

# Photoreceptor fine structure in the vervet monkey (*Cercopithecus aethiops*)

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**Summary.** The structure of the photoreceptors of the vervet monkey (*Cercopithecus aethiops*) has been investigated by light and electron microscopy. In this species the photoreceptors can be readily differentiated and adequately described by the classical terminology of rods and cones, with rods being the more numerous. Rods are long, slender cells while cones are shorter and stouter. Both rods and cones are highly differentiated cells and consist of an outer segment, a connecting cilium, an inner segment, a nuclear region and a synaptic process leading to a synaptic ending. Morphological differences are noted between rods and cones for the various regions of these cells.

**Key words:** Photoreceptors - Electron microscopy - Vervet monkey

## Introduction

The structure of retinal photoreceptors has been investigated in a variety of vertebrate species both because of their position as the first neuron in the visual pathway and also because of their heavy involvement in protein production as they continuously generate new outer segment discs (Cohen, 1972; Crescitelli, 1972; Rodieck, 1973; Braekevelt, 1973, 1975, 1983a,b,c; Young, 1974, 1978).

Traditionally retinal photoreceptors have been classified as either rods or cones on the basis of their morphological appearance (Walls, 1942; Stell, 1972). While in a few species the morphological criteria can become somewhat ambiguous (Sjostrand, 1958, 1959; Pedler, 1965, 1969) for most species and for mammals in

particular, the classification of rods and cones accurately and adequately describes these cells (Cohen, 1972; Rodieck, 1973; Braekevelt, 1975, 1983a,b, 1985).

As part of a comparative morphological study of vertebrate photoreceptors, this report describes the fine structure of the rods and cones in the duplex retina of the vervet monkey (*Cercopithecus aethiops*). While these cells are basically similar to the photoreceptors of other primate species, some differences are noted.

## Materials and methods

For this study the eyes of four healthy adult vervet monkey (*Cercopithecus aethiops*) were examined by light and electron microscopy. With the animal under deep surgical anesthesia, the eyeballs were quickly removed, opened at the equator and fixed for 5h in 5% glutaraldehyde buffered to pH 7.3 with 0.1M Sorensen's phosphate buffer at 4°C. 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 1mm<sup>2</sup>, taking care not to detach the retina. The tissue was then post-fixed for 2h in 1% osmium tetroxide in the same phosphate buffer, dehydrated through graded ethanols to propylene oxide and embedded in Araldite.

Pieces of plastic-embedded tissue were reorientated to desired angles by means of a wax mount. Thick sections (0.5µm) were cut, stained with toluidine blue and examined by light microscopy. Thin sections (600-700 Å) were then cut and collected on copper grids. These sections were stained in aqueous uranyl acetate and lead citrate and examined and photographed in a Philips EM 201 transmission electron microscope.

## Results

The vervet monkey (*Cercopithecus aethiops*) possesses a duplex retina with easily distinguishable rods and cones present in a ratio of approximately 20-25 rods for each cone (Figs. 1, 7). Rods are elongate, slender cell

*Photoreceptors of the vervet monkey*

which project through the external limiting membrane for 20-25  $\mu\text{m}$  (Fig. 1). Rod outer segments which are 8-10  $\mu\text{m}$  in length, reach to the retinal epithelial layer and their tips are enclosed by the apical processes of these cells (Figs. 3, 4, 7). The rod outer segment is composed of a stack of bitembranous discs mainly enclosed within the cell membrane. (Fig. 3). The rod outer segment discs display several marginal incisures to give a scalloped appearance in cross-section (Figs. 3, 7). An eccentrically placed connecting cilium is located between the inner and outer segments which are of approximately the same diameter at 1.5-2.0  $\mu\text{m}$  (Figs. 3, 4). The apical region of the rod inner segment is rich in long slender mitochondria aligned with the length of the rod. (Figs. 2, 4). Near the external limiting membrane, the rod inner segment is rich in rough endoplasmic reticulum, polysomes and Golgi zones but with fewer mitochondria (Figs. 4, 6). What appear to be autophagic vacuoles are noted in this region in both rods and cones (Figs. 4, 6). A prominent cross-striated fibril originates near the basal body of the rod connecting cilium and passes downward through the inner segment to terminate near the external limiting membrane (Figs. 2, 4). This fibril may split into two or more components and displays dark periodicity bands at approximately 0.05  $\mu\text{m}$  intervals (Fig. 2).

