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Retinal photoreceptor fine structure in the red-backed salamander *(Plethodon cinereus)*

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Summary. The retinal photoreceptors of the red-backed salamander *(Plethodon cinerus)* have been studied by light and electron microscopy. Rods and single cones are present in this duplex retina in a ratio of about 25:1. The photoreceptors in this amphibian species are much larger than is reported for most vertebrates. In the light-adapted state, rods reach deep into the retinal epithelial (RPE) layer. The rod outer segment is composed of discs of uniform diameter displaying several very deep incisors. The rod inner segment displays a distal elliposid of mitochondria and a short stout myoid region. Rod nuclei are electron dense and often protrude through the external limiting membrane. Rod synaptic spherules are large and display several invaginated synaptic sites as well as superficial synapses. It is felt that the rods do not undergo retinomotor movements. The cone photoreceptors are much smaller than the rods and display a tapering outer segment, an unusual modified ellipsoid and a large parabolid of glycogen in the inner segment. Cone nuclei are less electron dense than rods and are located at all levels within the outer nuclear layer. The synaptic pedicle of the cones is larger, more electron lucent and display more synaptic sites (both invaginated and superficial) than that of rods. It is felt that cone photomechanical responses are minimal.

Key words: Photoreceptors, Electron Microscopy, Amphibian, Red-Backed Salamander, *Plethodon cinerus*

Introduction

Retinal photoreceptors are extremely specialized and highly polarized cells which form the first neuron (receptor) in the visual pathway. They have been studied in a variety of animals and have been shown to be constructed on a basically similar plan throughout the vertebrate kingdom, consisting of an outer segment (light-sensitive region) joined to an inner segment (synthetic region) by a non-motile cilium, a nuclear region and a synaptic ending. (Cohen, 1963, 1972; Crescitelli, 1972; Rodieck, 1973).

Historically retinal photoreceptors have been classified as either rods or cones on the basis of morphological criteria at the light microscopic level (Walls, 1942; Duke-Elder, 1958; Cohen, 1972). With the advent of electron microscopy and the examination of more species it was felt by some workers that such a simplistic classification was inadequate and advanced other more complex forms of classification for photoreceptors. (Sjöstrand, 1958, 1959; Pedler, 1965, 1969). Despite these attempts however the terms rod and cone are still widely used and for the majority of species quite accurately and adequately differentiate these cells (Crescitelli, 1972; Rodieck, 1973; Braekevelt, 1983, 1985, 1988, 1990).

As part of an ongoing comparative morphological study of vertebrate retinal photoreceptors, this report describes the fine structure of the rods and cones in the duplex retina of a tailed amphibian (urodele), the red-backed salamander *(Plethodon cinerus)*.

Materials and methods

For this study the eyes from five adult red-backed salamanders *(Plethodon cinerus)* were examined by light and electron microscopy. The light-adapted animals were decapitated and the eyes quickly removed. The eyeballs were opened at the equator and immersion fixed for 5 h at 4°C in 5% glutaraldehyde buffered to pH 7.3 with 0.1 M Sorensen's phosphate buffer. The posterior half of the eyeball was then removed, washed in 5% sucrose in 0.1 M Sorensen's buffer (pH 7.3) and cut into pieces less than 1 mm^2. This tissue was then postfixed for 2 h in 1% osmium tetroxide in the same phosphate buffer (pH 7.3), dehydrated up through graded ethanols to methanol and then to propylene oxide and embedded in Araldite.

Pieces of plastic-embedded tisue were reoriented to

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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 EM 201 transmission electron microscope.

Results

The rods and cones of the red-backed salamander *(Plethodon cinerus)* are very large cells and are consequently present in fewer numbers than is normally the case in the vertebrate retina. This duplex retina is rod dominant with a rod : cone ratio of about 25 : 1. All photoreceptors are single with no double photoreceptors or a photoreceptor mosaic being observed..

In the light-adapted state, rod photoreceptors average about 40 µm in length from the external limiting membrane (ELM) to the tip of the outer segment (Fig. 1). In this condition the rod outer segments reach deep into the retinal epithelial (RPE) layer and are surrounded by the pigment-laden apical processes of the RPE cells (Figs. 1, 3, 5). These RPE processes reach almost to the ELM and in light-adaptation the rod photoreceptors are effectively isolated from one another by melanin pigment (Figs. 1, 3).

The rod outer segment measures about 9.0 µm in diameter and shows numerous deep incisures to give the outer segment discs a very scalloped outline (Figs. 3 - 5). The rod inner segment in light-adaptation is the same diameter as the outer segment and is composed mostly of an ellipsoid of large mitochondria (Figs. 2, 6). Below the ellipsoid is an accumulation of rough endoplasmic reticulum, numerous polysosomes with Golgi zones and autophagic vacuoles also present (Figs. 2, 6). The inner segment measures only about 3 - 4 µm in length and extends to and often surrounds the nuclear region of the rod photoreceptors (Figs. 1, 2). The rod nuclei in this species are large but display a dense heterochromatin pattern (Figs. 1, 2). Rod nuclei often protrude partially (Fig. 1) or wholly (Fig. 2) through the external limiting membrane (ELM).

A short wide process extends from the nuclear region to the synaptic spherule of the rod which in this species is quite large and electron dense (Figs. 12, 13) . Because of the large size of rod photoreceptors, the synaptic spherule displays 6 - 8 invaginated (ribbon-associated) synaptic sites as well as numerous conventional (superficial) synapses which appear to involve only membrane densification (Figs. 1, 12, 13). Rod synaptic endings are rich in synaptic vesicles (Figs. 12, 13) while Müller cells (and probably horizontal cells as well) are rich in glycogen deposits in the synaptic region (Figs. 12, 13).

