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Electron microscopic study of the occlusible tapetum lucidum of the southern fiddler ray (*Trygonorhina fasciata*)

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Summary. The choroidally located tapetum lucidum of the southern fiddler ray (*Trygonorhina fasciata*) has been examined by light and electron microscopy in both light- and dark-adaptation. 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 are situated immediately external to the choriocapillaris. These tapetal cells alternate with and are separated from one another by melanocytes which have an inner extension that curves and intervenes between the tapetal cells and the choriocapillaris. The tapetal cells and the melanocytes are flattened cells with their widest dimension facing the retina. Internally the tapetal cells display a peripherally-located, vesicular nucleus with most of the cell 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 small melanosomes of the melanocytes are widely dispersed and fill the portion of the cell intervening between the tapetal cells and the incoming light. This effectively occludes the tapetum as light is unable to reach the reflective material. In dark-adaptation the melanosomes withdraw from this location, exposing the tapetum to light and allowing it to act as a reflective layer. The retinal epithelium overlying the tapetal area is totally unpigmented so as not to interfere with the passage of light.

Key words: Tapetum lucidum, Electron microscopy, Elasmobranch, *Trygonorhina fasciata*

Introduction

The tapetum lucidum of the vertebrate eye is a reflective layer located external 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, thereby enhancing retinal sensitivity (Walls, 1967; Rodieck, 1973).

The reflective material of a tapetum lucidum may be located within the cells of the retinal epithelium (RPE) in which case it is referred to as a retinal tapetum. This type of tapetum is most commonly seen amongst the teleosts although it has been reported in other vertebrates (Pirie, 1966; Arnott et al., 1970; Braekevelt, 1976, 1977, 1980, 1982). The tapetum is however most usually located in the choroid immediately adjacent to the choriocapillaris. In this location it may be composed of closely arranged extracellular collagen fibres and be referred to as a tapetum fibrosum (Pirie, 1966; Bellairs et al., 1975; Braekevelt, 1983, 1984, 1986a). A choroidal tapetum may also be composed of an array of specialized cells containing a wide variety of reflective materials. This is referred to as a tapetum cellulosum and has been noted and described in a variety of species (Bernstein and Pease, 1959; Pedler, 1963; Denton and Nicol, 1964; Hebel, 1969, 1971; Braekevelt, 1981, 1986b; Lesiuk and Braekevelt, 1983).

As part of an ongoing comparative study of the tapetum lucidum, the morphology of the tapetum cellulosum of the southern fiddler ray (*Trygonorhina fasciata*) is described in both light- and dark-adaptation. The tapetum in this species is remarkable as it is occlusible and can be masked or exposed according to the environmental lighting.

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Materials and methods

For this study the eyes of four adult southern fiddler rays (or guitarfish) (*Trygonorhina fasciata*) were investigated by light and electron microscopy. Both light- and dark-adapted specimens were examined.

The animals were killed by severing the spinal cord and the eyeballs were quickly removed, opened at the

equator and fixed for 5 h in 5% glutaraldehyde buffered to pH 7.3 with 0.1 M Sorensen's phosphate buffer at 4° C. The posterior half of the eyeball was then removed, washed in 5% sucrose in 0.1 M Sorensen's buffer (pH 7.3), divided into tapetal and non-tapetal areas and cut into pieces less than 1 mm^2. The tissue was then postfixed for 2 h in 1% osmium tetroxide in the same phosphate buffer, dehydrated through graded ethanols to methanol and then propylene oxide and embedded in Araldite.

Pieces of plastic-embedded tissue were subsequently 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 (50-60 nm) of selected areas were then cut and collected on copper grids coated with a formvar film. These sections were stained in aqueous uranyl acetate and lead citrate and examined and photographed in a Philips EM201 transmission electron microscope.

Results

The tapetum lucidum of the southern fiddler ray (*Trygonorhina fasciata*) is located in the choroid of the superior fundus where it forms a horizontal band occupying about two-thirds of the fundus above the optic nerve head. In dark-adapted specimens it is easily seen in gross examination when it produces a golden-yellow reflectance.

