Electron Microscopic Study of the Vertebrate Retina

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Morphological studies of the vertebrate retina with the use of the electron microscope have been carried out by several authors since 1949, most of them in relation to the structure of the receptors. These studies were devoted at first to the outer and inner segments of the receptors (1-8). More recently, the interest has been concentrated on the synaptic connections of the receptors (9-12).

This work is intended to present a comparative study of all retinal layers of different vertebrates. A special attention has been given to a neuropile-like structure existing between the receptor endings and the bipolar cells.

MATERIAL AND METHOD

Retinae of the following vertebrates were used: monkey (Macaca rhesus, irus, and mulatta), chicken (Gallus domesticus var.), turtle (Pseudemys sp.), toad (Bufo marinus), and fish (Centropomidae). The animals were killed by decapitation, with the exception of monkeys, which were anesthetized with ether prior to decapitation. The retina, together with its pigment layer, was laid on a filter paper and cut into small pieces, together with the paper, and immediately immersed into an ice cold, buffered (pH 7.3 to 7.5) 2 per cent solution of osmium tetroxide for a period of 2 hours. Each fixative was diluted in the corresponding Ringer solution. After washing and dehydration through an ethanol series, small pieces were embedded in a prepolymerized mixture of n-butyl and methyl methacrylate. Ultrathin sections were made with a Moran ultramicrotome, equipped with a diamond knife (13), and examined with a Siemens Elmiskop I.

RESULTS

According to Walls (14), five layers can be distinguished in the retina (Fig. 1). The first is a receptor layer which comprises two zones separated by the outer
limiting membrane. The outer and inner segments of the cones and the rods are situated in the first zone, surrounded by the processes of the pigment epithelium. The second zone is occupied by the receptor bodies and their nuclei.

The second layer, corresponding in light microscopy to the outer plexiform layer, comprises the synaptic endings of the receptors and a neuropile-like structure formed by the dendritic processes of the bipolar cells.

The third layer is the zone of the bipolar and horizontal cells and possibly also the zone of the amacrine cells which so far could not be identified by the author with the electron microscope.

The fourth layer is formed by the connections between bipolar and ganglion cells.

Finally, in the fifth layer there are both ganglion cells and optic nerve fibers surrounded by the Müller cell cytoplasm.

The retina is bounded on its outer side by the pigment epithelium, the prolongations of its cells spreading in the direction of the receptors. These prolongations show two kinds of pigment granules: one is made up of numerous round formations filling the cytoplasm of the processes; the other consists of very dense spindle-shaped structures at the periphery of the prolongations. Since these processes completely surround the outer segment of the receptors, the spindle-shaped structure makes a good shield against the scattering of light (Figs. 2 and 3). The retina is bounded inside by the inner limiting membrane which appears as a double osmiophilic line separating the retina from the vitreous body.

The Müller cells, or Müller fibers, represent the supporting tissue of the retina. They appear as clear cytoplasmic portions containing both endoplasmic reticulum and mitochondria which are more numerous towards the portion contacting the inner limiting membrane (Figs. 1 and 17). The fibers extend from this membrane to the outer one, where they end with small finger-like processes inserted between the inner segments of the receptors. In the retina of the turtle the Müller fibers may be seen running almost across the entire retina. The same fibers can be seen in the monkey, where they extend across two or three layers.

Receptors

The general ultrastructure characteristics of the receptors were first described by Sjöstrand in the guinea pig, the fish (1–3), and the toad (4), and by De Robertis in the retina of the rabbit and the mouse (5–7).

Double cones appear in the fish, turtle, and chicken. The cones show very regular patterns in the fish (14), the most common one consisting of four double cones surrounding a single one (Fig. 3). The interlaminar spacing of the outer segment of different cones was measured and no difference was
found between the two members of a double cone (Fig. 4). The average inter-
laminar distance was 110 Å for double cones and 105 Å for single cones.

A connecting cilium, with a structure similar to that described by
De Robertis (6, 7), has been seen in the rods and cones of all the vertebrates
hitherto studied. The cilium is more evident in cross-sections of the ellipsoid
portion where it is situated laterally, surrounded in the fish rods by a crown of
7 to 8 mitochondria. In the monkey, the cilium is seen to be surrounded by a
limiting membrane making a meso-like structure on one side (Fig. 5).

The receptor bodies extend into the retina, penetrating the outer limiting
membrane which appears as a condensed ring embracing each receptor, as
described by Sjöstrand (3). In cross-sections of the monkey retina, it may be
seen that the condensed ring is a continuous double osmiophilic line surround-
ing the receptor bodies, which are connected to each other by meso-like
structures thus resembling unmyelinated nerve fibers inside a single Schwann

cell (Fig. 6).

