Addendum to Chapter 4
ELECTRON MICROGRAPHS SUPPORTING THE TEXT[1]

4.3.5.1 Mechanical formation of the disk stack (EXTRUSION AND COATING)

There is insufficient information on the precise method of protein secretion by the inner segment in *Chordata*. A question remains as to whether the protein is secreted in fine filaments from numerous individual points, as in spiders prior to winding the filaments into a cable, or whether the material is secreted as a liquid and pressed into a sheet. In *Mollusca*, it appears more certain that individual filaments are initially secreted. The question is then whether the filaments are formed into ordered sheets that still exhibit parallel striations reminiscent of their origin. The latter appear likely based on the sensitivity to light polarization found in various crustaceans and molluscs.

In *Chordata*, it is interesting to note that one of the lower members of the phyla leading to the vertebrates, the ascidians do not have disks aligned perpendicular to the axis of the inner segment. These animals have what appear to be parallel slabs of protein extruded from rows of secretory points much like in *Mollusca*[2]. See **Section 4.2.1**. This would suggest that the fundamental secretory process in all chordates is similar to that in Mollusca. However, in the immediately following process in vertebrates, the protein sheets are turned and broken into disks prior to extrusion through the calyx. Whether the disks of vertebrates are formed singly or in parallel may impact the portrayal used in this chapter based on Papermaster, et. al.

Figure 4.3.5-1, adapted from Papermaster, shows more clearly the dendritic structure of the neuron passing through the ciliary collar on the way to the outer segment. It also shows additional steps in the formation of the disks. Although not easily identified in electron micrographs, there are holes in the Calyx of the inner segment that allow the fluid of the IPM to enter this chamber. The raw protein material, opsin, is secreted into the cup of the Inner Segment behind the Calyx. The protein proceeds to form into a liquid crystalline sheet within the cup. However, it is forced to fold back and forth under the influence of the inner Calyx wall. The Calyx has a shoulder near the ciliary collar that forces the protein material to begin forming a strip of liquid crystalline protein. This sheet crosses the calyx and then forms a new fold. As the material enfolds and becomes more rigid in the new fluid environment of the IPM, it fractures into disks at the sharp bends. These disks are further shaped by the Calyx as shown below and then coated by the chromophoric material. The drawing shows the raw disks as straight lines. The emphasis in the drawing changes after coating. It has become conventional to emphasize the shape of the coating on the disks rather than the protein substrate. After disk formation and coating, the assembly leaves the Calyx. In the process of leaving the Calyx, the assembly becomes coated with metabolic material needed for the electrical operation of the dendrites. The metabolic material is a coating and not a membrane in the semantics of this work. This coating is maintained for the life of the disk. It is stripped away as the disks enter the digestive area of the RPE cells prior to phagocytosis of the disks themselves.

---

[1]Released: September 2, 2004

[2]Eakin, R. & Kuda, A. (1971) xxx *J. Ultrastruct. Res*. vol. 112, pg 287

The outer portion of the Calyx has another function. It forms a series of grooves in the periphery of each disk. The process is caricaturized in **Figure 4.3.5-2**. In (1), the Calyx is fluted in order to shape the substrate material as it is extruded. The Calyx maintains this shape for a distance equal to the thickness of an estimated 20-35 disks. In the next stage, (2), the individual dendrites are laid into the furrows formed earlier. The dendrites have previously exited the Inner Segment membrane wall and are physically immersed in the IPM. The dendrites enter ports in the Calyx above the furrows and are laid into the furrows much like a cable laying process (See figures in the next section). The Calyx becomes circular in this region to allow the dendrites passage, about Disk locations #36-50. The disk assembly now leaves the Calyx as shown in (3). The dendrites are in the furrows and are active. No indication could be found whether the dendrites are long lived and the disks pass by a fixed point on a dendrite as they progress toward the RPE. The alternate scenario is that the dendrites grow in length at the same rate as the disks travel toward the RPE. In either case, the dendrites do not appear to be continuous all of the distance to the RPE. Most electron micrographs showing the disks face-on display microtubules in only a few of the furrows. Functionally, the dendrites are in parallel and only a few are needed for normal photoreceptor operation.

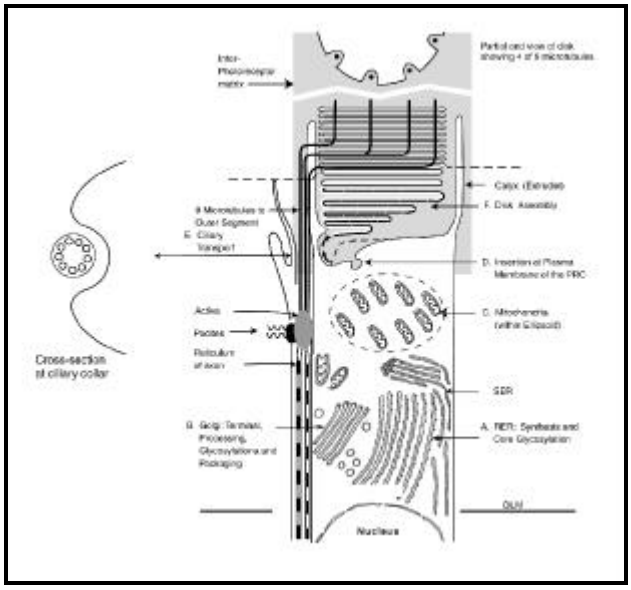

Figure 4.3.5-1 Formation of new disks at the OS/IS junction. A composite based on Papermaster (1985) in the area of disk formation.