The tapetum in this species is a tapetum cellulosum and is composed of a single layer of overlapping cells situated immediately external to the choriocapillaris (Fig. 1). These cells are positioned at an angle of about 30° to the retinal epithelial (RPE) layer although this does vary somewhat depending upon their location within the fundus (Figs. 1, 2). These tapetal cells are large flattened, plate-like cells with the widest surface facing the path of incoming light. The cells measure up to 30 μm in width and approximately 5.0 μm in thickness (Figs. 1-3). Internally these cells show a single large vesicular nucleus located peripherally (Figs. 2, 3, 5). Most of the cell organelles are clustered in a paranuclear location although small mitochondria, microbodies and short profiles of smooth endoplasmic reticulum are scattered through the cytoplasm (Figs. 2-5). The bulk of the cytoplasm is however packed with crystals of regularly spaced material. (Figs. 2-5). The reflective material was not chemically analyzed in this study as it is reported to be guanine (see Discussion). The reflective material is very brittle and for the most part chips out on sectioning, leaving behind empty membrane-bound, cisternae (Figs. 4, 5). When the reflective material chips out, a certain amount of artifactual widening of these cisternae occurs and it is difficult to obtain accurate measurements of the length, width and spacing of these crystals. In fortuitous sections however where no chipping and/or widening of the cisternae has occurred, measurements of these crystals were possible. The reflective crystals are approximately 0.10 μm in widht, 6.0-8.0 μm in length with a center-to-center spacing of about 0.14 μm between adjacent crystals (Figs. 4, 5). These crystals form 15-20 irregular layers across the width of a typical tapetal cell (Figs. 4, 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 2.0-4.0 μm in thickness (Figs. 1, 2, 4). The nuclei of these melanocytes are invariably located on the choroidal aspect of these cells where the cell body expands to accomodate the nucleus (Figs. 1, 2). The cell organelles of the melanocytes are not clustered near the nucleus but are scattered throughout the cell (Fig. 2). The cytoplasm of the melanocytes display numerous, small (0.2-0.5 μm) round to oval melanosomes (Figs. 1, 2, 4). Proceeding into the choroid layer away from the tapetal region, more melanocytes are present but these are now oriented parallel to the tapetum (Fig. 1). In this region an apparently displaced tapetal cell is occassionally noted, completely surrounded by melanocytes (Fig. 1).

The inner edge (i.e. closest to the RPE) of those melanocytes interspersed between tapetal cells is slightly curved and expanded and so arranged as to intervene between the tapetal cells and the incoming light (Figs. 2, 4). At least one and often two layers of melanocyte processes are thus present between the tapetal cells and the light (Figs. 4, 5). In the light-adapted condition these melanocyte processes are packed with melanosomes and hence effectively mask the tapetal cells by preventing light from reaching the reflective material (Figs. 4, 5). This effectively occludes the tapetum.

