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THE EFFECTS OF IONIZING RADIATION ON THE LIGHT SENSING ELEMENTS OF THE RETINA

Michael J. Malachowski (Ph. D. thesis)

July 1978

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THE EFFECTS OF IONIZING RADIATION
ON THE LIGHT SENSING ELEMENTS OF THE RETINA

Michael J. Malachowski
Ph.D. Thesis

August 1978

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ABSTRACT

This investigation was undertaken to quantitate possible morphological and physiological effects of particles of high linear energy transfer on the retina, in comparison with x-ray effects. The particles used were accelerated atomic nuclei of helium, carbon, and neon at kinetic energies of several hundred MeV/nucleon. These particles also occur in interplanetary space as "HZE" particles of galactic radiation and are present with low energies in solar particle emissions.

For morphological studies, scanning and transmission electron microscopy and light microscopy were used. Physiological studies consisted of autoradiographic data of the rate of incorporation of labeled protein in the structures (opsin) of the outer segment of visual cells. Structural changes were found in the nuclei, as well as the inner and outer segments of visual cells, rods and cones. Qualitatively these ranged from smoothing of the surface and changes in the size of the outer segments, loss of vertical and horizontal structure to deflation, phagocytation, and degeneration. At a low dose of 10 rad, x-rays and helium had no statistically significant morphological effects, but carbon and neon beams did cause significant degeneration of individual cells,
pointing to the existence of a linear dose effect relationship. At high doses of several hundred rads a "Pathologic Index" determined the relative biological effectiveness of neon against alpha particles to have a value of greater than 6. The severity of effects per particle increased with atomic number.

Labeling studies demonstrated a decreased rate of incorporation of labeled proteins in the structural organization of the outer segments of visual rods. The rate of self-renewal of visual rods discs was punctuated by irradiation and the structures themselves were depleted of amino acids. To explain the phenomena observed, a model of rod discs (metabolic and catabolic) was postulated for correlated early and late effects to high and low doses.
SECTION I. INTRODUCTION

Shortly after discovering x rays, Roentgen reported that a field of ionizing radiation could stimulate sensation of diffuse light ("visual phosphenes") in the human eye. In 1952, Tobias suggested that individual cosmic-ray particles might stimulate the visual apparatus and produce the sensation of light streaks. With the advent of space flight a variety of phenomena of visual stimulation were categorized. The astronauts of Apollo 11 experienced sensations of streaks and flashes of light. By the termination of the Apollo 15 mission, streaks, double streaks, stars, flashes, supernova, and luminous clouds had been categorized by the astronauts. It was postulated that different events, quality of irradiation, and energy deposition were responsible for the variety of phenomena observed. These events have been simulated by exposing human subjects to helium and neon particles. The results of these experiments confirmed the hypothesis that visual phenomena observed by astronauts are caused by cosmic rays, and relegated the primary site of interaction to the retina.

The chronic effects of heavy ions are not, however, well characterized. Morphological changes were observed after nitrogen irradiation in rods and cones; the reversibility of these changes, the damage caused by a single particle track, and the functional significance need investigation.

BACKGROUND

The Retina

The internal tunic of the eye is called the retina from rete
or net. Rufos of Ephesus used the term $a\nu\rho\alpha\chi\nu\omicron\sigma\iota\delta'\varsigma$ $\chi\iota\omicron\omicron'\nu$ meaning cobweb tunic. The great anatomist Herophilos of the Alexandrian school adopted the term $a\nu\mu\rho\iota\beta\lambda\eta\pi\sigma\rho\iota\sigma\iota\delta'\varsigma$ $\chi\iota\omicron\omicron'\nu$ (from $a\nu\mu\phi\iota\beta\alpha\lambda\lambda\epsilon\iota\nu$, to throw around) meaning an investing tunic. Galen adopted the secondary meaning of this term to mean a net that invests the captured fish (De usu partium corporis, Book 10, chapter 2). The Arabian translator Avecenna (Connon of Medicine, Book 3(3), chapter 1, page 23) took this meaning and adopted rescheth (net-like) in the sense that the retina encloses the vitreous and lens "as a fishing net holds the catch."

Gerard of Cremona made Latin translations of the Arabic texts using the Latin term retina—"The extremity of the optic nerve invests the vitreous body just as a net (rete) invests the catch, quapropter nominatus retina."

The concept of a netlike membrane is used in a number of languages to describe the retina—the German Netzhaut, the Russian syet' chataya abalochka, and the Japanese momaku.

Anatomically, the retina includes all of the tissues derived from the optic vesicle. The anterior portion has prolongations over the ciliary body and iris; the posterior portion is divided into the pigment epithelium and the cuticular layer of the membrane of Bruch, developed from the outer wall of the vesicle and the multilayered structure of the Pars Optica Retinae derived from the inner wall.

Evolution of the Light-Sensing Elements

Cilia are hairlike organelles that are commonly used to propel swimming cells or move liquids over fixed cells; they are composed
of sheaves of microtubules. Ciliary movement occurs when the microtubules, powered by ATP, slide past one another. The specialized cillum of an embryonic photoreceptor cell is covered by an outer membrane, which forms infolded convolutions during development. The protein opsin is incorporated into the infolding plasma membrane at the base of the embryonic cillum. Oxidized vitamin A, retinal, is supplied by the pigment epithelium layer of the retina and combines with opsin to form the purple visual pigment rhodopsin.

There is a remote phylogenetic connection between flagellate structures and photoreception. Protistic phytoflagella exhibit photosynthetic activity. In Euglena, Chromylina, and Chlamydomonas, a red pigmented eyespot is located in the kinetosome of the flagella containing (9 x 2) + 2 fibrils. The phytoflagellata exhibit positive phototaxis in the spectral region corresponding to the absorption spectrum of the carotenoid astacin. In Chromylina the pigment vesicles are formed along the ends of the one or two large photosynthesizing plastids, the chloroplasts. The photosynthetic and photoreceptor functions are structurally connected in the protist, where light stimulation is transmitted with the aid of microtubules. Divergent tracts of evolution are evidenced by the different chemical to mechanical energy transduction mechanisms for flagella-rotation and cillum-sliding filaments.

In vertebrates the outer segment disc develops at the expense of the plasma membrane coating the cillum of the embryonic visual cell. Future visual cells have an elongated shape, and with the aid
of desmosomes, adhere tightly to the pigment epithelium making the zona adherens. Numerous mitochondria are scattered throughout the entire cell but accumulate mainly around the nucleus. Mitochondria-like organelles divide at an intense rate by constriction and migrate to the area of the inner segments. Numerous smooth and rough endoplasmic reticulum, membranes and Golgi apparatus appear as offshoots from the nuclear membrane and correspond in productivity to the synthesis of the disc membranes of the outer segment. Cell differentiation toward the mature form proceeds at both ends, synaptogenesis proceeding at the opposite end from photoreceptor genesis. These opposite ends are separated by the tight junction, which delineates the inner segment-nuclear layer boundary.

A peculiar endoplasmic reticulum forms the basis of the developing paraboloid in the cytoplasm. Glycogen granules of 150 to 250Å surround the structure, and the myoid is oriented toward the nucleus. Metabolic synthesis of proteins, ribosomes, and lipids (formed in the soluble fraction of the cytoplasm), utilize glycogen as both a source of atoms and a source of energy, ATP. The basic membrane components are synthesized at the nucleus inner-segment level of the cell and transported to the inner-outer segment boundary where the disc membranes are assembled.127

The increasing complexity in the organization of the disc membranes and their differentiation from the original plasma membrane of the cilium in the embryonic visual cell can be linked to the integration of the rhodopsin molecule into the structure of the membrane. Vitamin A from the liver reaches the pigment layer where it
is oxidized into retinal (retenene). The mechanism that controls the induction of the growth of the outer segment has not been elucidated, however, the formation of rhodopsin from opsin and vitamin A is believed to induce the appearance of folds or discs in the outer segment. The growth of the plasma membrane of the cilium would appear to act as a "primer" for fitting the photosensitive membrane components. The deprivation of vitamin A from the developing retina disrupts the encrustation and intussusception of the membrane primer, and results in atresia of the membrane.

In insects, the photoreceptor membrane forming the rhabdomere of the ommatidium develops as a result of the growth of microvilli. The rhabdomere is formed by the development of microvilli on the lateral surface facing the center of the future retinula, when cytoplasmic vesicles move towards the base of the villi and attach themselves. The rhabdomeres of the ommatidium circle a common center. In cross section, the photopic unit appears quite similar to the cross section of the lower portion of the outer segments of amphibian retina—a central core of photosensitive discs and palisade of inner-segment filaments surrounding and containing the discs (Figure 1-1).

STRUCTURE

Eye Embryology

The eye develops from two germ layers, the ectoderm and the mesoderm. The elements of the visual system—the optic primordium and the lens primordium—are of ectodermal origin. The mesoderm furnishes the accessory eye structures. The lateral evagination of the optic
Figure I-1: Diagram of the outer segment of a photoreceptor rod from *Necturus* retina, after Brown, Gibbons, and Wald (1963). A palisade of dendritic fingers, calycle processes, originate from the inner segment. The discs of the outer segment are shielded from the processes by the intervening outer membrane. The top cross-section was made at the level of termination of the calycle processes. The outer segment outer membrane between the photoreceptive discs and the calycle processes continues distally into the pigment epithelium. Topologically the membrane is a sheet and maintains its continuity upon exocytosis and after phagocytosis of the discs.

The lateral section has exposed the parallel surfaces of the disc membranes which contain rhodopsin, the visual pigment. The spacing between the membranes, which determines the volume of the outer segment discs, varies with light cycling and ionic concentration.

The lower cross-section illustrates the proximity of the interior of the inner segment to the interior of the discs, 4-600 Å, in *Necturus*. These relations are species dependent, in *Mus* the processes surrounding the lower portion of the outer segments are derived from the Müller cells.
primordium appears very early (at 8 days in the mouse and at 18 days in man) before complete loss of the encephalic groove. It is induced by the prechordal plate and forms at the base of the future prosencephalon, a continuation of the third ventricle. The evaginated peduncle is opposite the cephalic surface ectoderm and induces the optic placode to form. The end of the peduncle, the primary optic vesicle, evaginates to form the neural elements of the retina and their support structures. The lens primordium is depressed into the secondary optic vesicle where it differentiates into the clear lens. This migration occurs over a four- to five-day period in the mouse and requires over a month in man. In the mouse, by the thirteenth day the nervous layer of the retina has formed to a thickness eight times as great as that of the pigment layer. By the fifteenth day, the neural layers of the retina have begun to differentiate, and by the eighteenth day the eye is essentially complete. In man, the eye has completed differentiation by the seventh month.

The optic stalk forms by encircling the hyaloid artery, which supplies the mammalian retina. The axons of the ganglionic cells progressively colonize the encircled spaces in the optic stalk as they progress toward the diencephalon. The axons cross over to form the optic chiasma and proceed to the CNS structures of the visual system—the external geniculate bodies and the optic area of the occipital cortex.

The anterior portion of the secondary optic vesicle tends to close in the front of the lens, delineating the pupillary orifice, when the internal and external layers of the retina unite. The internal layer, which remains thin and does not undergo sensory differentiation, gives
rise to the internal, nonpigmented layers of the iris. The external layer produces the pigmented epithelium of the iris.236

The posterior portion of the secondary optic vesicle differentiates into neural and glial cells, which are then invaded by the blood circulatory structures in mammals. Amphibian retinae are avascular and do not contain the support cells found in mammals. The external neuroblastic layer differentiates from the mantle layer next to the interretinal space into the light-receptive elements.148 The adjacent portion of the mantle layer gives rise to neurons, the internal neuroblastic layer, and the supporting cells. Cells of a column originate from the same matrix cells; the columnar organization of the retina is an expression of the clonal origin of the nerve cell types. The ependymal germinal epithelium, which borders the inter-retinal space, becomes the pigment epithelium. The ganglion cells form from the dorsal portion of the neuroblastic layer. The gap in the ventral side of the optic cup is carried back onto the optic stalk as a groove. It is through this opening in the walls of the cup and along the groove in the optic stalk that the optic nerve axon fibers grow.179

Membranes

The membrane components of the rod outer segments are synthesized in the cell soma and transported up to the outer segment, synthesized as disc membrane, and then pushed towards the pigment epithelium where the macrophages degrade the membrane. There are two types of membrane synthesized for the rod outer segment: the outer membrane, which surrounds the disc stacks and is responsible for initiating the cellular neural
impulse; and the disc membrane, which is responsible for initiating the response to light stimuli. Our knowledge of the interaction between these two membranes is vague, especially in relation to the approximately $10^6$ amplification factor observed from the reception of a photon to the propagation of an impulse.