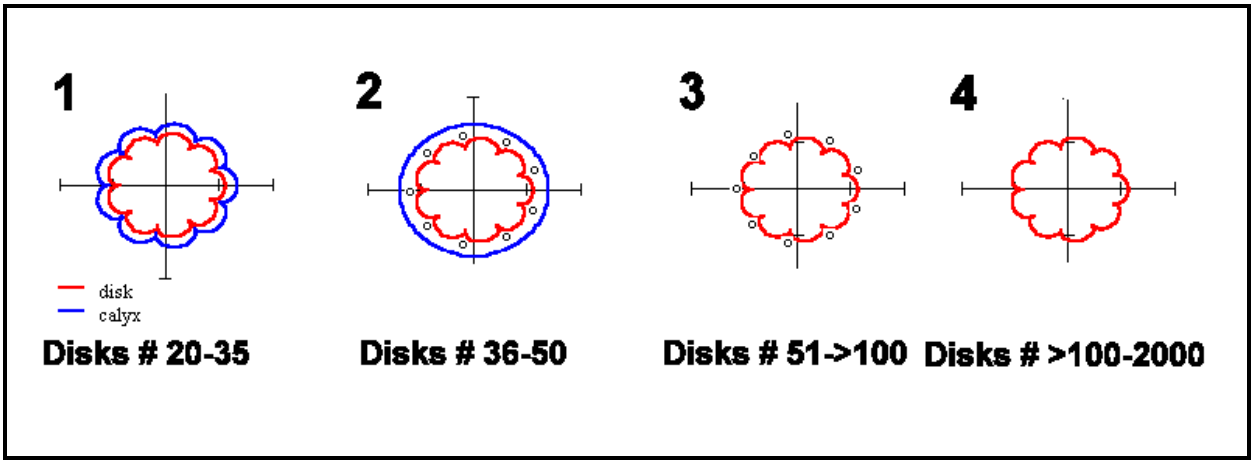

Figure 4.3.5-2 Disks during different stages of development

Figure 4.3.5-3 is one of the better electron micrographs available. There are usually nine grooves in the disks of

human; however, the number varies among species. Kroll & Machemer[3] provide good imagery of owl monkey disks with 29 grooves and petals. Some of the dendritic microtubules can also be seen filling these grooves. There is also a good micrograph of the frog outer segment[4].

Figure 4.3.5-3 Electronmicrograph of a human disk in plan view. The disk (a) was located at the outer extreme of the disk stack. Dendrites are marked by arrows at two points. The surrounding structure (b) relates to the RPE. (X 60,000) From Hogan (1971)

4.3.5.1.1 Electron micrographs of the Outer Segment

Figure 4.3.5-4 is a collage of electron microscope pictures from Tiering and Miller, writing in Autrum [5]. By going to the references cited, uncropped images of even higher resolution are available. Tiering's figure 35 from a pig (shown in upper left) is particularly useful in visualizing the length over diameter ratio of typical Outer Segments and locating the photoreceptors relative to the outer limiting membrane. A similar figure from Steinberg

[3]Kroll, A. & Machemer, R. (1968) Experimental retinal detachment in the owl monkey. *Am. J. Ophthal*. vol. 66, pp. 410-427 *in* Hogan fig. V-14

[4]Adler, R & Farber, D. (1986) The Retina: a model for cell biology studies. NY: Academic Press pg 211 *from J. Cell Biol*. 1978, vol. 78, 415-425

[5]Autrum, H. (1979) Comparative Physiology and Evolution of Vision in Invertebrates. NY: Springer-Verlag, pp 127-130

appears in Nolte and gives even more detail about the INM/IPM interface[6]. Figure 37 of Miller from a frog, *Rana Catesbiana,* (and shown in the remainder of the above figure) is at a higher magnification and shows many details. Frame **C** of that figure is shown without cropping and at a larger scale in Matsurra, et. al[7] where it is credited to Steinberg[8]. Note carefully the considerable surface roughness associated with the exterior of each Outer Segment. This roughness does not suggest the presence of a shrouding membrane. Careful examination of many of these figures will show a relatively smooth outer surface for the overall structure near the Outer Limiting Membrane that then becomes rougher and the furrows become more distinct. This interface is indicative of the end of the calyx. Steinberg has specifically noted this feature in his figure 6 where the beginning of the furrows is quite recognizable. Note also the multiple microtubules (many more than in humans) surrounding the Inner Segment near the Outer Limiting Membrane and prior to their entry into the calyx area. These appear to be being placed in the individual furrows of the disk stack. These furrows are generally narrower than the resolution of the image but they can be seen as long vertical lines at various points in the figure. Note also the twisting of the disk stack on the left in frame C as indicated by the curvature of the furrows. The photoreceptor stack in the center of the figure appears to have become disconnected from the inner segment and is probably no longer functional. The remaining connection to the disk stack is labeled a myoid (3) in the Miller figure but not in Matsuura or Steinberg. No microtubules are seen connecting to this disk stack, although there may be a remnant visible near the outer limiting membrane. Note also the deep indentations in the broken segment, in the upper left of the Matsuura and Steinberg images, which do not appear to be compatible with a shrouding membrane. Finally, there is no debris from torn exterior membranes in the vicinity of the ends of the disk stacks and the ends of some microtubules appear to be visible around the perimeter of some disk stacks. A picture is also available in Adler & Farber credited to Flannery[9]

.

---

[6]Nolte, J. (1999) The Human Brain. St. Louis, MO: Mosby pg 404

[7]Matsuura, T. Miller, W. & Tomita, T. (1977) Op. Cit. pg 769

[8]Steinberg, R. (1973) Scanning electron microscopy of the bullfrog's retina and pigment epithelium *Z. Zellforsch*. vol. 143, pp 451-463