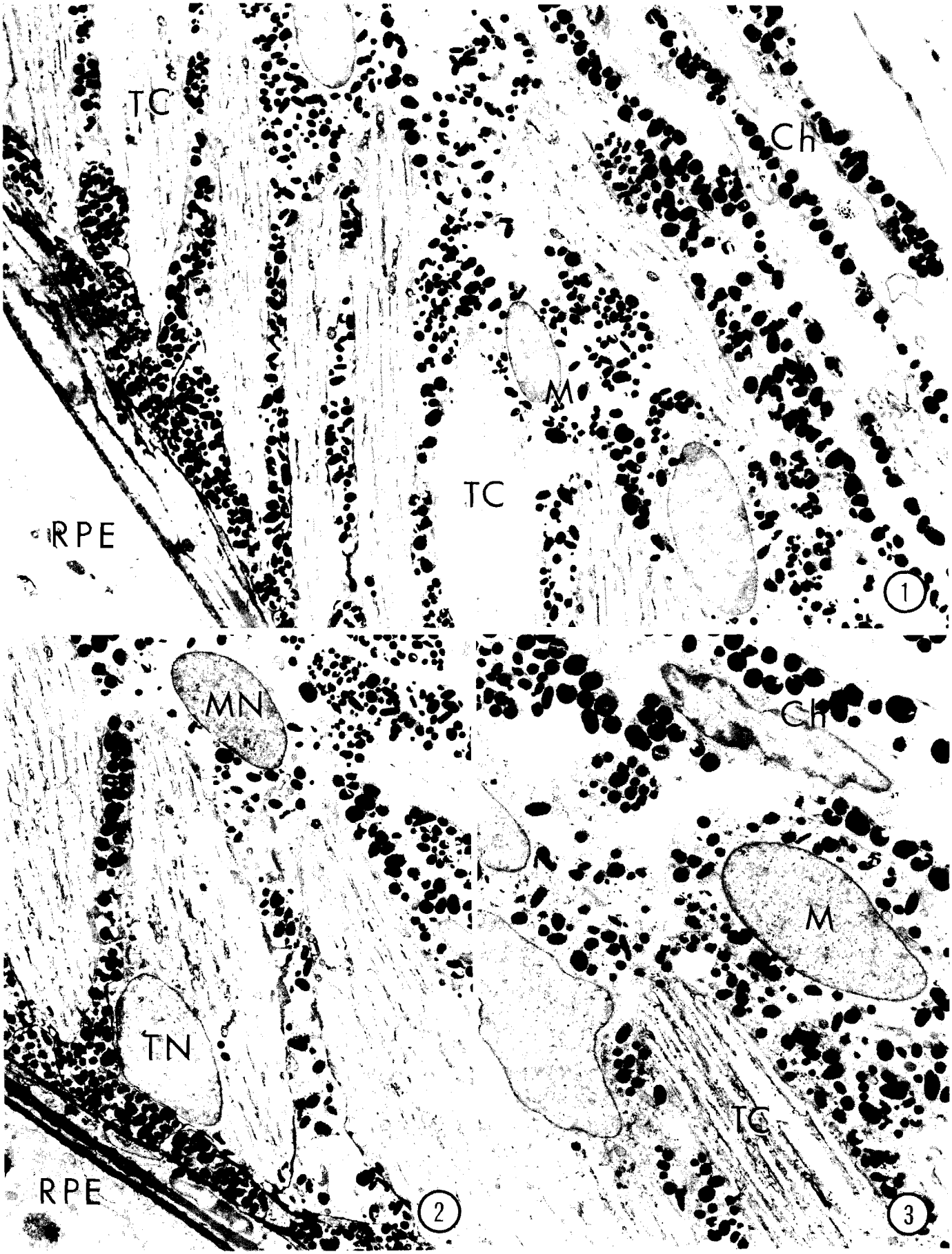
In dark-adaptation, the melanosomes retreat towards the nuclear region of the melanocytes and the melanocyte processes intervening between the tapetal cells and the incoming light become devoid of pigment. This exposes the reflective tapetal cells to light and thus forms a functional tapetum lucidum (Figs. 6, 7). While the melanocyte processes which intervene between the tapetal cells and the incoming light remain in place during dark-adaptation they appear to be somewhat thinner than when they are filled with melanosomes in light-adaptation (Figs. 5, 6).

The RPE overlying the tapetal area is of course always devoid of melanosomes so as not to interfere

Fig. 1. Low power electron micrograph of the tapetal region from a light-adapted southern fiddler ray. For orientation, the retinal epithelium (RPE), tapetal cells (TC), melanocytes (M) and the choroid (Ch) are labelled. × 3,900

Fig. 2. Electron micrograph of the tapetum lucidum from a light-adapted ray. The retinal epithelium (RPE), a tapetal cell nucleus (TN) and a melanocyte nucleus (MN) are indicated. × 4,200

Fig. 3. Electron micrograph of the choroidal edge of the tapetum in light-adaptation. A tapetal cell (TC), a melanocyte (M) and the choroid (Ch) are indicated. × 6,000