The receptor bodies appear as elongated structures with an enlarged zone
occupied by the nucleus, closer in the cones to the outer limiting membrane
than in the rods (Fig. 7). The cytoplasm presents a Golgi complex as well as
longitudinally arranged filaments 200 Å thick, which disappear towards the
synaptic enlarged section (Fig. 10). The synaptic endings of the receptors are
either pyramid- or ovoid-shaped and are usually much larger in cones than
in rods. The synapses appear darker in the cones, especially those of the fish
(Fig. 8), and are filled with synaptic microvesicles, about 300 Å in diameter,
with walls formed by small dense dots, each 50 Å in size. These small dots
were also found filling the cytoplasm of the synaptic endings scattered among
the microvesicles. The dendritic processes of the bipolar cells invaginate the
base of the synaptic endings of the receptors to a depth of approximately 1 μ.
In cross-sections (Fig. 9), the dendritic processes appear as numerous rounded
or elongated structures inside the synaptic ending, the cytoplasm of both cells
being separated by the corresponding plasmatic membranes as well as by a 100
Å wide space between these two membranes. The cytoplasm of the dendritic
processes is less dense than that of the receptor. Microvesicles have never been
observed in the dendrites.

The presence of large mitochondria, in the rod synaptic endings of the rat,
as described by Ladman (11), was confirmed by the author. Similar structures
exist also in the cone synaptic endings of the monkey, 3 to 4 μ above the base
of these endings (Fig. 10).

A structure resembling a big vacuole was seen near the vertex of the syn-
aptic pyramid in the cones of the fish. This vacuole contains microvesicles
and is surrounded by some 3 to 5 paired osmiophilic lines, running helically
around the vacuole and then descending towards the base of the synaptic
pyramid (Figs. 8 and 11).
Neuropile

Between the synaptic endings of the receptors and the bipolar and horizontal cells there is a complicated structure, 2 to 3 μ thick (Fig. 12). This structure is made up of an infinite number of thin dendritic processes of the bipolar cells which cross each other in all directions, forming a three-dimensional plexus before penetrating the base of the synaptic endings of the receptors. Each of these processes, having a diameter of 0.1 to 0.5 μ, is formed by a clear cytoplasm containing granules and a few mitochondria. These dendritic extensions are separated from one another by their own plasmatic membrane.

The above neuropile-like structure was observed in all the vertebrates studied and was also found to be present in the foveal region of the monkey (Fig. 13).

Bipolar Cells

The bipolar cells show a rounded nucleus, 4 to 5 μ in diameter, surrounded by a thin cytoplasmic strip, 0.2 to 1 μ thick, containing mitochondria as well as endoplasmic reticulum. The prolongations of these cells, spreading in the direction of the receptors, form the so called neuropile. The bipolar cells are arranged in 2 or 3 rows, except in the chicken where as many as 15 rows may be seen. In the monkey, three nuclear types are seen in the bipolar cells layer (Fig. 12). The first type comprises large grouped granules forming dense zones irregularly arranged, in contrast with less dense granular portions. The second type, with a pale appearance, has small granules homogeneously distributed. The third type of nuclei also presents a regular distribution of granules but its density is greater than that of the second type.

Horizontal Cells

The horizontal cells are also situated in the third layer of the retina. In the fish Centropomidae these cells are very large and have a filamentous cytoplasm with mitochondria at the periphery, almost touching the cell membrane. The nucleus is small, oval-shaped, situated in the center of the cell (Fig. 14). In cross-sections (Fig. 15) the horizontal cells appear star-shaped, 80 μ in diameter, with 3 to 5 processes 1.5 to 5.5 μ thick, making membrane-to-membrane connections at their distal ends with the processes of the other horizontal cells. In this way the horizontal cells form a structural unit, like a net, through which pass the prolongations of the bipolar and Müller cells.

Inner Plexiform Layer

This layer represents the fourth retinal layer and is formed mainly by the synaptic connections of the bipolar and ganglion cells. In the electron microscope this layer appears as a zone up to 40 μ thick (in chicken), formed by
numerous rounded sections of cytoplasmic processes of various thicknesses (0.4 to 1.6 \( \mu \) in diameter). Several irregularly shaped, dense cytoplasmic zones were noticed among them, corresponding to the Müller fibers (Fig. 14).

**Ganglion Cells**

These cells are rounded, having a cytoplasm rich in rough-surface endoplasmic reticulum, mitochondria, and vacuoles. The nucleus is almost as big as the cell body and appears to have the same density as that of the cytoplasm. The ganglion cells are not numerous and are scattered through the fifth layer except in the chicken where they form a single row, very close to the fourth layer (Fig. 16), and in the turtle where the cell bodies are tightly packed (Fig. 17).

