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Fine structure of the canine tapetum lucidum

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INTRODUCTION

Tapeta lucida are randomly distributed throughout the animal kingdom, being found primarily in animals that are dim light active (Walls, 1967). They are of diverse structure, organization and composition (Rodieck, 1973). Despite these differences, however, all act to increase retinal sensitivity by reflecting light back through the photoreceptor layer.

Two types of vertebrate tapeta lucida are distinguished. The reflecting material may be located within the retinal epithelium (retinal tapetum lucidum), or it may be located in the choroid, external to the retinal epithelium (choroidal tapetum lucidum). Within choroidal tapeta lucida, the reflective material may be an array of extracellular fibres (tapetum lucidum fibrosum), or layers of cells packed with organized, highly refractive material (tapetum lucidum cellulosum) (Walls, 1967; Rodieck, 1973). Amongst the reflective materials noted in tapeta cellulosa are guanine/hypoxanthine crystal plates, riboflavin crystal plates and rodlets of varying composition. The rodlet type of tapetum lucidum cellulosum is characteristic of all of the Order Carnivora except the Family Viverridae (Duke-Elder, 1958).

The bulk of the work reported on the rodlet type of tapetum lucidum cellulosum is on the cat (Murr, 1928; Lucchi, Callegari & Bartolami, 1978; Vogel, 1978; Bussow, Barmgarten & Hansson, 1980). It has been suggested that dogs, as well as possessing a well developed tapetum lucidum cellulosum, also have a retinal tapetum lucidum that may aid in the reflection of light by the choroidal tapetum (Walls, 1967). Histological studies of the adult dog tapetum are primarily restricted to light microscopy and are of a superficial nature. Because a thorough electron microscopic study of the adult tapetum lucidum is not present in the literature, this morphological study was undertaken to describe the fine structure of the canine tapetum lucidum.

MATERIALS AND METHODS

Eyes of six adult mongrel dogs were surveyed for this study. The dogs were anaesthetised with sodium pentobarbital, and, after loss of deep tendon reflexes, the eyes were rapidly enucleated. Each globe was incised and then fixed by immersion in 5% glutaraldehyde in 0·1 M Sorensen's buffer for 5 hours at 4 °C. The anterior hemisphere of each eye was carefully dissected away, in washing solution consisting of 5% sucrose in 0·1 M Sorensen's buffer, pH 7·3. The posterior hemispheres were then divided into pieces < 1 mm^2, post-fixed for 2 hours in 1% OsO_4 in 0·1 M Sorensen's buffer, dehydrated through a graded ethanol series to propylene oxide and embedded in Araldite 502. The embedded tissues were re-orientated to desired angles, using a wax mount, prior to sectioning on an LKB ultramicrotome with both glass and diamond knives. Sections on Formvar-coated copper grids were stained with aqueous

uranyl acetate and lead citrate, and viewed and photographed in a Philips EM201 electron microscope.

RESULTS AND OBSERVATIONS

The tapetum lucidum covered an area of approximately 30% of the superior fundus. It extended as a rounded equilateral triangle, with the base orientated horizontally (excluding the optic disc) and the apex directed upwards. In fresh preparations of the posterior hemisphere, the reflected colour of the tapetum varied from yellow–green to green–blue with an irregular marginal area.

The tapetum lay immediately external to the retinal epithelium, Bruch's membrane and the choriocapillaris. Central areas of the tapetum contained 15–20 layers of tapetal cells (Fig. 1), while towards the periphery the tapetum gradually thinned and eventually ended (Fig. 3). Tapetal cells appeared as polygonal plates and were arranged in each layer much like 'patio bricks' (Fig. 2). Layers were added to form a multilayered structure that in radial section resembled a 'brick wall' (Fig. 1). The lateral borders of the tapetal cells were tightly opposed, on the order of 0·02 μm with no obvious intervening material (Fig. 4). The large surface faces of tapetal cells were separated by a complex anastomosing network of elastic and collagen fibrils (Fig. 5). The surface faces of tapetal cells were indented to accommodate these extracellular fibres (Figs. 4,7). Between indented regions of the cell surface the two surface membranes again came to lie in close approximation, on the order of 0·02 μm (Figs. 7,8).