TC
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1
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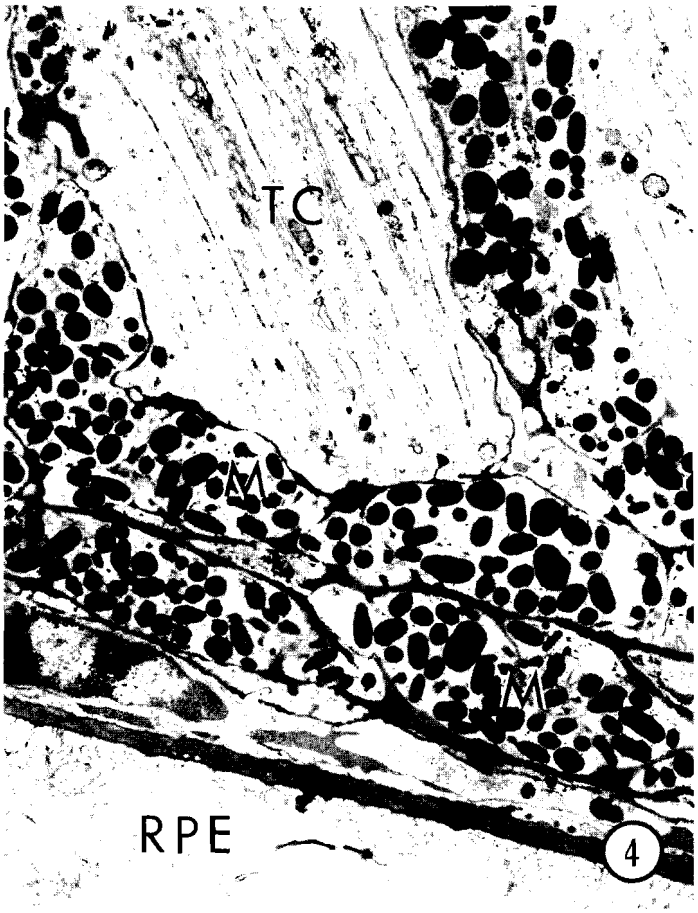
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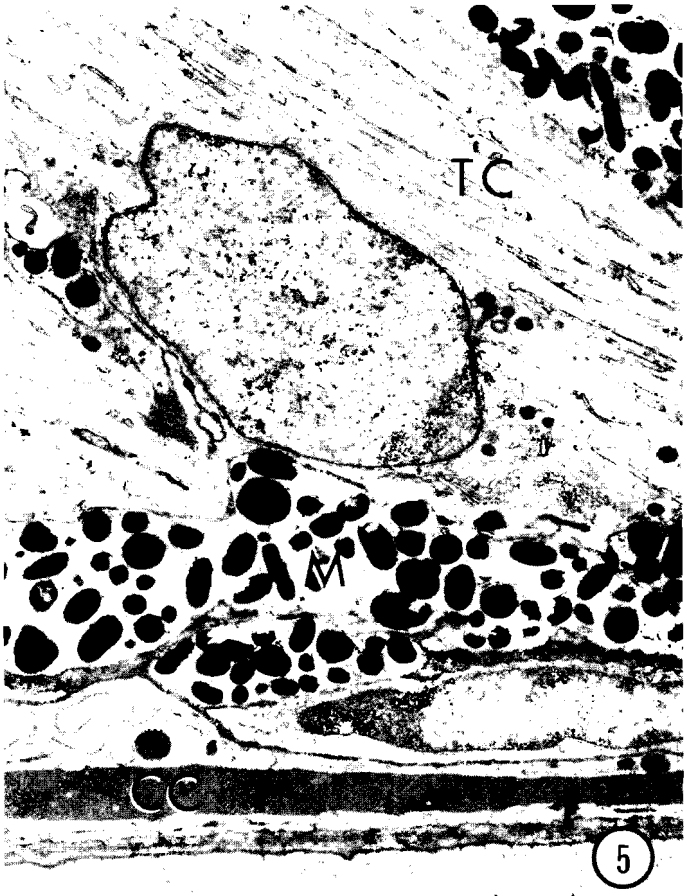
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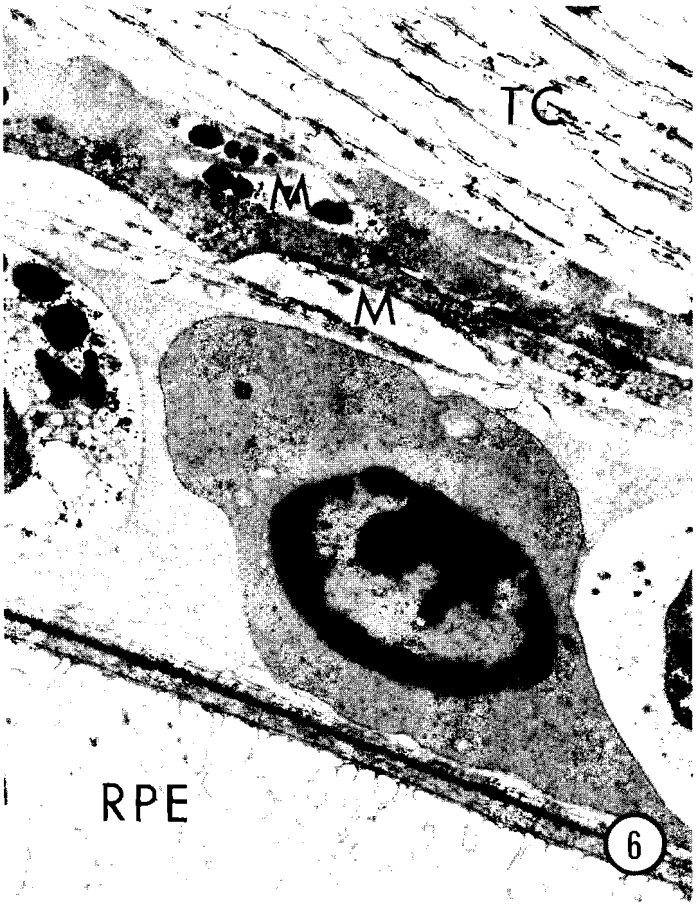