Below the external limiting membrane, rods are normally isolated from one another and from cones by the cytoplasm of intervening Muller cells. (Figs. 1, 6). Rod nuclei are located at all levels of the outer nuclear layer. They are round to oval in shape and display a dense heterochromatin pattern (Fig. 1). A synaptic process extends from the nuclear region to the synaptic ending or spherule. The rod spherule expands slightly and exhibits 2-3 invaginated (ribbon) synaptic sites (Fig. 7). Superficial (non-invaginated) synapses are also present on the rod spherule.

Cone photoreceptors are shorter and stouter than rod photoreceptors (Figs. 1, 5, 6). Cones project beyond the external limiting membrane for 10-12  $\mu\text{m}$  and display an outer segment which is 5-7  $\mu\text{m}$  in length and which tapers apically (Fig. 5). Cone outer segment discs show a fairly smooth perimeter as no incisures are present (Fig. 7). Cone inner segments measure 6-7  $\mu\text{m}$  in diameter at their widest but taper to about 3.0  $\mu\text{m}$  where the outer segment begins (Fig. 5). Cone outer segments do not reach to the retinal pigment epithelial cells. A prominent eccentrically located connecting cilium joins the inner and outer segments (Fig. 7).

The cone inner segment displays a large number of very elongated mitochondria just below the connecting cilium (Figs. 1, 5). Below this ellipsoid of mitochondria, the cone inner segment shows rough endoplasmic reticulum, numerous polysomes and Golgi zones but few mitochondria (Figs. 1, 6). As in the rods this region also contains much filamentous material (Fig. 6). The cytoplasm of the cone inner segment is typically slightly more electron lucent than that of the rod photoreceptor (Figs. 1, 6).

Cone nuclei form a single discontinuous row immediately below the external limiting membrane

(Figs. 1, 6). Cone nuclei are larger and more vesicular than the rod nuclei (Fig. 1). A long synaptic process extends through the outer nuclear layer to the cone synaptic pedicle. Within the synaptic ending, synaptic vesicles are abundant (Fig. 8). The synaptic pedicle of cones display 12-15 invaginated (ribbon) synapses as well as superficial synaptic sites (Fig. 8).

The external limiting membrane (ELM) is formed by a series of zonulae adherentes between rods, cones and Muller cells (Figs. 1, 6). For the most part photoreceptor cells are isolated from one another by Muller cell cytoplasm although areas of photoreceptor contiguity are occasionally noted. Fine processes of the Muller cells project through the ELM to surround the basal portion of the inner segments of both rods and cones (Fig. 6).

**Fig. 1.** Electron micrograph of retinal rods (R) and cones (C) of the vervet monkey. A cone nucleus (CN) and rod nucleus (RN) as well as the external limiting membrane (ELM) are indicated.  $\times 4,000$

**Fig. 2.** Electron micrograph of a rod photoreceptor. The outer segment (OS) connecting cilium (C) and inner segment (IS) are indicated.  $\times 13,200$

**Fig. 3.** Electron micrograph of rod outer segments (ROS) cut in cross-section. Pigment-containing processes (P) of the retinal epithelium are indicated.  $\times 19,500$