The layering of the tapetum was quite regular, both centrally and peripherally, at the inner border adjacent to the retinal epithelium. However, at the outer border of the tapetum, near the pigmented choroid whether centrally or peripherally, the layering was more irregular (Figs. 1,3).

Tapetal cells appeared as rounded polygons with diameters of 40–48 μm and a mean thickness of 3·28 μm (Figs. 1–3). Tapetal cells were tightly packed with bundles of membrane-bound rodlets that displaced other organelles and/or inclusions into the perinuclear or peripheral region of cytoplasm (Figs. 4,7).

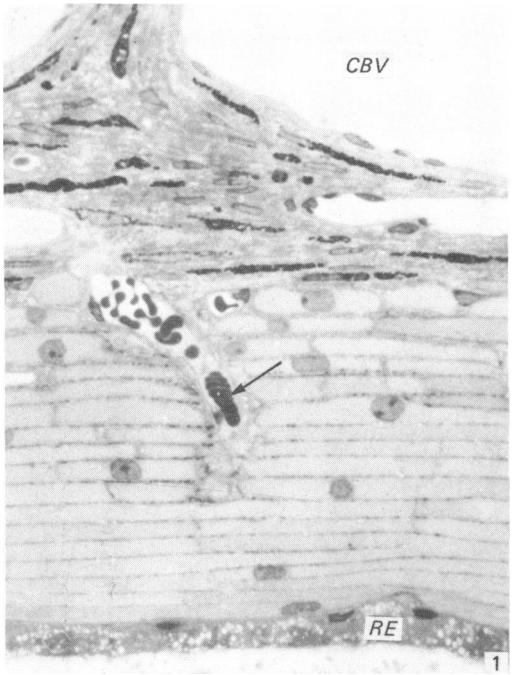
Individual tapetal rodlets were fairly constant in their dimensions, with a mean length of 4·0 μm and a mean diameter of 0·14 μm (Figs. 4, 7). Occasionally, however, rodlets as long as 6·5 μm were present, as well as very short rodlets, approximately 3 μm in length. The shorter rodlets tended to occur at the cell periphery and perinuclearly, whereas the longer rodlets tended to occur in the centre of rodlet bundles. Tapetal rodlets were enveloped in a unit membrane and appeared to contain a hard and apparently homogeneous core which tended to fragment out on sectioning.

Fig. 1. Light micrograph of a radial section of a maximal thickness region of the dog tapetum. Note the regular layering of tapetal cells and a portion of a tapetal penetrating capillary (arrow), choroidal blood vessel (*CBV*), and the retinal epithelium (*RE*). × 1500.

Fig. 2. Light micrograph of a tangential section of the tapetum illustrating the polygonal-shaped tapetal cells (*TC*) comprising each layer. Note the profiles of the tapetal penetrating capillaries, the lumina of some of which contain erythrocytes (arrow). × 1600.

Fig. 3. Electron micrograph of an intermediate thickness region of the tapetum. Note the location of the tapetomelanocytic interface (arrows), the displaced tapetal cell (*TC*), the retinal epithelium (*RE*) and a choroidal melanocyte (*M*). × 2200.