**Optic Nerve Fibers**

The optic nerve fibers are grouped into bundles which cross the fifth layer in the same direction. These fibers appear in the fish as all myelinated, and in the monkey as unmyelinated (Fig. 18), both kinds being present in the other vertebrates studied in this work (Figs. 16 and 17). The optic nerve fibers are most numerous in the chicken where they take up a space of up to 50 \( \mu \) in longitudinal sections (Fig. 16).

**DISCUSSION**

In the present electron microscopic study, the presence of a neuropile-like structure between the receptors and the bipolar cells has been demonstrated. Due to the complexity of this structure it has not been possible to determine clearly the connections between the receptors and the bipolar cells, since these appear to be diffusely connected even in the foveal region of the monkey. From the present study, and from that of Sjöstrand (12) who mentions the intertwining of the bipolar dendrites in the guinea pig, it is concluded that this neuropile is generally present in all vertebrates.

One of the three nuclei described in the bipolar cells layer was not observed in the parafoveal region of the monkey. Hence, it might be concluded that the type of bipolar cell, which is absent in this region, is connected to the rods.

Horizontal cells should be present in the third retinal layer, according to Cajal (15) who found them in all the vertebrate series. The author could identify the horizontal cells only in the retina of the fish and turtle. No apparent synaptic connections with the receptors were observed.

The Müller cells surround all retinal structures like glial processes, leaving only spaces of 100 Å between the membranes. Moreover, due to the special arrangement of the outer limiting membrane which forms meso-like structures around the receptor bodies, it is believed that the two limiting membranes are portions of the plasmatic membrane of the Müller cell, which would play
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the same role as the Schwann cell for the unmyelinated nerve fiber. This would modify the concept of Wald (16) according to whom the pigment cell is homologous to the Schwann cell.

The outer limiting membrane appears rather thick and there seems to be a resemblance between the meso-like structures, the desmosomes of the skin (17–20), and similar structures found by Maturana in the optic nerve (21).

SUMMARY

Retinae of several vertebrates were fixed in buffered 2 per cent osmium tetroxide and embedded in methacrylate. Thin sections were examined with the electron microscope. The findings have reaffirmed the structure of the receptors and provided new information about the other retinal layers.

Large mitochondria were found in the cone synaptic endings. A description is given of a neuropile-like structure 1 μ thick in the longitudinal sections, situated between the receptor synaptic endings and the bipolar cells. This neuropile is formed by the intertwining of the dendrites of the latter cells. Such a complicated structure provides a diffuse connection between the receptors and the bipolar cells, and appears to be constant in the vertebrate series.

The foveal and parafoveal regions of the monkey retina were also studied, showing two types of nuclei in the cells of the bipolar zone. The rest of the retina showed three types of nuclei in the same zone.

The horizontal cells, appearing as large star-shaped cells, have been found only in the fish. No direct connections were seen between these cells and the receptors. The ganglion cells are scattered through the fifth layer together with bundles of optic fibers. These fibers appear to be myelinated in the fish and unmyelinated in the monkey. Both types of fibers were seen in the other vertebrates studied. The cytoplasm and nucleus of the ganglion cells have the same density, the cytoplasm being very rich in endoplasmic reticulum, granules, and vacuoles.

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Figure 1. A low resolution composite electron micrograph of the retina of the monkey showing the five retinal layers. Cones (c) and rods (r) form the first layer which is divided into two zones by the outer limiting membrane (ol). The outer plexiform layer formed by the receptor synaptic endings (se) and the neuropile (N), can be seen lying between the receptor nuclei (n) and the bipolar cells (bc). The fifth layer, bounded on one side by the inner plexiform layer (ipl) and on the other side by the inner limiting membrane (il), shows the presence of ganglion cells (gc), optic nerve fibers (of) and the clear cytoplasm of the Müller cells (Mc).
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Figure 2. Longitudinal section through the outer and inner segments of the fish cones. The outer segments are surrounded by the processes of the pigment cells (pc). The outer segment is sheathed by the darker pigment structures while the other pigment structures, less dense, fill the cytoplasm of the process completely. The inner segment shows many rounded mitochondria (m) and the paraboloid body (pb), situated over the outer limiting membrane (sl). The receptor nuclei (n) appear below the limiting membrane.
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PLATE 7

Figure 3. Fish retina. Cross-section of the ellipsoidal portions of the cones showing the regular disposition of the double cones around the single ones. Cross-sections of rods (r), at different levels, can be seen among the cones, together with the processes of the pigment cells.