It is possible that the evolutionary origin of the photoreceptor membrane lies in the prokaryotic bacteria. The halophilic bacteria Halobacteria holobium contains photoreceptive portions of membranes, purple membranes, which function as a hydrogen-ion pump. The visual purple of the rod outer segment has a similar Vitamin A/protein structure. The bacterial purple membrane is integrated into the continuous bacterial membrane. Although the bacteria has a characteristic membrane lipid, it is not a critical component in the hydrogen-ion membrane pump. The protein has been found to be a triple-helix structure spanning the membrane. The photoreceptive retinaldehyde group lies in the center of the membrane completely surrounded by protein. A possible hydrogen-ion transport mechanism is a quanta mechanical transposition of the hydrogen ion through the hydrogen bonding of the alpha helix. Similar mechanisms for linear quanta transfer have been proposed to explain the forces involved in muscle contraction.

In addition to the rhodopsin complex of the outer-segment disc membrane, a large protein with a molecular weight eight times that of opsin (230 to 280 K Dalton) has been isolated. This protein is not required in the current models of the rhodopsin cycle and is found in a ratio of 1:300 to 1:900 rhodopsin molecules. Because this aqueous insoluble protein does not seem to be any of the other previously
described enzymes that are readily solubilized in aqueous buffers, its function remains unclear.

The site of synthesis of all photoreceptor cell protein must be the same opsin synthesizing ribosomes; the incorporation of membrane proteins is probably by a similar mechanism. Ultrastructure studies indicate that the disc membrane is a lipid bilayer with proteins running across it and on both sides. The inner (enclosed) side in the rod discs has an additional mucopolysaccharide coating. Because rhodopsin is incorporated into the membrane at the inner-outer segment boundary, it is likely that the other proteins and enzymes are incorporated into the membrane previous to this time. If the discs are formed essentially from an invagination of the outer membrane, the disc membrane would be similar in composition and function to the inner-segment outer membrane as would the outer membrane of the outer segment. These three membranes serve different functions, and require specialized mechanisms and structures to operate.

There are two basic models for membrane synthesis: a construction model, which requires exact positioning of all components; and the crystallizing model, whereby membrane components stack or fit together. Entropy considerations require a fit to be exothermic in order for an enhanced stability to be realized. The actual transport of the membrane components from the ribosomes to the inner-outer segment junction may be active or passive. The contractile and structural components (actin filaments and microtubules) could create a negative pressure by the phagocytosis of the end of the outer segment, or they may directly influence the membrane micelles formed from the combined materials.
Figure I-2: Retinal$_1$ and Figure I-3: Retinal$_2$. Retinal$_1$ and Retinal$_2$ are the antenna molecules of the visual pigments rhodopsin and phyropsin found in *Mus.* and *Necturus* respectively. A conformational change about carbon 11 (from cis to trans) occurs upon photon absorption. The addition of a second double bond to the ring structure between carbons 3 and 4 changes the spatial configuration of the ring and the molecular resonance values. In hexane the absorption peak is at 3680 Å for retinal$_1$. This shifts to 3850 Å for retinal$_2$. 
produced by the ribosomes and the soluble fractions of the cytoplasm.

Vitamin A

Two isomers of vitamin A are found in animal retina, vitamin A₁ and vitamin A₂. These isomers (and thus retinal₁ and retinal₂) can be distinguished from one another by their spectroscopic absorption characteristics. Vitamin A₁ is retinol, vitamin A₁ acid is retinoic acid, and vitamin A₁ aldehyde is retinene or retinaldehyde. Vitamin A₂ is 3-dehydroretinol, and retinene₂ is 3-dehydroretinal or retinal₂.

Vitamin A₁, found primarily in mammals (mouse), can be identified by its absorption spectrum, which peaks at 325 nm in either hexane or ethanol; the absorption peak is at 621 nm upon reaction with the Carr-Price reagent (SbCl₃ in CCl₃H). Retinal₁ has an absorption peak at 368 nm in hexane, 383 nm in ethanol, and 664 nm upon reaction with the Carr-Price reagent.⁹⁹

Vitamin A₂ and retinal₂, found mainly in amphibians (Necturus maculosus), is a chemical isomer that differs from vitamin A₁ by an added double bond in the ring at positions 3 and 4 (Figures 1-2 and 3). Retinal₂ is characterized as a deep yellow substance soluble in petroleum ether. It was crystallized by Salah and Morton,¹⁹⁹ and the maximum absorption peaks of crystalline retinal₂ were found to be at 385 nm in hexane, 388 nm in petroleum ether, and 401 nm in ethanol. Vitamin A₂ reacts with the Carr-Price reagent to give a maximum absorption peak around 693 nm, whereas retinal₂ peaks at 705 nm.²⁵⁹

Visual Pigment

The extracted visual pigments are identified primarily by their
absorption spectra to be either rhodopsin ($\text{retinal}_1 + \text{opsin}$) or porphyropsin ($\text{retinal}_2 + \text{opsin}$) for the retinal rods, and iodopsin ($\text{retinal}_1 + \text{cone opsin}$) or cyanopsin ($\text{retinal}_2 + \text{cone opsin}$) for the retinal cones.

**Retinal Rod Outer-Segment Lamellae**

Chemical analyses of the rod outer segment's weight show that rhodopsin accounts for 4 to 10 percent, protein accounts for 40 to 50 percent, and lipids account for 20 to 40 percent of the total dry weight. The cross sections in cattle and frogs were found to be 2500 and 2620 Å, which means that the diameter of the rhodopsin molecules should be on the order of 50 Å. Wald theoretically calculated the diameter as 40 Å. This and x-ray diffraction studies support a membrane model where rhodopsin molecules are packed on the lamellae membranes (Figure I-4).
Figure 1-4: Pictorial representation of stages in the bleaching of rhodopsin. Rhodopsin has, as a chromophore, 11-cis retinal, which fits closely to a section of the opsin structure. The action of light is to isomerize retinal to the all-trans configuration. The structure of opsin opens in stages (lumirhodopsin, metarhodopsin I and II), until finally the retinaldehyde is hydrolysed away from the opsin. The opening of the opsin structure exposes new groups, including two sulf-hydro and one $H^+$ binding groups.
Pigment Epithelium

Kölliker demonstrated that the pigment epithelium (PE) is retinal in origin because it forms the outer layer of the secondary optic vesicle. Anatomists had previously considered it to be a portion of the tunica ruyschiana (choroid). Wharton Jones was the first to adequately describe its morphology as a single layer of polygonal cells. Each cell contains a round or oval nucleus situated near the basement membrane and a large quantity of pigment called fuscin by Kühne. Fuscin collects preferentially in the inner part (base) of the cell, leaving the outer region (dome) with the nucleus next to the choroid comparatively free. Lipofuscin granules are membrane bound deposits that are thought to be the terminal stage of phagosomes. The common pigment granules found in the pigment epithelium are melanosomes. The processes of the pigment epithelium dip down between the ends of the rod and cone outer segments and pass through fenestrations in the cement substance condensed on the inner surface of the cells (the membrane of Verhoeff).

The pigment epithelium which is intimately interdigitated with the upper portion of the rod outer segment plays a number of roles in the visual system. It produces mucopolysaccharides; supplies and recycles vitamin A; regulates phagocytic activities; plays an active role in the dissociative shedding of outer-segment discs; and nourishes, attaches, and supports the light-sensing elements. Nutrients are supplied in the form of both energy and chemicals to the pigment epithelium. An intimate relationship must exist between the pigment epithelium cell filaments and the rod outer segment to ensure the continuity of the
rod outer-segment outer membrane during the phagocytic process. The rod outer-segment outer membrane regulates calcium transport between the interdisc spaces and the external milieu of the light-sensing elements. Ionic conduction between the outer-inner segments and the cell body is responsible for propagating the neural impulses across the tight junctions to the synapse of the light-sensing elements.

Müller Cells

Müller cells, the narrow, elongated, glial-type cells that span the entire thickness of the retina, are named after Heinrich Müller, a professor of comparative anatomy at Würzburg who proved that rods and cones are the photoreceptors. These cells have their nuclei in the inner nuclear layer of the retina. Their ventral fibers, the sustenacular fibers of Müller, terminate with their inner ends on the inner surface of the optic-nerve fiber layer at the internal limiting membrane. In the inner nuclear layer, the Müller cell forms multiple recesses that enclose a great number of somata of the bipolar cell. In the opposite direction, dorsally, their extensions pass through the densely arranged receptor-cell nuclei to the level of the photoreceptors. As the fibers pass the outer limiting membrane, they assume a brush-border or microvillous arrangement.

The central cytoplasmic area of the Müller cell is filled with microtubules; these microtubules are collectively known as the "Müller fiber." Glycogen synthesis occurs about the nucleus, and this material is contained as the outer area of the cell cytoplasm. The pigment epithelium, which interdigitates ventrally toward the end of
the Müller processes, is the only other retinal cell layer that exhibits the capacity to synthesize glycogen. Acid mucopolysaccharides, which are known to play a role in cellular adhesions, may function in outer-segment, pigment-epithelium adhesion; this material is synthesized in the Müller cells. The internal filaments of the Müller cell indicate a supportive role; the presence of glycogen suggests a nutritional function, however intercellular recording relates this cell to the b-wave of the electroretinogram (ERG). Because glycogen seems to be absent from the neurites embedded in the Müller processes, this glycogen may be a source of energy. The respiratory components of the Müller cell are located near the apical end or brush border near the choriocapillaris. This clustering of the mitochondria may reflect the most favorable position in relation to oxygen tension.

Fine and Zimmerman consider this cell to be a specialized form of astrocyte with a fibrous interior and protoplasm around its periphery. The Müller cell is astrocyte-like in its center and oligodendroglia-like in its periphery. This designation arises from the consensus that a glial cell containing a large number of intracytoplasmic filaments should be designated as a fibrous astrocyte; similarly, one with few such filaments is a protoplasmic astrocyte, and one lacking cytoplasmic filaments but containing quantities of ribosomes is an oligodendrocyte.

**FUNCTION**

The function of the retina is to recognize spatial and temporal patterns of light and to modulate neural message patterns. This process can be differentiated into two sequences of events. The first sequence
is the absorption of photons and the initiation of a potential change. The secondary sequences include many ERG source and modulating processes which cause transmission, propagation, summation and integration of the impulse. The first process occurs in the outer segments of the retinal rods and cones; the later steps occur in the other retinal layers.

The amacrine and horizontal cells allow spatial distribution of signals or summation of spatially distributed signals. The bipolar cells are organized into hyperpolarizing-depolarizing regions for signal processing, and the ganglion layer is for summation, integration, and propagation of neural impulses.153

The net result of the retinal-outer segment layer of interconnecting cells is an attenuation network that is functional over ten orders of magnitude. This latitude allows the eye to perceive a single photon under dark-adapted conditions131 or to operate effectively in full sunlight. Other phenomena such as edge detection, pattern recognition, and image resolution are more complex functions of the visual cortex.

