Processes in Biological Vision:

including,

Electrochemistry of the Neuron

This material is excerpted from the full β-version of the text. The final printed version will be more concise due to further editing and economical constraints. A Table of Contents and an index are located at the end of this paper.

James T. Fulton
Vision Concepts
1 (949) 759-0630
jtfulton@neuronresearch.net

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James T. Fulton
4. The Photo-Receptor/IPM/RPE complex of the Chordate Eye

There are a number of reasons for discussing the photoreceptor cell/IPM/RPE complex as an entity. The most important is that the operation of the photosensing function cannot be understood from a narrower purview. The above combination of functional elements constitutes the light sensitive portion of the retina. A second reason is to stress the fact that the outer segment, historically defined as an integral part of the photoreceptor cell, is in fact extracellular. Third, because of its extracellular nature, the outer segment is dependent on a variety of other elements for its proper operation. Finally, the continual high-rate generation and phagocytosis of the disks of the outer segment are probably unique within the body. Looking at the above elements as a group highlight the fact that a cone shaped outer segment of a photoreceptor cannot exist under the dynamic conditions present.

When exploring the entire photoreceptor cell/IPM/RPE complex, the reason for a variety of morphological (cytological) features can be explained and described in detail. In many cases, these features are due to electrical signaling requirements.

In a real sense, it is the outer segment that is the focal point of the photosensing function. It relies upon the glandular portion of the photoreceptor cell, the Mueller cells and the RPE cells for material support and the IPM for protection. In return, it generates the initial quantum mechanical signal, in response to light, that is transferred to the neural component of the photoreceptor cell. The IPM also plays a unique role in supporting the electrical requirements of the neural portion of the photoreceptor cell.

The neural component of the photoreceptor cell is probably unique in the animal neural system. It is also key to the operation of the photoreceptor/IPM/RPE complex. The electrophysiology of the cell will be introduced in Section 4.1.2.7 for purposes of continuity but not explained fully until Chapters 8, 10 & 12.

The goal of this chapter is twofold, first, to present a broader cytological description of the photoreceptor cell than previously available and second, to place the photoreceptor cell within the photoreceptor cell/IPM/RPE complex and describe the overall operation of that complex. While the chapter will focus on the structural and functional characteristics of the chordate photoreceptor/IPM/RPE complex, it appears that a similar analysis can be used to describe the non-chordate eye. Their primary difference is in their morphology (topography), not their topology.

4.1 Introduction

This chapter will concentrate on the functional aspects of the photoreceptor/RPE complex related to signaling. While it will address the general morphology of the cell for orientation purposes, it will be shown that its morphology is largely defined by functional requirements. Form follows function as in virtually all biological as well as man made systems!

In presenting this chapter, three principles will be relied upon that are developed in detail within the bulk of this work.

Principle one--The critical features of the photoreceptor cell related to signaling only appear at the level of cytology and molecular chemistry.

Repeating the position of Section 3.1.5.3, this work does not recognize any relationship between the functional performance of the photoreceptors of vision and the morphological concept of rods and cones. The only functional difference between the photoreceptors of vision is at the molecular level. It involves distinctly different chromophores coating the disks of the Outer Segment.

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As recently as 1987, Ahnelt, et. al2, said “The cones differ in having different photopigments and different neural connectivity, but no morphological differences with which to distinguish the three different spectral types have been reported yet.” They then attempt once again to identify a class of cones, using the term speculative, based on statistical morphological data and inner segment staining. It is proposed that even the distinction based on neural connectivity is spurious.

The most authoritative text on anatomical and clinical neuro-ophthalmology no longer supports a physical or electrophysiological distinction between rods and cones3.

This chapter will describe both the glandular and neural characteristics of the photoreceptor cell at a level never approached before. The glandular portion of the cell is conventional but largely unrecognized. The electrical characteristics of the neural portion of the cell have previously been unknown and have been basically ignored in the literature. The non-secretion related metabolic characteristics, previously labeled the functional aspects of the cell, will be largely ignored in this work. These characteristics are largely irrelevant to the performance of the cell in the visual system.

Principle two--Historically, the morphological literature has assumed the so-called outer segment of the photoreceptor cell was an integral part of that cell. The result has been that many investigators have assumed the outer segment was surrounded by the plasma membrane of the cell. Using the tools available at this time, no image of such a plasma membrane surrounding the outer segment has ever been published. In fact, the published images, and a variety of clinical conditions associated with the retina, refute this assumption.

This work does not recognize the existence of a plasma membrane surrounding the outer segment of the photoreceptor cell. The lack of such a membrane removes the foundation from under considerable conceptual work in the literature concerning the movement of molecular material through such a membrane. This is unfortunate. However, the lack of such a membrane leads to a much more understandable mechanism for incorporating the chromophores of vision onto the protein-based disks of the outer segment.

To bolster the position of the author in this regard, the web site www.4colorvision.com offers a $1000 reward to the first investigator that can provide documentation supporting the presence of a plasma membrane enclosing the central portion of the outer segment located beyond the confines of the inner segment and before the confines of the RPE cells.

The absence of a plasma membrane surrounding the outer segment leads to a major but alternate role for the GABAs, glutamates, glycines and other chemicals related to the glutamate cycle of biochemistry. These materials are shown to be the source of electrical power that drives the neural system. The absence of a membrane and the extra-cellular nature of the outer segment also explains how the disks of the outer segment can be phagocytized by the RPE without destroying the parent photoreceptor cell.

Principle three--The photoreceptor cell involves sophisticated electrical circuits within the cell that are based on the Activa, the active electrolytic semiconductor device that is the foundation of the neural system. The technical background necessary to define the features of the Activa and the neuron is too extensive to incorporate in this Chapter. It includes a broad range of disciplines not normally associated with biology. Not only must multiple disciplines within the field of biology be used in an unfamiliar way but many other disciplines that the biologist seldom encounters—radiation physics, quantum mechanics, optics, and analog electrical circuits—must also be brought into the picture. Using these, the subject can be addressed in a comprehensive scientific manner. It is this background that is absolutely necessary if a complete understanding of the operation of the photoreceptor cell is to be obtained. The required information is provided in other parts of this work. Appendix B develops the principles of the Activa and its occurrence in the neurons of animals. Chapter 8 develops the circuits, common to all neurons, that use the Activa and Chapter 9 addresses the neural circuits unique to the photoreceptor cell. Section 4.1.1 & 4.1.2 will present a few of the most critical concepts drawn from this material and needed to understand the remainder of the chapter.

This work proposes that the neural system is entirely electrolytic in its operation. All signal transfers between neurons and between the dendrites, podites and axons of neurons are electrical in character. The carrier of the signal between neurons is a charge, (an electronic neurotransmitter). While the chemistry of the glutamate cycle plays a major role in the operation of the neural system, it is in an electrostenolytic support role. There is no need for chemical neurotransmitters within the signaling functions of the neural system.

As indicated in Chapter 3, there are a wide variety of photoreceptor shapes in the animal eye, but a somewhat lesser number in the primate and human eyes. Prince⁶ has shown that the human eye displays a minimal number of different types of photoreceptor. Primarily because of this fact, this Chapter will concentrate on a typical photoreceptor of the human eye, how it is configured, how it performs its functions, and how it is supported by its local environment. Later material will discuss additional ramifications when broadening the discussion to include other animal eyes.

It must be stressed that the presentation of two-dimensional pictures in the literature representing a slice of a three-dimensional structure frequently leads to misinterpretation of the true situation. The three-dimensional structures of interest here are not axially symmetrical. Therefore, many features are omitted in any given two-dimensional representation of the actual situation. To create a comprehensive understanding of almost any function of a photoreceptor requires the study of a group of photographs as a minimum in order to create a three-dimensional concept in ones mind. Once this is accomplished, a realistic caricature of the subject can be presented.

Three works in the morphology literature are particularly noteworthy in the above context, Snyder & Menzel⁵, Yamada⁶ and Eakin⁷. These works provide important clues that can be assembled into a more appropriate composite picture if the variety of overlapping terminology can be distilled. Snyder & Menzel title their work “Photoreceptor Optics” but in the context of this work, it is limited to the physiological (geometry and structure of the photoreceptors) as opposed to the physical optics of eyes. Although many of their conclusions and/or proposals are archaic, the data and caricatures are important. It should be noted that they do not address the eye of Mollusca and their level of observed detail is limited to light microscopy. Their caricatures at the molecular level are marked hypothetical and their discussion of the orientation of the chromophore molecules in the surface of the disks can only be considered speculative. They were unaware of the importance of the liquid crystalline state of matter and discuss the chromophores in the single molecule form. Eakin provides some excellent scanning electron micrographs with detail at the 0.05 micron level or better. His figure 3 is an excellent example of a photoreceptor Outer Segment. It along with figure 1 and 2 show no sign of an outer plasma membrane surrounding the disk stack. Figure 4 shows excellent detail of the fissures in a disk as well as the location of several microtubules (dendrites) at the entrance to the fissures. Excellent imagery of the cilium (dendrite grouping) as it passes through the colax is also provided. Eakin has provided some excellent submicron level imagery of a variety of structures within various primitive eyes. He suggests that certain cilia (loosely defined) of the protist, Euglena viridis, were the earliest photoreceptors organized toward signaling the nature of the surrounding environment. He opens with his long held doubt about the constituents of the photo-pigments (chromophores) of vision.

A related work by Carthy & Newell⁸ discusses the structural aspects of the eye of the locust and also provides actual measurements on the polarization sensitivity of that eye. It also includes very early remarks by M. Land where he equates the eye of the scallop, Pecten, with the typical eye of Mollusca. This work clearly demonstrates the unique and specialized nature of the eye of Pecten that is completely compatible with all of Land’s remarks but shows that it is far from typical of Mollusca.

To understand the operation of the photoreceptor cell, and other neurological cells, it is critical that they are examined under electron microscopy at a minimum of 120,000x. At this level, differences in the structure of individual lemmas and the arrangement between adjacent lemmas can be analyzed. One of the most unexpected outcomes of these analyses has been the recognition that something that is not normally studied in the laboratory is very important. The space between the axon of one neuron and the dendrite of the next is a critical circuit parameter. If this space is of the correct dimensions, an Activa is formed between the two neurons. This Activa is of the simplest type and is the foundation of the so-called “gap junction.” Whereas the external morphology of the

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cell is largely irrelevant to vision, the internal cytology of the cell is critical. The photoreceptor cell includes a variety of internal lemma. These lemmas provide electrical isolation as well as an asymmetrical electrical impedance. When these impedances are combined with an appropriate “space” that is actually filled with hydronium, another Activa is formed. It is this Activa that is the amplifier found within all neural cells. The Activa is an active 3-terminal biological semiconductor, technically similar to an active 3-terminal solid state semiconductor known as a transistor. The Activa is a fundamental component of all animal neurons, regardless of phyla or family.

The true test of many of the caricatures to be presented below, as well as those in the literature, is whether they properly represent the actual biological situation in agreement with the photo-images found in the literature. A caricature must be compatible with these images. If a caricature fails this test, its appropriateness is highly suspect.

A major problem in the electrophysiological laboratory has been the failure to completely appreciate the nature of the electrical pathways of the neurological system. The electronic circuits supporting the Activa are electrolytic instead of metallic in nature. Some are conductive (analog circuit paths) and some are electromagnetic (phasic paths) in their character.

A similar problem exists in the understanding of the operation of the photoreceptor. Much work, both morphological and physiological, has been done on the static properties of the photoreceptor cell. However, insufficient work has appeared describing its dynamic aspects, particularly the anagene (the disks and signaling). This problem has been exacerbated recently by the assignment of some of the metabolic materials found in the vicinity of neurons to a signaling role. These materials include GABA, glycine, glutamic acid (glutamate), etc. The assignment has been made based on a floating model that has not been shown to be compatible with the overall operation of the eye. Several additional materials have been defined based on this floating model. Some of them are only defined conceptually and others appear to be illusory.

4.1.1 A roadmap of the photoreceptor/IPM/RPE complex

The photoreceptor cell is more than a cell. It is one of the most, if not the most, complicated cellular complexes in the animal system. It incorporates all of the functions found in, or associated with, any neuro-secretory cell while simultaneously including the most complex electronic circuitry found in any neural cell. The overall complex includes both the photoreceptor cell and at least one retinal pigment epithelium, RPE, cell. A crucial feature of this cell complex is the appreciation of the fact that it includes both cellular and extracellular components. Both the initial sensing element of the complex, the outer segment, and the mechanisms that provide electrical power to the cell are extracellular. The outer segment can be likened to a finger nail in that it is a structure secreted by the photoreceptor cell.

It is difficult to address all aspects of the photoreceptor cell without a background in the material in later chapters. This because much of the operation of the cell is not discernable from microscopic imagery without an extensive knowledge of what one is looking for. Because of the physical dynamics of many processes and the electrical character of the signaling elements, the imagery cannot be used as an effective exploratory tool. An investigator must know what he is looking for. To allow introduction of the photoreceptor cell at this point, the terminology and glossary section will be more extensive than normal. Sections 4.1.2.6 & 7 are important because they develop the electrical foundation for the operation of the photoreceptor cell and the neural system.

Following these introductory sections, Section 4.2 will address the morphology of the cell available from light microscopy. This material provides an inadequate view of the cell. Section 4.3 initially addresses the gross cytology of the cell using electron microscopy at 10,000x to 50,000x. This level of magnification begins to explain the structure of the cell. However, the section proceeds to the detailed level, based on magnifications of 100,000x or higher, required to actually understand the operation of the photoreceptor cell. Section 4.6 will introduce and discuss how the cell continually generates and regenerates the various elements associated with the outer segment of the cell. Section 4.7 will then introduce and briefly discuss the operation of the cell in signaling. The details of signaling will be found in Chapter 12. The cell plays two roles in signaling. It creates the initial electrical signal that is the key to the operation of the visual system and it processes this signal before passing it to other neurons of the neural system. The chapter concludes with two short sections addressing the differences among the photoreceptors of a given species or retina (Section 4.7.3) and the significance of the surrounding environment in the operation and experimental observation of the cell (Section 4.7.4).

4.1.1.1 The role of the RPE

The photosensing mechanism is completely nonfunctional in the absence of the RPE. The main function of the RPE
is to manufacture and re-manufacture, following phagocytosis of the Outer Segments, the chromophores of vision. As part of this process, it stores chromophores in pigment granules until they are required. The recycling of the chromophores involves an operating lifetime of at least a week. There is no recycling on the scale of seconds to minutes as envisioned, or required, by the stereoisomeric theories of photosensing.

The RPE accepts retinoid material from the choroid vascular system in the process of manufacturing the Rhodonine chromophores. It does not fabricate opsin. On the other hand, the photoreceptor cells accept amino acids from the retinal blood supply and create opsin. The photoreceptor cells do not accept retinoid materials from the bloodstream for the purpose of manufacturing Rhodonine.

4.1.1.2 The role of Mueller and other glial cells

The role of the various glial cells interspersed between the photoreceptors of the retina is still poorly understood. Many of these cells appear to straddle the Outer Limiting Membrane and may contribute to the isolation of the IPM from the INM. There are suggestions in the literature that these cells may play a role in secreting the IRBP type of retinoid binding proteins required to be present in the IPM. In this role, their location would be ideal.

4.1.2 Terminology

In describing the elements of the visual system at the histological, cytological and molecular level, it is important to have clear agreement on certain terms. These terms are defined in this section, Section 4.1.3 and the Glossary.

4.1.2.1 Relevant biochemistry of lipids and proteins

4.1.2.1.1 The lipids

The lipid family is unusual in chemistry. Members are not defined by their structure or their content. They are primarily defined by their solubility. Lipids are water-insoluble organic substances found in cells which are extractable by non polar solvents⁹. They are generally saturated but may contain double or triple bonds (which aid in semantic labeling). As a result of this definition, individual lipids can also be described by a variety of other designations. A variety of lipids play important roles in the operation of the photoreceptor-IPM-RPE complex. The lipids can generally be described graphically in a two-dimensional hierarchal format. Figure 4.1.2-1 illustrates the primary family relationships among the lipids of vision based on their prosthetic group. Those members on the left of the hatched line are also described in terms of their backbone structure. The members on the right of the hatched line can also be described by their structural organization but this involves a more complex breakdown than justified here.

Terpenes are a minor class of lipids. They are generally single chain hydrocarbons based on the isoprene structure and with a hydroxyl group as one terminal. Because of the isoprene structure, they are unsaturated by definition.

The fat soluble vitamins are a minor class in this family but a significant fraction of all vitamins. One group of this class is the A vitamin group. This group contains a variety of hormonally active chemical species including the common alcohol, aldehyde and acid derived from carotene and known collectively as the retinenes. Only the retinene containing the hydroxyl group as the prosthetic, the common Vitamin A, is diagramed in this figure. This vitamin is defined by its structure as shown at the lower center. Other members of the A vitamin group are based on different structures and prostheses.

Fat soluble vitamins are characterized by their insolubility in water. There are a large number of hormones in the A Vitamin family. One of the simplest and most common is known simply as Vitamin A. It is an alcohol with a hydroxyl group as one terminal. Common Vitamin A is also a terpene.

Fatty acids are a major class of this family. They are based on a carbonyl prosthesis. A subclass of this class is the fatty acids based on a carbonyl-ion system (where the two oxygen atoms are not associated with a common

Figure 4.1.2-1 Major classes and subclasses within the lipids and their relationships to vision. The diagram to the left of the hatched line is a two-dimensional matrix. The diagram to the right is a one-dimensional matrix. The glycerides have a different backbone structure than the other lipids shown. The potential chromophores of vision need not contain a β-ionone ring, but they all do.

carbon). This subclass contains the chromophores of vision, the Rhodonines. The Rhodonines are easily formed from the common Vitamin A by additional oxidation. The material then becomes a member of the carbonyl-ion system subclass. As in the case of the A Vitamins, many suitable chromophores can be formed in this way but only those that are fully conjugated (enclosed in the left dashed box) will exhibit the appropriate spectral response when in liquid crystalline state. Only those chromophores containing a β-ionone ring are used in biological vision.

Fatty acids are a very large class of lipids. They are generally single chain hydrocarbons with a carboxyl group as one terminal.

Carboxyl-ion systems can be considered a subclass of the fatty acids. In these systems, the two oxygen atoms are separated by a conjugated chain of carbon atoms. The Rhodonines, the chromophores of vision, are members of this subclass.

The other large class of lipids is the glycerides. A gigantic subclass is the phosphoglycerides as shown. This group contains most of the materials used to build plasma (and other biological) membranes. Only the two most prevalent members of this subclass are shown. These two members usually constitute a majority of the phosphoglycerides present in any tissue or cellular extract.

Acylglycerols are fatty acid esters of glycerol. They are neutral fats and the primary form of fat storage in plant and animal cells.
Triacylglycerols (formerly triglycerides) make up the great bulk of the neutral fats. By varying the individual fatty acids, and their molecular position in individual triacylglycerols, a very large family of compounds can be formed.

Phosphoglycerides (glycerol phosphatides) are formed when one of the primary hydroxyl groups of glycerol is esterified to phosphoric acid instead of a fatty acid. As in the case of the triacylglycerols, a very large family is formed. This family exhibits an asymmetrical carbon atom resulting in every member exhibiting two stereo specific forms. All phosphoglycerides possess a polar head and two non polar hydrocarbon tails and are called amphipathic or polar lipids. These materials are found almost entirely in cell membranes.

The most important phosphoglycerides in biological bilayer membranes are phosphatidyl choline (PC) and phosphatidyl ethanolamine (PE). Mention has been made in the literature of converting PE to PC by the addition of a methyl group. The process is considerably more complex. It involves the addition of three methyl groups according to Lehninger (pg 196). In both cases, the exposed terminal of the head group is polarized positive with a negative charge located between that terminal and the two glyceride chains.

4.1.2.1.2 The proteins

Proteins are the most abundant organic molecules in cells, typically constituting 50 per cent of their dry weight. They consist of a series of amino acids connected by amide linkages called peptide bonds. They are formed by the elimination of water from the carboxyl group of one amino acid and the a-amino group of the next. Many of them are easily hydrolyzed in acid back to their constituent amino acid building blocks. The proteins can be categorized in terms of their structure and their function. Recently, they have been subdivided into a family based only on their ability to react with guanine, the G-proteins. These will be discussed in the next section.

The proteins are divided into two major classes according to their structure, the simple and conjugated proteins.

Simple proteins, upon hydrolysis, yield nothing but amino acids.

Conjugated proteins, upon hydrolysis, yield a variety of amino acids and at least one other organic or inorganic group. This material is called the prosthetic group. The conjugated proteins are labeled according to the nature of the prosthetic group. The lipoproteins are one such large group.

Three major classes of proteins based on their function are uniquely important in vision. Opsin is a member of the structural protein class. It has a molecular weight of about 40,000. The second important class contains the transport proteins. There are nearly a dozen transport proteins involved in the creation and distribution of the chromophores of vision. They have molecular weights above 60,000. The third functional class of proteins provides the energy required to power the electrical operation of the neural system. These are of relatively low molecular weight, from 89 to less than 5,000, and are associated with the glutamate cycle.

4.1.2.1.3 The G-proteins

Recently, a large family of proteins have been segregated based on their ability to bind with the guanine nucleotide. These so-called guanine nucleotide-binding proteins (G proteins) are not characterized by their chemical formula, their chemical structure, or function10. Thus, the family is very heterogenous and care must be exercised when rationalizing their properties. Gomez & Benovic have provided references to several important papers in this area11. A large subfamily of the G proteins is the “seven-transmembrane-segment (7 TMS) proteins, or alternately seven-transmembrane-segment receptors12. The stereographic form of these proteins includes seven regions that are arranged roughly side-by-side with their long axes parallel. The common caricature of these proteins shows them embedded periodically in a plasma membrane. However, it will be shown below that in the case of the disks, they constitute the bulk of the disk surface and form a liquid crystalline bilayer. This bilayer may appear like a plasma membrane but in fact contains no phosphoglycerides. It is merely a bilayer structure formed with its hydrophilic surfaces exposed to the surrounding water-based environment.

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It is an early day in characterizing all of the known 7-TMS proteins. Currently, they are being described as constituted of three subunits, α, β, & γ where the names relate to the molecular weight of the subunits in descending order. It appears the α-subunit normally contains the amide terminal of the protein. However, this α-subunit has not been shown to be functionally significant. The β and γ subunits are less well characterized. The size of the α-subunit may be constant among a specific class of G-proteins or 7-TMS proteins but size is not constant among the various classes of G proteins within the 7-TMS sub-family.

The G proteins are frequently described as transducers of chemically and physically coded information. This is not the same meaning that is associated with the transduction of light in this work. Gilman specifically caveats his definition of the G proteins, in order to remain alert for such proteins that do not operate within the rules he defined for these materials in 1987.

Hargrave & McDowell surfaced some additional aspects of the visual proteins in 1992\textsuperscript{13}. They defined the pigment in the rods of vision as rhodopsin but the pigment in the cones of vision as opsin. They also differentiated between the two types of membranes in the Outer Segment. They said that 94-95% are “disk membranes” with the remainder being plasma membranes. They suggested that the difficulty of physically separating these two types of membranes experimentally has greatly impeded efforts to understand them. Quoting Boesze-Battaglia & Albert, et. al\textsuperscript{14}, they also suggested that “rhodopsin may only be present as a supply needed for the formation of disk membranes.” Hargrave & McDowell use a variety of percentages when discussing the rhodopsin content of the Outer Segments and the disks. It appears that more precise terms are needed to organize these percentages more specifically.

If one accepts the position of this work, that the disks of the rods are formed without the presence of retinal as a ligand, the 7-TMS proteins of the “cones” and “rods” of vision are both opsin (not rhodopsin in the case of the “rods”).

A review of the protein chemistry literature of the 7-TMS proteins uncovered two important facts.

+ Except for one group, no authors have ever confirmed the presence of retinol within the material they designated as rhodopsin prior to their experiments. As discussed in Section 4.6.2.3, that group found that retinol was not incorporated into the putative rhodopsin prior to its secretion by the photoreceptor cell.

+ In all of the sequencing work of recent years, no group could be found in the literature who confirmed the presence of retinol in any of the residues of its work or showed where the retinol ligand was attached to the protein.

All of the work and literature relied upon the location of a putative Schiff-base proposed by Bownds in 1967 which in turn relied upon the proposed existence of a Schiff-base joining the protein and retinol put forth by Collins in 1953. Neither of these proposals, upon which so much of the literature is based, have been confirmed in the laboratory. See Section 5.5.2.1.

Much of the research associated with the 7-TMS proteins, including the putative rhodopsin (claimed to be just opsin in this work), has been in-vitro and involved the hydrolysis and/or phosphorylation of the material. It is proposed that while much of this work is excellent, it is extraneous to the role of opsin in vision. Opsin is a benign substrate, external to the plasma membrane of the photoreceptor cell, in the protected chemical environment of the IPM.

4.1.2.1.4 The specific lipids and proteins of photodetection

The specific lipid involved in photosensing in vision is Rhodonine, a derivative of retinol, when in the liquid crystalline state of matter and condensed on a planar substrate. The molecular weight of the family varies between 285 and 299. This family of chemicals is so sensitive to chemical attack that it is frequently destroyed during typical biochemical extraction techniques. It is also so low in molecular weight that it is frequently overlooked in chromatography experiments to determine the molecular weights of the components of the disks of the outer segment.

The precise nature of the substrate on which Rhodonine is condensed is less clear and less important. The substrate is passive with regard to the photosensing function. Attempts to define the precise nature of the substrate have provided conflicting results. When the protein formed within the inner segment is analyzed, the findings tend to be

different from what is found when the material from the outer segment is analyzed. It is necessary to critically review the procedures used in such experiments.

In 1974, Bibb & Young provided a broad discussion of the characteristics of the substrates of the outer segments (colored by a variety of suppositions concerning the overall processes of vision that are not supported here)\textsuperscript{15}. The last paragraph of their introduction is noteworthy. They also concluded that 60% of the dry weight of the substrates was protein and about 40% were phospholipids. They state that “The protein content is unusually simple . . . where as much as 80% of protein appears to be opsin.” 80-85% of the phospholipids present were phosphatidyl choline and phosphatidyl ethanolamine. These two materials are normally associated with cell membranes. They therefore continue to consider this group of protein and phospholipid materials to be associated with a single cell membrane. However, it is not clear that they prevented the phospholipid membrane material from other sources to mingle with the protein of the substrates. They also provided additional definition of the character of the phospholipids. Their conclusion was unusual in that they say rhodopsin apparently floats in the lipid material that has a viscosity of a light oil, and the rhodopsin molecules are free to rotate while maintaining their long axis in the plane of the “membrane.”

Additional information was provided in a continuing paper\textsuperscript{16}. Figure 1 in both papers shows the RPE cells extending much further along the outer segment than in most similar figures. Birge put the percentage of opsin in the substrate at ~95% in 1981 and provided a large bibliography\textsuperscript{17}.

Fliesler, et. al\textsuperscript{18}. following Hargrave arrived at a different structure for the material they collected in 1985\textsuperscript{19}. They concluded that the opsin of vision (or rhodopsin depending on the author) is a conjugate protein with a prosthetic group consisting of two unusually short asparagine-linked oligosaccharide chains linked to the simple opsin by covalent coupling. This form may only be present as an intermediary, or apoprotein, prior to the secretion of the complete protein. Their statement that rhodopsin is a cell specific glycoprotein does not appear consistent with the above definition or their description of the underlying protein on page 575.

A team led by Nathans has presented a considerably different structure of the outer segments based on genetic principles and based on a variety of assumptions from the common wisdom of the time\textsuperscript{20}. Their position is that retinol is conjugated with the protein at an intermediate point along the length of the protein to form the chromophore but no other prosthetic terminal groups are present. It will be shown that neither this putative conjugate protein nor retinol alone is a chromophore of vision. Nathans, et. al. as well as Stryer\textsuperscript{21} have developed a caricature of the disks as consisting of a lipid (biological) membrane with the protein rhodopsin intertwined with this membrane in a very complex pattern. The intertwining maintains the putative retinol in a plane parallel to the surface of the membrane. There is no suggestion that any part of this structure has the fluidity of light machine oil. Whereas most authors have assumed the spectral differences of the putative rhodopsin have been due to singular, or at the most small, differences between the rhodopsins, Nathans proposes hundreds of differences in various amino acids within the opsin account for these differences. No experimental justification for this position was presented for this proposition as of 1987.

As the field of G protein research has advanced, the substrate found to form the disks of vision have been sequenced. They are members of the 7-TMS sub-family of G proteins. They are closely packed in a planar sheet that has been folded to protect (isolate) the hydrophobic termini of the proteins. The resulting bilayer appears similar to a plasma membrane but it contains no phosphoglycerides. The substrate is passive with respect to the signaling function in vision. It is not involved in the transduction of photons into an electrical signal.

Lacking direct correlation with the mechanism of photosensing, the discussions of phenotyping in the literature have not been correlated, beyond low level statistical inference, with actual vision defects.

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4.1.2.2 Membranes, films and coatings

Of particular importance at both the histological and cytological level are the terms, membrane, film and coating. In this work, the following meanings will be assigned to these words (see Glossary for more detailed definitions):

+ A membrane is a complex structure traceable to the tissue of a living cell or organism and known to have characteristic porosity to metabolic materials. More specifically, as in cytology, it is a three-layer structure consisting of two leaflets and the space between them. It is frequently labeled a bi-layer membrane, BLM, or less frequently a three-layer membrane after counting the space between the two layers.

+ A film is a complex structure of organic or inorganic molecules not necessarily traceable to living tissue. It exhibits tensile and compressive strength appropriate to its molecular structure. It is normally homogeneous at the molecular level.

+ A coating is a heterogeneous material found on the surface of another structure which is not traceable to living tissue and does not exhibit significant tensile strength.

These definitions differ somewhat from the vernacular. They must be more precise to avoid confusion.

4.1.2.2.1 Membranes as exterior cell walls

All neural cells employ an external cell wall (not to be confused with the cellulose based outer “cell wall” of plants) that can be described as a typical biological membrane. Such a typical membrane consists of two films of molecules separated by an extremely small space. It is frequently described as a three-layer structure. The two physical films are known as leaflets. Each layer consists of a liquid crystalline film of a phosphoglyceride. The hydrophobic ends of these molecules form one surface of each leaflet. The hydrophobic surfaces of the two leaflets are adjacent to each other. The resulting membrane is hydrophilic but it is impervious to water. Its porosity to other materials may be highly specialized, depending on the specific phosphoglycerides involved. Although the two films appear identical under the electron microscope, there is usually a critical difference between the phosphatidyl groups. The difference may involve only one atom in each molecule.

An asymmetrical biological membrane separating two different solutions may cause the solutions to exhibit a difference in potential. This potential difference is the foundation for the electrical operation of the neuron. By proper selection of the phosphoglycerides and solutes, different potentials can be obtained. By proper adjustment of the area of the membrane formed from a specific phosphoglyceride(s), the power handling capabilities of this source can be determined. If external test equipment is attached to the two solutions, the impedance of the configuration will be seen to be highly asymmetrical and hence nonlinear. In fact, the impedance is that of a perfect diode at low currents. The electrochemistry of such configurations is well understood and is reviewed in Appendix B. However, little emphasis has been placed on the dynamic characteristics of these configurations. In addition, it has seldom been recognized that there are additional charge carrying mechanisms besides electrons and ions. These include the holes of conventional semiconductor physics.

The semiconductor properties of BLM configurations are poorly understood. Figure 4.1.2-2 illustrates the fundamental situation. (a) shows the conventional electrochemical description of a single BLM immersed between two different solutions. (b) shows the same situation but with the BLM described at the molecular level.

(a) shows that no ions actually pass through the membrane. Instead, all reduction and oxidation occur at the respective surfaces with only charge actually passing through the BLM (indicated by the horizontal lines). This charge is composed of two components, electrons and “+” charges or holes. The net charge transfer is the algebraic sum of these two components. The ratio between these two components is an important characteristic of a semiconductor. In BLM’s of interest to neuroscience, the hole current dominates in one part of the membrane cross section and that material is described as “p” (positive) type material. The dominant current in another part of the cross section is dominated by electrons. It is described as “n” type material. The overall material is described as a “pn” junction and exhibits a perfect diode impedance characteristic under low charge flow conditions.

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(b) shows how this “pn” type material operates at the molecular level. The BLM consists of two phosphoglycerides arranged in two liquid crystalline films and a region of hydronium liquid crystal material associated with the hydrophilic (outer) surfaces of each film. Oxidation or reduction occurs at the respective exterior surfaces. The charge generated within the BLM is then transported within the BLM in the valence band of the liquid crystals by holes. These are empty electron sites in the crystalline lattice that are continually filled by electrons jumping from a filled site to the empty site. It is tempting to think of the holes moving along the glyceride chain of each molecule. However, this is a poor analogy. Each molecule occupies a lattice site in the liquid crystal. The charge moves from site to site, not atom to atom.

The electrical properties of the asymmetrical bilayer membrane are becoming clearer. Xxx, writing in Yeagle23, has discussed the interface between the hydrophobic components of the two molecular films in detail. He defines two major physical states. In the first, the two phospholipid films exhibit a clear bilayer midplane. In the second, the fatty acid chains of the phospholipids are of unequal length and interdigitation occurs between the two films. Three levels of interdigitation are defined. These levels of interdigitation probably account for the diode properties of the resultant membrane (See Section 8.3). Further discussion of the chemical properties of these interdigitated membranes is found in Slater & Huang24.

Figure 4.1.2-2. Bilayer lipid membrane configurations. (a) Conventional electrochemical model of a BLM separating two solutions. (b) Molecular level model of same BLM showing liquid crystalline structures.

The electrical characteristic of this configuration is that of a perfect diode in series with a battery. The operating characteristics of the diode and of the battery are determined by the area of the active portion of the BLM. Although cell walls may appear uniform under the electron microscope, detailed study would indicate they have a very spotted appearance at the functional level--different regions providing different capabilities.

The BLM making up an exterior cell wall is oriented in such a way that the interior of the cell can sustain a negative potential, i.e., the diode associated with the BLM is reverse biased. This condition is obtained by having the “n” type material of the junction in contact with the fluid surrounding the cell.

4.1.2.2.2 Juxtaposition of two membranes

[xxx text at end of this section 4.1.2.2.2 also appears in section 8.4.2.3 ]

[xxx edit one version with reduction/oxidation. Other with injected charge changing input side potential. ]

The juxtaposition of two biological membranes and the introduction of appropriate external electrical potentials, in addition to those generated by the individual membranes, can result in additional unique characteristics. The overall configuration becomes an active electrical device exhibiting what is called “transistor action.” Such a configuration is called an Activa. It is the key to the operation of the nervous system in animals. The electrochemical structure of the configuration, as used within a neuron, is shown in Figure 4.1.2-3.

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To obtain transistor action, both the spacing between the two BLM’s and the potentials applied to the two solutions, with respect to the potential of the hydronium liquid crystal, are critical. These are the net potentials combining the potential of external sources with the intrinsic potential created by the BLM itself in contact with the solutions on each side. The spacing between the two adjacent walls of the BLM must be less than 100 Angstrom. The net voltage across the BLM associated with the signal input must forward bias the diode characteristic of the BLM. The net voltage applied to the solution associated with the other BLM must be such as to reverse bias the diode characteristic of that BLM. Under these conditions, an electron injected into the hydronium liquid crystal in the junction area from the input BLM (along line #1) will diffuse across the liquid crystal and appear at the external side of the output BLM (at location #3) by transistor action. The insertion of an electron at a low impedance point, the input BLM, and its appearance at a high impedance point, the output BLM, constitutes signal amplification in the power domain. By appropriate circuit rearrangement, this amplification can be tailored to appear as a voltage gain or a current gain.

If the above polarity conditions are met, the active device created can be described in terms of the placement and type of semiconducting materials involved. For a simple active semiconductor, there are two possibilities, a “npn” or a “pnp” arrangement. Based on the voltages found associated with neurons in animals, all known active devices, Activas, found in biology are of the “pnp” type.

Figure 4.1.2-4 provides a similar presentation at the molecular level. The two solutes are labeled the dendroplasm and the axoplasm. The numbers 1 through 7 are those assigned by a cytologist to a seven-layer junction between two bilayer membrane walls. Note they usually see layers 1, 3, 5 & 7 as dark lines and assign 2, 4 & 6 to the light spaces between these lines. It is seen from this figure that the characters of these spaces are different. Whereas 2 & 6 appear empty, 4 has a distinct character. In fact, the material represented by 4 is critical to the operation of the neurons. A similar material that is performing a different function is found between layers 1 & 7 and their respective solutions. It would be advisable to number these regions 0 & 8 when speaking of the functional performance of such a sandwich.
Figure 4.1.2-4 The structure of the Activa at the atomic level. In operation, the configuration consists of two bilayer membranes (BLM) in close proximity and appropriate voltages applied between the dendroplasm, the axoplasm and the material in the junction area between the two bilayers (the podaplasm). The lattices in the junction area and on the extreme left and right surfaces are hydronium. Detailed atomic structure of an individual membrane from Pearson & Pasher, 1979.

Note the complex molecular structure at the interface between the plasma and the leaflet. This area is described in terms of hydronium ions. The structure in the junction area is also described in terms of hydronium. In this case, the material constitutes a hydronium crystal. There is no physical movement of ions within this overall structure. This is true even under the influence of external voltages.

Additional material related to the electrical topology of this seven-layer junction will be found in Chapter 8.

4.1.2.2.3 Electrical power derived from a coating on a membrane

If materials are deposited on the surface of a membrane of a cell, it is possible for the materials of the coating to participate in an energy exchange mechanism that does not require any ionic material to move through the cell wall. This mechanism can create an electrical bias between the electrolytes on each side of the membrane. In the neural system, the mechanism primarily involves the glutamate cycle of respiration and it is sometimes referred to as a GABAergic process. This mechanism is addressed again in Section 4.1.2.3.1 and explored more fully in Section 7.7.

4.1.2.2.4 Structural properties of liquid crystalline films/membranes

There is little information in the vision literature related to the complex structure of biological films and membranes. This is unfortunate in the light of their importance to an understanding of the vision process. The reason may be partly due to the very great complexity of the subject. Most biological materials of interest are polymorphic when deposited as liquid crystals. The deposited structure depends on at least six variables. Wolken provided a discussion of liquid crystals that is entirely too brief. His figure 5.3 seems to mix apples and oranges in a single figure, appearing to limit the terms nematic and smectic to non biological liquid crystals. Figure 4.1.2-5 provides an alternate, but still abbreviated, state diagram for biological liquid crystals. This diagram defines the impact of at least six parameters on the resulting structure. The first factor is whether the material is a single compound or a mixture (generally a solution). Thermally generated changes of state, usually involving only a single compound, are labeled thermotropic. In the more general case of deposition from a solution, the changes are described as lyotropic. The predominant factor in the formation of a liquid crystal is the electrical configuration of the material itself at the molecular level. It may be described as polar, non polar or amphiphilic. Since the material may have polar

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characteristics, the electrical characteristics of the substrate upon which it is deposited become important factors. Especially if only a monolayer of material is being formed. If this substrate is non-polar, it plays a more passive role than a polar substrate. The liquid crystal may be deposited with the major axis of the molecules parallel to the surface of a non-polar substrate. This is not the normal case with polar substrates. The molecules of a polar material will be deposited with their molecular axis such that the ligand of the molecule most compatible with the substrate will be nearest the substrate. This results in a liquid crystalline structure described as smectic. In this case, all of the like terminal ligands of the molecules are adjacent to each other on one surface of the film. Whether the major axis of the molecules is perpendicular to the substrate surface depends on the internal structure of the molecules. If the major axes are perpendicular, the material is described as Type A. If they are not perpendicular, the material is described as Type C.

**Figure 4.1.2-5** A biological liquid crystal state diagram. The paths to Rhodonine and to opsin are shown for reference and developed elsewhere in the text. By collecting the adjectives along the line leading to each material and placing them in front of the name of the material, a quite complete physical description of each material, as it is used in vision, is obtained.
An additional complication arises when the deposited material is multilayer. This can occur during initial deposition or by folding of a monolayer onto itself. For most amphiphilic materials, this results in the layers of the liquid crystal being arranged alternately so that hydrophobic surfaces face each other and hydrophilic surfaces face each other. However, the adjacent layers may exhibit lattice vectors that are not aligned layer-to-layer. If they are aligned, no special name is applied but the label rectangular is used in the figure. If they are not aligned, the material is described as either nematic-cholesteric or smectic-cholesteric. It is common in the non biological literature to shorten the nematic-cholesteric description to just cholesteric. However, this is not appropriate in the biological field. Many biological liquid crystals are smectic-cholesteric. Following the folding of an amphiphilic liquid crystalline film, the nature of the environment plays an important role. A folded film has regions where the exposed surface may be abhorrent of the liquid environment. In that case, the abhorrent surface will tend to withdraw. As a result, the more acceptable ligand will be pulled into a position to protect the abhorrent ligand. The result will be a micelle with a continuous outer surface compatible with the surrounding environment.

The figure shows that opsin is formed lyotropically into a micelle that is smectic-cholesteric and probably of Type A. The wall of the micelle is a monolayer film that is amphiphilic. It also shows that the chromophores of vision are deposited onto the above micelles lyotropically as a polar monolayer that is smectic, Type C. By depositing Rhodonine(7) on a substrate consisting of a micelle of opsin, the conceptual material rhodopsin is formed. The isotropic absorption spectrum of this material exhibits a peak at ~500 nm. The anisotropic absorption spectrum of this material when in the in-vivo state peaks at 532 nm and it has previously been labeled iodopsin in the literature. These descriptions will be justified in the following paragraphs and chapters.

### 4.1.2.2.4 Deposited films/membranes

The deposition of proteins and chromophores from a solution play an important role in vision. In the general case, the protein or chromophore has been secreted through a cell wall into an enclosed space containing a unique solution. This solution and the nature of the walls forming the space determine the nature of the product precipitated from the solution. In the case of proteins, which are generally amphiphilic, a polar substrate with its hydrophilic surface exposed will cause the proteins to aggregate into a smectic liquid crystalline film on the surface of the substrate (especially if the solvent is nonpolar). This is particularly true if the protein film is made to move along the surface of the substrate as in the extrusion cup of the Inner Segment. If this film is caused to fold due to the confining shape of the substrate, the result will be a sandwich of two amphiphilic films with their hydrophobic surfaces in close contact. Most of the periphery of the new two-layer membrane will be exposed to the solvent. If the solvent should become predominantly water based, the exposed hydrophobic surfaces along the periphery of the membrane will withdraw from the polar solvent and the hydrophilic surfaces will close on each other. The result is a thin membrane with a rounded fold along all of its periphery and only a hydrophilic surface exposed to the solvent. A micelle will be defined narrowly in this work as the above form of membrane.

The detailed crystalline form of the protein within the micelle will be determined by the specific properties of the protein. If the protein is initially deposited with its major axis perpendicular to the substrate, the resulting smectic structure is known as Type A. Otherwise, it is known as a Type C.

The relationship between the two films forming the micelle is also important. The surface of a protein film will frequently exhibit lattice axes. If the axes approaching the fold line is folded back parallel to itself, the resulting membrane will remain described as a smectic crystalline structure. If however, the axis is not folded back parallel to itself, the two films will exhibit lattices that are not aligned. The resulting structure is designated smectic-cholesteric.

If a micelle of the above form is now exposed to a supersaturated solution of polar chromophores, the chromophores will precipitate onto the surface of the micelle. The chromophores will form a smectic liquid crystalline surface that can cover the entire surface of the micelle.

### 4.1.2.3 Cells, micelles, & protocells

A biological cell invariably consists of a bilayer external membrane enclosing an electrolytic environment of immense cytological complexity. The fundamental bilayer consists of a lipid material. In more advanced cells, the character of the lipid may change with location on the cell surface. A fundamental cell for purposes of investigation
and further ramification has been defined by Wolken\textsuperscript{26} under the name protocell. A protocell is formed of a bilayer of uniform smectic lipid material enclosing a nematic electrolyte free of inclusions. This conceptual protocell can be extended, stepwise, to more fully define a conceptual cell equivalent to a real biological cell.

The term micelle first arose in organic chemistry to define a form taken by an amphiphilic material when introduced into a polar solvent, usually water. The material may be of many different forms, including protein. The material typically attempts to isolate its hydrophobic surface from the solvent by forming a fundamentally spherical surface with the hydrophilic terminals of the individual molecules all aligned to each other and in contact with the solvent. The result is a smectic liquid crystalline film enclosing an empty space. Alternately, it can enclose a hydrophobic material and this material may assume a liquid crystalline form. A flattened form of a micelle may result in the formation of a disk wherein the two hydrophobic surfaces of the amphiphilic layers are in contact with each other. However, the result remains a shape with an external wall that is a monolayer. If this monolayer encloses a second hydrophobic material that is nematic, a gross configuration very similar to the protocell is obtained. However, it is still a micelle with a monolayer for an exterior wall.

