

Fine structure of the tapetum lucidum in the short-tailed stingray (*Dasyatis brevicaudata*)

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Summary. The tapetum lucidum of the short-tailed stingray (*Dasyatis brevicaudata*) is located in the choroid of the superior fundus immediately external to the choriocapillaris. In this species the tapetum consists of a single layer of overlapping cells oriented at an angle of about 30° to the incoming light. These tapetal cells alternate with and are separated from one another by melanocytes which extend beyond the tapetal cells to intervene between the tapetal cells and the choriocapillaris. The tapetal cells and the melanocytes are flattened plate-like cells with their widest dimension facing the retina. Internally the tapetal cells display a peripherally located vesicular nucleus with most organelles in a paranuclear location. The bulk of the cell is packed with regularly spaced crystals reported to be guanine. The size and spacing of these reflective crystals is commensurate with constructive interference. In light-adaptation, the melanosomes of the intervening melanocytes are widely dispersed and for the most part block the passage of light to the tapetal cells. Although dark-adapted specimens were not examined, it seems reasonable to assume that in dark-adaptation, the melanosomes will retreat to unmask the tapetum and allow it to function as a reflective layer.

Key words: Tapetum lucidum, Fine structure, Elasmobranch, Short-tailed Stingray, *Dasyatis brevicaudata*

Introduction

The tapetum lucidum of the vertebrate eye is a reflective layer located external (choroidal) to the photoreceptors of the retina which by reflecting light back onto the photoreceptors provides these light-sensitive cells with a second opportunity for stimulation and hence increases retinal sensitivity (Walls, 1942; Rodieck, 1973).

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The reflective material making up a tapetum lucidum may be located either within the cells of the retinal epithelium (RPE) in which case it is referred to as a retinal tapetum or within the choroid immediately adjacent to the choriocapillaris. In this location the tapetum may be composed of an array of extracellular collagen fibers to form a tapetum fibrosum or consist of reflective material located within specialized cells to form a tapetum cellulosum. Retinal tapeta are most usually noted in teleost species but are reported in other vertebrates (Pirie, 1966; Arnott et al., 1970; Braekevelt, 1976, 1977, 1981, 1982). A tapetum fibrosum is characteristic of ungulates (Bellairs et al., 1975; Braekevelt, 1983, 1984, 1986a) while a tapetum cellulosum has been reported in a variety of species (Bernstein and Pease, 1959; Pedler, 1963; Denton and Nicol, 1964; Hebel, 1971; Braekevelt, 1981, 1986b, 1990, 1991, 1993; Lesiuk and Braekevelt, 1983).

As part of an ongoing comparative study of tapeta lucida, the morphology of the tapetum cellulosum of the short-tailed stingray (*Dasyatis brevicaudata*) is described in this report.

Materials and methods

For this study the eyes from two adult light-adapted short-tailed stingrays (*Dasyatis brevicaudata*) were examined by light and electron microscopy. The animals were killed by severing the spinal cord and the eyes quickly enucleated. The eyeballs were slit open at the equator and immersion fixed for 5 h at 4 °C in 5% glutaraldehyde buffered to pH 7.3 with 0.1M Sorensen's phosphate buffer. The posterior half of the eyeball was then removed, washed in 5% sucrose in 0.1M Sorensen's buffer (pH 7.3) and cut into pieces less than 1 mm². This tissue was then post-fixed for 2 h in 1% OsO₄ in the same phosphate buffer (pH 7.3), dehydrated through graded ethanols to methanol and then to propylene oxide and embedded in Araldite.

Pieces of plastic-embedded tissue were reoriented to desired angles by means of a wax mount and thick sections (0.5 µm) were cut, stained with toluidine blue and examined by light microscopy. Thin sections (60-70

nm) were then cut of selected areas and collected on copper grids. These sections were stained with aqueous uranyl acetate and lead citrate and examined and photographed in a Philips EM201 transmission electron microscope.

Results

The tapetum lucidum of the short-tailed stingray (*Dasyatis brevicaudata*) is located in the choroid of the superior fundus entirely above the optic nerve head. In this species it is a tapetum cellulolum composed of a single layer of overlapping cells situated immediately external to the choriocapillaris (Fig. 1). These cells overlap in a shingle-like fashion and are positioned at an angle of about 30° to the RPE although this angle varies somewhat depending upon the location within the fundus (Figs. 1, 6).