[9]Adler, R. & Farber, D. (1986) Op. Cit. pg 7

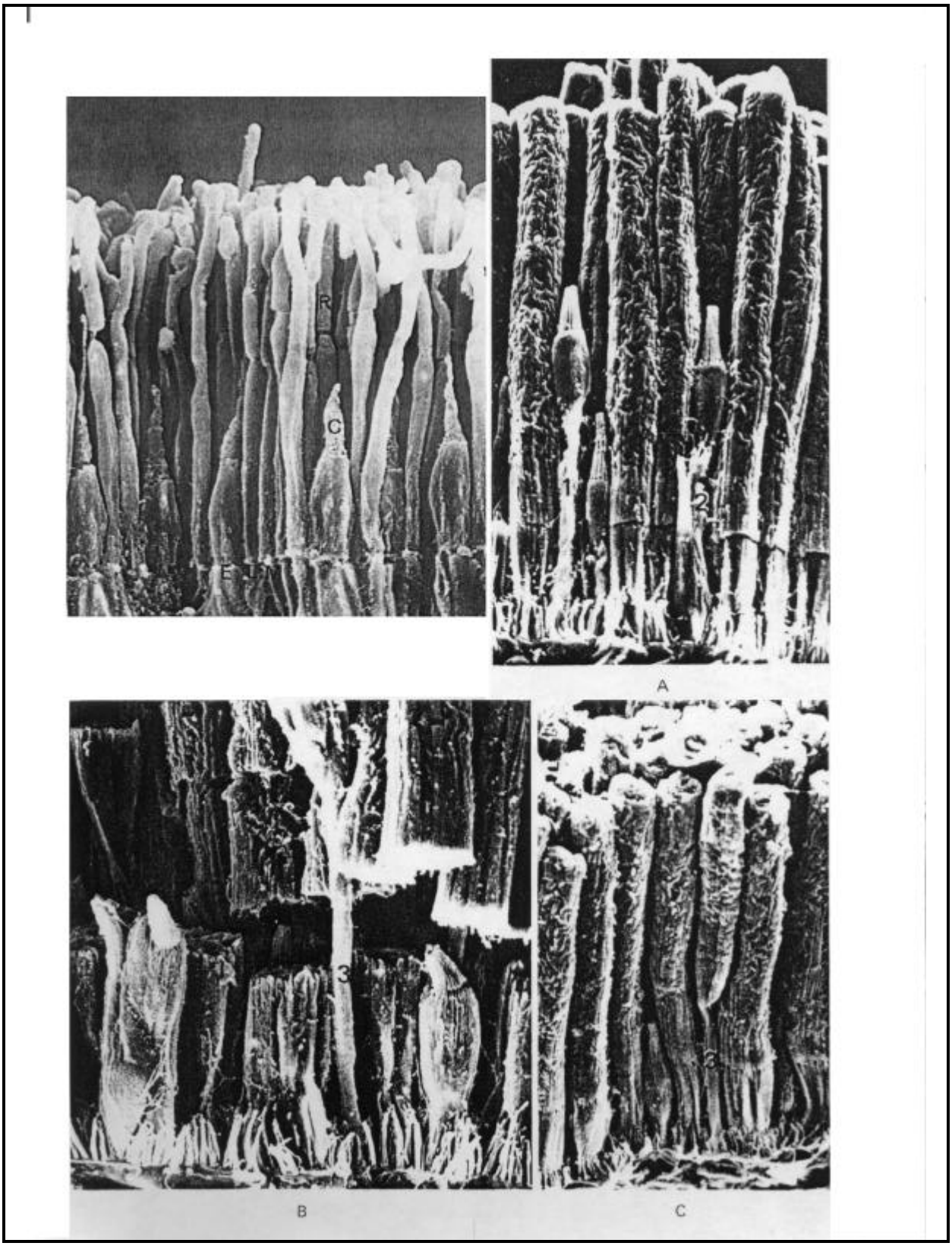

Figure 4.3.5-4 A collage of photoreceptor Outer Segments.

The electron micrographs originally by Steinberg are particularly revealing. Both frames **A** and **B** show the microtubules entering the disk stacks near the outer limiting membrane and before the calyx. Broken microtubules are very prominent in frame **B**. The microtubules can be seen emanating from the proximal surface of some of the broken disk stacks and from the distal surface, relative to the break, of other disk stacks. Again, there is no sign of outer membrane debris. In addition, many very fine structural features are shown that are not compatible with the shrouding effect expected of a smooth outer bilayer membrane.

The various authors in electron-microscopy referenced above assign names to the various Outer Segments in a strange way. In general, they do not perform any functional tests to determine the type of photoreceptor involved but rely upon their training in morphology. Steinberg, as an example differentiates between "red rods" and "green rods" by quoting Nilsson in 1964: "green rods have a shorter outer segment and a longer inner segment, with the outer-inner segment junction occurring more sclerally than in the red rods." He also notes the "red rods" are observed most often. This is in apparent conflict with most investigators in other fields who suggest the long wavelength photoreceptors are in the distinct minority.

Nearly all investigations into the phagocytosis of the Outer Segment near and in the RPE do not indicate the presence of any outer membrane surrounding the disks. Obviously, if the Outer Segment is in fact an integral part of the cell associated with the photoreceptor, it would be expected that there would be a continuation of the cell membrane wall enclosing the Outer Segment. However, this is not the case. The Outer Segment is located external to the cell membrane and is in fact formed in the tubule of the exocrine gland portion of the cell. It is then extruded in the direction of the RPE. **Figure 4.3.5-5**, from Hogan demonstrates this situation for the outer segment of the human eye. Even at the level of 300,000x, normally more than adequate to image the bilayer character of a plasma membrane, there is no suggestion that the interface between the material between the disks and the surrounding IPM consists of a bilayer plasma membrane. Note the thickness, structure and low fidelity of the line indicated by the arrows. Note also the termination of the inner segment stave in the upper right of the figure, above the arrow. Chen, Dong & Stark have recently provided a better micrograph (x22,600) showing both sides of the calyx and the disks of the outer segment exiting from the calyx [10]. There is no sign of the colax in the picture. There is no sign of a membrane surrounding the outer segment, particularly on the lower side. Excellent detail of the mitochondria is also shown. Molday & Molday have provided a similar electron micrograph of a *Bovine* Outer Segment that can be interpreted as not showing a plasma membrane surrounding the disks[11].

[10]Chen, D-M. Dong, G. & Stark, W. (1999) Ultraviolet light damage and reversal by retinoic acid in juvenile goldfish. *In* Retinal Degenerative Diseases and Experimental Therapy, Hollyfield, J. Anderson, R. & LaVail, M. *eds*, New York, Plenum,, 325-336.