Based on the fundamental difference between a biological cell and a micelle, it is important to examine the nature of the surfaces of these elements. Figure 4.1.2-6 presents some alternative structures frequently seen in the literature and related to the pharmacological aspects of vision. [\textit{This figure will be deleted in the next editing review.} xxx]

They also frequently appear in discussions of the synaptic junctions of neurons. They clearly apply to the cell with an external plasma membrane consisting of a bilayer of lipid material. It has not been shown that they apply to a micelle. It has not even been shown that these situations apply to an asymmetrical bilayer or that they apply to all areas of a given bilayer. There are major questions related to the electrical integrity of a bilayer that is penetrated by any material, such as represented in (d) of this figure. When discussing micelles, it is important to remember that the exterior wall is normally not a bilayer. If it is sufficiently flattened, small local areas may resemble a bilayer. However, the un-flattened ends define its true character. Adopting one of the forms shown in the above figure in discussions of a visual disk requires considerable substantiation. First; the micelle wall is not a bilayer. Second; the film, although formed by an amphiphilic material, is not formed of a lipid. Third; it is not a living cell and does not require penetration by even simple biological materials for its operation. Fourth; there is no known functional or metabolic reason for such a micelle to include protein material within it.

Much of the biochemical analysis of the Outer Segments has relied on techniques developed in the study of the surface structure and constituency of cells. This includes the solubilization and reconstitution of these surface features\textsuperscript{27}. The subject of receptorology has developed within this field. This subject refers to biological receptors on the surface of cells and does not relate directly to the field of vision. It will be shown in this work that it does not apply to signal transmission by the visual synapse either. Unfortunately, most of the solubilization and reconstitution processes in this field are not compatible with the liquid crystalline chromophores of vision. The detergents used in that field do not assure the reconstitution of a visual disk as found in the Outer Segment.

\textsuperscript{26}Wolken, J. (1986) Light and life processes. NY: Van Nostrand Reinhold pp. 54-55
The reconstitution of even a quasi-functional disk of the Outer Segment requires the deposition of a smectic liquid crystal of chromophore on a suitable smectic surface. Even if an appropriate detergent were used, the unique state of the molecules and oxygen atoms of the chromophores requires an additional mechanism for de-excitation of the chromophoric material after exposure to light. Absent this mechanism, the material will bleach rapidly. Such a mechanism must be provided to confirm the photosensitivity of the reconstituted structure.

4.1.2.3.1 Establishing the potential across a biological membrane

There are at least two conceptual methods of establishing a potential across a membrane surrounding a cell, or within a cell.

The first is the one addressed most frequently in the literature. It involves the transport of ions through a putative semiporous membrane by a putative ion-pump. While plasma membranes are obviously able to transport some ions, the nature of the ion-pump has not yet been defined at the operational level.

The second method is better understood but has not previously been applied to the neural process. It involves an electrostenolytic process on the surface of a membrane surrounded by two electrolytes. In this process, an electrogenic reaction occurs on one surface of an asymmetrical bilayer membrane. This reaction causes an electron or hole to transit the membrane. The result is an electrical potential between the two electrolytes. This is the normal method of polarizing neurons. The process is very rapid and it does not require the passage of any ions through the membrane. The transit velocity of electrons and holes is normally orders of magnitude faster than the velocity of normal ions present in the system. This mechanism will be discussed in detail in Section 7.7.

4.1.2.3.2 Coated membranes as electrical elements

The fact that a living membrane has the characteristics of a rectifier is generally recognized in the literature. However, the fact that when coated, it can act as a source of electrical potential is less well known. This combination can exhibit very specific and relatively unique electrical properties. When separating two electrolytes, this combination will exhibit the properties of a perfect battery and a perfect diode in series. The word perfect is used because at low current flow through this circuit, there is essentially no thermal loss due to resistive heating. The operation of the circuit is completely reversible under the influence of an additional external voltage. The result is a non-dissipative, thermodynamically reversible process. Any energy applied to the circuit is stored in an electrochemical battery for later release.

4.1.2.4 Cilia, flagella, villi and rhabdomere

The terms flagellum, cilium and villus have not been precisely separated in the literature of the photoreceptor cell.
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Using the definitions in the Merriam Webster Medical Dictionary, the term villus is constrained to an element of the vascular system. Both cilium and flagellum apply to hair like projections. A flagellum is always associated with motion and a cilium is frequently associated with motion. However, a cilium is frequently associated with a sensory nerve ending.

In discussing the photoreceptor cell, the term cilium appears appropriate. The question is what does it apply to? In the general case, the cilium is a proteinaceous material, secreted by a cell, that transmits a structural strain to a sensory nerve ending. This is the precise function of the coated disks of the outer segment. However, the “strain” (a quantum-mechanical excitation) is transmitted from the chromophoric coating of the cilium to the dendrite of the neuron. These dendritic structures, frequently called microtubules in the histological context, then pass through the colax into the soma of the photoreceptor cell. In this analog, the cilium has evolved into the disk stack by breaking and extrusion from the calyx. The colax is not associated with the cilium. It is a passthrough supporting the neural portion of the photoreceptor cell. The structures passing though the colax are the dendrites (frequently labeled microtubules) of the photoreceptor cell.

Akin introduced the term rhabdomere in 1963 to distinguish topographically between photoreceptor cells of two types. He describes his delineation as speculative and does not follow any recognizable phylogenetic tree. However, the terminology is now used quite widely. He derived two classes of photoreceptor cells, based on light microscopy, and employing a ciliary foundation and a none ciliary or rhabdemic foundation. For the purposes of this work, the rhabdemic photoreceptors are found in Arthropoda and Mollusca. The others are found in Chordata. The term rhabdomere derives from the Greek for a bundle of cylindrical rods. It is generally applied to the type of cell and not a specific configuration of the secreted protein material.

4.1.2.5 Morphogenesis through phagocytosis

The processes of photosensing, transduction and translation in vision rely upon an unusually active process of physical generation and regeneration that may be unique in the neural system. There is an obvious initial stage of morphogenesis that creates the fundamental structure of the eye and generates the initial physical elements. In the presence of disease, there are also mechanisms for isolating and digesting foreign material, usually defined as phagocytosis. However, the photoreceptor/IPM/RPE complex involves another chain of events involving genesis and phagocytosis that are not widely appreciated. There is an additional process of continual disk generation, chromophore deposition and subsequent phagocytosis and material recovery operating continuously within this complex. Large numbers of new disks are generated every minute and all of the disks of the retina are replaced on a weekly basis in warm blooded chordates. This situation calls for additional nomenclature.

**Anagenesis**–The continual creation of new disks at the proximal end of the Outer Segment and their coating with a chromophore in anticipation of operation.

**Migration**–The continual movement of each disk of each operational Outer Segment toward its distal end at a rate of 320 nm/hr for warm-blooded chordates.

**Phagocytosis**–As used here, the normal engulfing and digesting of the disks of the Outer Segment. This step is followed by the reclaiming of most of the amino acids and retinoids contained in those disks. Synonymous with endocytosis.

**Sloughing**–A term encountered in the literature to describe the falling away of part of the Outer Segment prior to its phagocytosis by the RPE cells. The term implies the part falling away is somehow isolated from the rest of the photoreceptor cell and is no longer within the plasma membrane of the cell. This term concept is not supported in this work. The Outer Segment is taken to be an extracellular structure secreted by the exocrine glandular capability of the photoreceptor cell.

4.1.2.6 Electrical currents, terminals and devices

4.1.2.6.1 Current versus electron flow

Figure 4.1.2-7 provides some important electrical terminology that will be relied upon in the electrical description of

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the visual system. There are important distinctions to be made between the “conventional current” and the electron flow within a circuit. There are additional distinctions to be made between the currents inside and outside of electrical sources. Finally, it will become important to define two different types of currents within a semiconductor material.

In (a), the circuit is divided into the “internal circuit” of a source and the external circuit connected to that source. Due to an error by the earliest experimenters (Messrs. Volta and Ampere had a 50:50 chance of being correct), the conventional current, \( i \), in an external electrical circuit has been and is defined as flowing from the positive terminal to the negative terminal. Unfortunately, with the discovery of the electron and its negative charge, the actual particle flow, \( e \), within an external electrical circuit is now known to flow from the negative terminal to the positive terminal. Since electrical sources will be an important component in the following discussion, it is important to note that the current flow, \( i \), is from the negative terminal to the positive terminal inside the source. The electron flow is also reversed compared to the external circuit.

It is important to distinguish between what is a source and what is a load. It is not sufficient to measure a voltage across a circuit element and proclaim the element is a source. One must demonstrate that a current was leaving an element in the presence of a voltage to label it a source. As will be seen below and in Chapter 12, the source of the c-wave of electrophysiology is the dendrites of the outer segment located in the IPM. This source causes a voltage to be measured across both the outer limiting membrane and the RPE cell layer of the retina. Earlier studies over the last 50 years have inappropriately proposed that the c-wave was generated within the RPE layer.29

When discussing semiconductor materials, an additional distinction must be made as illustrated in (b). The current flowing through the material can take two different paths. In both cases, the physical phenomenon involves the motion of electrons. When the electrons move through the material in the conduction band of the material, the flow appears normal. The electron flow, \( e \), through the material is fast and it is in the same direction as the electron flow in the external circuit connected to the material. The alternate path involves electron flow through the valence band of the material. This current is typically much slower than electron flow in the conduction band. Normally, it is also much smaller in absolute number of electrons passing through a given cross section in a unit of time. However, in many useful semiconducting devices, this last condition is not true. The apparent slow motion of electrons through the valence band of a crystalline material is defined as “hole motion.” It can be modeled as the flow of “holes,” \( h \), moving in opposition to the conventional electron flow in the conduction band. The net electron flow within the semiconductor material is seen to equal the external electron flow. Similarly, the net current

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within the material is seen to equal the external current. Holes play an important role in the operation of neurons.

### 4.1.2.6.2 Terminals of relevant devices

A second set of terms relates to the internal and external terminals of the neuron and the Activa. These involve the three terminals associated with each Activa and each neuron.

The similarity of the Activa and the Transistor makes it appropriate to adopt similar names for their terminals to avoid confusion. From a quantum-physics viewpoint, they are functionally identical.

From a signal perspective, the fundamental neuron can be considered a three terminal black box with at least one Activa inside of it. The poda is an area on the surface of the external neuron membrane where current is observed to leave or enter the cell concurrently with other currents entering or leaving a dendrite or axon. It would be considered the “common” connection in most analogous man-made circuits. In each case, there may be electrical impedances located between the internal terminal of an Activa and the corresponding external terminal of the neuron. The conductive impedances are electrolytic in nature and do not involve metallic paths.

The fundamental neuron has three additional terminals besides those discussed above. These have to do with the power sources involved in the overall neuron. **Figure 4.1.2-8** provides a diagram defining all six of the fundamental terminals. Each of the three signal handling structures, the dendrite, the axon and the poda have a power source associated with it. These power sources are all connected to the interneural plasma. They derive power from redox reactions occurring on each side of the cell membrane. Note that the signal input terminals of the dendrite are shown with a hollow diode (a white center) to indicate it is reverse biased in the absence of an external, transistor based, connection. Therefore, no current passes through the dendroplasm, this “open” connection, to the interneural plasma in the absence of a separate mechanism.

In the case of the photoreceptor cell, the networks shown as #1 are replicated nine times and converted into individual Activas. When modified morphologically as part of the replication process, these individual structures are converted into the microtubules extending from the Inner Segment to the Outer Segment of the photoreceptor cell. As a result of this action, the photoreceptor cell is seen to consist of a number of individual signaling paths, containing their own Activa, converging on a second Activa within the same cell. This more complex circuit will be presented in **Section 4.7**. The detailed operation of this circuit will be discussed in detail in **Section 9.2.3** and **Chapter 12**.

Each Activa exhibits a unique electronic phenomena known as the “transistor effect.” This effect is only realized when two rectifying devices are connected back to back and share a common region of a very small but finite size. In addition, one of the devices **must** be electrically biased so as to conduct current while the other device is biased to oppose the flow of current. Under these conditions, a charge or current introduced at the emitter terminal (associated with the conducting device) will appear at the collector terminal even though the collector is biased to oppose current flow. The result of this process can be signal (although not necessarily current) amplification.
The terminals of the Activa and the fundamental neuron will be defined according to the following table in accordance with the above figure.

<table>
<thead>
<tr>
<th>Terminal of the Activa</th>
<th>Associated terminals of the neuron</th>
<th>Signal terminals</th>
<th>Bias terminals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emitter</td>
<td>Dendrite</td>
<td>(No formal name yet)</td>
<td></td>
</tr>
<tr>
<td>Base</td>
<td>Poda</td>
<td>&quot;</td>
<td></td>
</tr>
<tr>
<td>Collector</td>
<td>Axon</td>
<td>&quot;</td>
<td></td>
</tr>
</tbody>
</table>

Although it has generally been accepted in the electrophysiology of the photoreceptor cell that it is a three terminal device, the terminals have not been formally named. Hagins\textsuperscript{30} and many others have shown caricatures of photoreceptor cells with current emanating from the axon and from the Inner Segment while re-entering the cell through the Outer Segment. Rodieck\textsuperscript{31} and many others show a similar event occurring with respect to a junction between two cells. However, the actual situation is much more complex. The photoreceptor cell incorporates a complex electrical circuit consisting of multiple Activa. The electrical configuration of this cell will be discussed in detail in \textbf{Chapter 12}.

4.1.2.7 Locations of Activa within the neural system

Activas are found at three locations within the overall neural system of an animal. Some of these locations are easier to recognize than others.

\textsuperscript{30}Hagins, W. (1979) Excitation in vertebrate photoreceptors.  4th Study Program in Neurosciences pg. 183 BML WL 100 N494-8
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The gap junction between two neurons is actually an Activa. Shepherd\textsuperscript{32} describes the physical characteristics as “the outer leaflets are separated by a gap of 2-4 nm, to form a seven-layered complex.” He is discussing the three leaflets associated with each of two membranes and the gap between them. Unfortunately, he did not recognize the dynamic electrical properties of such a junction. He did note their variation in diameter, ranging from 0.1 to 10 microns. This is a factor of 100 in area. The actual area used by each class of Activa will be correlated with other performance parameters in Section 9.6.

Each Node of Ranvier is a visible representation of an Activa. Although this feature can be seen at the anatomical level, it requires the resolution of an electron microscope to distinguish the construction of the Activa. Ottoson has provided such an image\textsuperscript{33} and described the “seven-layer complex including a 2-4 nm gap.” Figure 4.1.2-9. Ottoson’s original caption for this figure is: “Ranvier’s Node in living tissue isolated by dissection. The ‘internal synapse’ between the two segments of the axon is clearly seen. It is also clear that the point of contact is extremely small and that this region has direct conductive contact with the medium surrounding the nerve at this ‘void’ in the myelin sheath.” One might argue with the claim that the tissue in the photograph is still living. It is also quite unlikely that the Node is still operational due to the disturbance of the medium surrounding the nerve as well as the plasmas in the two sections of the axon.

![Electronmicrograph of the Activa within a Node of Ranvier. From Ottoson (1953) via Waxman fig 10.6.1](image)

By connecting a series of membranes in a single electrical circuit, it is possible to transmit currents through the network without significant thermal dissipation. This is a key feature of the animal neurological system. In the overall circuit, the electrons created at one location by forward chemical reaction (battery action) are free to flow through a circuit to a second location where they are absorbed in a second reverse chemical reaction. The reactants in the second reaction are able to flow by diffusion to other locations where they can participate in another forward reaction. The result is an overall neurological system that is to a large extent closed and extremely efficient with regard to thermal energy dissipation. What chemical energy is dissipated in the process is removed from the system by expiration.

4.1.2.9 Diffusion versus electrostenolysis

Electrostenolysis Effect-- A process of chemical oxidation or reduction on the surface of a substrate that affects the local electrolytic environment. If the substrate is conductive to electronic charges, a potential may be created across the substrate.

There are many discussions in the visual literature concerning diffusion at a cell membrane and the ability of the diffusion of charged particles to create a potential across that membrane. There is considerably less discussion of a related process that plays a very important role in the neurological system. Electrostenolysis is the generation of free electron charges at a cell membrane, through a chemical reaction, that are able to diffuse through the cell and establish a potential between the two sides of the membrane. No ions are required to pass through the membrane in

\begin{footnotesize}
\textsuperscript{33}Ottoson, D. (1953) XXX as referenced in Waxman? Figure 10.6.1 [see wpb by fig num]
\end{footnotesize}
The Photoreceptor Cell 4- 23

this process. Both of these processes are important in the operation of the neurological system.

4.1.2.10 Definition of the OS/IS interface

In the past, it has been conventional to consider the OS and IS adjacent internal parts of a single photoreceptor cell. In this interpretation, a group of cilia was found to exit the cell membrane at one point near the OS/IS demarcation and either disappear or re-enter the cell membrane again. Where the group of cilia left the cell membrane has generally been called the colax but has been described by a variety of other names. These include the ciliary stalk, ciliary transport and calyx. In this work, the root calyx takes on a more significant meaning. Therefore, the structure surrounding the cilia at the point where they leave the cell membrane will be called the colax. The name calyx will be reserved for the larger collar or lip of the cup forming the distal end of the IS. This calyx acts as an extrusion die associated with the above extrusion cup. The die forms the furrows in which the dendrites from the ciliary transport, i.e., colax, are placed.

4.1.2.11 Dimensions at the atomic and molecular level

It is important to recognize there are no sharp corners at the molecular and atomic levels of material. It is very difficult to define the edges of various surfaces, which are generally quantum-electronic in nature, and to define the centroid of a given feature based on those edges. In this Chapter, all numeric values should be considered nominal or mean values subject to an error term. In general, two decimal digit accuracy is the best that can be specified in the absence of very specific definitions of surface or edge locations and supporting statistical calculations. Most authors quoted here have not provided either detailed definitions or adequate statistical data to support more accurate numerics.

4.1.3 Additional glossary entries

The previous section has defined a wide variety of terms. This section includes additional miscellaneous definitions.

Lipofuscin - A generic term for age-related lysosomal residual bodies of classical pathology and cell biology (Eldred, 1982). The material is generally identified via light microscopy. It is usually characterized by its fluorescence using a large area aperture that integrates light from many smaller sources. The typical spectrum is broad 540-640 nm but is historically quantified at 470 nm. In the case of RPE cells, the material is an aggregate of pigment granules and other materials.

There are a variety of terms in the literature used to describe the components of the photoreceptor cell. Most of them stem from light microscope-based morphology. The following table proposes alternate names for several of these components based on their function.

<table>
<thead>
<tr>
<th>OLD NAME</th>
<th>PROPOSED NAME</th>
<th>FUNCTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calyx</td>
<td>Calyx</td>
<td>Extrusion of the outer segment disks</td>
</tr>
<tr>
<td>Ciliary Stalk \ Cilium / Cilium Transport Colax (dendritic Channel)</td>
<td>Dendritic Bundle Dendritic Path from Is to OS Channel enclosing Den. Bundle</td>
<td></td>
</tr>
<tr>
<td>Microtubules Outer Segment</td>
<td>Dendrites (or item in dendrites)</td>
<td>Sensing State of Chromophores Photon Detection/Nerve Excitation</td>
</tr>
<tr>
<td>Midget Bipolar Cells Horizontal Cells Most amercine cells Ganglion Cells Stellate Cells Node of Ranvier</td>
<td>Analog summing neurons Analog differencing neurons Analog to Pulse encoding neurons Pulse to analog decoding neurons Signal repeater internal to an axon</td>
<td></td>
</tr>
<tr>
<td>Ribbon, axonal</td>
<td>Conduit, axonal</td>
<td>Electrical channel to gap junction</td>
</tr>
<tr>
<td>Gap</td>
<td></td>
<td>Base region of an Activa</td>
</tr>
</tbody>
</table>
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4.2 Typical morphology of a photoreceptor (using light microscopy)

Sections 2.2.4 & 2.3.3 addressed the morphogenesis of the eye and retina. This section will only address the mature photoreceptor cell-IPM-RPE complex. However, the morphogenesis of the individual disks of the outer segment is a major activity within this complex. The level of detail concerning this complex available through light microscopy is quite limited. The diameter of a typical photoreceptor cell approximates the limit of resolution of a typical light microscope. Although some additional resolution can be obtained via phase-microscopic techniques, the relative improvement is small. This section is included for continuity only. It can be ignored in favor of material in the next section gained from electron-microscopy. Even the early electron-microscopes, introduced in the 1950’s, were unable to resolve the membrane surrounding cells. This limit on resolving power led to considerable emphasis on extrapolating what is seen to what is probably present, a poor scientific technique that has led to years of discussion concerning the actual morphology of the photoreceptors. Sir Arthur Conan Doyle, in the persona of Sherlock Holmes provided cryptic advice in this area:35:

It is a capital mistake to theorize before one has (adequate) data. Insensibly one begins to twist facts to suit theories, instead of theories to suit facts.

The commonly accepted representation of the photoreceptors since 1970 has been due to R. W. Young (not to be confused with J. Z. Young, another leading physiologist of the time) who worked up to then without the aid of the advanced electron microscopy available today. His caricatures of a cylindrical rod and a conical cone with the outer segment encased in an unresolved and highly convoluted external membrane have appeared in textbooks for two generations. However, these caricatures were based on the conventional wisdom and were not based on imagery resolving the putative plasma lemma surrounding the outer segment. This caricaturization began to change in the late 1970s when Hogan, et. al. noted that the disks in human outer segments were not connected to the putative cell membrane. Townes-Anderson et. al. have recently provided similar imagery and discussion concerning the complete separation of the disks from the surrounding structure in rabbit. Hogan, et. al. also noted (on the same page) the stave-like prolongations which extend externally around the inner one tenth of the outer segments. These prolongations can be better described as the staves of a barrel terminating in a hoop that forms the extrusion die, the calyx, of the inner segment. This overall feature is labeled the extrusion cup in the remainder of this work. Some of the staves are missing in the extrusion cup to allow the entry of the fluids of the inter-photoreceptor matrix (IPM) into the cup. This feature is recognized in the early caricature of Brown, Gibbons & Wald (if the putative membrane surrounding the disks is ignored). Section 4.3.5 will present electron microscope pictures that show there is no bilayer membrane surrounding the outer segments of chordates.

The two-dimensional caricatures of Young during this period also highlighted the putative coupling between the inner and outer segment exclusively via a small diameter (largely unresolvable at the time) cilium. This feature was used in the radiographic work of R. W. Young as an aid in caricature. However, this resulted in further perpetuating this feature as a nutritional path in the conventional wisdom, rather than an electrical path. Young frequently omitted the cilium in other contemporary work that will be discussed in Section 4.6.2. This omission clearly recognized the fact that when sectioning samples with a microtome, he only occasionally obtained a section that contained the cilium. The true nature of the cilium has only become known recently.

On page 430, the imagery of Hogan, et. al. at 31,500x showed the cilium typically had a diameter of 0.3 microns and a length of about one micron. The cilium included a circle of nine individual structures whose purpose was unknown. These are now known to be electrical conductors and will be labeled dendrites in this work. The cilium transport or colax is now known to be the feedthrough for this bundle of nine dendrites leaving the inner segment of the photoreceptor cell on the way to the furrows of the disk stack.

In this work, all of the opsin produced in the inner segment is secreted into the IPM within the secretion region of the

35 A scandal in Bohemia (1891)
extrusion cup. Several authors have noted the prodigious production rate of opsin required to maintain the disks in the outer segment in the face of phagocytosis by the RPE. The cross sectional area of the colax is less than one fortieth of the diameter of the disks. If the Golgi apparatus, the mitochondria and the secretion region of the inner segment must work mightily to produce sufficient opsin, imagine the flow rate of opsin through the small area of the colax that would have to be maintained under Young’s conceptualization.

4.2.1 Photoreceptors of Chordata versus those of Arthropoda and Mollusca

The photoreceptors in the chordate retina are topologically similar to those in the retinula of arthropods and molluscs. The major difference between them is topographical. This difference leads to small physiological differences as well. Both types of differences are discussed in this section. Laughlin40 provides a summary discussion of the common principles between the two systems after a broad discussion of more than 100 pages. His summary stresses the commonality of processes in animal vision and does not support the signal polarity difference assumed by many (and frequently credited to him). There are many differences between the interpretation of this work and his at the detailed level. His work assumes chemical synapses except “(Limulus is an exception to this).” He also stresses the dense packing of the photo-pigments. This is true at the light microscope level of morphology but is not true at the level of cytology and quantum mechanics. Similarly, he discusses the transducer function in a bulk sense whereas here it will be subdivided into more discrete processes.

Laughlin and many others have adopted a lexicon based on some terms defined by Eakin. These terms in morphology suffer from the same problem as “the poverty of our neural language” proclaimed by Zeki41. In this case, the terms flagellum, cilium and villus have not been precisely separated. See Section 4.1.2.4.

4.2.1.1 Morphological differences

Figure 4.2.1-1 provides a collage highlighting the primary differences between the chordate and non chordate photoreceptor cells. The variety at the detailed level among species can be amazing. The figure can be compared to the figure of Thurm42, who trace the structure all of the way back to Planaria, and the discussion and references in Autrum43. However, a key element in the discussion should be one of the invertebrate chordates, the tunicates. Eakin & Kuda have provided interesting electron micrographs of this animal that place it as an intermediary between Mollusca and the remainder of Chordata44. The disks of Chordata are generally assumed to be formed serially by secretion from a single region of the IS, followed by bending and extrusion to form disks aligned with their surface perpendicular to the axis of the inner segment. In Mollusca, the photosensitive surfaces appear to be formed by the creation of multiple filaments that extend radially from a central column. However, the resulting surfaces are still perpendicular to the axis of the bulk of the inner segment. In the case of the tunicate tadpole, the photosensitive surfaces are formed from multiple secretion sites, as in the case of the Mollusca, but are not bent and extruded as in the case of other members of Chordata. In the “retina” of the tunicate tadpole, the laminas of

Figure 4.2.1-1 Major differences between the photoreceptor cells of Chordata and Mollusca and showing the intermediate state represented by the tadpole of tunicate. Left; outer portion of Mollusca photoreceptor from Goldsmith (1973) merged with inner segment of this work for purposes of orientation. Right; photoreceptor of Chordata from this work. Top; photoreceptor of ascidian tadpole from Eakin and Kuda, 1971. In all cases, the irradiance is applied perpendicular to the chromophore coated substrates. Whereas both Mollusca and the tunicates employ multiple filament secreting vesicles, it is not known whether this approach to disk formation is used in Chordata.
the Outer Segment are frequently aligned at a significant angle relative to the inner segment of the cell.

4.2.1.1 Formation of the Outer Segments of Mollusca & Arthropoda

The rod-shaped protuberances of Mollusca appear to be formed in “parallel.” There appear to be two scenarios leading to their formation. In one case, they could be formed by the secretion and extrusion of protein material as in the case of Chordata. In the second case, they might be formed by the individual microtubules (dendrites) of the neural portion of the photoreceptor cell.

Looking at the secretion scenario, the rod-shaped protuberances would be formed in vast numbers by individual secretory sources on the cell wall. These might be described as vesicles.

There is inadequate data on how this secretion occurs at the detailed level in all phyla. Most of the figures in the literature are caricatures. The question in both Mollusca and Chordata is whether the protein material is extruded from essentially point sources and then formed into sheets, or whether, at least in Chordata, it is formed in sheets initially.

A case can be made, based on the above figure, that the opsins substrate is extruded as individual filaments in all eyes of Mollusca and Chordata and that these filaments are grouped into a larger mass, as in spider spinnerets. In the case of photoreceptor cells, the resulting material can be viewed as forming a series of parallel cylindrical rods or as a ribbed plank of rectangular rods (with rounded corners as artifacts). Whereas the parallel rod approach is common in caricatures of the photoreceptors of Mollusca, the polarization sensitivity of these eyes would suggest the plank approach is a better representation. It presents more surface area perpendicular to the incident light. However, the grooves remaining on the surface of the planks may be sufficiently prominent to act as a grating polarizer. The precise method of substrate formation appears to play a significant role in the polarization sensitivity of the various photoreceptors to light. Polarization sensitivity will be discussed in Section 4.2.1.1. A case can also be made for the formation of multiple disks in parallel in the advanced eyes of Chordata. This subject of disk formation in Chordata will be addressed more thoroughly in Section 4.3.5.1.

In both of the above cases, the chromophore is transported through the IPM to the protein substrates, from the RPE in the case of the typical chordate and from the “pigment cells” of the tunicate tadpole and other non chordates. The dendritic structure of the chordates is readily recognized by its discrete location with respect to both the IS and the OS of the chordates. In the case of non chordates, the location of the dendrites has not been studied as intensely. The dendritic structure may be inside the overall cell wall, similar to the case in a chordate amercine cell, or it may be a very fine structure external to the main cell wall and paralleling the region of diffusion. No explicit electron-micrographs were found of in-situ non chordate microtubules. In both cases, the chromophores are deposited on protein substrates that insure the average index of refraction of the chromophores (measured at the wavelength of light) is held low enough to avoid total reflection due to a large difference in that index relative to the surrounding materials.

In the second scenario, the individual rod-shaped protuberances (aka microvilli) are taken to be individual microtubules (dendrites) that are directly coated with chromophore. There is no protein substrate. Hamdorf & Schwemer have addressed this scenario and provided dimensions. The interpretation provided in that paper and in Hamdorf are quite different from the analyses to follow. However, the data is excellent.

Hamdorf describes the protuberances as similar in many species of Arthropoda and Mollusca. They remark on the uniformity in diameter with length and give a length of 45-80 microns depending on species for this core. The diameter of the core is given as 42 Angstrom. Hamdorf describes this core as a “more or less crystalline cylinder built of tightly packed membrane "bricks."” They suggest these bricks are chromophoric material and that the core is surrounded by phospholipid material with a thickness of 14 Angstrom.

This work suggests an inverted arrangement with the core a microtubule of phospholipid material surrounded by a monolayer of chromophoric material. Their 14 Angstroms agrees quite well with the best estimates of the length of the chromophores, 15 Angstrom. Under this interpretation, each microtubule is a dendrite emanating directly from the inner segment and there is no need for a colax as found in Chordata. There is no need for the secretion of a protein substrate under this interpretation.

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45Hamdorf, K. & Schwemer, xxx (1975) xxx
4.2.1.2 Polarization sensitivities of the photoreceptors

The chordate eye is insensitive to the polarization of light, except due to secondary effects due to the physical optics of the eye. These secondary effects have been reviewed by Waterman47. The eyes of *Mollusca* frequently demonstrate polarization sensitivity, as do many crustaceans within *Arthropoda*. In some cases, this sensitivity is correlated between multiple spectral bands within the same eye48.

In experiments, it is important to differentiate between the functional absorption of the various photoreceptors versus the observable but non functional absorption at 500 nm.

In chordata, the long axes of the chromophores are aligned with the long axes of the Outer Segments. These chromophores exhibit a highly anisotropic absorption spectrum that is also aligned with the long axes of the Outer Segments. In this so-called end-fire absorption mode, no preference is shown to the orientation of the E-field of the incident light. As a result, the chromophores are not sensitive to the polarization of light arriving parallel to the long axes of the Outer Segments.

In the laboratory, experiments are occasionally performed where the Outer Segments are illuminated from the side. This illumination does not excite the functional absorption spectrum of the photoreceptors because of their anisotropic absorption which exhibits a null at 90 degrees from the long axes of the Outer Segment. However, the light can excite the non functional isotropic absorption spectrum (with a peak response near 500 nm). This absorption spectrum is maximum when the E-field of the light is parallel to the long axes of the chromophores. The early suction pipette experiments of Baylor used this type of illumination. The experiments did show a polarization sensitivity as described above. However, this is not a functional characteristic of the chordate eye as illuminated through the normal pupil.

In the case of the mollusc, polarization sensitivity is achieved through two fundamental features. The individual rows of rhabdomeres form a grating that is sensitive to radiation with its E-field parallel to the long dimension of the individual rods (or grooves as the case may be). By interdigitating the rhabdomere of two photoreceptors at right angles, the information needed to determine the polarization angle of incident light is available to the nervous system.

4.2.1.2 Physiological differences

Conventional wisdom since the work of Laughlin & Hardie49 in the 1970's has indicated that the vertebrate and invertebrate eyes responded differently to light intensity. The vertebrate photoreceptor was known to exhibit an axon voltage which rose with illumination, considered hyperpolarizing since it became more negative. On the other hand, Laughlin & Hardie showed waveforms for currents in the IPM of insects that appeared to be of the opposite polarity, becoming more positive with illumination, considered depolarizing. By comparing their work to what was known at that time about the output of chordate axons, an “apples and oranges” comparison indicated there was a fundamental polarity difference between species. As will be shown in Chapter 12, this error was due to the use of a “floating model” that did not recognize the third signal terminal, the poda, of the photoreceptor at all. The data may have been collected without the pedicle being terminated properly from the electrical perspective.

Shepherd50 draws the conclusion (as have many others) that the invertebrate and vertebrate photoreceptors exhibit a different polarity on page 341 even though he reproduces a figure from Laughlin (1981) on page 342 showing the same polarity output signals for both classes of animal. Unfortunately, the output signals are not taken at the axons. Careful review of that figure shows that the probe points for the two cells are different. Recall that the probes used in this type of work are normally an order of magnitude larger in diameter than an individual cell. For the invertebrate, the probe is clearly in the dendritic area of the IPM. For the vertebrate, the probe is somewhere in the vicinity of the poda terminal. The location of the return lead is not shown. It would be useful to repeat the experiments of Laughlin & Hardie using a more complete electrical model of the cells, accounting for all each of the six electrical terminals of the cells and insuring that the entire cell is functioning normally during the tests.

All photoreceptors exhibit a current entering the poda connection of the cell. The total current entering the poda is nominally a constant under physiological conditions. In the absence of illumination, there is no current exiting the cell via the IPM. The IPM near the OS will exhibit a zero potential relative to the IPM in the vicinity of the poda. On the other hand, there is a large current exiting the axon and flowing into the INM. The current exiting the axon establishes the quiescent voltage level of the axoplasm relative to the poda. Under physiological conditions, the voltage remains considerably below the negative voltage supply of the axon. Upon illumination, current begins to exit the cell through the adaptation amplifier and into the IPM. This current causes the voltage of the IPM near the dendritic tissue to rise relative to the voltage of the IPM near the poda. Simultaneously, the current exiting the axon is reduced by a similar amount. This causes the axon voltage to rise toward the supply voltage and become more negative.

4.2.2 Typical photoreceptor of Chordata

Van Sluyters, et. al. have provided a discussion incorporating two important drawings defining the external characteristics of chordate photoreceptors in vivo. The caricature from Hendrickson & Yuodelis stresses the curved nature of the axon segment, labeled the Fiber of Henle, and the curvature between the IS and the OS for typical photoreceptors not located within the fovea. The other caricature from Banks & Bennett stresses the uniform diameter of the OS, and the IS, in a so-called foveal cone of a human. The OS is shown as a true cylinder with a constant diameter of 1.2 microns over a length of 50 microns. The IS is shown as an extended cone with a taper of only 2 percent (diameter varying from 1.9 to 1.2 microns over a distance of 32 microns). This caricature also shows the “cones” of neonates as having a much larger diameter, but shorter length, IS while the OS remains at a constant diameter of 1.2 microns as it grows from an initial length of near 3.1 microns.

Recognizing the similarities and differences developed above, Figure 4.2.2-1 will serve as a typical caricature of a photoreceptor positioned within a larger context. It bares a family resemblance to a figure by Fliesler & Anderson. It is also similar to a simpler caricature by Holtzman, except the line surrounding the disks does not represent a plasma membrane. Holtzman shows all of the disks as independent of any surrounding structure. Because of the complexity of the figure, an exploded view of the same cell will be presented later in this section. This caricature will be further developed into a block diagram and then further into a schematic diagram as the discussion below develops.

The drawing has labeled three distortions. The so-called ROS has a typical aspect ration of 25:1. Similarly, the distance between the nucleus and the pedicle and the distance between the nucleus and the inner segment can both be much longer than the entire inner segment. Confusion has arisen in the literature when the inner segment and the nucleus are in close proximity. They have sometimes been described by the single label “inner segment” under this condition. This has aggravated the discussion concerning the location of the true inner segment. In the figure presented by West & Dowling, it is clear that all of the inner segments are found in a single plain. However, the nuclei are staggered in depth to take advantage of the available real estate.

The caricature does not display the narrow neck between the Outer and Inner Segments that has become an icon based on an original caricature of Young. This narrow neck does not correspond to our current knowledge. His drawing was prepared by an artist and was apparently based on only a few images of photoreceptors, or limited consultation. The neck probably would have been presented differently if more information had been available to the artist. Young worked with a team and the neck was presented inconsistently by that team. His paper of 1967

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56 West, R. & Dowling, J. (1975) Anatomical evidence for cone and rod-like receptors in the grey squirrel, ground squirrel and prairie dog retinas J Comp Neurol vol. 159, pp 439-460
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(unwithout coauthors) does not show a neck at all58. Unfortunately the neck has become a means of quick recognition in the literature. More recent electron micrographs show the neck playing a more limited role. No nutritional or metabolic functions have been associated with this neck, now labeled the Ciliary Collar. Its role is to facilitate the passage of the dendrites of the photoreceptor neuron through the outer cell membrane. The problem is complicated by the asymmetric three-dimensional structure of the OS/IS junction. Images chosen to clearly show the ciliary collar area frequently do not show the circularly symmetrical calyx area equally well. Richardson59 provides several good electron micrographs and a drawing to explain his imagery. Unfortunately the drawing does not show the dendrites passing up through the ciliary collar although the electron micrograph clearly does.

59Richardson, T. (1969) Cytoplasmic and ciliary connections between the inner and outer segments of mammalian visual receptors. Vision Res. vol. 9, pp. 727-731 (Also in Hogan, pp. 73-75)
Figure 4.2.2-1 Caricature of a photoreceptor cell with RPE interface and Outer Limiting Membrane. Note the length to diameter ratios given for several elements, and the presence of the Activa and the peditic terminal on the left of the inner segment. Note also the calyx (extrusion cup) and the colax (ciliary transport) formed by the cellular membrane of the inner segment. The colax provides passage of the dendrites from the Activa to the disk stack of the outer segment. Compare to Fliesler & Anderson (1983) and the following figure from Miller & Newman.
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Young’s artist also introduced a conceptual outer cell membrane on the assumption that the Outer Segment was an internal part of the photoreceptor cell. Considerable artistic license was taken in sketching this putative membrane. The membrane as drawn has the characteristics of an Escher drawing. The nature of the “membrane” was not discussed or its content defined. The “membrane” enclosing the Outer Segment will be shown to be a coating as defined in this work, a heterogeneous conglomerate of materials involved primarily in generating an electrical potential by metabolic means. Kuwabara\textsuperscript{60} has provided an excellent electron micrograph showing the micelles of the disks to be in direct contact with the extracellular space of the IPM. There is no sign of an external membrane enclosing the disk stack. Bownds & Brodie have also provided an excellent micrograph showing the Inner Segment/Outer Segment interface when a portion of the Outer Segments has been sheared away\textsuperscript{61}. There is no sign of any debris relating to the fracture of membranes putatively surrounding and containing the disks of the Outer Segment.

The Outer Limiting Membrane, OLM, is also shown explicitly in the figure because of the important functional role it plays in vision. Ong\textsuperscript{62} indicates the OLM was named based on light microscopy. Electron microscopy shows that it is formed by a multi-holed membrane (similar to the lamina cribosa of the optic nerve) as well as Mueller cells packed in between the photoreceptor cells. The overall membrane is important for its hydraulic and electrical impedance. This would place the Muller Cells in a role similar to that of the pigment cells surrounding the retinula of compound eyes. Note the length to diameter ratios provided for several features in the figure. Some of the nomenclature shown (with letters in front of the caption) is from Fliesler & Anderson.

4.2.2.1 The Outer Segment

In the above caricature, the Outer Segment is defined as a series of disks stacked like compact disks (CDs) in a column some two thousand disks high. The diameter of this column is typically 2 microns in diameter and 50-100 microns long. The extreme end of this column is located near and partially surrounded by the RPE. The RPE provides at least two functions important to the operation of the photoreceptor, producing the chromophoric material for new disks and phagocytosis of the old disks. The end of the Outer Segment adjacent to the Inner Segment is seen to be in intimate contact with and to be partially surrounded by the IS.

4.2.2.2 The Inner Segment

The inner segment of the photoreceptor is structurally much more complex than the outer segment, and appropriately so because of the many functions it will be seen to perform. Nearest the Outer Segment, the Inner Segment is shown to provide a “pocket” from which the Outer Segment emanates. It also exhibits a ciliary structure that seems to connect to the Outer Segment at or beyond the area of the “pocket.” From detailed study, the Inner Segment is known to contain all of the functional parts of a typical nerve cell.

Various researchers have, however, gone farther and defined many additional features to the cell beyond those of a nerve cell. As an example, it is frequently claimed that the photoreceptor Inner Segment has significant contractile properties as shown in O’Day & Young\textsuperscript{63}. If true, the exterior shape of the Inner Segment could change with time under various experimental conditions. This would be a very important characteristic to note since it might have significant bearing on the categorization of photoreceptors into bins related to the shape of the main body of the Inner Segment, i.e. “rod” shaped or “cone shaped” inner segments. The possibility that the photoreceptor would be categorized differently at a different time cannot be ignored and a researcher who did not make note of the contractile state of the photoreceptor would be seriously amiss. This work does not address these potential contractile properties except to introduce two questions:

\begin{itemize}
  \item whether significant movement of the optically sensitive portions of the cells is compatible with maintaining their position in the focal surface of the optical system
  \item whether contraction between the main body of the cell and the foot is compatible with nerve tissue connecting the two structures.
\end{itemize}

\textsuperscript{60}Kuwabara, T. In Bloom & Fawcett (1968) A textbook of histology. Philadelphia, PA: W. B. Saunders pg. 801


4.2.2.2.1. Motion of the Inner Segment relative to the nucleus

Various investigators have spoken of a motion between the Inner Segments and the nuclei of photoreceptor cells as a function of their state of illumination prior to dissection. These discussions have been based on the variable distance between Inner Segments and nuclei found upon dissection. To account for this motion, some of the tissue of the Inner Segment is assumed to be muscle and is defined as the myoid. As Rodieck says, the literature on this subject is large but relatively sparse in recent times. The investigations are generally based on light microscopy and seldom define the precise location of the investigation within the retina. The presentations are nearly all caricatures and they frequently refer to the OLM as a reference surface instead of the Petzval surface which is more relevant. O’Day & Young illustrate motions of the photoreceptors upwards of 50 microns when referenced to the OLM. As shown elsewhere in this work, the depth of focus of the optical system is on the order of +/−8 microns. Similarly, the change in myoid length of +6:1 would imply a significant stretching or coiling of any neural tissue traveling along the length of the inner segment. The requirement for and/or practicality of this amount of motion needs to be established. No electron micrographs have shown any coiling of the reticulum structures of the Inner Segment. The likelihood that such material is stretchable to the above extent appears low.

Such gross motion may be sought as a putative mechanism for moving the putative rods and cones in and out of the focal plane depending on the illumination level. However, such a mechanism is not needed since this work shows that all photoreceptors have the same dynamic signal range and there are no achromatic photoreceptor cells.

Penn & Hagins have shown that an effective packaging of nuclei of adjacent photoreceptor cells calls for a variable length between the individual nuclei and their associated Inner Segments. In their caricature, all of the Inner Segments, and particularly, the Inner Segment/Outer Segment interface maintain a constant distance from the OLM (at least in a local sense). At the same time, the “myoid” lengths are quite variable but not dynamic. It is proposed that this is the more likely scenario for explaining the variable distances between nuclei and Inner Segments. Under this scenario, the photoreceptors would maintain a constant position relative to the Petzval surface.

No reference to a cell exhibiting both neural and muscular properties could be found in the neurological literature. Thus, the term myoid is believed to be archaic in reference to the photoreceptor cells. This morphological structure is more appropriately described as the ellipsoid. Daw dropped any reference to either the myoid or ellipsoid in his 1995 reproduction of figure one of Papermaster, et. al. On the other hand, many types of neural cells exhibit both neural and glandular properties. The photoreceptor cells are one of these types.

4.2.2.3 The Junction of the Outer & Inner Segment

At the end of the Outer Segment nearest the Inner Segment, several processes are observed in the biological sense of the word and several processes are also observed in the operational sense. There is clearly an extrusion function going on that is generating the disk material. This will be discussed further in a following histological section. Similarly, there is an electrical signal path connecting the Outer Segment to the Inner Segment via the ciliary collar.

4.2.2.4 The Junction of the Inner Segment and the soma

4.2.2.5 The proximal terminal of the photoreceptor cell

The figure shows an extended distance between the inner segment of the photoreceptor cell and its pedicle. This feature is seldom illustrated in textbooks. However, Lolley, et. al. show a variety of cells exhibiting this feature at their true scale. Note the distinct separation frequently found between the inner segment and the nucleus of foveal photoreceptors as well as the significant length of the axon between the nucleus and the pedicle in order to cross the outer fiber layer of the retina. The length-to-diameter ratios shown in the figure are taken from the figures of Lolley.

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The pedicle is generally more than 20 times the diameter of the axon.

At the proximal end of the Inner Segment, the cell does exhibit an ending not unlike other nerve cells. Different photoreceptor cells do exhibit variations in this area. However, the most critical circumstance here is the width of the cleft between the foot of the photoreceptor cell and its associated nerve cell. Several teams, especially Vardi, et. al. and Shein have provided excellent data in this area. Whereas Vardi, et. al. have decorated a pedicle caricature frequently presented in the literature, Shein has provided computer generated tomographs of actual pedicles. Tsukamoto, et. al. have provided actual electron micrographs that underlay these interpretations. See Section 4.3.6.1.1.