These tapetal cells are large flattened plate-like cells with their widest surface facing the path of incoming light. These cells measure up to 35 µm in width and about 5 µm in thickness. Internally these cells show a single vesicular nucleus located peripherally (Figs. 2, 3). Most of the cell organelles are clustered in this paranuclear region although small mitochondria and glycogen-like particles are scattered throughout these cells (Figs. 2, 3). The bulk of the cytoplasm of these cells is however packed with crystals of fairly regularly spaced material (Figs. 1-7). The reflective material was not analyzed in this study but is reported to be guanine (see Discussion). This reflective material is very brittle and often chips out on sectioning, leaving behind empty membrane-bound cisternae (Figs. 1, 6). When the reflective material chips out a varying amount of artefactual widening of these cisternae occurs and its difficult to obtain accurate measurements of the length, width and spacing of these crystals (Fig. 4). In areas where the reflective material has not chipped out however, the crystals were about 0.10 µm in width, 5.0-8.0 µm in length and with a center-to-center spacing of about 0.15 µm between adjacent crystals (Figs. 5, 7). A typical tapetal cell will have 15-20 layers of these reflective crystals across the thickness of the cell (Figs. 2, 5).

The tapetal cells alternate with and are separated from one another by melanocytes which are also flattened plate-like cells measuring about 35 µm in width and 2-4 µm in thickness (Figs. 1-3). The nuclei of these melanocytes are invariably located on the choroidal aspect of these cells where the cell expands to

accommodate the nucleus (Fig. 1). The organelles of the melanocytes are scattered throughout the cell but the cytoplasm is dominated by numerous small melanosomes (Figs. 1, 2). Proceeding into the choroid layer away from the tapetum, more melanocytes are present but these are oriented parallel to the tapetum and do not interdigitate with the tapetal cells (Figs. 1, 4). In this region are also occasionally noted what are presumed to be displaced tapetal cells, not oriented with the main mass of the tapetum (Figs. 1, 4).

The inner edge (closest to the RPE) of the melanocytes which are interdigitated with the tapetal cells is slightly longer than the tapetal cells and curves slightly to lie between the tapetal cells and the incoming light. In many, but not all, locations therefore melanocyte processes are present between the tapetal cells and the light (Figs. 3, 5, 7). In the light-adapted specimens examined in this study this region of the melanocytes normally contains melanosomes which would prevent light from reaching the tapetal cells and hence effectively occlude the tapetum (Figs. 3, 5, 7). In some locations however even in light-adaptations there are no melanosomes situated between the tapetum and the incoming light (Figs. 2, 6). As only light-adapted specimens were available it is not known to what extent the melanosomes retreat to expose the tapetum to the incoming light and produce a functional and effective tapetum.

The RPE overlying the tapetal area is of course always devoid of melanosomes so as not to interfere with the passage of light to the tapetal cells and the return of light being reflected back from the tapetum to the photoreceptor cells (Fig. 1). In this species however even in non-tapetal locations the RPE contains very few melanosomes that probably do not seriously interfere with the passage of light.

