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Histological study of choroidal melanocytes in animals with tapetum lucidum cellulosum

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Abstract. The distribution and morphology of choroidal melanocytes in dogs and cats which have a tapetum were compared with those of humans who do not. In dogs or cats, tapetal cell-like melanocytes were arranged in layers on the scleral side of the tapetum and underneath the choriocapillaris in the non-tapetal area. Although the tapetum of the dog occupied a smaller area than that of the cat, the tapetum plus the area of tapetal cell-like multilayered melanocytes occupied most of the fundus in the dog in almost the same way as in the cat. These multilayered melanocytes contained few intracytoplasmic organelles except for melanin granules, and some had regularly arranged melanin granules. In human eyes tapetal cell-like melanocytes were not found anywhere. It was concluded that the morphology and structural architecture of choroidal melanocytes of dogs or cats are different from those of human eyes and closely correspond to the tapetum.

Introduction

The tapetum lucidum cellulosum is composed of several reflecting cell layers that are present in the eyes of dogs and cats [1, 5, 8, 9]. It enhances scotopic sensitivity by reflecting the light that has passed through the visual cells to them once again [9]. Some investigators have hypothesized that the tapetal cell and choroidal melanocyte have a common origin because of the coexistence of the tapetal rodlets and melanin granules in the same cell [1, 8] and a similar course of development of tapetal rodlets to melanosomes [3, 4, 6]. However, Tjalve [10] contends that the matrix of tapetal rodlets are not melanin because of changes seen with glutaraldehyde fixation. Bernstein et al. [1] observed the tapetal cell-like melanocytes at the periphery of the cat's tapetum. However, no detailed study on the distribution and morphology of melanocytes in the whole choroid has been carried out. In the present study, we compared the distribution and morphology of choroidal melanocytes of dogs and cats whose tapetum had a different size and thickness. In addition, the structural features of choroidal melanocytes of these animals were compared with those of human eyes. Particular attention was focused on the variety of distribution and the morphology of the choroidal melanocyte.

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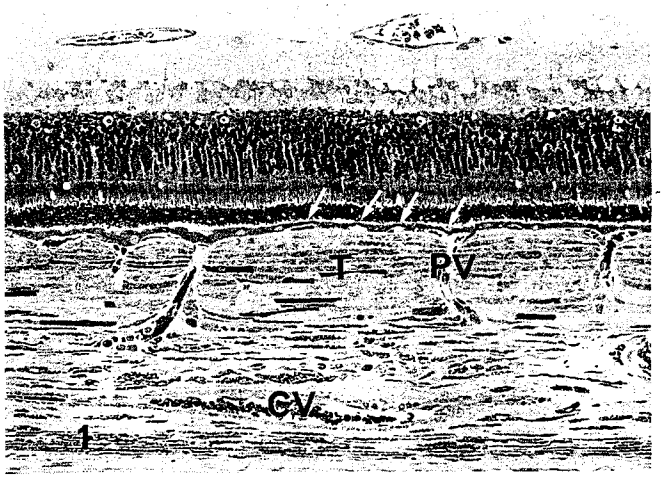
Materials and methods

Three dog eyes, three cat eyes, and three human eyes were used for this study. After anesthetizing the animals with sodium pentobarbital, both eyes were removed, and then the animals were killed with an overdose of potassium chloride. The two human eyes were from eye bank donors (38 and 64 years old), and one eye was enucleated due to choroidal melanoma (44 years old). The anterior hemisphere of each eye was cut away after being kept in a fixative of 4% glutaraldehyde buffered at pH 7.2 with 0.1 M cacodylate until the eyeball became hard enough to cut without distortion. The choroid with retina and sclera were dissected into small pieces, keeping track of their location in the ocular fundus. The tissue pieces were again immersed in the same buffered fixative for 12–24 h. Following several washings with a fresh buffer solution, the tissues were postfixed by 1% osmium tetroxide in the same buffer solution. After dehydration by using a graded series of ethanol and then clearing with propylene oxide, they were embedded in Epon 812. The embedded tissues were reoriented to the desired angles prior to sectioning on a Sorvall MT-IIB ultramicrotome. Sections were cut in two planes: parallel to and at right angles to the long axis of the visual cells. The first is referred to as the vertical section and second as the flat section. Semithin sections were stained for 1 min in 0.1% Azur II with borax at 90° C. Ultrathin sections were stained with uranyl acetate and lead citrate and examined with a JEM-100CX electron microscope.

Results

The tapetum of dogs was seen as a rounded triangular area that occupied about half of the superior fundus. The base of the tapetum was oriented horizontally, with the apex of the triangle pointing upward. The tapetum of cats occupied most of the superior fundus, exluding the periphery, and extended to the posterior pole of the inferior fundus. Histologically, the tapetum had a multilayered structure of parallel sheets of tightly packed rodlike cells in the vertical section (Fig. 1) and rounded polygons in the flat section (Fig. 2). It was sandwiched between layers of choriocapillaris, which indented into the retinal pigment epithelium, and choroidal vessels (Fig. 1). The central areas of the tapetum contained 10–15 layers of tapetal cells in dogs and about 30 in cats. The tapetum gradually thinned and eventually disappeared towards the periphery in both animals.