Two tasks are relegated to the inner-outer segments of the photoreceptors. One task is the adaptation to the general intensity of light, i.e., light and dark adaptation, and is accompanied by structural changes of shape and movement. The second task is that of discriminative vision and is related to discrete changes in the light-sensing element's outer segment membrane permeability affecting photon absorption. This portion of the visual pathway leads from the outer segment to the inner segment and then to the synapse of the photoreceptor.
Figure I-5 and I-6: The ε-amino lysine nitrogen of the opsin protein undergoes a Schiff-base reaction with the carbon, C_{15}, of the retinal molecule. The displaced aldehyde oxygen proceeds from a hydroxyl to water molecule. The site of the reaction in vivo is on the surface of the rod disc where the ring moiety rotates on its tail about the Schiff-base linkage. A stable state is reached (Fig. I-4A). The attachment of the retinal group to the opsin initiates functional potential in newly synthesized discs.
Photochemistry

The isolated rod visual pigment bleaches upon exposure to light, yielding retinal and its carrier protein opsin. Retinal is bound in a Schiff-base linkage. Morton and Collins have shown that retinal is attached to an amino group of opsin.

\[
\text{Retinal - C = O} + \text{N - Opsin} \quad \text{Retinal - C = N - Opsin} + \text{H}_2\text{O}
\]

Bleaching produces an all-trans retinal, which must be isomerized to the 11-cis form in order to recombine with opsin. Although steric interference between the methyl group at carbon 13 and the hydrogen at position 10 would prevent the molecule from becoming entirely planar, Wald found this 11-cis form to be the functional isomer. Because of its hindered configuration, the 11-cis form is the least stable of the possible isomers; it is most easily formed upon irradiation, and the most sensitive to temperature and light. This molecule is then, appropriately, very unstable in the light and stable in the dark.

The bleaching of the rhodopsin proceeds through a series of steps, outlined in Figure I-7.

Biopotentials

In rod cells, the "early" receptor potential is generated by the rhodopsin molecules on the surface of and within the outer-segment disc membrane. In cones, the "early" receptor potential is generated
Figure I-7: Schema of photochemical events and the dark reactions of rhodopsin associated with the visual process. Rhodopsin undergoes a series of transformations during the bleaching process. At reduced temperatures a number of states of different absorption peaks have been identified. A transition to the Metarhodopsin III or sometimes to the Metarhodopsin II state results in a Schiff-base bond rupture. The trans-retinal is either converted to the all trans-retinol by an oxidoreductase or is converted to 11-cis retinal which bonds to the opsin molecule. Once the bleaching process is initiated, photochemical events are capable of cycling lumirhodopsins and metarhodopsins to the rhodopsin configurations. The lifetimes for a particular state are identified by shifts in absorption peaks.
by the visual pigment fitted into the enveloping membrane of the outer segment. An "early" receptor potential occurs soon after a light flash, 25 to 50 microseconds, whereas a "late" receptor has a latent period of 5 to 7 milliseconds. In rods, the late receptor potential decays slowly and yields a long trace potential; in cones, it decays rapidly, and the more intense the stimulus, the later the trace potential is observed.

The action of light decreases the sodium permeability of the outer segment membrane to induce hyperpolarization of the cell. Penn and Hagins believe that the absorption of one light quantum controls the flow of 60,000 sodium ions whereas Korenbrot and Cone claim that the excitation of a single rhodopsin molecule transiently stops up to $10^7$ sodium ions. Because the membrane is hyperpolarized in the light state, this state is the resting state, and the dark state is the excited state.

The reduction of the number of photons causes an increase in membrane permeability; the absorption of a photon causes a transient decrease in ionic flow, which is converted into a neural impulse at the rod or cone cell synapse.

Ouabain suppression of the sodium-potassium pump has no effect on current conduction when the external milieu of the outer-segment discs is kept at an appropriate sodium concentration. It is probable that the inner segment, endowed with energy and undergoing structural changes during illumination, must contribute to the act of stimulating the visual cell. In the inner segment, the response is not propagated by typical neural membrane permeability change. Because a reversal of the inner segment response to stimuli is found at approximately -85 mV, Werblin
suggested that an electrogenic membrane pump, i.e., the K+ battery, is involved. Hubbell's work with outer-segment disc membranes has lead to the belief that either a cyclic nucleotide or calcium or both are involved in the signal transmission from the disc receptor site to the outer segment membrane. The membrane has some unknown intricate relationship between the protein mucopolysaccharide and the phospholipid bilayer; functionally, a thicker bilayer prevents regeneration of the visual pigment. The polar head groups of the lipids were found to be unimportant in the process.

The molecular structure of the rod and cone cell's synaptic transmitter is also unknown, although it is possible to interfere with the release of the transmitter which is calcium+2 activated. Cobalt+2 competes with calcium+2 at the membrane and prevents the release of the transmitter. The action of cobalt towards the synaptic transmitter in the dark is similar to that of the synaptic transmitter on the synapse caused by the hyperpolarization effect of light. Marshall and Werblin have found that both glutamate and the synaptic transmitter have the same effect although the same site of action was only inferred. Burnside has found that the actin-myocin filamentous processes in the intracellular matrix interdigitating with the outer segment become unstable with the addition of calcium.

The necessary change in the permeability of the membrane required to generate an action potential can be caused, in theory, by a single event of molecular scale, e.g., a conformational change in a protein molecule. Jahn believes that vitamin A, being a carotenoid, undergoes a structural change on exposure to light, and, by taking part in electron
transfer, may be responsible for visual-cell stimulation.\textsuperscript{101}

Illumination of the light-sensing element also stimulates a change in the RNA content of the cell (both cytoplasmic and nuclear) and probably in protein metabolism.\textsuperscript{240}

Under the influence of photon energy, rhodopsin "straightens" to the all-trans form separating it, in part, from the opsin molecule (Figure I-4). This is the beginning of the stimulation of the visual cell. The critical photochemical event takes place sometime between the initial cis-trans isomerization of the retinal groups and the generation of meta II. Figure I-8 plots photocurrent latency and meta I to meta II transition, associating the photochemical event with a conformational rather than a chemical event.\textsuperscript{195} This conformational change is accompanied by the rupture of the Schiff-base bond to the epsilon-aminolysine group, bleaching the rhodopsin complex.

The rod disc membrane is thought to cause transient charge relocation upon photon capture.\textsuperscript{195} This is reflected in the $R_1$ (initial positive transient) and $R_2$ (large negative deflection) of the early receptor potential (ERP). This signal is amplified on its passage to the outer membrane of the outer segment, propagated by cable property transmission through the inner segment to the synapses on the opposite side of the tight junction, and processed through the neural layers to the ganglion cell. The overall electrical effect of a visual stimulus observed by recording the retinal potential is the ERG, both the early and late potentials are apparent in the ERG; chemical alterations of synaptic potential such as the addition of cobalt, aspartate or glutamate alter the ERG.
PARTICLE RADIATION

Background

The primary biologically hazardous components of cosmic rays are high-energy heavy ions, which have come to be known as HZE (high-Z and energy) particles. The biological effects of this component of space radiation are the least adequately measured and understood. With the advent of accelerators capable of producing significant fluxes of these particles and the probability of extended space flight, the interest in HZE particles has increased.

It was not until the summer of 1948 that the HZE component of galactic radiation was discovered. A stack of nuclear emulsions exposed during a high-altitude balloon flight exhibited what are now known to be characteristic heavy-ion tracks. An early observation of an HZE particle effect was five years later. Black mice were sent aloft in a balloon and incurred gray spots and streaks in their fur, which were effects of damaged melanocytes along the tracks of HZE particles. Ground-based research in this field was initiated in 1957 with the development of the heavy-ion linear accelerator (HILAC) at the Lawrence Berkeley Laboratory, University of California, and at Yale University. These accelerators allowed for systematic investigation of HZE particles and provided the experimental information required for the construction of the Bevalac at the Lawrence Berkeley Laboratory. The Bevalac is a unit consisting of a HILAC module injecting heavy ions into a synchronous-ring accelerator, the Bevatron. The successful fusion of these two machines has created a new ground-based research effort utilizing particles of energies comparable to those found in galactic cosmic rays.
Figure I-9: Sea level measurements of cosmic ray ionization with a shielded ionization chamber.

Fig. 1-9
Figure I-10: Absorbed dose rates in free air from cosmic ray charged particles in the lower atmosphere at geomagnetic latitude 50°N.
Absorbed dose rates in free air from cosmic-ray charged particles in the lower atmosphere at geomagnetic latitude 50°N (from Lowder and Beck, 1966). (10 mrad y⁻¹ = 1.14 µrad h⁻¹.)

Fig. I-10
It has become customary among radiation physicists and radiobiologists to classify cosmic-ray particles having a charge \( Z \) in excess of 2 as HZE particles, even though such a classification is merely operational. Usually particles with a range in excess of 1 mm are considered HZE particles. This requires particles to have a minimum initial energy of 10 to 35 MeV per amu, which corresponds to energy deposition rates of 100 kilo electron volts (keV) per micrometer near the end of a particle's range. (Bevalac energies used to investigate retinal effects were on the order of 300 to 400 MeV/amu.) For consistency, this classification has been followed throughout this thesis.

Interplanetary cosmic rays are either of galactic origin or they are emitted by the sun during solar flare activity. Near sea level, HZE particles are absent owing to either fragmentation and absorption by the atmosphere, or exclusion, deflection and trapping by the geomagnetic fields of the earth (Figures 1-9 and 1-10). Galactic cosmic rays entering the solar system are modified in flux and energy by interplanetary magnetic fields, but nuclei having a broad distribution of energies are always present in space.

The largest proportion of incoming galactic particles is due to protons; heavy charged nuclei comprise a small fraction of the total cosmic-ray flux. Compared to other particles, HZE particles have much higher values of linear energy transfer and are therefore sometimes referred to as high-LET particles. The heavier particles have such high LET that they account for 60 percent of the free space dose from galactic cosmic rays; protons and helium particles account for the other 40 percent. Less than one tenth of one percent of the cosmic
rays with Z less than 6 have a LET value greater than or equal to 200 keV per micron. Most of the HZE particles have an atomic number between 6 and 26 and account for the majority of high-LET events. More than 90 percent of particles with a charge greater than 6 have energies greater than 100 MeV/amu. HZE particles have rather unique physical properties exhibited only to a lesser extent by other types of ionizing radiations. They undergo very little scattering from their initial trajectory and have precise range/energy relationships. The extent of ionization by heavy penetrating particles increases with decreasing velocity and shows a maximum effect just before these particles are brought to rest.

Radiation from HZE particles is theoretically and experimentally potentially damaging to biological systems. In space, this potential for damage is a challenge to long-term space travel. As a therapeutic modality, high-LET HZE particles provide a unique weapon against cancer.

**Dosimetry**

The rad is the classical physics parameter used to measure energy absorbed per unit mass (1 rad equals 100 ergs absorbed per gram). Therapeutic doses may be measured in hundreds of rads or the Gray (1 Gray = 100 rads), while maximum allowable doses and radiation effects upon the population are measured in millirads (1000 millirads = 1 rad). For radiations that can be described by classical field theory, for example electromagnetic waves such as x rays, this measure is an adequate description of the system. High-LET radiations cover a spectrum of mechanisms of particle and photon energy transfer, and the end point is
not a single-valued function of dose. The use of the rad alone as a description of dose for HZE particles is insufficient for a reproducible characterization of microscopic or nonhomogeneous systems.

In a living biological system where both micro and macro environments are critical, the problem of exact dosimetry is compounded by the statistical nature of the de-excitation mechanisms. To facilitate an understanding of the problem, a number of simplifications of the system are possible under experimental conditions; restraints placed on the system have permitted the creation of mathematical models. For particles the concept of track structure was originated and has been refined to account for many of the observed biological phenomena.

The distribution of energy in space by the passage of a heavy particle is rather non-uniform, hence the chemical stage that follows immediately after the initial absorption of energy has to deal with reactive species that are distributed in a nonhomogeneous manner. The chemical species (from the radiolytic decomposition of water) are most densely populated close to the trajectory. Any quantitative evaluation of radiobiological effects must be integrated over the whole space in which energy is deposited with due consideration to the proper distribution of damaging chemical species. Since such an evaluation is at present fragmentary and is rather difficult, it is necessary to depend upon experimental results for both the evaluation of the hazardous aspects of heavy particles and for understanding the basic mechanisms involved in radiation damage.