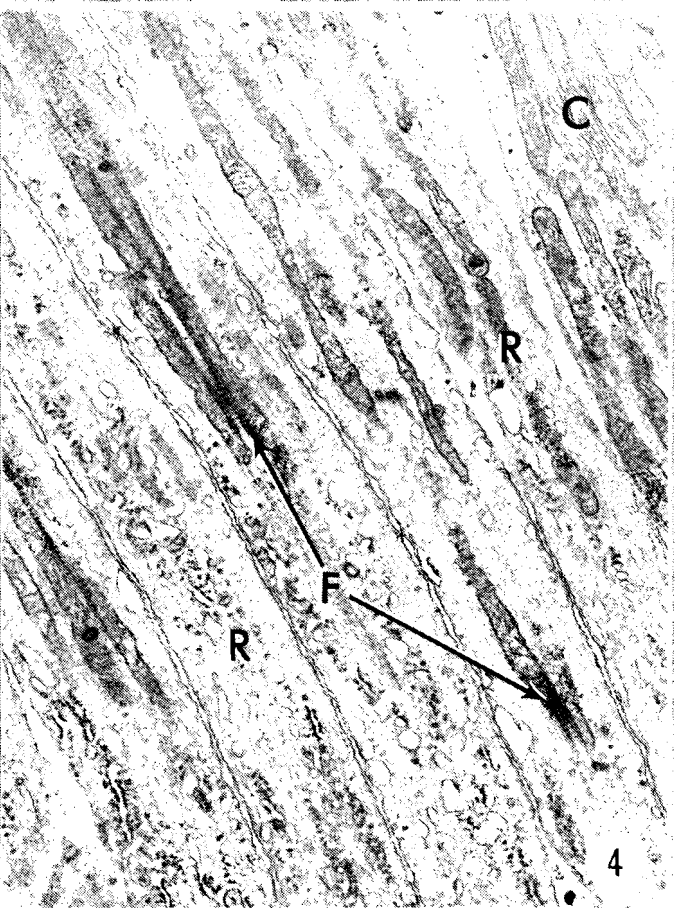
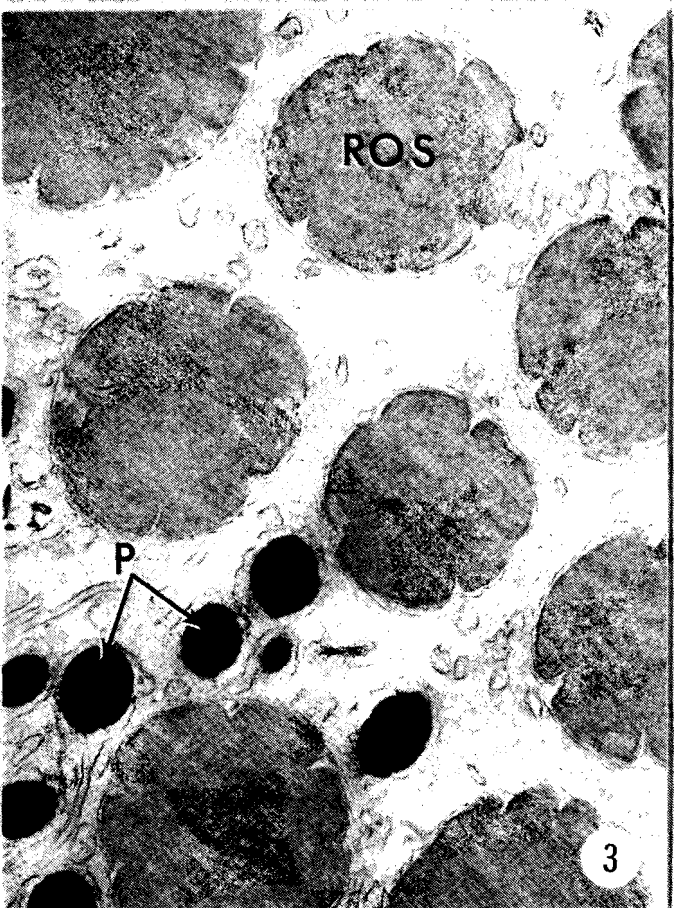
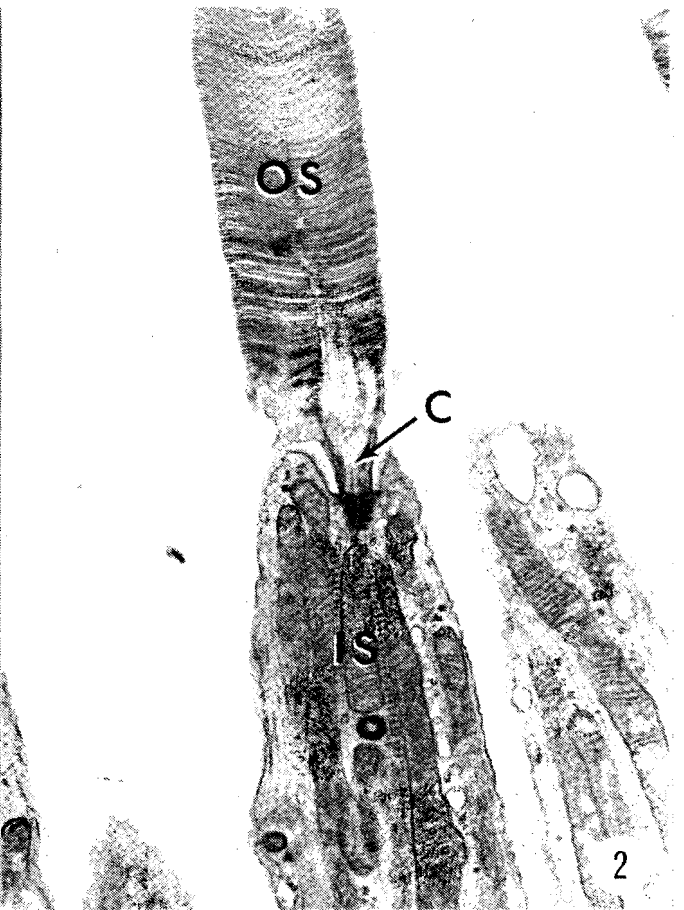
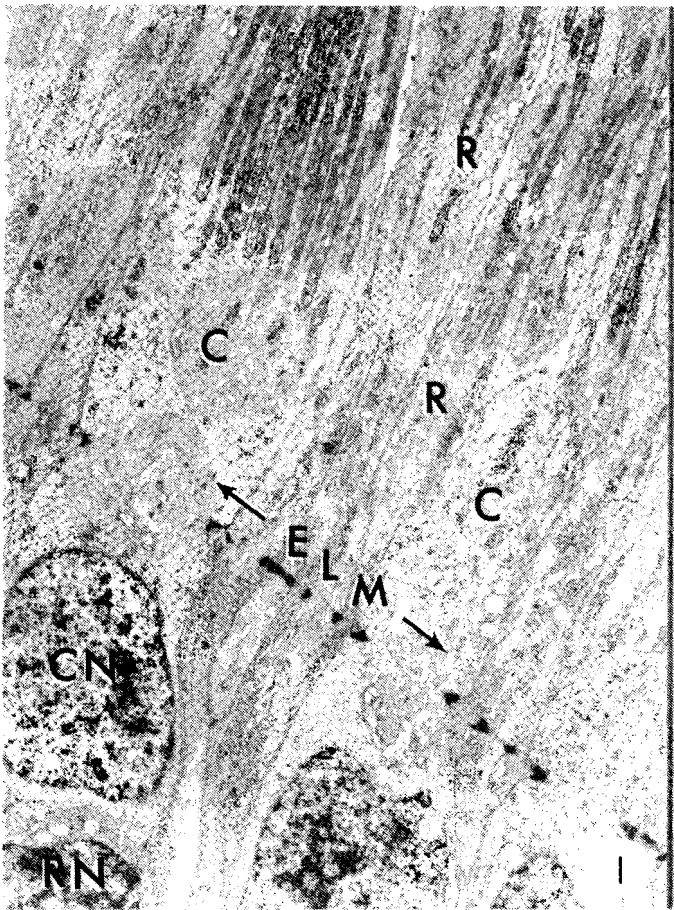
**Fig. 4.** Electron micrograph of the inner segments of several rod photoreceptors (R). The cross-striated fibril (F) is indicated as is a cone (C) cell.  $\times 10,000$