Figure 4. Longitudinal section through the outer segments of a double cone of the fish retina showing the lamellar structure. Both members are separated by a double membrane 115 to 120 Å thick, formed by the apposition of the two cellular membranes. The interlaminar spaces are equal for both cones.
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Figure 5. Monkey retina. Cross-section through the ellipsoidal portion of one cone, showing the mitochondria (m); some of the lamellae of the outer segment, and on one side, the connecting cilium (arrow) surrounded by a cavity. A meso-like structure is seen to connect the cilium to the cavity membrane as will be seen more clearly from the insert at the top right hand corner.

Figure 6. Electron micrograph of the monkey retina, showing a cross-section of rods (r) and cones (c). The thick membrane surrounding the receptors corresponds to the outer limiting membrane (ol). The latter is formed by double osmiophilic lines, which pass from one receptor to another forming meso-like structures (arrow), see insert.
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PLATE 9

Figure 7. Longitudinal section through the receptor layer of the monkey retina. One of the three cones (c) can be seen running from the ellipsoid (e) to the synaptic ending (cse). The nuclei (n) in the cones are closer to the outer limiting membrane (ol) than they are in the rods. The rod synaptic endings (rse) appear as rounded structures among the larger ones belonging to the cones. Bipolar cells (be) are seen in the lower part of the picture.
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FIGURE 8. Longitudinal section through a cone synaptic ending of the fish, showing the dark cytoplasm filled with microvesicles. The synaptic ribbons (arrow) can be seen radially arranged from the base. A big vacuole with numerous microvesicles is seen at the vertex of the pyramidal synaptic ending. Cross-sections of the bipolar dendrites (bd) appear at the base of this pyramid.

FIGURE 9. Cross-section through the synaptic endings of the receptors of the turtle, showing the penetrating dendrites of the bipolar cells (bd) as clear cytoplasmic portions. In the cytoplasm of the receptor ending (re) there appear many microvesicles, besides the synaptic ribbons (arrow). No connections can be seen between these ribbons and the plasmatic membrane of the receptors.

FIGURE 10. Longitudinal section through the synaptic endings of the receptors of the monkey showing the pyramidal shape of the cone endings (cse) and the ovoid shape of the rod endings (rse). The filaments of the receptor body cytoplasm can be observed at the vertex of the pyramid. The rest of the cytoplasm is filled with the synaptic microvesicles. Big mitochondria (m) appear in both kinds of receptor endings. The neuropile (N) is seen lying between the receptor endings and the bipolar cells (bc). The clear cytoplasmic portions belong to the Müller cells (Mc).

FIGURE 11. Vertex of a pyramidal cone synaptic ending of the fish, showing the system of paired osmiophilic membranes helically arranged.
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Figure 12. Oblique section through the neuropile (N) of the monkey retina. The receptor synaptic endings (se) appear on one side, the bipolar cells (bc) on the other side of the neuropile. Three types of nuclei (a, b, c) can be seen in the bipolar zone.
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PLATE 12

FIGURE 13. Longitudinal section through the parafoveal region of the monkey retina showing on the left side, tangential sections of the bodies ($f_c$) and the synaptic endings ($cse$) of the foveal cones. On the right side are the bipolar cells with only two types of nuclei ($b, c$). The neuropile ($N$) can be seen between receptors and bipolar cells.
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PLATE 13

FIGURE 14. Composite electron micrograph showing a longitudinal section of the fish retina. The synaptic endings of the cones (c) appear on the right as dark pyramidal structures, placed among the receptor nuclei (n). The horizontal cell bodies (hc) can be seen as large cytoplasmic portions among which appear the bipolar cells (be). The inner plexiform layer (ipil) is seen on the left.
PLATE 14

FIGURE 15. Composite electron micrograph showing a cross-section through the third layer of the fish retina. A horizontal cell appears as a big star with five prolongations and a small dark nucleus. The arrow shows the connection between the prolongations of the horizontal cells. The bipolar cells (bc) are small dark cells with big nuclei, while the Müller cell processes (Mc) appear as clear cytoplasmic portions.

FIGURE 16. Longitudinal section through the fifth layer of the chicken retina showing numerous optic nerve fibers (of), most of which are myelinated. Ganglion cells (gc) appear on the left forming a simple row which separates the optic fibers from the inner plexiform layer (ipl). The inner limiting membrane (il) can be seen as a dark line bounding the retina on the right.
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PLATE 15

FIGURE 17. Section through the fifth layer of the turtle retina, showing numerous ganglion cells (gc) with their nuclei (n); bundles of optic fibers (of), some of them myelinated, and Müller cell cytoplasmic portions (Mc). The inner limiting membrane (il) appears as a dark line on the right.

FIGURE 18. Section through the fifth layer of the monkey retina, showing bundles of optic fibers (of) separated by cytoplasmic portions belonging to the Müller cell processes (Mc). It should be noted that all the fibers are unmyelinated.
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