Fig. 4. Electron micrograph of several layers of tapetal cells illustrating the regular hexagonal array of tapetal rodlets. Note the close approximation of tapetal cells of the same layer (arrows) and the interposed collagen (*C*) and elastic fibre (*E*) network between cell layers. × 17000.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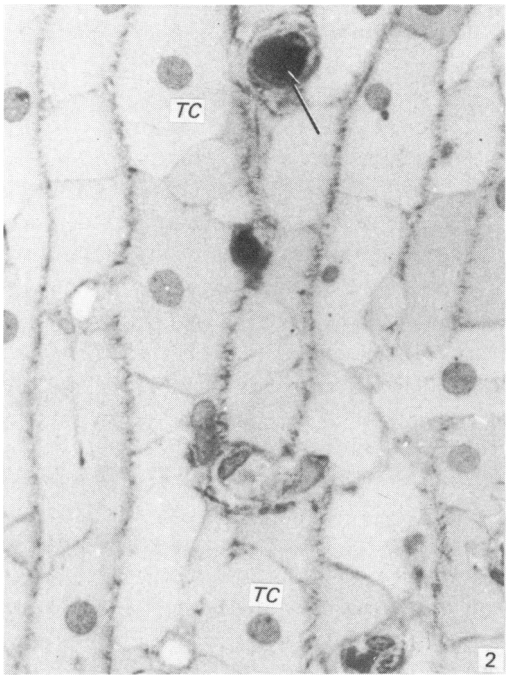
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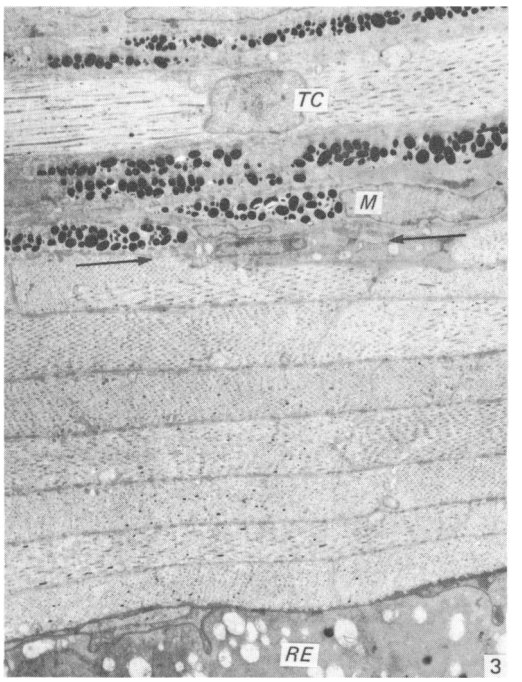
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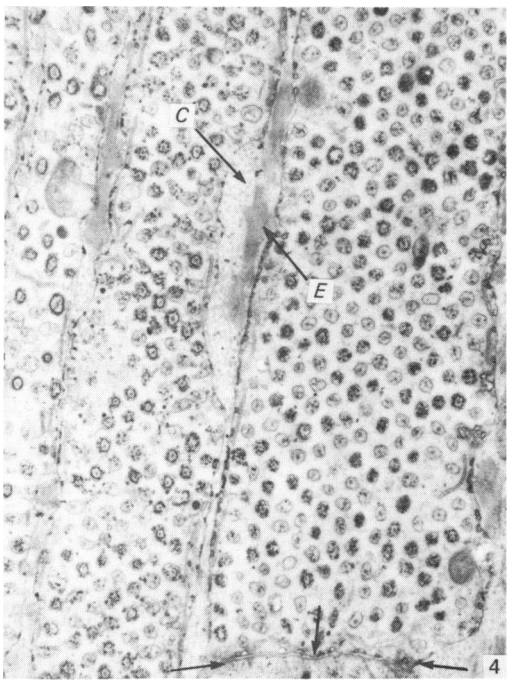

C
E
4

The tapetal rodlets were arranged into separate bundles within the cytoplasm, with no distinct material separating the bundles (Fig. 6). Each tapetal cell might contain as many as ten separate bundles of rodlets, each bundle orientated at a different angle around the nucleus (Fig. 6). Analysis of partially serial sectioned tapeta revealed that the majority of rodlet bundles displayed the same longitudinal axial orientation throughout the entire thickness of a tapetal cell (Fig. 4). The bundles were located so that the individual rodlet longitudinal axes were roughly tangential to the cells' large surface face and hence tangential to the retinal surface (Fig. 5).

