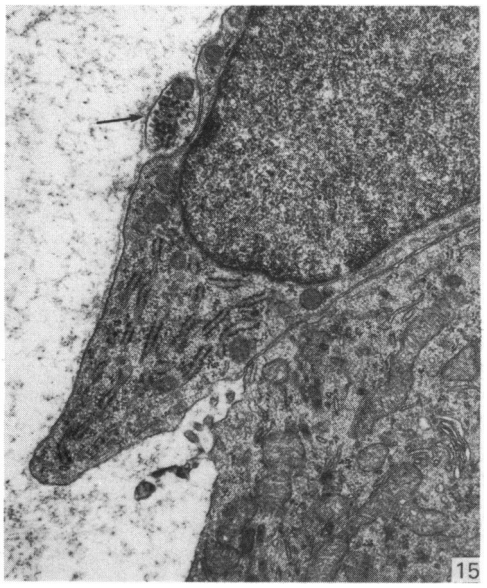
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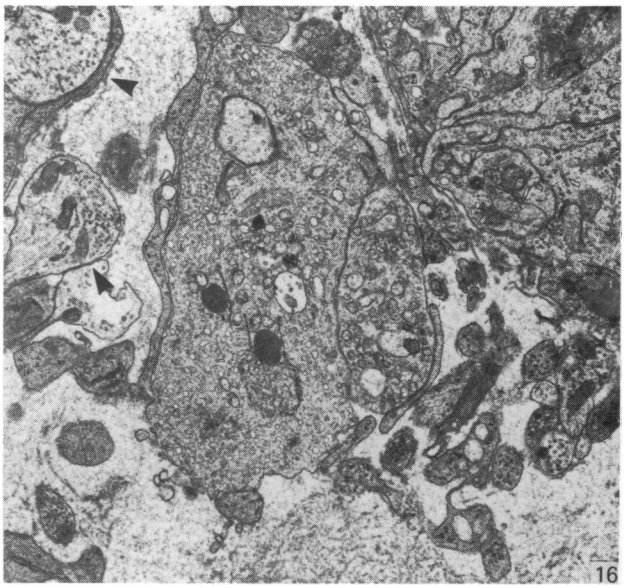
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sudden appearance of rather well differentiated Type II pinealocytes coincided with the penetration of nerve fibres into the pineal gland. From the beginning and through later stages, these fibres were closely related to Type II pinealocytes. Therefore, it is possible that the differentiation of this cell type may be induced by pineal nerve fibres. Steinberg *et al.* (1981) have studied the development of neonatal pinealocytes (non-innervated) in monolayer culture. At 5, 11, and 17 days of culture, the cells showed features of Type I pinealocytes. At 21 days they described clear and dense cells. However, as mentioned above, these dense cells seem to correspond to pinealoblasts. This would thus mean that, in the absence of innervation, typical Type II pinealocytes do not develop.

The differentiation of pinealocyte cell types which had begun at three days of age, continued until 15 to 20 days, by which time all parenchymal cells could be included in one of the two cell types described. Biochemical studies also indicate that most pineal indole-amines and related enzymes develop in the first three weeks after birth (Hakanson, Des Gouttes & Owman, 1967; Ellison, Weller & Klein, 1972; Klein, Namboodari & Auerbach, 1981).

Along with Type I pinealocyte hypertrophy, important changes were found in the appearance of the nucleus and in the number and type of organelles. There are few previous descriptions of these changes. According to Bayerova & Malinski (1972), most changes in cytoplasmic volume and organelles take place between 5 and 10 days with few differences between 10 days and the adult stage. Steinberg *et al.* (1981) also found few changes in organelles between 4 and 28 days, which was the last day studied. Our results, however, indicated a gradual transformation of Type I pinealocytes during the first 60 days of age. At 60 days, all the ultrastructural features described for the adult rat (Wolfe, 1965; Arstila, 1967) were already present.

Another characteristic of the Type I pinealocyte during postnatal development is its tendency to adopt a polarity around a cavity incompletely surrounded by junctional complexes. These structures have been described in the adult rat (Wolfe, 1965) and during postnatal development (Zimmerman & Tso, 1975; King & Dougherty, 1980; Steinberg *et al.* 1981). The only extensive study of these structures has been made by Zimmerman & Tso (1975), who compared them to photoreceptor cells during development of the retina. Our results differed somewhat from theirs. Zimmerman & Tso (1975) found cavities with terminal clubs located in the lumen in 4–12 days old rats. From 17–96 days they found groups of junctional complexes, but neither lumina nor terminal clubs. According to our findings, the groups of junctional complexes were only slightly differentiated in the first 15 days, reaching their maximum development at 45–60 days. Terminal clubs were observed during all

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Fig. 13. Day 60. A junctional mechanism is shown, between an evagination and an infolding of two adjacent Type II pinealocytes. The content of the evagination (*) is more lucent than the remaining cytoplasm. × 11 500.

Fig. 14. Day 60. A connective tissue space containing unmyelinated axons is shown. In the upper right hand corner there is a Type II pinealocyte process containing the typical vacuoles of this cell type. One of these (framed) itself contains another vacuole (inset). Arrow, discontinuity of parenchymal basement membrane. × 12000.

Fig. 15. Day 60. An axon is located in a depression (arrow) on a Type II pinealocyte. × 18000.

Fig. 16. Day 60. A terminal club is located in a connective tissue space in which nerve fibres (arrowheads) may also be observed. The club contains abundant clear vesicles, lipid droplets and multivesicular bodies. × 14500.

the stages studied. Thus, our results indicate that junctional complexes and their related terminal clubs undergo a development and not an involution with increasing age. Zimmerman & Tso did not find any relation of these structures with the connective tissue spaces. Our results, however, indicate that there is a frequent relation between them, which would explain the location of terminal clubs inside connective tissue spaces during development and in the adult stage (Wolfe, 1965; Arstila, 1967). Finally, Zimmerman & Tso (1975) established a relation of centrioles and cilia to the central cavity, locating the cilia in the terminal clubs. In our material, most centrioles observed were located in the perinuclear cytoplasm. Cilia were located in intercellular spaces not enclosed by junctional complexes. Centrioles and cilia disappeared after 15 days, when centrioles became a cluster of microtubules joined by a dense material, as described also by Lin (1970).

Type II pinealocytes were joined by structures which resembled gap junctions. No junctional mechanisms were observed between Type I and II pinealocytes. Junctions between Type II pinealocytes were occasionally found between a wide evagination of one cell located inside an infolding of another nearby cell. A transverse section of this junction would appear similar to the clear vacuoles described in Type II pinealocytes. However, the number and the usually deep location of the clear vacuoles would make it difficult to admit that they are all transverse sections of an intercellular junction, although with the techniques used this may not totally be excluded. The vacuoles of Type II pinealocytes have been described in the adult rat by Wolfe (1965), but their nature, their possible relation to junctional complexes, and their meaning still need further study.

SUMMARY

The morphological development of the rat pineal gland has been studied from 1 to 60 days of age. During the first days, undifferentiated cells (pinealoblasts) with scanty cytoplasm and frequent mitotic figures were observed. The differentiation of cell types (Types I and II pinealocytes) began on the third day after birth and was completed by days 15–20. At 3 days of age, nerve fibres were first observed, both in the connective spaces and in the parenchyma. After 5 days, an important hypertrophy of pinealocytes began, mostly Type I, which continued until 60 days of age. After 45 days, all the ultrastructural features described in the adult pineal gland were already present. The findings are discussed.

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