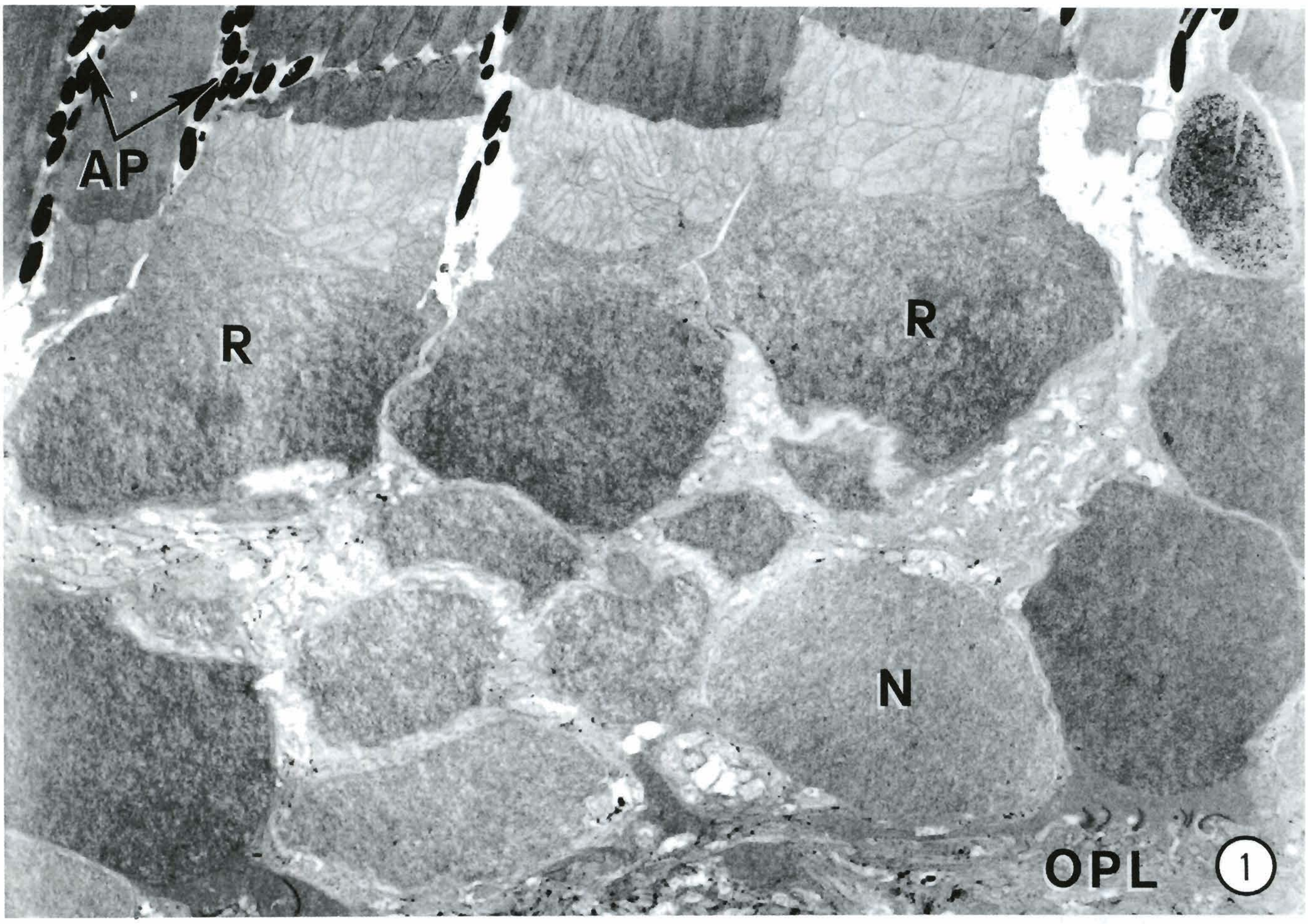
Cone photoreceptors in this species while fairly large cells in their own right are dwarfed by the rod photoreceptors (Compare Figs. 2 and 8; 3 and 10). Cones measure about 20 µm from the external limiting membrane to the tip of the outer segment. The cone outer segment is about 12 µm in length and tapers distally from a basal width of about 5 µm (Fig. 8). Cone outer segment discs have no peripheral incisures and hence have a smooth outline in cross section (Fig. 10). The pigment-laden apical process of the RPE enclose the cone outer segments and reach to the inner segment region of these cells which measure about 7.0 µm in diameter (Figs. 7 - 9). Below the connecting cilium which joins the inner and outer segment of both rods and cones (Fig. 6) is a large ellipsoid of often very large mitochondria which show much matrix and relatively few disorganized cristae (Figs. 7 - 9, 11). These mitochondria are very tightly packed and often appear almost confluent (Figs. 7 - 9, 11). Below the ellipsoid is a large and very compact accumulation of glycogen particles (the paraboloid) (Figs. 7, 8, 11). Below and surrounding the paraboloid are profiles of rough endoplasmic reticulum, numerous polysomes, Golgi zones and occasional autophagic vacules (Figs. 7, 8).

Cone nuclei are found at all levels of the outer nuclear layer and display a less dense chromatin pattern than that of the rod photoreceptors (Fig. 1). The cone cytoplasmic area is more electron lucent than that of the rods (Fig. 13) and the synaptic pedicle of cones displays more synaptic sites (both invaginated and conventional) than that of the rods (Fig. 13).

As is noted in many other species, fine finger-like processes of the Müller cells project through the external limiting membrane to surround the basal region of the photoreceptor inner segments (Figs. 2, 6). Also as is the case in most species, the external limiting membrane in the red-backed salamander is formed by a series of zonulae adherentes (Figs. 1, 2).