The rodlets of each rodlet bundle were primarily arranged into a compact regular hexagonal array, or 60° lattice, with mean rodlet centre to centre spacing of 0·20 μm (Fig. 4). However, the two dimensional lattice structure might vary, with a 'square' array, or 90° lattice, occasionally observed.

Tapetal cells contained a large disc-shaped nucleus, averaging 3·1 μm in thickness and 8·3 μm in diameter (Figs. 6, 7). The nucleus was usually positioned centrally within the tapetal cell and occupied practically the entire thickness of the tapetal cell, with only a small amount of cytoplasm between the nucleus and the adjacent surface membrane, which lacked rodlets and other organelles (Fig. 7). Mitochondria were the most abundant of the other organelles and were located in the perinuclear cytoplasm or displaced to the cell periphery (Fig. 6). Characteristically, the mitochondria appeared crucible-shaped, with a mean diameter of 1·0 μm (Fig. 7). The Golgi apparatus was extremely attenuated and, along with fragments of granular endoplasmic reticulum and isolated polyribosomes, was located adjacent to the nucleus (Fig. 7). Only a small amount of smooth endoplasmic reticulum was present in tapetal cells, modified as subsurface cisternae, paraplanar to the cell surface (Fig. 7). Incomplete and/or abnormal rodlets and vesicles were often present at the peripheral edges of rodlet bundles (Fig. 6).

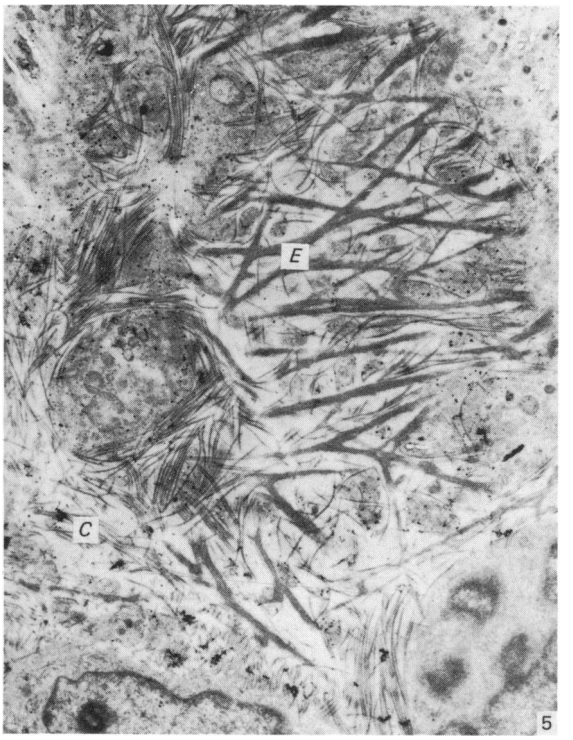
Normal appearing tapetal cells were occasionally found displaced among the melanocytes of the pigmented choroid (Fig. 3). Tapetal cells that appeared 'abnormal' might also be found within the tapetum, at the tapetomelanocytic interface or between melanocytes of the pigmented choroid. These abnormal appearing tapetal cells characteristically contained either a disorganized arrangement of rodlets and/or abnormal appearing rodlets. Melanosomes might also be present in these abnormal tapetal cells. Grossly atypical tapetal cells frequently occurred at the tapetal periphery near Bruch's membrane.

Fig. 5. Electron micrograph of a tangential section between cell layers of the tapetum. The complex elastic (*E*) and collagen fibre (*C*) network located between the cell layers is obvious. × 6000.

Fig. 6. Electron micrograph of a tangential section of a tapetal cell illustrating the large prominent nucleus containing a nucleolus, the perinuclear cytoplasm containing mitochondria, rodlets and vesicles, and the surrounding rodlet bundles. × 6600.

Fig. 7. Electron micrograph of four layers of tapetal cells. Note the prominent nuclei extending from surface to surface with very little intervening cytoplasm, the attenuated Golgi apparatus (arrow), the crucible shaped mitochondria (*M*), the regular arrays of rodlets, the subsurface cisternae (arrowheads), and the collagen and elastic fibre network occupying the spaces between the cell layers. × 13000.