[11]Molday, L. & Molday, R. (1987) xxx *Biochem. Biophys. Acta* vol 897, pp 335-340

Figure 4.3.5-5 The disks of the photoreceptor outer segment at 30,000 & 300,000x. A, The arrows show the interface surrounding the disks at 30,000x. Note the end of the plasma membrane stave of the calyx of the Inner Segment at the upper right. B, Magnified view of the edges of the disks and the interface at 300,000x. Note the interface between the IPM and the disks does not exhibit the bilayer structure associated with a plasma membrane. If there was a plasma membrane surrounding the disks, its bilayer structure should be easily visible at this magnification as it is for the disks. From Hogan, 1971.

Figure 4.3.5-6 reproduces an electron micrograph of frog that appears in Adler & Farber[12]. It shows two adjacent disk stacks and is quite clear There is no sign of a plasma membrane surrounding either stack.. There certainly are not two membranes passing down between the two stacks. The arrow points to a feature called a filament. It typically appears recessed from the disk edge by 15 nm. These filaments occur at 14 nm spacing along the perimeter of the disks. A similar picture that did not capture the filaments is available from Molday[13].

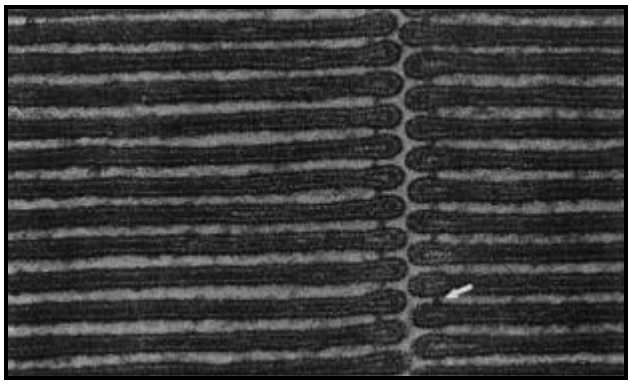

Figure 4.3.5-6 CR Electron micrograph of two adjacent disk stacks of frog. The rims of the disks are clearly visible but there is no sign of two, or even one, plasma membranes separating the stacks. The arrow points to one of tthe filaments frequently noted between disks at their edge. From Usukura & Yamada, 1981.

4.3.6.2.1 The distribution amplifier

In the case of the distribution amplifier, the material separating the dendrolemma from the axolemma is a liquid crystal of hydronium. This is the same material forming the synapse between two separate neurons. As in the case of the plasmas associated with the external synapses, these plasmas are each connected to an electrostenolytic power source on dedicated surfaces of their respective lemmas that are in contact with the fluid matrix surround. Also, as in the case of the external synapses, each neuroplasm is capable of supporting a connection to a separate nearby neuron or other signal source, and each axolemma is capable of making connections to one or more orthodromic neurons. It will be shown in **Section 4.7** and discussed in detail in **Chapter 11** that the poditic terminal of the distribution amplifier does not support a signal input. The dimensions of the internal junction associated with this amplifier are essentially the same as that for the external synapses required to carry the same

---

[12]Xxx (1982) xxx *J. Cell Biol*. vol. 95, pp 487-500

[13]Ogden, T. & Hinton, D. ed. (2001) Retina, 3rd Ed. Vol. 1, St. Louis, MO: Mosby pg 124

signal current. The precise physical size of the Activa associated with the distribution amplifier will be described in **Chapter 9**.

The actual circuit topology of the amplifier is shown in **Section 4.7.1** and the details of the circuit are discussed in **Chapter 11**.

4.3.6.2.2 The common and poditic terminals of the photoreceptor cell

Because of the unique internal structure of the photoreceptor cell, both the dendroplasm and the podiplasm of the cell must be in electrical contact with the surrounding fluid matrix via their own electrostenolytic power source. **Figure 4.3.6-4** from Lolley, et. al. is an electron-micrograph published in 1986 that appears to show the "common" terminal (associated with the dendroplasm) and poditic terminal of a photoreceptor cell[14]. The entwined strands appear to be slightly distal of the outer limiting membrane. Their surface area would provide a lower impedance electrical connection to the IPM than that of a flat area of lemma. A scale was provided but no magnification was given for the microscope.

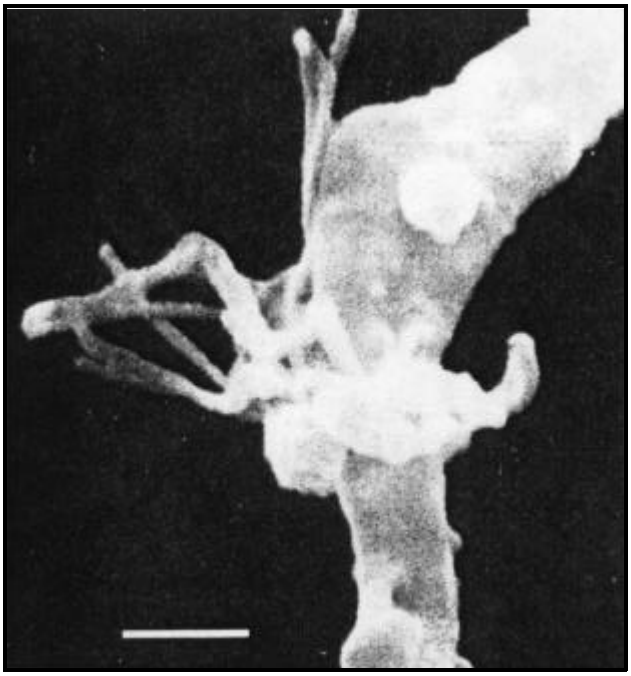

Figure 4.3.6-4 An electron-micrograph believed to show the poditic terminal structure of a photoreceptor cell. The strands are believed to provide more surface area and provide a low impedance electrical connection to the IPM. The clumped material is presumed to be residual matter from the outer limiting membrane. Bar is one micron. From Lolley, et. al., 1986.

As will be discussed in **Chapter 12**, there may actually be two distinct terminals of the photoreceptor cell shown in this picture. One would be a "common" terminal connected to the emitters of both the adaptation and distribution Activa and the second would connect to the base of the distribution amplifier.

