Journal of Pineal Research 1:83–89 (1984)

Structure and Ultrastructure of the Aging Rat Pineal Gland

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The pineal gland of rats 12–28 months old was studied with light and electron microscopes. All pineal components exhibited regressive changes of different intensity with age. In type I pinealocytes, there was a marked increase in dense bodies as well as the occasional appearance of wide cell profiles full of vesicles. Type II pinealocytes showed nuclear infoldings and cytoplasmic deposits of lipofuscin. Pineal stroma displayed an increase in connective tissue fibers, both collagen and oxytalan, as well as remains of basement membranes and other materials of unknown nature. Calcareous concretions were also found, mostly in the pineal capsule. All regressive changes were more intense with increasing age.

Key words: pineal gland, pinealocytes, aging, ultrastructure

INTRODUCTION

The appearance of regressive changes in the pineal gland during old age is a phenomenon already known in the human species [Arieti, 1954; Scharenberg and Liss, 1965]. However, it is unknown if this process is common to other species. In the albino rat, few studies have been published on the ultrastructure of the pineal gland in old animals [Bondareff, 1965; Johnson, 1980; Allen et al., 1982]. Bondareff [1965] found an increase in the number of dense bodies in pinealocytes of rats 25 months old. Johnson [1980] gave a more detailed description of regressive changes. This author described a collagen infiltration and changes in the main pinealocytes, mostly referring to a decrease in lipid droplets and an increase in dense bodies. Allen et al.

Received November 3, 1983; accepted January 31, 1984.

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[1982] studied the adult and aged rat pineal gland with scanning and transmission electron microscopy, finding, in addition to the above-cited changes, an increased number of striated muscle fibers and calcareous concretions as well as modifications in pinealocyte mitochondria. The purpose of the present study was to confirm and extend the changes described in the old rat pineal gland, using light and electron microscopy.

MATERIALS AND METHODS

We used 24 albino rats (Wistar) of both sexes and of ages ranging between 12 and 29 months, maintained under routine laboratory conditions (LD 14:10). The age intervals studied were 12, 16, 22, 24, 28, and 29 months. Six animals were taken for each age interval (three of each sex); two were used for light microscopy and four for electron microscopy. The rats were anesthetized with ether and decapitated between 1100 and 1300 h. For the light microscope, the glands were fixed in Bouin's fluid, embedded in paraffin, and serially sectioned 7 μm thick. The staining techniques used were hematoxylin-eosin, phosphotungstic acid hematoxylin, and, the silver method of Gordon and Sweets for reticular fibers [Cook, 1974]. For electron microscopy, the pineal glands were fixed by immersion in 0.1 M phosphate-buffered 3% glutaraldehyde, pH 7.4. After washing in buffer, they were postfixed in phosphate-buffered 1% osmium tetroxide and embedded in Vestopal. Ultrathin sections were stained with uranyl acetate and lead citrate and examined in a Philips 201 EM.

RESULTS

The changes found in the different age intervals and sexes differed only in quantity. With increasing age there was a gradual increase in the intensity of regressive changes. Thus, our results will be described as a whole, indicating in the text those characteristics more clearly related to age and sex.

Light Microscopy

The most obvious change was an increase in the size of connective tissue septa, mostly in the periphery of the gland (Fig. 1). Fibrosis was greater with increasing age of the rat. Using techniques for oxytalan fibers, the number of these fibers appeared to be much greater than in adult animals [Calvo and Boya, 1983a].

Striated muscle fibers and calcareous concretions (Fig. 2) were found in the pineal stroma. The latter were mostly located in the pineal capsule. One to five calcareous concretions were usually found per pineal gland, the higher numbers corresponding to older rats.