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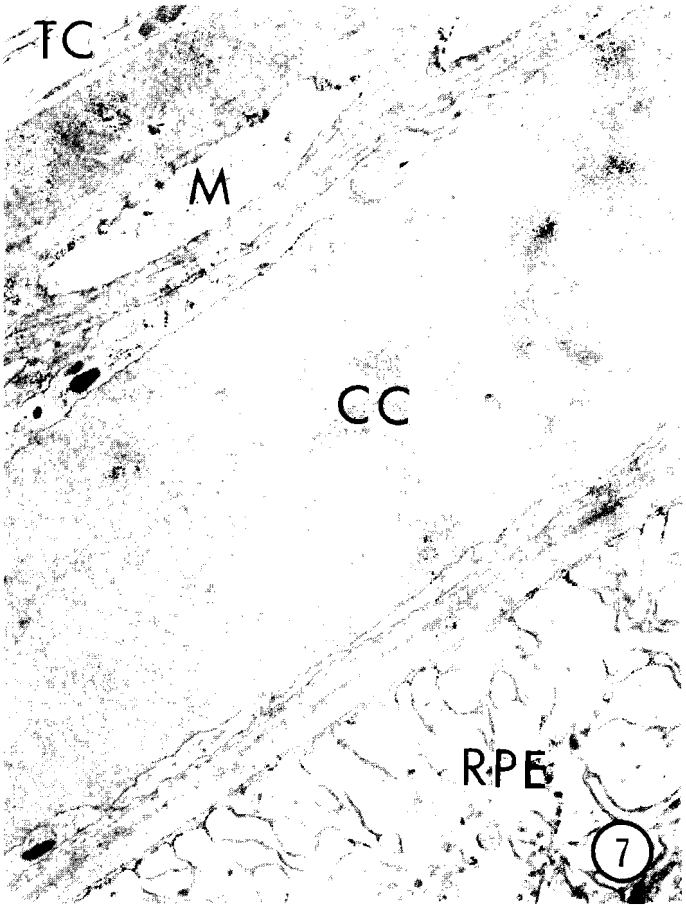
TC
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Fig. 4. Electron micrograph of the retinal side of the tapetum in light-adaptation. A tapetal cell (TC), two layers of melanocyte processes (M) and the retinal epithelium (RPE) are labelled. × 6,400