[14]Lolley, R. et. al. (1986) Op. Cit. inset to figure 4

4.5.1.3

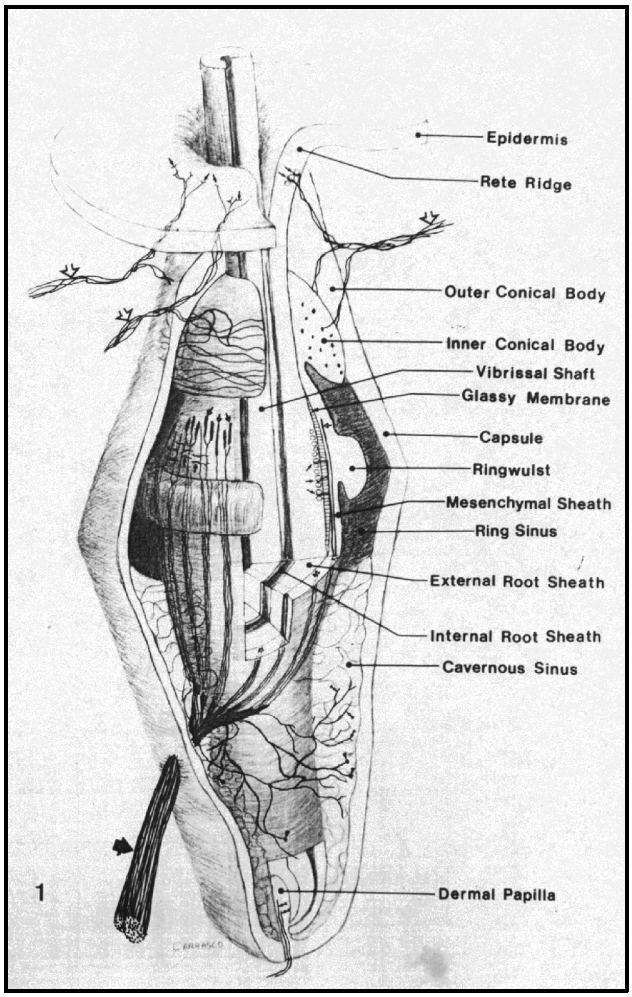

Figure 4.5.1-3 Caricature of the follicle-sinus complex of a myastacial vibrissa (whisker) of a rodent. Note the "deep vibrissal nerve" (heavy arrow) entering the capsule and differentiating in order to surround the vibrissal shaft (hair). The source of the protein for the hair is not shown. However, if the root sheaths were shaped to "form and break" the protein of the shaft, disks would be formed as in a photoreceptor cell. From Rice, et. al. 1986.

The photoreceptor cells are neuro-secretory and quite similar to their analogs elsewhere on the ectodermal surface, hairs. In fact the similarity between the caricatures of a photoreceptor cell in this work and a caricature in Rice, et. al. is striking[15]. Although representing a considerably different size skin-hair combination, **Figure 4.5.1-3** shows a follicle-sinus complex, F-SC, with the deep vibrissal nerve entering the capsule (arrow at lower left) much like in a photoreceptor extrusion cup and differentiating into a series of individual dendrites surrounding the vibrissal shaft of protein. (Note the vibrissal shaft is not surrounded by a plasma membrane.) Ectoderm usually differentiates into multiple dermal and neural layers. While the ectoderm of the retina has differentiated into neural layers, it has not differentiated into dermal layers. As a result, the epidermis is missing and the dendrites of the photoreceptor cell are in direct contact with the chromophore coated protein of the shaft. In the drawing, the Ringwulst would perform the same physical strengthening as the tissue of the calyx (the extruding die) of the photoreceptor cell. Based on these similarities, a photoreceptor cell, a quantum-mechanical receptor, can be considered an analog of other mechanoreceptors of the somatosensory and auditory systems.

[15]Rice, F. Mance, A. & Munger, B. (1986) A comparative light microscope analysis of the sensory innervaton of the mystacial pad. *J. Comp. Neurology* vol. 252, pp 154-174

4.6.1.3 Formation of the Outer Structure

The formation of the outer segments cannot be easily understood from just the histological data. The process is too dynamic, both during initial formation and during the ensuing lifetime of the animal. However, combining this data with experiments with nuclear tagged amino acids and nuclear tagged chromogens provides a very convincing story for the formation of the outer segments, both at the cytological and histological levels. The formation of the Outer Segment is based on the fact that the photoreceptor cells are ectodermal, as shown above, and operate similarly to hair cells. Their specialization in *Arthropoda* and *Mollusca* appears designed to generate multiple shafts of protein from a single cell. In *Chordata*, it appears they are designed to convert the vibrissal shaft of protein into a series of discrete disks. What is not yet clear is whether this is accomplished using a series of individual secretory tubules to initially form a parallel series of protein filaments before they are merged into the substrate of a disk.

Figure 4.5.1-4, from Dowling (1967) shows a mature photoreceptor cell from a squirrel at relatively low resolution for an electron-micrograph. While Dowling shows a structure surrounding the Outer Segment within and beyond the staves of the Inner Segment clearly defined by the picture, neither structure is resolved adequately to demonstrate that it is a plasma membrane (a bilayer membrane). It is clear from the figure that the Plasma membrane surrounding the Inner Segment is not contiguous with the material surrounding the Outer Segment. The important details, which will be demonstrated below, relating to the dynamic situation are:

+ the apparent emergence of the material forming the disks from a pocket or cup in the inner segment cell wall.
+ the apparent folding of this material to form a continuous serpentine strip of material

+ the apparent transformation of this continuous strip into a series of double walled disks

+ the apparent fact that the outer segment material is extra-cellular with respect to the plasma membrane of the inner segment

It is not well accepted and could be considered controversial to describe the photoreceptor cell as operating at least in part as an exocrine gland. However, this situation is:

+ quite compatible with a tissue related to nerve tissue,
+ the only explanation for the data showing the outer segment being formed from material provided by the inner segment
+ the only logical explanation for the outer segment material congregating in the obviously crowded space of the cup formed by the calyx and staves of the inner segment.