Electron Microscopy

The most striking effect of aging on the type I pinealocyte [Calvo and Boya, 1983b] was the increase in number and size of dense bodies (Figs. 3, 8). They generally presented elongated shapes and sometimes appeared in groups (Fig. 3) in relation to autophagic vacuoles, multivesicular bodies, and occasionally lipid droplets. The lipid droplets of the type I pinealocytes were fewer and larger with advancing age. Rats of 28 and 29 months displayed a single or very few large lipofuscin granules in their type I pinealocytes (Fig. 4).

Old rats, mostly females, presented very wide cytoplasmic processes, generally forming groups in the vicinity of the parenchymal basement membrane (Figs. 5–7). Their content varied according to the size of processes. The content of the smaller ones reminded us of the terminal clubs in type I pinealocytes [Calvo and Boya, 1983b]. Moreover, they displayed membranous elements in the form of very short and sinuous tubules. Other times the membranes were wider and they tended to fuse, forming lamellar structures. All these elements were sometimes arranged concentrically in such a way that the membranous structures (vesicles, tubules) were placed in the center with the mitochondria and microtubules around them in the periphery (Fig. 7). The wider processes showed a disorganization of the vesicles and tubules, thus displaying a material of a reticular appearance which reminded us of membrane remnants (Figs. 5, 6). Ribosomes were usually absent. The diameter of these processes was in many cases greater than 5 μm.

Groups of type II pinealocytes were frequently found (Fig. 8). Their surface displayed infoldings and junctional complexes. In old rats, numerous type II pinealocytes were located inside connective tissue spaces and connected through their processes (Fig. 8). They thus formed a network which frequently occupied a great part of the connective tissue space.

Some type II pinealocytes showed deep infoldings in their nuclear envelopes. Small and increasingly numerous lipofuscin granules were observed in the cytoplasm of this cell type (Fig. 9).

At low magnifications, connective tissue spaces of old rats were different from those of young ones. They appeared almost completely occupied by cellular, fibrillar, and amorphous structures (Fig. 8). Among the cell components were blood vessels, unmyelinated nerve fibers, connective and migrant (lymphocytes, monocytes) cells, the network of type II pinealocytes, and striated muscle fibers. In addition to these components, old rats displayed a cell type characterized by a massive deposit of inclusions similar to lipofuscin (Fig. 10). These granules were not numerous but they were very large. Their diameter was sometimes more than 5 μm.