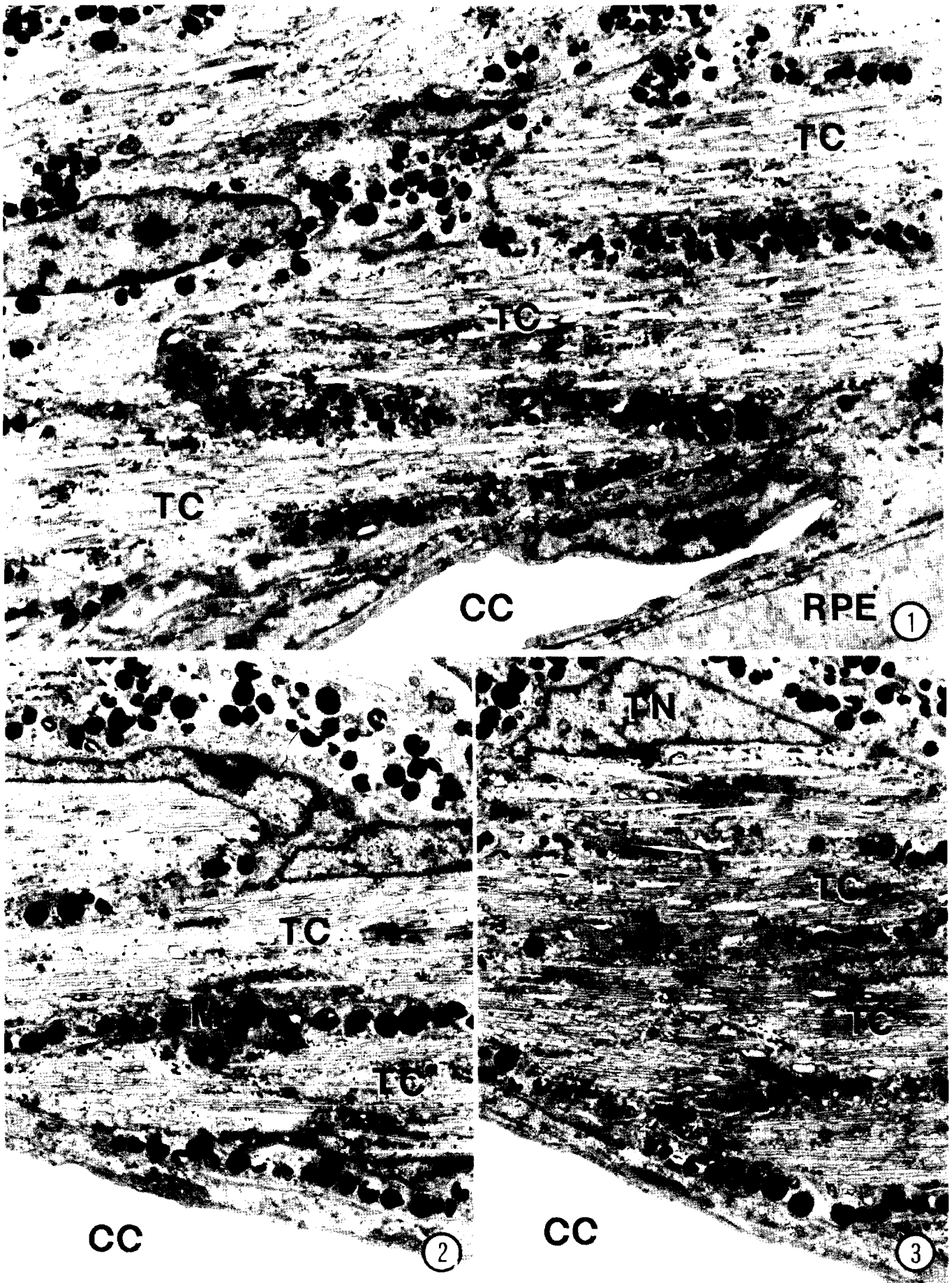
Discussion

A tapetum lucidum is a relative common feature in the eyes of vertebrates whose habitat is ordinarily poorly illuminated (Walls, 1942; Rodieck, 1973). The presence of a tapetum greatly increases the sensitivity of a retina although presumably worsens acuity. Gunter et al. (1951) for instance have calculated that in the cat the presence of a tapetum lowers the threshold for light stimulation by a factor of six and allows for the detection of light that is imperceptible to the human eye. Rodieck (1973) has indicated that the cat's eye reflects about 130 times more light than the human eye.

Fig. 1. Low power electron micrograph to illustrate the tapetum lucidum of the short-tailed stingray. Three tapetum lucidum cells (TC) are labelled. For orientation the retinal epithelium (RPE) and choriocapillaris (CC) are also indicated. $\times 5,600$

Fig. 2. Electron micrograph of the tapetum lucidum to indicate tapetal cells (TC) separated by processes of melanocytes (M). The choriocapillaris (CC) is also labelled. $\times 7,100$

Fig. 3. Electron micrograph to illustrate the abundance of reflective crystals within the tapetal cells (TC). The nucleus of a tapetal cell (TN) is indicated as is the choriocapillaris (CC). $\times 6,400$