For a beam of charged particles, the dose at a point in a medium
is proportional to the flux of particles at the point multiplied by the LET and divided by the density of the medium. In radiobiology and health physics, LET is measured in keV/micron of tissue. The physics and nuclear engineering term dE/dx, or the energy change per unit distance, is measured in MeV per gram per cm$^2$ of matter (MeV cm$^2$/g). For a homogeneous sample, the dose at any given depth from the entrance point of a beam of known energy can be measured. For biological systems this is approximated by water or aqueous solutions. Tissue density is on the order of 1 g/cm$^3$, and therefore 1 keV/µ = 10 MeV cm$^2$/g.

A depth-dose or Bragg curve has a characteristic shape for HZE particles (Figure 1-11). The curve may be measured experimentally or calculated by finding the product of the flux of the beam and the LET at any particular depth.

**Stopping Power Formula**

For a fast charged particle moving through a medium, $z$, $v$, and $E$ are the atomic number, velocity, and energy of the charged particle, and $Z$ is the number of electrons in the medium.

The LET of a charged particle has been approximated following the work of Bohr and Bethe in a model known as the stopping power formula:

$$-\frac{dE}{dx} = \frac{1}{2} \frac{A}{M} \frac{2e^4}{\gamma^2} \frac{N}{Z} \left\{ \ln \frac{2\beta^2}{I(1-\beta^{-2})} - \beta^{-2} - \delta/2 \right\},$$

where $I$ = mean excitation potential, $\beta^2 = v^2/c^2$, $\delta$ = density correction, and $M$ = mass of the electron.

Energy may be lost through either elastic (kinetic energy is conserved)
Figure I-11: Particles have different depth dose characteristics due to the variety among energy loss mechanisms. Heavy ions fragment into atomic and subatomic particles (e.g. lighter elements, pions or muons) which either decay or suffer further collisions with the medium in the core of the track. Excited electrons are generally the end product of energy cascades and form a penumbra around the core of the track. The Bragg curves for pions and nitrogen ions are related to the track structure of energy dispersion. The plateau region are areas of low linear energy transfer (LET) due to particle passage while the Bragg peak is a region of stopping particles and high LET. The steepness of the backside of the Bragg peak and the length of the tail is related to the amount of beam fragmentation. LET varies as the square of the particle's charge. An uncharged particle such as a neutron has a linear absorption coefficient.
Figure 1-11

Bragg Peaks

30 MeV Electrons

14 MeV Neutrons

50 MeV π^-

4 GeV Nitrogen Ions

% Depth Dose

0 100

0 5 10 15

Depth in Tissue (cm)

XBL 789-10771

Fig. I-11
or inelastic (kinetic energy is not conserved) collisions. The former is a resonant energy loss process which creates states of excitation between the lowest electronic excited state and about 100 eV; the latter creates knock-on electrons with energies between 100 eV and a maximum energy that depends on particle velocity. Inelastic collisions with electrons and the nucleus account for primary excitation, ionization and bremsstrahlung energy mechanisms. Cherenkov radiation is a relativistic phenomenon of photon creation. It generally results in a loss of energy to the system and is therefore not a local damage mechanism.

Rarely for any one particle do two de-excitation methods compete; different processes, or mechanisms of energy transfer, occur at different energies. In a large system a spectrum of particle energies occurs and a number of processes occur simultaneously. Energy loss in the core is initially located close to the trajectory of the particle; energy loss in the penumbra leads to delta rays and branch tracks.

At higher energies, the probability cross section for an interaction decreases, while with increased Z the energy deposition increased. The distribution of the energy about any point in an HZE particle track can be considered in its own space-time continuum. Acceptable transitions include charge transfer, nucleon transfer, fragmentation, and nuclear exchange reaction as well as radiative energy exchanges. An initial HZE particle de-excitation event progresses in a "dome" type of distribution of events around a central point in a time-space coordinate system. This dome bounds the de-excitation event horizon and produces a track structure for HZE particles.²³⁴
A generalized model of energy loss distribution is the string-of-beads hypothesis. This model assumes a radially dependent spherical energy distribution about each point of energy transfer along the HZE particle track. Because the event probability is a function of time, the beads are spaced along the track. The spacing tends to decrease as the energy of the HZE particle decreases, and as the energy deposited decreases (Figures 1-12 and 1-13). As the spacing decreases, the spheres merge into a cylindrical type of energy dissipative model, which is also radially dependent.

The string-of-beads, or spurr, model was initially conceived for low-LET radiation; for HZE particles the LET is so high that spurrs overlap from the beginning of their interaction with the system to form cylinders.

The patterns of energy deposition are determined by the physical interactions of the particle with the medium. One mechanism leads to the creation of a "core" and another to a well-defined region of lower energy density, the "penumbra."

The radius of the track core is given by

$$ r_c = \frac{\beta_c}{\omega_p}, $$

where $\omega_p$ is the plasma frequency of the medium. This equation gives the Bohr adiabatic criterion: matter that is farther away from the particle trajectory than $r_c$ has time to follow the impulse adiabatically and does not absorb energy directly. The radius of the penumbra, $r_p$, was found empirically to be
Figure I-12A: Diagram of spur overlap at the end of a particle's range to form a cylindrical track. Based on the spur model energy is deposited periodically, as a function of particle energy, along the track. As the particle slows, loses energy, the separation between spurs decreases until the spherical energy distribution of each sphere merges to form a cylinder of energy deposition.

Figure 12B: Diagram of spur showing inner-core and outer penumbra regions. The core region absorbs energy directly and cannot respond adiabatically to a particle passage. The outer penumbra region is permeated by electrons arising in the core and executing random walk patterns at the ends of their trajectories.
Figure I-12A

Spurrs

Region of overlap

Figure I-12B

Penumbra
Core
Random Walk Electrons

XBL 789-10770

Fig. I-12A & 12B
Figure I-13: Plot of average distance in angstroms between spurs as a function of MeV/amu for HZE particles Carbon +6, Nitrogen +7, Oxygen +8, Flourine +9, and Neon +10. A is for an average energy loss of 40 eV in each spur while B is for an average energy loss of 27 eV in each spur. Note that for heavier ions the maximum distance between spurs for the same energy per amu decreases. This feature limits the usefulness of this model for heavier ions.
Fig. I-13
where the particle velocity is megameters per second and $r_p$ is in angstroms. The penumbra is not uniformly saturated but rather is permeated by electrons executing a random walk motion. This empirical formula for $r_p$ underestimates the lateral expansion of the track at energies above 400 MeV/amu.

The core structure is a consequence of the initial deposition of energy and has a half-life on the order of $10^{-16}$ seconds. The energy deposited results in the formation of chemically reactive species, with lifetimes on the order of $10^{-16}$ to $10^{-9}$ seconds. The chemical actions of high-energy radiations occur through transient reactive intermediates created in the tracks. It is assumed that the reactive intermediates are $\cdot H$ and $\cdot OH$ radicals that are diffusion limited. Biological effects are registered on a longer time scale (the earliest effects occur within $10^{-10}$ seconds), and the sequences of processes are complicated.

Mechanisms in radiation biology involve interplay between chemical and biological processes.

The stopping power formula gives the LET for a medium and gives the total range for a particle. Knowing the energy deposition in the sample, even as a function of depth, is necessary but not sufficient to reproduce the energy distribution in the biological system; where and how the energy is deposited is still uncertain. Each biological end point that can be measured may have a different interaction cross section. Furthermore, two particles $z_1$ and $z_2$ with velocities $v_1$ and $v_2$, may have identical LET values and yet not exhibit identical biological end
Interaction models of biological radiation damage generally involve the effects upon DNA strands. DNA damage in mitotic cells either immediately kills the cells or makes them unable to undergo successful cell division. Postmitotic cells undergo immediate death, a delayed cell death, or cellular dysfunction. Since this model of biological radiation effects postulates a target region within a cell, target theory may be used to predict biological damage.

For some of the radiation effects, e.g., lethality and mutation, the maximum measured cross section is approximately equal to the size of the cell nucleus. A cardinal point for the HZE radiobiologist is: Can the cross section be larger than that of the cell nucleus, i.e., is DNA the target? Consider the question of a single HZE particle inducing membrane injury and causing cell death. In this case the cross section might be equal to the projected area of the entire cell but the cause of cell death would have to be differentiated from one particle damaging a number of cells. The macroscopic energy loss does not predict the biological effect, the biological mechanisms of damage can only be elucidated by the use of microscopic distributions of energy. However, cross sections are a useful method of statistically calculating energy deposition.

In the classical sense target theory is derived from the two body collision problem with a characteristic interaction cross section determined from the impact parameter. Radiobiologists use statistical methods for relating dose-effect (survival curves) to target size and molecular weight. These formulations are unsuited
Figure I-14: Log-log plot of calculated energy density versus radial distance for neon particles in water (from Magee and Chatergee). The core and penumbra regions are separated by a one to two log unit step in energy densities. The energy density of the core is uniform while that of the penumbra region decreases with radius. The radius of the core and penumbra increases with particle energy.
Fig. I-14
for low doses of HZE particles for two reasons. First, small numbers of events per cell are dealt with and require skewed single event statistical analysis (Appendix II); second, the interaction cross section is unknown because the effective radius, in terms of biological effect, of the incoming particle remains uncertain. This leaves the value of the impact parameter as a variable. Figure I-14 relates some of the known dose-particle-radius data.

Target theory allows the calculation of the probability of a "hit" on a critical target. The distribution of particles penetrating a cell is given by the Poisson distribution. To obtain dose-effect relationships from HZE particles experimentally, the biological responses to irradiation are evaluated. The comparison of the calculations with the actual data gives a measure for the validity of the model and helps to interpret physical, chemical and biological phenomena.

PATHOLOGY

Background

An understanding of the pathological changes that affect the body can link ultrastructure morphology with function. Dysfunction and death are two general divisions of pathology. Cellular dysfunction may be caused by external factors (injury or disease) or environmental factors (hormonal or circulatory). In humans, disease and injury account for an understanding of many of the gross pathological states observed. Genetic alterations have led to an understanding of underlying pathological mechanisms. Radiation can induce a wide range of cell dysfunctions and/or death, and accounts for another body of information on
physiological mechanisms, both morphological and pathological.

Pathological responses in the nervous system involve both the nerve cell itself and the glial accessory cells. These accessory cells will respond to changes in the nerve cell caused by injury or stimulation by attempting to repair or nurse the "sick" postmitotic nerve cells; glial cell proliferation is the classic response.

Proliferation of flat glial cells is commonly seen on the inner retinal surface of the inner limiting basement membrane in degenerative disease. They can also be found on top of hemorrhages or foci of inflammation in the retina. Blood can pass or break through the inner limiting membrane to form subhyaloid or vitreous hemorrhage, and retinal microglia will migrate across the membrane from the retina into the vitreous. Folds in the inner limiting membrane are seen as folds of the whole retina around retinal scars or tumors, in retinal detachment, or when the eye is compressed by space-taking processes in the orbit. Rupture of the membrane as a result of contra-coup injuries leads to craterlike central retinal "holes."

Simple atrophy of the neural layers can be found after occlusion of the retinal arteries, in glaucoma, in diseases of the ganglion cell layer, as a result of retrograde degeneration, after trauma or other kinds of interruption of neurites in the optic nerve or optic tract, or in combination with hemorrhage after occlusion of the retinal veins. Local ischemia or incomplete microinfarction of the neural layer causes changes known clinically as "cottonwool" spots. These are globular bodies with a dark-staining central pseudonucleus and lighter-staining pseudoprotoplasm. Cytoid bodies are the terminal
swellings of Cajal at the end of interrupted nerves. Retrograde degeneration causes the cottonwool spots to disappear after a few weeks, and a glial scar is left in the area. Such degeneration generally does not impair vision because of the multiple neural paths for the visual process.