In the above figure, the cell wall of the inner segment is seen to form a floor for the formation chamber and to extend up along the sides of the outer segment forming the calyx. Equally important, the cell wall folds back away from the outer segment to continue the wall of the calyx and become the outer wall of the inner segment itself. Brown, Gibbons and Wald provided a caricature of this area of the photoreceptor cell which can be interpreted as showing the calyx of the inner segment reinforced by the staves but being entirely separate from the Outer Segment[16]. The jagged line along the outside of the outer segment is not contiguous with the cell wall associated with the inner segment and in fact does not appear continuous within the cup formed by the calyx. Its nature and function are not clear from these pictures. This picture is particularly useful since it slices through the OS/IS junction area in a plane that did not intersect with any of the nerve tissue associated with the Cilium. If the

[16]Brown, P. Gibbons, I. & Wald, G. (1963) The visual cells and visual pigment of the mudpuppy (*Necturus*), *J. Cell. Biol*. vol. 19, pp 79-106

photoreceptor had been sliced through the Cilium area, the interpretation of the structure would be more complex.

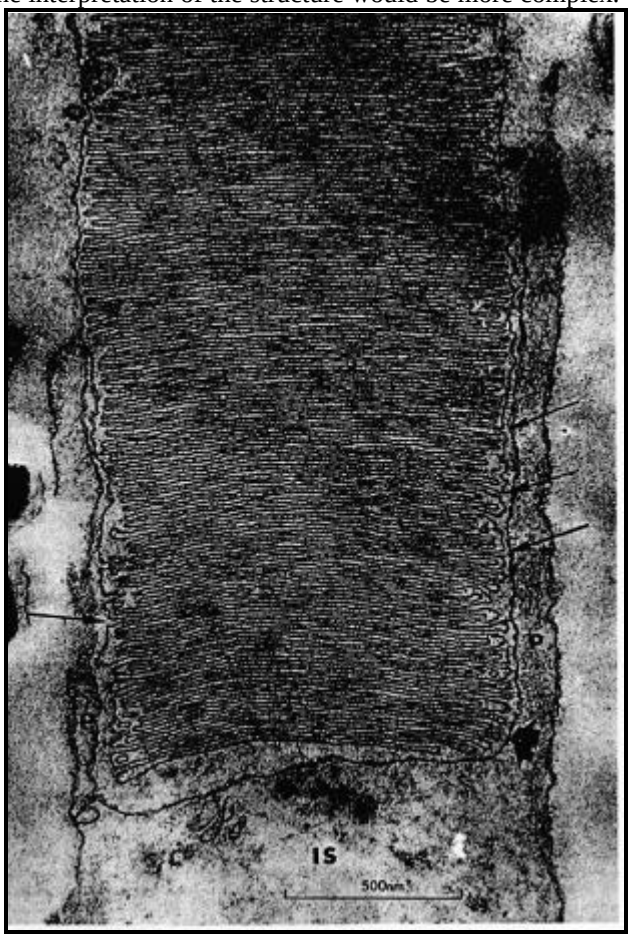

Figure 4.5.1-4 Electron micrograph of photoreceptor outer segment from the squirrel retina. Toward the base of the outer segment, continuity of saccule and plasma membrane is clearly seen (thin arrows), and at the very base the structure appears as one continuously folded membrane (thick arrow). The processes from the inner segment (IS) extend distally along the length of the outer segment (p). From Dowling (1967)

4.6.1.4 Migration

There is considerable data showing that the generation of the Outer Segments is a dynamic process, at least in the case of *Chordata*. The rate of replacement is a function of the body temperature of the chordate. In humans, the rate of replacement has been found to be approximately every 7-10 days. This mode of operation remains consistent with the ectodermal source of the photoreceptor cells. The details of this process will be discussed in **Section 4.6.3**.

In 1967, Young reported on his migration studies with rats. He injected tritium labeled methionine into the rats. He found that in rats injected on the 7th postnatal day, relatively little of this amino acid was utilized by the developing photoreceptor cells. The photoreceptors apparently had not reached that period in their development where they could utilize this material. However, in a 4 to 8-week-old rat, this amino acid was rapidly taken up by the inner segment and some hours later newly synthesized material was found to be concentrated in the "reaction band" at the base of the outer segment. Within the week, the tagged material had moved along the length of the outer segment; on the 9th day, it reached the end of the segment and on the 10th day, it disappeared.

White, et. al[17]. give the rate of migration as 2-3 weeks in the rat based on 1,000 thin disks with 30,000 rhodopsin molecules per disk. However, their comment about the orientation of the retinoids is not consistent with this work and they did not quantify the age of the rats precisely.

Further work reported by Young et.al. provides a picture of the disks progressing along the animal outer segment as a function of the temperature of the animal. They report the rate of disk formation for a frog at 22.5 Celsius. For a "red rod," it is approximately one every 40 minutes. They give one disk every hour as the rate of formation for a "green rod." Hall, Bok, & Bacharach claimed in 1968-69 that the mean travel time for an adult frog was about 8.5 weeks.

Figure 4.6.1-2 Electron microscope radioautogram of frog outer segments, four days after injection of tritieated amino acids. Incomplete caption From Young (1969)

[17]White, A. Handler, P & Smith, E. (1973) Principles of Biochemistry, 5th ed. McGraw-Hill, pp. 1015-1016

Figure 4.6.1-5 is also very instructive. It shows phagocytosis/digestion of several segments of the OS being performed simultaneously. It is informative to note that the various segments lose contrast and definition in the process of phagocytosis; specifically, it appears the chromophore material is removed early and transferred to the storage sites (the pigment granules) while the protein structure is digested separately.

Figure 9-19 of Hogan et. al. is also instructive. It shows two pigment granules at x60,000 and the caption points out the granules are surrounded by a single membrane and have very little internal structure. This would be expected if the material was essentially a store of the chromophores alone in liquid crystalline form or if the material was the chromophoric material esterfied with palmitic or stearic acid and stored in either a liquid crystalline or plain liquid form.

Bridges and others do state, rather emphatically, that during phagocytosis, all trans-retinol {Rhodonine} is transferred from the end of the OS to the RPE. If this is correct, and it is very likely that the material transferred is in fact the complete chromophore since its color and contrast are maintained as indicated above during the transfer, then it is likely that the pigment granules are filled with the Rhodonines and not their precursor chromogen, all trans-retinol.