Tapetum of short-tailed stingray

The design of a tapetum lucidum is basically quite simple, consisting of a reflective layer located scleral to the photoreceptor cells. This reflective layer is usually placed in the choroid immediately adjacent to the choriocapillaris (choroidal tapetum). In this location the tapetum may be composed either of cells packed with reflective material (referred to as a tapetum cellulosum) or an accumulation of extracellular collagen fibres (a tapetum fibrosum) (Walls, 1942; Rodieck, 1973; Braekevelt, 1981, 1983, 1986a,b, 1990, 1991, 1993). The tapetal reflective material may also be located within the RPE cells to form a retinal tapetum (Arnott et al., 1970; Braekevelt, 1977, 1982).

In the short-tailed stingray (*Dasyatis brevicaudata*) the tapetum is a tapetum cellulosum located in the choroid of the dorsal fundus of the eye. While only light-adapted specimens were available for this study, it is felt that this elasmobranch possesses an oclusable tapetum lucidum. In the light-adapted state the melanocytes which are interspersed with the tapetal cells do have their melanosomes positioned so as to block the passage of light to the reflective material of the tapetum. Although no dark-adapted specimens were available for study, it would be unusual if the melanosomes did not migrate in dark-adaptation to allow light to reach the elaborate reflective tapetum which is present. Also other elasmobranchs are reported to have oclusable tapeta lucida (Nicol, 1989; Braekevelt, 1991). In the light-adapted specimens studied for this report, some areas of the tapetum were not totally masked by melanosomes. Pigmentation within the RPE was also reduced however and it was felt that the inability to totally cover the tapetum with melanosomes was simply a factor of reduced numbers of melanosomes.

The problem of producing efficient reflective surfaces in a biological context is most often solved by arranging material with a high refractive index (such as guanine) in a low refractive medium such as the cytoplasm of a cell (Huxley, 1968; Coles, 1971). This will produce numerous small reflections from the highly refractive material. If the reflective material is randomly arranged, a diffuse reflectance will occur. This is generally the case in retinal tapeta where the reflective material is located within the RPE cells and is not usually high ordered (Braekevelt, 1976, 1977). If however the highly refractive structures are of a constant size and separated by a constant spacing, the small reflections will sum in a constructive fashion and a higher reflection will be

achieved (Denton, 1970, 1971; Denton and Land, 1971; Land, 1972). For optimal constructive interference to occur, the optical thickness (actual thickness $\times$ refractive index) of the refractive material should be a quarter of the wave length of the incident light and the reflective units should be separated by the same distance (Coles, 1971; Land, 1972).

The reflective material within a tapetum lucidum (either choroidal or retinal) can vary widely in chemical composition between species. Amongst the compounds reported are guanine, cholesterol, zinc cysteine, riboflavin, pteridine and a variety of lipids (Weitzel et al., 1954; Pirie, 1966; Arnott et al., 1970; Nicol, 1989). While the chemical nature of the reflecting crystals in the short-tailed stingray was not analyzed for this study, Nicol (1989) indicates that only guanine is reported in the tapeta of selachians. Since it is felt that a tapetum lucidum has been evolved on a number of occasions in response to a dim environment, it is perhaps not too surprising that both its location and structure as well as the choice of reflective material should show such a wide variation (Walls, 1942).

In this study the reflective crystals are measured at about 0.10 μm in thickness and they are separated by about 0.15 μm . This is very similar to the values reported in another elasmobranch, the southern fiddler ray (Braekevelt, 1991b) and these dimensions for thickness and spacing are commensurate with the principles of constructive interference. In the cat the size of the reflective rodlets is also reported at 0.10 μm in diameter and separated by 0.25–0.30 μm (Braekevelt, 1990). In the dog they are reported at 0.18 μm in width and spaced at about 0.20 μm (Lesiuk and Braekevelt, 1983), while in the grey seal tapetum they are again reported at 0.10 μm in diameter with a spacing of 0.15 μm (Braekevelt, 1986b). These relatively small differences in reflective material thickness and spacing may indicate small differences in the optimal wavelength of the incident light in these species.