In retinal diseases with destruction or atrophy of the astroglia, the perivascular glia undergoes extensive hypertrophy with the formation of bizarre spiraling processes around blood vessels. The perivascular glia degenerates, hyalinizes, and finally becomes round or star-shaped hyaline bodies in the retina. With destruction and degeneration of the neurons of the inner retina the retinal astroglia found in the inner retinal layer becomes hypertrophic and proliferates. The free-moving microglia "gitter" cells that phagocytize necrotic neurons degenerate to form free pools of lipid, which cause deep, hard exudates in the nuclear and plexiform layers. This is seen with hypertension, collagen diseases, long standing papilledema, and diabetes.

The changes seen in ganglion cells are few and nonspecific—swelling, destruction of processes, and various stages of shrinkage and disintegration. A special type of lipoidal ganglion cell degeneration may be observed in familial amaurotic idiocies (Tay-Sachs, Bielschowsky, and Nieman-Pick syndromes).

Degenerative changes of the pigment epithelium and the outer retinal layers, degeneration of rods and cones, and diffuse pigment deposition have also been seen. Retinitis pigmentosa, a hereditary degenerative disease, starts with bilateral degeneration of peripheral rods and cones coupled with pigment migration from degenerating pigment epithelium.
into the involved area of the retina.\textsuperscript{238}

Retinoblastoma, a possible genetic dysfunction, may be endoplytic or exoplytic, growing on the anterior or posterior of the retina. The rosettes that form in this condition are considered to be an abortive attempt of primitive neuroectoderm to form a layer of rods and cones, a primitive neural tube,\textsuperscript{154} or primitive optic vesicles.\textsuperscript{268} Nematode endophthalmitis,\textsuperscript{7,35,57,143,241,253} Coat's disease,\textsuperscript{38,191,258} retinal dysplasia, and traumatic or endogenous endophthalmitis are causes of the clinically similar "pseudoretinoblastoma."\textsuperscript{154}

Genetic Disorders

Differences among various cellular genetic diseases in the retina may result from either differences in time of onset or fundamentally different mechanisms. The distinction between these possibilities might be made by examining progressively earlier stages of the retina, searching for abnormalities that precede the observed functional, morphological or histopathological expression of the disorder. Studies with mice, rats and dogs\textsuperscript{2,108,109,122,224,227,242} established that both the mechanisms of dysfunction and the time of onset can be genetically altered and that special inbred strains can be produced by accentuating a particular mechanism.

La Vail characterized a number of degenerate changes in the photoreceptor cells of the retinal degenerate (rd) mutant mouse, C57 BL/6J. Typically, the rod outer segments reach their mature length before degeneration begins. With severe degeneration and dissolution of the outer and inner segments, the outer nuclear layer rapidly diminishes.
In normal eye development a single row of pyknotic of hyperchromatic nuclei is all that is retained when a normal eye just reaches maturity. In rd mutant mice, the nuclei remaining after maturity slowly and progressively disappear over a matter of months. Subtle changes occur in the ganglion layer with the loss of some cells and a reduction in the volume of others. Irregular gliosis and disruption of the inner nuclear and inner plexiform layers appears within six months. 122

Chimeric rats of the retinal dystrophic (RCS) strain undergo local retinal degeneration in a manner that implicates the pigment epithelium rather than the photoreceptor cell as the cause. The photoreceptor cells degenerate because they are not phagocytized. Thus not only is the genetic defect localized in the pigment epithelium, but the crucial nature of this layer is established--dysfunction of the pigmental epithelium precurses degeneration of the light-sensing elements. 159

The development of glutamine synthetase activity in the embryonic chick retina is a useful indication of physiological control mechanisms. 95,192 La Vail has shown that glutamine synthetase is distributed throughout the retina, and that a relationship exists between the development of glutamine synthetase activity and the final structural and functional maturity of the mouse retina. Final synaptogenesis (the appearance of electrical activity and the formation of photoreceptor outer segments) occurs during the time of the initial increase in glutamine synthetase activity. 42,108,170,254 The causes and effects of this correlation have yet to be determined.

Lolly found elevated levels of cyclic guanosine monophosphate (GMP) in degenerating C3H mouse retina as well as alterations in
adenosine monophosphate (AMP) metabolism.\textsuperscript{63,135} An early defect in C3H mice affects the control mechanism for cyclic nucleotide phosphodiesterase, which seems to be involved in membrane and cell growth regulation;\textsuperscript{178} this may affect DNA and RNA.\textsuperscript{21} It is possible that the cyclic nucleotide accumulation is in some way responsible for photoreceptor cell dysfunction and death. Blanks found a virtual absence of triad synaptic configurations in photoreceptor terminals of rd mutants. Although he cannot distinguish the genetic from the functional cause, he does relate it to the photoreceptor cells.\textsuperscript{21}

Work with rd chimeric mice indicates that the pigment epithelial cell is not the site of the primary genetic defect, and the site of dysfunction is the neural retina, presumably the photoreceptor cell. Electron microscopy has shown that there is no lack of normal outer segment phagocytosis in the rd mutant.\textsuperscript{194,202,211} Although other functions of the pigment epithelium could be responsible, chimeric data do not support this supposition.\textsuperscript{124} In the normal retina, 8 to 10 rows of photoreceptor nuclei were evident; mutant retinal areas had fewer. In effected animals, the rod outer segments were shorter than normal, a common phenomenon in progressive retinal atrophy in Irish setters,\textsuperscript{3} rd rats,\textsuperscript{242} hypertensive cats, and Purkinje-cell-degenerate (pcd) mice.\textsuperscript{159}

Nonionizing Radiation

The absorption of a photon in the retina leads to a variety of consequences depending upon where the photon is absorbed. With the exception of pigments (melanin, hemechrome, fuscin, rhodopsin, and the
color chromophores) the retina is transparent to "visible" light and it should not absorb energy in this range. As the frequencies broaden to include the infrared and ultraviolet ranges, a greater portion of the retina becomes opaque, absorbs greater amounts of energy, and exhibits primary photic damage. Secondary mechanisms become important at energies and densities capable of forming reactive chemical species, free radicals, or dielectric breakdown. Pathology may be due to varying degrees of primary and secondary mechanisms dependent upon the energy spectrum enacting damage.

Visible light is ambivalent in its effect on chromophores; it destroys chlorophyl while being their prime source of energy. Light is necessary for sight but simultaneously bleaches the pigments. Not only the frequency but the intensity, i.e., the number of photons, is important to the visual process. The eye has the ability to adapt within certain thresholds, higher intensities, cause damage while lower intensities are not perceived. Visible light has a number of functions in the eye; the different pigments probably play different roles in the physiology and mechanisms of the eye.

Hormonal and humoral factors control cell proliferation, as does cell contact and communication between neighboring cells. External factors are important stimuli to the control mechanisms. Light exposure affects the developing synapses in the retina and the lateral geniculate nucleus. Visual deprivation decreases the number of synapses in the inner plexiform layer. The circadian light cycle has been shown to be responsible for the shedding of the outer segment discs, and thus regulates the process of growth and phagocytation of the
photoreceptors. Light stimuli are responsible for the movement and pressures of interdigitation of the zone between the tight junctions, the outer limiting membrane, and the pigment epithelium. Light causes transitions in the backbone structure of rhodopsin; light is also responsible for the severance of the Schiff-base bond with the epsilon amino group of lysine.

Doses as low as 70 μjoule of 6943 Å coherent light have been shown to produce ultrastructural alterations in rod and cone outer segments. After prolonged exposure, ambient laboratory light causes fragmentation, disorientation, and destruction of the outer segments in albino rats. Fluorescent light exposure (140 foot-candles) causes the displacement of the bipolar cells from the region of the outer nuclear layer along the blood vessels, and decreases the inner nuclear layer. No photoreceptor cells were found, and the villous processes of the pigment epithelium were constrained by the outer limiting membrane and appeared compacted. The bipolar neurons were close to the pigment cells; the Müller cells continued to form a boundary membranous structure between the inner retinal layers and the pigment epithelium. The outer limiting membrane was no longer visible after the pigment epithelium was destroyed by intense illumination.

The Müller cells rapidly respond to light-induced damage by increasing the number of their filaments, microtubules, glycogen granules, mitochondria, and oxidative enzyme activity. During the early stages of retinal degeneration, phagocytes invade the retina and engulf debris into cytoplasmic vacuoles. With intense light, the endothelial cells of the choriocapillares lose their normal fenestrations and are
separated from the basal membrane by collagen and ground substance. In pigeons exposed at doses of 13,500 nits for a full day, the cone outer segments were almost completely lost, and the cone lamellae assumed a vesticulate or tubular form. The glycogen granules of the myoid increased dramatically while a smaller increase in the number of phagosomes was observed. Pathological changes could be produced by doses of less than 3000 nits. 145

Hanson found that light irradiation causes cytochrome oxidase, diaphorase, and dehydrogenase activity in the nonpigmented retinal epithelial cells and in the inner segments of photoreceptor cells. Müller neuroglial cells became more prominent during the first few days after exposure; their radial processes were observed in cells stained for enzymatic activity. These cells have a tendency to confine the light-damaged portions of the retina by extending microvilli to surround degenerating cells; they also tend to have increased enzyme activity in the peripheral, newly established border processes. There is a marked inhibition of the transport of peroxidase across the epithelial cells. 92

It is possible that the rods and cones are not the only sources of light reception. Bennett found that nine out of ten rats with no intact photoreceptor cells could perform light-dark discrimination with 77 percent to 97 percent of the responses correct. None of the rats could distinguish light and dark following bilateral enucleation; this relegated the reception site to a nonphotoreceptor sensing source. On morphological grounds, the Müller pigment cell junctional complex of the degenerated retina was proposed as the site on which the demonstrated visual function was dependent. When rats with light degenerated rod
cells were placed in a circadian daily light cycle they showed a diurnal variation in nonstress plasma corticosterone levels. The Müller cell has been identified as the generator of the ERG beta wave, and Noell has shown that the beta wave is lost in rats with retina damaged by light. Light appears capable of producing a visual response even beyond the point of inducing normal photoreceptor degeneration, but it would not be unreasonable to assume a dramatic loss of resolution in such an altered system.

Ionizing Radiation

Roentgen was among the first to observe that x radiation could produce a visual stimulus. By 1897 detrimental effects such as pain in the orbit and globe were noticed along with the visual effects at doses estimated at 200 to 300 rad. A common measure of dysfunction in experimental animals is loss of the ERG. This has been shown to be irreversible in rabbits and cats at doses of 3000 to 8000 rad. Lipetz observed reversible ERG responses in humans given doses of 0.5 to 1.0 millirad. In general, it has been found that x rays cause a significant decrease in the threshold intensity of a light flash capable of evoking a response for periods of at least six hours. When the eye was light adapted and then dark adapted after x irradiation, the light threshold returned to the normal level. This indicated that the x rays had acted on the photochemical system of the eye.

X rays appear to selectively affect the rod cells of the retina in rabbits, rats, dogs and monkeys. The rods are also characteristically sensitive to iodoacetate, a poison with inhibitory effects on
glycolysis, and of oxygen at high pressure.\textsuperscript{163,164} The amount of radiation necessary to produce retinal changes in the rabbit depends on the LET.\textsuperscript{11} Pyknosis, the first sign of cell death, occurs one to two days after irradiation, and it is followed by chromatolysis and cell disintegration. Ganglion cells and bipolar cells are almost twice as radioresistant. Cells that survive a single dose show characteristic degenerative changes—thinning and partial or complete disintegration of the outer segments, and swelling of the inner segments.