Discussion

The traditional division of vertebrate retinal photoreceptors in to either rods (stäbchen) or cones (zäpfchen) 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 by some workers that not all photoreceptors were adequately described by the simplistic terms of rod or cone (Dowling, 1965). This led to new categories of photoreceptor classification based on criteria other than just cell shape (Sjöstrand, 1958, 1959; Pedler, 1965; 1969). While these more elaborate classifications are perhaps more accurate and in some cases even preferable, in most species retinal photoreceptors are still adequately described and differentiated by the classical terms of rods or cones. This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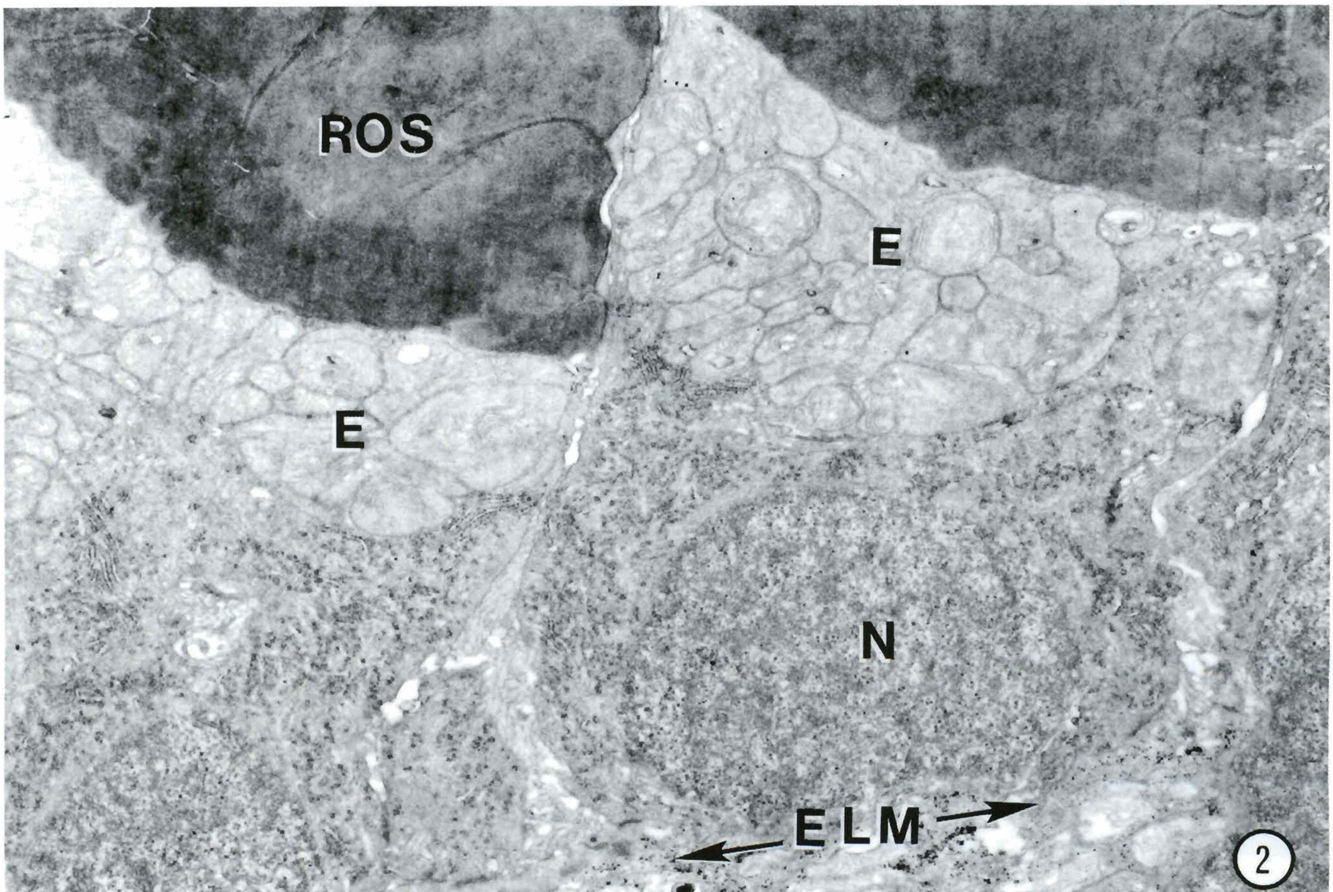
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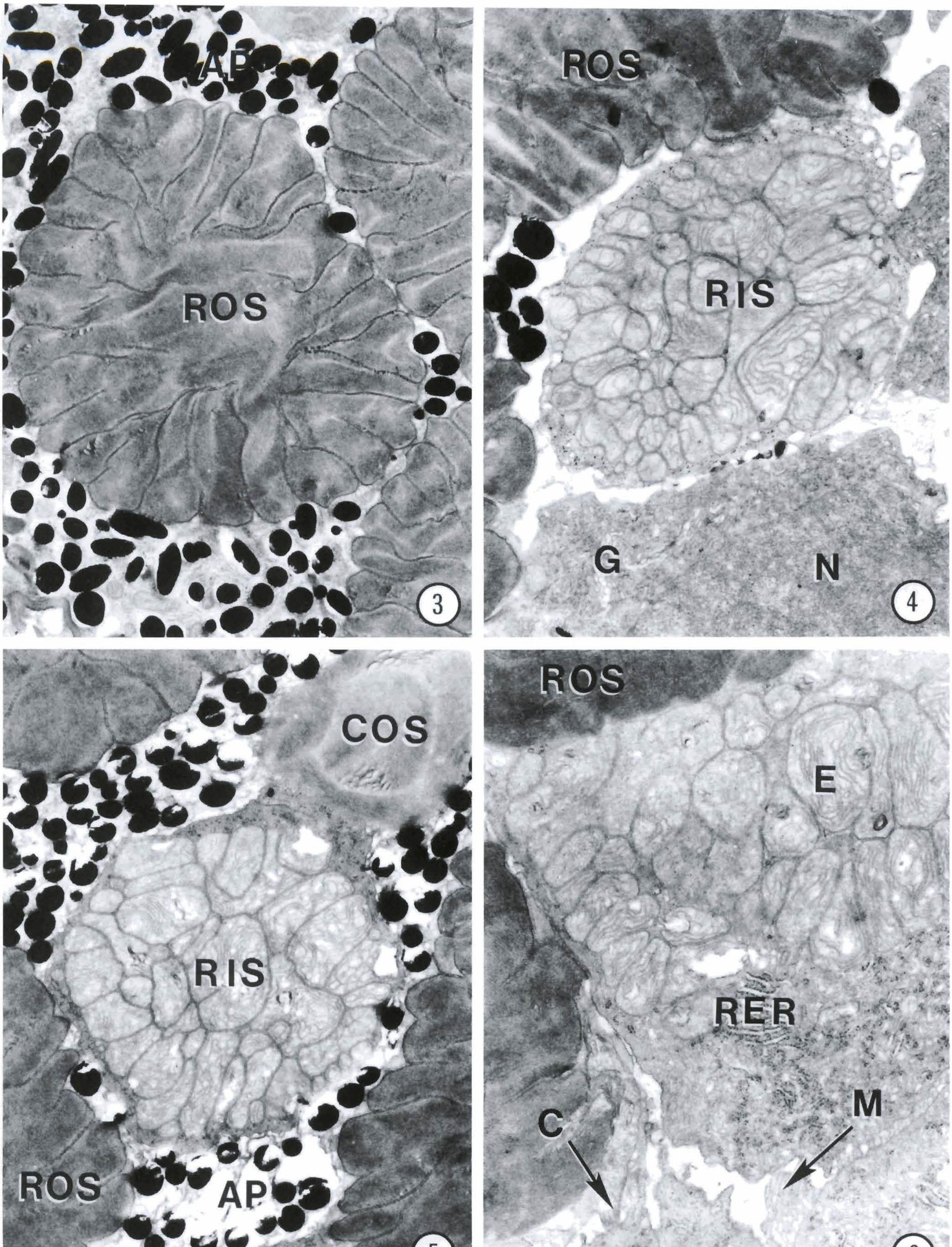
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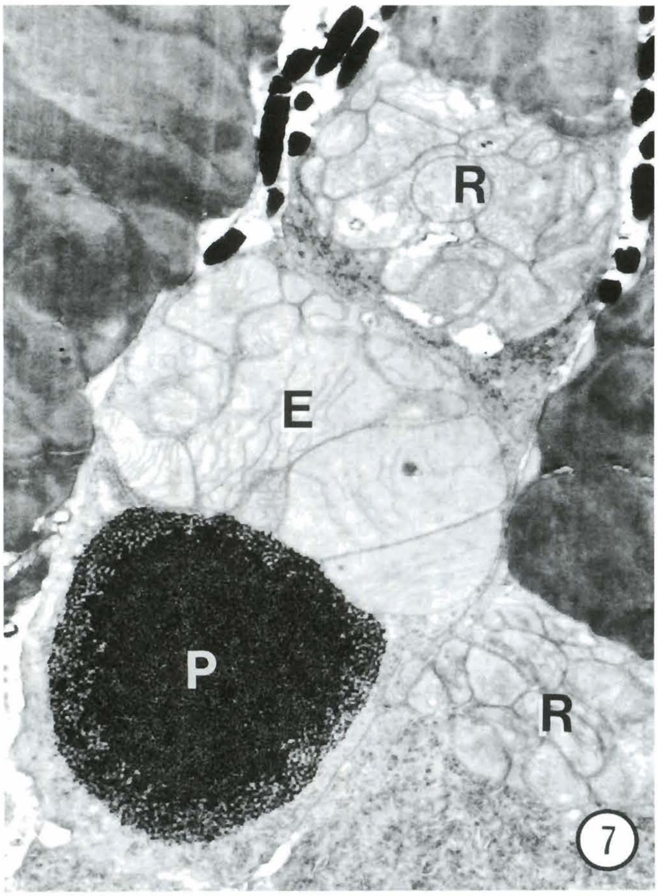