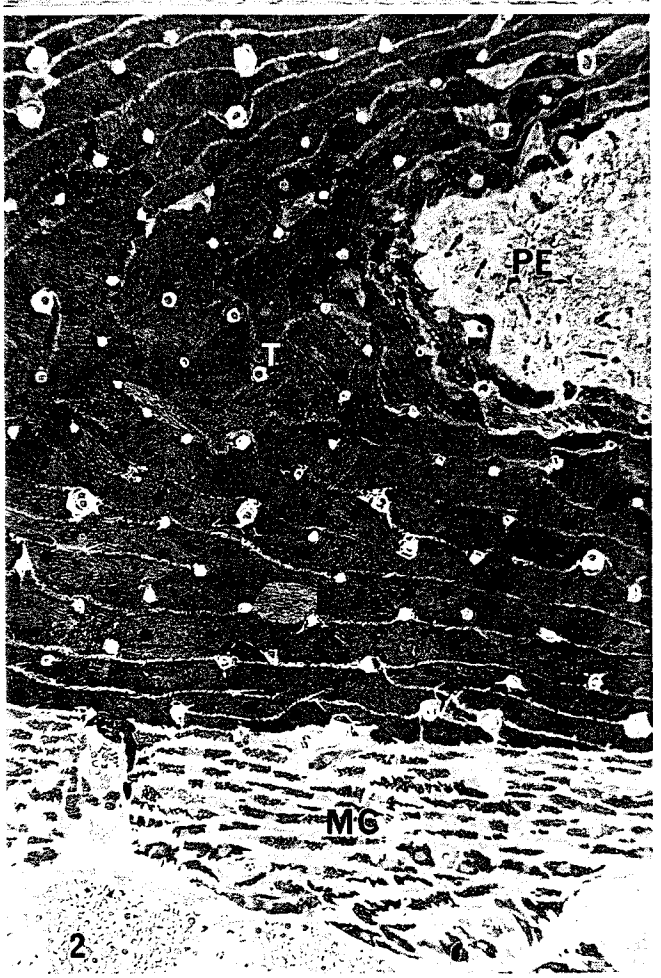


Fig. 1. Light micrograph of the retina and choroid of the dog. The tapetum (*T*) is located between the choriocapillaris (*arrows*) and the choroidal vessels (*CV*). Penetrating vessels (*PV*) connect the choriocapillaris with the choroidal vessels. × 190

Fig. 2. Light micrograph of flat section of the dog tapetum. The rounded and polygonal tapetal cells are arranged in onionlike layers. Note the multilayered melanocytes (*MC*) on the scleral side of the tapetum. *T*, Tapetum; PE, pigment epithelium. × 380

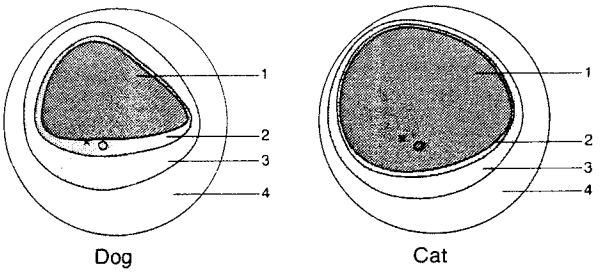


Fig. 4. Schematic drawing of the distribution of the tapetum and tapetal cell-like multilayered melanocytes. *1*, Tapetum (+); *2*, tapetum (+/−) and tapetal cell (+); *3*, tapetum (−) and tapetal cell (−); tapetal cell-like multilayered melanocytes (+). *4*, Tapetum (−) and tapetal cell (−); Tapetal cell-like multilayered melanocytes (−)

Melanocytes with the structural features of tapetal cells were seen on the scleral side of the tapetum and showed a layered arrangement (Fig. 2). The layers of melanocytes gradually increased toward the periphery of the tapetum. At the border region of the tapetum, the tapetum became indistinct macroscopically, but 1–3 layers of tapetal cells were observed microscopically; moreover, multilayered melanocytes were distinctly seen on the scleral side of the tapetum. In particular, there were many layers of melanocytes replacing the tapetal cells from the inferior border region to the posterior pole of the inferior fundus (Fig. 3a–d). The melanocyte layers gradually decreased in number as they went away from the tapetum and the arrangement became irregular to some extent. About 3–6 layers of melanocytes were found in the middle of the inferior fundus. The multilayered melanocytes that replaced the tapetal cells were also found in the peritapetal region of the superior fundus, although it was confined to a limited area. These tapetal cell-like multilayered melanocytes extended to the retinal side of the large choroidal vessels; however, the arrangement gradually became irregular as it went away from the choriocapillaris. On the suprachoroid they were densely packed again although no tapetal cell-like melanocytes were found. At the periphery of the non-tapetal area, the tapetal cell-like melanocytes were no longer observed. They were densely packed on the suprachoroid and decreased in number going toward the retina (Fig. 3e). The tapetal cell-like melanocytes in the inferior border region observed in a flat section showed polygons similar to tapetal cells (Fig. 3f). There were more layers of melanocytes in dogs than in cats on the scleral side of the tapetum and underneath the choriocapillaris in the non-tapetal area (Fig. 3a–d). The tapetum of the dog occupied a smaller area than that of the cat; however, the area of the tapetum plus the area of tapetal cell-like multilayered melanocytes occupied most of the fundus just as in the cat (Fig. 4).