ERG loss requires a minimum dose; an ERG that is preserved 24 hours postirradiation indicates that a fraction of the visual cells will survive up to two weeks. No metabolic changes are apparent after doses that fail to reduce the ERG, but there is a strong correlation between ERG effect, respiration, and glucose oxidation.\textsuperscript{165}

The work of Lipetz,\textsuperscript{132} and Bachofer and Wittry\textsuperscript{9} supports the hypothesis that x-ray "phosphene" is the result of a stereoisomeric change in rhodopsin; however, the major site for x-ray stimulation does not appear to be identical to that for visible light and may be separate from the photochemical system.\textsuperscript{46} It is not necessary that x-ray stimulation of the retina be initiated in the rod outer segment to account for ERG beta-wave activity. The x-ray effect may relate to the energy transfer system between the photopigment isomerization and the triggering of the neural discharge in a primary afferent neuron.\textsuperscript{47,48} In the rabbit, changes that appear following 50-nsec x-ray flashes are not incompatible with ERG results after extremely weak visible stimuli, but the hypothesis that organized receptor cells are necessary for transduction of ionizing radiation into visual information is not
Barnes found that presynaptic inhibition may be particularly sensitive to x rays. X irradiation was found to increase the retinal inhibitory component and decrease the lateral geniculate or supraretinal inhibitory component to cortical evoked potential (CEP) in cats. Gaffey found that the ERG loss in cats was not due to vascular damage from either x rays or alpha particles. Leith and Schilling have shown that ionizing radiation affects reaggregation of embryonic mouse brain cells. Since the phenomenon of reaggregation is thought to depend on membrane integrity, and in particular, the presence of certain glycoprotein or mucopolysaccharide moieties at the cell surface, it is possible that radiation is interfering with the biosynthesis of materials needed for proper cell interaction. Philpott found a loss of capillary mucopolysaccharides postirradiation with both x rays and heavy ions.

The effects of radiation are probably due to radiation lesions in the membranes of the retinal cells. Changes observed on mammalian membranes include the following: changes in passive ionic permeability; leakage of K\(^+\), Ca\(^{2+}\), and Na\(^+\); changes in active transport; initiation of action potentials; disclosure of sites for lysolytic and proteolytic actions of enzymes and toxins; release of enzymes; and changes in the longevity of cells in the G\(_0\) state. X rays appear to have the ability to stimulate both primary and secondary visual events as well as the ability to initiate pathological cellular alterations.

Neary found that chromosomal aberrations in plant cells were dependent on the square of the LET, but the effectiveness of 250-keV
x rays was approximately the same as the effectiveness of high-energy alpha particles (which also have a low LET) in blocking the ERG in the cat. The correlation between x-ray and alpha-particle irradiation data suggests the feasibility of using alpha particles as a control for irradiation with particles of various LET.

The advantage of alpha-particle irradiation over x rays for studying the effects of radiation is the directional control over the beam passage. The use of 910-MeV helium ions to radiosurgically separate cerebral hemispheres in cats is evidence of both the effectiveness of radiation to cause cell death and the manipulatory ability of the beam in biological tissue. Orton reviewed the suitability of high-LET radiation over low-LET particles and x rays, and he concluded that high-LET particles were superior in causing cell death. In general, the relative biological effectiveness (RBE) increases as the LET increases.

Zeeve performed initial studies on vertebrate retinal interactions with the nitrogen beam. Two weeks postirradiation gross morphological damage was observed in the outer segments of Necturus maculosus. Irradiated receptor cells appeared to have lost part of their volume; some either shrank or collapsed. Studies were conducted on pocket mouse and rabbit retinae with x-ray, nitrogen, and oxygen irradiations. In mice sacrificed three to five days postirradiation, the outer segments responded in a patchy fashion, producing areas of altered receptors scattered among normal appearing receptors. The rods were swollen near the inner segments, and, although the outer membrane surrounding the rods appeared intact in most places, the discs within
the rods appeared disrupted. The pigment epithelium showed signs of alteration with vacuole formation in the cytoplasm. Scanning electron microscopy (SEM) of rabbit retina two days postirradiation showed a patchy response, and localized swelling and membrane rupture after 2000 rad of x rays.

Eyes from rats flown on Cosmos Flight No. 782 as well as eyes irradiated with nitrogen, oxygen, neon, and argon beams have been investigated.\textsuperscript{185,186} In the low-dose retina (123 particles per eye) the cells of the outer nuclear layer appeared most affected by HZE particles. Occasionally cells in the outer nuclear layer became swollen and exhibited a clearing of the cytoplasm. In some instances, the inner segments directly above these necrotic cells were also swollen, and contained myelin figures and swollen mitochondria. Disruption of the outer segments also occurred. A few nuclei from the outer nuclear layer were displaced into the inner segment area apparently by passing through the outer limiting membrane. Occasional pools of glycogen were seen in the inner segments. Exposures of high doses at the Bevalac (greater than 1000 rad) resulted in positive alterations, sporadic cytoplasmic swelling, clearing, and cell death.\textsuperscript{185} Damage to the outer rod segments seems to indicate that the rod cells were particularly sensitive, possibly because a particle would traverse a minimum of five or six nuclei if it was traveling at right angles across the retina. A greater number of nuclei would be hit if the trajectory were more horizontal.

Beckman found that retinal damage in the monkey produced by HZE particles is quite severe and extends through the full thickness of
the retina as evidenced by vessel obliteration, capillary permeability changes, and pigment-epithelium damage.\textsuperscript{15} In contrast, 3000 rad of x rays at the retina appear to be well below the threshold for vascular damage demonstrated with fluorescein angiography and fundus photography. Because HZE-particle damage is initially in the form of small discrete foci of capillary disruption with a sharp margin of demarcation about the lesion's periphery, it may be that damage to the retinal vasculature occurs microscopically at discrete points as a highly localized discontinuous process.

The ERG inactivation dose of neon irradiation in cats was found to be on the order of 4.0 to 4.5 thousand rad. Doses of 1200 rad abolished the ERG 12 to 24 hours after exposure. At lower doses, the b-wave amplitude of the ERG was attenuated as a monotonic function of irradiation. In general, cats lost their a-wave ERG response before the b-wave was lost. Double light flash experiments, which test the electrophysiological recovery of the eye, indicated that the ERG is lost at neon dose levels that do not appear to block the ERG response to the first flash.\textsuperscript{72} These results may be indicative of the dual nature of the visual system; the Müller and other cells responsible for phototrophic responses may be more radioresistant than the discriminatory visual system of the rods and cones.

RETINAL DEGENERATION

The pathology of retinal degeneration has been explored with a number of different systems and causes. Typical results are decreases of outer segment length, pyknotic nuclei, cell degeneration, and in-
increased phagocytic activity. Genetic factors, disease, and ionizing radiation can cause the same general degradative process; light and x rays cause similar effects.\textsuperscript{54,165} Evidence for a nonpigment-epithelium genetic defect responsible for the loss of the light-sensing elements in the retina suggests the light-sensing element itself becomes dysfunctional.\textsuperscript{125} The similar appearance of genetic synthetic dysfunction and radiation pathology argue that similar inactivation mechanisms may be in operation. A variety of mechanisms may be responsible for retinal dysfunction as evidenced by the variety of changes categorized for different qualities of ionizing radiation.

HZE particles may produce a potentially irreparable nucleic acid or membrane injury that may reduce the neuron lifespan and temporarily or permanently impair cell function. Alternatively, the effects may be manifested as the effects of other types of degenerative injury, such as reflexes, coordination, communication, and storage. The primary effects of low doses are not readily observable even with the present ultrastructure techniques--partly because of not knowing where to search and partly because of the inability to observe without destruction.\textsuperscript{76} Observations of secondary effects allows categorization of morphological changes after biological amplification of primary effects. The study of secondary effects has two purposes. The first is the assessment of dysfunction due to various quantities and qualities of radiation by the observation of pathological changes. The second is to postulate the primary sites of damage and the subsequent changes by modeling and reconstructing the sequence of events producing biological amplification of light and genetic damage.
SECTION II. MATERIALS AND METHODS

ANIMALS

**Necturus maculosus**

The retina of the amphibian **Necturus maculosus** (Mogul Ed Company, Oshkosh, Wisconsin), the common mudpuppy, was selected because the cells are large (ten times the size of most mammalian retinal cells) and thus ideal for scanning electron microscopy and physiological studies. The response of the retina to light had been well characterized, both for each individual nerve cell layer and for the retina as a whole. The animals were raised in a special tank at $5^\circ$ to $10^\circ$ C. Prior to an experiment, a specimen was anesthetized by immersion in a tricain-methylsulfanate (TMS) solution (1:1000).

**Mus nigerus #C57**

The retina of the C57B mouse (Jackson Laboratories, Bar Harbor, Maine) was selected because of the data available on its genetic composition and the literature available from ultrastructure research on these strains (see Pathology-Genetic Disorders, Section I). Animals were raised in a temperature-controlled ($20^\circ$C), light-cycled (12:12) room. Prior to an experiment, animals were anesthetized with diabutal (1 gm/ml) diluted 13:1 (Saline: Diabutal), 60 mg/kg body weight (0.3 to 0.4 ml per animal).

IRRADIATION TECHNIQUES

X irradiations of both eyes were performed with a Phillips therapeutic x-ray machine operated at 230 kV, 15 mA, with an inherent filtration
of 1.1 mm Al and added filtration of 0.25 mm Cu and 1.0 mm Al. The half value layer (HVL) was 0.83 mm Cu, the dose rate was 250 rad/min as detected by a Victoreen condenser ionization chamber. A roentgen-to-rad conversion factor of 0.96 corrected to standard temperature and pressure (STP) was used. The dose was delivered to both eyes by exposing the anterior portion of each animal, from several mm posterior of the eyes to the tip of the nose.

The 184-inch cyclotron was used to accelerate alpha particles. The plateau region of the full energy accelerated helium-ion beam (910 MeV) was used to deliver doses of 0, 10, 50, 100, 500, and 1000 rad. The beam was low-LET radiation (16.67 MeV cm²/gm) and at this energy has a maximum range in water of about 32 cm.

Neon ions were produced at the HILAC with energies of 8.5 MeV/nucleon and injected into a beam transfer linear line, terminating in a 50-MeV proton injector. The ions were then introduced into the Bevatron and accelerated further to final energies between 0.25 and 2.6 GeV/amu. Neon ions were extracted from the Bevatron at 400 MeV/amu and transported to the biomedical irradiation area. (The energy after dosimetry into the retina was 375 MeV/amu, high LET radiation (320 MeV cm²/gm); carbon ions were extracted at 260 MeV/nucleon; dose rates varied from 300 to 400 rad/min for high doses to 5 rad/min for low doses as measured with a parallel-plate ionization chamber. Lyman has described the HZE dosimetry for the biomedical facility at the Bevalac.¹³⁸) For Necturus, doses of 0, 10, 50, 100, 500, and 1000 rad were used. For mice, doses of 0, 50, 100, 300, 600, and 1000 rad were used. For the x-ray studies at 7, 10, 14, 19, and 40 days postirradiation,
Figure II-1: Diagram of restraining apparatus for Necturus irradiations. An optical bench parallel and below the beam held a number of devices for modulating and measuring the beam and exposing specimens. These experiments used a water column set to zero to provide a plateau Bragg curve exposure LET. A six-inch long cylindrical brass collimator provided a beam of \( \geq 80\% \) uniformity over a 3/8 inch diameter. Passage of the beam through an ionization chamber allowed for calculations of dose at the eye of the animal. Animals were mounted on an adjustable stand and aligned by means of a pointer (not shown) which was inserted between the ionization chamber and the animal, but removed prior to radiation when the animal was placed as close to the ionization chamber as possible.
Figure II-1

*Necturus maculosus* irradiation apparatus

Water Column and Calibrator

Ionization Chamber

*Necturus maculosus* Holder

Optical Bench

Beam Path

Fig. II-1
Figure II-2: Diagram of restraining apparatus for *Mus* irradiations.

An aluminum block covered by a wide plastic strap was used to mount mice. The strap was permanently attached at one end and was adjustable to the size of the animal by means of a series of holes which were slipped over screw heads on the opposite side. The apparatus was mounted on top of fixed pins to a translator. This arrangement allowed for alignment of the animal in the preparation room and therefore a rapid change of animals in the bio-medical cave.
Figure II-2

Mus niger C57 irradiation apparatus

Mus niger

Beam Path

Pin Fitted Aluminium Block

Mounts to Pins on Translator

The Translator mounts on the Optical Bench behind the Ionization Chamber

Plastic Restraining Strap

Screws to Hold Strap

Holder
one animal at each of three doses, 0, 1000, 5000 rad was sacrificed. For the Necturus helium and neon particle studies, animals were sacrificed two weeks (12 to 16 days) postirradiation. For the mouse studies, animals were sacrificed at 5, 7, 10, 12, 14, 17, 60, 62, 65, 67, 69, and 72 days postirradiation.