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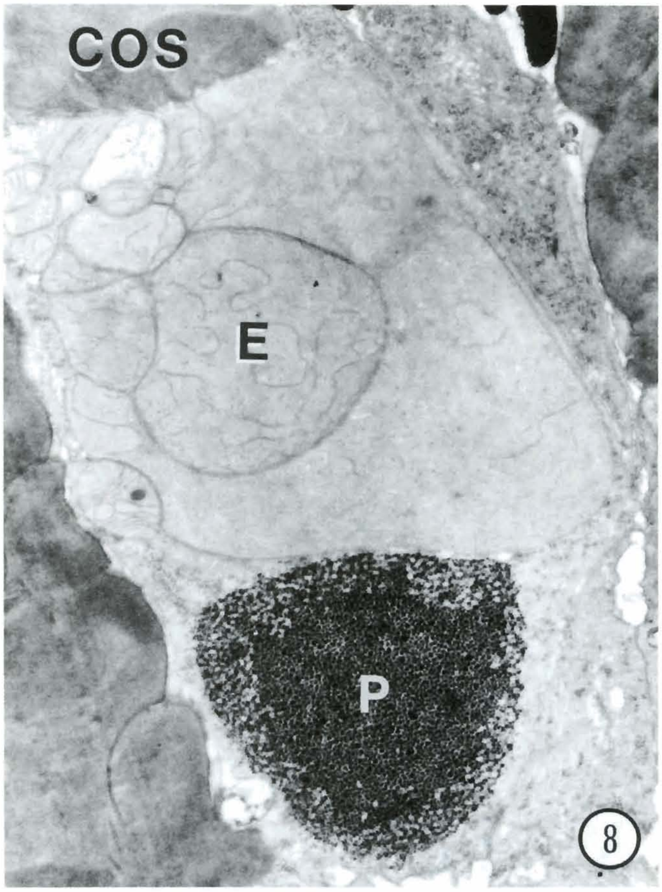
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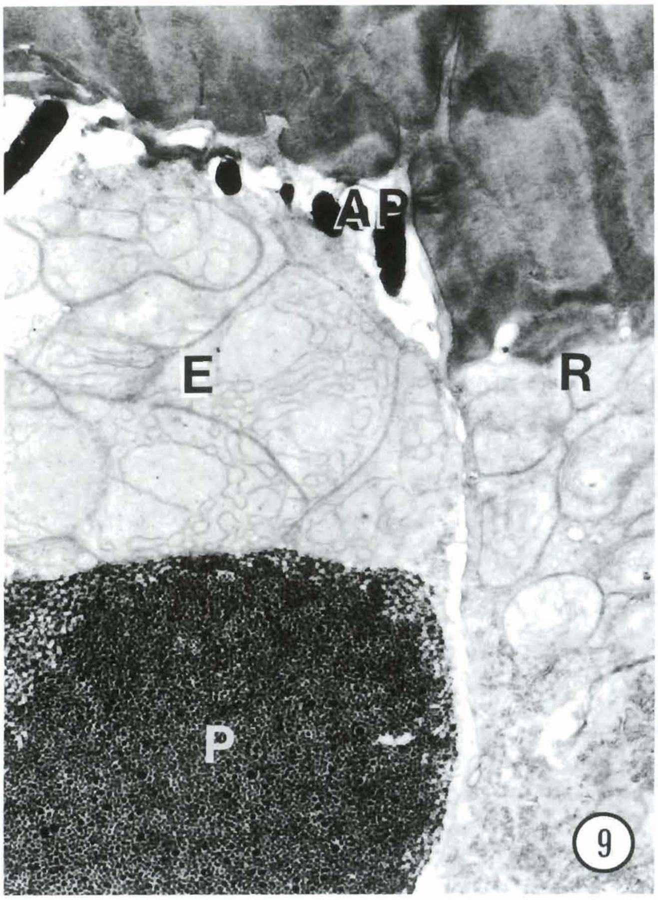
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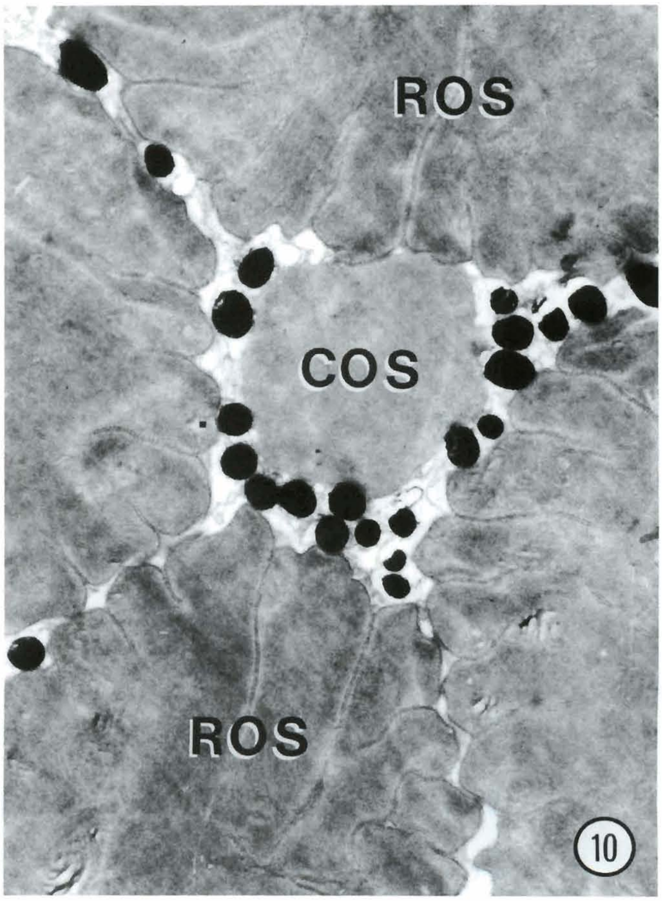
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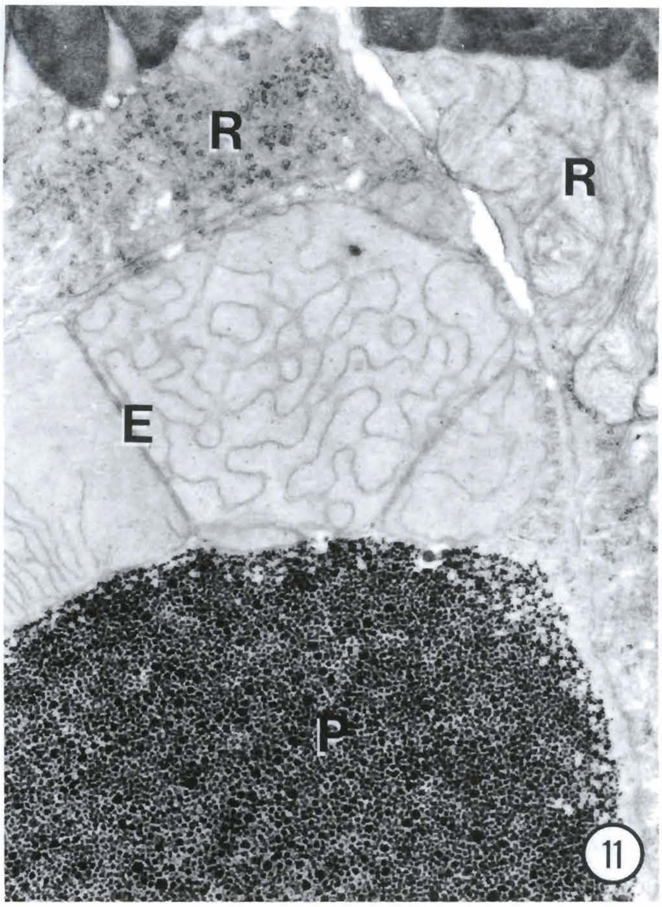
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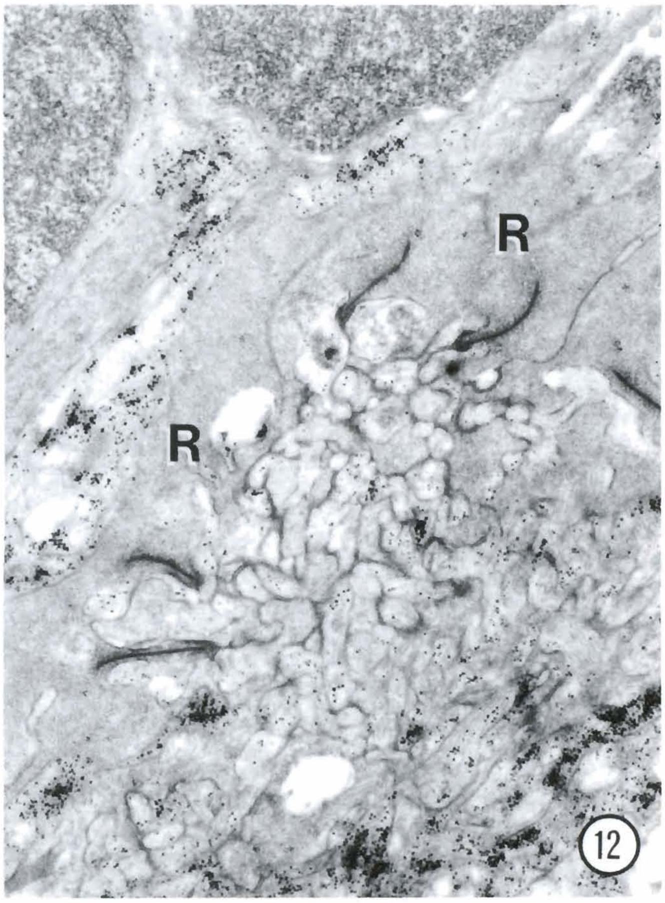