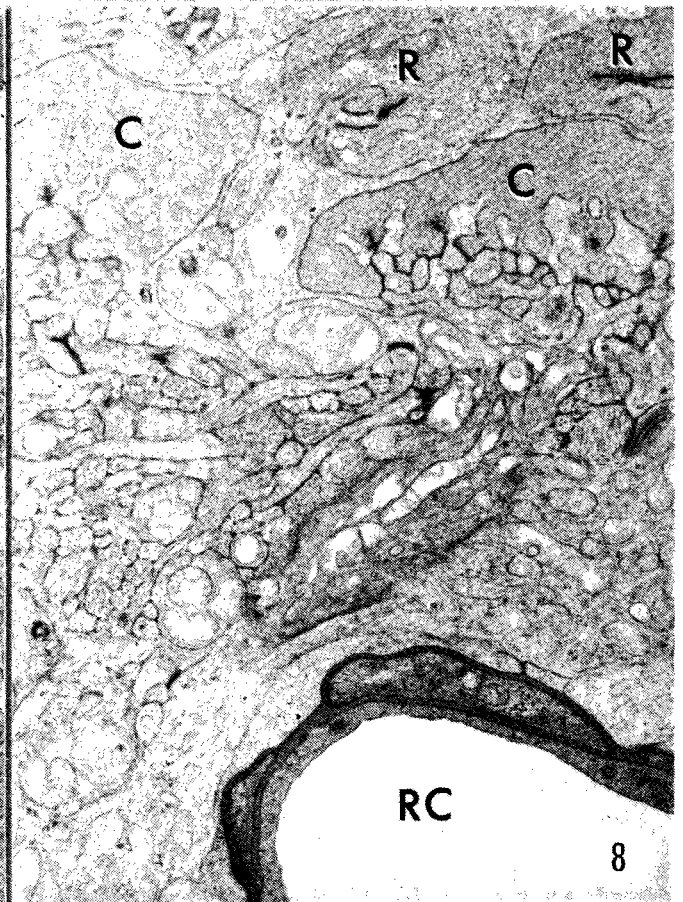
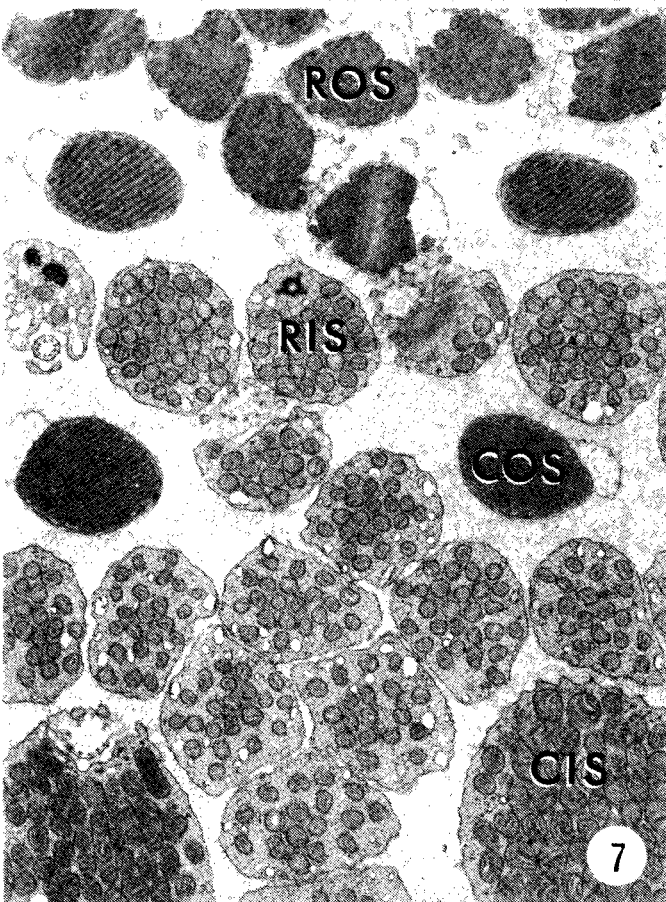
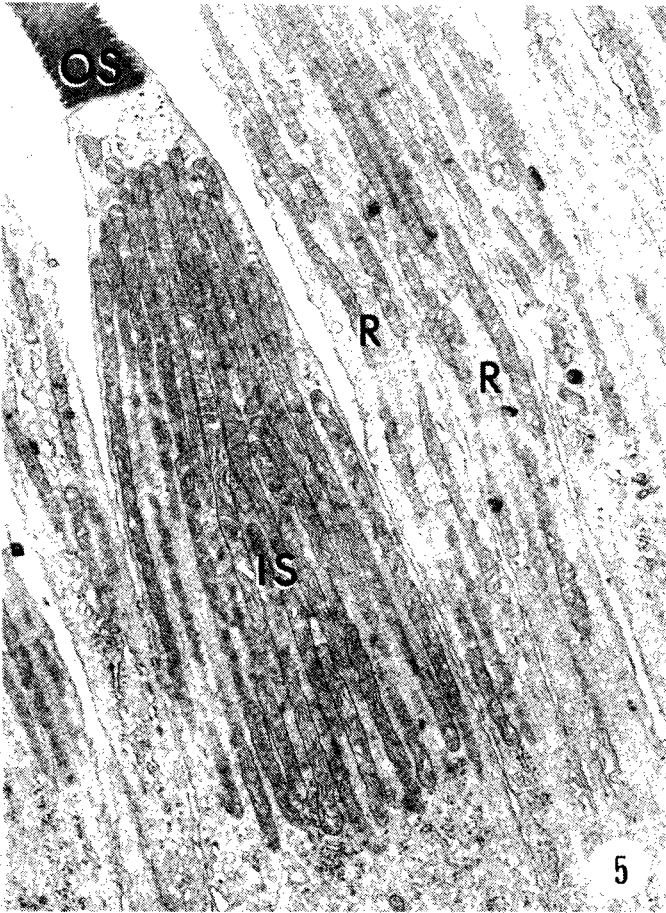
**Fig. 5.** Electron micrograph of a cone photoreceptor to indicate the outer segment (OS) and inner segment (IS). Several rod cells (R) are also present.  $\times 6,400$