In the area where the tapetal cell-like melanocytes were arranged in layers underneath the choriocapillaris, the choriocapillaris was indented into the retinal pigment epithelium like in the tapetal area (Fig. 3c, d).

Electron microscopy showed that the tapetal cells contained mostly tapetal rodlets that occurred in a regular configuration, forming a 60° lattice (Fig. 5). The cell nucleus was flat and centrally located and extended the full thickness of the cell. The layered melanocytes resembled tapetal cells in their outward form and in the shape of their nuclei

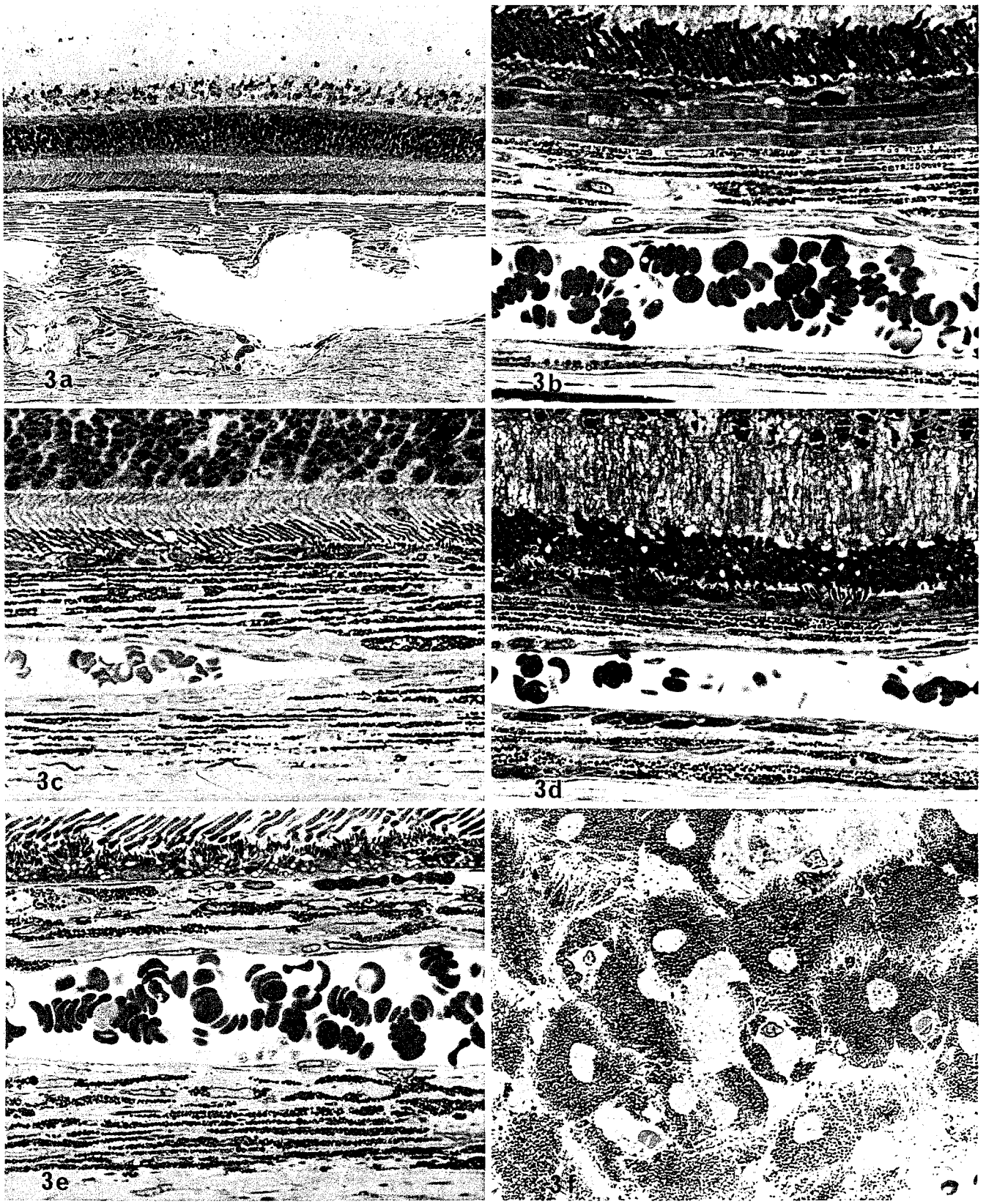
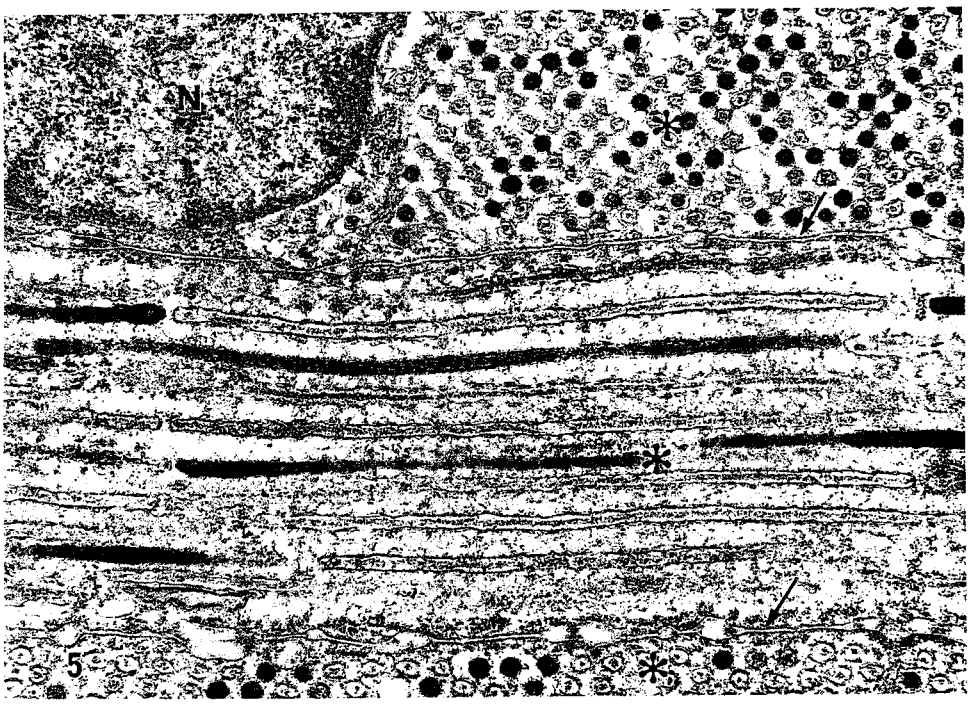
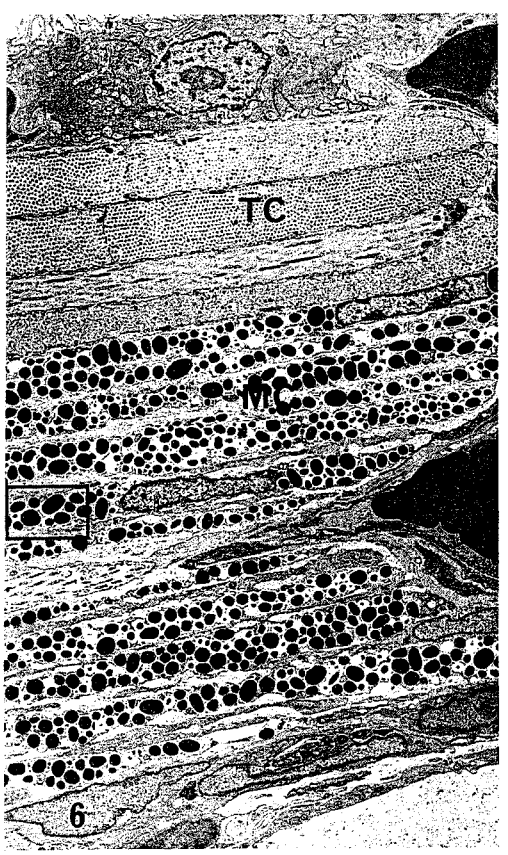


Fig. 3a–f. Light micrograph of the choroid of dogs and cats. **a** Inferior border region of the tapetum (dog), ×160. **b** Inferior border region of the tapetum (cat), ×1040. **c** Inferior region of the optic disc without the tapetum (dog), ×440. **d** Middle of the inferior fundus without the tapetum (cat), ×960. **e** Periphery of the inferior fundus without the tapetum (dog), ×860. **f** Flat section of the melanocyte at the inferior border region (dog). ×1300