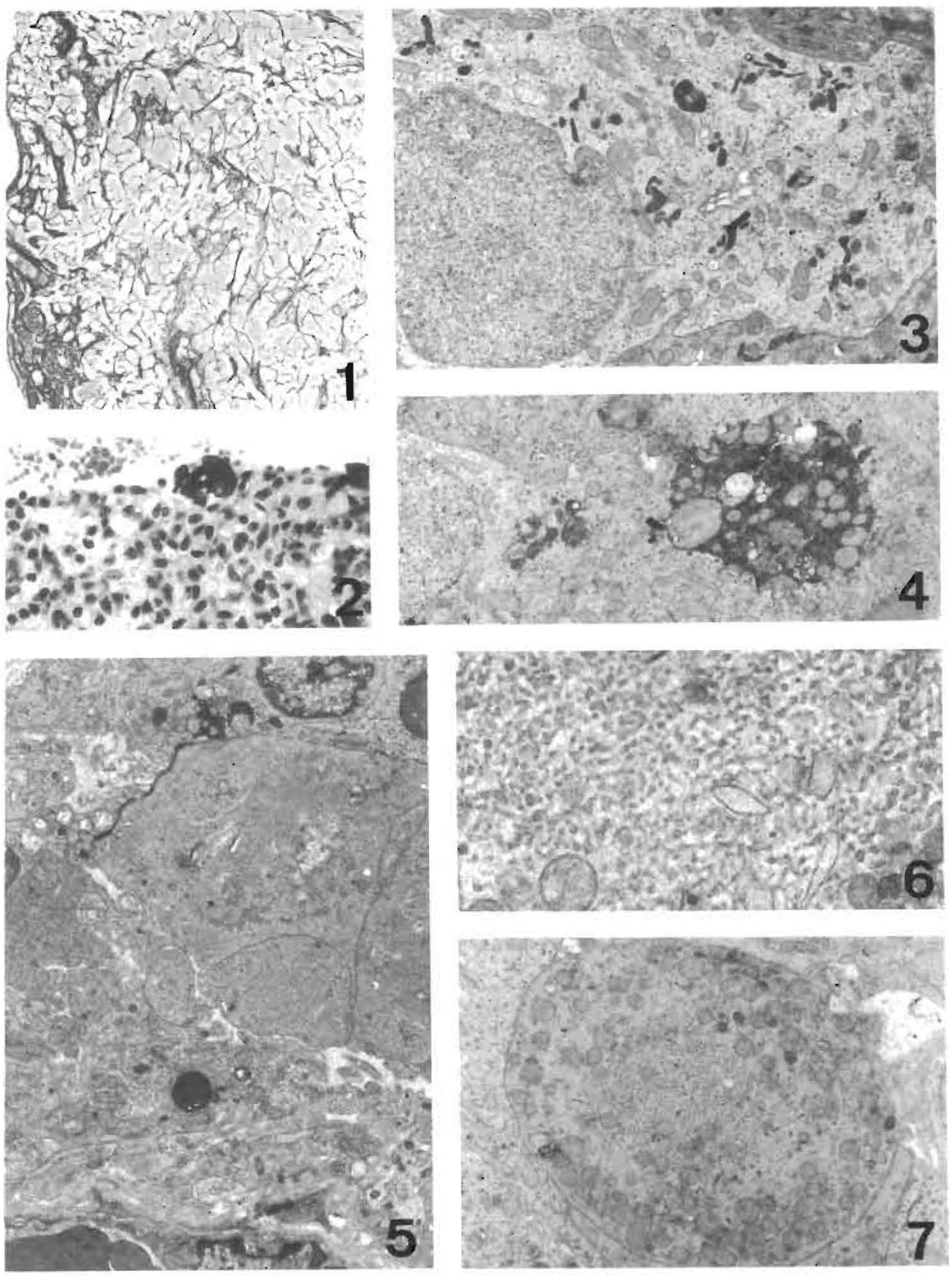
There was a clear increase in the number of connective tissue fibers in old rats. Among these, the thicker septa contained mostly collagen fibers and the thinner septa mostly oxytalan ones (Fig. 11). In addition to connective tissue fibers there was other material whose nature was not clear. The most frequently found were irregular fragments of a dense material similar to basement membranes (Fig. 11). The paraenchymal basement membrane, clearly wider in old rats, was incomplete and often partially detached from the parenchyma (Fig. 11).

DISCUSSION

The results of this study show the appearance of many changes in the old rat pineal gland which support previous investigations [Bondareff, 1965;

Johnson, 1980; Allen et al., 1982], suggesting an involution of the gland in the aged rat.

Fibrosis was the most evident change under the light microscope. There was a marked increase in the number of collagen fibers in the thick connective tissue septa of the periphery. However, in the thinner septa the most increasing fibrillar component was oxytalan fibers, confirming our previous results [Calvo and Boya, 1983a].



Calcareous concretions seemed more frequent in the pineal gland of old rats. They were few and small, and generally located in the capsule. No calcareous deposits were observed in the parenchyma. Several authors [Gardner, 1949; Quay, 1974] deny the existence of calcareous concretions in adult rat pineal gland. Diehl [1978] describes these deposits in 90% of adult rats. Although our results agree as to shape, size, and location of the concretions described by Diehl [1978], we have found these deposits to be very scanty when they exist in 1-yr-old rat, increasing only in oldest rats.