**Fig. 6.** Electron micrograph of rod (R) and cone (C) photoreceptors near the external limiting membrane (ELM).  $\times 8,900$

**Fig. 7.** Electron micrograph of rod and cone photoreceptors in tangential section. Rod inner (RIS) and outer (ROS) segments as well as cone inner (CIS) and outer (COS) segments are indicated.  $\times 9,000$

**Fig. 8.** Electron micrograph of rod (R) and cone (C) synaptic endings. A retinal capillary (RC) is also indicated.  $\times 9,900$





## Discussion

The traditional separation of retinal photoreceptors into either rods («Stabchen») or cones («Zapfchen») was originally proposed by Schultze (1866). In this classical division, typical rods have cylindrical inner and outer segments of much the same diameter while typical cones have a shorter conical outer segment and an inner segment of greater diameter. This classification was used exclusively in numerous light microscopic studies (Walls, 1942; Polyak, 1957; Duke-Elder, 1958). With the advent of electron microscopy and the more detailed examination of these cells in more species, it was felt that not all photoreceptors were adequately described by the simplistic terms of rod or cone (Dowling, 1965). This led various workers to propose new categories of photoreceptor classification based on morphological criteria other than just cell shape. (Sjostrand, 1958, 1959; Pedler, 1965, 1969). While these more elaborate classifications are perhaps more accurate and in some non-mammalian species may be necessary, in most cases retinal photoreceptors can be adequately described and differentiated by the classical terms of rod or cone. The non-foveal photoreceptors of the vervet monkey for example fit the traditional definitions very well as do a number of other species (Rodieck, 1973; Braekevelt, 1973, 1975, 1983a,b,1985).

The outer segment of both rods and cones consist of a stack of bimembranous discs. In rods normally only a few of the more basally located discs are not enclosed by the cell membrane while in cones a number of the discs along the length of the outer segment may be open to the exterior (Sjostrand, 1958; Okuda, 1961; Cohen, 1964, 1970). In rod photoreceptors the outer segment discs are all of the same diameter while in cones the more apical discs are smaller than those of the basal region, giving the outer segment a tapering or conical shape (Cohen, 1972).