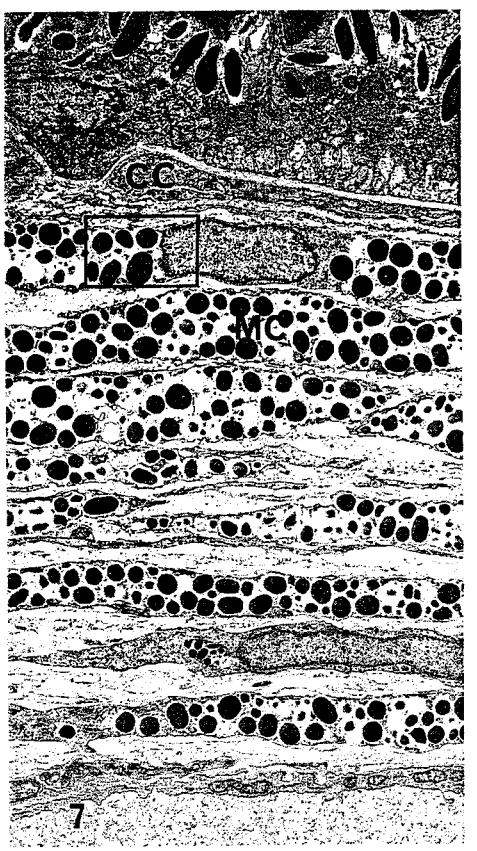
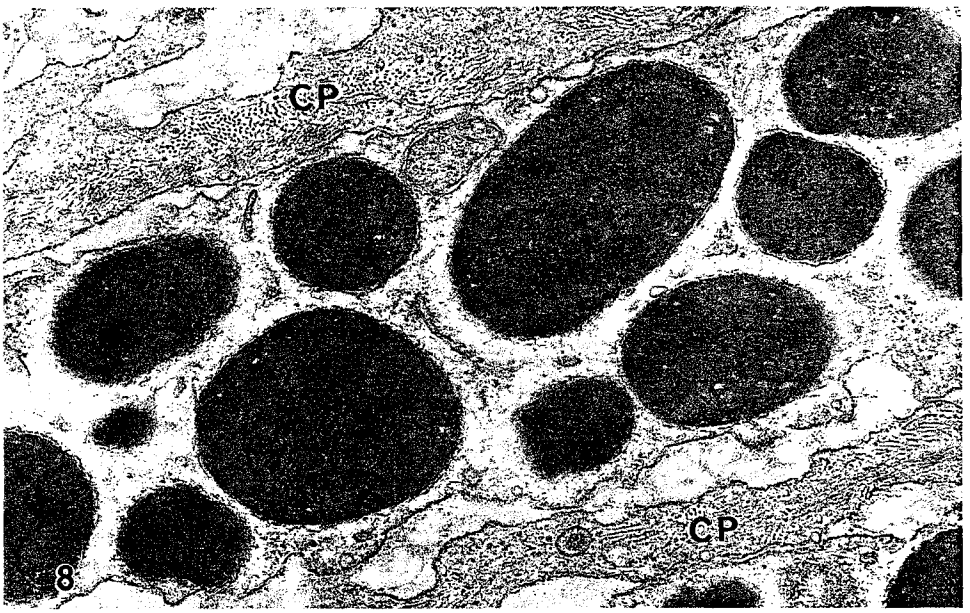
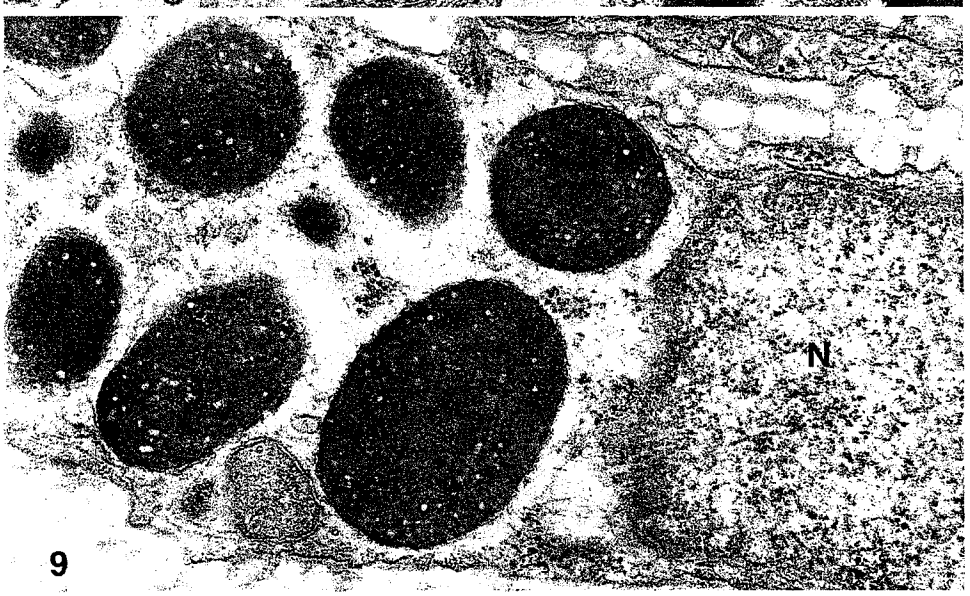