The pedicle of a photoreceptor cell plays an unusual role in the neural system of vision. It acts as a current-to-voltage converter prior to participating in the synaptic process. Werbin et. al. report measuring a voltage at the foot of the photoreceptor. They were making measurements under large signal conditions and the waveforms shown are not typical of normal eye operation, i.e., small signal conditions. This subject as well as the detailed structure of the photoreceptor pedicle will be discussed in detail in Chapter 12.

4.2.3 Other cells in the photoreceptor laminate

Both light and electron micrographs of retinas frequently display unusual morphological forms of what are believed to be photoreceptor cells. This is particularly common in non primate chordates. One form that is common even in primates is what appears to be a photoreceptor cell with a cone shaped Outer Segment that does not extend to the interface with the RPE. Knowing that normal photoreceptors in these animals are typically growing toward the RPE at a nominal rate (7.2 microns per day for humans), the reason for the existence of these cells must be determined. The entire disk stack of the Outer Segment (nominally 50 microns long) is normally replaced in 7-10 days. O'Day & Young, as late as 1978, said “evidence of disk shedding by cones is very rare, and has not been shown to be a regularly occurring process.” Based on the expected growth rate of all human photoreceptors, a “cone” with a conical Outer Segment cannot exist under normal conditions for more than a week. Morphologically, it appears that a short cone shaped Outer Segment must belong to either a juvenile cell or a cell that has been stunted in its growth. If it is juvenile, it would not be expected to begin shedding disks until the OS reaches the RPE and phagocytosis begins.

4.3 Histology of photoreceptor and RPE cells (Introducing electron microscopy)

The advent of the electron-microscope in the 1950-60's marked a turning point in our knowledge concerning the photoreceptor cells. As performance improved to about 100,000x in the 1970's, it even became possible to resolve the image of the plasma lemma of the cell as a line or pair of lines. More recent improvements have actually allowed the resolution of the bilayer structure of a lemma. At this level of performance, it became possible to demonstrate that the outer segment of the photoreceptor cell is actually an extra-cellular structure.

This section will only touch on the histology of the photoreceptor. It will address what might be called the gross cytology of the cell. A more inclusive discussion of the detailed cytology, based on much higher magnification electron microscopy, will appear in Chapter 10.

The participation of the chromophores of vision in the formation of the complete disks of the outer segment will begin to appear in this section. This is necessary to provide continuity in the discussion. Details of the formation of the chromophores of vision will be developed primarily in Chapters 5 & 7. The basic premises assumed here and demonstrated there include:

+ the chromophores are formed in the RPE and not the photoreceptor cells.
+ the chromophores are deposited on the disks in the extrusion cup which is external to the photoreceptor cell.
+ once the chromophores are deposited on the disks, they remain there until phagocytosis of the disk.
+ the chromophores are functionally active and reusable without traveling to the RPE for renewal.

4.3.1 Recent morphology based, chronology of the view of a photoreceptor cell

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The conventional view of a photoreceptor cell is changing rapidly in the literature. Figure 4.3.1-1(A) shows a histological caricature of a pair of photoreceptor cells taken from the new 5th edition of Walsh & Hoyt’s Clinical Neuro-Ophthalmology. The figure shows cells labeled rod and cone but virtually no discernable morphological or cytological difference between them. The figure is accompanied by virtually no explanation or discussion of the difference. There is no discussion of the spectral performance of the putative “rod” or “cone.” The obvious difference is the location of the nucleus of the two cells. However, this difference is ephemeral. In the actual retina, such a difference is a function of location in the retina and not a function of the spectral characteristic of the cell. Figure 4.3.1-1(B) shows a complete photoreceptor teased from the retina of a rabbit. The structures labeled outer and inner fiber are dominated internally by the axon of the cell. The cell body is essentially extraneous to the signaling function of the cell. Townes-Anderson et. al. also provided a variety of higher magnification images of parts of the cell. A similar figure, showing a photoreceptor teased from the actual retina of a mouse was published in 1986. Although the precise orientation of the parts of the photoreceptor cell may have changed, it is not likely that their relative sizes or lengths changed. The separate axon segments between the inner segment and nucleus and pedicle are clearly seen. Lolley, et. al. also provided a variety of images in their 1986 paper. In one, it is claimed that one cell is a cone, apparently because its nucleus was relatively larger than the others and the inner segment was not attached (and may never have been attached) to an outer segment. The inset to figure 4 of Lolley, et. al. shows the first published picture of the poditic terminal of a photoreceptor cell (unrecognized by them as to function). This figure will be discussed in Section 4.3.6. The limitations of the differential interference contrast microscope should be reviewed when examining their figures in detail.

Neither of the authors presenting these images discussed the spectral performance, the internal cytology nor the electrophysiology of any of the cells shown. The right side of the caricature drawn by Hengst is noteworthy for the space shown between the basal bodies of the cilium and the ciliary rootlets. This space corresponds to the base of the Activa proposed by this work. Recognizing this fact, the so-called basal bodies are actually the emitter terminals of the Activa and the ciliary rootlets are the collector terminals. These latter terminals represent the start of the reticulums within the axon leading to the pedicle of the cell.

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Many of the previous discussions in the literature concerning photoreceptor cells, and using caricatures based on light microscopy, can be resolved if their shape as a function of their location in the retina is examined. Such an
examination is awkward to illustrate for three reasons:

+ because of the large difference in scale between the retina and an individual cell,
+ because of the fact that the light transverses the inner segment in order to reach the outer segment, and
+ because of the unique geometry, particularly the axons, of the cells located in the foveola.

Figure 4.3.1-2 shows the gross shape of several different photoreceptor cells located around a cross section of a retina. When combined with figure 13.9 in Oyster, a better perspective emerges relative to the shape of photoreceptor cells. This perspective aids in reinterpreting several of the proposals found in the pre electron microscope literature (such as the putative change in length of the axon as a function of light level dating from 1915 and republished as recently as 1973). As discussed more fully in Chapter 3, the outer segments of all cells point toward the center of the pupil while the inner segments tend to point more toward the center of the optical sphere. As shown in Oyster, the length of the axon of a photoreceptor in the foveola can exceed the length of the rest of the cell by a factor of two in order to traverse Henle’s fiber layer on a diagonal. Similarly, the distance between the nucleus and the inner segment can change by a factor of three due to the need to arrange the larger diameter nuclei in close proximity to the remainder of the cell in the outer nuclear layer.

Hageman & Kuehn have included many images of the arrangement of the outer and inner segments of the photoreceptor cells74, including the excellent set of images attributed to Uehara et al. The latter multiple images of the retina of albino rat show the significant angular difference between the axes of the inner segments and the axes of the outer segments. The angles are frequently over 45 degrees and occasionally reach 90 degrees.

Most attention in the literature is given to the internal structure of the Inner Segment with some additional specialized attention given to the internal structure of the disks of the Outer Segment. Only limited attention has been given to the foot of the cell and to the ciliary region at the junction between the two major components. The names used in this figure are essentially those accepted in the literature, there seem to be only minor inconsistencies.

The area of the OS/IS interface is particularly poorly characterized due to the experimental methods used. By examining slices through an asymmetrical structure obtained with a light microscope, a variety of caricatures of the complete structure have appeared in the literature. Most of them continue to show a single fragile, eccentrically located connection between these two structures. It is usually labeled either as a ciliary connection or as a colax through which cilia pass.

Lolley, et. al. have provided some excellent images of

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the shape of actual photoreceptor cells. While most investigators have concentrated on one aspect of the photoreceptor or another and seldom display the overall cell from a histological viewpoint, Lolley, et. al have provided one of the few photomicrographs available of complete photoreceptor cells. The difference relative to the conventional caricatures is startling. They also image the arborization of the poditic terminal on the IPM side of the OLM as expected by this work. They describe the podite arbor as strands of unknown origin near the OLM. By isolating complete cells, they were able to provide preliminary values for the volume, weight and other parameters of the cells.

The use of the term rod in the title of their paper is curious. They did not address the subject of cones or how they separated the cones from the rods in their samples. It is proposed that the adjective rod is superfluous to their subject matter.

Madreperla & Adler provide a caricature that is unusual. It differentiates between a calyceal process on one side of a photoreceptor cell and a ciliary connection on the other side, both in the vicinity of the OS/IS junction. It shows no extrusion cup for forming the disks. Richardson also discusses two physical connections between the IS and OS but located in close proximity. These approaches all stress a mechanical connection between the two sections within a common cell. Papermaster, has taken a different approach and suggested that the transport of opsin between the IS and OS is by secretion into a cup formed between the two sections. Figure 1 of that paper is not drawn to scale and a subsequent paper suggests a different set of proportions. The transport of opsin by this method is the approach supported in this work.

4.3.2 Proposed internal organization of the photoreceptor cell

Little is available in the literature that attempts to differentiate the different functional portions of the photoreceptor cell in an explanatory way. As suggested above, it has become more and more difficult to describe the photoreceptor cell based on historical morphological grounds as new information appears. The functional elements of the cell do not correlate well with these historical descriptors based primarily on morphology.

Figure 4.3.2-1 shows the photoreceptor cell and RPE separated into functional components using an exploded view format. The extracellular Outer Segment (the disk stack) is shown in the center of the figure. The glandular portion of the cell (the inner segment) is shown in the lower right along with the nucleus. A major function of this portion is the production of opsin by the Golgi Apparatus and the mitochondria. This material is then secreted into the extrusion barrel where it is formed into furrowed disks by the calyx. The neural portion of the cell is shown in the lower left. The photoreceptor cell is one of the most complex neurons of the nervous system. It contains a host of individual Activa providing both signal processing (adaptation) and signal distribution. The reticulum (the

77Richardson, T. (1969) Cytoplasmic and ciliary connections between the inner and outer segments of mammalian visual receptors. Vision Res. vol. 9, pp. 727-731
conductor within the morphologically designated axon) connects the distribution amplifier to the pedicle of the cell. In this view, the (typically) nine dendrites of the neuron exit the cell at the colax (the basal body of the cilium) and fan out around the Outer Segment before being placed in the furrows of the disk stack (where they have previously been called microtubules). Below the cilium, the dendrites merge into a single connection to the distribution amplifier, or Activa, shown. The poditic terminal of this Activa is shown explicitly. The adaptation amplifier Activa cannot be shown at this scale. They are represented by the microtubules. Full details of the elements of the photoreceptor cell will be described in Chapter 12. The individual structures associated with these portions of the cell can only be imaged at 120,000x or higher.
Figure 4.3.2-1 An exploded view of the baseline photoreceptor cell configuration. An RPE cell is shown at upper right. The central image is the extracellular Outer Segment, i.e., the disk stack immersed in the IPM (foreshortened for convenience). The typical disk stack consists of 2000 disks, formed into a "spaceframe" structure and immersed in the IPM. The lower left shows all of the neural elements of the photoreceptor cell as a group (including both the distal and proximal axon segments). The distribution Activa is shown explicitly. The microtubules represent both the "quasi-dendrites" of the distribution Activa as well as the Activa associated with the adaptation amplifiers. The adaptation amplifier Activa cannot be shown at this scale. The lower right image shows all of the glandular portion of the photoreceptor cell (exemplified by the inner segment) as well as the other housekeeping functions (exemplified by the nucleus).
In the exploded view two different names are given for a number of elements to ease correlation with the literature and language of various disciplines. A discussion concerning the most appropriate names to be used in further (and future) investigations will also be found in this Chapter.

4.3.3 An overview of the structure of the Inner Segment

Because of its complexity, an overview of the gross cytological structure of the inner segment will be examined first. The following sections will concentrate on the individual functional portions of the inner segment and the rest of the cell. Figure 4.3.3-1 assembles data from a number of investigators to provide a comprehensive view of the inner segment. For further details concerning individual features of this figure, see the papers noted below.

The 1969 paper of Richardson presented the clearest electron micrographs (at 54,000x) and caricatures of the ciliary collar and the extrusion cup of the inner segment available at that time\(^8\). The imagery is from the rhesus monkey. The two structures are shown as completely separate with an extracellular space, es, where the IPM can flow between them. It also delineates the nine individual dendrites passing through the “typical” mammalian ciliary collar. These conduits resemble a stranded electrical cable made up of individual insulated wires. In this picture, the total collar diameter is approximately 400 nm and each dendrite has a diameter of approximately 50 nm. He notes the fuzziness associated with the material surrounding the cable. He also takes exception to Young’s view regarding protein transport to the outer segment via the colax, suggesting that the protein material transfers via the cytoplasmic bridge. The micrographs in his paper illustrate the difficulty of imaging an entire structure. Some features appear to enter and disappear from the plane of the image in a random manner. In some images, the disks seem to originate from the microtubules passing through the colax. In others, they clearly originate within the extrusion cup.

4.3.3.1 The electrolytic circuit associated with the inner segment

The inner segment contains the Activa known as the distribution amplifier in this work. This Activa is located within that part of the inner segment located within the IPM and its base connects electrically to the IPM. The dendritic structure passing into the inner segment, from the IPM, through the colax terminates at the emitter of the Activa. The reticulum forming the conduit that eventually emerges as the axon begins at the collector of the Activa. The Activa is essentially transparent to both light and electron microscopy (unless variations in charge density can be imaged as in some electron micrographs).

In his caricature, Richardson shows the dendrites (microtubules) ending at a point below the extracellular space and the reticulum of the axon beginning in the same region. It is proposed that the space between these two locations is the site of the Activa forming the distribution amplifier within the photoreceptor cell and shown in the above figure. This proposal is supported by the electron micrograph of xxx showing the location of the proposed poditic terminal adjacent to the above area (See Section XXX). The reticulum associated with the axon is shown as a dashed channel following the convention of Borwein in 1981. See Section 4.3.5.

Figure 4.3.3-1 Composite caricature of the inner segment stressing the functional elements present as well as certain electrically important features. It is based on earlier caricatures and micrographs expanded to include the functional electrical features defined in this work. The nucleus frequently stands alone and may be farther removed from the inner segment than suggested here. See text.

Papermaster et. al. 1985 provide the clearest caricature of the secretion process. It is shown in the center of the above figure modified slightly to be compatible with the more detailed electron micrographs showing dendrites passing through the ciliary collar. The detail on the left of the ciliary collar is a caricature of the actual electron micrograph in Bunt-Milam, et. al. The outside diameter of this structure is about 0.25 microns.

Papermaster, et. al. showed the protein opsin being extruded into an area labeled D. The exact method of movement

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of the protein through the cell wall remains a question. Jamieson has described his concept in caricature\(^83\). The Papermaster figure has been extended to suggest how the secreted opsin is forced to form a continuous sheet by the shape of the extrusion cup cavity. This cavity is nominally circular. While the protein is being folded back and forth, two steps occur. The material is coated on its hydrophilic side with one of the Rhodonines, the chromophores of vision. The Rhodonines enter the extrusion cavity from the Inter-Photoreceptor Matrix, IPM, through ports in the wall of the cavity. The material also begins to harden due to the chemical character of the environment within the cavity. As the strip of opsin is compressed, it breaks into small pieces prior to extrusion from the cavity. In the process of extrusion, furrows are introduced into the periphery of the disks (prior to crossing the dashed line) by the Calyx, or extrusion dye. These furrows are subsequently filled by the individual dendrites emanating from the ciliary collar. This detail is shown in the cross section. Two separate reticulums (electrical conduits) are shown within the structure that will become known as the axon when it emerges from the inner segment. The location of the Outer Limiting Membrane (OLM) is shown explicitly because it plays a role in the electrical performance of the photoreceptor cells and the retina. In this figure, the ellipsoid is seen to be a morphological designation for an area with a different optical index in light microscopy.

It is important to note that the disk formation process may appear quite differently depending on the plane of the section taken relative to the circular cavity. Whereas the disk forming shoulder is shown in the same plane as the midline of the colax, this is not a condition in real cells.

Ottoson, discusses microtubules and neurofilaments in the generic sense\(^84\). He describes them as slightly larger than those of the colax and the outer segments. He lists the microtubules as typically 240 Angstrom in diameter. They are frequently stranded and filled with neurofilaments on the order of 100 Angstrom in diameter. The specific dimensions of those structures associated with the outer segment are critical to the operation of the visual system and will be discussed further in Section 4.3.5.

Brown, et. al have provided an interesting image of the Calyx\(^85\). It shows the reinforcement of the Calyx typical of what would be found in a man-made extrusion die. A heavy external band is placed around the die and connected to the staves of the extrusion cup. Where staves are missing, the fluids of the IPM can enter the extrusion cavity.

In many caricatures, it is claimed that there is a “membrane” surrounding the Outer Segment. However, no electron microscope picture or any other evidence could be found that shows the material surrounding the OS to be a membrane in the technical sense. There is excellent evidence that there is no membrane (See Section 4.3.5). In the absence of evidence to the contrary, the material surrounding the OS will be considered a coating and not a membrane.

Dowling (1967) shows the disks of the OS extending beyond the end of the calyx at a resolution appropriate for the time. However, the words in the caption relative to the membrane cannot be supported at this resolution. There is no suggestion that the lines were resolved adequately to determine whether they are a membrane or a coating. In this figure, the coating present around the disks after leaving the calyx, is not present deep in the seat of the calyx.

4.3.3.2 The glandular function associated with the inner segment

It is extremely difficult to find a single electron-micrograph that completely describes in a single two-dimensional image the fundamentally three-dimensional cytology and secretion processes associated with the IS. An image worth studying appears as Figure 9-23 in Hogan. It is at x36,000 and appears to show that even in Chordata, the disk stack may be formed from multiple secretion sources. It is quite clear that these sources are not related to the colax (ciliary collar) and the dendrites passing through the colax. The termination of the calyx is shown clearly on the left side of the Outer Segment but less definitively on the right because of the presence of the dendritic bundle. The figure also appears to show the location of the Activa within the IS. It is proposed that the base of the Activa is formed at the upper end of the two dendrites shown passing through the colax. Immediately above this base region, and before the top of the image, the axolemma and axoplasm can be seen in faint outlines. Being a section through a three-dimensional shape, the image does not show any furrows within the disks or the insertion of any dendrites into these furrows. A discontinuous boundary is shown on the left side of the OS but no similar boundary is obvious on


\(^85\)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 Also Fig. V-5 in Rodieck
the right side. Neither the boundary nor the bilayer nature of the disks is resolved at this magnification.

The above figure represents the best available representation of the secretory process based on the review of a large number of electron micrographs. The protein material is secreted by the cell into the bottom of the extrusion cup (outside the cell wall) where it is first formed into a strip. This strip is then folded repeatedly and then put under pressure until it breaks into approximately square flakes. These flakes are then put under pressure and extruded first into circular disks and then further extruded into disks with grooves spaced about equally around the outside edge, the grooves extending inwardly approximately 50% of the radius of the disk. At this point, the dendrites from the cilium are laid into these grooves and the total structure enters the region filled with the material of the IPM, the Inter-Photoreceptor Matrix.

There seems to be no difficulty in describing the basic manufacturing portion of the photoreceptor cell in the manner described above. It is the typical situation found in the exocrine glands where a cell creates and exudes a material either autonomously or under central nervous system control. This subject is treated in most Histology textbooks. Figure 4.3.3-2 shows the “manufacturing facilities” in a typical cell, how the material is produced and how it moves toward the surface of exocytosis. The figure is a composite of figures from Peters, et. al and Papermaster, et. al. At this point, there are two open questions. First, how does the protein material get from the mitochondria to its position in the disks? Peters, et. al. addressed the two options, transport through the cilium transport (the colax) or directly to the disks through the calyx (pg 274). Unfortunately, they settled on the colax route without understanding the neural purpose of this structure. They defend their choice based on two points. First is their estimate of the low concentration of opsin in the apical membrane of the inner segment. Second is their high estimate of the concentration of opsin in the colax. These points are contrary to the radiographic data of Young as reviewed and interpreted in Section 4.6.2.3.1. One of Young’s goals was to show that opsin passed through the colax. However, his data showed the contrary. It passed to the disks via the calyx.

In the second case, there is the question of how the basic protein substrate as well as the chromophore material are both produced and brought into close contact with each other. This work adopts the view that the protein is secreted into a space connected to the IPM within the calyx. From there, it is extruded into individual disks as shown in the figure. As discussed in Section 4.6.2.3, there is convincing evidence that only the protein material is secreted by the photoreceptors. While the total protein molecule does contain a ligand derived from retinol, this ligand does not participate in the photodetection process. The chromophores of vision consist of another material derived from retinol. As discussed in the same section, the chromophore material is secreted by the RPE cells. The RPE provides a “closed loop” manufacturing capability. It provides new chromophoric material for the disks and at the same time participates in the phagocytosis of the old chromophoric (and protein) material from the outer end of the Outer Segment.

Bloom & Fawcett point out that it is common for a nerve cell to possess some secretory capabilities. Furthermore, the IS is shaped very much like the basal region of a tubular type exocrine gland in the electron-micrograph of their figure 4-2, a pancreatic acinar cell. A neuron can secrete protein material in one of three ways. It is described as merocrine if the secretion involves release of material through the cell membrane with the cell remaining intact. There are also glands that secrete a single strip of material and glands that secrete multiple strips of material. They are called unicellular and multicellular respectively in the literature. The latter is most clearly seen in the arthropod and mollusc photoreceptors. It is also seen

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in arachnoid web spinners. However, better names are probably monofilament and multifilament since the materials may be formed in fine ducts or by multiple vesicles, all within a single cell. See Section 4.2.1.

A reinterpretation of figure 4 in Peters, et. al. (in line with their discussion on page 267) would suggest that the nine ridges (or grooves) of the frog inner segment are each sources of opsin, and the nine dendrites passing through the colax align themselves in the outer portion of the grooves between the individual strands of opsin. As suggested in their text, the colax is frequently less centered than shown in their figure. In fact the stereotypical image of the colax shows it located external to the calyx. Many micrographs actually show a void between them. The adjacent portions of the material secreted from the apical membrane of the inner segment grooves tend to merge (although they frequently remain visible in plan-view pictures of disks). See the plan view of a human disk in [Figure 4.3.5-3].

4.3.4 Gross structure of the outer segment

There is considerable inconsistency in the literature concerning the detailed structure of the Outer Segment. This is particularly apparent between the early caricatures, based on light microscopy, and more recent electron-micrographs. Much of it seems to relate to insufficient scope in the original investigations or to attempts to tie the data to preconceived ideas about what to expect. As an example, there is considerable discussion of an outer membrane enclosing the disks; however, several caricatures show the membrane in question disappearing within a short distance of the junction with the Inner Segment--and some of these imply that the outer membrane becomes an integral part of the disks or is absorbed by the disks. Most electron-micrographs do not show any trace of an outer membrane. These images will be discussed in Section 4.3.5.1.1.

4.3.4.1 The Outer Segment as a passive element

In the electronics field, there are two broad classes of passive elements, the simple elements such as the resistor, capacitor, inductor and diode, and the more complex elements such as transformers and piezoelectric crystals. Only the resistor, among the above elements consumes power in its normal operation. The transformer can change the impedance level between two circuits and thereby cause a change in voltage or current. However, it cannot cause a change in signal power.

The Outer Segment is involved in a two-step passive process, the transduction of photons into excitons and excitons into free electrons. This latter function is performed in close association with the dendritic system of the photoreceptor cell and is called translation in this work for purposes of clarity.

4.3.4.2 The Outer Segment from a light wave perspective

As in conventional antenna theory, the OS can be described in terms of a group of individual resonators forming a two or three-dimensional array. Because of the sharing of electrons by the molecules of an individual disk coating, each coating looks like two clouds of electrons separated by a conductor of finite length. Each cloud is approximately the diameter of the disk. The resonant frequency of each coating is determined by the effective distance and the velocity of electron propagation between the two clouds. The velocity of propagation of exciton energy has been shown to be quite slow in a resonant conjugated structure. The propagation along the resonant axis of such a structure is approximately 230 times slower than free space propagation (the speed of light)\(^9\). A slow wave factor is defined as the ratio of this velocity to the free space speed of light.

To determine the exact relationship between the wavelength of excitation and the ability of the chromophore to detect it efficiently, a number of factors are involved. The principal factors are the actual slow wave factor and the exact geometry of the chromophores. Detailed knowledge of the stereo geometry of the chromophores in the liquid crystalline film on the surface of the opsin substrate is required. Neither of these values is known precisely at this time. Figure 4.3.4-1 presents a nomograph illustrating the relationships involved. Wolken has given a value of five Angstrom for the diameter and ~15 Angstrom for the length of the retinal molecule (in some publications, he dropped the approximation sign). It can be assumed that the Rhodonine molecule will not be significantly different in length although it may be slightly less circular. All of the available data suggests the chromophores of vision are deposited on the opsin substrate as a liquid crystalline film. These chromophores are defined as Rhodonine, are derivatives of retinol, and are discussed in Chapter 5. If Rhodonine is deposited on the substrate as a Type A smectic film, then the molecules would be arranged perpendicular to the surface of the opsin and parallel to the

stimulating radiation. Their auxochromes can be assumed to be closer together than the maximum dimension of the molecules. The dotted lines project the effective electronic distance between the auxochromes for each of the four spectral bands of interest in a polymethine based on the Amidinium ion. Using the theoretical slow wave factor of 230 from Platt, the effective wavelength of the energy absorbed by the molecule is between seven and thirteen Angstrom. For a molecule acting as a dipole antenna, the resulting effective spacing between the auxochromes is 3.5 and 6.5 Angstrom. These values are comfortably within the potential maximum of ~15 Angstrom of Wolken. Figure 2-2 in Platt shows how accurately his analysis predicts the actual wavelength of the Carboxylic-ion-based polymethine. It is particularly good for the odd conjugation values used here and appears to be +/-5% for conjugation levels between 5 and 11. Platt’s equations based on the free-electron model are independent of the type of heavy atoms in the conjugated system. He states that a carboxylic ion system would be expected to show the same performance. He also stresses that the cis- form of a resonant-conjugated molecule has a significantly lower absorption coefficient and absorbs at a significantly shorter wavelength.

The similarity between the spectra of Platt and some of the spectral absorption figures in Mees⁹⁰ is striking. A transform line for a Type C smectic film at 45 degrees is also shown to bound the possibility that the chromophores are of Type C. If they are, their angle is probably less than 45 degrees. As discussed later, it is common in photography to find the chromophores deposited at an angle of about 35 degrees from the normal in planes containing the heavy atoms that are separated by 4.5 Angstrom.

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Figure 4.3.4-1 Slow wave structure nomograph. Dotted lines indicate most likely effective electronic length between the auxochromes for dipole resonance and a Type A smectic structure (see text). Conjugation length is 1.40 Ångstrom for all atomic pairs. Also shown is a predicted transform for a Type C smectic structure at an angle of 45 degrees.

It appears that a Type C smectic structure, a slow wave factor of 230:1, and a full-wave resonance condition will easily satisfy the maximum molecule length criteria at the worst case (625 nm.) wavelength. These lengths and geometries are also compatible with those shown in Figure 4.3.2-3 and in later figures for actual photoreceptors. The active elements of each disk can be represented by two of these “sheet” resonators separated by about 150 Ångstrom (center to center). The disks are in turn spaced at about 250 Ångstrom, a small fraction of the peak absorption wavelength of the coatings.

This configuration should be considered in terms of two configurations from Electromagnetic Theory, an end fire type of antenna and a power dissipating termination in a waveguide. Since the sheets, and the disks, are not interconnected in an electrically coherent manner, they do not form an effective antenna array. However, as a series of absorbing surfaces separated by a material of approximately the same index of refraction as found at the entrance to the absorber, they do represent an effective absorber without causing excessive reflection of energy back toward the source.

4.3.4.2.1 The Outer Segment as a waveguide

Miller has provided a discussion of the Outer Segments as waveguides⁹¹. His approach is not rigorous from an engineering perspective. He does not differentiate clearly between the reflectance at the entrance versus the internal

reflectance along the guide following acceptance of the energy. He also does not consider the detailed nature of the material within the waveguide.

More recently, Stacey & Pask have provided a mathematically rigorous description of the performance of the photoreceptor cell treating the inner segment as a waveguide of 3.2 microns diameter feeding a one micron diameter outer segment\textsuperscript{92}. They show a resolution limit, driven by the photoreceptors alone, of 130-165 cycles per degree, and show that the level of coherence between two beams arriving at opposite sides of the very limited acceptance angle is unimportant. These values were obtained by considering the individual photoreceptor and sweeping the wavefronts (assumed to be flat) across the aperture. It appears there modeling would be applicable to an outer segment alone but different values would be obtained. However, there specific model of the inner segment differs considerably from the less optimum shape of that element explored in Section 2.4.6 of this work. This is particularly true with respect to the uniformity of the index of refraction of various parts of the inner segment. Stacey & Pask did not describe in detail the structure of the outer segment in their model.

From the perspective of Electromagnetic Theory, the OS can be considered a cylindrical waveguide of approximately 3-5 wavelengths in diameter and filled with about 2000 sheets of closely spaced highly absorbing material. The sheets are not in contact however. The waveguide is surrounded by a coating of metabolic material and the IPM. These materials also fill the space between the coated disks. Within the OS, all dimensions are considerably smaller than a wavelength of visible light. The effective index of refraction under this condition is the mean of the index of all of the materials within the optical path of the OS. The spacing and the intrinsic index of refraction of the fill material, the protein material, and the chromophores lead to a very low reflection of energy at the entrance aperture. When looked at as a waveguide, this structure is a very effective photon collector. It exhibits a diffraction limited optical acceptance angle as calculated by its diameter and the indexes of refraction for the surrounding medium. This angle is broader than that required to capture all of the light emanating from the pupil of the eye.

Figure 4.3.4-2 highlights these characteristics. The chromophoric molecules are shown arranged as would be expected for a material forming a smectic type A liquid crystal. The chromophore molecules need not be perpendicular to the surface of the disk. It is likely they are at an angle as usually found in photography. In that case, the material would be smectic type C. Each face of each sheet of this liquid crystalline material appears electrically as a cloud of randomly positioned \(n\)-electrons. The distance between the two \(n\)-electron clouds associated with each film, and the transport velocity of charges between these clouds, make the materials highly absorbent at wavelengths within the UV to L-spectral range.

The above configuration is diametrically opposite to one frequently found in the literature showing the long axis of the chromophore molecules in the plane of the disks\textsuperscript{93}. These configurations assume the chromophores are maximally sensitive to light impinging perpendicular to the molecular axis. This is only true for molecular absorption (with a peak wavelength at 500 nm). This absorption is not used in vision although it has frequently been measure in dilute solutions of chromophores and chromogens. It has been frequently measured in early suction pipette experiments. The absorption used in vision is related to the “slow wave” absorption. This absorption occurs at wavelengths of 342, 437, 532 or 625 nm and is optimally effective when the light impinges parallel to the axis of the individual chromophores. The orientation of the chromophores presented above leads to a different interpretation of the material in Davson (with largely the same results)\textsuperscript{94}.


\textsuperscript{93}Knowles, xxx Dartnall, xxx. ( ) The Eye in Davson pg 233.

The spectral characteristics of this configuration when examined in the laboratory are complicated due to the many special properties of the chromophores. The individual chromophore molecules exhibit an isotropic spectral characteristic related to “molecular absorption.” This absorption is directly related to the physical size of the entire molecule. This is the spectrum usually reported in the literature, based on measurements of dilute solutions, and on electrophysiological experiments on Outer Segments with transverse illumination, either \textit{in-vivo} or \textit{in-vitro}. The wavelength observed (~500 nm) is characteristic of retinol and all retinenes and Rhodonines. This absorption is strongest when the Poynting vector of the incident light is perpendicular to the long axis of the molecules and the electric field is parallel to the length of the individual molecules. This is the absorption traditionally reported in the literature and associated with the radiation from or to a dipole antenna. It is not the primary absorption characteristic of vision.

There is a second highly anisotropic absorption characteristic associated with the spectra of vision. This absorption spectrum is maximum when the Poynting vector of the illumination is parallel to the axis of the resonant retinoid. Under this condition, absorption is maximum when the frequency of the illumination is the same as the resonant frequency of the resonant liquid crystalline structure. This spectrum is normally only measured in retro-reflection absorption experiments on \textit{in-vivo} specimens. Most recently it has also been measured in quasi-in-vivo experiments using suction-pipette techniques. It is only exhibited when the chromophoric material is in the liquid crystalline state. In addition, it shows no polarization preference. This is why the face of the disks in the above figure shows an array of dots instead of an array of bars as found elsewhere in the literature. The dots do not suggest any polarization preference.
4.3.4.3 The chemical composition of the outer segment

There is little data on the composition of the outer segments and most of the data is questionable based on the extraction techniques used to obtain it. Brown & Wolken have provided an early table based on a variety of sources that they did not identify. Nor did they describe the techniques used to collect the data. They averaged the collected values. Lacking a model, it is not clear whether the data incorporates parts of photoreceptor cells, Mueller cells or RPE cells. Nor does it indicate whether the materials found within the IPM were included in the values. No category was provided in the table for retinoids or a putative rhodopsin containing a retinoid.

While the molecular weight of the Rhodonines (285-299) is much less than the protein of the substrate (opsin = ~41,000), and they are both present in a monolayer configuration, it is important that a value be determined for the amount of chromophore present in any analysis of the outer segments. In 1982, Corless, et. al. indicated that more than 90% of the mass of the outer segment is protein. Based on the relative thickness of the chromophore layer and the protein layer of this work, and excluding any other material from the IPM, the number should be on the order of 80%. Section 4.3.5 addresses this subject in more detail.

4.3.4.4 Summary of the Outer Segment Histology

In summary, the component commonly called the outer segment in the literature is not a component part of the photoreceptor cell at all. It is not contained within the cell membrane. It is an exocellular element that after formation acts as a transducer for the excitation of the nerve part of the photoreceptor cell. In this sense, the neuro-anatomist would label the Outer Segment a capsule in intimate contact with the nerve endings. The combination of a capsule and a neuron in intimate contact is frequently defined as a mechano-receptor. This definition appears to apply in the photoreceptor case if the capsule is considered a quantum-mechanical transducer stimulating a neuron.

In the vertebrate and some other eyes, this capsule is surrounded by the dendrites of the neural portion of the photoreceptor cell. The capsule consists of a stack of disks. In other species, the capsule surrounds the neural dendrites in which case, it is called a retinula--although the literature is inconsistent in this name. The retinula is frequently made up of a set of sectors, as in an orange, instead of disks. However, the functional purpose is still achieved, separating the chromatophoric liquid crystalline material into a number of individual, electrically isolated, elements.

The nerve portion of the photoreceptor cell is conventional, receiving “irritation” from the “outer segment” and synapsing with another nerve cell at its foot. The variation in the shape of the pedicles among the photoreceptor cells serve only to accommodate the variable number of synapsis involved.

The principal components of the outer segment are the disks which support the chromophores that absorb the incident light. These disks are coated on their exterior surfaces with the chromophore. The substrate of the disks is a well-characterized protein material, opsin. There is significant space between the coated disks, even near the edges where they are most closely packed. The available data indicate considerable experimental range in the 20-50 Angstrom thickness of the substrate, while indicating the auxochrome pitch to be 50 Angstrom and the chromophore pitch to be 60-70 Angstrom. In his Figure 9-24, Hogan portrays the major difference between his two cases, presumed to be from a morphological rod and a morphological cone, as due to the difference in thickness of the substrate layer. This may be the situation, however, his broad brush in the schematic does not provide the desired degree of authenticity for these numbers. Both Vanderkooi & Sundaralingam and Nilsson provide a significantly more detailed interpretation of these dimensions with both leading to a lower auxochrome spacing. Another interesting observation is that the space between the disks is quite considerable, some 50 Angstroms near the thickened edges and as much as 165 Angstroms near the center of the disks. This spacing leaves room for considerable hydraulic and metabolic activity to occur within the space between the disks.

The conventional wisdom, as quoted by Rodieck, is that an effective “… chromophore absorbs light to the degree that its conjugated chain lies (1) perpendicular to the light ray and (2) parallel with its electric vector.” This “wisdom” is not based on a model of the underlying process. It appears to be based on his interpretation of the parameters compatible with common crossfire antenna theory. This work does not support this position.

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The configuration proposed in this work places the axes of the chromophores, the conjugated carbon chains connecting the two auxochromes, parallel to the expected direction of the incident light ray in chordates. This configuration is called the end-fire condition in antenna theory. It is compatible with the slow-wave theory of photoexcitation of Platt. It also results in a highly anisotropic absorption pattern.

The proposed structure is compatible with all of the orientation data referenced by Rodieck. Of some possible significance is the reference to Liebman\(^7\) wherein Liebman discusses the possibility that the axis of an individual chromophore may not be exactly perpendicular to the surface of the substrate and therefore exactly parallel to the direction of the incident light ray. This may be true for at least three reasons. There is an angular dispersion to the light rays entering an outer segment and the light rays within this bundle are reflected down the length of the disk stack, acting as a waveguide, at a variety of angles. More importantly, as frequently found in photography, the chromophores making up the liquid crystal may assume an angle other than 90 degrees to the substrate in order to satisfy packing factor requirements. This is a small but probably a measurable factor. Finally, there is no assurance or requirement that the two liquid crystalline layers on opposite sides of a substrate have the molecular axes of the chromophores parallel.

Most of the literature has examined and discussed the structure related to photo-transduction in a static context. Thus they speak of the disks or rhabdome as being invaginations of a cell wall without concern as to how they got there. Some authors speak of these photo-transduction structures as based on cilia and other speak of these as based on microvilli. Such differences are not easily defensible because of the wide variety of shapes found for these structures in different animals. This work proposes that the protein portion of all disks and/or rhabdome is formed by a secretion from the photoreceptor cell. Whether the resultant material is formed as a single strip or as multiple adjacent rods is not of great consequence as far as the basic extrusion process is concerned.

It is clear that different mechanisms are involved in processing the protein material of the disks and the retinoid material of the chromophores. \textbf{Section 4.6.2.3} will discuss the autoradiographic data supporting the separation of the disk formation and the disk activation processes.

\textbf{4.3.5 Detailed structure of the Outer Segment}

Nilsson provided a series of papers during the 1960’s that are probably the most comprehensive studies available of the Outer Segment at sufficient resolution to answer many questions\(^98,99,100\). He achieved a resolution on the order of 20 Angstrom in that time period. That resolution should be sufficient to resolve a plasma membrane of some 150 Angstrom. Although he speaks of a plasma membrane surrounding the Outer Segment, his images do not resolve such a bilayer structure. His images do show numerous structural features on the order of 50-60 Angstrom. His referral to magnifications of x745,000 and x800,000 may involve photographic enlargement as much as electron-micrographic enlargement. These numbers do not infer higher limiting resolution than his work at x300,000.

\textbf{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 \textit{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 \textit{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 \textit{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 \textit{Mollusca}\(^101\). See \textbf{Section 4.2.1}.

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\(^{98}\) Liebman, P. (1962) \textit{In Situ} microspectrophotometric studies on the pigments of single retinal rods. \textit{Biophys. J.} vol. 2, pp. 161-178 \textit{Also in Rodieck} (1973) pg 140


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.
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.
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\textsuperscript{104}. 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 appears in Nolte and gives even more detail about the INM/IPM interface\textsuperscript{105}. Figure 37 of Miller from a frog, \textit{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\textsuperscript{106} where it is credited to Steinberg\textsuperscript{107}. 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 Matsurra 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 Matsurra 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\textsuperscript{108}.

\textsuperscript{104}Autrum, H. (1979) Comparative Physiology and Evolution of Vision in Invertebrates. NY: Springer-Verlag, pp 127-130
\textsuperscript{105}Nolte, J. (1999) The Human Brain. St. Louis, MO: Mosby pg 404
\textsuperscript{107}Steinberg, R. (1973) Scanning electron microscopy of the bullfrog’s retina and pigment epithelium Z. Zellforsch. vol. 143, pp 451-463
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. There is no sign of the calyx 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.

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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 shows the best available imagery of the actual outer segments of a vertebrate species by Bok\(^\text{111}\). After incorporating the caricature of Young (which was de rigueur during that period), he showed many images that did not show a bilayer cell membrane surrounding the disks forming the outer segments. Interestingly, Bok asserts (caption to his figure 1-1) the virgin disks are formed outside of the bilayer membrane of the photoreceptor cell and then suggests they are somehow reincorporated into the cell. In this figure Bok credited to J. Usukura, it is clear that there is no bilayer cell membrane surrounding the disks of the outer segment.

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The disks have easy chemical axes to the Interphotoreceptor matrix (IPM). The individual disks are clearly folded bilayer membranes, and compatible with the disk creation process discussed below.

Similar imagery is also shown in Adler & Farber\textsuperscript{112}. As in the above imagery, there is no sign of a bilayer plasma membrane surrounding either stack. There certainly are not two membranes passing down between the two stacks. A similar picture that did not capture the filaments is available from Molday\textsuperscript{113}.

The above figures are taken as the baseline for this work that no plasma membrane enshrouds the Outer Segments of chordate photoreceptor cells. The author awaits conclusive evidence to the contrary and offers a financial reward on his website for such evidence.

As noted in Rattee & Breuer, whenever one is dealing with high molecular weight natural proteins in an aqueous environment, a lower molecular weight material is always present\textsuperscript{114}. The composition of this material is similar to the parent material. This “wool gelatin” material may be one of the materials forming the coating on the exterior of the disk stack that is frequently assumed to be a biological membrane.

Before leaving the extrusion cavity, the extruded material is formed into disks consisting of a protein layer coated over all or a majority of its surface with the chromophore, Rhodonine(n), to be introduced in \textbf{PART B}. These disks are stacked like poker chips to form a column. The literature is beginning to agree on the dimensions of these disks; they are variable in diameter depending on location in the retina but typically 2 microns in diameter. The disks near the inner segment end of the column tend to have serrated edges in order to accommodate the adjacent microtubules of the nervous portion of the photoreceptor cell. Further out in the column, their shape is generally reported as more circular. The pitch of the disks is generally reported as 250 Angstroms, but with less certainty; similarly the thickness of the disks and the space between disks is not known with great precision. This is partly due to the lack of definition as to where the boundaries are that are involved in these dimensions. Hogan presented a table of dimensions in 1971 that are shown in \textbf{Figure 4.3.5-7}.

![Figure 4.3.5-7 Drawing of two different sets of photoreceptor disk dimensions. From Hogan (1971)](image)

Hogan shows the dimensions of the disks in schematic as:

\textsuperscript{112}Xxx (1982) xxx \textit{J. Cell Biol.} vol. 95, pp 487-500
Case 1 is labeled “Cones” and Case 2 is labeled “rods” in the original presentation although no further information was given as to if their spectral sensitivity was determined, how many layers were examined or what were the statistical value of these dimensions.

Dimensions measured in Angstroms must be carefully defined. The edges of a 20-Angstrom dimension may frequently be sharper than the resolution of the device used to measure them. This leads to inaccuracy. It is likely that these dimensions have a statistical error of +/- 5 Angstrom or more. In particular, the thickness of the substrate will be considered imprecise until the next paragraphs are reviewed.

Based on the following material, the average thickness of each bilayer of the opsin substrate will be taken as sixty five Angstroms and the average coating thickness will be taken as fifteen Angstroms.

Note that the total thickness near the edge of the disks is usually shown as greater than in the interior regions. This is most likely due to structural forces associated with the molecules of the coating attempting to maintain their structural relationships with their neighbor chromophores while trying to simultaneously adhering to the substrate as tightly as possible. In the later case, it is most likely that only hydrogen bonding or Van der Waal forces are involved in attaching the chromophores to the substrate.

4.3.5.2 Physical characteristics of the disks

While much of the literature associates the disks of the outer segment with a putative plasma membrane surrounding, forming and incorporating the disks, this work does not support this premise. In this work, the disks are essentially lipid free, and free standing, protein structures coated with a chromophore in electrical contact with the neural system via the ciliary collar. Neither the subject of how the protein rhodopsin is embedded in a plasma membrane, or the subject of how heavy ions pass through a plasma membrane arise under this interpretation.

Most of the material in the literature related to the structure of the disks at the sub-histological level assumes the primary material involved was defined properly by Bownds. The assumption is that the disks are made up of a heterogenous material consisting of the 11-cis isomer of retinal bound as a prosthetic group to a (lipo-)protein moiety opsin. Bownds worked under the additional assumptions that the binding was via a Schiff Base (as suggested by Hubbard) and the chromophoric material was unique in presenting an absorption spectrum with isotropic peaks at 382 and 498 nm. Therefore, the work is usually reported under a heading including the name rhodopsin. As shown in Chapter 5, the structural requirements on the chromophores of vision are not compatible with a Schiff Base. Furthermore, their spectral peaks of interest are not isotropic and do not occur at 498 nm, although they do exhibit a (non-functional) peak near 498 nm when in dilute solution. An astronomical number of materials, of many types, exhibit an absorption peak at 382 nm. The peak at 498 nm is also shared by a large number of chromogens of vision along with many other conjugated non-resonant molecules.

Corless, et. Al questioned the assumption that the disks were formed of rhodopsin and left open the option that they were actually studying simple opsin. They also focused on the material under study as a protein that made up more than 90% of the disk mass. The fact that the chromophores of vision are not combined with the opsin before being secreted from the Inner Segment has been confirmed by the work reviewed in Sections 4.6.2.3 & 7.1.