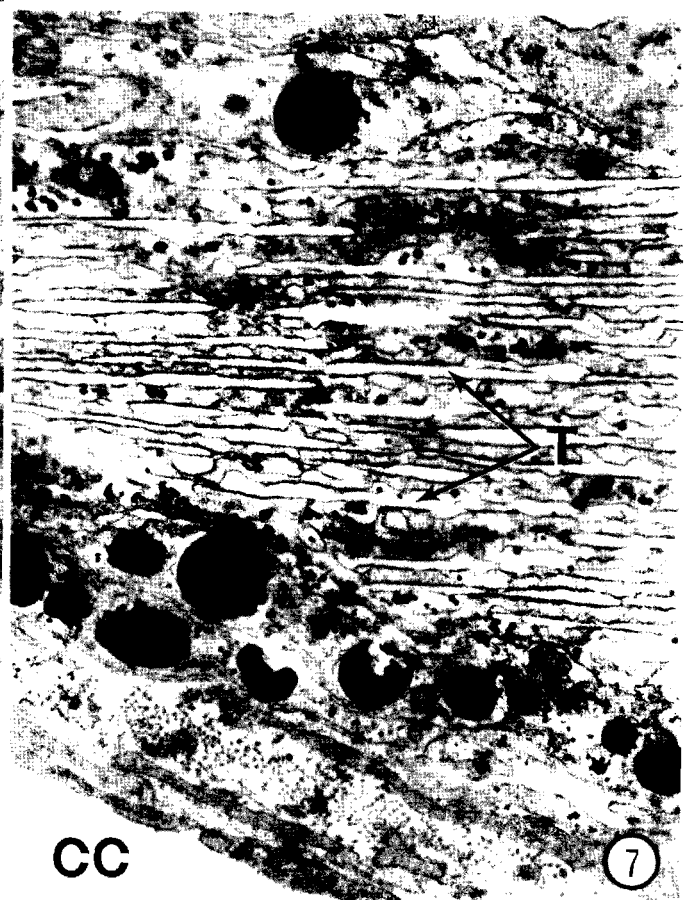
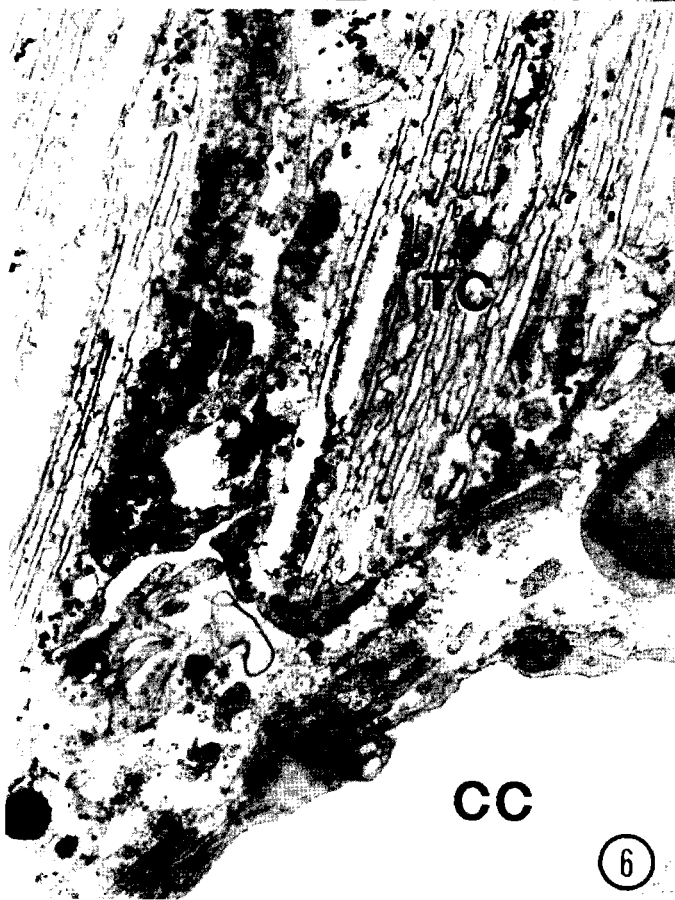
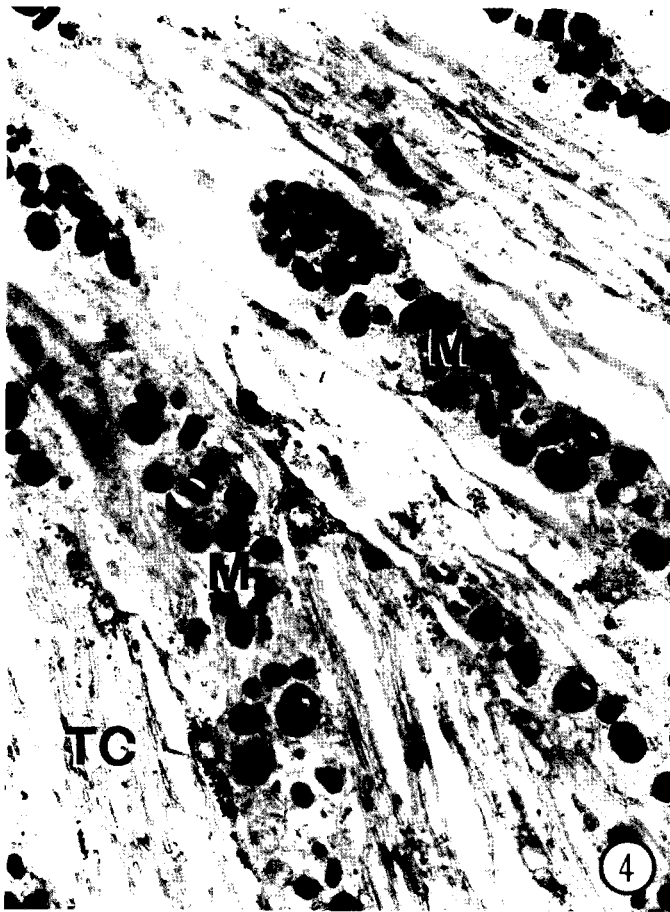
The thickness (number of layers of reflective light cells and the number of reflective layers within these cells) of the tapetum also varies from species to species. The dog's tapetum is reported at 9–15 layers (Wyman and Donovan, 1965), 10–12 layers (Hebel, 1971), and 15–20 layers (Lesiuk and Braekevelt, 1983) while the ferret is reported to have 10–12 layers (Braekevelt, 1981) and the grey seal at 30–35 layers (Braekevelt, 1986b). The cat tapetum is variously reported at 6–10 layers