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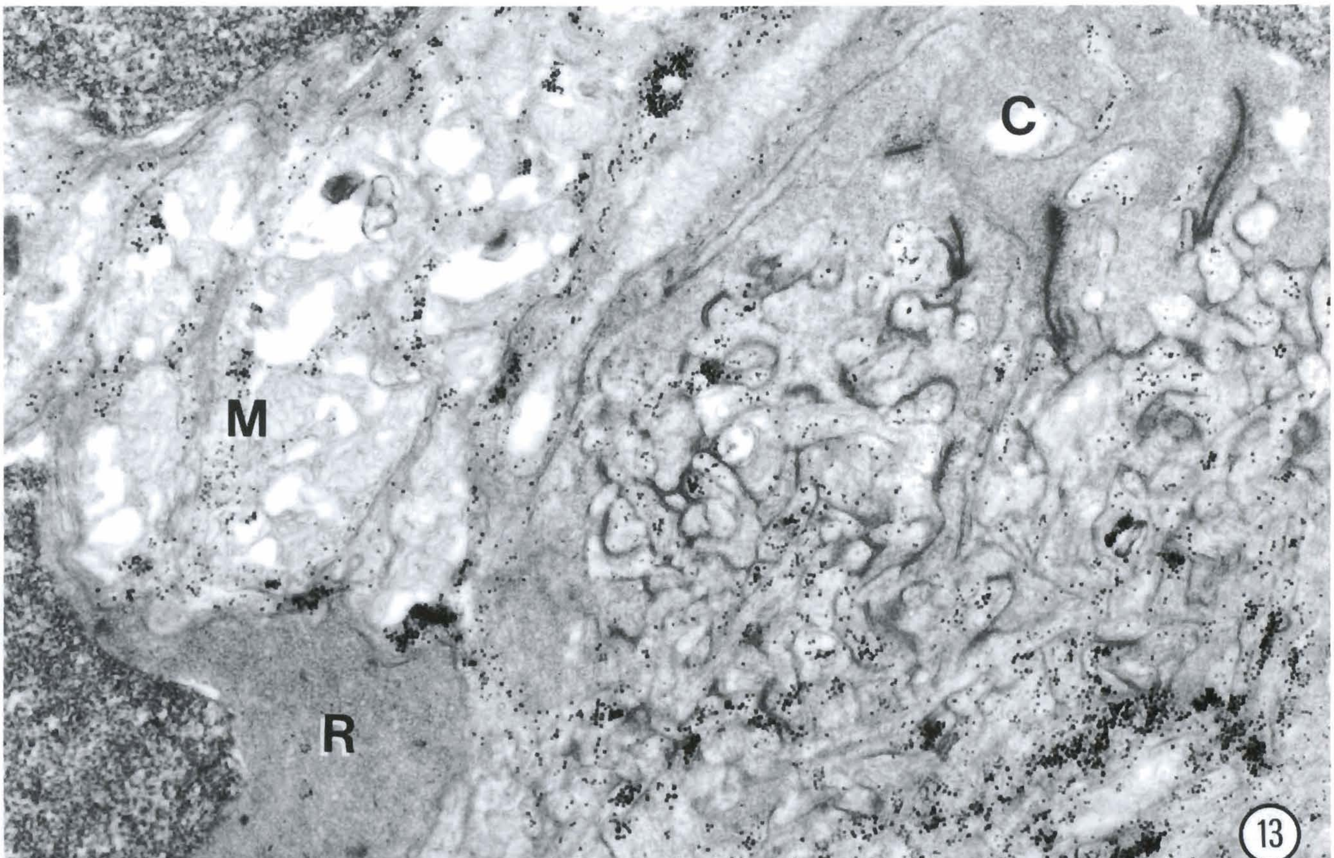
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Fig. 1. Electron micrograph of the retinal photoreceptors of the red-backed salamander. Two rod photoreceptors (R) and a cone nucleus (N) are indicated as in the outer plexiform layer (OPL). Pigment laden apical processes (AP) from the retinal epithelium are also indicated. x 3,800