Between Bownds (1967) and Corless et al. (1982), Singer & Nicolson reviewed the work of another group supporting the Corless et al. position. They made the following observations:

- “This membrane system is unusual in that it contains as its predominant, if not only, protein component the pigment rhodopsin.”
- “These studies are particularly noteworthy because they involved a membrane which, by conventional electron

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microscopic techniques, appears to show long-range periodicity over its surface.”

The physical characteristics of the disks are dominated by the structural properties of the opsin substrate. The thin coating of chromophoric material is frequently removed in the process of preparing the disks for study.

As indicated above, the disks in their virgin form are made of protein secreted by the Inner Segments of the photoreceptor cells. The secreted material appears to form a planar liquid crystal based on the Nilsson electron micrograph. This strip of material is folded prior to breaking and extrusion by the calyx. The nature of the material provides an insight into this folding and breaking. Low resolution electron micrographs of Outer Segment cross-sections frequently indicate a folding at one side of a disk but not at the other. This is probably due to the asymmetrical internal structure of the protein, opsin. It appears that it bends easily back upon itself to form a bilayer with a hydrophilic external surface. It does not easily bend to form a bilayer with a hydrophobic external surface. Thus a disk is generally formed that exhibits a shape like the eye of a needle along one edge when sectioned. The other sides may not exhibit this feature. More study is needed of the fate of any exposed hydrophobic surface.

As initially formed, each disk is approximately 2.0 microns in diameter and on the order of 165 Angstrom thick. Immediately after formation, they are grooved by the calyx before coating with chromophore and having the dendrites laid into the grooves. As developed in Section 4.6.2.3, the disks do not contain nor are they coated with chromophore when formed. No chromophoric material is found in the Inner Segments, although small amounts of the growth vitamin and chromogen, retinol, are found in the segment. The actual chromophores, the Rhodonines, are transported from the RPE via the IPM to near the Inner Segment/Outer Segment interface. They pass into the cup of the Inner Segment and are coated onto the appropriate disks at that location. The coating may completely enclose the protein substrate in order that can transport excited electrons from their point of generation to the dendrites.

4.3.5.2.1 Structure of the disk substrates based on the literature

It is routine in laboratory studies of the Outer Segment, to employ detergents to separate the disks. This theory proposes that the chromophoric and substrate material are normally solubilized separately. There are several crucial results of this process. First, the chromophoric material is separated from the substrate material. Second, the chromophoric material is frequently oxidized back to a non chromophoric state. The resulting liquid mixture is then processed further to obtain a precipitate consisting primarily of the substrate. The process frequently does not isolate and preserve the chromogenic component of the mixture.

The in-situ structure of the protein substrate is not known because of experimental complexity. However, it appears to be ordered. On the assumption that it is ordered and that it will assume the same order after its removal with detergent and reconstitution, Schertler & Hargrave have provided some useful material117. Their figure 1 shows reconstituted substrate material from frogs. The images and substrate dimensions look very similar to images by others obtained after less complete solubilization of Outer Segment disks. However, the images only show rod or strip shaped crystals, although it is possible that they selected images showing only cross sectional views. Schertler & Hargrave proceeded to use optical diffraction techniques to determine the liquid crystalline structure of these reconstituted substrates. They showed that there were subtle differences in the crystalline structure of the reconstituted material depending on the concentration of detergent in the solution. In one case, the crystals were of form p2. In the second, they were of form p221. They also noted that in some cases, they obtained planar sheets of material consisting of “two superimposed layers.” In their figure 2, they showed the actual diffraction patterns. These show a pattern distinctive of a multilayered material that has been folded along a particular crystalline axis. In this case, the pattern is hexagonal with four of the six sides more distinct than the other two. Corless et. al. have shown two cases of a similar diffraction pattern obtained from more obviously planar crystals. They also found the crystalline form to be p221. In their figure 1, no fold is obvious in the planar crystal and no fold is indicated by the diffraction pattern. In their figure 3, they show a square diffraction pattern indicative of a single fold, in agreement with the electron-micrograph image.

By using larger liquid crystals of the reconstituted material (but still smaller than the diameter of an in-situ disk), Corless was able to determine additional information about the unit cell of the crystal. They found the two-dimensional unit cell to be orthorhombic with dimensions of 47 ± 1 by 151 ± 2 Angstrom. They also found that in their planar sheets, the molecules are arranged in a herringbone pattern, undoubtedly due to the affinity between their

asymmetrical terminals. There is general agreement that the dimensions of the individual molecules are 28 by 39 by ~64 Angstrom\textsuperscript{118}. The work of that group also shows that the material studied was clearly a protein and not a lipid or lipoprotein.

Corless, et. al. did not present any material concerning the formation of liquid crystalline sheets in the orthogonal direction to the sheets formed on a carbon-coated grid suitable for electron microscopy. They did report a variety of other crystalline structures based on the same unit cell that occurred during the precipitation procedure. There is a strong possibility that the actual liquid crystals used in the disks are folded from a planar material where all of the polar molecules have their hydrophilic terminals on one surface associated with a temporary deposition substrate. This arrangement would result in a folded bilayer sheet thickness on the order of 128 Angstrom before coating with a chromophore. This arrangement would not necessarily conform to the unit cell described above. After coating on both sides with a Rhodamine chromophore (of about 15 Angstrom thickness), the total disk would have a thickness of from 150 to 165 Angstrom. This range is in excellent agreement with the above data at the histological level. This arrangement would also be analogous to a typical lipid membrane found in a cell wall. It may follow that the polar protein-based disk substrate was formed on a temporary polar substrate consisting of the Inner Segment plasma membrane.

None of the above crystallographers presented any information showing their reconstituted disks exhibited any photosensitivity.

Hargrave has presented several papers describing a reconstruction of the rhodopsin molecule. In 1982, both skeletal and space filled models were presented based on a partial sequencing of the rhodopsin molecule\textsuperscript{119}. The 1982 representations were described as hypothetical in the text but not in the captions to the figures. In 1983, he and Dratz presented a paper containing a group of four caricatures\textsuperscript{120}. For the first time, he assigned dimensions to the putative lipid membrane of the disks. However, the dimensions were based on gross calculations related to assumed molecular and disk volumes. The thickness of the putative lipid based disk does not agree with the electron microscope images of Nilsson (See Section 4.3.5.3.1) or the estimates by other investigators. The estimated center-to-center spacing of protein molecules of 56 Å does not appear consistent with the caricature in their Fig. 2. The 2001 paper of Hargrave provided a more complete space filled model of the molecule that appears to supercede the 1982 results. It gives the dimensions of the rhodopsin molecule of frog as 28 Å x 39 Å x 64 Å in height. While figure 1 of that paper shows the molecules of the protein imbedded in the structure of a disk membrane containing a significant fraction of smaller molecules, it did not give the thickness used in the 1983 paper. An alternate interpretation of the structure of the disk will be presented below. In this alternative, the protein molecules (present at a concentration of 94% by some estimates) are placed side by side in a sheet and folded to form the disk. There is no requirement for any lipid material in this configuration. The thickness of this configuration agrees well with the measured thickness of the disks.

4.3.5.2.2 Conflict between crystallographers, microscopist and physical chemists

There appears to be significant conflict between the material of the crystallographers in the above section and the results presented by Nilsson in Section 4.3.4.4. It is important to distill the situation addressed by the two groups. Nilsson presented a high magnification (transmission) electron-micrograph of the cross section of an intact frog disk. His resolution was limited to 20 Angstrom and he did not discuss the material shown as electron dense areas within the layers of the disks or compare that material with the material causing similar contrast areas external to the disks. He showed two rows of electron dense areas within each disk layer with the two rows of high electron density aligned into columns. He provided considerable statistical data based on his experiments. The imagery does not suggest an orthorhombic crystalline lattice within the individual layers.

Corless, et. al., on the other hand, present mathematically reconstructed images of the crystalline structure within a reconstituted single (and folded) planar film of putative rhodopsin (possibly only opsin). The reconstituted film was crystallized onto a non polar substrate by painting with a fluid mixture containing the solubilized protein. The results were critically dependent on the concentration of the detergent (Tween 80) in the fluid mixture. The resultant monolayer of liquid crystalline material exhibited an orthorhombic unit cell consisting of molecules described as

\textsuperscript{119}Hargrave, P. et. al. (1982) The carboxyl-terminal one-third of bovine rhodopsin, Vision Res. vol. 22, pp 1429-1438
\textsuperscript{120}Dratz, E. & Hargrave, P. (1983) The structure of rhodopsin and the rod outer segment disk membrane. TIBS, April, pp 128-131
dimeric. The dimeric characteristic suggested that the underlying molecules were amphiphilic. The thickness of the individual layers given by Nilsson agrees quite well with the molecular length of the material given by Corless, et. al.

Dratz & Hargrave have presented a quite different picture based on low-angle x-ray crystallography\textsuperscript{121}. They suggest the individual layers of the disk are about one half as thick as the above authors while giving a slightly larger disk spacing for the frog rod Outer Segment. Based on a single reference to Miljanich, they also provide a set of computed values with regard to the physical spacing of the constituents of the individual layer. It is hard to follow the calculations provided and it appears they are not consistent with the caricature provided (which is labeled arbitrary but follows the conventional wisdom). The caricature shows the rhodopsin molecules widely spaced. However the numerics given are for a surface area of the rhodopsin molecule (assumed to be only opsin in this work) of 3400 sq. Angstrom (radius 68 Angstrom) and a most probable distance between neighboring rhodopsin molecules of 56 Angstrom. These numbers suggest the layer is composed entirely of rhodopsin with no room for other lipids.

Section 5.5.3 of this work reviews the knowledge base relevant to liquid crystals. An important fact is that liquid crystalline materials are polymorphic. The precise crystalline form of a liquid crystal is highly dependent on the environment in which it is precipitated. Both the temperature, the solvent and the substrate are critical parameters. Most biological materials are amphiphilic and very sensitive to the substrate upon which they crystallize. It appears that the results of Corless, et. al. concerning the unit cell would be different if the material had been precipitated on a polar substrate. Precipitation at an air-water interface would probably have resulted in a crystalline structure in agreement with Nilsson’s imagery. In this case, the chromophoric material would be found in the smectic type A liquid crystalline formation. Such a crystal could be imbedded and sectioned to provide an electron micrograph very similar to that of Nilsson.

Future investigators may need a more refined model to use in experiment design. Nilsson was unable to resolve the chromophores proposed here. Corless, et. al. probably discarded the chromophores in their selective purification process. Dratz & Hargrave appear to have only considered the substrate on the assumption, from the common wisdom, that it contained the chromophore within the protein. It appears that the imagery of Nilsson presented the protein material as black with white areas of high electron density usually associated with heavy atoms. The Corless, et. al. imagery shows the protein molecules as white on a black background with no internal detail indicative of heavy atoms.

The large amount of imagery in Nilsson show an extremely well ordered substrate lattice. Although there are occasional dislocations, there are no signs of any inclusions or “gates” passing through these lattices. Unger discusses the potential locations of the carboxyl and amidic terminal of the proteins but does not demonstrate the nature of the high electron density regions of the lattice shown by Nilsson. He does state that these regions cannot be interpreted in terms of trans-membrane helices. Schertler\textsuperscript{122} has recently provided a review. While this author disagrees with a majority of the background material in this review, Schertler does describe a ribbon diagram for the protein that might suggest that the high electron density regions are due to observing helices #4 and #7 head on. However, this interpretation would require that Nilsson’s imagery fortuitously recorded all of the lattices from the same angle relative to the orientation of the protein molecules. In some recent papers, each protein molecule of the single disk layer has been assumed to contain a “gate” between certain of the helices. This interpretation would allow for the presence of gates in the absence of other lipids. However, the mechanism of gate operation remains undefined and a gate mechanism is not required by this work.

In summary, the predominant element of the disk is the folded layer of the passive material opsin. Opsin is found to be in the liquid crystalline state of matter and to be amphiphilic. The hydrophobic terminals of the opsin molecules are found facing each other on the inside of the folded structure. The exact nature of the space between the two molecular layers is not known but appears to be unimportant to the operation of vision. The hydrophilic terminals are facing outward and form a surface compatible with the hydrophilic character of the chromophores. One of the chromophores of vision forms a film on the surface of each disk. All of the disks in a single Outer Segment are coated with the same chromophore. The choice of specific chromophore may be determined at an initial “seeding” of the disk stack during incubation of the fetus. This seeding may be temperature sensitive and account for the normal, and possibly abnormal, distribution of the specific chromophores within the retina. Alternately it may be controlled by or supported by the material found within the inter-disk matrix, IDM. The complete disk stack is


immersed in a material that differs sufficiently from the IPM to cause a distinct boundary to be observable along the outside perimeter of the disk stack. This boundary has frequently been defined as a plasma membrane in the general vision literature. The assumption being that this boundary is part of the plasma membrane of the photoreceptor cell. However, there is no substantiation for this designation in the cytological literature. Beyond the fiftieth disk in the stack from the Inner Segment, there is no published record of a biological membrane surrounding the Outer Segment.

4.3.5.3 Other characteristics of the disks

There are many characteristics of the disks that do not relate to the physical arrangement of the substrate, opsin. These include the molecular, quantum mechanical and semiconductive properties associated with the chromophores and their relationship to the microtubules (dendrites) of the photoreceptor cell.

Table 4.3.5-1 provides a summary of the dimensions associated with the disks. While this figure is compatible with the Hogan and Nilsson figures, it provides considerable more detail that illuminates the overall operation of the Outer Segment.
TABLE 4.3.5-1

NOMINAL DIMENSION OF HUMAN DISKS & DISK STACK

Overall dimensions of a disk stack

<table>
<thead>
<tr>
<th>Dimension</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length</td>
<td>50 microns, nominal</td>
</tr>
<tr>
<td>Diameter</td>
<td>2 microns, nominal</td>
</tr>
<tr>
<td>Number of furrows in stack</td>
<td>9</td>
</tr>
<tr>
<td>Furrow depth in disk stack</td>
<td>0.5 microns, from periphery, (~50% of the radius)</td>
</tr>
</tbody>
</table>

Overall Dimensions of disks

<table>
<thead>
<tr>
<th>Dimension</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter</td>
<td>2.0 microns</td>
</tr>
<tr>
<td>Depth of furrows</td>
<td>0.5 microns</td>
</tr>
<tr>
<td>Thickness</td>
<td></td>
</tr>
<tr>
<td>At fold</td>
<td>220 Angstrom</td>
</tr>
<tr>
<td>At center</td>
<td>160 Angstrom</td>
</tr>
<tr>
<td>Disk to disk spacing</td>
<td>250 Angstrom</td>
</tr>
<tr>
<td>Disk velocity along stack</td>
<td>300 nm/hr. (7.2 microns/day)</td>
</tr>
<tr>
<td>Nominal disk lifetime</td>
<td>7 days/ 1 week</td>
</tr>
<tr>
<td>Nominal disk formation rate</td>
<td>10-12/hr/cell</td>
</tr>
</tbody>
</table>

Associated Dendrites

<table>
<thead>
<tr>
<th>Dimension</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length</td>
<td>50 microns max.</td>
</tr>
<tr>
<td>Diameter of dendroplasm</td>
<td>250 Angstrom</td>
</tr>
<tr>
<td>Diameter of reticulum</td>
<td>100 Angstrom</td>
</tr>
<tr>
<td></td>
<td>Also called microfilaments</td>
</tr>
</tbody>
</table>

Dimensions of liquid crystal monolayers

<table>
<thead>
<tr>
<th>Element</th>
<th>Thickness</th>
<th>“a” vector</th>
<th>“b” vector</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (Opsin)</td>
<td>lattice</td>
<td>64 Angstrom</td>
<td>47 Angstrom</td>
</tr>
<tr>
<td></td>
<td>molecule</td>
<td>64 Angstrom</td>
<td>28 Angstrom</td>
</tr>
<tr>
<td></td>
<td>feature spacing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chromophore (Rhodonine)</td>
<td>lattice</td>
<td>15 Angstrom</td>
<td>~5 Angstrom</td>
</tr>
<tr>
<td></td>
<td>molecule</td>
<td>~3-12 Ang. (variable)**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>auxochrome spacing</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* The relationship between the “a” & “b” vectors of lattices and those of molecules are not known
** based on peak absorption wavelength divided by ratio of speed of light to slow wave velocity: 250. The spacing is asymmetrical with respect to the length of the molecule.

4.3.5.3.1 Arrangement of the disks at the molecular level from the literature

Livrea & Packer made an observation in 1993 based on the conventional wisdom. “Studies of the details of the positioning of retinoids within lipid bilayers and the specific interactions of retinoids with the various lipids that comprise biological membranes are scarce\textsuperscript{123, 124}. This is understandable in the light of this work. The retinoids comprising the chromophores of vision are not found in association with lipids. They are intimately associated with the protein double layer forming the disks.

Figure 4.3.5-8(1) shows a caricature by Vanderkooi and Sundaralingam\textsuperscript{124} showing their conception of the location of chromophores in relation to the substrate of the disks. There have been many similar caricatures in recent years. They generally show a complex immersion of chromophores within the chemical milieu of the substrate. Although the dimensions of the above authors are similar to those of Hogan, they are different (and more comprehensive):

<table>
<thead>
<tr>
<th>Case 1</th>
<th>Pitch</th>
<th>275-300 Å</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coating thickness</td>
<td></td>
<td>75-80</td>
</tr>
<tr>
<td>Substrate thickness</td>
<td></td>
<td>0-10</td>
</tr>
<tr>
<td>Max. thickness @ edge</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nom. thickness (ctr.)</td>
<td></td>
<td>150-160</td>
</tr>
<tr>
<td>Auxochrome spacing (c/c)</td>
<td></td>
<td>40</td>
</tr>
<tr>
<td>Chromophore pitch (c/c)</td>
<td></td>
<td>60-70</td>
</tr>
</tbody>
</table>

This work suggests the shaded area labeled lipid in the caricature of Vanderkooi and Sundaralingam is primarily the protein material, rhodopsin. It is proposed the circles in the upper caricature and the white blobs in the lower electron-micrograph are concentrations of charge within the molecular structures of the protein. The support for this proposal follows in Sections 4.3.5.4 and 4.3.5.5. Rodieck\textsuperscript{125} provides a discussion of the structural form of the disks. His figure V-31, interprets this data to show two rows of auxochromes with the auxochromes staggered as in (1) above. The interpretation is that the auxochromes are on separate discrete molecules and not associated with a liquid crystalline array. The two rows are shown at a spacing of almost exactly 4.0 nm (40 Ångstrom being the equivalent dimension used at the molecular level). However, since the data underlying this figure is highly inferential, as opposed to direct, alternate interpretations must also be considered.

For unclear reasons, Dratz & Hargrave give much smaller dimensions for the disk substrate (that they show as independent of, but enclosed by, a putative surrounding membrane\textsuperscript{126}). They give the overall thickness of each layer of the disk as only 45 Ångstrom with what they call the bilayer hydrocarbon layer of only 28 Ångstrom. They give the total center-to-center disk spacing as 295 Ångstrom. This would leave the space between layers of the same disk at about 200 Ångstrom.

There is a major problem when attempting to rationalize these caricatures with the electron micrographs (2) from Nilsson\textsuperscript{127}. These show a much more uniformly structured substrate with no incursions. The resolution of the image is about 20 Ångstrom. This view clearly shows two rows of features aligned in columns perpendicular to the substrate surface, although there are clearly faults in the structure. These features are usually described as electron opaque in electron microscopy. They are separated by about five nanometers or 50 Ångstrom. Such a large dimension is far longer than the chromophores defined in PART B and more likely represent features within the opsin substrates. They could be the carbonyl ends of the protein molecules since oxygen atoms are electron opaque.

Rodieck’s discussion highlights the fact that the spacing between the rows is a variable among different investigators. Values of four, five and seven nm have been given by different sources.

It is clear that there are two prominent areas of high electron density separated by about 40-50 angstroms and aligned perpendicular to the surface of the substrate. It is also clear that the intervening space between the substrates is occupied by an electron opaque material. It is proposed that it is this area that contains the actual chromophores and chromogens of vision.

Vandrkooi & Sundaralingam give 40 Angstrom as the nominal spacing of the electron opaque concentrations while Nilsson gives 50 Angstroms for this same dimension. The measurements may be for different conformations of the substrate material or relative to different locations on poorly resolved features.

It should be noted that the orderly lattice structure recorded by Nilsson does not support many of the caricatures showing a highly convoluted series of helices of rhodopsin interdigitated among the molecules of a lipid comprising the surface of a disk. These caricatures generally trace back to a proposed model of a disk by Hargrave. Similar models frequently appear in relation to the putative gates in the putative plasma membrane surrounding the Outer Segments.

4.3.5.3.2 Molecular level characteristics based on radiography

Blasie, et. al\(^{128,129}\) have studied the structure of the disks in some detail using both electron-microscopy and low-angle x-ray diffraction. The title of their second article is informative. They found the photopigments to be arranged in a planar, liquid-like arrangement. An array of molecules that is liquid-like is now known as a liquid crystal. Although they frequently describe the pigment molecules as within the membrane, at other times they use the expression within the disk structure (which more easily accommodates the concept of a liquid-like array on the surface of the disk membrane). It is this latter interpretation that is more consistent with the high resolution electron-micrographs of Nilsson (1965). Their findings that: the individual particles “have a core of uniform electron density 38-40 Angstrom in diameter with a total diameter of 44 to 46 Angstrom, and occur in a planar or two-dimensional liquid-like arrangement within the wet frog retinal receptor disk membranes.” is completely consistent with this work if the word structures replaces the word membranes. Their paper describes these particles as ~40 Angstrom in diameter with a cell side of ~70 Angstrom. These numbers are completely consistent with the previous discussion. Since they were working at the resolution limit of their electron-microscope and the taking of Fourier Transforms of X-ray diffraction patterns does not give precise dimensional data, it is not clear that the term ~40 Angstrom should be considered the dimension of a spherical particle. It could just as well be considered the mean diameter of a highly ellipsoidal particle. The Blasie articles provide their original imagery. Their estimates of the molecular weight of these particles is entirely dependent on their assumption that the particles are either lipid or protein. The values were determined based on the bulk densities of these materials and the use of the 40 Angstrom number as if it was a non quantum-mechanical, hard, value. It is expected that the molecular weight based on the gross density of a long chain resonant retinoid such as the Rhodonines in a planar lattice with a cell side of ~70 Angstrom would be quite different. They conclude: “If our interpretation of the low-angle patterns are correct, the 40 to 50 Angstrom particle is not a spherical lipid micelle.”

4.3.5.3.3 Quantum level characteristics

The arrangement of the chromophores in a liquid crystalline film on the surface of the opsin substrate has a profound impact on the quantum mechanical properties of the chromophores. The energy levels of the individual molecules are shared and thereby broadened. This accounts for the broadened and anisotropic spectral absorption of the structure compared to that of the chromophores in dilute solution. This subject will be discussed in detail in Chapter 5.

4.3.5.3.4 Solid state characteristics

The outer segment does not appear to have a known analog elsewhere in the animal body. There is an underlying simile to a hair follicle and there may be similes to the outer structure of the taste buds. Its proposed properties are all familiar in other scientific contexts but do not appear to have been recognized in the literature related to vision, certainly not in-group. The outer segment is a marvelous example of optimal use of a broad range of tools. To appreciate the functional


operation of this structure, it is necessary to have a familiarity with a number of concepts from quantum electronics, thermodynamics, noise theory, liquid crystal chemistry and of course dye chemistry. The actual dye chemistry aspects, related to the absorption properties of the photoreceptor will not be discussed until PART B. This section will begin with the excitation of the liquid crystal coating the substrate and made up of the chromophores that actually sense the photons. This liquid crystalline mass has several unique features of interest here. First, in the plane perpendicular to the long axis of the outer segment, the overall liquid crystal can be considered a “space frame” made up of a series of sheets of absorbing material separated from each other by alternate sheets of the substrate material and an interstitial fluid. The result is that the photons encounter a dissipative form of a Luneberg lens, and similar to what is used in the termination of a waveguide by a dummy load. The object is to absorb the incident radiation energy in a progressive fashion without causing a significant step function in the dielectric constant of the medium. A step function in the dielectric constant of the medium would cause a reflection of the unabsorbed photons at the step, a re-emergence of the photons at the entrance surface and a reduction in the overall absorption coefficient of the structure. In the present case, the variation in the index (if definable at these dimensions) is occurring at a pitch which is shorter than the wavelength of visible light. If the index is averaged over a distance equal to the wavelength of visible light, the average index would approximate that of the interstitial fluid and the substrate, i.e., 1.33.

The ridge on each side of the individual disk at its edge is a very important feature with at least three significant functions. First, it helps establish the spacing of the liquid crystalline material in the space frame. Second, it brings the separate layers of liquid crystalline material into intimate contact so that quantum level energy particles (they will be assumed to be electrons here but they could be described as excitons or phonons in the analytical contexts) can be conducted along the length of the outer segment. Third, on the other hand, these same ridges can act as a relative impedance to the unrestricted transfer of electrons. This results in a restriction in the number of particles able to pass a given point and which could become limiting at high flux levels.

And finally, the serrations in the edges of the disks near the inner segment end provide room for the dendrites of the nerve to make intimate contact with the space frame of the chromophores. As indicated above, the refraction time of the single nerve (or the dendrite), limits the rate that the excited electrons can be de-excited at the nerve/outer segment interface. The space frame consists of a very large number of chromophore molecules that each contain multiple n-electrons that can be excited by the incident radiation. If this total number of n-electrons, are excited at a higher rate than the nerve is able to de-excite them, a large number of excited electrons will exist in the space frame and the number of electrons remaining subject to excitation will be decreased, i.e., the absorption cross section of the space frame will be decreased significantly (in the lexicon of the vision researcher, the outer segment will become bleached). This mechanism is one, but not the only, mechanism responsible for the “dark adaptation” process in the eye.

One additional property of this space frame of chromophores in the liquid crystalline state which is probably unique in the animal system is the ability of the quantum level energy particles in the liquid crystal to be added together in order to accumulate enough energy to overcome the minimum energy threshold. This is the same capability that has been so successfully exploited in the silver halide photography world wherein dyes sensitive to longer wavelengths than the associated silver halide crystals are able to absorb the incident radiation, collect the energy into 2, 3 and even 4 quantum packets and then excite a single silver sulphide atom, located on the surface of the silver halide grain, to produce an atom of free silver.

4.3.5.3.5 Estimates of the number of chromophores per disk

Without knowing the exact lattice constants and inclination angle of the chromophoric material, it is not possible to calculate the exact number of molecules in a monolayer of chromophore contained within an area given by the lattice dimensions of the protein substrate. The range would be 44 molecules per unit area based on the molecular dimensions to as high as 88 based on the lattice spacing of the protein and the minimal lattice spacing of the chromophore when arranged with axes perpendicular to the surface. Shichi\(^{130}\) gives the molecular weight of Opsin (his bovine rhodopsin, 41399, less retinal, 284) as 41115. He also provides a tabulation and a sequence for the constituents of the protein. Using the lower number of molecules of chromophore per molecule of protein, the percentage of protein in the complex is about 70% with the remaining 30% being chromophore. This number is in reasonable agreement with the 80% given by Shichi without discussion.

There are a variety of estimates of the number of chromophoric molecules per photoreceptor in the literature. Leibovic (1972) says 10\(^6\). The method of arriving at these other estimates is nebulous.

Using bulk sizes, Oyster has given a number of 150,000 molecules of the chromophore covering both sides of each two-

micron diameter disk\textsuperscript{131}. He comes to the interesting conclusion that without regeneration of the chromophores, they would all be bleached away in about 2.5 minutes in bright ambient sunlight. How the excited molecules are removed from the disk and recirculated to the RPE and back in less than a fraction of 2.5 minutes, as called for in his figure 13.29 and featured in the conventional wisdom of the literature, is not addressed.

Based on the above analyses, and the orientation of the chromophore molecule axes as perpendicular to the surface of the disk, each of the sheets of absorbing material, taken as 2.0 microns in diameter contains approximately 10 to the 7th power of chromophore molecules (considerably more than calculated by Oyster), each with at least 3 n-electrons subject to excitation. The overall mass of chromophore available in a single Outer Segment of 50 microns length is therefore on the order of 4 times 10 to the 10th power of chromophore molecules, each with multiple n-electrons available.

4.3.5.4 Sequencing and mapping of the structural protein Opsin

[XXX consolidate with 5.5.10.6 or reference. one section applies to the substrate and cell, the other to the chromophores]

It is an early day in the sequencing and mapping of proteins like opsin. Rarely, if ever, do two investigators report the same sequence for even part of the presumed same opsin molecule. Dratz & Hargrave provided the first complete sequence of a putative rhodopsin in 1983. Rarely do they agree on the exact constituent structures and, to date, no investigator has found any retinoid as a ligand in their residues. As the rules related to polymorphism have become better known, some of these differences have become understandable.

4.3.5.4.1 Sequencing of Opsin

Section 5.5.2.1 reviews the search for the chemical connection between opsin and a retinene in the putative compound, rhodopsin. This section will review the sequencing of various proteins in the context of opsin being only a structural protein. Rhodonine is deposited on this protein as a liquid crystal. The two materials are only joined by hydrogen bonds. In this sense, the two materials form a composite and not a compound.

Without any retinoid being found in a residue of the putative rhodopsin, the materials investigated will be assumed to be only opsin.

Heller described opsin as a structural protein consisting of 235 amino acid residues with a molecular weight of 26,400. He said that 118 of those residues had nonpolar side chains\textsuperscript{132}. He performed an exquisite set of experiments, based on the knowledge-base of the era. They were designed to overcome some of the compromises of earlier work. At that time, he was unable to determine the terminal amino groups of what he described as bovine visual pigment. However, he could explore all of the protein fragments comprising opsin (consistent with a yield of 70-90%).

Heller was unable to purify native visual pigment as a complex based on gel filtration. However, good results were obtained using liquid chromatography and other techniques. He stressed that native visual pigment and the denatured apoprotein displayed different signatures using chromatography.

Heller made several points: “The term ‘native’ is somewhat ambiguous and ill defined. It is used generally in the sense that, as far as can be determined experimentally, the protein under consideration has the same conformation and the same properties it had \textit{in situ}. However, if a rigorous proof is asked for the native state for proteins that are part of an assembled complex, it turns out in many cases that this is more of an assumption than of a proven fact.”

The native material showed peaks at 500 and at 280 nm. Although he anticipated a peak at 350 nm based on earlier Wald data, it was not found. This work suggests the so-called β-peak at 350 nm was due to the presence of a pure retinoid such as retinol in the earlier work. Heller was unable to determine either the amino or carboxyl terminal residues in the 1968 time period. However, he did establish the general composition of the protein. His data reported the presence of 0-2% phospholipids and evidence of carbohydrates in the form of sugar. He subsequently described the material as a glycoprotein. He did not observe any residue containing a retinoid. However, he did present a proposal on page 2917 that perpetuated the Schiff-base proposal of earlier workers. He proposed the linkage was a protonated Schiff-base (he described it as a substituted aldimine) between retinol and lysine. Based on this assumption, he went on to describe possible methods and caveats concerning the excitation of the chromophore.

\textsuperscript{132} Heller, J. (1968) Structure of Visual Pigments, \textit{Biochem.} vol. 7, pp 2906-2920
In his 1970 paper, Heller focused on a single glycopeptide nearest the amine terminal of the bovine visual pigment. This material consisted of nine amino acid residues. The terminal residue was found to be methione. He found that all of the sugar contained within the molecule was associated with aspartic acid at position 2 of the glycopeptide. He suggested the sugar complex acted as a stereo chemical marker during the assembly of multiple proteins into the disks of the Outer Segment. If correct, the sugar would ensure the amine terminal was oriented toward the aqueous surround. He did not address how the sugar isolated the amine terminus from the aqueous surround.

Schwemer, writing in the same year gave different results. He described the opsin from compound eyes, giving the molecular weight range as between 32 and 32 kDa. He describes the protein as containing 348 residues versus the 348 in bovine opsin. He describes the polypeptide chain as folded into seven hydrophobic segments of 24-28 amino acids each and suggests these are primarily nonpolar. These segments are connected by hydrophilic segments. He repeats the position that retinol is attached to lysine, 296 in vertebrates based on Bownds claim. The site of retinoid binding in the compound eye is given as lysine(K)319, although the site is not marked in his figure. An alternate is reported by other investigators is given as lysine 328. His figure 1 treats the visual pigment molecule as embedded in a plasma membrane rather than forming one surface of a disk. Six possible sites of phosphorylation are shown near the amide terminus of the molecule. No location is defined for the sugars found by Heller. The figure apparently originated elsewhere but no citation was provided. The caption was abridged.

Hargrave has written extensively on opsin. His figure 3 in a review shows a resemblance to the figure 1 of Schwemer but it is clearly different. The sequence of amino acids shown for bovine rhodopsin is distinctly different. His figure is for one layer of a disk instead of a plasma membrane. In 1983, Hargrave, et. al. gave a tentative sequence for the complete structure of bovine opsin (putative rhodopsin but without a trace of any retinoid). They claimed their material had a molecular weight of 39,000 and consisted of 350 amino acids. With the exception of only three areas, they claim to have sequenced the whole molecule for which they provided a tentative sequence. By coordinating their findings with those of Ovchinnikov, et. al., a complete sequence was then proposed containing only 348 amino acids. Hargrave, et. al., like Heller, found sugars attached to two amino acids (Asn and Asn) near the amide end of the protein. Hargrave, et. al. placed the amide terminal of the protein in contact with the “aqueous interior of the disk membrane.” The carboxyl terminal is shown in contact with the putative cytoplasm of the photoreceptor cell.

Hargrave & McDowell provided a comprehensive list of previous investigations and introduced additional nomenclature. They numbered the seven helices of the bovine protein they were studying. They also illustrated the two sugars near the amide terminal. These were originally introduced by Ovchinnikov, et. al.

In a more recent lecture, Hargrave did not specify his current estimate of the number of amino acids in the putative protein, rhodopsin (aka opsin in this work).

4.3.5.4.2 Mapping of Opsin

Hargrave has been working on three-dimensional reconstruction of bovine and frog rhodopsin. In 1997, Unger, Hargrave, Baldwin & Schretier provided four views of a reconstruction of the protein at 9 Angstrom. Figure 5 in his 2001 review shows a reconstruction of frog rhodopsin at a contour interval of 2 Angstrom. No location is defined graphically for any sugar or retinoid in these figures. The 2001 figure apparently supercedes the work of Hargrave, et. al. of 1982 where they reported, the retinoid was attached to a lysine 53 residues from the carboxyl terminal based onBownds claim. The frog rhodopsin reconstruction in 2001 followed it’s extraction with a weak detergent, crystallization, electron micrography and then optical diffraction of the electron micrographs. The maps show the molecules filling a space frame of nominally

28 x 39 Angstrom with a height of 64 Angstrom. Using the earlier calculation of the average protein molecule spacing by Hargrave, 56 Angstrom, it is seen that the molecules are very close to each other (depending on the exact array factor and measurement criteria). Using the square root of the sum of the squares, the above molecule has a diameter of 48 Angstrom.

Based on the above numbers, and assuming the protein is free of any retinoid, the surface of a disk can be described by a surface as shown in Figure 4.3.5-9 that is 64 Angstrom thick. The view is created by replicating the energy-density profile presented by Unger, et. al. assuming a simple rectangular molecular array. The circle is 56 Angstrom in diameter. Note how little space is left for any other molecular species. If the average spacing of 47 Angstrom, given by Corless, et. al. (see next section), were used the circle would be smaller and the opsin molecules would need to be brought even closer together. It should be noted that no space is provided for any phosphorylation of the molecule. Neither is any space provided for a ligand of sugar attached to the molecule. This representation is quite different from the commonly reproduced caricature of the surface as a sparse array of protein embedded in a continuum of lipid material (Example, fig. 2 of Dratz & Hargrave, TIBS, April 1983).

Figure 4.3.5-9 A reconstruction of the surface of a disk based on replicating the mid-section energy density profiles of Unger, et. al. The numbers identify the seven helices of the protein. The circle represents a 56 Angstrom diameter.
Based on the above interpretation, **Figure 4.3.5-10** shows the proposed structure of the protein part of the disk surface. The spacing between opsin molecules is between 47 and 56 Angstrom. Both the height and diameter of each molecule depend on the definition of their boundaries at the atomic field level. The helices are not smoothed sided. They show many protrusions and pockets as shown by Hargrave's reconstructions. Hargrave typically shows these molecules with the amide terminal at the bottom. If followed in this caricature, two sugar ligands would be found associated with the bottom of each molecule (Compare to fig. 9 in Hargrave & McDowell). These ligands contain many oxygen atoms. Similarly, the carboxyl terminal at the top includes oxygen. These atoms probably account for the two polar locations found in electron micrographs of the disks.

In attempting to follow Young's concept of the disks as an integral part of a convoluted plasma membrane, Hargrave's group has described the environment above the proteins as that outside of the cell or as the cytoplasm. Similarly, the environment below the proteins is described either as the intradisk space or as the extracellular space. This problem is avoided in this work by considering the disks as free standing structures.

### 4.3.5.5 Proposed composite structure of a disk and its environment

Based on the above discussion, **Figure 4.3.5-11** presents a proposed cross-section of that portion of the disk-chromophore-dendrite complex found in the Outer Segments after fabrication of the disks within the cup and calyx of the Inner Segment. The figure is idealized to show both the structure of the disk stack and the interface with the dendrite of the neuron found within the photoreceptor cell. Because of the aspect ratio of the individual disks, only a small fraction of an entire disk can be shown at this scale. The chosen fraction is that associated with the dendrite interface. The dendrite is shown at an expanded scale to highlight the internal elements of interest. It is virtually impossible to section both the disks and the dendrite (microtubule) in order to obtain a single electron micrograph capturing the detail shown here. This figure is meant to replace the "speculative organizational scheme" of Molday & Molday. It eliminates the phosphoglycerides from the disks, and places the opsin molecules adjacent to each other. One end of each protein is occupied by the sugar ligand. The other end is occupied by the carboxyl ligand. The twisted vertical strands are defined as the microtubules (dendrites) associated with the neural portion of the photoreceptor cell. Hargrave & McDowell noted the presence of these thin filamentary structures in their 1992 paper.

The following paragraphs will address each element of this figure beginning with the disks.
The disks of the “fundamental photoreceptor complex” are taken from the middle of a mature disk stack, from positions greater than #51. Each of these disks consists of two circular liquid crystalline films of the protein opsin merged along their periphery by a fold designed to isolate the hydrophobic ligand of the molecules from the surrounding water-based solution.
Cross sections of disks shortly after formation and/or before shaping by the calyx frequently show edges of the two films of opsin that are not connected by a fold. These appear to be the characteristics of an immature disk. It was frequently claimed in the literature during the 1960-70s that these free edges are contiguous with the exterior plasma membrane of the Outer Segment. However, no electron micrographs have been found of sufficient resolution to demonstrate there was in fact a plasma membrane surrounding the Outer Segment. It is more likely that the free edges temporarily terminate near the transition between the IPM and IDM to be discussed below.

It is also likely that the two films form a fold along the edges of the fissures created in the disks by the calyx.

The opsin film is shown in the figure as a regular liquid crystalline monolayer with a hydrophilic ligand, shown by the large white circles, and an associated hydrophobic ligand, shown by the large white squares. It may be that sugar ligands are located quite close to the hydrophobic terminal. The shape of these molecules are distorted in the area of the fold. It would be expected that the liquid crystal would also be distorted in this area. The lattice spacing of the opsin monolayer is probably similar to that given by Corless, et. al. for a different crystalline form of opsin, 47 Angstrom. Each opsin molecule has a mean length transverse to the surface of the film of about 64 Angstrom. In the plane of the film, the dimensions of the molecule are believed to be 28 by 39 Angstrom. The assumed crystalline form of the monolayer is type A smectic as expected from a monolayer formed by sliding along a smooth hydrophilic surface. Alternately, it could be formed by sliding along a hydrophobic surface with a hydrophilic solution on the other side of the film. It is not the form found by Corless, et. al. for deposition on a non polar substrate. The bilayer substrate is formed by folding the monolayer leaving two hydrophilic surfaces exposed. The upper and lower exposed hydrophobic edges turn inward and the outer hydrophilic surfaces join. This action results in a disk-shaped micelle with a raised edge along the periphery and an exterior that is totally hydrophilic. The resulting bilayer can be considered as of the smectic-cholesteric type with an angle between the two crystalline planes of either 0 or +/-120 degrees. In this figure, as in that of Nilsson, the white areas are proposed to be areas of high electron density. The circular area would logically be associated with the carboxylic group and the square would be associated with the amidic group of the native protein.

The apparent width of the space between the two hydrophobic surfaces is less than a few Angstroms. Nilsson gives the transverse length of each molecule as 63 angstroms, the thickness of the overall bilayer as 123 Angstrom. And the distance between the uncoated hydrophilic surfaces of adjacent disks (neglecting the folds) as 114 Angstrom. The 123 and 114 numbers are slightly smaller than the analogous numbers given by Hogan (see Section 4.3.5).

Nilsson indicates that a precision of two places after the decimal (frequently four or five decimal places in total) was carried in his calculations. A statistical error analysis would show that the results discussed in this Section are good to a maximum accuracy of two digits. The number of measurements is small and, in some cases, data from different species were averaged. In this theory, the specimens described as cones by Nilsson are considered immature or malformed photodetectors that should be discarded. Note that a cone shaped outer Segment cannot be sustained by the disk stack growth process for more than 7-20 days depending on species.

The folded substrate formed by the protein is coated on the outside by a liquid crystal monolayer film of one of the four possible chromophores, Rhodone(n). This is the active photodetection layer of vision. This layer can be as thick as 15 Angstrom if of the type A smectic form (as shown) or as thin as 10 Angstrom if of the type C smectic form. It is believed that the material is type C. If the angle associated with type C is 45 degrees, the diagram would still show the high electron density areas aligned perpendicular to the substrate surface. In both cases, the nominal diameter of the chromophore molecule is five Angstrom. This diameter is well below the resolution of Nilsson’s imagery and absent from the reconstituted material of Corless, et. al.). The electron micrographs of Nilsson (1964) and Robertson (see Nilsson, 1964) may have shown the faint pattern on the surface of the disks due to this liquid crystalline coating. There orderly arrangement may also account for the extensive Moire Patterns usually seen in similar but lower resolution micrographs of the surface of a disk.

It is the liquid crystalline material of the chromophores that is in quantum contact with the dendritic structure of the photoreceptor cell. At higher resolution, it is predicted that this coating will be resolved into a rectilinear array similar to the high electron energy locations in the protein crystal of the disk substrates. However, the two high electron energy locations in each molecule of the liquid crystalline coating will correspond to one of the heavy atoms of oxygen forming the resonant conjugated structure that is sensitive to light. These two atoms will be separated by a distance that will vary with the chromophore. The distances between the auxochromes of the four chromophores of vision will be in the ratio of 4:5:7:9 depending on the chromophores present, UV:S:M:L. The chromophore molecules are shown with their long axis perpendicular to the surface of the disks. In the actual situation, they are probably formed at a more acute angle to the surface as is typically found in the chromophores of photography (10 to 20 degrees from perpendicular).
The viscous but liquid material (the inter-disk matrix, IDM) in the space between the disks consists of a saturated solution of at least one of the chromophores of Rhodonine, and a highly concentrated solution of the metabolic materials used in the electrostrolytic process of vision. The un-precipitated chromophoric material in the inter-disk space is not in the liquid crystalline state and is transparent to radiation in the visual region of the spectrum. While in the liquid state or complexed with a transport protein within the IPM, the Rhodonines are not photosensitive in the visual spectrum. It is only when they are in the liquid crystalline state and the liquid crystal is in contact with an appropriate quantum interface that the material is an effective photodetector.

It is likely that the surface, usually described as a plasma membrane surrounding the disk stack, is in fact a non biological transition between the precipitated electrostrolytic material, the protein wool noted above, and the rest of the IPM.

The nature of the electrostrolytic material is discussed in Section 7.7.

Two of the important features depicted are the presence of the interface between the chromophoric coating and the underlying protein substrate and the location of the microtubules constituting the dendrites of the photoreceptor cell. The chromophoric material is attached to the opsip substrate by hydrogen bonding. This bonding method does not disturb the chemical nature, the stereo conformation, or the electronic configuration of the chromophore. There is negligible physical space between the opsip layer and the chromophore layer. The liquid crystal of the chromophore associated with each disk is in intimate contact with the adjacent dendrite at the quantum level.

Only one half of a dendrite, commonly labeled a microtubule in the literature, --or a cilium as it approaches the colax-- is shown in this figure. It is shown at an expanded scale in this figure so that the important cytological structures can be seen. Ottoson gives the typical diameter of a microtubule in this area as 240 Angstrom. He says it is filled with neuro-filaments on the order of 100 Angstrom in diameter. These dimensions are in good agreement with those needed to create an active semiconductor device in this area. The dendrite is shown at the extremes of the disks. However, a dendrite is usually found tucked into each of the fissures around the periphery of the disks. A single dendrite traverses essentially the entire length of the active part of the disk stack. It consists of a concentric structure consisting of the smooth dendrolemma, a layer of hydronium, and a reticulolemma enclosing the dendroplasm. It is the combination of the dendrolemma, the hydronium crystal and the reticulolemma that forms the Activa of the dendrite. It is the hydronium crystal, serving as the base of the Activa, that is stimulated by the excitons formed in the chromophoric material by light. This stimulation generates a free electron in the base that is amplified by the Activa. The resulting electron current flows out of the emitter terminal of the Activa into the dendroplasm where it travels through the calyx area of the photoreceptor cell to the Activa that acts as a distribution amplifier terminating in the pedicle of the cell. The region surrounding the dendrite is filled with metabolic material used in the electrostrolytic process powering the Activa.