It is also clear that an all-*trans* form of a retinoid is transported by the Interstitial Retinol-Binding Protein (IRBP) from the RPE to the vicinity of the newly formed OS disks. IRBP is found only in the eye--and only within the IPM of the eye. It is believed to only transport the all-trans form of retinoids. Fong et. al.[18] have provided two data points. They have determined that IRBP binds with 2.2 molecules of all trans-retinol {Rhodonine}--apparently measured on dark adapted eyes. In the light-exposed eyes, the value was only 0.6 molecules of retinoid per molecule of IRBP. It is not clear how these data points should be interpreted since light exposure should not have a major affect on the IRBP transport rate.

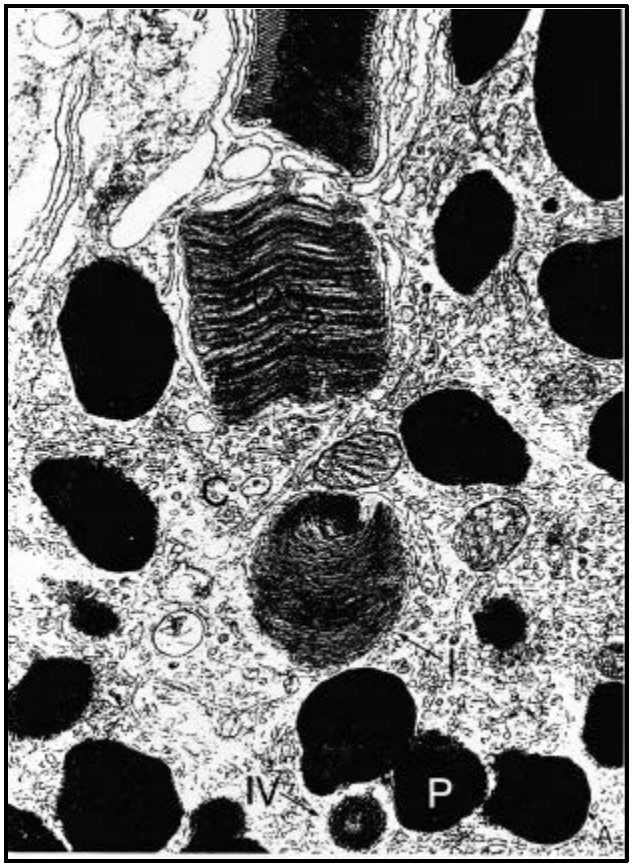

Figure 4.6.1-5 Stages in the phagocytosis of the disks of a photoreceptor Outer Segment. The RPE cell cytoplasm is shown as (c) and a pigment granule at (P). A recently formed phagosome (I) contains rod discs. IV is a phagosome in the end-stage of hydrolytic digestion. OS_1 and OS_2 show groups of discs in earlier stages of digestion. (X 28,800) From Hogan, Alvarado, & Weddell (1971)

[18] Fong, S. Liou, G Landers R. Alvarez, R. & Bridges, C. (1984) Purification and characterization of a retinol-binding glycoprotein synthesized and secreted by bovine neural retina J. Biol. Chem. 259, 6534

4.6.2.2.2 Unique chemistry of the retinoids of vision

Retinol is easily converted into retinoic acid; a structure with a functional end group containing a single atom of carbon associated with an oxygen atom through a double bond (a carbonyl group)and an OH group through a single bond (a hydroxyl group). Thus, the overall molecule has many properties of both an alcohol and an aldehyde. In a simple ester of retinoic acid, the functional end group contains a single atom of carbon associated with an oxygen atom through a double bond (a carbonyl group)and an OR' group through a single bond.

The resonant retinoids proposed as the chromophores in this model contain a carboxyl ion system. This system separates the hydroxyl group and the aldehyde group associated with a single carbon in an organic acid. As a result, the Rhodonines exhibit the properties of both an alcohol and an aldehyde simultaneously; however, the two ligands are associated with different carbon atoms at the ends of a resonant conjugated chain. The Rhodonines can also mimic many of the properties of retinoic acid. These features of the Rhodonines can lead to a great deal of confusion in the laboratory environment if they are not anticipated.

4.6.2.2.3 Material concentrations in the OS/RPE environment

There are a variety of percentages given in the literature for the amount of opsin, chromophore, rhodopsin and other materials related to vision present in the various areas of the eye. Currently, the numbers do not paint a coherent and explicit scenario of the location of all the retinoids in the eye. In general, there has been a lack of specificity with regard to the exact compound and the exact location of the material within the photoreceptor/RPE complex being discussed. Ganguly[19] says "The photoreceptor cells contain about 4% of the total vitamin A of the eye . . . " and "Excluding the retinoids present in the rhodopsin in the retina, this amount (in the RPE) represents about 87% of the total retinoids in the eye." It is not clear whether he includes the retinoids of the Outer Segments in his photoreceptor cells or treats them separately. If he treats them separately, the remaining 13% of the retinoids would presumably be found in the IPM. These values indicate the ratio of retinoids in the RPE to retinoids in the OS is either about 5:1 or 20:1. He goes on to quote Bridges as "the RPE from a pair of human eyes contains on average 4.5 μg of retinol and retinyl esters. Most of the vitamin A found in the RPE is in the form of retinyl esters and the major esters are palmitate and stearate . . . In the humans, the ratio of palmitate to stearate is about 5:1 . . . in human RPE cells the retinyl palmitate is present in the forms of both all-*trans* and 11-*cis* isomers."

Based on the model of this work and the experimental results in the literature, the chromophores of vision are not processed within the photoreceptor cells. They are processed within the RPE cells. This situation does not support the putative description of rhodopsin as a molecule containing both opsin and retinol (buried deep within the structure of the protein) that is formed within the photoreceptor cell. Although this configuration was commonly shown in caricature in the literature of the 1970s and 1980s, the work of Bridges & Yoshikami show it must be abandoned.