Fig. 8. Electron micrograph of the retinal–tapetal junction depicting an indenting choriocapillary (*CC*). Note Bruch's membrane (arrow) which, in this region, consists of the fused basal laminae of the retinal epithelium (*RE*) and the endothelium of the choriocapillaris. × 8600.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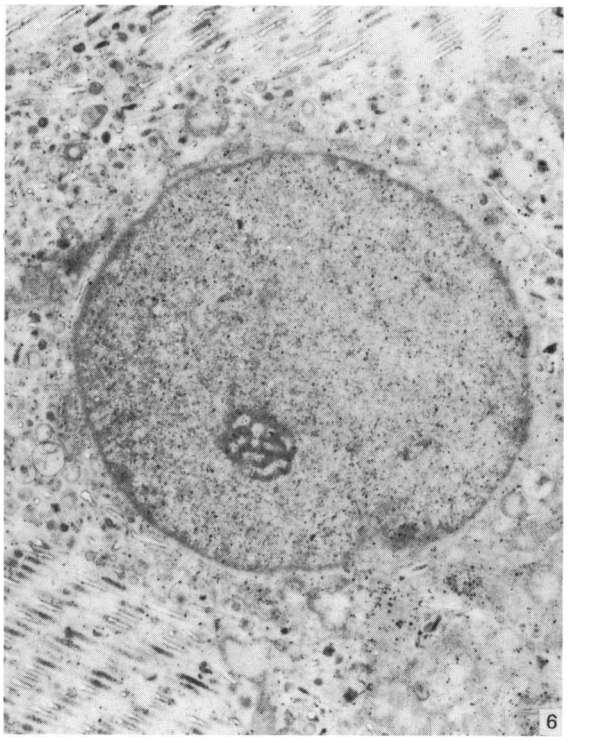
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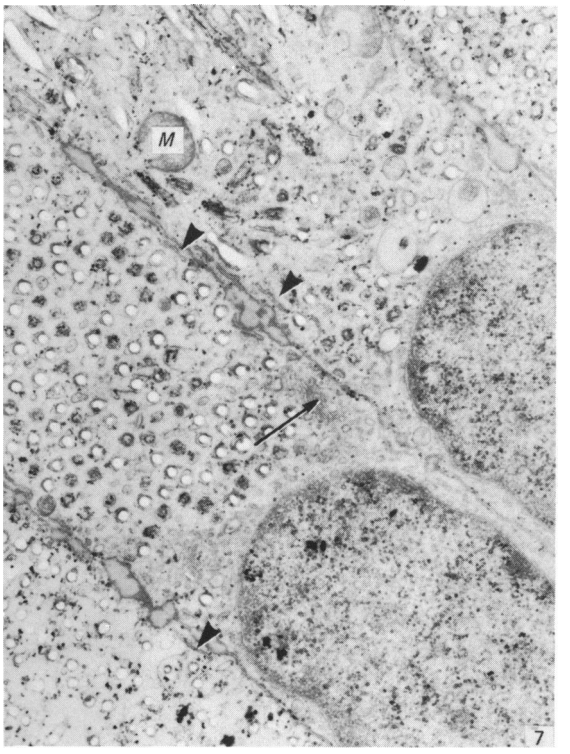
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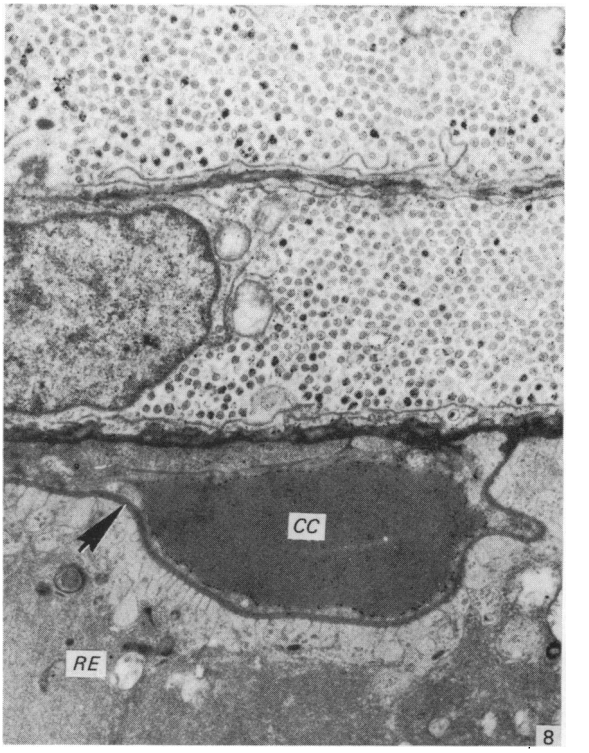
CC
RE
8