4.3.5.6 Summary of Section 4.3.5

In summary, the outer segment consists of a very complex and very highly optimized structure for the efficient absorption of photons, the collection of excited electrons (and their short term storage if necessary), the transmission of such excitons to the vicinity of the nerve portion of the photoreceptor cell, and the sequential de-excitation in a rate-controlled quantum level process (that amounts to irritating the nerve in the vernacular). As will be shown in Chapter 12, the nerve portion of the photoreceptor cell converts this excitation into free electrons, amplifies the resulting current and delivers the resulting signal to its synapse with the signal processing neurons.

A disk structure is developed that successfully meets the functional requirements of the disks without the presence of any non-opsin, non-chromophore, lipid material except possibly as a lubricant during extrusion. The disks are formed in a manner answering questions #3 & 4 presented by Shichi and showing that his questions #1 & 2 are poorly constructed.

4.3.6 The cytology of the neural portion of the photoreceptor cell

This section will discuss anatomical structures that cannot be discussed completely without reference to imagery obtained at 100,000x or higher. Such imagery, along with the overall cytology and electrophysiology of the individual neural structures, is discussed in detail in Chapter 10. Findings at this resolution introduce profound distinctions between the classical morphology-based descriptions of neurons and the current electrophysiological description of the individual structures. These structures determine the functional performance of individual neurons and neural system. Discussions

in the pre-1980 literature should be viewed, and reinterpreted, based on the more recent findings obtained at this
resolution.

One profound finding regards the fundamental biological structures. While the cell remains the nominally smallest living
entity within a larger animal, the neural system is based on a different minimal configuration. This configuration is
developed in detail in Section 10.6. The neural system is based on a chain of alternating conduits and Activa-based
amplifiers (Figure 10.10.1-1). In this chain, the housekeeping functions associated with the nuclei are largely
superfluous to the signaling function. More significant is the fact that the so-called plasma membrane of the cell is not
a continuous outer enclosure when examined at magnifications of 100,000x or higher. The fundamental enclosures
surround the plasmas of the individual conduits. Even these enclosures are found to be inhomogenous at the molecular
level in order to accommodate the electrostenolytic and other specialized processes. These inhomogeneities at nearly
every scale make it very difficult to make sweeping statements concerning one or more observed features of the neural
system.

Looking at the nervous portion of the photoreceptor cell in [Figure 4.3.2-1(a)], many definable features and functions
become understandable, although some changes in terminology may be necessary for those working in different
disciplines. The first thing to notice is that this sub-portion of the overall photoreceptor cell looks quite conventional.
It has an axon leading to a specific type of termination. The axon is connected to a dendritic structure and both of these
are supported by a perikaryotic structure.

The only thing that might be considered unusual in this view is the way the dendrites are grouped in a bundle (a cable)
and passed along the side of the area where the outer and inner segments join before spreading out into the structure of
the outer segment. However, this is the very same kind of structure seen in the field of insect vision as part of the
rhabdome. After passing into the area of the outer segment, the cable fans out into a series of microtubules which have
the same size and configuration as typical dendritic endings, a thick walled cylinder or “tubule” with a diameter of
about 200 angstroms and a wall thickness of about 60 angstroms. These are shown clearly in Figure 4.3.xx where
they could be considered as inside the primary dendrite at this point. The histologists will need to get together and decide
whether to call these final structures tubules, canaliculi, neurotubules, microtubules or whatever.

It should be noted that Vinnikov144 in (1965) indicated: “A number of processes--”dendrites “-- of the inner segment are
directed toward the outer segment. The inner and outer segments are joined by a stem provided with 9 pairs of peripheral
fibrils, . . .”

Borwein145 provided one of the few caricatures in the literature that show a signal path from one end of the photoreceptor
cell to the other (although the reticulum of the axon is labeled with the morphological name striated rootlet). It is also
one of the very few caricatures that shows the direction of the incident light and the direction of signal flow. He also
named a variety of internal components but some of the names appear to be based on low resolution micrographs. It is
noteworthy that Borwein did not show a signal path from the basal body of the cilium to the pedicle of the “cone” as he
does for the “rod.”

This section will proceed to discuss the individual morphological features of the neural portion of the photoreceptor cell.
It will begin with the pedicle and proceed distally.

4.3.6.1 The cytology and function of the pedicle

Being one of the more isolated, and hence less integrated, portions of the photoreceptor cell, the pedicle has been studied
for a very long time using light microscopy. The results have not been very rewarding at that resolution. It became
common to speak of two forms of synaptic terminations, the spherule and the pedicles. The increasing complexity of
each of these structures has led to their being described more generically as synaptic bodies but the morphological
designation pedicle is still common.

With the advent of low resolution electron microscopy, some internal structures could be identified as well as more

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Biology, pp. 293-300
Also in Am. J. Anat.  vol. 159(2) pp 125-146
details related to invaginations along the periphery. Boycott & Kolb provided a widely reproduced caricature of the spherule and pedicle in 1973\textsuperscript{146}. They displayed the “synaptic ribbon” connecting the interior of the axon to the surface of the synaptic body. They also showed a scattering of vesicles within the axon terminal and a variety of largely conceptual interfaces with bipolar and horizontal cells.

Only analysis of a set of higher resolution electron micrographs taken from a variety of planes could clarify the above situation. With the availability of computerized tomography, this has now been done. The work of Schein will be discussed below.

4.3.6.1.1 The structure of the pedicle and the associated synaptic complex

A number of researchers have recently provided high resolution microimages of the pedicles and their interaction with the dendrites of the subsequent bipolar and horizontal cells. They show a maturing of knowledge in this area.

Schein has recently prepared a series of tomographic reconstructions of complete synaptic complexes associated with a single pedicle in the fovea of macaque\textsuperscript{147}. The images are stereographic and in color. His work demonstrates that a typical pedicle;

+ may be 5 microns across (2.5 times the diameter of the typical Outer Segment),
+ contains about twenty individual “ribbons,”
+ may have as many as 400 individual basal contacts with neurite spines,
+ may make many individual basal contacts with spines that converge into a single dendrite.

His data reflects at least one type of “ON-cone” interfacing with about 125 ON-midget bipolar neurons. He describes these as all of the Blue-ON type. This data is indicative of the degree of spatial encoding relative to the scene and dispersive encoding relative to the passage of signals along the optic nerve that can be expected in macaque. This latter encoding will be discussed in Chapter 13.

It is likely, based on this work, that the number of spines converging into a single dendrite from a single pedicle is an indication of the net impedance of that connection as represented by the coefficients in the fundamental equations of vision to be presented in Chapter 16.

Vardi, et. al. have provided an excellent caricature of a simplified synaptic complex (only one ribbon and only in one plane) with various notations based strictly on the conventional view of the synaptic junction as a (possibly electrically driven) chemical signal transfer device\textsuperscript{148}. Rodieck has recently provided a caricature of a simplified pedicle in two orthogonal planes\textsuperscript{149}. However, based on Schein’s work, it is primarily of value in pedagogy. Vardi, et. al. obtained a large number of individual images based on 50-100 micron thick sections and examined them at a magnification of up to 42,000x. This is not sufficient magnification for drawing many conclusions. However, it is one of the early studies reporting the “electron dense” regions of tissue near or including the plasma membranes. The lower portion of their figure suggests that all signal processing neurons approach the junction axially. Saito, et. al. show caricatures of this synaptic complex at similar magnification but with the dendrites intertwined with the pedicle in a more three-dimensional configuration\textsuperscript{150}. The dendrites approach the junction perpendicular to the synaptic ribbon. A detailed comparison of the two papers is useful. Saito’s OFF-B type bipolar cell would correspond to the central bipolar shown in Vardi’s figure and the following illustration. This cell exhibits its reverse polarity because of its connection to an amercine type of horizontal cell. Their ON-B(II) bipolar would correspond to a bipolar contacting the axon of the photoreceptor directly. Their work did not discuss or illustrate electron dense areas but they did provide data on the size of the

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arborizations of some of the orthodromic neurons.

Going beyond the caricature and reconstruction, the electron micrographs of Tsukamoto, et. al. are useful in quantifying the above discussion\textsuperscript{151}. They were obtained at x80,000 magnification. While they show detail at the 10-30 nm level, the magnification is not high enough to show the character of the gap junctions clearly. Tsukamoto, et. al. did not differentiate clearly between their adherent junctions and their gap junctions. They did not address the electrical biases related to the junctions. In the absence of this data, their discussion is most likely to apply to inactive adherent and gap junctions. It is not likely to apply to the active junctions of interest in signal transmission.

Figure 4.3.6-1 presents a significantly modified caricature incorporating features from the above sources. This variant is compatible with the totally electronic signaling proposed for the synaptic junction and to be discussed in the next section as well as in Chapters 10, 11 and 12. In this figure, the pedicle is the morphological designation for a very complex termination of the morphologically designated axon. It is part of a very complex physical, chemical and electronic interconnection, known as a synaptic complex. It interconnects one or more presynaptic axons and an unlimited number of post synaptic dendrites. It is also a region of chemical activity associated with the generation of electrical potentials to power the neurons present. The figure consists of a number of important individual areas:

+ The body of the pedicle is included within the membrane containing the word pedicle in the figure.

+ The body of two embedded horizontal cells, one on the left labeled HD and one on the right containing the labels HP and HA.

+ The dendritic structure of one bipolar cell interfacing within the synaptic complex and shown in the center of the lower portion of the figure.

+ A large reservoir of chemicals shown by the horizontally striped area in the center of the figure and labeled M for manifold.

+ A series of small black areas, known as synaptic disks, shown between the membranes of the various cells present.

+ A circular arc shown within the pedicle just above the manifold, M.

+ A series of terminal vesicles, shown as open circles, along the inner edges of the axon terminations.

+ A variety of other vesicles shown by shaded circles that are not relevant to the signaling function.

+ A black vertical bar, designated the ribbon in most drawings, representing the electrical conduit within the axon and connecting the terminal vesicles to the interior of the neuron.

+ A series of open areas bordered by either plasma membranes or interior elements shown by dashed lines.

Several other dendrite elements are shown in the lower part of the figure that are passing near the pedicle but are not involved in signal transmission between this synaptic complex and their related neurons.

To interpret this figure completely requires a detailed description.

knowledge of the potentials of each element in the figure. This discussion will occur in Chapter 10 and 12 and be summarized in Section 4.7. This section will only discuss the physical parameters of the synaptic complex related to a photoreceptor cell. In such a complex, the manifold is largely surrounded by the neural tissues. In other synaptic complexes related to Nodes of Ranvier and most projection signal paths the reverse is true, the neural tissue is surrounded by the manifold.

The various cell membranes are shown in close but unspecified spacing relative to each other except in the small areas defined as synaptic disks. Under these conditions, the membranes form excellent insulating barriers between the adjacent cells as well as between the cells and the extracellular manifold. This is because of the asymmetrical electrical conductivity of the membranes. Each membrane can be represented by a nearly perfect diode that is reverse biased by the potential between the plasma of the cell and the manifold. Such reverse bias causes the diode (membrane) to act as an excellent insulator.

Within the synaptic disks, the membrane spacing is closely controlled to between 45 and 100 Angstrom as will be discussed in the next section. This spacing supports the creation of an active electrical signal connection between the two membranes when those membranes are at appropriate electrical potential. At a spacing of 45-100 Angstrom, very few molecules are small enough to exist in this space. The rules of Brownian Motion call for all but the smallest molecules to exit this region if they are free to move during the genesis period of the network. As a result, a liquid crystalline structure of hydronium forms between the two plasma membranes within these synaptic disks. When appropriately biased, the combination of the two membranes and the hydronium liquid crystal form an Activa. Although actually a three-terminal electrolytic device, the Activa can be described as a nearly perfect diode in this situation. This Activa provides a very low impedance path for electrical charges passing between the axoplasm on one side and the dendroplasm on the other.

The synaptic vesicles, shown by open circles, connect to the reticulum of the axon of the photoreceptor cell represented by the heavy vertical black line (and frequently labeled the synaptic ribbon). These vesicles provide electrical conductivity between the potential of the plasma of the reticulum and the pedicle membrane within the synaptic disks. It is the potential between this plasma and the manifold fluids that determine the electronic state of the membrane in this area. These vesicles also play a structural role in determining the spacing between the axonal and dendritic membranes.

The ultimate source of the electrical potential within both the axons and the dendrites is provided by an electrostenolytic reaction occurring along portions of the membrane surfaces not involved in forming Activas. These areas act as chemical substrates for reactions related to the glutamate cycle of biochemistry. The reactions occurring at these locations transfer free electrons across the membrane in the direction to bias the inside of the cells negatively relative to the manifold. The resulting charges are found to congregate along the inner surfaces of the plasma lemma unless an additional structure is present to aid in their rapid transfer to the bulk electrolyte in each cell. This appears to be the purpose for the arcuate structure shown by the heavy curved line at the base of the synaptic ribbon. The charge densities resulting from these reactions are documented in Vardi, et. al. (pg 1364).

Section 4.1 of Vardi’s paper explored the inconsistencies they found when attempting to relate GABA receptors (labeled GABA<sub>A</sub>) to the areas near the vesicles they expected to be involved in the release of chemical neurotransmitters. They found that all of the GABA<sub>A</sub> sites they located were in apposition to areas of high electron density within the cells. These inconsistencies related to a chemical neurotransmitter hypothesis disappear under an electrical transmission hypothesis. Under this hypothesis, the GABA<sub>A</sub> sites are expected to coincide with the electrostenolytic sites. These sites are expected to be located in apposition to and to cause the areas of high electron density within the cells. Since quantum-mechanical constraints imposed by the narrowness of the gap between the membranes in the areas of signal transmission prevent any chemicals from entering these gaps, no GABA<sub>A</sub> sites would be expected within the region of the individual synaptic disks.

The white area within the dendrite of a horizontal cell (labeled HD) on the left defines the electrical conduit within that dendritic structure. To the right of that area are the synaptic disks connecting it to the photoreceptor axon. At the upper left of the area is the area of high electron density associated with the membrane and the area of electostrenolytic activity on the outside of the membrane. This is the area where one expects to find GABA<sub>A</sub> sites.

The horizontal cell shown on the right of the figure is structurally more complex. The structure shown is the axonless (americine) portion of a horizontal cell. It actually consists of both an input terminal, labeled HP (for a poditic terminal), and an axon terminal, HA, confined within a common outer membrane but separated by an internal membrane as shown. The remainder of this horizontal cell is out of plane and does not appear in this caricature. The upper half of this horizontal cell is arranged and operates in the same way as the horizontal cell on the left. The lower half of this structure is arranged and operates in the same manner as the pedicle of the photoreceptor cell. It includes prominent vesicles that...
create a synaptic junction, represented by the synaptic disk, with the bipolar dendrite at the bottom center of the figure.

Functionally, there is a major difference in the two horizontal cells shown. The dendrite on the left is the non-signal-inverting input to the Activa within that neuron. Conversely, the podite terminal on the right is the signal-inverting input to the Activa within that neuron. As a result, the axon labeled HA on the right exhibits a signal that is of opposite phase to the signal presented by the photoreceptor cell. As a result, great care must be taken to distinguish between the electrical signal at the output terminal of the photoreceptor cell and the electrical signal at the output terminal of the amercine horizontal cell nearby. The polarity of the bipolar cell associated with this configuration, and shown in the lower part of the figure, may or may not be in phase with the photoreceptor cell. Its polarity depends critically on which axon it contacts.

As will be discussed in Section 4.3.6, the bipolar cell at the bottom of the figure makes synaptic contact with only the right horizontal axon, HA, and also exhibits an area of electrostenolytic activity and high electron density on the upper left portion of its plasma membrane. Its output is directly related to, and in phase with, the output of the right-hand amercine horizontal cell. On the other hand, the axon of the left-hand horizontal cell (out of plane and not shown) is directly related to and in phase with the output signal from the photoreceptor cell.

Although not obvious from this two-dimensional presentation, the manifold, M, is in good hydraulic communication with the extracellular matrix via the third dimension. It is able to efficiently exchange chemical reactants required by the electrostenolytic mechanism with other cells and with the vascular system.

4.3.6.1.2 The role of glia in the synaptic junction

There is good data supporting the role of glia (Schwann cells in the PNS and astrocytes in the CNS) as active manufacturing support to the neurons in the vicinity of a synapse. Because of the role they play, this role is developed in Section 15.1.8.

4.3.6.1.3 Simplified caricature of the synaptic complex

There are a great many caricatures of the synaptic complex in the literature, mostly based on the assumption of a chemical neurotransmitter moving between the pre and post synaptic terminals, and mostly archaic. They are widely reproduced for pedagogical purposes. They are not based on our present understanding based on current electron micrographs. Unfortunately, these caricatures leave an image in the mind of a student that is difficult to replace. Figure 4.3.6-2 presents an alternate simplified caricature. The figure is divided into two major zones. Those activities below the center line related to signaling, and those above the line related to support functions.
4.3.6.1.4 The cytology of the synaptic disks

The photoreceptor cell pedicle, like the pedicle of all neurons, makes contact with orthodromic neurons through a synaptic complex. The complexity of this complex depends primarily on the current carrying capacity required of the connection. To allow scalability of this capability, the synapse exhibits a modular structure that will be developed in Chapter 10. As indicated in the previous section, each synapse of the pedicle synaptic complex employs a group of synaptic disks as the connection between an axon and a neurite (dendrite or podite). Each of these synapses involves a synaptic disk with a diameter of 0.3-0.5 microns. Each of these disks is structured as shown in Figure 4.3.6-3. The sizes shown are in Angstrom and these features can only be resolved by high magnification electron microscopy. The structure can be compared to that visualized by the group led by Robertson\textsuperscript{152}. The vesicles shown along the upper edge by open circles correspond to those of the previous drawing. They are connected to the reticulum of the axon through the synaptic ribbon. A similar row of vesicles is shown along the bottom edge. In fact, both of these arrays are two-dimensional as shown in the cutaway view at lower left. The vesicles place structural pressure on the two bilayer membranes so as to establish a fixed spacing between the membranes. In the regions between the upper and lower vesicles, this space is so small that only water molecules can fill it. These molecules assume a liquid crystalline structure in these areas known as hydronium. The development and operation of this structure will be discussed in detail after a discussion of transistor action in PART C of this work.

4.3.6.2 The structure of the internal amplifiers of the photoreceptor cell

The photoreceptor cell is probably the most complex neuron in the neural system of any animal. Its configuration may be unique to the visual system. As in all neurons, there is an internal electrolytic amplifier circuit within the cell. There is an Activa at the center of this circuit. It is formed at the junction of the dendrolemma, the axolemma, and the podilemma as shown in [Figure 4.1.2-5]. The basic details of this structure are presented in Section 10.6.1. However, the photoreceptor cell is more complex. Each of the input structures shown at the upper left in the above figure have been modified to create additional Activas. These additional Activas are associated with the adaptation amplifiers of the photoreceptor cell. The original Activa is associated with the distribution amplifier within the cell. The details associated with these amplifiers will be addressed in detail in Chapter 12. This additional electrolytic complexity makes the definition of the dendroplasm more difficult. It actually becomes the conduit between two separate amplifiers within the same cell.

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

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\textsuperscript{152}Zampighi, G. & Robertson, J. (1973) Fine structure of the synaptic discs separated from the goldfish medulla oblongata. J. Cell Biol. vol. 56, pp92-105
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 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. 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.

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.

4.3.6.3 The structure of the dendrites of the photoreceptor cell

As shown earlier, the typical input structure to the distribution amplifier of the photoreceptor cell consists of nine separate and distinct dendrite branches with a common external lemma. The distal terminals of the dendrolemma of the photoreceptor cell obviously do not connect to another neuron. They are the initial elements of sensory neurons and must participate in the sensory process. To do this, the dendrites (frequently described as microtubules at their extreme ends) are cytologically unique. Their modification to provide this unique sensing function is both simple and exquisite.

Whereas normal dendrites associated with signal processing and signal projection have dendrites of relatively large diameter relative to the reticulum within the dendrite, this is not the case for those of the photoreceptor cells. Here the dendrites that are laid into the furrows along the sides of the disks are much smaller in diameter. As they become smaller, they eventually reach a size where they are only larger in diameter than the reticulum by less than 200 Angstrom. At that point, the quantum-mechanical rules related to Brownian Motion again come into play. They force all large diameter molecules out of the space and the remaining water molecules form a liquid crystal of hydronium. In this case, the liquid crystal is contiguous over the majority of the length of the microtubule. As a result of this process, each microtubule forms a continuous Activa within an electronic circuit. This continuous Activa is very sensitive to quantum-mechanical stresses as well as electrical inputs and it is in intimate physical (quantum-mechanical) contact with the disks of the outer segment. As will be discussed in Chapter 5 and 11, the de-excitation process in the chromophores of vision creates a quantum-mechanical energy

153Lolley, R. et. al. (1986) Op. Cit. inset to figure 4
packet that can stimulate this Activa. This is the method of electrical signal generation in the photoreceptor cells of vision.

4.3.6.3.1 The source of the adaptation mechanism

The plasmalemma of the microtubules is thinner than in a conventional dendrite. This thinness introduces another mechanism unique to the photoreceptor cells. This lemma exhibits a relatively low electrical breakdown potential relative to the potential established by the electrostenolytic process on the outer surface of the dendroplasm. As a result, an additional amplification mechanism is introduced into the photoreceptor cell. This mechanism depends on avalanche current multiplication that is sensitive to the potential across this lemma. By optimizing the impedance of the electrostenolytic source supporting this membrane, the amplification becomes a function of the average current passing through the Activa. This mechanism is the source of the adaptation mechanism associated with vision. See Chapter 11.

4.3.7 The RPE cell

The RPE cells are not neurons. They are highly specialized cells designed to extract the chromogens of vision from the bloodstream, process them further, store them if needed and transfer them to the IPM in conjunction with a specialized group of transport proteins. The so-called pigment granules of these cells are multicolored and are locations of chromophore storage. The cells are pressed together in a layer in order to provide both a hydraulic and an electrically insulating barrier between the IPM and the choro-vascular system of the eye. The American Academy of Ophthalmology has provided a brief description of the functions of the RPE. It clearly recognizes the role of the RPE in the “uptake, transport, storage, metabolism, and isomerization of vitamin A.” The operation of the RPE cells will be addressed in detail in Section 7.1.2.

4.3.8 Environment between the photoreceptor and RPE cells

The environment surrounding the photoreceptor cells is required to meet a variety of requirements. Because of this, the photoreceptors are positioned uniquely with respect to the other structures of the retina. To satisfy the requirement to protect the chromophores of vision from any oxidizing chemicals, the outer segments and most of the inner segments are located within the Inter-Photoreceptor-Matrix, IPM, located between the layer of RPE cells and the Outer Limiting Membrane, OLM. While protecting the operational disks from oxidizing chemicals, the IPM also provides an avenue for the chromophores to be transported from the RPE cells to the extrusion cup where they are initially deposited on the disks. The remaining part of the inner segments, all of the nuclei and the axons of the photoreceptor cells are located within the Inter-Neural Matrix, INM.

The portions of the photoreceptor cells within the IPM and the INM must both be surrounded by fluids with a reasonable degree of electrical conductivity. These fluids provide both the electrical return path for the electrical currents resulting from the operation of the amplifiers and the electrostenolytic processes supporting this operation. It is worth noting that the OLM is both a chemical barrier and an electrical barrier. It is a good insulator. It is this insulating property that defines many of the electrical waveforms measured by both external ERG techniques and local ERG techniques based on an invasive probe. This mechanism will be developed more completely in Section 4.7.4.

4.3.8.1 The electrical performance of the fluid surround

The environment surrounding the photoreceptor is divided into two different zones by the Outer Limiting Membrane. Some authors show this membrane as actually consisting of a great number of Muller Cells packed in between the Inner Segments of the photoreceptor cells. In either case, there is a barrier at this location that forms two zones.

There are two different fluids surrounding the photoreceptors. The IPM surrounding the Outer Segment and the distal part of the Inner Segment, and the fluid found saturating the neural laminate. These materials play distinctly different roles.

The Outer Segment and most (if not all) of the Inner Segment are immersed in the Inter-Photoreceptor Matrix, IPM. The

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IPM is confined between the Outer Limiting Membrane and the surface of the RPE. The IPM may play several roles. It plays a major role in transporting the chromophoric material from the RPE to the disks of the OS. It also plays a major electrical role in providing a return path for the electrons emanating from the dendrites along the sides of the disks. It also appears to provide a metabolic role by supporting the transport of metabolites from the RPE to the surface of the disk stack. At this location, they can provide the electromotive force (voltage) needed to sustain the operation of the Activa(s) located in the dendritic structure. It also appears to provide similar support to the poda region of the photoreceptor cell located on the surface of the IS. As indicated earlier, the fact that these metabolic materials can diffuse through the IPM between the poda terminal of the IS and dendritic terminal of the OS contributes to the very efficient energy conservation properties of the eye.

The nucleus and axon of the photoreceptor cell reside in the neural laminate on the other side of the Outer Limiting Membrane from the remainder of the cell. This laminate plays much the same role as the IPM except it does not support the transport of chromophores. Metabolites are provided to the photoreceptor cell wall near the axon terminal. And, as in the case of the IPM, the metabolites are able to diffuse between the various electrical terminals of the neurons to support the recovery and reuse of chemical energy by the various batteries. The actual conductivity of the neural fluid may be lower than that of the IPM since most of the current flow in the area is within the neural conduits of the various neurons. This lower conductivity may support a higher electrical breakdown potential for the medium which would in turn allow higher packing density for the neurons.

4.3.8.2 The chemical performance of the fluid surround

There are a number of materials found in high concentration on the surface of the photoreceptor cells, near the Outer Segments, near the inner segment within the IPM and along the cell membrane in the neural laminate. A series of glutamic-acid-rich-proteins, GARP, are found along the length of the microtubules of the photoreceptor cells\textsuperscript{155}. These appear to be the source of the glutamic acid found at these locations. GABA (\(\gamma\)-aminobutyric acid), glycine and glutamic acid are generally found at high endogenous levels along with the presence of GABA synthetic enzyme, glutamic acid decarboxylase and calcium\textsuperscript{156}. The conventional view is that some of these materials are the chemical messengers traveling between neurons. The rest of the materials facilitate the process.

Recently, the same materials have been assigned a role in the interface between the disks and the IPM. The assumption being that since they are there, they probably control the permeability of the putative membrane wall surrounding the Outer Segment.

This work takes a different approach and assigns these materials to the function of providing electromotive force, a voltage, between the surrounding fluid and the inside of the membrane wall of the neurons. This is accomplished via an electrostenolytic process. Based on this model, these materials do not directly participate in the signaling function of the neural system. The voltage generated is a function of the specific chemistry of these materials, the characteristics of the membranes involved and the chemistry of the fluid surround. The voltage generated is generally in the range of 20-150 millivolts and may vary between the dendritic, poditic and axonal plasmas of the same cell. See Section 7.7.2.

4.3.9 Correlation of old names with actual functions (see 4.xx at end)

Referring again to the exploded view of the baseline photoreceptor “cell,” each of the cell portions can be examined independently at the histological level. Because the entire model has yet to be quantified in this work, several nominal values will be specified in this section which are only for illustrative purposes.

The big surprise is of course the fact that the outer segment is not an integral part of the “cell.” The outer segment is not living material and in its fully formed configuration is actually a kind of piezo-photo-electric transducer. The incident photons excite the liquid crystal layer of the chromophore and the energy is transmitted through the crystal to the area of the nerve cell dendrites. At that site, the energy is transferred to the dendrite at a quantum level. This is an important criterion because the noise performance of a nerve cell is critically dependent on there being a specific threshold below which it will not respond. Alternately, the energy received from a photon is distinctly limited. In this case, the threshold level is in the vicinity of 1.9 electron volts, a very small but critical parameter.

The next important property of the photoreceptor cell is that it exhibits two distinctly different characters. The remainder of the photoreceptor cell is functionally divided into two parts; a nerve portion of relatively conventional

\textsuperscript{155}Korschen, H. et. al. (1999) Interaction of glutamic-acid-rich-proteins with the cGMP signaling pathway in rod photoreceptors Nature, vol. 400, pp 761-766
function and a gland function which is also relatively conventional but may have a few special features if it in fact creates and exudes two different and distinct organic molecules. If it only exudes one molecular species, it would be considered a relatively ordinary exocrine gland.

Looking at the nerve portion first, the irritation of the nerve by the outer segment results in a signal which is transferred by the nerve cell to its foot where it interfaces with one or more nerve cells of the inner nuclear layer. The nerve portion of the cell appears quite ordinary. However, it is important to determine how fast this type of nerve can transmit information and what refractory time is associated with this type of cell. This will be discussed later after a more complete modeling case is available. This model will allow the available data to be interpreted properly and provide guidance and/or data to quantify these characteristics of the photoreceptor class of cells. As a typical case, the nerve is able to transmit this signal at a velocity of about 10 meters/sec and at a rate of about 100 samples per second. These properties may vary significantly with the size of the cells and possibly with their position in the retina independent of size, due to the metabolic parameters of the INM in the local area.

The foot of the nerve portion of the cell (the pedicle) is believed to transmit its information to the first signal processing group of nerves by electrical means. This proposition which will be defended later is chosen because of the unusual and possibly unique nature of the signal processing nerves of the retina. They are known to operate in an analog, as opposed to a binary—all or nothing, mode. This is almost surely an electronic mode which does not rely on chemical changes. The literature presents many signals that are offered as analog in nature and as emanating from the photoreceptor cells.

The gland like portion of the photoreceptor cell is relatively conventional once its function is accepted. Its overall purpose is to assimilate materials and secrete them into the IPM space. Since this space is independent of the blood stream, this type of secretion is known as exocrine secretion. The secreted material is formed into a continuous strip of material which can be easily configured into the disks of the outer segment. Bloom presents a figure attributable to J. Gross showing a glandular cell producing molecules which after emiocytosis form up into a higher level structure. In this case, tropocollagen molecules aggregate extracelluarly into collagen fibrils. The words presumed and believed are used liberally in the original caption for Bloom’s figures. However, the nomenclature appears appropriate to the operation of the glandular part of the photoreceptor cell.

The above description may appear superficial at this stage because enough details of the overall model have not been presented to justify it completely. This will occur below and allow further specific characteristics of the photoreceptors and their functions to be quantified.

4.4 Functional Description of components at the cytological level and beyond

The housekeeping functions (the only functions usually discussed in the anatomical literature) will not be discussed here. It is the neural and glandular functions that are important in signaling. The nerve portion and the gland portion of the photoreceptor cell operate at and below the cytological level much as other body cells do. Their functional characteristics will be reviewed after discussing the outer segment.

4.4.1 Outer segment

4.4.1.1 Overview

Snyder & Menzel provided an important and comprehensive series of papers on the performance of the Outer Segment from the perspective of electromagnetic wave theory. About one-half of the material applies to Arthropoda and will not be discussed here. Some of the papers relied upon the conventional wisdom of the time that the orientation of the major axis of the chromophores were randomly arranged on the surface of the disks. This assumption does not conform with the present current wisdom that the chromophores form a ligand located deep within the opsin molecule. Nor is it consistent with the proposal of this work that the chromophores are located on the surface but with their major axis of the chromophores aligned with the long axis of the Outer Segment. Other papers are very useful in quantifying the optical performance of the Outer Segments.

An interesting conjecture appears in Liebman on page 203 of Snyder & Menzel. He finds that lipids and proteins make up 85-90% of the dry mass of the Outer Segments and speculates on what makes up the other 10-15%. He does not

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157 Bloom, xxx [not in files ]
discriminate between soluble and insoluble lipids and proteins. In this work, the actual chromophores account for that missing mass. They are an easily disturbed liquid crystalline coating on the disks that is frequently washed away after being attacked by a detergent. When in-vivo, they exhibit unusual electronic properties similar to those described by Liebman.

In another paper in Snyder & Menzel, Fischer & Rohler discuss the light absorption properties of an idealized photoreceptor on the basis of waveguide theory. They treat the Outer Segment as a cylinder of homogeneous dielectric material surrounded by another homogeneous material. The third paragraph of their theory explains how their model is unrealistic and how they had to violate the boundary conditions associated with the application of Maxwell’s Laws. This work avoids this problem by removing any assumption of homogeneity and introducing instead, the concept of a spaceframe. In both works, the conclusions are the same. The waveguide properties of the Outer Segments are key to the absorption of light by the photoreceptors. The Outer Segments act like directional antennas. The properties of these antennas are developed in detail beginning on page 38 of Snyder & Menzel.

4.4.1.2 Growth cycle

See Section 4.6.2 & 7.6.

4.4.1.3 Physical analog of the Outer Segment at “human scale”

The imagery of the previous section doesn’t give a very good perspective to the reader concerning the relative geometry of the Outer Segment and the disks of which it is composed. The following brief sections are meant to give a perspective to the size and geometry of the Outer Segment and its components.

4.4.1.3.1 The Outer Segment as an Assembly

If the typical Outer Segment is taken to be 2.0 microns in diameter and 50 microns long, it has an aspect ratio of 25:1; a long thin (and in fact, fragile) rod--rod because it is in the form of a solid cylinder. The inside of this space is filled with a stack of essentially planar disks which are extremely thin and have a lip around the outside edge. Ignoring the lip for the moment, the individual disk is approximately 165 angstroms, or 16.5 nanometers, thick with a diameter of 2.0 microns. This disk has an aspect ratio of about 120:1. Until recently, it was difficult to find something in everyday life with this high an aspect ratio. A compact disk (CD) is very close to this aspect ratio; it is 1.0 millimeters thick and 120 millimeters in diameter, an aspect ratio of 120:1. Therefore an “optical disk” of the retina could be represented by a “compact disk” which is of coarse an optical disk of a different technology. To complete the emulation, each of these CD disks would have to be made with a lip at the outside edge which was symmetrical in height and width and raised about 1/3 the thickness of the disk on both top and bottom. In addition, the disks should exhibit an edge resembling a chrysanthemum of nine petals, which would provide space for the dendrites of the inner segment to establish intimate contact with these disks. This feature results in incisions into each disk which are approximately 30-50% of the disk diameter. The source of this feature is almost surely the Calyx which acts as an extrusion die.

To emulate a typical outer segment, of 50 microns length, would require that a group of these CD disks, each with a total thickness including the lips of 1.66 millimeters, be stacked about 2000 units high--for a height of about 3.3 meters or 11 feet. This stack is considerably higher than the 8-foot ceiling in an average room.

Note that the above stack is not solid, the lips cause the body of the disks to be separated by about 2/3 of their basic thickness. Only about 66% of the space within the stack is occupied by the material of the disks. The remaining space is filled with a fluid. In the actual Outer Segment, this space is almost certainly occupied by the interstitial fluid, known as the Inter-Photoreceptor Matrix or IPM, surrounding the Outer Segment. If this space was actually empty, i.e., a void, it would have a very deleterious effect on the index of refraction of the stack. A truly empty space has an index of refraction of 1.00. To be an effective photon absorber, the index of refraction of the stack should remain essentially constant throughout its length; where constant is interpreted to mean variations no greater than 5%, within distances of a few wavelengths of light.

4.4.1.3.2 The Disk as a Component

Continuing the above analogy, the individual disk is 1.0 mm in thickness and 120 mm in diameter with a symmetrical lip around and including the outer edge definable as a torus of 0.8 mm radius. The planar portion of the disk is made up of a stiff transparent sheet that is glazed with a film on each side. The sheet is actually a laminate of two 0.5 mm thick sheets corresponding to the individual opsin layers.

4.4.1.3.3 The Chromophore as a Coating
The substrate defined above is glazed on all sides (including the torus) by a film with the thickness of about one quarter of that of the individual sheet. This very thin film is equivalent to the chromophore material. It is approximately 0.1 mm thick. The films are in fact one single layer which is continuous around the torus at the edge of the substrate. The disks are separated and supported by a thin layer of fluid, basically gelatinized water. This glaze is highly structured, as might be produced by a chemical deposition process as opposed to a painting process, and provides an attractive appearance due to its specular scattering of light of certain colors. The film is a liquid crystal. It is quite soft.

### 4.4.1.3.4 The neural connections

The disks in the above stack are aligned so that the incisions in each disk form nine nominally vertical furrows along the stack. Within each of these furrows is an electrical cable approximately 1.5 mm in diameter. These cables represent the microtubules (dendrites) of the photoreceptor cell.

### 4.4.1.3.5 The Overall analogy

The resulting analogy of an Outer Segment is a stack of highly colored CD like disks stacked about 11 feet high and separated by a thin film of gelatinized water. The disks are physically supported in two ways. Both the bottom and top of the stack are covered by a tight fitting sleeve about three inches long. The sleeves are nailed to the floor and ceiling. In between the sleeves, the disk stack is primarily supported by the proximity of similar stacks and the fact the interstitial space is also filled with a viscous material. The stack is dynamic. Every five minutes, a new disk is introduced at the bottom of the stack and one is removed at the top. The resulting disk stack slides up relative to the nominally stationary microtubule “cage” surrounding the disks.

### 4.4.2 Inner segment

There are two major functional mechanisms related to the inner segment. The easiest to understand are the chemical aspects associated with the routine generation of the opsin used as a substrate in the disks and the formation of these disks. The second is the electrical performance of the structure based on its inclusion of the first electronic amplifiers of the neural signal paths.

In discussing the chemistry of the segment, little can be added regarding fabrication of the disks to the work of Papermaster, et. al. As indicated earlier, the terms myoid and ellipsoid should be dropped from their figure 1 as being extraneous\(^{159}\). Furthermore, it should be noted that their description concerns the fabrication of a protein, opsin, that is in no way associated with any chromophore or chromogen of vision.

The discussion of the electrical performance of the inner segment of the photoreceptor cells is one of the most important areas in vision. However, it cannot be developed based on morphological and cytological studies. It will be postponed to PART B, Chapters 8 & 9 where it will be developed in detail.

### 4.5 The complete Photoreceptor-IPM-RPE Complex

The individual photoreceptors described above are assembled into a complex structure designed to support the continuous operation of the photodetection function. This photoreceptor/IPM/RPE complex both provides and maintains the chromophores of vision and also supports the electrical performance of the operating system. Figure 4.5.1-1 provides a composite view of this interface showing a variety of cells. It attempts to highlight a variety of conditions found within this complex. These conditions will be discussed individually by reference to the cell number along the left margin.

A critically important feature of this figure is its dynamic character. As indicated at the bottom, the disks move continually toward the RPE cells at a rate of about 300 nm/hr. With a disk pitch of about 250 Angstrom, this rate suggests a disk generation rate of ten disks per hour per disk. At this rate, each disk stack is completely replaced weekly in humans. The generation of new disks at a rate of ten per hour per cell suggests a prodigious manufacturing capability for each photoreceptor cell and a prodigious phagocytotic capability for the RPE cells. The RPE cells on the right are shown storing a variety of chromophores in so-called color granules. Those marked U represent stores of the ultraviolet chromophore, Rhodamine(11). The other granules represent the observed color of the granules, which are the complements of the absorption spectra of the individual chromophores. These granules are formed as a result of the

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absorption of the chromophores of vision at the interface of the RPE cells and the vascular matrix adjacent to the choroid.

The disks of the outer segment continue to be shown without an enclosing membrane. This condition can be seen relatively clearly in a micrograph at 36,000x in Hogan, et. al\textsuperscript{160} and in the other micrographs of Section 4.3.5. The plasma membrane of the inner segment has clearly folded back on the left and does not continue to enshroud the outer segment. The caption, arrows and text clearly indicate that the membrane folds back on both sides. However, the additional structure associated with the dendrites emanating from the cilium make this less obvious on the right. The outer segment does remain shrouded by a barrier of limited integrity. It is proposed that this is not a biological membrane but a chemical density difference.

Nilsson provided a caricature in 1964 that is in agreement with this interpretation. He showed the Outer Segment without a plasma membrane associated with the photoreceptor cell. However, in some figures within that caricature, he showed the calyx of the inner segment extending nearly to the end of the Outer Segment and the “processes” of the RPE cells extending beyond the Outer Segments to enclose parts of the Inner Segments. In other figures within the caricature, the calyx is shown extended only along one side of the Outer Segment. Steinberg chose to de-emphasize the role of the “processes” in his reproduction of the figure\textsuperscript{161}.

Bruch’s membrane is shown explicitly near the RPE cells. Also shown explicitly are the cells located between the RPE cells and known variously as zonulae occludens, terminal bars, and Verhoeff’s membrane. This barrier is known to prevent passage of all proteins and may add to the electrical isolation of the inner and outer segments of the photoreceptors.

Also shown explicitly, but less obviously, is the Outer Limiting Membrane (OLM). This membrane is represented by the black rectangles between the inner segments of the photoreceptor cells. The OLM is sometimes labeled the External Limiting Membrane (ELM) or as formed from zonulae adherentes. The rectangles are shown long enough to also accommodate a variety of Mueller Cells in the space between the photoreceptor cells. Hogan presents a figure at 24,000x showing major components (baskets) of the Mueller cells located on the IPM side of the OLM\textsuperscript{162}. These two membranes provide both chemical and electrical isolation between the IPM and the INM and between the IPM and the vascular matrix. Both the dendrites and the poditic terminal of the photoreceptor cells are in electrical contact with the IPM while the axon connects electrically with the INM and other elements within the INM. This difference plays an important role in the electrical waveforms generated by the visual system.

Cell 1 is shown in partial detail and stressing its generation of the raw disks. These disks are formed from the secreted protein, opsin, at the location represented by the black dot. The material is shaped into disks within the extrusion cup and calyx of the inner segment. The disks are pushed toward the RPE cells by the secretion of subsequent protein material. Upon reaching the RPE, the disks are phagocytized as shown.

Cell 2 is drawn in more detail. It shows the cell creating the disk stack within the extrusion cup and calyx. However, it also shows the presence of the IPM in the space between the plane of the inner segments and the plane of the RPE cells. This matrix is saturated with chromophoric material being transported by the IRPBs. In cell 2, it is assumed this material corresponds to the ultraviolet sensitive chromophore. It is shown as transparent. The material is shown as coating both sides of each disk formed as in Cell 1. Such a cell coated with Rhodonine(11) may not be found, or may be only residual, in the human retina.

Cell 3 repeats much of the detail of cell 2 while assuming the chromophore coating the disks corresponds to the long wavelength chromophore, Rhodonine(5). This material appears azure by reflected light but absorbs maximally at 625 nm. An additional feature is shown in this view. This is the presence of a concentration of material near the disk stack. This material contains both excess chromophore present in low concentration within the vicinity of the disk stack and the materials needed to provide electrostabilizing power to the dendrites to be discussed in conjunction with Cell 4. There is no cell membrane shown surrounding any of the disk stacks in this figure.

It should be noted that the functional (anisotropic) absorption spectrum of the chromophores when deposited on the disks can only be observed by light applied axially to the disk stacks. If transverse illumination is applied to a disk stack, the peak absorption will always occur at the isotropic absorption peak of the Rhodamines at 500 nm regardless of the chromophore present. This phenomenon is due to the molecular alignment of the liquid crystalline chromophores on

\textsuperscript{160}Hogan, et. al. (1971) Op. Cit. pp 425-426  
\textsuperscript{161}Steinberg, R. (1973) Op. Cit. pg 454  
\textsuperscript{162}Hogan, et. al. (1971) Op. Cit. pg 435
the individual disks and the resultant enhanced absorption due to the larger absorption cross section of the resulting configuration.

Cell 4 shows the disks coated with the mid wavelength chromophore, Rhodone(7) which appears magenta by reflection or transmitted light due to its strong absorption at 532 nm. It also shows the dendrites (microtubules) within the furrows of the disk stack. These dendrites emanate from the inner segment via the cilium transport and interface with the Activa within the inner segment (not shown) prior to connecting with the pedicle of the cell via the axon shown as an arrow.

Cell 5 is shown for completeness. It is morphologically and cytologically identical to cells 3 & 4. The only difference between them is the presence of a different chromophore coating the disks of the outer segment. In this case, it is Rhodone(9) which appears yellow-orange by reflected or transmitted light and absorbs maximally at 437 nm.

Cells 6 & 7 show the consequences of a retinal tear. While the Outer Segment in 6 remains aligned and will reconstitute itself over time, the Outer Segment of cell 7 has become misaligned. Although it will probably realign itself over time, there may be residual material left in the IPM space and/or the IPM may show a bulge in this area (which is generally unacceptable because of the lack of proper focus of the image projected onto the retina). It should be noted that such a tear is particularly likely because of the lack of membranes surrounding the individual disk stacks. The plane intersecting the disk stacks between the outer extremities of the inner segments and the outer extremities of the RPE cells forms the structurally weakest area of the retina. This interpretation conflicts with that of Feeney-Burns & Berman. They assume that the weakest point is at the disk stack-RPE interface because of the structural strength provided by a putative membrane surrounding the outer segment. Anderson, et. al. have explored the results of retinal detachment in some detail. See also Chapter 18.8.4.