Fig. 2. Electron micrograph of two rod photoreceptors to indicate the outer segments (ROS), ellipsoids (E) and nuclei (N) protruding through the external limiting membrane (ELM). x 8,600

Fig. 3. Electron micrograph of rod outer segments (ROS) to indicate the plentiful deep incisures. Apical processes (AP) of the retinal epithelium are also indicated. x 6,400

Fig. 4. Electron micrograph of a rod inner segment (RIS) at the ellipsoid level. The nucleus (N), a Golgi zone (G) and an outer segment (ROS) from other rods are also labelled. x 9,500

Fig. 5. Electron micrograph of a cone outer segment (COS) and rod inner (RIS) and outer segments (ROS) separated by retinal epithelial apical processes (AP). x 9,500

Fig. 6. Electron micrograph of a rod photoreceptor to illustrate the outer segment (ROS), ellipsoid (E) and profiles of RER and numerous polysomes in the inner segment. Müller cell processes (M) and a connecting cilium (C) are also indicated. x 13,800

Fig. 7. Electron micrograph to indicate the ellipsoid (E) and parabolid (P) of a cone photoreceptor. Note the different mitochondrial morphology from the ellipsoid of adjacent rods (R). x 6,400

Fig. 8. Electron micrograph to illustrate a cone outer segment (COS), ellipsoid (E) and parabolid (P). x 8,900

Fig. 9. Electron micrograph of a cone ellipsoid (E) and parabolid (P). Retinal epithelium apical processes (AP) and a rod photoreceptor (R) are also indicated.

Fig. 10. Electron micrograph to illustrate the differences in size and morphology of rod (ROS) and cone outer segments (COS). x 9,500

Fig. 11. Electron micrograph to indicate the unusual mitochondria morphology in the cone ellipsoid (E). The cone parabolid (P) and two rod photoreceptors (R) are also indicated. x 12,900

Fig. 12. Electron micrograph of the synaptic region of two rod photoreceptors (R). Both invaginated and superficial synapses are obvious. x 13,800

Fig. 13. Electron micrograph to illustrate a cone synaptic pedicle (C) and a rod synaptic spherule (R). Note the more electron dense cytoplasm of the rod. Both invaginated and superficial synapses are present on both rods and cones. Glycogen is plentiful within Müller cell cytoplasm (M). x 18,700

is particularly true in the case of species such as the red-backed salamander *(Plethodon cinerus)* that possess only rods and single cones with no multiple receptors present. This is in contrast to the newt *(Triturus viridescens)* and the leopard frog *(Rana pipiens)* where double cones are reported (Nilsson, 1964; Keefe, 1971; Dickson and Hollenberg, 1971).

The very large photoreceptors observed in this species have been noted in other urodeles (Walls, 1942; Dowling, 1969; Werblin, 1969). As the rods particularly are very large with long wide outer segments and are in a preponderance over the cones (about 25 : 1) this produces a very large light-capture area and implies a highly sensitive retina in this species. The presence of numerous deep peripheral incisures in the outer segment discs is presumably an attempt to even further increase surface area of the outer segment.

The outer segments of both rods and cones consist of a stack of bimembranous discs (Cohen, 1972). In rods these 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 (Crescitelli, 1972). In most species, cone discs display a circular outline while rod discs usually show a scalloped or lobulated perimeter due to the presence of one or more incisures and that is also the case in this species (Nilsson, 1965; Cohen, 1972; Braekevelt, 1983, 1985).

In retinas which undergo photomechanical or retinomotor movements in response to changes in environmental lighting, cones are most contracted and rods are most elongated in the light-adapted condition (Walls, 1942; Rodieck, 1973; Burnside and Laties, 1979). In the light adapted specimens examined in this study the myoid region of the rods is very short and stout and it is doubtful that the rods can shorten any further in dark-adaptation. Cones also have a very stout inner segment and do not appear to have lengthened in respose to light. While it is felt that the melanin granules of the retinal epithelium in the red-backed salamander do undergo retinomotor movements (Braekevelt, 1992), judging from light-adapted specimens only it is felt that the rods do not undergo photomechanical changes and cones only slightly or not at all.

The inner segment of a photoreceptor cell is known to be the synthetic center of the cell and it is here for instance that the materials for new outer segment discs are produced (Young, 1976). The large accumulation of mitochondria at the apex of the inner segment (the ellipsoid) is a constant feature of all photoreceptors (Cohen, 1972; Rodieck, 1973). The mitochondria in the rod ellipsoid of the red-backed salamander are unremarkable but those of the cone ellipsoid are unusual in that they appear to have much matrix and few cristae and actually appear to be confluent to form a single large structure. Oil droplets have been reported in this location at the apex of the inner segment of cone photoreceptors in a variety of vertebrates (Walls, 1942; Rodieck, 1973) including amphibians (frogs) (Nilsson, 1964). Berger (1966) has shown that in fish the origin of these oil droplets is converted or modified mitochondria. Although true oil droplets are not reported in the cones of the red-backed salamander, the mitochondria of the cone ellipsoid do display a morphology reminiscent of the changes described by Berger (1966) and may perhaps give rise to a structure that behaves somewhat like an oil droplet.

The paraboloid which is an accumulation of glycogen found in the cone inner segment of some birds, fish, amphibians and reptiles (Cohen, 1972; Rodieck, 1973) is also present in the red-backed salamander. While early workers felt that this glycogen body was a refractile structure, it is now felt that this concentration of glycogen acts as a ready source of energy for visual cell metabolism (Meyer, 1977). The presence of an ellipsoid and parabolid, numerous polysomes and profiles of rough endoplasmic reticulum, Golgi zones and autophagic vacuoles as noted in this species are all indicative of metabolically very active cells (Cohen, 1972; Remé and Sulser, 1977).

The positioning of the rod and cone nuclei is a bit unusual in this species in that cone nuclei are not invariably found closest to the external limiting membrane (ELM) which is normally the case (Cohen, 1972; Braekevelt, 1983, 1989, 1990). In this species many rod nuclei are situated immediately adjacent to or even scleral to the ELM while cone nuclei can be found at all levels of the outer nuclear layer. This unusual arrangements of nuclei may be due to the unusually large rod photoreceptors present in the red-backed salamander.