Fig. 5. Electron micrograph of the three layers of tapetal cells in dogs. In the top and bottom cells, cross-sectioned tapetal rodlets can be seen, while in the middle, longitudinally sectioned tapetal rodlets are present. The tapetal rodlets are arranged forming a 60° lattice. Each cell layer is indicated by * and the cell membranes by *arrows*. N, Nucleus. ×19000

Fig. 6. Electron micrograph of melanocytes at the inferior border region of dog tapetum. The multilayered melanocytes (*MC*): the nearer the location of the melanocyte to the tapetal cell (*TC*), the more similar the structure to the tapetal cell. ×2400

Fig. 7. Electron micrograph of multilayered melanocytes at the middle of the inferior fundus without the tapetum in the cat. Melanocytes (*MC*) with a structure similar to tapetal cells are arranged in layers underneath the choriocapillaris (*CC*). ×3900





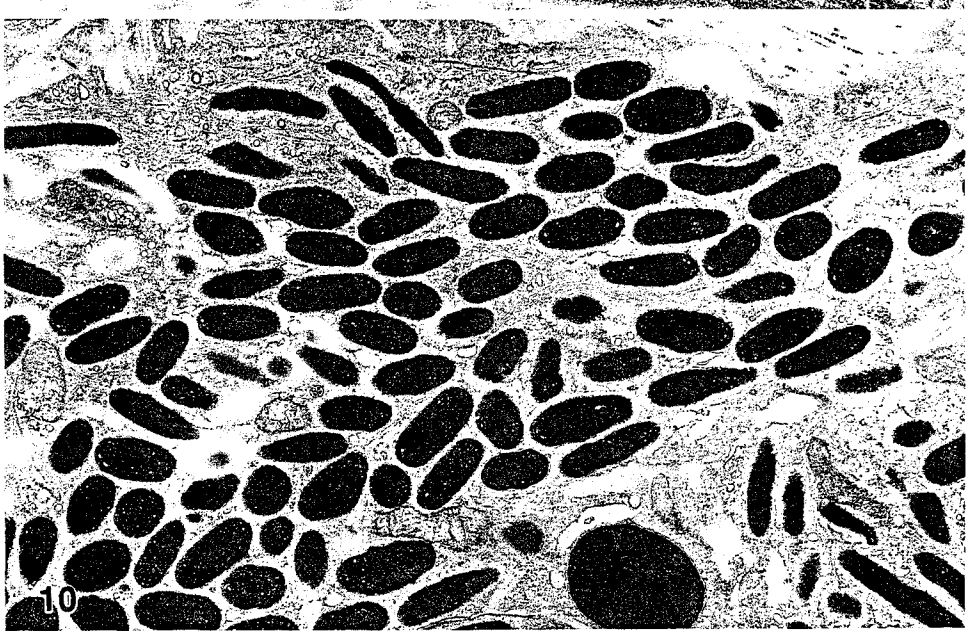


Fig. 8. High-power view of area indicated by square in Fig. 6. The melanin granules occupy most of the cytoplasm, while other intracytoplasmic organelles are found mainly in the periphery. The multilayered melanocytes are bordered by fibroblast cell processes (*CP*). ×36000

Fig. 9. High-power view of area indicated by square in Fig. 7. The shape of cell and nucleus (*N*) look like those of the tapetal cell. Note the sparse intracytoplasmic organelles of the cells except for the melanin granules. ×36000

Fig. 10. Electron micrograph of flat section of tapetal cell-like multilayered melanocyte at the inferior border region of dog. Melanin granules are arranged in regular order. ×11000