Cell 8 is shown for completeness. It represents a set of possible conditions. It is most representative of an immature cell. Until such a cell reaches the stage of creating disks of full diameter and nominal spacing, it may exhibit a cone-shaped disk stack. This is an abnormal condition over the long term. Once the calyx is fully formed and sufficient protein material is present in the extrusion cup, the formation of a fully formed stack of constant diameter disks can be expected. It is possible that this will not occur until the additional back pressure provided by the presence of the RPE cells blocking the growth of the disk stack becomes important. In any case, a conical shape for the outer segment of a photoreceptor cell cannot be sustained over a period longer than a week due to the growth of the stack.

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Details of photoreceptor-RPE interface

Colors are residual after absorption & as observed in Lab. Absorption spectrum of Outer Segments only observable in axial view. Transverse spectrums always have peak at ~500 nm. and appear magenta.

Figure 4.5.1-1 The photoreceptor cell-IPM-RPE interface. See Text
4.5.1 The morphogenesis of the photoreceptor-IPM-RPE complex

It is common to find a caricature in the literature describing the morphogenesis of the eye by analogy to the formation of a cup. However, the actual embryology is considerably more complex. Hogan, et. al. have provided a comprehensive description of the process but they did not provide a visual aid. Coulembre provided a tabular flow chart of the process. However, it contains a large number of dashed lines. Goldsmith and Miller & Newman have provided caricatures (including the formation of the lens). However, they are not detailed enough for the goal of this section. Figure 4.5.1-2 provides a cross-sectional view of the eye during morphogenesis. The key is to recognize the continuous nature of the ectodermal surface forming the eye cup and beginning with the outer surface of the optic nerve. The details of the lamina cribosa shown in Chapter 3 aid in this interpretation. Two nested cups are actually formed. Part of the outer surface of the inner cup (heavy lines) differentiates into the neuro-dermal layer associated with the photosensitive part of the retina. Part of the inner surface of the outer cup (heavy dotted lines) differentiates into RPE cells.

Whether the lens is formed by a merging of the two edges of the outer cup shown or from a distinctly separate layer of ectodermis separate from the optic cup is incidental to this discussion. However, a separate origin does not appear consistent with the operational nature of the system. No tissue arising from, and metabolically supported by, a source other than the optic stalk is normally associated with the optical globe.

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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. 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.

The RPE cells are glandular-digestive and analogous to cells within the alimentary tract. The RPE cells secrete the chromophores of vision and digests the disks of the outer segment.

The inter-photoreceptor matrix, IPM, is formed by the cul-de-sac between these two cups and is entirely extradermal. This area becomes isolated when the outer cup contracts to form the pupil at the top of the figure. This causes the apical area of the inner cup to make contact with the outer cup. Once isolated, the IPM is able to control both the presence of oxidative material that might damage the disks and the presence of material that might hydrolyze the proteins present. The biological activity within the IPM is supported through secretions from the two adjacent surfaces. Once isolated by the electrically insulating dermal layers, the IPM exhibits an electrical isolation relative to the other surrounding structures and the rest of the body.

4.5.2 The character and content of the Inter Photoreceptor Matrix
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Johnson, et. al. have discussed the IPM\textsuperscript{170} but their introductory material is equivocal. In the abstract, they say the IPM appears to be a major route for metabolic support to the photoreceptors. Then, after saying little is known about the function of the IPM in the first paragraph, they say it is a major route of nutrients and metabolites. As suggested above, this work takes a more restricted view. Nearly all of the active metabolic processes of vision occur within either the INM or the choroidal-RPE space. Except possibly for the diffusion of materials associated with the electrostenolytic processes, the contents of the IPM are supported exclusively by secretions from either of these two surrounding structures. Johnson, et. al. note the absence of both fibronectin and collagen from the IPM. They also indicate the soluble fraction of bovine IPM has been shown to contain a high proportion of protein and glycoprotein (98%), ”a property that also differs from connective tissue extracellular matrices.”

The Johnson paper concentrates on what they define as extracellular domains within the IPM and generally found adjacent to the disks of the outer segments. They stress the distinctness of these domains relative to the bulk of the IPM. They suggest these domains contain carbohydrates that are significantly different in composition, and concentration from those found elsewhere in the IPM. Although they define these domains as sheaths, they carefully differentiate them from the cellular sheaths adjacent to the outer segments and originating with the inner segments and with the RPE cells. They suggest these sheaths exhibit sufficient morphological integrity to allow their separation from the retina. While providing interesting photomicrographs of the photoreceptor cells fluorescing, they did not resolve the character of the material in their domains in detail. This material appears to correlate with the GARP’s found by Korshen, et. al\textsuperscript{171}. They also discuss the presence of the protein IRBP in the IPM and indicate it appears to concentrate directly adjacent to the surface of the RPE cells. This is their area of major chemical activity. However, it is not clear that they are secreted by the RPE cells. They may be secreted by the Mueller cells located between the photoreceptor cells.

The above findings are consistent with this work. It is proposed that the extracellular domains of Johnson, et. al. are the materials associated with the electrostenolytic process supporting the electrical activity of the neural portion of the photoreceptor cells. Under this hypothesis, these materials are primarily GABA, glycine and the glutamates associated with the glutamate cycle of nutrition. Unfortunately, the use of formaldehyde in the experiments of Johnson, et. al. probably obscure the presence of such materials as the aldehyde of glutamic acid. The concentration of their material in domains near “cones,” as opposed to “rods,” is explained by the lower packing density of disk material in these areas. The electrostenolytic material is merely present in higher volume in these relative voids.

4.5.3 The character and content of the RPE layer

Considerable work has been performed to determine the character and content of the RPE layer because of its presumed involvement in aging. Much of it has been done using light microscopy and associated visual band fluoroscopy. The work of the Feeney-Burns teams is particularly large with dates ranging from 1965 to 1992\textsuperscript{172}. Weiter, et. al. have also provided useful data\textsuperscript{173}. Early work began using fluoroscopy at an excitation wavelength of 365 nm and an observation wavelength of 470 nm. This procedure has continued to be followed even though the fluorescence of the RPE is known to exhibit a broader spectrum (Typically 430 to beyond 700 nm) with a peak in the 540-640 nm range\textsuperscript{174}. The data of Elder, et. al. is based on difference spectra between old and young eyes which may introduce a problem in interpretation. Much of the work has involved integrating the fluorescent from a cross section of an entire RPE cell or row of RPE cells. Areas of 30 by 3 microns are common. Older imaging was based on tungsten illumination and is generally at 800x with a few visual observations at 2000x. This level of performance is not able to resolve the cytological activity occurring within the samples and fails to reflect much of the blue character of the materials. The two pigment materials normally observed using these techniques are lipofuscin and melanin. It is not believed that the chromophoric pigments granules of vision present in the RPE can be observed by these techniques. The granules are quite small and the chromophores do not normally fluoresce due to their dipole structure. Some of the investigations employed electron microscopy where no chromatic information can be obtained. No rigorous definition of melanin could be found in these papers nor was there any discussion of whether the melanin was multicolored. The On-Line Medical Dictionary defines melanin as a mixture of red, yellow and violet colored materials without defining these colors rigorously. This set does not conform to the conventional notation of either the RGB or the CMYK system. It is a mixed set. See the highlighted sentence

below.

Hogan, et. al. provided additional information in 1971, including electron micrographs of pigment granules\(^{175}\). They also provided a caricature suggesting that “fingers” from the RPE cells may extract the chromophores from the disks before the terminal group of disks are engulfed by the RPE plasma membrane. This extraction would be easier if the putative membrane surrounding the disks, shown in their caricature, were absent as proposed here.

The 1988 paper by Feeney-Burns, et. al. provides electron microscopy of RPE and Bruch’s membrane cells ranging from 16,000x to 32,000x\(^{176}\). This paper explores the digestion of the phagosomes, engulfed from the outer segments of the photoreceptor cells, by lysosomes. The imagery also shows the presence of many individual granules identified only as “lysosomes (incipient lipofuscin granules)” in the investigation. Their introduction supported the view that most RPE phagolysosomes (combinations of phagosomes and lysosomes) are reduced to less than half their initial cross section within 16 hours after a shedding event in young animals. They also support the view that the process goes to completion leaving negligible residue. However, they propose that residues do accumulate in older eyes leading to lipofuscin granules and that some residues may accumulate in cells of Bruch’s membrane due to apoptosis, i.e., the shedding of RPE cytoplasm into Bruch’s membrane.

No reference was found in the above work to the possible presence of a variety of retinol binding proteins within the RPE space! This seems remarkable in light of other work going on during the same time span defining the composition and concentration of these materials in this space. Similarly, no reference could be found to the spectral properties of the melanin granules observed or whether they were all of the same color. It is interesting to note that Elder, et. al. presented a “corrected” fluorescent spectral emission curve for non polar chloroform extracts of whole human RPE that looks remarkable similar to the photopic luminosity function of human vision (the detailed function of this work, not the smoothed CIE variant). It is possible the extract consisted primarily of the chromophores of vision in significant concentration. The Elder curve is a small difference between two larger functions. It may deserve more detailed interpretation based on the procedure used to obtain it.

4.5.4 The Inner segment(EMPTY)

4.6 Development & Life Cycle of Photo-Receptors (Physical dynamics)

Other than some comparative material, this section focuses on describing the eye of Chordata. More research is needed to determine whether it is applicable to Arthropoda and Mollusca. It is likely that some similar mechanism is operable for the longer life species in these phyla. The material applies to the tetrachromatic eye. However, the schematics are drawn for the trichromatic eye for convenience. They can easily be extended.

There is a large amount of very valuable data in the technical literature on this subject. However, most of it has been interpreted differently than it is here. Rodieck\(^{177}\) has provided an extensive discussion and an excellent reference list up to 1973. However, there are many inconsistencies at the detail level. Stein, et. al.\(^{178}\), writing in 1991, provide a more concise discussion and additional references. The discussion addresses both disk formation and disk shedding. Lam & Bray have provided a discussion on the embryology of the various cell types in the retina\(^{179}\).

Very important work in this area was performed at the nucleotides during the 1980's. Those works were designed to determine the dynamics of the disks of the outer segment of the photoreceptor cells. This work demonstrated clearly that the opsin substrates were formed in a process entirely independent of the process that formed the chromophoric material. The only conclusion is that rhodopsin is a conglomerate and not a chemical compound. It is a conglomerate of the chromophore, Rhodamine, deposited as a liquid crystalline film on an inert protein substrate, opsin. This designation will be used here to differentiate the complete protein molecule from the chromophore, even though the protein incorporates a retinal ligand (that does not act as a chromophore). The photosensitivity of this conglomerate to visible light is changed greatly when the neural connections to the parent photoreceptor cell are disturbed.


\(^{179}\)Reh, T. in Ryan, et. al. (2001) Retina, 3\(^{rd}\) ed. Vol. One, St. Louis, MO: Mosby, Chap 1
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More recently, detailed description of the opsin found in the disks of chordates have become available. These have shown the precise location and structural arrangement of a N-retinoid ligand totally enclosed within the overall protein. This ligand does not appear to participate in the signaling function. However, its presence appears to explain the need to replace the disks in chordate eyes on a regular basis and at a rapid rate. Okada, T. Le Trong, et. al. have found that solid crystals of opsin (incorporating the non-chromophore -N-retinoid) are sensitive to, swell under, and are destroyed by visual light \(^{180}\). It appears that the light causes an isomerization of the retinoid and this change in structure is sufficient to damage the structural integrity of the crystal. It is not known whether a similar isomerization within a liquid-crystal of opsin would have the same effect. If it does, the damage caused to the opsin substrate by the small amount of light absorbed molecularly (as opposed to resonantly) by the retinoid would generate the requirement to replace the disks on a routine basis. No other reason was found in the literature for the routine replacement of the disks.

4.6.1 Overview

In the last few years, much new information has become available concerning the life cycle of the photoreceptors. It is now possible to definitize and quantify many of the processes occurring in this regime; specifically, the rates of growth, the methods of material formation, the transport paths involved and, in a preliminary fashion, the transport mechanisms involved. It is also clear that there is a series of feasible experiments that could be performed to fill holes in the data. A specific group of experiments would be to repeat the work of the Young\(^{181}\) team and the Bridges team in the autoradiography of the photoreceptor-IPM-RPE complex discussed below.

Another important area would be to continue the work of understanding the processes of esterification of retinol occurring in the RPE and of understanding the details of the transport of the resultant retinoids (Rhodonines) through the inter-photoreceptor matrix (IPM). The recent work in these areas is summarized in Ganguly\(^{182}\).

With the elucidation of this model, some of the ideas presented in the literature are best described using a more precise syntax. The ideas are frequently correct but are found to utilize words which lack sufficient precision. There are also some inconsistencies that can be eliminated if this model is adopted.

There are a number of ideas in the literature referring to processes that occur when the retina is illuminated and others that occur when the retina is not illuminated. These ideas do not fully recognize the continuity of the visual process with respect to illumination. If there was actually a process that consumed or de-activated material during illumination without allowing its restoration, the eye would show fatigue effects which would actually lead to blindness under continuous use. This does not occur except in certain cases of fatigue related to inadequate metabolic supply from the bloodstream (i.e., transient tunnel vision) and this condition is rapidly eliminated without requiring the retina be placed in the dark. It is important to recognize that all processes in the eye operate continuously.

There are also a number of statements in the literature that speak of “oxidizing All-trans-retinol to 11-cis-retinaldehyde by the membrane-bound retinol dehydrogenase of the ROS”\(^{183}\) and “Used retinoids leave the photoreceptors, cross the sub-retinal space, and are stored in the RPE, where all-trans retinal is re-isomerized to 11-cis retinal.”\(^{184}\) When associated with any model, these statements are seen to be inconsistent. These statements are inconsistent with the model used here for several reasons. First, it is not specified which “membrane” is being discussed, the outer membrane surrounding the disks or the “membrane” forming the disks themselves. Second the model does not require and there is no traceable evidence for a membrane surrounding the OS. Third 11-cis-retinaldehyde is not required to explain the phototransduction processes using this model. In this model, the Rhodonines are transported from the RPE to the photoreceptor cells in all-trans form by the Interstitial Retinoid-Binding Protein (IRBP) and utilized in that configuration. The term retinoid is used in the above label instead of the conventional retinol since it has not been demonstrated that the material transported by the protein is retinol. To be even more specific, it should be changed to Rhodoline when speaking specifically of the retinoids transported to the RPE and IPM to implement vision. As shown below, this latter change would open a discussion on whether a single IRBP is capable of transporting three different

\(^{180}\) Okada, T. Le Trong, I. Fox, B. et. al. (2000) X-ray diffraction analysis of three-dimensional crystals of bovine rhodopsin obtained from mixed micelles J. Struct Biol vol. 130, pp 73-80


Rhodonines or whether there are three different IRBP's which either create or select the specific Rhodonines in conjunction with their transport.

Heckenlively has provided a caricature and an overview of pathogenesis related to the operation of the photoreceptor cell, other than the signal generating functions\textsuperscript{185}. It appears his discussion is enhanced when reinterpreted in the context of this model.

Because of the importance of relating the following discussion to the literature, quotations will frequently be used. However, this author would choose to use more precise labels in many cases. In those cases, the quotation will be used but a different label will be suggested by its inclusion in curved brackets immediately after the label of the original author. Generally, it will be left to the reader to determine which label is more appropriate. [xxx was this technique used ]

4.6.1.1 Formation of the photoreceptor cells and Outer Segments

There is a variety of data available on the formation of the animal eye and when it becomes visually functional. From this data, several propositions can be drawn. There are a number of interesting questions about the eye of the human baby shortly after birth. Is the baby able to sense light when it first opens its eyes? Can it see colors properly at a very early stage? Is the signal processing function of the retina fully functional when the eyes open?

The question of signal processing and seeing colors at an early stage can be answered fairly well for various animals. It appears obvious that if a four legged ungulate (such as a deer) can get up on its feet within minutes of birth and scamper around after its mother, the signal processing function of the retina and brain must be pretty well developed. This would indicate much of the signal processing circuitry must be genetically encoded. If the geometric circuitry is genetically encoded, it is probably true that the color sensing circuitry is also genetically encoded. If this is true, then the color sensitivity of the various photoreceptors must also be genetically encoded or else incorrect color information could be provided to the color processing circuitry. This would lead to the conclusion that there is a genetically controlled mechanism in the photoreceptor that defines what retinoid will initially form a liquid crystal in the OS. This genetic encoding would therefore control the specific color sensitivity of each photoreceptor in the eye as a function of its position in the eye.

Recent autopsy data suggests that the Outer Segments may not have attained their nominal length at birth in humans but do so within a week or so.

As we learn more about the mosaic properties of the eye, it appears likely that a clearer understanding of the initial capability of the eye at birth will arise.

There is an interesting question about the two forms of Vitamin A. Given a choice, which form of Vitamin A will be used to form the chromogens that are eventually deposited on the disks as chromophores? Bridges\textsuperscript{186} provides an interesting discussion that says the type of Vitamin A used is determined primarily by the environment of the animal. In migratory fish, both types are used at different times in their lives. In mixed environments, the chromophores appear to be based on a mixture of Vitamin A’s. The ratio of Vitamin A\textsubscript{1} to Vitamin A\textsubscript{2} can be seen to vary in the disks along the length of given photoreceptor Outer Segment. If measured precisely versus an abrupt change in the environment, this data could provide another avenue for determining the rate of growth of the disks. In human, only retinol associated with Vitamin A\textsubscript{1} is usually found.

4.6.1.2 Initial formation of the basic structure

There are a number of tantalizing reports in the literature concerning the formation of the photoreceptors but very little that attempts to put the data into an overall context. It is clear that there is a wide range of conditions that can affect the formation and organization of the photoreceptors. Some of these conditions could have significant impact on the health and operation of the photoreceptor over the long term. These include the environment during initial formation, during maturation and obviously during long term operation where the system is subject to damage from a number of sources.

In reviewing this material, it became important to segregate that related to the visual system in tadpoles from that of the later infant form of the mature animal. The data frequently suggested the 6-day-old tadpole visual system resembled that of the intermediate tunicate form shown in [Figure 4.2.1-1] more than it did the adult form. However, it was difficult

to be sure as the location of the lens was not specified. By eight days, a more conventional chordate Outer Segment was observed\textsuperscript{187}.

The literature generally places the formation of the retina and its basic components during the seventh to fourteenth weeks of gestation in the human. There is speculation on how completely the retina and its interconnections are formed at birth. There is little data in the literature concerning humans in this respect. However, there is the interesting observation that the ungulates and other animals who are capable of rising to their feet and running within a few minutes of birth are not known to run into things for lack of vision. In these cases, primarily relating to warm-blooded animals, it is clear that the retina is operational at birth--at least with respect to luminance information. It is also clear that the interconnections and data reduction capability are mature enough to reduce the data acquired by the retina. This leads to the conclusion that much if not all of the interconnections are genetically programmed. In small mammals that are born with their eyes closed, it has been observed that their photoreceptor cells frequently lack an outer segment. This segment forms during the first few days of post-natal life; with the eyes opening and functioning properly only a few days later. Recent studies of human infants also suggest immature Outer Segments at birth.

There is data on the speed with which the outer segments are formed for some warm blooded and some cold blooded animals. This data indicates the growth rate is specifically related to temperature as would be expected; the warm-blooded rate being significantly higher than for the cold blooded.

No data was found related to any difference in speed of outer segment growth for different types of photoreceptors in the same organism. No data was found relating to how the different types of photoreceptors in a given organism are formed relative to location or spectral sensitivity. It appears that these parameters, size and spectral sensitivity are related to their location in the retina by relatively simple geometric rules--but they have not been enunciated in the literature.

For the human, there is reason to believe that the “seeding” of the individual photoreceptors in order to determine their spectral sensitivity is strongly influenced by the temperature of the fetus during the seventh to fourteenth weeks of gestation. Excessive temperature of the fetus during this interval appears to correlate with abnormalities of color vision in the mature eye. This situation will be addressed more completely in the chapter on color abnormalities.

Once formed, the photoreceptor cells of the eye appear to be relatively fixed structures for the life of the animal except for the outer segments; the outer segments are dynamic structures with a continual and a rather rapid cycle of renewal. The formation, operational life and destruction of a given structure of the outer segment have been investigated from many aspects and a reasonably coherent story emerges.

The static structure and function of the outer segments have been discussed above. The interesting thing is that the individual photoreceptor functions normally while in a structurally transient state. New disks are formed at the base of the outer segment nearest the inner segment and older disks are removed from the outer segment at the RPE interface while the receptor is operating normally; absorbing light, transporting excitons through its structure and exciting the dendrites of the photoreceptor. It is not clear yet from a physical point of view whether the dendrites grow and move along with the disks or whether the dendrites are of fixed length and make contact with the moving disks.

The case where the dendrites are of fixed length is simple. The dendrite-outer segment relationship can be pictured similarly to the small brushes used on a copier machine to remove static electricity from the paper emerging from the machine. The charge is transferred to the brush after the paper has been processed even while the paper is moving past the dendrites continuously.

The alternate mechanism would involve the dendrites growing in length at the same rate as the disk stack moves toward the RPE. In this scenario, the dendrites would have a permanent contact(s) to each disk. However, the terminal end of the dendrites would probably be phagocytized continuously at the RPE.

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

\textsuperscript{187}Nilsson, S. (1964) Receptor cell outer segment development and ultrastructure of the disk membranes in the retina of the tadpole (Rana pipiens) \textit{J. Ultrastr. Res.} vol. 11, 581-620
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 4.6.1-1, 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 Segment188. 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 photoreceptor had been sliced through the Cilium area, the interpretation of the structure would be more complex.

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\textsuperscript{189}, 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.

\subsubsection*{4.6.1.5 Phagocytosis}

If the disks of the Outer Segment are produced continuously in an enclosed space, it is obviously necessary for there to be a mechanism to remove the older material from that space. The digestive capability of the ectodermal tissue facing the photoreceptor cells, the so-called retinal epithelium cells, is optimally located to accomplish this process. They are able to digest the disks routinely when the disks reach the immediate region of the RPE cells. This process is called phagocytosis.

There is some question in the literature as to how many disk stacks terminate on a single RPE cell. Various cartoons suggest a one-to-one relationship. Some texts suggest a ratio of 24-44 disk stacks per RPE cell\textsuperscript{190}. In either case, the RPE cells as a group perform a daunting disk digestion and chromophore salvage task.

Holtzman has shown a cross-section of phagocytosis using autoradiography which he credits to R. Young\textsuperscript{191}. The image shows no sign of any plasma membrane destruction during the destruction of the disks.

Steinman, et. al\textsuperscript{192}, and Muller, et. al\textsuperscript{193}, have presented reviews with large bibliographies that go into many aspects of endocytosis.

\subsubsection*{4.6.1.6 Regeneration}

As part of the phagocytosis process within the RPE cells, a large amount of protein material is reduced once again to individual amino acids and other residues. Simultaneously, a large amount of chromophoric material is recovered from the disks. As will be shown below, the RPE cells are the original source of the chromophoric material of the disks. The RPE cells are ideally suited for reclaiming this chromophoric material, storing it temporarily if required, and redelivering

\textsuperscript{190}Xxx. pg 399 (can’t find reference by typeface, page is in flexible filefolder.)
\textsuperscript{191}Holtzman, xxx. (1975) Op. Cit. pg 165
it to the area of initial disk formation and activation within the extrusion cup of the Inner Segments of the photoreceptor cells. The RPE cells are not the original source of the protein material used to form the opsin substrates of the disks. Opsin is created within the inner segment, or the extrusion cup of the Inner Segment, of the photoreceptor cell. Therefore, this material is returned to the bloodstream by the RPE cell for transport to other locations for further processing or disposal.

### 4.6.2 Normal operation of the photoreceptors--metabolism & growth

The operation of the photoreceptors of the eye is a continuous and steady process when viewed from any of many perspectives, illumination, chemistry, metabolic transport, and the transport of functional materials.

Figure 4.6.2-1 presents an overall caricature of the Photoreceptor-RPE environment as it applies to any photoreceptor cell in the chordate retina (although it requires further expansion when applied to some of the multiple layer retinas found in some deep ocean fish).

In this caricature, there is no external membrane around the disks (d) of the OS. A calyx, an extrusion cup labeled (e), is formed in the distal end of the Inner Segment. The dendrites of the nerve pass through the calyx, previously and frequently labeled the cilium, (c) before being deposited in the fissures of the disks (not shown). The electrolytic conduit, known as the axon (a) of the nerve, passes beside the nucleus on its way from the Activa to the pedicle (P). Finally, the RPE forms a receptacle around the terminal end of the disk stack (d) to aid in ingestion and subsequent digestion.

The figure is superficially related to that of Fliesler &
Anderson\textsuperscript{194} and shown in Ganguly. However, several differences are important. The area shown here as the extrusion cup is interpreted differently and there is no photoreceptor cell membrane enclosing the OS. In addition, the area from the cilium to the photoreceptor axon ending is rearranged and interpreted differently. Although not shown at this level of detail, a feature described by Richardson\textsuperscript{195} appears relevant and interpretable. There appear to be apertures in the wall of the extrusion cup providing a hydraulic connection between the newly formed disks and the IPM.

Many of the following caricatures will not display the neural aspects of the photoreceptor since these aspects are not involved in the fluid and chemical dynamics of the ROS.

In several of the following schematics, the total blood flow to the retina is divided into the neural laminate portion and the RPE laminate portion (the choro-vascular system). For orientation purposes, the total blood supply to the eye is given by Hogan\textsuperscript{196} in the following statements:

The retinal rate of flow is estimated at 1.6 to 1.7 ml per mm per gm of the retina.

The mean retinal circulation time in man is 4.7 +/- 1.1 sec.

The mean transit time is 3 to 4 seconds.

He provides references for these statements. There is clearly a time constant associated with transient operation of the eye with a value on the order of 3-5 seconds. Hogan does not discuss the division of the blood flow between the two laminates, concentrating primarily on the neural laminate, that is easy for the investigator to see.

4.6.2.1 Details of the OS

The OS consists of a stack of disks that are formed by extrusion from the exocrine gland portion of the photoreceptor inner segment. These disks are extracellular with respect to the IS. Although parts of the disk stack are surrounded by membranes associated with the calyx or the RPE, they do not reside with the plasma membrane of the photoreceptor cell. The disks are in physical and electronic contact with each other and are immersed in a liquid medium defined as the inter-photoreceptor matrix (IPM). The disk stack is mechanically supported by the extrusion cup of the IS and the receptacle cup of the RPE. This support is structurally quite adequate during almost all animal activity. However, the disk stack is a fragile structure which can be damaged in human sports and accidents; resulting in a “separated or torn retina.” In fact, this structural weakness is used to advantage in the experimental laboratory where the retina is usually separated from the RPE by shearing off all of the photoreceptors in the region of the disk stacks.


\textsuperscript{195} Richardson, T. (1969) Cytoplasmic and ciliary connections between the inner and outer segments of mammalian visual receptors. Vision Res. 9, 727-731 [V-A]

During normal operation of the eye, the disks move continually toward the RPE until they enter a terminal phase where they are separated from the OS stack in groups and are phagocytyzied by the constituents of the RPE. The rate of this movement is known from the literature of Young et al.\textsuperscript{107} and Hall, Bok and Bacharach.\textsuperscript{108} This model also proposes a monomolecular layer of a retinoid essentially covering the surface of the disks. Most of the literature does not indicate the presence of a separate film of retinoid material since this material is usually removed by the aggressive "washing" used to separate the disks and remove "extraneous material" in the laboratory. In this model, the disks are coated with a monolayer of liquid crystalline material consisting of one of the Rhodones. This material not only acts as the light-sensitive material of the eye, it provides a mechanical and electronic interconnection between the individual disks and the neural system. The monolayer represents only a very small fraction of the total disk mass, a fraction easily dismissed in the laboratory analysis of the disks if its presence is not anticipated. During washing, the monolayer is frequently dissolved or hydrolyzed (destroying its spectral absorption characteristics) and becomes another constituent (along with materials from the IPM) of the wash solution.

4.6.2.2 Background related to the dynamics of the material processing loops

The ideas introduced in the preceding discussions can be assembled into an overall view of the creation, operation and renewal of the photoreceptor/RPE complex which can be very informative. The overall processing loop is shown schematically in Figure 4.6.2-2. The separate paths followed by the protein material, opsin, and the retinoid material, Rhodone, are clearly differentiated. These will be discussed in detail below.

In this figure, the protein generation and disassembly process is essentially isolated from the retinoid generation and reuse process. As shown, and well verified by laboratory experiments, the necessary materials to create the structural protein, Opin, are drawn from the blood stream of the neural laminate at the photoreceptor cell. It then passes through the Golgi apparatus (not shown) and the Ribosomes (RI) of the Inner Segment (IS) of the photoreceptor cell where the protein is either partially or fully assembled. The material then passes through the cell wall into the IS extrusion cup where it is found in completed protein form. This material is folded and cut (probably broken by bending forces) into disks and extruded as a stack of disks comprising the outer segment (OS). The disks are separated by a physical space required to satisfy optical absorption requirements. The resulting stack of disks can be considered a spaceframe composed of individual disks.

The disk material is extruded continuously resulting in a mean disk velocity away from the IS and toward the RPE of about 300 nm/hr or 7.2 μ/day. After approximately seven days, 1 week, the disks reach the RPE and are phagocytized by the RPE. The components are then disassembled and the constituents are returned to the blood supply.

Simultaneously, but separately, the chromophores of vision are prepared in the RPE cells. The retinoid material is delivered to the RPE laminate by a serum-retinoid-transport-protein (SRBP) via the choro-vascular bloodstream. It is then transported to the Ribosomes of the RPE cell for processing by the protein, CRBP. The material is then transported to the pigment granules (PG) for temporary storage by the protein, CRAIBP. When required, the material is then transported to the RPE/IPM interface by the protein, CRAIBP, where it is released to the protein, IRBP. IRBP then supports the final transport of the chromophores to the

\textsuperscript{107} ibid, Young

disks of the Outer Segment. This process is discussed in detail in Sections 4.6.2.4 & 7.1.2.

It is likely that, after the protein material is shaped into strip form and before it exits the IS extrusion cup, it is exposed to the fluid environment known as the IPM (shown between the double barred lines) which is saturated with the chromophoric material attached to the binding (and transport) protein IRBP. The IRBP interacts with the protein spaceframe and performs an initial coating of the protein. IRBP releases the chromophore and coats at least one surface of the protein with the chromophoric material. This material is deposited in a monolayer that forms a liquid crystal of the chromophore. The chromophoric material is probably held to the spaceframe opsin by very weak forces, typically Van der Waal forces or hydrogen bonds based on similar situations. As will be addressed completely in Chapter 5, the use of a Schiff base in this situation to combine the chromophore and the protein substrate would destroy the electronic configuration of the chromophore necessary for its enhanced spectral performance. This initial coating is necessary to insure the coating chromophore is the same chromophore (1 of 3 carried by the IRBP molecules in long wavelength trichromats) as earlier deposited for that particular photoreceptor. The chromophores are assumed to have sufficient stereo-specific properties to insure this chromatic purity in the resulting outer segment.

As the protein material is broken and formed into disks, the resulting spaceframe of protein material is still in the IPM bath and any ruptures in the initial chromophoric coating are repaired by additional deposition of chromophoric molecules, building on the existing liquid crystal. The overall result is individual disks that are completely—only about 60% coverage is believed adequate in similar silver halide based photographic applications—with the proper chromophore type for that particular photoreceptor. The liquid crystal even coats around the edge of the protein disk so that the two sides are in electronic communications. The liquid crystal appears to follow the lip formed around the edge of the disk due to a limited ability of opsin to curve around a corner. This lip effectively acts as a spacer between the faces of adjacent disks.

This liquid crystal material, a gel, may act as a glue and aid in holding the disks in position within the OS after extrusion is completed.

The liquid crystalline material is now in the proper condition to exhibit the necessary enhanced photo absorption properties and be raised to an excited state by the absorption of energy from the photons. If in close proximity to appropriate neural tissue, it can transfer that energy to the neural network. As developed in Appendix A, this cycle of excitation and de-excitation, described by the Photoexcitation/De-excitation Equation, occurs continuously throughout the life of the disk at rates measured in microseconds to milliseconds depending on the capability of the neural system to absorb the energy.

As the disks of the outer segment pass into the receptacle of the RPE, they leave the saturated solution environment of the IPM and enter an environment which is conducive to the solvation of the liquid crystal. The chromophoric material may be treated as a particle and participate in the process of phagocytosis or it may be treated as a solute and be absorbed through the wall of the RPE cells. In either case, the material is bound to the binding protein CRAIBP and transported back to one of the pigment granules appropriate for that chromophore. This same binding protein is also responsible for transporting the chromophores to the RPE cell boundary for acquisition by the IRBP, thus completing the “loop” for the retinoid materials. Ganguly\(^\text{199}\) says it in another way, “The retinol, whether it enters the RPE cells from the blood plasma or whether it comes from the retina . . . is esterfied in the RPE cells.” It is proposed that retinol should be broadened to retinoids in the above statements, and probably changed to refer to the Rhodonines.

It is possible that some of the chromophoric material is damaged during its existence on a disk. If this happens, the CRAIBP will not be able to bind to it; it will be excreted back into the blood supply. To account for this loss, the manufacturing facilities of the RPE cell are able to produce new chromophoric material by extracting all \textit{trans}-retinol from the blood stream and processing it into the Rhodonines that are the actual chromophoric materials. The retinol is transported to the manufacturing area by CRBP which is specific for all \textit{trans}-retinoid material. After manufacture and before use, chromophoric material is transported to the pigment granules by CRABP which is believed to be capable of binding to the “conjugated retinoid acid” although this is yet to be demonstrated in the literature. Alternately, CRAIBP could also perform this function. However, it appears that CRAIBP is specifically designed to attach to only one end of the chromophore so that it can perform a handshaking operation with both the palmitic (stearic) acid found in the pigment granules and the IRBP at the cell boundary. See Section 4.6.2.3.3.

\textbf{4.6.2.2.1 Caricatures from the literature}

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  \item \textsuperscript{199} ibid, Ganguly, pg 156
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Figure 4.6.2-3 is a caricature of the visual cycle similar to that of Bridges\textsuperscript{200} but with some significant differences. It is assumed that vascular flow occurs within the RPE laminate independent of the choriocapillaris on the other side of Bruch’s membrane. The arrows indicative of the flow of the simple nonresonant retinoids have been dropped from the figure. A slight rearrangement of the location of the binding proteins has been made. A diagonal line has been introduced to indicate the foreshortening of the OSP’s in the figure. The photoreceptors should be shown with no horizontal spacing and OSP’s with an aspect ratio of 25:1. IRBP is shown circulating between the RPE interface and the base of the OSP’s in the vicinity of the Image Focal Plane. As indicated elsewhere, the Outer Limiting Membrane may actually consist of Muller cells packed in between the PC’s. The location of the Petzval surface, the image focal plane of the optical system, is also shown for reference.

The figure does not show the individual Pigment Epithelial cells (PE) explicitly. They would be shown immersed in the RPE laminate. This laminate includes the vascular diffusion bed that brings holo-SRBP to the external wall of each PE cell and returns no longer vital SRBP to the bloodstream. SRBP is a one time use binding protein. It is created in the liver and must be complexed with all-trans-retinol (only) on a 1:1 basis before being released into the bloodstream. After release of the retinol to the consumer cell, SRBP is catabolized by the kidney.

For purposes of later discussion, CRBP is shown as the binding protein that extracts retinol from the RPE diffusion bed by performing a handshake with the holo-SRBP. It is also shown delivering the retinoid to the Ribosomes of the PE cells. CRABP is shown accepting the chromophores manufactured in the Ribosomes and delivering it to the Pigment Granules for storage. CRAIBP is shown accepting the chromophores for transport to the cell wall where they are passed to the apo-IRBP for transport to and deposition on the disks of the OSP. CRAIBP is also shown recovering the retinoids from the phagocytosis process and returning them to the Pigment Granules. Ganguly speaks of the presence of an isomerase found exclusively in the RPE and responsible for the esterfication of retinol. He uses this to support the role of the RPE as opposed to the IS in preparation of the chromophores.

Figure 4.6.2-4 is a caricature taken from the work of Hogan, Alvarado & Weddell\textsuperscript{201}. They provide a valuable view of the overall phagocytosis process in vision. They show the thick sheaths (a), typically 4 microns tall in humans, that capture the end of the OSP for both physical support and for purposes of isolating the end of the OSP from the IPM. The sheath allows phagocytosis to begin without chemical competition from the materials of the IPM. Note the words of the caption. These words do not describe a cell membrane enclosing the disks (b). Note also the call-out (l) which describes microtubules originating in the basal body of the rod [photoreceptor] cilium (sic). If these microtubules are active neural tissue as at the IS end, it implies that the entire disk stack is in good electronic communication with the neural dendrites of the IS. Hogan implies by showing the finger like villus process [structure] (c) contacting the OSP before the OSP reaches the sheath (right side) that some phagocytosis may occur before the OSP reaches the sheath. Hogan’s text suggests that these villus structures are beginning the phagocytosis (solvation or hydrolysis) of the chromophores from the OSP and transferring the chromophores to the “numerous pigment granules” found nearby in the RPE cells. This figure is consistent with the proposals of this work.


Figure 4.6.2-4 A three-dimensional caricature of the process of phagocytosis showing the relation of the outer segments of the rods to the retinal pigment epithelial cells. Thick sheaths (a) of the pigment epithelium enclose the external portions of the rod outer segments (b). Numerous finger-like villous processes (c) are found between the photoreceptors; they contain pigment granules (d). The apical portion of the pigment epithelial layer of cells is seen at the bottom. It contains numerous pigment granules (e), mitochondria (f), a well developed, smooth-surfaced endoplasmic reticulum (g), a poorly developed, rough-surfaced endoplasmic reticulum (h) and scattered free ribosomes. The stacks of the rod outer segment discs are depicted in meridional section at (i) and in cross-section at (j). There is scalloping of the periphery of the discs (k). Microtubules origination in the basal body of the rod cilium extend externally into the outer segment; one such microtubule is shown in cross-section at (l). From Hogan (1971)
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Figure 4.6.2-5 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 chromophoric 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 esterified 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.\textsuperscript{202} 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.

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\textsuperscript{203} 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-\textit{trans} and 11-\textit{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 4.6.2-6. There are three clearly delineated types of globules of chromogenic if not actually chromophoric material visible in this image from Wolken\textsuperscript{204}. 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\textsuperscript{205}. More recently, Nakanishi has described the at least one of these chromogenic globules within the RPE as the fluorescent pigment lipofuscin\textsuperscript{206}. His slide was based on two references\textsuperscript{207,208}. Sakai, et. al. define a chemical structure of a material that Nakanishi associates with one of the colored globules in the RPE. Sakai, et. al. describe the material in the title to the paper as “an unprecedented pyridinium bisretinoid.” The chemical structure of the material appears incomplete. The material as described is almost certainly transparent at visible wavelengths.

\textsuperscript{201} ibid, Ganguly, pg 153 & 156


\textsuperscript{206} www.columbia.edu/cu/chemistry/groups/nakanishi/vision/Slide1.GIF (case sensitive address)

\textsuperscript{207} Ben-Shabat, S et. al. (2001) Fluorescent pigments of the retinal pigment epithelium and age-related macular degeneration Bioorg Med Chem Lett vol 11, pp 1533-1540

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 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.

Figure 4.6.2-7 provides a more complete caricature of this area of the RPE based on this work. It shows all four chromogen sources, although the UV– supply cannot be photographed with conventional photographic film or observed through the microscope by humans. It will appear as a transparent cell.

4.6.2.2.4 The presence of “Gates,” and materials crossing membranes in the IPM

It should be noted that the data, collected by Ganguly and others, does not report a significant level of either sodium or
calcium in the IPM or its individual constituents. It is difficult to interpret this lack of reported data as an oversight on the part of the researchers. It is also important to note that most authors who have attempted to make measurements on the putative plasma membrane surrounding the Outer Segment have not been sufficiently careful. They have not differentiated between the membrane and staves of the Inner Segment, the sleeve of the RPE that tend to enclose significant parts of the Outer Segment, and any putative plasma membrane surrounding only the disks. In the absence of significant quantities of calcium and/or potassium in the IPM and the absence of any documented proof of a plasma (bilayer) membrane surrounding the disk stacks of the Outer Segment, it is difficult to give any credence to the various “gate” models of the photo-transduction process.

There have been two schools attempting to explain the change in potential of the axoplasm based on the assumption that the change is due to the physical conductance of particles through a membrane. One school has assumed the principle particles were Ca\(^{2+}\) ions Minke has led this effort and presented a large number of papers over the years. Yau has been the main proponent of the cGMP gate model\(^{209}\). In this model, cGMP is the particle moving through the membrane and interpreted as causing a change in electrical conductance. Yau, et. al\(^{210}\). made an interesting comment in 1987. They said: “The two ideas are not necessarily mutually exclusive.” This statement could have been broadened to say, these two ideas, accounting for the change in axoplasm potential, are neither mutually exclusive or exclusive of other ideas. In fact, this work shows that there is no change in membrane conductance due to ion transport. The change is entirely accounted for by electron transport by an entirely different mechanism, transconductance, at related to an Activia. The following paragraphs are presented in the interest of completeness.

An early paper by Fesenko, et. al. has been relied upon by later authors discussing cGMP gates\(^{211}\). That paper illustrates the difficulty they encountered in obtaining the expected (desired) results. Out of a larger sample, they “obtained 136 patches of material stable enough to allow changing of fluids on one side of the material. Of these, only 70 responded to the application of cyclic GMP by an increase in conductance, while the current fluctuated in a random manner without any recognizable steps which could represent single-channel activity.”

As Yau notes in his comprehensive paper of 1994, there are still a variety of conflicts within individual gate models and between different gate models. The paper contains a large number of putative relationships, develops a number of reversals in position with time, and continues to rely upon the existence of an undefined “Na\(^{-}\)-Ca\(^{2+}\) exchanger” (apparently the still mythical ion pump of Hodgkin and Huxley). He also looks forward to the day the work in the gate field will transition from its current conceptual level “to describe the entire phototransduction process in a quantitative way.” In the meantime, his figure 12 continues to contain a number of question marks. These same question marks appeared in his paper of five years earlier, along with a similar list of unanswered questions about the proposal\(^{212}\).

His figure 14, reproduced from Lam, shows two distinctly different responses to a “single photon” by amphibian rods and cones. He does not define the state of adaptation of the cells involved. The responses appear to be two samples of


a continuous response that is a function of the excitation state within the chromophores of vision exactly as predicted by the P/D Equation of this work. While addressing the previously held view that mammalian rods did not adapt to light level, Yau concluded on page 22 that: “counter to previous belief, mammalian rods indeed adapt to light much like, for example, amphibian rods.”

Molday & Molday have recently provided their description of the cGMP gated channel\textsuperscript{213}.

It is important to note the separation between the terminology used in genetics and that used in physiology. Because of the order of research in reverse genetics, variants of the terms from physiology are frequently used in genetics. In this case, cGMP is defined as a protein that contained 690 amino acids and was first isolated in bovine\textsuperscript{214}. Kaupp, et. al. first isolated a cDNA clone for a subunit of the bovine gene associated with the putative rod cGMP-activated channel in the wall of the putative plasma membrane surrounding the Outer Segment\textsuperscript{215}. This caused a flurry of activity during the 1990's. The work of Sundin, et. al. and Kohl, et. al. are the latest reports on the genetic aspects of this work (See Section 18.8). There are now two recognized and differentiated genes of the above group associated with achromatopsia. Whereas Sundin, et. al. only associated these genes with the photoreceptor cell, Kohl, et. al. made a leap of faith and associated these with putative cGMP gates. An alternate and more specific association is discussed in Section 18.8.

The cGMP model relies upon the putative rhodopsin activation sequence first proposed in the 1950's but not supported here. This foundation requires that the chromophore of vision be an integral part of the protein secreted by the inner segment. This is not the situation illustrated above and confirmed experimentally below.

The only conclusion that can be drawn from a coherent reading of the complete literature is that the Outer Segments associated with the photoreceptor cells exist in extra-cellular space. It is not surrounded by a cell membrane and there is no source of or requirement for Calcium, Sodium ions or cGMP to participate in a neural transmission event. Transduction does not rely upon an isomeric transformation of a chromophore buried within a protein and does employ exciton transfer or phonon (mechanical) transfer of energy to the neuron as discussed earlier and developed in detail in Chapter 11 & 12.

**4.6.2.3 Details of the material processing loops of the Photoreceptor-IPM-RPE complex**

This material is critical to the defense of the ideas here in Chapter 4. It eventually will be moved to Chapter 7.

The data derived from nuclear labeling in studies of the photoreceptor-IPM-RPE complex is of crucial importance to the understanding of the visual system and the clarification of earlier concepts.

**This section will demonstrate conclusively that the classic concept of rhodopsin as a chemical compound formed within the cytosol of the photoreceptor cell is incorrect.** It will be shown that the appropriate material is a structured chemical conglomerate of an active Rhodonine deposited on an inert opsin surface. This material only exists within the IPM and external to the photoreceptor cell. It only exists within the RPE during the process of phagocytosis.