In most species studied, cone discs display a circular outline while rod discs have a lobulated perimeter due to the presence of one or more incisures (Cohen, 1963; Nilsson, 1965; Braekevelt, 1983a, b). The presence of incisures in rod photoreceptor discs is presumed to be a method of increasing surface area. Although some species display incisures in both rods and cones (Braekevelt, 1983c) in the vervet monkey, cone discs are not indented while rod discs show several incisures.

It is a fairly consistent finding that cone photoreceptors are shorter than rod cells (Cohen, 1964, 1972; Braekevelt, 1973, 1983,a,b). Both rods and cones are now known to periodically shed apical portions of their outer segments which are then picked up and degraded by the retinal pigment epithelium (RPE) (Young and Bok, 1969, 1970; Young 1976; Bok and Young, 1979). While rod outer segments are normally in intimate contact with the RPE cells, cone cells being shorter are not. In a number of species larger elongated processes of the RPE extend to and enclose the tips of cone outer segments (Scullica and Tangucci, 1968; Fine and Yanoff, 1972; Steinberg and Wood, 1979; Braekevelt, 1983c). This is believed to be a method of

maintaining a close relationship between retinal epithelial cells and cones. No such processes are noted in the vervet monkey retina and it is not known how outer segment material shed from the cones is picked up by the RPE layer.

The connecting cilium located between inner and outer segments is a constant feature of all vertebrate photoreceptors described (Sjostrand, 1953; Cohen, 1960, 1972; Braekevelt, 1973, 1983a,b,c). In this species the connecting cilium is typical of that described in other studies in that it is eccentrically located and displays the peripheral arrangement of nine pairs of microtubules and lacks the central pair typical of motile cilia (Dowling and Gibbons, 1961). In some species a prominent cross-striated filamentous structure referred to as a rootlet fibril descends from the basal body of this connecting cilium (Uga et al., 1970; Cohen, 1972; Braekevelt 1982). In the guinea pig this fibril is very long and extends as far as the synaptic terminal of the rod. (Spira and Milman, 1979). Such a cross-striated fibril is seen in the rods of the vervet monkey but it terminates at the level of the external limiting membrane. The function of this fibril is speculative but is felt to be either structural or perhaps contractile.

The inner segment is known to be the synthetic center of the photoreceptor cell (Cohen, 1972; Rodieck, 1973). Numerous mitochondria (collectively referred to as the ellipsoid), polysomes, profiles of RER and a prominent Golgi zone are normally located in this region (Young and Bok, 1970; Young, 1976). The presence of autophagic vacuoles in the inner segment region as noted in the vervet monkey appears to be a regular occurrence in all species and probably indicates normal turnover within these metabolically active cells (Reme and Sulser, 1977; Reme and Knop, 1980).

As is the case in most other species, the external limiting membrane (ELM) in the vervet monkey is formed by a series of zonulae adherentes (Uga and Smelser, 1973). Also as has been noted in other species with a duplex retina, the larger, more vesicular nuclei of the cone cells invariably occur immediately below (vitreal to) the ELM, while the more numerous rod nuclei are scattered throughout the outer nuclear layer (Walls, 1942; Cohen, 1972; Braekevelt, 1983a,b). The process extending from the nuclear region to the synaptic ending in both rods and cones appears similar to the axon of conventional neurons.

Within the outer plexiform layer, the synaptic pedicle of the cone is usually larger and displays more synaptic sites than the rod spherule (Cohen, 1972; Crescitelli, 1972). Synaptic sites on retinal photoreceptors are either invaginated and associated with a synaptic ribbon (Missotten, 1965) or are superficial synapses involving only membrane densification (Dowling, 1968; Cohen, 1972). While bipolar and horizontal cells are both involved in invaginated synapses (Stell, 1965; Kolb, 1970), superficial synapses may be between photoreceptors and bipolars or between photoreceptors themselves (Missotten, 1965; Cohen, 1964; Kolb, 1970). In a few species, only superficial contacts are reported

(Dowling and Werblin, 1969). The vervet monkey displays both typical invaginated (ribbon) and superficial synaptic sites on both rods and cones.

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