Most of the regressive changes were ultrastructureal. The cell apparently less affected by the regressive process was the type I pinealocyte. Even lipofuscin, which is considered a characteristic feature of senile involution, is relatively scarce in this cell type. From a morphologic point of view there seemed to be no cytologic signs indicating a clear depression in the activity of type I pinealocytes. Reiter et al. [1980] found a decrease in the nighttime production of melatonin in old hamsters and gerbils. However, the daytime levels of melatonin, hypothalamic amines, hypophyseal hormones, and the appearance of the reproductive organs showed few changes as compared to adult animals. Greenberg and Weiss [1978] found a decrease in the density but not in the affinity of beta-adrenergic receptors in the pineal gland of old rats. Therefore, it would seem that the decrease in pineal function with age could be due to a change in regulatory mechanisms and not to a decrease in pinealocyte activity itself.

Type I pinealocytes of old rats showed a marked increase in the number of dense bodies. This confirms the previous observations of Bondareff [1965], who also found acid phosphatase activity in these bodies, and others [Johnson, 1980; Allen et al., 1982]. In the pineal gland of hens 1–5 yr old there was also an increase in the number and volume of pinealocyte lysosomes

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Fig. 1. Male; 24 months. Pineal stroma. Larger connective tissue septa in the periphery of the gland. Gordon and Sweet silver technique. Original magnification, ×140; reduced to 85%.

Fig. 2. Male; 28 months. Calcareous concretions located in the pineal capsule. Hematoxylin eosin. Original magnification, ×350; reduced to 85%.

Fig. 3. Female; 12 months. Type I pinealocyte. Dense bodies arranged in groups. Original magnification, ×7,800; reduced to 85%.

Fig. 4. Male; 29 months. Type I pinealocyte. Large lipofuscin droplet (compare with the nearby mitochondria). Original magnification, ×7,500; reduced to 85%.

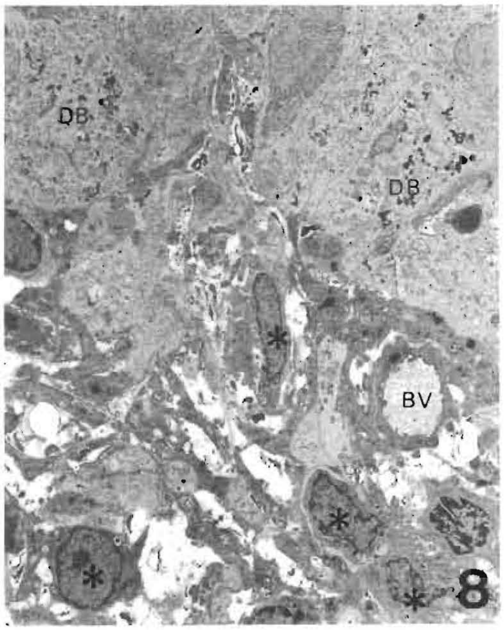
Fig. 5. Female; 24 months. Several wide cytoplasmic processes located near a connective tissue space. Original magnification, ×6,000; reduced to 85%.

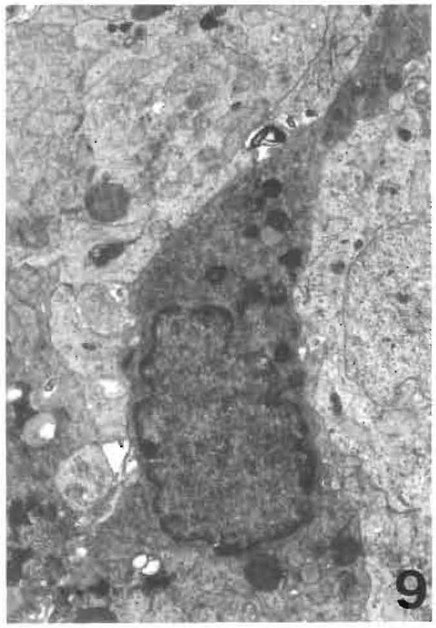
Fig. 6. Female; 24 months. Membranous material located in a wide cytoplasmic process. Original magnification, ×27,000; reduced to 85%.