Recent experiments using nuclear isotope labeled materials have provided valuable information on the operation of the photosensitive functions of vision. The experiments have involved both in-vivo and in-vitro introduction of the isotopes labeled materials. The in-vivo experiments have taken advantage of the extremely high level of metabolic activity within both the photoreceptor cells and the RPE. These levels of activity have allowed the insertion of nuclear isotopes into an animal followed by observation of their physical concentration within the retina prior to their general distribution throughout the animals body. Two distinctly different (classes of) materials have been used in these experiments. In one case, a nuclear labeled retinoid has been introduced into the system, typically all-trans-[11,12-\textsuperscript{3}H]retinol. In the second case, a nuclear labeled amino acid has been introduced into the system, typically tritium labeled L-leucine or L-phenylalanine. The work has been dominated by the team led by R. W. Young. This team has generated a considerable bibliography in the 1967-78 period. Most of the work was exploratory as suggested by the time intervals used and the small sample sizes. Summaries were prepared in 1978, including a change in position relative to cones\textsuperscript{216}. Most of the

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\textsuperscript{215}Kaupp, U. et. al. (1989) Primary structure and functional expression from complementary DNA of the rod photoreceptor cyclic GMP-gated channel. *Nature*, vol. 342, pp762-766

\textsuperscript{216}Young, R. (1978) Visual cells, daily rhythms, and vision research. *Vision Res.* vol. 18, pp 573-578
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in-vivo work is believed to be archaic since it had not introduced the cytology of the photoreceptor cell presented by Papermaster and others in the 1980s\textsuperscript{217}. This is particularly true for the assumption that opsin traveled from the inner to the outer segment via the cilium. That assumption will be countered in this work. The early work of Hall, et. al. is discounted for reasons that involve the awkward wording in the last paragraph of their discussion\textsuperscript{218}. The complete explanation will become obvious below.

The totally in-vitro experimental activity, such as by Bok, et. al.\textsuperscript{219}, has generally been more indirect, has involved many uncontrolled variables and hence has been more controversial. Another type of study has investigated the requirement for fatty acids to be present in order to create photoreceptor membranes. These have taken two avenues. One involved the assumption that fatty acids are included in the basic disk structure. The other has involved the requirement for certain fatty acids to be present during protein manufacture\textsuperscript{220}. These studies are ancillary to the focus of this work.

There are two primary conclusions of these studies. First, the protein opsin is secreted, without any retinoid being present, by the photoreceptor cells. Second, the retinoids, Rhodamine( ), are secreted by the RPE cells. These materials are then combined in the inter-photoreceptor matrix to form the Rhodamine coated Opsin disks that are sensitive to light and capable of causing an electrical signal in the dendrites adjacent to the disks. Although this conglomeration, not compound, can be referred to as Rhodopsin for historical continuity, it is not recommended.

It must be stressed that all of the work sited in the literature has been based on morphology and biochemistry estranged from any attempts to demonstrate a physiological relationship of the materials to the spectral absorption of the chromophores of vision. Most of the work has implicitly assumed that the chromophore associated with opsin was present within the inner segment of the photoreceptor cell. Only in 1974 did Bok, et. al. allow that the chromophore might be added after protein fabrication.

This section will adopt the cytological model of the inner segment developed by Papermaster, et. al., and the cytological models of the IPM and the RPE developed in previous sections of this chapter. This includes a cilium (more properly a colax) that is totally dedicated to the neural function and does not transport protein for the disks. The overall situation is as illustrated in Figure 4.6.2-8. This figure highlights a single photoreceptor cell and the associated elements of the photoreceptor-IPM-RPE complex. For purposes of discussion, each disk shown in this figure represents 100 actual disks in the human photoreceptor outer segment. The necessary amino acids and other required materials are drawn from the vascular matrix of the INM through the plasma membrane of the photoreceptor cell either near the nucleus or near the inner segment. The material is processed initially in the Golgi Apparatus and then migrates past the mitochondria to the secretion point of the inner segment at the bottom of the extrusion cup. This is the model proposed by Papermaster and contained within [Figure 4.2.2-1]. There is no suggestion that the opsin forming the disks is recovered and reused within the PC/IPM/RPE complex. It appears the protein is hydrolyzed completely during phagocytosis and the residues returned to the vascular matrix of the RPE/Choroid space. The chromophore processing loop is fundamentally different. While the initial chromogen material is drawn from the vascular network of the RPE/Choroid, after the first traverse of the loop, the chromophoric material is not destroyed. The material is re-circulated continuously in the chromophore loop between the RPE cell layer and the extrusion cup of the inner segment of the photoreceptor cell (without ever crossing

the plasma membrane of that cell). Reserves of the chromophoric material are maintained within the RPE cells.

The data supporting the proposal associated with the caricature, from isotope-labeled experiments, will be presented in the following sections.

4.6.2.3.1 The secretion and absorption of the protein, Opsin (extended loop process)

Young performed a wide-ranging set of radiographic experiments during the 1970's exploring the movement of amino acids and proteins through the photoreceptor cell and the Outer Segment. While Young was meticulous in describing his studies as involving the movement of amino acid nucleotides, his work has been widely interpreted as applying to the putative compound and hence the chromophores of vision. His many caricatures have been widely reproduced. Although they changed over time, the concept of a ciliary neck transporting the protein material from the Inner to the Outer Segment has been widely adopted.

Contemporaneous radiographic tests by Bridges & Yoshikami were designed to demonstrate that retinoids passed through the same manufacturing channels as the tagged amino acids. However, this was not found to be the case. The retinoid material did not pass through the photoreceptor cell. The retinoids arrived at the Outer Segments via the RPE cells. See Section 4.6.2.3.3.

Young first reported the movement of isotope-labeled amino acid material from within the inner segment to the basal location of the disk stack in 1967. The caricatures in that paper did not illustrate a cilium. In 1968, additional data was presented and the discussion suggested the protein material traveled from within the inner segment to the disk stack via the cilium of the cell. This cilium had been defined morphologically in the 1953-56 time period. The technology of the day did not allow it to be characterized in detail. Young proposed that it was a channel for the transport of protein and prepared a widely reproduced caricature supporting this assumption apparently based entirely on morphology. The assumption was based on electron microscopy at 20,000x to 40,000x. Later investigations by Papermaster reviewed this assumption221, using newer equipment (magnification up to 74,000x) and presented the position that secretion of the protein, opsin, occurred within the extrusion cup of the inner segment (at least in frogs). He also proposed that the assembly of opsin was not completed until the opsinogen reached the vesicles along the plasma wall inside the extrusion cup. This position was taken because no holo-opsin was found in soluble cytosol fractions.

Since the desired experiments necessarily involve injection of isotope-labeled amino acid(s) followed by sectioning of the retinal tissue after a prescribed period, rats, mice and frogs are favorite subjects. Cold-blooded frogs can be used to easily explore the effects of temperature. The caricature in Figure 21 of Young’s 1967 paper shows this variation in the location of the isotope tagged opsin disks 10 days after introduction of 3H-methionine into the blood stream. His discussion says the first band of tagged material appeared in the basal disk within 24 hours. His figure 8 shows a micrograph after the subject was given two tagged doses 96 hours apart. While each band is not a solid line due to the low particle emission rate (half life of tritium, 12.26 years), they do exhibit a well-defined median perpendicular to the axis of the outer segment (at least after two days). Young’s published work in 1967-68 does not show a concentration of labeled amino acid passing through the cilium. On the contrary, figures 5 & 12 (and most likely 9) of his 1968 paper show a conspicuous absence of such material in the plane of the sectioned cilium222. In his 1968 paper with Droz, frame

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2C of figure 2 changed style to avoid showing a concentration of radiation activity within the cilium. The caricature in Frame 2d shows the radiation distributed uniformly across the disk at the bottom of the extrusion cup, as do the radiographs in Figures 11, 13 & 15.

It appears that the interpretation used here, based on Papermaster, is more realistic than that proposed earlier by Young. This interpretation assigns the role of the cilium to the neural role as found elsewhere in neural sensor cell biology.

**Figure 4.6.2-9** presents this interpretation which will be discussed in terms of the data of the Young team. Note. The cilium plays no role in the protein replacement loop.

The time for a disk to travel from the point of formation to the point of phagocytosis varies significantly by species and body temperature. Miller & Newman give a value of 7 to 60 days\(^{223}\). For human, it is nominally seven days for a disk stack length of 2000 disks. This is a production rate of about 12 disks per hour per cell. Young & Droz (1968) gave a production rate of one and one half disks per hour for frog photoreceptors at 22.5°C. Following this time, the disks are engulfed prior to phagocytosis in groups of 30-50 disks. Within 16 hours, the engulfed mass, a phagosome, is reduced to one half its diameter in micrographs of sectioned material. The literature suggests there is virtually no observable residue following the phagocytosis. The chromophore material is placed in storage within the RPE cells and all of the protein material is returned to the bloodstream as amino acids. It appears the phagocytosis process is basically one of (1) hydrolyzing the protein material and (2) returning the liquid crystalline chromophore material to a state of matter where it can be easily transferred to pigment granules within the RPE cells.

Based on the work of Papermaster, a major reinterpretation of several caricatures of Young is appropriate. **Figure 4.6.2-10** employs the data of

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Young but does not accept his concept that all of the disk material passes through the cilium. Instead, the roles of the cilium as an electrical feed-through for the dendrites of the neural portion of the photoreceptor, and the extrusion cup as the site of secretion of protein material into the IPM cell are recognized. The disks are at full diameter and grooved for dendrite insertion before leaving the extrusion cup (See Section 4.3.5.1.1). With these changes, the remainder of the Young caricature can be used as originally portrayed.

Figure 4.6.2-10 Caricature of the creation and transport of disk substrates based on an injection of radiolabeled amino acids. The path of the dendrites from the Activa within the inner segment through the colax to the outer segments is shown on the left of each frame. Frame A shows two of the dendrites in furrows passing up along the disks. The caricature is a reinterpretation of a Young caricature based on knowledge of the secretory properties of the photoreceptor cell. See text for details. Reinterpreted from Young 1976.

To explicitly illustrate the dendritic path from within the photoreceptor cell, the left frame of the figure shows two of the nine dendrites as they pass up along the furrows in the disk stack. In the other frame, only the easily identifiable
The Photoreceptor Cell 4- 119
dendrite bundle is shown. As shown, the disk stack is entirely external to the photoreceptor cell and, beyond the staves
and calyx of the Inner Segment, there is no membrane surrounding the stack. The heavy line surrounding the stack is
a contrast edge formed by the difference in material concentrations within the stack and the IPM.

Frame A shows the presence of nucleotides scattered uniformly within much of the inner segment, except within the
nucleus.

The technique of labeling certain amino acids and tracing their passage through the visual
system is only possible because of the prodigious acquisition and consumption of these materials
by the inner segment (in the short term) in order to continually prepare the protein required by
new disks for use in the outer segment. This concentration of the nucleotide labeled amino acid
is far greater than in any nearby tissue. Over the long term, the distribution of the nucleotides
becomes uniform throughout the body tissue as shown in frame 2d of Young & Droz, 1968.

A similar technique is available using tritiated retinol. It results in the accumulation of the
nucleotide material in specific locations within the RPE cells prior to its transport to the disks.
The prodigious rate of processing is again the reason for the high and rapid concentration of the
nucleotide labeled material.

Young differentiates between these two modes of material deposition. He defines that involving amino acids and the
formation of the substrates as “membrane replacement.” He defines the action related to tritiated retinol as “molecular
replacement.” These are acceptable as long as two points are understood, the term membrane refers to the substrate
of the disks and not a plasma membrane, and the term molecular refers to the chromophores of vision.

In frame B, the nucleotides have coalesced within the Golgi apparatus. Following preliminary processing, the
nucleotides pass on to the mitochondria for final processing before being secreted into the extrusion cup as shown in
frame C. It is in frame C where the protein is assembled into the substrates of the individual disks. Frame D shows the
loss of disks containing the nucleotides passing up along the length of the OS. Frames E and F show the initial
engulfing of the disks in groups of about 50 and their phagocytosis within the RPE cell.

It is important to study the radiographs of Young, et. al. relative to the cilium in detail. His images represent a time
sequence involving considerable time between samples. This situation makes it necessary to interpolate the position of
the nucleotides at intermediate times. Although this author found many images showing concentrations of nucleotides
near the cilium, no images were found in the published literature that showed a concentration of nucleotides within
the cilium as shown in the caricatures of Young.

It is not clear, from the literature related to vision, exactly how the opsin or its components are passed through the
membrane wall of the IS.

4.6.2.3.2 Disk formation and transport parameters

In the retina of a living animal, under constant light stimulation, a steady state must exist under which the rate at which
the photochemicals are excited (observed as bleaching) is equal to the rate at which they are de-excited (generally
described as regeneration).

The data in the literature concerning cell growth is of limited precision, mostly order of magnitude estimates. However,
even these estimates stress the remarkable capability of the photoreceptor cells to produce and the RPE cells to
phagocytize disks. The best available estimates, based on a disk pitch of 250 Angstrom, are:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disk formation rate</td>
<td>12 per hour per cell</td>
<td>nominal / warm blooded mammals</td>
</tr>
<tr>
<td>Disk operating life</td>
<td>166 hrs/7 days/1 wk.</td>
<td></td>
</tr>
<tr>
<td>Disk transport velocity</td>
<td>300 nm/hr--7.2 μ/day</td>
<td></td>
</tr>
</tbody>
</table>

The rate of disk formation and transport result in a typical lifetime for a disk of 1 week. This lifetime is incompatible
with the hypothesis in the isomerization theory that 11 cis-retinol is converted to all trans-retinol by light and remains
in that form until the disk containing it is phagocytized. If this were true, based on the photon fluxes involved in vision,
the normal eye would consume all of the transducer material in the eye in a matter of minutes and require the complete
exclusion of all light for up to 7 days to reacquire full sensitivity. Alternately, the all-trans retinol would have to be
removed from each disk after its formation, be transported to the RPE for regeneration as 11 cis-retinol, and then be
transported back to the disks. It is not reasonable to expect this procedure to occur within the time constants of the
adaptation process.
Based on the literature values, the amount of retinoids stored in the RPE is between 5 and 20 times larger than that in the outer segments. If this is correct and the mean disk operating life is one week, the human eye stores enough retinoids to supply itself for between one and six months without requiring external resupply. This estimate is compatible with Section 4.6.3.3.

The process loop presented in this chapter may give important insights into diseases of the retina. In the case of retinitis pigmentositis, it offers a number of possibilities for better understanding. Is the disease related to the buildup of debris in the RPE due to a failure in phagocytosis or is it due to an overproduction and subsequent storage of excessive amounts of the retinoids? Optimal treatment would be quite different under these two different situations.

4.6.2.3.3 The secretion and absorption of the chromophore, Rhodonine (local loop process)

To the surprise of many, the studies of retinoid transport and of the uptake of isotope-labeled retinoids have failed to show any appreciable amount of the retinoids entering the inner segment of the photoreceptor cells. The data is convincing that the retinoids of vision enter the IPM via the RPE cell layer. There is no indication that a chemical reaction involving opsin and retinol occurs in the photoreceptor cells or anywhere else in the visual system. This position excludes considering hydrogen bonding a chemical reaction.

The conventional wisdom concerning Vitamin A processing into a chromophore, before the late 1980s, assumed it happened in the IS of the photoreceptor cell. The assumption has been that it was then transferred to the OS as part of a complex molecule of the protein, Opsin, and the chromophore.

Pepperberg, et. al.225 provided a review in 1993 that began to change the emphasis toward fabrication of the chromophores in the RPE. However, lacking a working model, they continued to assume that the chromophore was involved in an isomerization and required physical transport to and from the RPE via IRBP.

Saari226, has provided a good summary of the conventional view of the retinoid loop and the enzymology involved as of 1994. He presented a “parsimonious model” of retinoid transport and concluded saying: the paper “serves to bring into focus unanswered questions and puzzling features of the cycle, and emphasize [sic] that we have merely touched the surface in our understanding of this complex process.”

These authors have suffered from the assumption that the Outer Segment was an internal element of the photoreceptor cell. They were not able to give convincing evidence how the chromophore entered the photoreceptor cell from the IPM or how the disks within the cell were later phagocytized without destroying the cell in the process.

The experiments of Bridges & Yoshikami, the data in Ganguly and this work have shown that the conventional wisdom did not describe the actual case. Careful measurements following ingestion of Vitamin A showed two things. Nearly all of the Vitamin A transported to the eye of the animal was transferred to the RPE. Only a minuscule amount was transported to the photoreceptor cells. This was the amount of vitamin needed to support fabrication of new protein.

Bridges & Yoshikami performed the earliest isotope experiments tracking retinoids within the photoreceptor-IPM-RPE complex space of rats227 and freshwater fish228. In both papers, they injected all-trans-retinyl-11,12-3H2-acetate. Recently, Okajima, et. al. have examined the similar processes in the toad229. Their work was all in-vitro and used all-trans-[11,12-3H]retinol. Ganguly provided a general background on the nutrition and excretion of isotope-labeled Vitamin A230.
In the 1969 experiments, Bridges & Yoshikami injected dark adapted rats with tritiated all-trans retinyl-11,12-3H2-acetate and found the radioactive content increased in the outer segments after 12 days whether the rat was kept in the light or dark. They did not use autoradiographic techniques to locate the precise sources of the radiation. Although their results appear valid, the authors did not speculate on any intermediate chemical processes involving the acetate, i.e., conversion into an aldehyde or alcohol.

More recent autoradiographic experiments have been performed to show the retinoid material believed to be the chromophores (or at least chromogens) are accumulated in the RPE, passed to the IPM and reach the newly formed disks in the area near (or in) the extrusion cup. This action strongly suggests the principal function of the protein ( Opsin) extruded from the cup is that of a structural protein and not necessarily related to the photosensing and transduction processes at all. In the proposed model, the chromophores utilize the structural protein as a substrate only and do not involve it in the signal generation process.

There is an interesting side-note to the 1970 Bridges paper. While they espouse the theory that they were replacing porphyropsin (an archaic synonym for the mid wavelength visual pigment with anisotropic absorption at 532 nm) with classical rhodopsin (peak isotropic absorption at ~500 nm exhibited by all visual chromophores), they did not specifically state the source of their retinyl acetate. This work would argue that they were replacing the Vitamin A2 based mid-wavelength chromophores of vision associated with fresh water fish with a Vitamin A1 based mid-wavelength chromophores of vision associated with marine fish (formed from the retinyl acetate obtained from their supplier). They were essentially inducing a transition encountered periodically in euryhaline fish. They did not provide any visual band spectrographic data to support their interpretations. Their UV spectrographs did confirm the switch between common Vitamin A1 based chromophores (peak isotropic absorption at 332 nm) and Vitamin A2 based chromophores (peak isotropic absorption at 340 nm), including a transition mixture containing 68% Vitamin A2 based material (See Chapter 6). Of particular interest are their comments on page 1341 concerning the “vitamins A” (quotes in the original) and their reference to Wald’s work in 1939. This work and the Wald paper interpret the changes reported by Bridges as the replacement of the Vitamin A2 based chromophores with Vitamin A1 based chromophores. Although not confirmed in the printed material, the change in peak absorption of the individual spectral chromophores in the visual spectral range should have been negligible.

Caricatures 1, 2 & 3 in Figure 5 of Bridges, et. al. (1970) are in total graphic agreement with this work. Only the interpretation is different. They do not show the presence of any material derived from retinyl acetate within the plasma membrane of the inner segment. They show the orderly replacement of the chromophoric material derived from retinyl-11,12-3H2-acetate on the assumption that it is part of the rhodopsin molecule. This assumption by Bridges, that rhodopsin is formed in the RPE, conflicts directly with the data of the Young team showing that the protein opsin originates in the inner segment and not the RPE, Section 4.6.2.3.1. However, the first sentence in their discussion is more explicit. They speak of replacing the retinol-based prosthetic group of rhodopsin. If the term rhodopsin is eliminated from their nomenclature in favor of Rhodonine, the conflict is resolved. When they speak of changing the retinol-based prosthetic group, it can be interpreted as changing the prosthetic group, the auxochrome location, within Rhodonine. They use the term condense to describe the way the chromophore forms on the substrate. It is suggested the form is more complex and should be described as adsorption. Rhodopsin can then be used to describe the conglomerate consisting of the chromophore, Rhodonine, adsorbed on the surface of the adsorbent, opsin, as a liquid crystal. Figure 4.6.2-11 shows this reinterpretation.

Trobe illustrates this same interpretation of chromophore flow, but including the archaic conventional view relating to other features (gates and rhodopsin activation).

In this work, the retinyl acetate chromogen is convertible to retinol in the liver and then converted into the chromophores of vision during transport to the RPE cell layer. The chromophores are transferred from the RPE cells to the IRBP in the IPM and transported to the interior of the extrusion cup. It is then adsorbed on the surface of the disks of the outer segment as shown in their figure and the figure above. The chromophore material then proceeds to move with the disks toward the RPE cells and phagocytosis. Upon phagocytosis, the chromophore material is transferred, without destruction, to the pigment granules for subsequent re-transfer to IRBP molecules and re-transport to the interior of the extrusion cup.
The closed loop cycling of the chromophores of vision continue within the RPE-IPM-outer segment complex without the chromophores ever passing through the plasma membrane of the inner segment, (or the photoreceptor cell itself). There appears to be no internal degradation of the chromophore material. However, it is subject to damage by external cosmic rays and man-made X-rays. Thus, the cycling of the material does provide the opportunity to remove non vital chromophoric material.

An assumption in the figure is that the supply of tagged chromogen can be turned on and off rapidly at the RPE-INM interface in order to create a sharp demarcation between tagged and untagged disks. This action cannot be achieved by controlling the ingestation of the retinoid by the animal or by injection of the retinol into the general circulation. This is due to the amount of stored material in the system. Because of the large reservoir of retinoid in the RPE, the nearly indefinite re-circulation of the retinoid and the long half-life of the tagged isotope, the entire outer segment will eventually become uniformly radioactive. The experiment will be further impacted by the storage of chromogens in the liver. The Bridges data needs to be reviewed further to determine the time required for the interface between the intrinsic chromophores and the tagged chromophores to reach a given position in the disk stack relative to the inner segment. Compare this figure with the earlier and similar one for the replacement of opsin in the disks.

Using mice, Redmund, et. al. have recently provided information on the accumulation of esters within the RPE and the failure of the chromophores to transfer from the RPE to the disks of the outer segment related to a series of diseases. Although their introduction and interpretation are based on the conventional wisdom of 11-cis-retinal, their article suggests the protein RPE65 is a factor in the transfer of the actual chromophores of vision to the disks. They noted that the transfer of chromophore material from the RPE to the disks did not involve the putative cones, only rods. Van Hooser, et. al. have provided additional information based on mice. Acland, et. al. claim RPE65 is a membrane associated protein involved in retinoid metabolism. They have restored the vision of dogs exhibiting a mutation in this gene by infection with a virus, AAV-RPE65, injected intra-retinally. In similar work with monkeys, Bennett, et. al. achieved improvements lasting more than one year following injection.

### 4.6.2.3.4 Individual steps in The Retinoid Loop

235Acland, G. et. al. (2001) Gene therapy restores vision in a canine model of childhood blindness Nature Genetics, vol. 28, pp 92-95
Section 4.1.2.1.1 develops some important information concerning the retinoids of vision. It shows that retinol should not be considered *identical* to Vitamin A. The Vitamin A group now contains about 50 biologically active members. The generic name for this group is provitamin A. It also develops the fact that the retinoids play two separate roles in the retina.

To discuss the retinoid loop related to vision, it is necessary to review the list of “binding proteins” thought to be involved in retinoid transport and storage within the loop. Table 4.6.2-1 based on Ong\(^{237}\) summarizes the retinoid binding proteins involved in vision. The names of these materials were all developed to be self explanatory based on the limited knowledge of the retinoids of the day. They were usually defined based on *in vitro* experiments using retinoids prepared in the laboratory. It will be shown that many of these names are not as precise as might be thought. This becomes obvious when it is recognized that the resonant character of the chromophores provides them with an ambidextrous quality, exhibiting the properties of both the alcohol and aldehyde forms of the simple retinoids simultaneously. An *in vitro* laboratory test using only simple non-resonant retinoids is not conclusive of the actual binding ability of these proteins *in vivo*. Ong used a different title for this chart than shown above (from Ganguly). He indicated these proteins bound the retinoids non-covalently. As in the previous statement, it is not clear that this earlier work could definitively determine the actual method of chromophore binding using only non-resonant retinoids. The column labeled Endogenous Ligand has not been shown to be exclusive with relation to the complete retinoid family.

**TABLE 4.6.2-1**

<table>
<thead>
<tr>
<th>Name</th>
<th>Abbreviation</th>
<th>Endogenous Primary Ligand</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retinol Binding Protein</td>
<td>(S)RBP*</td>
<td>All-trans retinol</td>
<td>Bloodstream</td>
</tr>
<tr>
<td>Liver Retinol Binding Protein</td>
<td>LRBP</td>
<td>All-trans retinol</td>
<td>Liver</td>
</tr>
<tr>
<td>Cellular Retinol Binding Protein</td>
<td>CRBP</td>
<td>All-trans retinol</td>
<td>RPE &amp; PC laminates</td>
</tr>
<tr>
<td>Cellular Retinoic Acid Bind. Prot.</td>
<td>CRABP</td>
<td>All-trans retinol acid</td>
<td>“</td>
</tr>
<tr>
<td>Cellular Retinal Binding Protein</td>
<td>CRALBP</td>
<td>11-cis-retinol**</td>
<td>Specific cells of eye</td>
</tr>
<tr>
<td>Interstitial retinol Bind. Prot.</td>
<td>IRBP</td>
<td>All-trans &amp; 11-cis retinol</td>
<td>IPM of PC laminate</td>
</tr>
</tbody>
</table>

* More recent studies suggest that the label RBP is not sufficiently precise. There appear to be several different RBP’s in the bloodstream. Some carry all-trans retinol to all cells of the body indiscriminately for purposes of growth and metabolism. A more specific, more sophisticated, and serum-based variety complexes with all-trans-retinol for purposes of transport to the RPE for use in vision. As discussed below in Section 4.6.3, this variety, to be labeled SRBP, operates in conjunction with another protein labeled TTR.

** This work does not support the use of 11-cis-retinol in the visual process. In this work, CRBP, CRABP, CRALBP and IRBP all transport one of the resonant (trans) forms of retinol, the Rhodonines. These forms contain both alcohol and aldehyde ligands simultaneously, causing great difficulty in analytical testing based only on sensitivity to a specific ligand.

The right-hand column of the Table needs further development. In the text associated with the same Table in Ganguly\(^{238}\), it was pointed out that CRBP and CRABP are retinoid binding proteins known to exist throughout the organism. CRAIBP and IRBP are similar retinoid binding proteins but are believed to occur only in the eye. CRAIBP is found in the RPE exclusively and IRBP is found in the IPM exclusively. This suggests these materials play unique individual roles in the processing of the retinoid into chromophores within the retinoid loop of vision.

Ganguly gave only one paragraph to the description of the “Visual cycle of Vitamin A.” However, a great deal of information is provided about the static conditions related to this loop. This includes the proposition that the retinoids when bound to CRBP are easily esterified to the palmitate. There is also discussion concerning the transportability of the retinoids when in the alcohol, aldehyde and ester form. The likely hood that the esters are the form stored within the RPE will be discussed below.

Ong did not approach the subject from the perspective of a loop. Near the end of his paper, he dropped the terms retinol


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and retinal from his discussion regarding vision in favor of the more generic retinoid. He did provide more information concerning the transport of retinol in the blood. Goodman has also provided more information concerning the relationship between the retinol to be used in vision and RBP (hereafter SRBP).

The retinoid loop will be discussed at a more detailed level than in the above works. It will also be discussed from the perspective of the four different chromophores of vision, the Rhodonines. And, it will be discussed in terms of the resonant nature of these chromophores.

The retinoid loop is a “local” loop in the sense that once retinol is delivered to the RPE from the blood stream, the chromophores manufactured there re-circulate continuously within the RPE and photoreceptor laminates. The literature reports re-circulation times exceeding 5 weeks in white rats and 12 weeks in humans. These numbers probably refer to the time constant of an exponential. Some of the chromophores are probably decomposed over time in which case their remnants are catabolized in the RPE and returned to the bloodstream. No estimate of the lifetime of a chromophore could be deduced from the literature.

Retinol can be considered the precursor of two different families of retinoids. It is the hormonogen of the provitamin A group (and its most important member). It is also the chromogen of the Rhodonine group of visual chromophores. These families are handled differently in the animal. Retinol, being insoluble in water, is complexed with a retinol binding protein, SRBP, in the liver to facilitate its transport via the blood stream. Other members of the provitamin A group are transported by other means.

Every living animal cell requires a member of the provitamin A group to support its growth and general manufacturing. The cell can obtain this material from the blood stream in whatever form it is transported. In addition, the cells of the RPE require retinol for special manufacturing purposes, creating the chromophores of vision. The RPE cells are only able to obtain retinol from the blood stream for this purpose when it is complexed with SRBP. The consumption of provitamin A to support general cell metabolism will not be pursued here. The focus will be on the consumption of retinol in the manufacture of the chromophores of vision.

The mechanics of retinol transport to the RPE will be detailed in Section 4.6.2.3.3. It involves requirements related to conformal chemistry that the other members of the provitamin A group cannot satisfy. There is a requirement that the molecule be all-trans and that the hydrogen ion in the hydroxyl ligand be very loosely bound.

The retinoid loop is quite different from the protein loop. Where the IS in the protein loop relies on the bloodstream as the storage medium for the material to build the proteins, the RPE provides a major storage facility for the retinoids used in vision. It is very clear that the bulk of the retinoid material in the eye is stored in the RPE and on the disks themselves; the material is transported from the RPE to the base of the OS disk stack near the extrusion region of the IS, the retinoids are incorporated into the disk assembly, the retinoid material is then physically transported back to the RPE by the continuing extrusion process. When a disk reaches the RPE, it is phagocytosized and the chromophores are recovered and returned to the pigment cells of the RPE prior to reuse. This entire process occurs within the confines of the RPE-OS-extrusion cup space. It has available the manufacturing capability of the RPE cells. It does not involve the capabilities of the IS or penetration of the IS membrane wall. Each of the steps described above will be examined in more detail.

4.6.2.3.5 Possible alternate paths for individual chromophores

At the present time, the model of this work can support several retinoid transport methodologies between the RPE and the disks of the OS. The fundamental methodology was shown schematically in [Figure 4.6.2-1] and is reproduced here for convenience as Figure 4.6.2-12. The figure is divided into two parts to emphasize the functional difference between the retinoids supporting the IS and those supporting the RPE. That retinoid supporting the IS is ingested for the purpose of manufacturing the substrate protein, Opsin, and other normal cellular functions. Manufacturing is assumed to occur in the Ribosomes (RI). Following secretion of the protein, formation of the disks and following phagocytosis of the disks, the protein is seen to be catabolized further (the square loop). Its constituents are then returned to the blood stream.

Although the cells of the RPE absorb small amounts of a retinoid for normal cellular functions, they take up large amounts of retinol exclusively from holo-SRBP for the purpose of chromophore manufacture. The second part of the figure concentrates on this process. It assigns roles to the various binding proteins (sometimes labeled enzymes) in accordance with the recent literature.

There are several additional complications not addressed in this figure related to the existence of three different spectrally selective types of Outer Segments. Since it is obvious that the different chromophores delivered to the disks must segregate and adsorb on the appropriate Outer Segment to preserve the spectral selectivity of a given photoreceptor cell, there must be a mechanism to provide this capability. These complications center on where the retinol is first converted to the resonant retinoids of vision (chromogens) and how these chromogens travel to their respective spectrally selective Outer Segments where they are adsorbed to form chromophores.

There appear to be two options related to the first complication that can be supported by the data in the literature and the theory of the chromophores presented here.

1. The retinol extracted from the liver is delivered to the surface of the RPE cells by the SRBP in unaltered form. No change in the SRBP has occurred and it is reusable.

2. The retinol extracted from the liver is delivered to the surface of the RPE cells by the SRBP in one of three (four in the case of tetrachromats) resonant retinoid forms. The conversion from retinol involves a chemical reaction with the SRBP. The post-transport SRBP is no longer viable and it is removed from the vascular system by the kidneys.

In the first option, the retinol received at the RPE cells must be converted to one of the required resonant retinoids by the ribosome apparatus of the RPE cells before passing to the pigment granules for storage. In the second option, the retinoids have already been transformed into the resonant form and can be transferred directly to the pigment granules for storage.

The first option only requires one CRBP to transport the retinol from the surface of the RPE cells to the ribosome apparatus. However, it is possible that three CRABPs are needed to transport the resonant chromogens to the pigment granules. It is possible that the second option requires three separate retinoid binding proteins to transport the three resonant chromogens from the RPE surface directly to the pigment granules. Whether these are variants of CRBP or of CRABP is unknown at this time. The ribosomes of the RPE cells appear to play no role in the second option. It is known that SRBP is not reusable. This strongly suggests that option 2 describes the nominal mechanism of vision.

Ganguly discussed the three relevant enzymes that have been isolated in the RPE, CRBP (cellular retinol-binding protein), CRABP (cellular retinoic acid-binding protein) and CRALBP (cellular retinaldehyde-binding protein). Note that the resonant retinoids proposed in this work are carbonyl systems and not simple carbonyls (organic acids). Therefore, these terms may not be sufficiently precise to describe a binding protein used to transport resonant retinoids within the RPE and differentiate them from other binding proteins used to transport non-resonant retinoids in other cells of the body.

In both of the above options, three different resonant chromogens must be transported from the pigment granules to the RPE/IPM interface onto the disks of the photoreceptor cells. This introduces the second complication. How is this transport accomplished? Are three separate CRALBPs required within the RPE cells? Are three separate IRBPs required within the IPM? Figure 4.6.2-13 addresses these questions. The frame on the left is drawn as if a single CRALBP and a single IRBP are used to transport all of the required chromogens from the spectrally selective pigment granules to the spectrally selective Outer Segments. This approach assumes the chromogens deposit themselves on the appropriate spectrally selective disks due to their unique stereographic geometry. The frame on the right makes an
alternate assumption that the chromogens are transported from the three spectrally selective types of pigment granules to the disks by separate paths using spectrally selective forms of both CRALBP and IRBP.

Section 4.6.2.3.6 will develop a series of equations to describe the passage of the retinoids around the retinoid loop within the retina. These equations will use the historical names for the various binding proteins. However, it will assume the more unique binding described above relative to the resonant retinoids. That section will also present the most recent information concerning the transport of retinol from the liver to the RPE cells.

Although superficial, the above description is satisfying since the two enzymes of the above group outlined as most important in the function of the eye, IRBP and CRALBP, are found only in the eye240. Furthermore, the IRBP is found in the IPM but has not been found in the RPE241. Ganguly references six sources when he draws the conclusion that “the IRBP is mainly responsible for transporting the retinol [retinoids] back and forth” within the IPM. Ganguly states without attribution that “Most of the CRBP of bovine eyes has been found in the RPE, with very little of it being associated with the retina.” And finally, Ganguly states that CRALBP is found in both the RPE and the retina but gives no source. He may be following the work of Bridges et. al.242 who show CRALBP in the area of the retina vitral to the

240 ibid, Ganguly


external limiting “membrane” in a figure that Ganguly also used in his work. CRALBP located in this remote area would not significantly affect the above transport processes but it would support normal cell growth and metabolism as it does elsewhere in the body of the organism.

4.6.2.3.6 The Retinoid Loop Model

Based on the above ideas, Figure 4.6.2-14 illustrates the most plausible overall situation for the retinoid loop. The storage capability of the RPE is large compared to the instantaneous needs of the OS. Therefore, the loop can operate over intervals of days to weeks without significant amounts of new material being drawn from the bloodstream in the adult animal. To maintain the necessary supply of chromophoric material to the new disks being formed, a significant portion of the available chromogens are probably stored and circulating in the IPM (that includes the inter-disk space). They are probably stored as a saturated solution of each of the three Rhodonines (probably) complexed with individual forms of IRBP. Aphakic human eyes suggest that all four IRBP’s and visual chromogens/chromophores are present to at least some extent in humans but only three are used effectively due to the spectral limitations of the lens group.

![Diagram of retinoid loop model](image)

Figure 4.6.2-14 Proposed protein and retinoid transport loops. The square outer loops are three similar protein paths to three spectrally different Outer Segments. The curved inner loops are the multiple retinoid loops. Whether multiple forms of CRALBP and IRBP are used to deliver chromophores to the disks remains an open question. However, the stereographic properties of the chromogens are probably adequate to insure spectrally selective adsorption on the appropriate disks.

It is assumed that each disk stack was initialized to accept only a particular Rhodonine during morphogenesis. Henceforth, only that type of Rhodonine will be deposited in a liquid crystalline monolayer on the structural protein forming the individual disks of that stack. It is not clear whether the monolayer is deposited on the protein frame during initial forming, before folding and extrusion of the individual disks, but this would be the expected situation if deposition, based on the initial seeding, is to be maintained.

Referring to the binding capacity of IRBP as presented by Fong et. al.\textsuperscript{243}, the value of 2.2 is interesting if confirmable since it would provide data on how the retinoid binds to the IRBP and on the maximum capability of IRBP to transport retinoids in the IPM. The alternate value of 0.6 would be expected if the eye was in the process of rapid building of new disks or if the transport capability of the total IRBP in the IPM was limited to only slightly more than the average requirement. In either case, this lower value would suggest that some of the IRBP had deposited its retinoid and was returning to the RPE surface empty-handed under the conditions observed by Fong. It would be expected that the average IRBP load would be only a fraction of its maximum capacity.

4.6.2.3.7 The individual mechanisms of retinoid transfer

This section appears here for continuity. It assumes the chromophores of vision are resonant retinoids, the Rhodonines, containing two atoms of oxygen separated by a conjugated carbon backbone. Justification for this assumption will be provided in Chapter 5.

It is useful to describe the topological aspects of retinol and the Rhodonines, their special characteristics in conformal chemistry and the special resonant properties of the Rhodonines. Starzak\textsuperscript{244} has addressed the problem of two different cations attempting to bind to an organic molecule with two identical binding sites, Figure 4.6.2-15(a). The basic molecule is shown in the center of the figure, single binding on the left, double binding on the right. The occurrence of each configuration is based on simple probability. In biologicals, the likelihood of occurrence is more complex. Enzymes require that a binding site have a precise topology. Furthermore, complex molecules may have a higher number of sites. Retinol appears to have five identifiable sites. The least well defined site is the binding site of SRBP. When retinol is combined with SRBP, the combination is soluble. It is likely that this combination involves the hydrophobic, \(\beta\)-quinone, portion of the molecule. The hydroxyl ligand on the other end of the molecule makes an obvious binding


site. In addition, each of the hydrogen and methine sites along the conjugated carbon chain can be considered an enzymatic target site. The sites at carbon #5, 7, 9 & 11 are special in that they correspond to the sites used in the chromophores of vision. These sites are compatible with extending the resonant structure of the conjugated carbon to include the polar atoms. Neglecting the site specific to SRBP, Figure 4.6.2-15(b) illustrates these sites. When site #15 and any one of the above sites are both occupied by polar atoms, the structure becomes resonant. It is then a member of the Rhodonine family of retinoids. These molecules are represented by the formula $[O \Rightarrow(-C\Rightarrow-C)\Rightarrow-O]^-$ where the location of the $\beta$-quinone ring, and any non-resonant residual of the conjugated chain of retinol, is indicated by the bold bracket. The resonant bonds are indicated by the symbol $\Rightarrow$. $n$ indicates the number of vinylene groups present. Finally, the molecule carries a single delocalized negative charge.

In its ionized form, each of the Rhodonines is capable of binding with a protein at either of the polar atom locations. The possibility that it may transition from binding with one protein at one location to binding with a second at the other location allows for a simple description of the processing of retinol into the chromophores of vision. Figure 4.6.2-16 illustrates this possibility in concept and without regard for the charges involved. Addition of a hydrogen ion to either binding site would produce a neutral molecule.

The following conceptual equations involve the putative properties of the various binding proteins in-vivo, not as measured using a non-exclusive test procedure in-vitro.

(1) shows the resonant, but neutral, molecule. (2) shows the Rhodonine combined with an enzyme capable of transporting it along a diffusion gradient. (3) shows the molecule being passed from one enzyme to another material. If the second material is an organic acid, an ester is formed that is easily stored. If the material is a second enzyme on the other side of a cell wall, further transport may occur in a second diffusion gradient. Thus, (4) may represent either a storage situation or a transport situation. (5) represents the molecule in its neutral form at its point of destination.

Assuming the above model of the retinoid loop and the method of chromophore transport are correct, the partial equations in Table 4.6.2-2 are offered to explain how the loop operates. In these equations, the $\beta$-ionone ring of the retinoids is omitted for simplicity. Its presence is indicated by the bold bracket on the right of the formulas. The value of $n$ can range from 2 to 5 and is indicative of the peak spectral absorption of the chromophore when in the liquid crystalline state. 2 corresponds to the UV, 3 to the S-, 4 to the M- and 5 to the L-channel chromophore. When $n$ is 2 or 4, a methine, Me, on the conjugated chain of retinol has been replaced by an $O$; when $n$ is 3 or 5, an $H$ has been replaced by $O$. The bond symbol, $\Rightarrow$, indicates a resonant bond, sometimes thought of as 1.5 bonds in a conjugate chain. The $\Rightarrow$ symbol indicates a hydrogen bond or a temporary bond in transition.
Table 4.6.2-2

Conceptual Equations of the Retinoid Loop
(shown for n = 2 or 4 in Liver, otherwise Me is replaced by H)

<table>
<thead>
<tr>
<th>Location</th>
<th>Store/Use</th>
<th>Transition</th>
<th>Transport</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>[O=C((-\text{C} = \text{C})_n \text{-Me})]</td>
<td>[O=C((-\text{C} = \text{C})_n \text{-O})] SRBP +Me</td>
<td></td>
</tr>
<tr>
<td>Blood</td>
<td></td>
<td>[O=C((-\text{C} = \text{C})_n \text{-O})] SRBP</td>
<td></td>
</tr>
<tr>
<td>Cell Wall</td>
<td>CRBP [O=C((-\text{C} = \text{C})_n \text{-O})] SRBP</td>
<td></td>
<td>CRBP [O=C((-\text{C} = \text{C})_n \text{-O})] SRBP</td>
</tr>
<tr>
<td>RPE Laminate</td>
<td>CRBP [O=C((-\text{C} = \text{C})_n \text{-O})] Palmb</td>
<td>CRBP [O=C((-\text{C} = \text{C})_n \text{-O})]</td>
<td></td>
</tr>
<tr>
<td>RPE IPM Interface</td>
<td>CRALBP [O=C((-\text{C} = \text{C})_n \text{-O})] IRBP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PC Laminate</td>
<td>H [O=C((-\text{C} = \text{C})_n \text{-O})] IRBP</td>
<td>[O=C((-\text{C} = \text{C})_n \text{-O})] IRBP</td>
<td></td>
</tr>
<tr>
<td>Phagocytosis</td>
<td>CRALBP [O=C((-\text{C} = \text{C})_n \text{-O})] H</td>
<td>CRALBP [O=C((-\text{C} = \text{C})_n \text{-O})]</td>
<td></td>
</tr>
</tbody>
</table>

This sequence of equations highlights a variety of features. In;

(2) Apo-SRBP combines with retinol and releases either water or a methyl compound. The hydrophobic portion of retinol, which is soluble, is protected by SRBP and a “cap” of TTR (not shown). TTR is the abbreviation for transthyretin (also known as prealbumin).

This is the location where the retinol is converted to a resonant structure and the value of n determined.

(3) The SRBP-retinoid-TTR complex is transported through the bloodstream to the RPE.

(4) apo-CRBP is more aggressive than the SRBP-TTR complex with regard to the resonant moiety.

(4a) At the end of the transition, the SRBP no longer carries an oxygen that can be used to join with retinol in the liver. It is therefore not reusable and is discarded in the kidney.

(5) The retinoid has now passed through the RPE/blood boundary. CRBP is shown combined with a resonant chromogen.

(6) The retinoid is transferred to the palmitate and the CRBP is available for reuse.

(7) The individual chromophores are stored as esters, generally a palmitate, in the pigment granules of the RPE. Depending on the molarity at which they are stored, they may exhibit their enhanced spectral absorption characteristics.
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This is the first time the potential chromatic spectra of the animal can be determined.

(8) apo-CRALBP is more aggressive than the palmitate

(10) apo-IRBP is more aggressive than apo-CRALBP

(12) The chromophore is attracted to its place in the liquid crystal of the disk at the expense of IRBP.

(13) The chromophore exists on the surface of the disk in the liquid crystalline state. It is held to the substrate, opsin, by a hydrogen bond, i.e., the Van der Wall force.

(14) & (15) there are three options to deal with the chromophore during phagocytosis. (14) and (15) shows the path when the material is to be used immediately by transfer to the IPM. Line (15) is identical to line (9). (14a) and (15a) shows the path when the material is to be returned to storage within the RPE. Line (15a) is identical to line (5). If the material is structurally damaged (possibly by UV or cosmic rays), it is no longer viable as a chromophore and it is disposed of via the blood stream.

(14) apo-CRALBP is more aggressive than the liquid crystal.

(15) CRALBP complexed with the chromophore is ready to repeat step (10).

(14) apo-CRBP is more aggressive than the liquid crystal.

(15) CRBP complexed with the chromophore is ready to repeat step (6).

The fact that an all-trans-retinoid enters the RPE from both the IPM (at the apical surface) in step 15a and from the bloodstream (at the basal surface) in step 4a is consistent with the statement of Crouch, et. al. in 1996. The only difference is that in this work, the actual material is all-trans-Rhodonine.