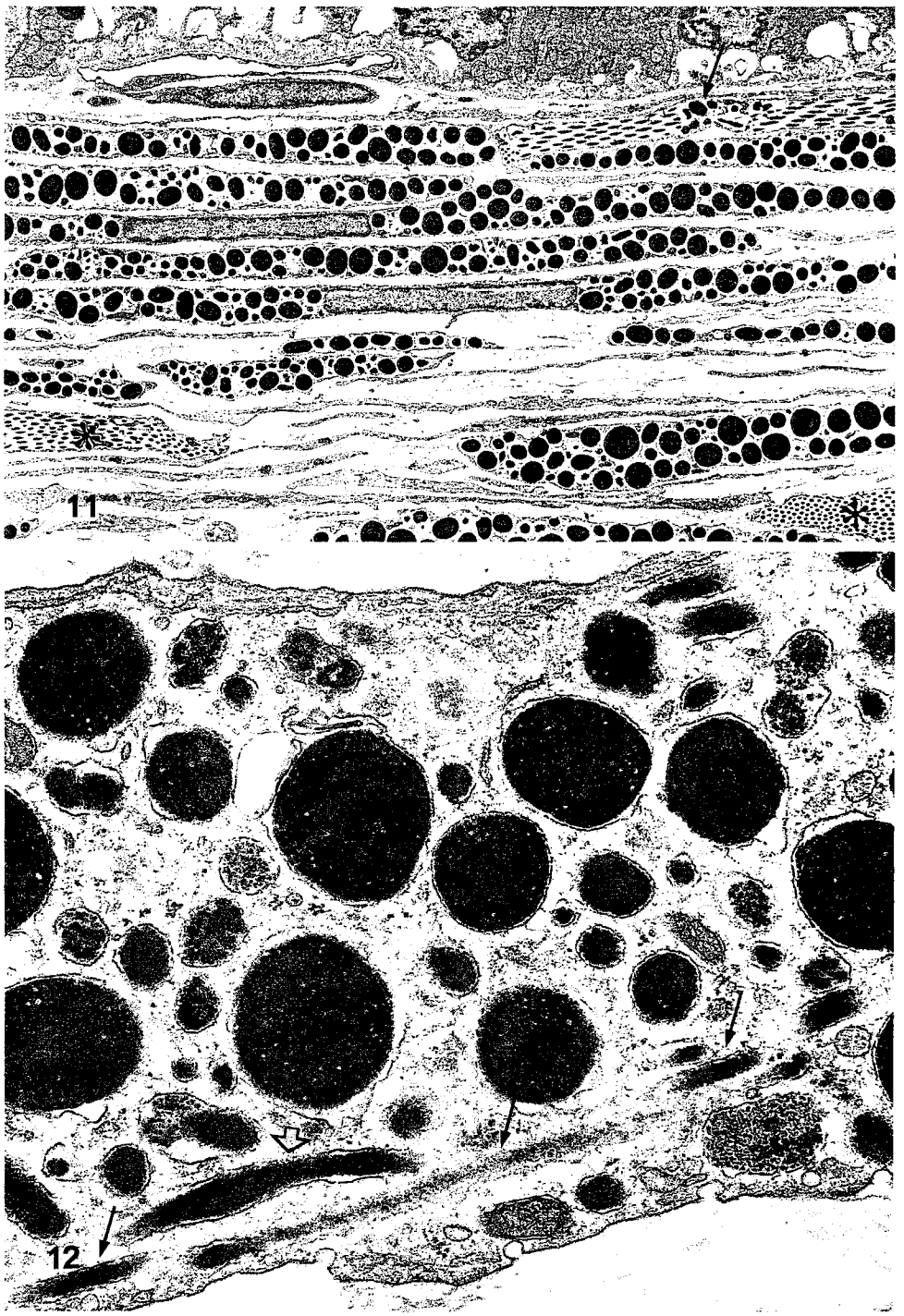


Fig. 11. Electron micrograph of tapetal cells and tapetal cell-like multilayered melanocytes at the inferior border region of the cat tapetum. Melanin granules (*arrow*) are seen in the tapetal cell. Going away from the choriocapillaris, melanocytes lose their specific rectangular cell outline and are less tightly packed. Interposed tapetal cells (*) around this area show a similar tendency. × 3500

Fig. 12. Electron micrograph of tapetal cell-like multilayered melanocytes at the inferior border region of cat tapetum. Tapetal rodlets (*arrows*) and the morphological types intermediate between the melanin granules and tapetal rodlets (*open arrow*) are found in the cell. × 32000

both in dogs and cats (Figs. 6, 7). In these melanocytes, melanin granules occupied most of their cytoplasm, while mitochondria, ribosomes and intracytoplasmic filaments were found mainly at the peripheral portions of the cells (Figs. 8, 9). In the tapetal cell-like multilayered melanocytes at the inferior border region of the tapetum, the layers were bordered by fibroblast cell processes (Fig. 8), and some melanocytes had regularly arranged melanin granules like those of tapetal rodlets (Fig. 10). The tapetal cells sometimes contained melanin granules and, similarly, some tapetal cell-like melanocytes contained tapetal rodlets at the border region of the tapetum (Figs. 11, 12). Morphological types intermediate between melanin granules and tapetal rodlets were also observed in these melanocytes (Fig. 12). On the other hand, no tapetal cell-like morphological features were found in the choroidal melanocytes on the suprachoroid and in the periphery of the fundus.

In human eyes, melanocytes were most abundant in the macular region and gradually decreased toward the periphery. They crowded closer to the suprachoroid and gradually decreased in number going toward the retina where they were seen in limited numbers underneath the choriocapillaris in all regions (Fig. 13a). They contained abundant mitochondria, ribosomes, and intracytoplasmic filaments, as well as randomly arranged melanin granules (Fig. 13b). Tapetal cells or the specific arrangement of melanocytes like that of tapetal cells were not found anywhere.