Within the outer plexiform layer, the synaptic pedicle of the cone cell is typically larger, and displays more synaptic sites than that of a rod spherule (Cohen, 1972; Crescitelli, 1972). While that is still true in this species, because of the large size of the rod photoreceptors, this is not as striking as in most vertebrates. Synaptic sites on retinal photoreceptors are of two types: either invaginated and associated with a synaptic ribbon (Missotten, 1965) or of the more conventional superficial type involving only membrane densificiations (Dowling, 1968; Cohen, 1972). While bipolar and horizontal cells are both involved at invaginated synapses (Kolb, 1970), superficial synapses may be between photoreceptors and bipolars or between photoreceptors themselves (Cohen, 1964; Missotten, 1965; Kolb, 1970). The red-backed salamander displays both typical invaginated (ribbon) and superficial (conventional) synaptic sites on both rods and cones.

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References

Berger E.R. (1966). On the mitochondrial origin of oil drops in the retinal double cone inner segments. J.Ultrastruct. Res. 14, 143-157.

Braekevelt C.R. (1983). Photoreceptor fine structure in the domestic ferret. Anat. Anz. 32, 33-44.

Braekevelt C.R. (1985). Photoreceptor fine structure in the archerfish *(Toxotes jaculatrix)*. Amer. J. Anat. 173, 89-98.

Braekevelt C.R. (1988). Retinal fine structure in the European eel *Anguilla anguilla* VI. Photoreceptors of the sexually immature silver eel stage. Anat. Anz. 166, 23-31.

Braekevelt C.R. (1989). Photoreceptor fine structure in the bobtail goanna *(Tiliqua rugosa)*. Histol. Histopath. 4, 281-286.

Braekevelt C.R. (1990). Retinal photoreceptor fine structure in the mallard duck *(Anas platyrhynchos)*. Histol. Histopath. 5, 123-131.

Braekevelt C.R. (1992). Retinal pigment epithelial fine structure in the red-backed salamander. *(Plethodon cinerus)*. Histol. Histopath. 7, 471-477.

Burnside B. and Laties A.M. (1979). Pigment movement and cellular contractility in the retinal pigment epithelium. In: The retinal pigment epithelium. Eds. K.M. Zinn and M.F. Marmor, Harvard Univ. Press, Cambridge. pp 175-191.

Cohen A.I. (1963). Vertebrate retinal cells and their organization. Biol. Rev. 38, 427-459.

Cohen A.I. (1964). Some observations on the fine structure of the retinal receptors of the American gray squirrel. Invest. Ophthal. 3, 198-216.

Cohen A.I. (1972). Rods and cones. In: Handbook of sensory physiology. Vol. VII/2. Physiology of photoreceptor organs. Ed: M. Fuortes, Springer-Verlag, Berlin. pp 63-110.

Crescitelli F. (1972). The visual cells and visual pigments of the vertebrate eye. In: Handbook of sensory physiology, Vol. VII/1. Photochemistry of vision. Ed. H.J.A. Dartnall. Springer-Verlag, Berlin pp 245-363.

Dickson D.H. and Hollenberg M.J. (1971). The fine structure of the pigment epithelium and photoreceptor cells of the newt, *Triturus viridescens dorsalis* (Rafinesque). J. Morph. 135, 389-432.

Dowling J.E. (1965). Foveal receptors of the monkey retina: fine structure. Science 147, 57-59.

Dowling J.E. (1968). Synaptic organization of the frog retina: an electron microscopic analysis comparing the retinas of frogs and primates. Proc. Roy. Soc. B. 170, 205-228.

Dowling J.E. and Werblin F.S. (1969). Organization of the retina of the mud puppy *Necturus maculosus*. Synaptic structure. J. Neurophysiol. 32, 315-338.

Duke-Elder Sir S. (1958). System of ophthalmology. Vol. I. The eye in evolution, Henry Kimpton, London.

Keefe J.R. (1971). The fine structure of the retina in the newt *Triturus viridescens*. J. Exp. Zool. 177, 263-294.

Kolb H. (1970). Organization of the outer plexiform layer of the primate retina: electron microscopy of Golgi-impregnated cells. Phil. Trans. Roy. Soc. B. 258, 261-283.

Meyer D.B. (1977). The avian eye and its adaptations. In: Handbook of sensory physiology. Vol. VII/5. The visual system in vertebrates. Ed. F. Crescitelli, Springer-Verlag, Berlin. pp 549-612.

Missotten L. (1965). The ultrastructure of the human retina. Arsica, S.A., Brussells.

Nilsson S.E.G. (1964). An electron microscopic classification of the retinal receptors of the leopard frog *(Rana pipiens)*. J. Ultrastruct. Res. 10, 390-416.

Nilsson S.E.G. (1965). Ultrastructure of the receptor outer segments in the retina of the leopard frog *(Rana pipiens)*. J. Ultrastruct. Res. 12, 207-231.

Pedler C. (1965). Rods and cones. A fresh approach. In: Biochemistry of the retina. Ed. C.N. Graymore, Academic Press, New York. pp 1-4.

Pedler C. (1969). Rods and cones. A new approach. Inter. Rev. Gen. Exp. Zool. 4, 219-274.

Polyak S.L. (1957). The vertebrate visual system. Univ. Chicago Press. Chicago.

Remé C.E. and Sulser M. (1977). Diurnal variation of autophagy in rod visual cells in the rat. Graefe's Arch. Ophthal. 203, 261-270.

Rodieck R.W. (1973). The vertebrate retina. Principles of structure and function. W.H. Freeman, San Francisco.

Schultze M. (1866). Anatomie und Physiologie der Netzhaut Arch. Mikros. Anat. Entw. mech. 2, 175-286.

Sjöstrand F.S. (1958). Ultrastructure of retinal rod synapses of the guinea pig eye as revealed by three-dimensional reconstructions from serial sections. J. Ultrastruct. Res. 2, 122-170.

Sjöstrand F.S. (1959). The ultrastructure of retinal receptors of the vertebrate eye. Ergeb. Biol. 21, 128-160.

Walls G.L. (1942). The vertebrate eye and its adaptative radiation, Cranbook Press, Bloomfield Hills.

Young R.W. (1976). Visual cells and the concept of renewal. Invest. Ophthal. 15, 700-725.

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