Crouch, et. al. presented a flow diagram that may be appropriate for a review article but is believed, based on this work and the above material, to be archaic and may lead to misinterpretations. Their figure 1 does not address many of the mechanical aspects of chromogen transport and storage. It also does not address the transformation of retinol into four separate chromophores. Leaving this latter function to a group of four putative opsins appears unrealistic. Based on these points, alternate flow diagrams should be considered.

This work has suggested that, for purposes of forming the chromophores of vision, there are two possible paths. The formation of Rhodonine can occur within the RPE or it can occur within the process of transporting the retinoids to the RPE. The first method suggests elimination of a methyl or hydrogen from the retinoid and its replacement by oxygen at the RPE/bloodstream interface. This approach suggests there are at least two different forms of CRBP within the RPE and that these forms were destroyed in the process of conversion of the retinoid. The alternate approach would suggest there are at least two different forms of SRBP within the bloodstream (possibly originating in the liver) and that these forms were destroyed in the process of conversion of the retinoid. Since it is known that SRBP is not reused but is decomposed in the kidney after use, it appears likely that post transport SRBP differs from the pre-transport SRBP and this alternate approach is more likely. In this approach, the pre-transport apo-SRBP would provide an oxygen atom as the coupling element in the process of forming the holo-SRBP. After reaching the bloodstream/RPE interface, the decoupling would occur on the opposite side of the oxygen in the process of transferring the retinoid, now Rhodonine, to the initially apo-CRBP within the cell. The post-transport apo-SRBP is no longer viable, since it has lost an oxygen atom, and it is decomposed or excreted via the liver. This methodology is attractive if the removal of the methyl group or hydrogen, and their replacement with an oxygen is realistic. Obviously, the replacement of the hydrogen is not difficult. The replacement of the methyl group is more difficult. However, it is a known process among hydroaromatic compounds such as the retinoids. Replacement of the methyl group would depend on the structure of the binding site of the SRBP. If this site contained an oxygen, elimination of a so-called angular methyl group might be the avenue.

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The chemical structure of apo-SRBP is known in detail. As in all proteins, it has a carboxyl group as one termination. Of much greater importance is the fact it contains multiple carboxyl groups (45) associated with its aspartic and glutamic acid residues. It is believed that only three of these residues can normally react with retinol in humans. However, the possibility of four sites cannot be ruled out. The natural reaction of such a molecule is to replace the –OH group associated with a carboxyl ligand, exactly the reaction required to convert the retinol to the chromophore Rhodochrome. As a result, the exo-SRBP becomes a functional derivative of the original apo-SRBP. The fact that such an –OH replacement could occur at three different locations within SRBP is in excellent agreement with the structure of Rhodochrome and its formation as discussed in Section 7.1.2.

Figure 4.6.2-17 is offered as a substitute for the figure of Crouch, et al. The figure has been expanded to begin with storage of the retinol in the liver, generally as an ester. It is based on the assumption that the retinol to be used as a chromogen is converted to one of the Rhodones when first extracted from the liver by SRBP & TTR. This conversion is not used for the retinol to be transported through the bloodstream for purposes of growth and metabolism in cells.

The process, involving the movement of the chromogen, appears to involve the formation of a carboxyl ion system, that is potentially tautomeric, within the “SRBP/TTR bottle.” This tautomer continuously changes form during its transfer from the bottle to its final deposition on the surface of the disks of the Outer Segment. During these transfers, the crucial material exists as a carboxyl ion system with a negative charge. When ultimately deposited in the liquid crystalline state as the chromophore, it is the resonant character of the carboxyl ion system that is critical to the sensitivity of the photoreceptor.

The overall transport process involves seven major steps and a number of sub-steps. Initially, retinol is ingested in the form of Vitamin A as shown in (A). Upon absorption, most studies have concluded that it is attached to an LRBP and transported to the liver (not shown) via the lymphatic system where it is stored, probably as a palmitate, as shown in (B). Crouch, et al. use a slightly different nomenclature. They describe the binding protein used in lymphatic transport as LRAT (lecithin: retinol acyl transferase).

More careful definition of the terms and the mechanisms involved is required before discussing whether any discrete enzymes are required to support the complexing and transport employed in the processes discussed here. It may be that a binding protein should be looked at as an enzyme. Alternately, most of the reactions occur at or on the surface of a cell membrane. Such a surface could be considered as acting as an enzyme.

Upon demand, the retinoid is absorbed into the blood stream in a multi-step process that is unique and key to the overall visual process. Rather than be transported in the bloodstream as a simple alcohol, it is first complexed with apo-SRBP by esterification. The literature reports that this apo-SRBP has a molecular weight of about 21,000.

Some investigators have suggested that this complexing involves acylation. However, such a mechanism does not establish a conjugated condition between the two oxygen atoms of the retinoid and is not supported by this work. A different mechanism is proposed that shares the oxygen of the apo-SRBP with the retinoid. This introduces a second oxygen into the structure of the retinoid. This step causes the retinoid fraction of the complex to become chemically resonant. To protect this structure as it leaves the liver, the palmitate ligand is replaced by TTR, (C). The surrounding of the chemically resonant retinoid by SRBP and TTR protects it from deterioration during transit to the RPE.

When first complexed with the SRBP, there are four possible locations where the oxygen can be added to the retinoid while creating a conjugated condition with respect to the original oxygen. These locations are illustrated in (C). These locations correspond to the fifth, seventh, ninth and eleventh carbon of the retinoid. The resulting retinoid can be described in terms of a carboxyl ion system with a level of conjugation of five, four, three or two respectively. These four systems (the chemically resonant structure between and including the two oxygen atoms) are the four basic forms of Rhodochrome that provide the spectral absorption characteristics found in vision. From this point forward, the ionone ring of the original retinoid is extraneous to the visual process. The total SRBP-retinoid-TTR complex is believed to be combined with albumin that is present in blood serum (total mol. wt. ~80,000 to 90,000).

For the remainder of the figure, it will be assumed that the oxygen was attached to the fifth carbon, resulting in a chromogen capable of supporting the long wavelength spectral absorption found in the L-channel of vision.

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Figure 4.6.2-17 Overall scheme for retinoid transformation and transport (both chromogens and chromophores) in the visual cycle. The specific form of the retinoids participating in the process are shown on the left. In B, the oxygen of the SRBP can react at any one of four sites. The numerals in parentheses indicate the resulting individual levels of conjugation. See text for details. Compare to Crouch, et. al. (1996)
Upon arrival at the RPE interface, the TTR (on the outside of the membrane) is first released and replaced by apo-CRBP as the retinoid transitions through the RPE membrane. The SRBP is then released in a destructive manner. The oxygen contributed by the apo-SRBP stays with the retinoid as part of the carboxyl ion system. The modified material, now described as the exo-SRBP, is no longer functional and is removed from the bloodstream by the kidney. Valquist, et al. described this exo-SRBP as lacking retinol and the normally encountered COOH-terminal amino acid and unable to bind to prealbumin (TTR). This description is consistent with this work. However, it may not be exhaustive in that there are such a large number of COOH ligands associated with this material (See Rask, et. al. above). Valquist discusses the relative concentration and production rates of these materials.

The holo-CRBP, CRBP + resonant retinoid shown in (D), now moves to one of four locations of temporary storage (the pigment granules) within the protected environment of the RPE cell. In a step that is not illustrated, the CRBP releases the Rhodonine as it is converted into a palmitate within the granule. The CRBP is returned to a viable apo-CRBP condition for reuse.

When needed, the specific Rhodonines are extracted from the pigment granules by a binding protein known as CRALBP, a convenient name for a second RBP closely related to CRBP. The name was originally assigned based on the assumption that it preferred to bind to the aldehyde form of retinene. As indicated here, the AL in the label is essentially meaningless when used to describe a protein capable of binding to Rhodonine. This chromogen is a tautomer containing both a keto- and enol- form at all times.

By binding at C15, only one apo-CRALBP appears necessary to transport the four individual Rhodonines while within the RPE. At the RPE/IPM interface, a transfer is performed that is simpler than at the bloodstream/RPE interface. At the RPE/IPM interface, the Rhodonine is passed through the cell wall and is accepted by one of four IRBPs compatible with the slightly different location of the oxygen found along the backbone of the molecule nearest the ionone end of the molecule. This situation is shown in (E). The IRBP transports the Rhodonine( ) through the similarly protected IPM environment to its proper spectrally tailored Outer Segment. The complete Rhodonine molecule takes its proper stereometric position as part of the liquid crystal on the surface of a newly formed disk. The IRBP is released in its original apo-IRBP form for reuse. The resultant condition of the chromophore (no longer a chromogen as a result of its deposition as part of a liquid crystal) is illustrated in the upper half of (F).

The most important feature of Rhodonine( ) in its stereometric configuration is the presence of the conjugated structure of the carboxyl ion system within its overall structure. When deposited on the disks in a liquid crystalline form as shown in the upper half of (F), this system is both physically resonant, and chemically resonant. As a result, the physically resonant structure is spectrally sensitive to a wavelength determined by the physical length of the carboxyl ion system. It can be excited by radiation with a Poynting vector parallel to the long axis of this structure. The requirement that the Poynting vector be parallel to the long axis of the chromophore is unique to the visual process. It introduces an absorption spectrum that is highly anisotropic. This absorption spectrum peaks at a different wavelength than that associated with the normally isotropic absorption spectrum associated with the retinenes. In the retinenes, the non physically resonant structure between the ionone ring and the terminal oxygen associated with the fifteenth carbon exhibits a peak spectral wavelength at 500 nm when in the liquid crystalline condition. However, this absorption spectrum is not usually used in the visual process. It is not the absorption spectrum responsible for the scotopic luminous efficiency function of the eye.

Besides the liquid crystalline system being both physically and chemically resonant, any electrons exited into an excited

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state of the system is highly delocalized. The excited form of the molecule when in the liquid crystalline state is shown in the lower half of (F). Upon excitation by a photon of the appropriate wavelength, an electron is excited and becomes an exciton. Furthermore, because of the nature of the liquid crystal, this exciton is delocalized and free to move within the physical dimensions of the excited state associated with the entire crystal. Excitons associated with dipolar chromophores of oxygen are usually very long lived because of a unique property of oxygen discussed in Chapter 5. They normally do not return to the unexcited state via fluorescence.

The unexcited n-electrons associated with a liquid crystal are also delocalized. This condition greatly expands the absorption cross-section of the material to radiation.

Although the above exciton will not will return to the unexcited state via fluorescence, it is easily de-excited upon transfer of its excess energy to another suitable medium. Such a transfer occurs at the dendritic surface of the photoreceptor cell associated with the disk. As a result, the electron is instantly returned to the unexcited state within the overall liquid crystal and it is immediately available for re-excitation by another photon.

Several experimenters have attempted to measure the excitation time of visual chromophores. The experiment is difficult. However, the process is known from quantum physics to occur in less than a picosecond. The de-excitation process is similarly rapid. The time-consuming portion of the overall process is the transfer of the exciton from its location of creation to its location of de-excitation. This usually takes 1-3 milliseconds at biological temperatures as discussed in detail in Appendix A as part of the Photoexcitation/De-excitation process. Once de-excited, the electron rejoins a large pool of unexcited n-electrons associated with the liquid crystal.

The process illustrated in (F) can continue as long as the disk is viable and associated with a de-excitation mechanism such as provided by the dendrites of the cell. However, as part of the life cycle of the overall system, the process has a specific limitation. After a nominal one week in the case of human, the disk containing the Rhodonine(n) molecules have traveled from their point of formation in the Inner Segment extrusion cup to the RPE where phagocytosis occurs. During phagocytosis, the disks and their liquid crystalline coating are disassembled at the molecular level. The Rhodonine(n) is recovered as illustrated in (G). A majority of the Rhodonine is recaptured by either CRBP or CRALBP depending on whether it is to be returned to storage within the RPE or whether it is to be transferred immediately to IRBP at the RPE/IPM interface.

This alternative to the presentation of Crouch, et. al. appears to satisfy significantly more technical requirements than addressed in their Figure 1. It is:

+ consistent with the fact that SRBP is not reused.
+ consistent with the observation that the genetic code assigned to IRBP shows a fourfold replication that could be responsible for four separate IRBP molecules or for one IRBP molecule with four distinct binding sites.
+ consistent with photo-chemistry in providing the correct spectral characteristics found in vision.

This alternative does not require:

+ the presence of any enzymes beyond the retinoid binding proteins themselves.
+ the re-isomerization of a molecule following its excitation and subsequent isomerization.
+ the long time period of incapacitation associated with the re-isomerization of a molecule at a location removed from its point of initial excitation.

The above equation set does not use CRABP. In the literature, CRABP is claimed to only associate with retinoic acid and no retinoic acid is used in the chromophore manufacturing process.

As noted in an earlier chapter, the structure of the Rhodonines is unique. The conjugated carbon chain of the molecule with an oxygen atom on each end has many of the properties of an acid of retinal. In the limiting case, where n=0, (and
ignoring the stabilizing β-ionone ring) these molecules become formic acid. In the general case, the conjugated carbon chain of the Rhodonines may be able to act much like a single carbon atom as far as forming a functional group is concerned.

These equations are intellectually satisfying since they:
+ provide a consistent and simple set of formula that describes the operation of the “chemical” part of the retinoid loop,
+ use the various enzymes in the portions of the loop where they are known to exist,
+ do not require unreasonable transport velocities for the materials in the loop
+ are consistent with the findings of Dowling & Wald concerning Vitamin A deprivation\textsuperscript{249}
+ involve no difficult energy problems such as those that have always plagued the all-trans- to 11\textit{cis}- conversion in the current theory of the simple retinenes, and
+ do not conflict with the literature if:
  ++ the terms used to describe the retinene family can be stretched to include one of the other subsets of the retinoid family, the dual auxochrome molecules– the Rhodonines.
  ++ CRABP is not needed in this loop or the capabilities of CRABP are found to include binding to the chromophores in their “conjugated retinoid acid” form.
++ the Rhodonines can be considered “conjugated retinoid acids” for the purpose of forming esters.

Bernstein, Law & Rando\textsuperscript{250} have presented results where they dual-labeled the fifteenth carbon of all-trans-retinol with \textsuperscript{14}C and \textsuperscript{3}H. They came to several conclusions that appear consistent with the above discussion. They concluded:

+ The chemical manipulations they carried out did not disturb the presence of the tritiated hydrogen in the retinoids involved.
+ All-trans-retinol was processed into large amounts of all-trans-retinyl palmitate in the RPE of amphibians.
+ Appreciable quantities of all detergents tested interfered with their experimental results.
+ Free all-trans-Retinaldehyde is not the substrate used by their putative isomerase.
+ All-trans-Retinyl Esters are not directly isomerized to 11-cis-Retinyl Esters.

The most interesting of their conclusions is the creation of large amounts of what they call all-trans-retinyl palmitate within the RPE. It is proposed that this material was actually all-trans-rhodonine palmitate (the retinoid contained two auxochromes at this stage) in one of its four chromogenic forms prior to being transported to the Outer Segments of the retina.

4.6.3 Other aspects of metabolic dynamics of photoreceptors

This work does not recognize any chemical dynamics, such as isomeric changes, related to the photodetection process. However, there are a variety of important mechanisms and processes that do cause changes on a time scale of seconds and longer. Some of these involve physical changes and others can involve chemical kinetics. They fall into two main categories as described above, the formation of the protein-based disk substrates and the activation of those substrates through the coating with a Rhodonine. Section 4.6.2.3 reviews the excellent research data available from isotope tagging. That data is consistent with the mechanisms defined in this work. In some cases, the interpretation of the data is quite different from that of the original authors.

4.6.3.1 Additional details relative to SRBP

The participation of SRBP, and the other RBPs, in the visual process appears more complicated than described in various individual papers. In order to understand their overall participation, considerable semantic precision is required when discussing the metabolites used in the retinoid loop. Clear distinctions need to be made between the holo- and apo- forms of the various transport proteins.

The discussion regarding SRBP in Ganguly will be used as an example. In 1989, Ganguly, referencing Valquist, spoke of the biological half life of RBP (SRBP) in human as approximately 11.1 to 11.7 hours. Expanding the discussion to include the various conditions of RBP, it becomes clear that the above time interval applies to the life cycle of apo-SRBP after delivery of its retinoid to the RPE cells. This material might better be labeled exo-SRBP. SRBP in this condition has completed its metabolic role and is subject to removal from the bloodstream by the kidney. There are four conditions needing analysis with regard to SRBP; pre-transport apo-SRBP, holo-SRBP without TTR and holo-SRBP with TTR and post-transport apo-SRBP (exo-SRBP). TTR is the abbreviation for transthyretin. Goodman provides extensive material on the complete holo SRBP complex.

The SRBP-retinol- TTR combination is believed to consist of a 1:1:1 molar complex. Although Ganguly says changing the functional end group of retinol does not affect the efficiency of binding to the apo-protein, Ong says this is only true in-vitro. He says only retinol (and only all-trans retinol) binds to SRBP in-vivo. When released from the liver, the complete complex probably has a very long lifetime compared to apo-SRBP. Valquist is a source of data in this area.

Two time intervals are not well documented. First is the time required between the arrival of SRBP-R-TTR complex at the RPE cell and when a chromophore becomes available at the RPE cell IPM interface for use, or arrives at the pigment granules for long term storage. Second is the time for the chromophore to transit from the RPE cell IPM interface to the IS/OS junction area.

This loop can operate continuously for the life of the organism without difficulty. It is completely consistent with the known locations of the various binding proteins. CRAIBP does not appear to participate directly in the chromophore forming process. The presence of a small amount of CRAIBP in the IS would not be surprising since it appears throughout the body and is widely used to support cell nutrition/metabolism.

### 4.6.3.2 Putative diurnal secretion and absorption by the outer segment

Both Bridges & Yoshikawa and O’Day & Young addressed the possibility that the extrusion of protein disks and/or the coating of those disks might occur with a diurnal variation. The total available data is not large. Firm conclusions may not be warranted. Bridges & Yoshikawa found little evidence suggesting a diurnal variation in the rate of coating of the disks in rats. O’Day & Young addressed the question in goldfish where they found a more statistically significant variation. However, their definition of rods and cones was based strictly on morphology. Their figure 1 assumes a considerable movement of the cells by the myoid that does not appear to be compatible with the optical requirements of the lens system of the eye. The “cone” in their figure 7 has a distinctly hour-glass shape. More experimental work, based on a stronger model, is needed in this area.

### 4.6.3.3 Vitamin A stress and starvation

Dowling & Wald provided Figure 4.6.3-1 in a day when it was thought there was only one biological form showing the “growth factor” property associated with Vitamin A. Since then, a number of retinoids have been isolated with hormonal properties. Ganguly provides a comprehensive source. Dowling performed experiments that led to a reinterpretation of the above figure. Dowling & Wald showed that animals deprived of Vitamin A but supplemented with retinoic acid continued to develop but their vision degenerated. This was due primarily to changes in the OS. Thus, the loss of opsin in the above figure can be ameliorated by the presence of retinoic acid in the diet. However, retinoic acid cannot compensate for the loss of all-trans retinol. Their 1958 paper also showed that 11-cis-retinol (neo-b retinol) cannot be injected into the blood stream or ingested in order to overcome dietary deprivation of all-trans-retinol. They

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note that 11-cis-retinol is not found anywhere in the body except for its putative presence in the retina. They also note the significant amounts of all-trans-retinol stored in the liver and the retina of healthy humans. In some cases, the stored supplies are adequate for years.

The original interpretation of their figure can be summarized as follows. Relatively few data points could be collected with the small sample size available. The data was fitted to straight lines. Fitting the data to exponential curves would be an alternate possibility. However, the results appear clear. The Vitamin A level in the liver dropped substantially within three weeks. The vitamin level in the blood remained at normal levels until the level in the liver became very low, supporting the conclusion that the liver stores considerably more Vitamin A than required by daily metabolism. The Vitamin A level in the blood dropped precipitously when the liver could no longer supply this material. Since the disks of the OS remain operational for some time after creation, the amount of rhodopsin present fell gradually after the blood became unable to supply Vitamin A. It took more than five weeks for all operational disks to reach the RPE and be phagocytized. The decrease in rhodopsin was linear as expected when due to a transport phenomena. An ERG was used to measure the level of rhodopsin present. This test probably represents the amount of operational chromophore present.

4.6.3.3.1 A reinterpretation

We now know that protovitamin A plays two important roles in the biological system. Any member of this group can provide enzymatic functions that are critical to the operation of all neurological cells, if not all cells. However, only all-trans-retinol can be extracted from the liver for use in forming the chromophores of vision.

It is now known that retinol is the hormonogen for approximately 50 compounds exhibiting biological activity related to growth and generally known by the generic name provitamin A. Generally, members of this group must contain at least one β-ionone ring that is not hydroxylated258, 259, page 5.

All-trans retinol is also the chromogen for four naturally occurring chromophores found in animal vision. These all-trans compounds make up the Rhodones. The chromophores rely on the presence of a conjugated all-trans carbon chain terminated on each end by a polar atom, oxygen. The length of this resonant structure is the mechanism for achieving the individual spectral sensitivities of the family. The β-ionone ring in the chromophores plays a role similar to that of rings in photographic “dyes.” It acts as a stabilizing agent against the effect of temperature on the long conjugated chains.

All-trans retinol is the predominant form of Vitamin A in the blood stream (90%) where it is complexed with a retinol binding protein (RBP) to allow the transport of a material which is normally insoluble in water (page 13).

While all-trans retinoic acid possesses some of the biological activity of retinol, it is not transported by the SRBP employed in vision (page 15).

The above distinction appears to be significant in terms of the transport of the retinoids from the blood stream to specific cells for specific purposes. For purposes of growth, any provitamin A can be absorbed into any cell. However, for purposes of chromophore production, the presence of the complete acid ligand prevents retinoic acid from being absorbed by the RPE cells. Only the alcohol or aldehyde can be moved into the RPE cells for purposes of modification into a chromophore.

258 Page numbers in the next few paragraphs refer to Machlin, see below
This background supports a reinterpretation of the figure by Dowling & Wald. The original caption was not specific as to the form of Vitamin A involved. While any type of provitamin A could lead to the levels of vitamin A in the liver and the blood shown, and could account for the amount of opsin present, this is not true for the chromophoric material. Only Vitamin A in the form of retinol can be extracted from the liver for use in the photoreceptors of vision. Thus, the curve labeled rhodopsin is only a function of the amount of retinol in the diet. This curve should be renamed chromophore or Rhodonine as it is specific to the amount of chromophore present and is not directly related to the amount of opsin present. The reduction in the chromophore content was determined via an ERG. The curve is most indicative of the inability of the RPE to extract the chromogen retinol from the blood stream due to its low concentration in the blood and deliver the chromophore to the OS.

The last line, the presence of the structural protein substrate of the disks, opsin, requires careful interpretation. It is correct if the animals are deprived of all members of the provitamin A group. Under this condition, the photoreceptor cells are deprived of the “growth factor” provided by provitamin A and needed to produce new substrates. This deprivation applies to all cells of the animal and it dies relatively quickly. Alternatively, if the animal is provided another provitamin A, retinoic acid as an example, instead of retinol, the animal is able to maintain general growth and disk substrate production. However, the animal still becomes blind because retinoic acid is not transported through the blood by SRBP and it cannot support the production in the RPE of the chromophores required for vision.

These facts lead to the understanding that retinal and retinol, along with other retinoids like retinoic acid, play a significant hormonal role. However, it appears that only retinol is transported to the cells of the retina, from the liver, for purposes of chromophore creation.260

4.7 Operation of the photoreceptor in signaling (Electrical dynamics)

Based on the above histological, cytological and quantum level analysis, the operation of the photoreceptors in the visual process is seen to be more complicated than generally viewed. But these additional complications at the cytological level are primarily associated with the metabolic and growth processes in and near the photoreceptor cell.

The photoreceptor cell plays a unique and uniquely integrated role in vision. The role is entirely characterized by its electrolytic characteristics. It includes the individual functions of photosensing, signal amplification and stabilization, and current to voltage conversion. The photosensing mechanism is usually subdivided into one of transduction of light into excited but bound electrons followed by the conversion of these excited electrons into free electrons constituting a current. The amplification and stabilization mechanism is designed to accept an extremely wide dynamic range input signal and produce a nominally constant output signal amplitude. This constancy is in terms of both the DC and AC components of the signal. The current-to-voltage conversion mechanism is critical in the signal distribution role of the photoreceptor cell. This mechanism allows the cell to distribute the signal to multiple orthodromic amplifiers without sharing the limited current available between them.

This work is based on the electrolytic signaling theory of vision. It does not support the biochemical signaling theory of vision. The biochemical theory is still highly conceptual and very complex with multiple authors providing multiple chemical paths to the generation of a signal. None of these biochemical approaches provides a mathematically defined avenue to the creation of an electrical signal, much less the precise description of the luminous efficiency function or the dark adaptation function of human vision. They remain conceptual in the absence of the electrolytic theory presented here.

4.7.1 Terminology and fundamentals

The point has been reached in this work where the recognition of the fundamental electronic nature of the nervous system is necessary. The commonly described photoreceptor cell consists of two major elements, the external Outer Segment and the Inner Segment, which rely on two significantly different electronic processes. The Outer Segment relies on quantum mechanical processes to generate “bound electrons” occupying the excited energy states of a liquid crystalline material. This process is properly described as transduction. The Inner Segment uses conventional semiconductor physics in an unconventional semiconductor device to generate “free electrons.” These electrons are then manipulated in conventional electronic circuits prior to delivering a signal current to the output terminal of the cell, known as the axon. The manipulation involves a significant level of amplification, again using well-known techniques. The initial manipulation of free electrons will be discussed under the general descriptor, translation.

The processes used in the OS are entirely passive. There are no metabolic processes occurring in the OS. No power is supplied to the OS for the purposes of transduction or translation.

The situation in the IS is quite different. The IS, which includes the dendritic structures passing along the sides of the disks of the OS, is best described as a six-terminal electrical circuit where three of the terminals are related to the supply of electrical power to the active devices of the cell. The other three terminals are related to the signal creation, amplification and distribution by the active devices within the cell.

Because of the detailed nature of the quantum level structure of the transducers and translators used within the photoreceptor cell, much of this fundamental material appears in Appendix A and B of this work. This Section will concentrate on the functional features of the cell, rather than the quantum mechanics used to perform the functions.

Appendix A provides a detailed description of the quantum mechanics of the transduction process and the complete solution to the Photoexcitation/De-excitation Equation that has not appeared elsewhere as of this date.

Appendix B provides a detailed discussion of transistor theory and its application to a new class of active electrolytic semiconductor device known as an Activa. There is a variety of Activa used in the animal nervous system. Chapter 9 of this work provides a detailed description of each type of Activa found in animal vision.

In electronic terminology, all of the Activa of the visual system (and by analogy all neurons in animals) are of the PNP type. This means two things. The electrical potential of the collector terminal must be negative with respect to the base terminal. The electrical potential of the dendritic terminal must be positive with respect to the base terminal.

In biological terms, this also means two things. The electrical potential of the axoplasm must be negative with respect to the podaplas. The electrical potential of the dendroplasm must be positive with respect to the podaplas. If these conditions are not realized, “transistor action” does not occur and the unique active characteristics of the neuron are lost. By applied logic, the above requirement says the dendroplasm must be positive with respect to the axoplasm. These requirements are absolute. However, the voltages involved may be quite small. The maximum voltage between the axoplasm and the podaplas is normally less than the voltage between the axoplasm and the surrounding media. This voltage is typically less than 0.200 volts.

### 4.7.2 Introductory description

While the operation of the photoreceptor cell involves a variety of mechanisms and techniques not previously discussed in much of the biological community, the individual techniques are well known and understood in related communities. Some of the techniques are widely used in these communities. A few are niche techniques. One has been known for years and is actually obsolete within the electronics community at the current time.

**Figure 4.7.1-1** illustrates the overall topology of the electrolytic circuit of the photoreceptor cell overlayed on its cytology. The circuit is only presented for orientation here. Its performance will be discussed in detail in Chapter 12. The drawing is meant to define the neural portion of the photoreceptor cell as enclosed by the three rounded sections, that containing the dendroplasm, that containing the axoplasm and that structure between them containing a podaplas. Each of these regions is in electrical contact with the surrounding IPM or INM. The input structures between the Outer Segments and the dendrites are shown in the upper left. The output structure between the axoplasm and one orthodromic circuit is shown at the right. Three distinct electrosteno electrolytic power sources are shown polarizing the individual plasmas. The diode in the power source supplying the axoplasm is critically sized to provide the current to voltage conversion. This conversion is required to allow the output portion of the cell to act as a distribution point.
It is worth noting that the sum of the currents emanating from the dendroplasm plus the current emanating from the axoplasm of the cell is equal to the current entering the podaplasm of the cell. The reason for this will be explained in detail in Chapter 12 and can be deduced from the next figure. The current emanating from the dendroplasm when measured across the impedance of the IPM forms the Class C waveform (the leading edge of the a-wave of the conventional ERG). The current emanating from the dendroplasm, at a measurably later time, forms the Class D waveform (a component of the trailing edge of the a-wave and the leading edge of the b-wave of the conventional ERG).

Figure 4.7.1-2 illustrates the electronic circuitry of the above photoreceptor cell in a simpler form recognizable by most electronics engineers. The left side of the schematic is descriptive of one of the multiple dendrites (microtubules) associated with a single photoreceptor cell. The currents from all of the Activas within the dendrites pass through the ciliary collar and are summed along with the current from the Activa on the right. The total current then passes through the dendritic power supply shown at the bottom of the figure. For simplicity in further description, all of the individual Activas within the individual microtubules and individual dendrite leads will be replaced by a single equivalent amplifier that will be called the adaptation amplifier. Thus, the photoreceptor cell can be considered to consist of a single adaptation amplifier (on the left) connected to a single output amplifier (on the right). This configuration with a common power supply at the bottom is known as a differential pair in electronics. A key feature of this circuit is that the current in the output amplifier is a mirror image of the current in the adaptation amplifier (it is of opposite polarity). Shaw studied the currents related to the photoreceptor cell of the locust, S. gregaria261. He reported certain anomalies based on his assumption that the cell consisted only of a photoconductive impedance in series with a second impedance.

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straddling the “basement membrane,” a two terminal network. He noted the reversal in polarity of the current associated with the Outer Segment and the pedicle. To account for this, he introduced a virtual ground connection between the middle of the cell and the local surround. In this work, this connection is the poditic contact of the three terminal photoreceptor cell. With this reinterpretation of the nature of the photoreceptor neural circuit, Shaw’s data appears highly supportive of the model developed here.

While the Activa on the right acts as a constant gain amplifier, the Activa on the left does not. Because this Activa is formed in the confined space of the microtubules, its collector (the upper most terminal) exhibits a very low electrical breakdown voltage. Simultaneously, the high voltage across this circuit element can cause avalanche current amplification. As a result, this Activa exhibits a very large current gain at low levels of excitation and lower gain at higher levels of excitation. This is the source of adaptation in vision.

Before proceeding, note the crucial fact that the total direct current entering the poda connection is equal to the sum of the currents leaving the dendritic region and the axon. This fact is usually missed in laboratory experiments using low noise AC coupled amplifiers. The total signal current leaving the axon is normally the negative of the signal related current leaving the dendritic structure for all but the most excessive illumination levels.

It should be noted that no metallic conductors are used in the visual system, or the nervous system, of animals. All of the currents are carried by electrolytic conductors consisting of conduits filled with fluid. The conduits are generally described (inconsistently in the literature) as microtubules or groups of microtubules. The velocity of signal propagation in these conduits is relatively low. The lead proceeding to the right extends to the pedicle of the photoreceptor cell. There is a significant time delay associated with this lead. Because of the difference in time between the occurrence of the Class C waveform and the Class D waveform, the current entering the podaplasm is not a true constant.

Additional details regarding the signaling operation of the photoreceptor cells will be presented in Chapter 12. The following summarizes the performance characteristics developed there.

The structural optimizations found in the region of the OS result in an even distribution of both photon absorption and signal current release as a function of the distance along the OS.

The electrically passive chromophores of vision sense photons linearly over an enormous dynamic range.

The translation process between excited bound electrons within the chromophores and free electrons within the conduits of the photoreceptor cell exhibits a variety of nonlinearities.

The signal voltage sensed in the region of the IPM becomes more positive with illumination while the signal voltage sensed in the region of the axon becomes more negative with illumination—for the same photoreceptor cell.

The adaptation amplifier incorporates avalanche gain, a high degree of negative feedback and a band-limiting filter in the feedback loop. As a result the amplifier provides a large variation in gain while essentially removing the DC component associated with the input signal.

The voltage sensed at the axon is a highly compressed function of the illumination. The DC component of the signal is almost completely removed and the AC component is of nearly constant amplitude.

Both the current and the voltage at the axon conduit are functions of the illumination that are accurately described mathematically by this model.
The neural circuits proximal to the pedicles of the photoreceptor cells operate in a constant amplitude large signal mode.

All signaling prior to the ganglion cells occurs in the analog mode. There are no action potentials associated with signal sensing or signal manipulation. Action potentials are introduced by the ganglion cells prior to signal projection to the brain.

4.7.3 Variations in photoreceptors

4.7.3.1 Variations related to color vision

Up to this point in the development of the model, it has not been necessary to hypothecate any variation in the operation of the photoreceptors to account for photopic, scotopic or other variations of a spectral nature in the operation of a photoreceptor. A little ground work was laid that will have a significant impact on the performance of the long wavelength sensitive photoreceptors. This will become much clearer when the mathematical portion of this work is reached. The conclusion is that the only functional difference between the photoreceptors of animal vision relates to the particular chromophore glazing the protein substrates of the Outer Segments. The only measurable and spectral performance related physical difference between photoreceptors in retinas of Chordata is in the spacing between the auxochromes of the chromophores glazing the protein substrates. This difference can be measured using high magnification electron-microscopy. Neglecting the large mass of the auxochromes, their physical spacings would be expected to range from about five Angstrom for Rhodonine(11) operating at 342 nm in the ultraviolet to about twelve Angstrom for Rhodonine(5) operating at 625 nm. As noted in Section 4.3.4, the actual spacing between the centers of mass is reported as 40 Angstrom for an unspecified chromophore. All other morphological differences are for purposes of efficient packaging and appropriate output signal distribution.

4.7.3.2 Variations related to routine growth

Up to this point in the development of the model, it has not been necessary to hypothecate any variation in the operation of the photoreceptors to account for photopic, scotopic or other variations of a spectral nature in the growth of a photoreceptor. This is because the differences involved are basically related to the detailed makeup and stereo-chemistry of the chromophoric molecules.

4.7.3.3 Variations related to retinal position

There are three significant variations in photoreceptors relative to retinal position. The most significant variation is in the shear-angle between the Outer Segment (and the adjacent portion of the Inner Segment) and the tangent to the surface of the retina at the RPE/choroid interface. This shear-angle is established to maintain the axis of the Outer Segment parallel to the light emanating from the exit pupil of the objective optical group. The diameter of the Outer Segments does not vary significantly in retinas without a fovea. For foveal eyes, the variation is relatively small, about 3:1 over the surface of the retina in a given species. The third variation associated with foveal eyes is the slight difference in the length of Outer Segments. They are slightly longer in the foveal area to compensate for the distortion of the Petzval surface due to the thickness variation of the neural laminate.

4.7.3.4 Other classifiable variations in photoreceptors

The most significant variation in the photoreceptors of the eye at the histological level is in the axon endings and how they relate to the neurons of the inner nuclear layer. This variation may not have much to do with the spectral performance of a specific photoreceptor; but it may have an indirect relationship if the neurons of the inner nuclear layer have specific requirements with respect to the photoreceptors sensing a specific wavelength region, i. e. if more neurons must connect to the mid-wavelength photoreceptors, it is plausible that these photoreceptors may develop larger feet to accommodate the larger number of neural connections.

4.7.4 Operation of the photoreceptors in the local environment

As eluded to in an earlier section, the individual photoreceptors in a given zone of the retina do draw their nutrients from a common reservoir provided by the local cardio-vascular mesh. Under large signal conditions, particularly when applied
to a carefully defined group of photoreceptors, the defined group may draw down more than their share of nutrients; the neighboring photoreceptors will then exhibit an impact on their performance (resulting in various edge and “zone” effects as reported by the psycho-physicist). The principal impact on their performance is represented by the well-known adaptation curve. It describes the transient performance of the combined signal processing and metabolic processing capability of the eye. Many of these effects will be defined and documented in PART C below.

4.7.4.1 Experimental techniques

Experimental design must carefully consider the normal operating environment of the eye and take precautions to duplicate the conditions or to note changes used in the test configuration. In-vivo experiments must take particular note of the physical size of the probes relative to the size of the structures being examined and the surrounding fluid matrix. In-vitro experiments that do not simulate the “system environment” of the particular cell must recognize the limited relevance of the collected data.

The most significant problem with signal recording is the extremely high impedance level of the neuron electrical circuits. A typical neuron will exhibit a change in voltage relative to the INM of about 40 mV for a current change of about 20 pA. This corresponds to a differential resistive impedance of about 2000 megohms. Most laboratory grade oscilloscopes will exhibit a shunt resistive impedance of about 100 megohms. To maintain a bandwidth of at least 100 Hertz at the 2000 megohm level, the capacitance associated with this resistive impedance must be less than about 0.4 pF. This is a very small value. A typical laboratory quality oscilloscope probe cannot achieve a shunt capacitance below 5.0 pF. Thus, when attempting to measure the electrical characteristics of a single neuron, the probe loads the circuit under test considerably. It introduces a shunt impedance of about 100 megohms in parallel with a capacitance of at least 5.0 pF.

Some experimenters have used a special electrometer probe. However, it is difficult to achieve the necessary operating bandwidth with such a probe. This probe provides a direct connection to the gate of a very high input impedance MOSFET transistor. This type of probe exhibits a resistive input impedance that can be as high as $10^{15}$ ohms or 1000 megohms and a capacitance that may be as low as 0.1 pF. However, the cable connecting the probe to the subject frequently introduces a much higher capacitance, seldom less than 15 pF. A successful probe assembly, for purposes of interrogating a single neuron, should have the unpackaged MOSFET transistor mounted in the very tip of the probe with its gate terminal exposed to the test environment.

When designing an experiment involving a neuron, it is important to review in advance what can cause a neuron to change operating modes. What can make it stop generating action potentials if it is or what can make it begin generating action potentials if it is not? This change of operating mode is commonly encountered in experiments that have not been adequately researched and planned.

As will be shown in PART C, a neuron operating in the electrotone mode is frequently driven into producing action potentials by the simple process of attaching a test set probe to the neuron. The excessive capacitance associated with the probe and the inherent negative impedance of the Activa causes the neuron to go into oscillation. This oscillation can be continuous or appear as intermittent action potentials.

Conversely, any electronic probe that contacts a neuron that is generating action potentials may cause those pulses to cease if the probe causes the bias conditions of the circuit to change, moving the Activa out of the negative impedance condition.

Any voltage clamp technique is liable to introduce problems of the above types.

The waveforms generated by both classes of neurons can also be altered by disturbing the power supply serving the neuron. This can be done by introducing anoxia, removal of the bio-energetic materials such as GABA, introduction of excessive glutamates, the introduction of a wide variety of pharmaceuticals, or excessive change in the condition of the electrolyte external to the cell.

4.7.4.1.1 Effect of capacitive loading

The presence of a probe can affect the operation of a typical neuron in two important ways. It can limit the frequency passband of the overall circuit. It can also change the operating mode of the overall circuit. Although the reduction in the bandwidth of the overall circuit is seldom recognized by the investigator, it is very obvious to the trained eye in many published oscillograms related to vision. Of more obvious significance is the effect of the probe capacitance on the operating mode of the neuron. This effect has caused consternation among many investigators and the assignment of
Figure 4.7.3-1 Typical neuron test circuit configuration. See text

As indicated in the previous section, the capacitance associated with the dendritic terminal of the active device, $C_d$, is usually less than 0.4 pF in neurons of the bipolar and lateral types. The associated resistive impedance is typically 2000 megohms. A typical probe and electrical test instrument are shown in the lower left corner. This circuit includes the resistive and capacitive input impedance of the instrument, $R_{in}$ and $C_{in}$. It also includes the obvious resistive and capacitive elements of the probe, $R_p$ and $C_p$. What is not so obvious but also shown are the capacitances related to the probe cable, $C_c$, and the probe tip, $C_t$. $C_p$ is normally made adjustable for two reasons. It is used to maximize the flat portion of the passband associated with the probe and instrument combination and to compensate for the additional capacitance, $C_c$, due to the variable length of the cable used between the probe and the instrument. The uncontrolled variable in this configuration is the capacitance of the probe tip, $C_t$.

This circuit is very sensitive to the capacitance associated with the emitter and collector terminals of the active biological semiconductor device. There are four situations to consider based on the total capacitance in shunt with the resistive impedance shown at the emitter terminal.

- **The total shunt capacitance is small.** In this case, the circuit acts as a wide band amplifier capable of manipulating the applied signal without distortion.

- **The total shunt capacitance is larger but still small.** The output of the circuit can now exhibit two different output conditions depending on the electrotonic conditions present at the other terminals of the Activa. It can exhibit ringing due to the high frequency peaking introduced into the passband of the amplifier circuit. It can also exhibit a lower than normal passband for signals applied to one of the two input terminals of the Activa, the emitter and the base.

- **The total shunt capacitance is still larger.** The output of the circuit may now exhibit one or more “action potentials” that are a function of the electrotonic signal level applied to the emitter terminal.

- **The total shunt capacitance is excessively large.** The output of the circuit will exhibit oscillations which resemble a continuous series of “action potentials.”

The above conditions describe the operation of a single neural circuit as a function of the capacitive loading of the emitter and/or collector circuit. This variation accounts for much of the versatility of the neuron. It simultaneously explains the frequent references in the literature to “oscillations” recorded within the signal processing circuits of the retina distal to the ganglion cells. The mere contact of a lead, representing a significant shunt capacitance, with an individual neuron can impact the operation of that circuit significantly.

### 4.7.4.1.2 Signal recorded in-vivo

When recording signals *in-vivo*, the above discussion indicates how critical it is that the experiment is carefully planned. Is the intent to interrogate a single active circuit, i.e., a single neuron or a single synapse, which involves a very high
impedance level? Or, is the intent to interrogate a group of neural circuits in parallel, which lowers the impedance level of the overall test circuit drastically and may avoid many extraneous recorded measurements? It is extremely important to define exactly what the intent of the experiment is and what the actual accomplishments were. This can be done by describing the impedance conditions involved in the experiments explicitly.

### 4.7.4.1.3 Signal recorded in-vitro

Where *in-vivo* electrophysiological tests normally involve compromises with respect to physical dimensions (probes ten to one hundred times larger than the targeted elements) and impedance mismatches, *in-vitro* tests frequently do not incorporate the entire circuit environment in the test design.

Whereas *in-vivo* signal recording causes minimal disruption of the local signal environment, this is not normally true in *in-vitro* recording. Care must be taken to preserve all of the functional circuit elements involved in the *in-vivo* situation. Otherwise, the experimental results will be corrupted. Clearly, the fluids surrounding the photoreceptor cell must be of the same relative impedance as the in-vivo situation, including the effect of the outer limiting membrane on the signal path length. The data of Penn and Hagins\(^\text{262}\) is very useful. However, their procedures do not maintain the operating integrity of the photoreceptor cells. Their data does not show any electrical activity near the pedicel of the photoreceptors.

If the photoreceptor cell is washed, the chemicals on the surface of the cell will probably be removed or disturbed. This will immediately change the electrostenolytic conditions and thereby change the voltages applied to the circuits of the cell in a generally unappreciated way. What is more important, washing will remove the bio-energetic chemicals supplying energy to the cell and its performance will degrade rapidly with time (seconds to minutes).

Current attempts to lengthen the functional life of in-vitro neurons involve the use of a variety of solutions, such as glucose in water and various Ringer’s solutions. These attempts may also involve perfusion of the solutions with oxygen and/or carbon dioxide. Consideration should be given to the provision of, and maintaining the balance in the bio-energetics used by the electrostenolytic process, generally those of the glutamate cycle.

The separation of the RPE from the photoreceptor cell does not seriously impact the short term operation of the cell. However, over a period of days, its absence would become apparent.

### 4.7.4.1.4 Signal recorded by the ERG

[ xxx limit the discussion here to the Class C and Class D waveforms of the ERG. This seems to duplicate an earlier paragraph]

The ERG will be discussed in detail in Chapter 17. It will become clear at that time that there are two separate and distinct electrical waveforms that are directly associated with the photoreceptor cells. However, these do not correlate directly with the so-called a-wave and b-wave. The first waveform relates to a current emanating from the dendritic structure of each cell. This waveform is labeled the Class C waveform and corresponds to the early photoreceptor potential of the literature. The second waveform emanates from the axon structure of the cell. It is defined as a Class D waveform in this work and corresponds to the “generator potential” of the literature. These two waveforms are very similar in shape but occur at two different times due to the finite transit velocity of current passing through the cell and down the length of the axon. How these waveforms contribute to the ERG is addressed in Section 4.7.2.

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(Inactive) DEFINITIONS INDEX (Use individual marks)

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Retinal illuminance
Transport delay
net photoreceptor