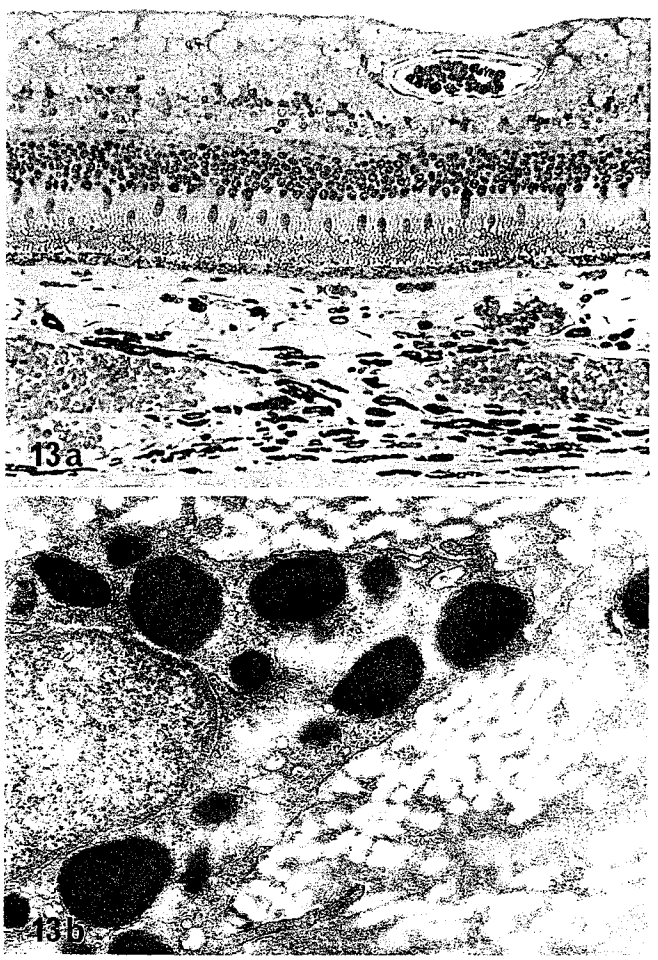


Fig. 13a, b. Melanocyte of the human eye at the inferior region of the optic disc. **a** Light micrograph of the retina and choroid. The melanocytes crowd toward the suprachoroid and gradually decrease in number toward the retina with just a few underneath the choriocapillaris, × 220. **b** Electron micrograph of melanocyte. The melanocyte has a lot of intracytoplasmic filaments, ribosomes, and randomly arranged melanin granules. × 25000

Discussion

In the present study, we demonstrated that melanocytes resembling tapetal cells exist in regions of the choroid of dogs and cats elsewhere than at the periphery of the tapetum, as reported by Bernstein et al. [1]. Moreover, it was shown that the total area of the tapetum plus the area of tapetal cell-like multilayered melanocytes of dogs and cats occupy most of the fundus, although there is a difference in the ratio of the area of the tapetum to total area of the fundus – that is, in the cat whose tapetum occupied most of the fundus, there were fewer tapetal cell-like multilayered melanocytes than in the dog whose tapetum was limited to the posterior pole of the superior fundus. The number of layers of tapetal cells in dogs was much fewer than in cats, and there were more layers of tapetal cell-like melanocytes on the scleral side of the tapetum. These melanocytes contained very few organelles except for the melanin granules mentioned by Bernstein [1]. In addition, the multilayered melanocytes from the inferior border region of the tapetum that extend slightly toward the posterior pole of the inferior fundus resembled tapetal cells in their outward form in both animals; they had a flat nucleus, and some of them had regularly arranged melanin granules. Moreover, the cell layers in these areas were bordered by fibroblast cell processes. Similarly, tapetal cells were bordered by the cell processes of the mesenchymal cells in the course of development of the tapetal cell [4, 6]. As mentioned by Bernstein et al. and Pedler [1, 8], we also found the coexistence of tapetal rodlets and melanin granules in the same cell, i.e., there are some tapetal cells in which some melanin granules can be found, and there are also some melanocytes in which tapetal rodlets can be found. These varieties of melanocytes were never observed in the choroid of the human eye, which has no tapetum; they were observed only in the area between the choriocapillaris and large choroidal vessels where the tapetum was located in the choroid of dogs and cats.

It is known that the choriocapillaris is indented into the retinal pigment epithelium and that Bruch's membrane is reduced to only the two basal laminae of the choriocapillaris and retinal pigment epithelium in the tapetal area [2, 5, 7]. Some researchers believe that the indentation of the choriocapillaris is due to the rigidity of the reflective material of the tapetum [2, 7]. However, the indentation was also found in the area where the multilayered melanocytes replace tapetal cells. This means that the indentation of choriocapillaris is caused not only by the rigidity, but also by the special structural architecture of the choroidal melanocyte.

The present study supports the contention that the tapetal cell and choroidal melanocyte have a common origin [1, 4, 6, 8] because of the following three points: (1) their morphological similarity; (2) the coexistence of tapetal rodlets and melanin granules in both tapetal cells and melanocytes; (3) tapetal cell-like melanocytes extend over a larger

area in dogs than in cats. In addition, it is supposed that the internal and external arrangement and specialization of the ancestor melanocyte related to the development of the tapetum occurred between the choriocapillaris and large choroidal vessels in most of the fundus of both dogs and cats.

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