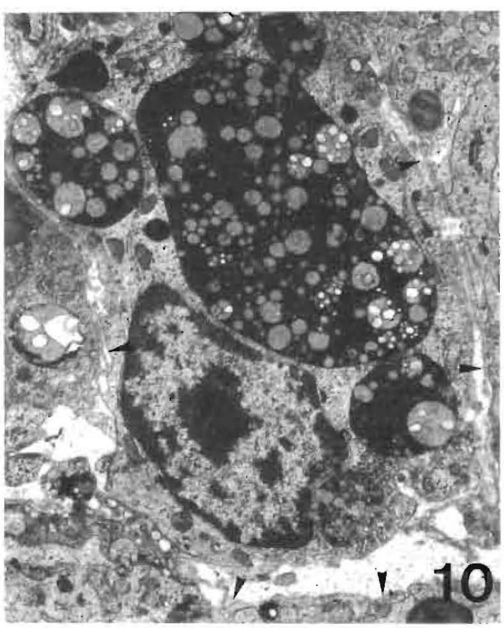
Fig. 7. Female; 16 months. Wise cytoplasmic process whose components are arranged concentrically. In the center there is a membranous material. Mitochondria are located in the periphery. Original magnification, ×10,500; reduced to 85%.

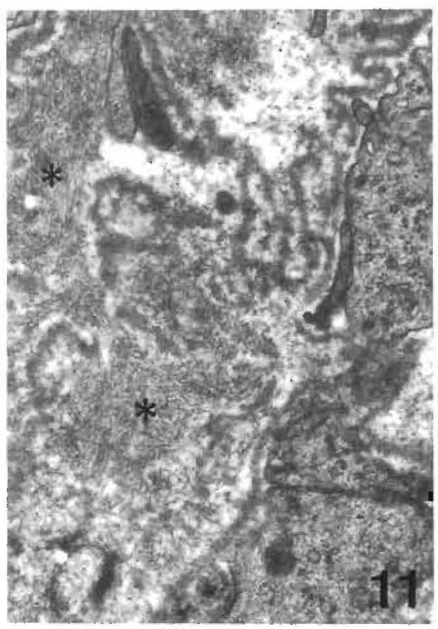
[Boya and Calvo, 1979]. This increase in the lytic activity with age could suggest an increase in destruction rate of cell components.

The wide cytoplasmic processes found in old female rats probably belong to type I pinealocytes. These processes have not been previously described. Their content, rich in vesicles and lacking ribosomes, their location close to the parenchymal basement membrane, and their frequent ap-









pearance in groups, seem to suggest that they may be expanded type I pinealocyte terminal clubs. However, there are no definite proofs of this hypothesis. The occasional appearance of these wide processes tends to indicate that they must have no important function although they do constitute a characteristic regressive change in old female rat pineal gland.

Type II pinealocytes show many changes. The deposit of lipofuscin is greater than in type I pinealocytes. There are also changes in the appearance of the nuclei, location of the cells (pinealocyte II network in the connective tissue spaces), etc. The degree of regressive changes in type II pinealocytes, greater than those of type I, suggests that these cells could possibly have a more important functional role than just elements of support.

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Fig. 8. Male; 22 months. Low-magnification image of the limit parenchyma-stroma. In the connective tissue space there is a network of type II pinealocytes (asterisks). DB, dense bodies in type I pinealocytes. BV, blood vessel. Original magnification, ×2,500; reduced to 84%.

Fig. 9. Male; 28 months. Lipofuscin deposits in type II pinealocytes. Original magnification, ×8,500; reduced to 84%.

Fig. 10. Female; 24 months. Cell located in a connective tissue space containing large granules similar to lipofuscin. Arrowheads, basement membrane. Original magnification, ×9,000; reduced to 84%.

Fig. 11. Male; 24 months. Connective tissue space. Fragments of basement membranes located among numerous oxytalan fibers (asterisk). Original magnification, ×25,000; reduced to 84%.