Fig. 5. Electron micrograph of the tapetum lucidum in light-adaptation to illustrate a tapetal cell (TC) nuclear region, melanocyte process (M) and the choriocapillaris (CC). × 9,500

Fig. 6. Electron micrograph of the inner edge of the tapetum in partial dark-adaptation. Some melanocyte processes (M) still contain melanosomes while others do not. A tapetal cell (TC) and the retinal epithelium (RPE) are also indicated. × 9,500

Fig. 7. Electron micrograph of the inner aspect of the tapetum in dark-adaptation. Melanocyte processes (M) contain no melanosomes. A tapetal cell (TC) the choriocapillaris (CC) and the retinal epithelium (RPE) are also indicated. × 13,800

with the passage of light to the tapetal cells and/or light reflecting back from the tapetum to the photoreceptor cells (Figs. 1, 4, 6, 7).

Discussion

A tapetum lucidum is a relatively common feature in the eye of vertebrates whose habitat is ordinarily poorly illuminated due either to nocturnal conditions or a turbid aquatic environment (Walls, 1967; Rodieck, 1973). The design of a tapetum is basically quite simple, consisting of a reflective layer located sclerad to the photoreceptor cells. This reflective layer is most usually found in the choroid immediately adjacent to the choriocapillaris (choroidal tapetum). In this location the tapetum may be composed of either cells packed with reflective material (tapetum cellulosum) or an accumulation of extracellular collagen fibres (tapetum fibrosum) (Walls, 1967; Rodieck, 1973; Braekevelt, 1981, 1983, 1986a,b). The reflective material may also be located within the RPE cells to form a retinal tapetum (Arnott et al., 1970; Braekevelt, 1977, 1982).

In the southern fiddler ray (*Trygonorhina fasciata*) the tapetum is a tapetum cellulosum located in the dorsal fundus of the eye which corresponds to the lower or foreground visual field (region below the visual horizon) in which prey is usually detected. The presence of a tapetum greatly increases the sensitivity of a retina. Gunter et al. (1951) for instance have calculated that in the cat the 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.

The reflective material within a tapetum lucidum (either choroidal or retinal) varies widely in chemical composition from species to species. Amongst the compounds reported are guanine, cholesterol, zinc cysteine, riboflavin, pteridene and a variety of lipids (Weitzel et al., 1954; Pirie, 1966; Arnott et al., 1971; Nicol, 1989). While the chemical nature of the reflecting crystals in the fiddler ray was not analyzed for this study, Nicol (1989) indicates that only guanine is reported in the tapeta of selachians. As 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 wide variation (Walls, 1967).

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 appears to be the case in retinal tapeta where the reflective material is not usually highly 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 X refractive index) of the refractive material should equal a quarter of the wavelength of the incident light and the reflective units should be separated by the same distance (Coles, 1971; Land, 1972).

In this study the reflective guanine crystals are measured at approximately 0.10 μm in thickness and they are separated by about 0.14 μm. These dimensions for thickness and spacing are commensurate with the principles of constructive interference. In the cat the size of the reflective rodlets are reported as 0.10 μm in diameter with a distance between rodlets of 0.15 μm (Braekevelt, 1990). In the ferret tapetal rodlets are 0.20 μm in diameter and separated by 0.25-0.30 μm (Braekevelt, 1981). In the dog they are reported at 0.18 μm in diameter and spaced at about 0.20 μm (Lesiuk and Braekevelt, 1983) while in the grey seal they are 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 wavelengths of reflection between these species.

The thickness (number of layers of reflective 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 (Rodieck, 1973) through 15-20 layers (Pedler, 1963; Donovan, 1966) to a high of 35 layers (Bernstein and Pease, 1959). While species differences certainly exist, the intraspecies differences reported are probably due to breed variance, sampling of different areas and indeed to developmental stages. In the fiddler ray while the tapetum is composed of only a single layer of cells, these cells are arranged in an overlapping fashion such that light might traverse 4 to 5 layers

of reflective 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 differences 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 probably required to create an effective tapetum.

An occlusible retinal tapetum is reported in some teleosts (Duke-Elder, 1958; Arnott et al., 1971, 1972) but it is only in selachians that an occlusible choroidal tapetum (cellulosum) is reported (Nicol, 1989). While it is difficult to envision the evolutionary pressures that favoured the production of such an elegant mechanism for occluding the tapetum of the southern fiddler ray, the value of a tapetum that can be masked or unmasked in response to environmental lighting is obvious.

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