In the description of the retinoid processing loop presented here and the numbers in the literature, the reserves of the chromophores are so high in the RPE that the RI is only required to acquire small amounts of all trans-retinoid per day and process it into Rhodonines. This makes radiographic confirmation of this process awkward. However, it has been accomplished by Bridges & Yoshikami (**See Section 4.6.2.3.3**). Wald employed a simpler approach based on restricted metabolism (under starvation conditions) to obtain useful data. It will be discussed below.

The manner in which the chromogens are stored in the RPE is well documented by **Figure 4.6.2-2**. There are

---

[19] ibid, Ganguly, pg 153 & 156

three clearly delineated types of globules of chromogenic if not actually chromophoric material visible in this image from Wolken[20]. No globule representing the L–chromophore appears in this figure. There was no discussion of the specific colors in the original figure. It is suggested that the red globules are actually more magenta and the yellow globules contained more green than portrayed here. The observed color of these globules is the complement of their absorption spectra. The globules marked UV– were colorless in the original microphotograph. No dimensions were provided for this image. Marmor has provided a similar color picture of the RPE[21].

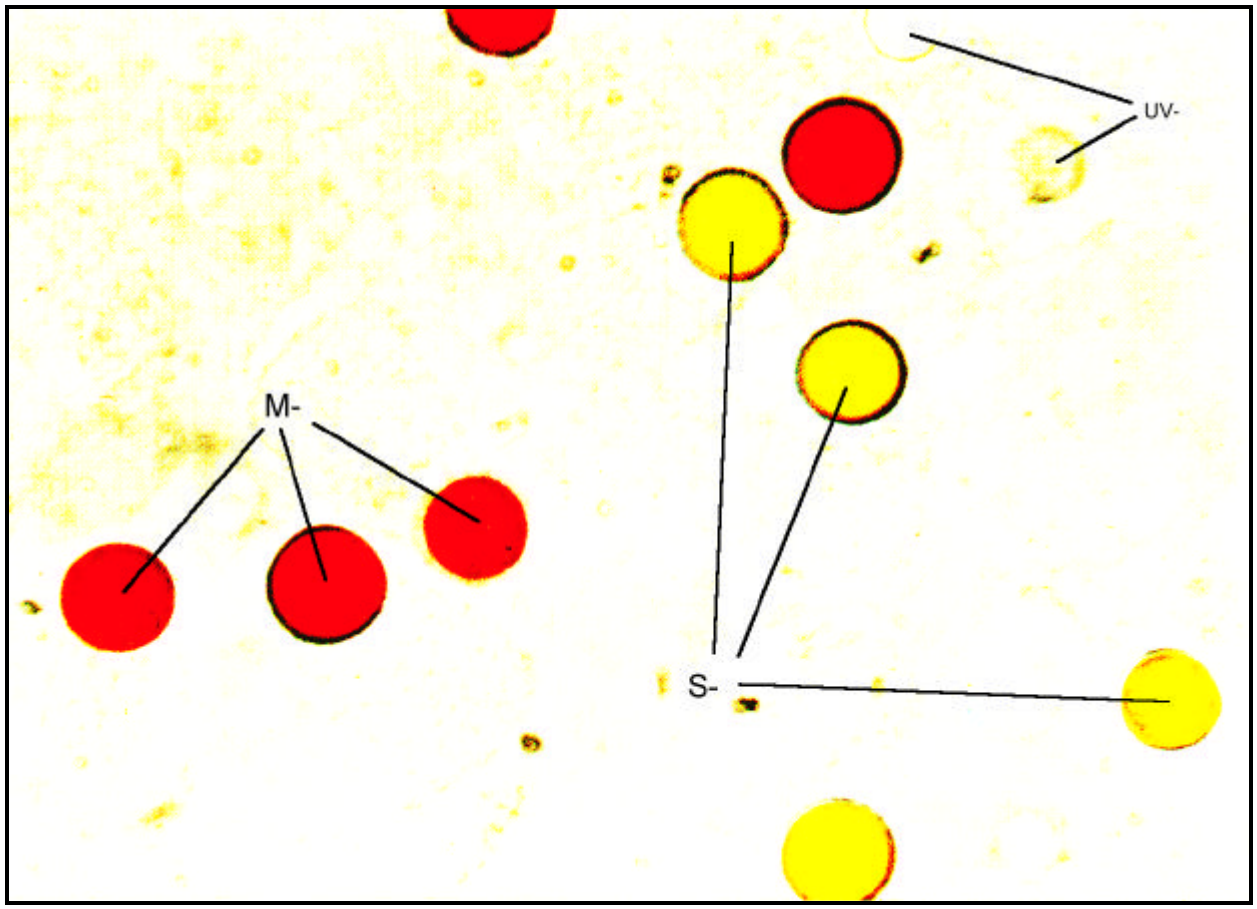

Figure 4.6.2-6 Color picture of chromophoric globules stored in the RPE of the swamp turtle, *Pseudemys scripta elegans*. No scale provided. Globules indicative of three separate chromophores are noted. Their recorded color is the complement of their peak absorption. Globules of UV– chromogens would appear transparent using normal photography and as observed with a microscope. Few color pictures of the RPE are available in the literature. From Wolken 1966.

Wolken also provided a micrograph of a chicken retina showing globules located between the outer and inner

[20]Wolken, J. (1966) Vision. Springfield, Il: Charles C. Thomas pp 70-73

[21]Marmor, M. (1959) xxx *Surv. Ophthalmol*. Vol. 4, pp 279-285

segments of photoreceptor cells. There are individual globules associated with individual photoreceptors. The color of a globule is suggestive of the specific (complimentary) absorption spectrum of that photoreceptor. A scale was provided for that picture and the globules appear to approximate the diameter of the outer segments, about three microns. This work would suggest that the globules photographed in the extrusion cup area were a result of in-vitro processing. In-vivo, the material would be expected to be in solution until deposited in ultra thin layers on the opsin substrates. These layers would not be observable individually by the techniques available to Wolken.

Table of Contents

Appendix R COLORIMETRY AND VISION 1
R.1 Background 1
R.1.1 Historical perspective 1
R.1.2 Conventional scientific description of colorimetry 1
R.1.3 Historical perspective 1

List of Figures