The tapetum appeared to be supplied by branches of blood vessels of the pigmented choroid. These tapetal penetrating capillaries were fenestrated and were aligned perfectly radially, with tapetal cell lateral borders lined up against their outer walls (Figs. 1, 2).

The choriocapillaris formed an extensive anastomosing network of fenestrated capillaries, located adjacent to the retinal epithelial layer. In tapetal and peritapetal areas the choriocapillaris indented the retinal epithelium while in peripheral non-tapetal areas the retinal epithelium was not indented by the choriocapillaris (Fig. 8). The nuclei of the endothelial cells of the choriocapillaris were characteristically located in the cytoplasm on the side of the vessel opposite the retinal epithelium.

In the dog, Bruch's membrane over tapetal areas was extremely attenuated, appearing as a single homogeneous layer (although it is bilaminate) with a mean thickness of 0·16 μm (Fig. 8). At the periphery in non-tapetal areas Bruch's membrane was thicker at 0·54 μm and was pentalaminate with a central core of loose elastic fibres.

Centrally, overlying the tapetum and sometimes slightly beyond its margins, the retinal epithelium was devoid of melanosomes while peripherally (in non-tapetal regions) it was pigmented (Fig. 8).

DISCUSSION

Arhythmic animals, such as the dog, characteristically display adaptations of sensory organs that act to expand their potential activity periods. For example, the dog's eye possesses a tapetum lucidum and correspondingly can function at extremely low levels of light. Tapeta lucida are known to be effective in increasing retinal sensitivity. In the cat, the tapetum lucidum acts to lower the threshold for light stimulation by a factor of six, and allows for the detection of light that is imperceptible to human eyes (Gunter, 1951). The tapetum lucidum of the dog is located in the dorsal fundus which corresponds to the lower or foreground visual field (region below the visual horizon) (Rodieck, 1973) in which prey is usually detected (Pirie, 1966).

The central region of maximal tapetal thickness in carnivores appears to correspond to the fixation area and displays the highest degree of reflectivity (Weale, 1953; Wyman & Donovon, 1965). It is known that for a multilayer reflector, the reflectivity increases as the number of layers increases (Born & Wolf, 1964). In an ideal multilayer thin film reflector using the principles of constructive interference, only ten alternating low and high refractive index $\frac{1}{4}$ wavelength films are required for essentially 100 % reflectivity (Land, 1972). The tapetum in the centre of the tapetal fundus of the dog, however, consists of 18–20 layers of cells, with each layer containing approximately 15–18 ranks of rodlets with as many intervening layers of cytoplasm. Apparently the arrangement, the optical thicknesses of the layers and the refractive indices of the components are not exactly those of the ideal $\frac{1}{4}$ wavelength thin film multilayer reflector and must be compensated for by an increased number of layers.