Fig. 4. Electron micrograph of the choroidal edge of the tapetum lucidum. Tapetal cells (TC) and melanocytes (M) are indicated. Note the different orientation between cells of the tapetum and of the choroid. $\times 7,100$

Fig. 5. Electron micrograph to illustrate melanocyte processes (M) intervening between the tapetal cells (TC) and the incoming light. The choriocapillaris (CC) is also indicated. $\times 15,000$

Fig. 6. Electron micrograph illustrating an area where melanosomes are not interposed between the tapetal cells (TC) and the incoming light. The choriocapillaris (CC) is again indicated for orientation. $\times 15,000$

Fig. 7. Electron micrograph to illustrate the packing and width of the tapetal crystals (T). Most cell organelles are displaced peripherally. The choriocapillaris (CC) is also labelled. $\times 20,000$



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(Rodieck, 1973) through 15-20 layers (Pedler, 1963; Donovan, 1966) to a high of 35 layers (Bernstein and Pease, 1959). While species differences probably exist, the intraspecies differences reported are possibly due to breed variance, sampling of different areas and indeed to developmental stages. In the short-tailed stingray and the southern fiddler ray (Braekevlt, 1991) while the tapetum is composed of only a single layer of cells, these cells are arranged in an overlapping fashion such that light may traverse 3 or 4 layers of cells in the dark-adapted condition.

Denton (1971) has indicated that in an ideal theoretical model of constructive interference, 5 layers of reflective material would give rise to 75% reflection. Land (1972) states that for most biological systems, the reflectance will approach 100% after 10-20 layers. The difference between actual and ideal reflectance would seem to indicate that constructive interference in a biological context is far from perfect. Structures such as blood vessels, nuclei of cells and imperfections in spacing and thickness probably all interfere with reflectance and therefore more layers are presumably required to create an effective tapetum lucidum.

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