Irradiation at both the 184-inch cyclotron and Bevalac was administered to a single animal mounted in specially constructed holders. The holder was oriented in the beam path with the body of the animal perpendicular to the beam and tilted so that either one or both eyes were exposed to the beam (Figures II-1 and 2). The eyes were centered with the aid of an aligned pointer and monitored on closed-circuit television.

The plateau portion of the Bragg peak was used. For alpha particles, Figure II-3, the ionization ratio was one-third that of the peak and constant for both eyes.

AUTORADIOGRAPHY

Mice were pulse-labeled with equal portions of tritiated leucine and phenylalanine (L-(4,5-³H) leucine, 38.6 Ci/m mole and L-(3-³H) phenylalanine, 15.7 Ci/m mole). Animals were injected intraperitoneally with 25 microcuries per gram body weight 3 and 58 days postirradiation. Six animals at each dose were labeled and sacrificed over a two-week period, starting at 5 and 60 days postirradiation. Dose points were #1-0 rad, #2-50 rad, #3-100 rad, #4-300 rad, #5-600 rad, and #6-1000 rad (Table II-1).
Figure II-3

ALPHA PARTICLE BRAgg CURVE

Unmodified Helium Beam

Water Column Scan

Window = 1.3 cm of water

Ionization Ratio

Centimeters of Water

Fig. II-3

XBL 789-10767
Figure II-4

400 MeV/nucleon Neon
Bragg Curve in Water

Ionization Ratio

Gm of Water

Fig. II-4
TABLE II-1. SACRIFICE AND LABEL TABLE

<table>
<thead>
<tr>
<th>Mouse No.</th>
<th>Sacrificed Days Postirradiation</th>
<th>Postlabeling</th>
</tr>
</thead>
<tbody>
<tr>
<td>11-16</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>21-26</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>31-36</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>41-46</td>
<td>12</td>
<td>9</td>
</tr>
<tr>
<td>51-56</td>
<td>14</td>
<td>11</td>
</tr>
<tr>
<td>61-66</td>
<td>17</td>
<td>14</td>
</tr>
<tr>
<td>71-76</td>
<td>60</td>
<td>2</td>
</tr>
<tr>
<td>81-86</td>
<td>62</td>
<td>4</td>
</tr>
<tr>
<td>91-96</td>
<td>65</td>
<td>7</td>
</tr>
<tr>
<td>101-106</td>
<td>67</td>
<td>9</td>
</tr>
<tr>
<td>111-116</td>
<td>69</td>
<td>11</td>
</tr>
<tr>
<td>121-126</td>
<td>72</td>
<td>14</td>
</tr>
</tbody>
</table>

HISTOLOGY

*Necturus maculosus*

Animals were sacrificed in the dark, and the eyes were removed under red light. The eyes were removed by first making a circular incision around the eye socket, lifting the eye, and cutting the connecting tissue with a scissors from below. Samples were prepared for scanning electron microscopy and transmission electron microscopy.

A syringe full of gluteraldehyde fixative was fitted to a small-gage
needle inserted into the vitreal space behind the lens. After a small incision was made on the opposite side of the iris from the needle, a flow was established and the interior of the eye was perfused for two to three minutes. An iris scissor was used to remove the anterior of the eye; the retina was removed by scooping and detaching it with a small spatula.

The retina was fixed in 2.5 percent gluteraldehyde for 60 to 90 min at room temperature and then washed twice (15 min) in cacodylate buffer (67 mM Na cacodylate with 45 mg/ml sucrose). The retina was postfixed in 2 percent osmium tetroxide buffered with Veronal acetate for 1 to 3 hours at room temperature. The retina was dehydrated through an ethanol series and then through an ethanol-Freon TF (Dupont) mixture series to a double wash in 100 percent Freon TF. The retina was critical-point dried (CPD) using a Freon 13 CPD machine. Alternately samples were dehydrated from ethanol to acetone and then CPD using CO₂.

**Mus nigerus #C57**

Animals were anesthetized with diabutal as described previously. A V-shaped incision was made into the abdominal cavity through the lateral aspects of the ribs to the neck, exposing the thoracic viscera. A needle was inserted into the left ventricle, and, as perfusion was initiated, an incision was made in the right atrium. When the tail became stiff, perfusion was stopped and the animals were placed on ice for 15 min to several hours. The top of the skull was then removed, and the optic nerve was severed. The skin between the eyes was slit to the bone, and as the skin was tensioned, the eye was freed from the
socket, cut from the connecting tissue, and placed in fixative.

Mice were perfused first with physiological saline and then with a solution of 4 percent gluteraldehyde, 2 percent formaldehyde in 0.075 M cacodylic acid buffered to pH 7.4 with calcium chloride. Alternately triple fix, using 1,5 difluoro 2,4 dinitro benzene (0.5 grams/liter) as the third fixative, was utilized for mice. After fixation the eyes were bisected on the anterior to posterior plane through the optic nerve, dehydrated through a ethanol-propylene oxide series, and then embedded in Spurrs low viscosity embedding medium (Polysciences, Warrington, Penn.)

ULTRASTRUCTURE

Light Microscopy (LM)

Thick (1 to 2 micron) and thin (800 to 1100 Å) sections of material were cut using glass knives with a Porter Blum MKII ultramicrotome. One-quarter or one-half inch knives were broken daily on either a LKB or Sorval knife breaker.

Scanning Electron Microscopy (SEM)

Ethanol-infiltrated retina was cryofractured while in a Prafilm cylinder immersed in a liquid nitrogen bath. A fresh single-edged razor blade held in a forceps was used to fracture the specimen.

Critical-point dried samples were mounted on the appropriate SEM stud with silver paint. Samples were coated with gold, paladium, or other heavy metal before viewing in a scanning electron microscope in the secondary emission mode for high-resolution, high voltage work (20 to 25 kV, 40 to 90 microamps). Uncoated samples were viewed at lower
potentials (5 to 10 kV). The Cambridge Stereoscanner, JEOL (Type JSM), the Coates and Welter field emission scope, and the AMR 1200 SEM were used.

Transmission Electron Microscopy (TEM)

Grids were examined with the Zeiss 9A, Seimens 1A, or Philips 300 TEM (60 keV) electron microscopes. Sections were stained by floating on 1 percent uranyl acetate solution for 30 min, at 60°C, rinsed with doubly distilled water, floating on saturated lead citrate solution for two to three minutes, and then placing on a 100-mesh grid.

Autoradiography

For autoradiography four to five thick sections were placed on a microscope slide and allowed to dry before being coated. Kodak NTB-2 emulsion was melted at 41°C. Slides were dipped, drained, and allowed to dry for one hour before they were sealed in light-tight, dessicated boxes for periods of 1, 2, 3, and 4 weeks of exposure. Exposed slides were developed, fixed, washed, and allowed to dry. Light microphotography was used to examine the sections. Phase contrast was used to identify the morphology of the retinal layers, and normal micrographs were taken to exhibit exposed grains from autoradiography.

SCORING

Necturus maculosus

Relative biological effectiveness (RBE). Pictures of retina enlarged 1000 to 3000 times were used to quantitate the effects of radiation. A photographic field of retina consisted of between 50 and 200 individual
Table II-2. Qualitative criteria for radiation damage to rods and cones of *necturus maculosus*.

<table>
<thead>
<tr>
<th>Pathological Index (PI)</th>
<th>Severity of Injury</th>
<th>Numerical Scale</th>
<th>Quality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>A smoothing of surface</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>B decrease in diameter</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>C loss of vertical structure</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>D loss of horizontal structure</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>E skewed vertical serration</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>F filament breaks and dissociation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>G deflation</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>H phagocytation</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>I degeneration</td>
<td></td>
</tr>
</tbody>
</table>
rods and cones (in a ratio of approximately 50:50); a 12 to 16 photograph matrix was taken of the retina of each animal scored. Selection criteria were the following:

1. the area was coplanar within 35°;
2. there was little net change over surface;
3. there was visibility for a 5° deviation perpendicular to the surface;
4. the matrix area was free of loose pigment epithelium tissue;
5. there was no gross mechanical damage due to tissue preparation; and
6. the area was representative of the sample on the whole.

Elements were characterized in terms of first-, second-, or third-stage radiation damage (Figure III-2) scored numerically, averaged, and normalized for each dose and animal. A score, termed the "pathological index" (PI), was developed. Table II-2 correlates the use of damage criteria to the stages of radiation damage. Photographs were mixed and then scored blind by the author and an assistant. Numerical values ranging from 0 for an undamaged photoreceptor to 30 for a degenerated photoreceptor were given. Individual rods and cones were evaluated to arrive at a numerical value for each photograph. Statistical analysis of each matrix of photographs provided range and mean values. The data to generate the dose effect curves was obtained by assuming that a PI value of 0 signified a functioning photoreceptor, while a PI value of 30 signified no function. A functioning photoreceptor would have 100% efficiency (BE), while a nonfunctioning photoreceptor would have 9% BE.

The following nine criteria were used to gage the quality of damage to the photoreceptors in evaluating the effects of radiation:
1. smoothing of the surface of outer segment outer membrane;
2. decrease in the diameter of the outer segment;
3. loss of the vertical fine structure (filaments);
4. loss of the horizontal fine structure (disc membranes);
5. the skewedness of the vertical serration (disc rotation);
6. the breakage, dissociation, and retraction of filament structure;
7. deflation of the photoreceptor;
8. phagocytation of photoreceptor material; and
9. degeneration of the entire structure down to and below the outer limiting membrane.

Mus negrus \( \theta C57 \)

Normalization and dimensionless methods were used to offset the errors induced by sections that were not perpendicular to the curve of the retina.

**Protein synthesis**  A normalized rod outer-segment area was computed from photomicrographs taken of autoradiographic slides. A measurement was generated for each data point by multiplying the width of ten outer segments times their length and dividing by the cosine of \( \Theta \), a normalization factor:

\[
\text{area} = \frac{a \times b}{c/d \cos \Theta},
\]

where \( a \) is the width of ten outer segments, \( b \) is the length of the outer segment, \( c \) is the inner segment-outer segment combined length, and \( d \) is the length of the shortest inner-outer segment length. Theta, the angle from the perpendicular, was determined by measuring the sum of the inner-
outer segment length for each particular eye and dividing this by the shortest measured inner-outer segment length.

Another measurement calculated was the fraction of the sum of the inner-outer segment length that was composed of the outer segment. This was done by measuring the length of the outer segment and dividing by the length of the inner and outer segment.

**Outer-segment protein transit.** A two dimensional relative activity rather than three dimensional specific activity was used for the autoradiography. Relative activity of protein synthesis was determined by grain count from autoradiographical light-photomicrographs taken at a magnification of 1863 times. A 2.5-cm by 6.5-cm cutout was overlayed on the photomicrograph covering portions of the outer nuclear layer, the inner segment layer, and the outer segment layers. A normalized average grain count was taken for each data point. This method was used to determine both label uptake in control and irradiated animals, and to follow the activity in the retina proteins as a function of time post-labeling.

The movement of the labeled outer segment discs from the inner segment to the pigment epithelium was followed by observing the band of labeling transiting the outer segments in a sequence of photomicrographs taken of sections on the slides that were autoradiographed. The center of the band of labeled discs was measured and used to determine the fraction of the total outer segment length traversed.
SECTION III. RESULTS

NECTURUS MACULOSUS

Ultrastructure

Light microscopy. Control retinæ exhibited normal morphology under the light microscope. Figures III-1 and III-2 of *Necturus* retina consist of the nuclear, plexiform, and inner-outer segment layers. The rod and cone cells are in the layer bordering the posterior side of the retina while the ganglion cells border the anterior side. The amacrine, horizontal, bipolar, and Müller cell nuclei are localized anterior to the rod and cone nuclei.