A complex anastomosing network of collagen and elastic fibrils is interposed between the tapetal cell layers and the surfaces of the cells are indented to accommodate this network. The origin of the network is not determined in the dog or in any carnivore. It is possible that, as in the cat, the mesenchymal cells that effectively compartmentalise the embryonic or prospective tapetum lucidum in the early postnatal period and subsequently regress (Bussow, 1974) lay down this fibril network. The functional significance of this fibril network, which appears to occur generally in other choroidal tapeta lucida (Dartnall *et al.* 1965) is uncertain, but it may be that

the network is involved in maintaining the structural integrity of the tapetum lucidum.

Tapetal cells are densely packed with bundles of rodlets, almost to the exclusion of other organelles and inclusions. There is some variation in the size of tapetal rodlets, especially in their length, but on the whole they are fairly uniform. The shortest rodlets (those approximately 3 μm in length) tend to predominate at peripheral edges of rodlet bundles, possibly due to the limited space these areas provide for elongation during development, or because these rodlets may be newly forming ones, as suggested for rodlets of this size and location in cat tapetal cells (Bernstein & Pease, 1959). Conversely, the longest rodlets (as long as 6·5 μm) tend to be found in the centre of rodlet bundles, presumably because this location affords the least space constraints during development.

Although the dimensions of the tapetal rodlets of the dog are similar to those of other carnivores, the interplanar spacings in the cat, at 300–500 nm (Coles, 1971), are greater than those determined in this study for the dog (200 nm). The most striking difference between the cat and dog tapetum lucidum is the difference in reflectivity. The dog tapetum, besides reflecting light of a different wavelength, does so less efficiently. This may be due to the spacing and arrangement of the reflecting rodlets. Canine tapetal cells when viewed in tangential section are not uniform in shape and contain rodlet bundles arranged at several angles approximately perpendicular to incident light. In the cat, the rodlet bundles are more precisely orientated and occupy a greater proportion of the cytoplasm (Pedler, 1963). In addition, the hexagonal (or 60° lattice) arrangement of the rodlets is frequently disrupted in canine tapetal cells, but is only rarely so in the cat (Pedler, 1963).

The radial alignment of the tapetal penetrating capillaries appears to be the best way to connect the choriocapillaris to both its supplying and draining systems, because this results in the least interference with tapetal reflectivity (Walls, 1967).

The choriocapillaris indents the retinal epithelium in tapetal regions of the eye in dogs and other tapetalised animals, such as the cat (Nakaizumi, 1964*b*) and the bushbaby (*Galago*) (Braekevelt, 1980). This indenting is apparently due to the rigidity of the tapetum lucidum (Nakaizumi, 1964*b*) and possibly also to the desirability of a relatively smooth reflecting surface (Braekevelt, 1980).

In the dog and in other carnivores, Bruch's membrane over tapetalised regions is attenuated, lacking a central elastic lamina. In peripheral pentalaminate regions of Bruch's membrane, the central elastic lamina is believed to provide support for the retinal epithelium and adjacent photoreceptors (Nakaizumi, 1964*a*). It may be that this supportive function is usurped by the tapetum, and hence Bruch's membrane over these regions does not require an elastic core. It is of obvious advantage to the eye for Bruch's membrane to be as thin as functionally possible (only as thick as necessary for support), so as to have the least influence on exchange processes.

The reflective particles, previously described as being present in dog retinal epithelium (Walls, 1967), were not observed in this study. It may be that their presence in the retinal epithelium was due to a pathological condition, because various disease processes, such as retinal degeneration, are common in dogs (Santos-Anderson, Tso & Danwolf, 1980) and in the early stages display a large accumulation of vesicles.

SUMMARY

The structure of the canine tapetum lucidum appears to be basically similar to that described in some other carnivores, but specific distinctions are present. The reflect-

ing rodlets of the dog are similar in their dimensions to those of the cat, but they are more closely packed, resulting in a unique value for the wavelength of maximal reflectance. In addition, the rodlets are less precisely orientated, as compared to those of the cat, a condition which appears to result in a less efficient reflectance from the dog tapetum. It has also been shown that the dog does not possess a retinal tapetum lucidum, as had previously been suggested.

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