Scanning electron microscopy. SEM showed that the control cone was indeed cone shaped in appearance. The light-sensing elements arise from the outer limiting membrane and stand 30 to 40 microns high. The inner segment projects from the line of tight junctions that separates the nuclear layer from the segment layers of the retina having a diameter of 10 to 15 microns and a length of 30 to 40 microns. The outer segments begin at the end of the inner segment, with a similar diameter, and extend from the plane of the membrane another 15 to 20 microns forming a rounded point at the apex of the cone. About 25 filaments originating from the inner segment below the inner-outer segment boundary, stand around each outer segment at regular intervals about the nonfissured surface. The outer surface of the outer membrane had a rough or mottled appearance (Figure III-3).

The control animal retinal rods were rod-shaped, extending 35 to 40 microns from the outer limiting membrane to a point generally slightly above the
Figure III-1: A light micrograph of the retina from *Necturus maculosus* (x 700). The nuclei are stained darkly and are best distinguished by their spatial relationships (Fig. III-2). Because the retina was mechanically separated from the pigment epithelium (PE), the retinal-PE boundary, the inner and outer segment layers, and specifically the outer segments, show trauma not observed in intact tissue. Individual rods and cones can be observed in the outer-segment layer. The inner plexiform layer appears as a light staining ribbon down the left side of the picture.
Figure III-2: Light micrograph of *Necturus maculosus* giving the relationship of the retinal cells and the retinal cell layers (x 1000).
Necturus maculosus Retina

XBB 776-5481

Fig. III-2
Figure III-3: Scanning Electron Micrograph (SEM) of *Necturus maculosus* retina, control cone cells (x 1500). Various top and side views of cones are visible. Located in the middle, left and right are cone cells in profile. Below and above these two cone cells are seen from a top, pigment epithelial view. Surface topology includes nodules; knoll' rings, approximately 10 percent of the cone diameter in size; and a uniform fuzzy coating, mucopolysaccharides, over the whole of the surface.
Figure III-4: Transmission Electron Micrograph (TEM) cross section of *Necturus maculosus* rod outer segment (OS). Dendritic filaments (DF) were located in the triangular notches around the parameter of the outer segment. The disc lines observable in the outer segment give a topographical indication of disc "flatness".
Figure III-5: Selected freeze fracture photos of *Necturus maculosus* retina. Center: traverse fracture of the inner segment (IS), ellipsoid (E), parabaloid (P) and outer segments (OS). Left top: inner-outer segment junction area of membrane synthesis. Left center: ellipsoid area. Left bottom: nucleus (N) and endoplasmic reticulum (ER). Right top: membranous micelle (MM). Right center: continuity of the inner segment membrane (ISM) and the outer segment membrane (OSM). Right bottom: the parabaloid (P) region of the inner segment. (Photos by Fred Abrahms)
Fig. III-5
Figure III-6: Schematic drawings of *Necturus maculosus* rod cells illustrating the first, second, and third stages of radiation damage. The rod cell is orientated with a synapse facing the light source. The receptor region is facing away from the light source and embedded in the light absorbing pigment epithelium. Separation of the retina from the choroid occurs at the outer segment layer which facilitates the observation of the rod and cone outer segments with the scanning electron microscope. Three stages of radiation damage were categorized. In the first stage the fuzzy mucopolysacharide coating came off the outer segment outer membrane and the diameter of the discs synthesized postirradiation decreased in size. In the second stages of damages there were continuity changes. At this stage the filaments detached, the convolutions smoothed out, and the upper portion of the outer segment assumed a deflated appearance, as if turgor or material were lacking. The third stage of radiation damage there definite phagocytic activity with the loss of portions or all of the inner and outer segments.
2.1 ROD

2.2 FIRST STAGE RADIATION DAMAGE

2.3 SECOND STAGE RADIATION DAMAGE

2.4 THIRD STAGE

Fig. III-6
Figure III-7: TEM of *Necturus maculosus* rod cell. N: nucleus; R: ribosomes; P: parabaloid; E: ellipsoid; and OS: outer segment (x 7500). The spatial arrangements of the components of a rod are shown starting from the nucleus, going through the parabaloid and the ellipsoid of the inner segment, and ending with the discs of the outer segments. Compare to Figures III-5, IV-2, and IV-3.
Figure III-8: TEM of the interface between the paraboloid and the ellipsoid. Synthesis and aggregation sites are noted by arrows, in the lower portion of the micrograph numerous stained granules form linear arrays which aggregate in the middle of the photo. This boundary between the paraboloid and the ellipsoid is probably of a dynamic nature and would therefore be formed when a critical concentration has been reached.
level of the cones as observed from a pigment epithelial view. The diameter of the inner segments was uniform, 10 to 15 microns, as were the diameters of the outer segments. The outer segments were generally a few microns larger in diameter than the inner segments, giving a steplike appearance to the inner-outer segment junction. Vertical structure in the rods was defined by 25 to 30 fissures which run the length of the outer segment. The rod filaments, originating from the inner segment below the inner-outer segment boundary, were typically triangular in cross section, lying in and conforming to the shape of the mouths of the fissure. Figure III-5 shows the different photoreceptor intercellular components exposed after freeze fracture. Figure III-6 is a schematic representation of a normal rod; the horizontal fine structure is generated by the outer outer segment membrane shrinking inwards upon the rod discs during fixation and drying. Shrinkage due to the methods of preparation used has been found to be 10 to 20 percent. Figure III-6 also gives details of the morphological aspects of radiation damage.

Transmission electron microscopy. Transmission electron photomicrographs, Figures III-7 through 12, detail the nuclear, inner segment, and outer segment areas. The ellipsoid and paraboloid areas appear between the nucleus and the outer segment. Figure III-7 is a composite of the total area with ribosomal activity superior to the nucleus below the ellipsoid. Figure III-8 is localized at the nuclear facing, inferior, paraboloid-ellipsoid boundary. This position is then superior to the dense granular opsin-synthesising ribosomal area below the membranous micelles of the ellipsoid.

A cross section through the outer segment, Figure III-4, reveals
the triangular shape of the dendritic palisade surrounding the outer-segment disc membranes. The three-dimensional nature of the outer-segment disc topology is evidenced by the appearance of parallel disc outer-segment membranes regardless of the plane of section.

Figures III-9, 10, and 11 are of areas located at the outer-segment facing ellipsoid boundary. The discs of the outer segment are shown in Figures III-9 and 10 and the membrane sheets of the processes of outer segment disc formation can be seen in Figure III-11. The microtubules transporting opsin may be apparent in Figure III-10 (starred arrow); the activity of membrane growth is suggested by the membrane continuity between the inner and outer segments (arrows). Figure III-12 details the relationship of the outer-segment outer membrane, the inner-segment membrane, and the disc membranes.

Damage Evaluation and Scoring

Damage and changes in structure were attributed to radiation. Nine indicators of qualitative change were characterized (Table II-2); with the exception of skewedness of the discs which is not present in cones, the same criteria were used to judge both rods and cones. The sequence of appearance of the indicators was not necessarily the same in both rods and cones, but the indicators observed in each stage of damage were found to be consistent. The severity of damage was scored on a numerical scale of 0 to 30 (Table II-2) and from this the Pathological Index was calculated. Visually, when shrinkage was present a 20 percent decrease in the diameter of the outer segment synthesized postirradiation was observed over time; by following this change over a postirradiation
Figure III-9: TEM of the inner-outer segment junction region, the arrow indicates an area of disc membrane growth from a micelle. The starred arrow points to an unknown body, possibly a microtubule or growth region.
Figure III-10: TEM of the inner-outer segment growth region. In the middle, low power, micrograph, numerous disc edges may be distinguished in the upper left hand corner. The intradiscal space increases with decreasing distance to the inner-outer segment boundary. In the area of the lower micrograph numerous filaments, f, exhibit a continuity between the invaginating disc membrane and the micelles of the inner segment. Arrows, upper and lower micrographs, indicate the areas of the continuity between membranes of the invaginating discs and of the micelles (M).
Figure III-11: TEM of a membrane layer originating in the inner segment and being laid down as rod disc membranes in the outer segment. The space between inner and outer segments may be an artifact.
Fig. III-11
Figure III-12: TEM of the confluence of the rod inner segment, outer segment, and disc membranes (arrows). Unknown structures (microtubules and micelles) occupy the edge of the inner segment. This region is similar to that in the center left of Figure IV-2. A fold of membrane continuous with the outer segment outer membrane lies over the inner segment membrane. The junction between this fold and the inner segment membrane, center right, appears to blend with the disc membranes. A possible microtubule sandwiched between two micelles and the inner segment outer membrane, about 1200 Å from the inner segment arrow, may be involved in a growth center (Figure IV-3).
Figure III-13: Elements are smooth and have deflated appearance.
Dose = 1,000 rads neon x 1500. This micrograph relates to second stage radiation damage and typifies the difficulty of distinguishing between rods and cones. Compared to Figure III-3, these elements appear to have lost the fuzzy outer coat as well as much of their overall structure.
Figure III-14: Dissocation of filament material at inner-outer segment junction of cone Dose = 1000 rads x ray (x 1500). This cone was in a transition between first and second stage damage. The outer fine structure has been lost but rings of graded size may be seen in the upper portion of the cone. Lower along the segment numerous filaments, some having been detached from above, were indicative of a rejection, retraction or disassociation of neural processes.
Figure III-15: Two rods side by side; the left-hand rod exhibits radiation damage. Note the decrease in diameter of the lower portion of the outer segment and the loss of filaments postirradiation. 

Dose = 5000 rads x ray (x 2100). Radiation appears to affect a number of independent synthetic processes or control mechanisms. A frequent effect was manifested as a decrease in the diameter of the OS synthesized postirradiation. Protein labeling studies showed a significant decrease in the protein incorporation postirradiation. Filament detachment, contour variation, and deflation were also evident at this stage. The variations in damage from photoreceptor to photoreceptor suggests that each cell has independent control over a number of functions.
Figure III-16: Control rod having a rough surface with horizontal and vertical fine structure. Vertical serrations were aligned (x 13,200). At high magnification the outline of individual discs was distinguished. At the top of the photo is the area of phagocytic activity of the pigment epithelium. Observe that the stacking arrangement and the alignment of the discs persists at the distal portion of the outer segments. This persists although the palisade of calycle process has terminated at some point below the top of the stack.
Figure III-17: Skewed vertical serrations. Dose = 5000 rads x ray (x 30,000). As with Figure III-16 individual discs were clearly discernable, the black triangle in the upper left hand corner. A significant alteration in the vertical alignment was apparent. The shift observed by individual discs suggested a rotation about a central axis. A number of factors may have contributed to this phenomenon including the loss of the filaments; the loss of the membrane surface proteins or the mucopolysacharides; the changes in the outer segment outer membrane; and the perturbations in the synthetic or the phagocytic rates, associated with concommitant differential pressure along the rod outer segment axis.
Figure III-18: Top view of holes through the substratum, outer limiting membrane, which represent a degenerated element. (There are four such holes in this view.) Dose = 500 rads neon (x 1200). In this example of third stage radiation damage the outer limiting membrane was clearly distinguishable between the individual elements. The large holes, 20 - 30 microns in diameter, were all that remain after photoreceptors degenerated. Insufficient amounts of the other elements remained to distinguish between rods and cones.
period of 40 days, it was established that in about two weeks the
rod outer segments had synthesized approximately half of their total
length.

A change in the appearance of the surface of the cone was observed
in irradiated retina over a period of a few weeks. With increasing
doses of radiation, a noticeable number of cones had markedly smooth
surfaces; this varied from a few percent at 10 rad to 100 percent at
500 rad (Figure III-13).

In helium-ion irradiated retinae, changes visible at lower doses
were the smoothing of the surface of cone outer segment (Figure III-14),
decrease in diameter of the portion of the rod outer segment synthesized
postirradiation, loss of horizontal fine structure, and loss and dis-
organization of filaments (Figure III-15).

The loss of vertical fine structure following irradiation was
unique to the rods. Figure III-16 is a portion of a control rod outer
segment showing both horizontal (disc) and vertical fine structure.
Figure III-17 exhibits loss of straight vertical lines, and skewedness
of the linear arrangement. Note that the magnification is different
for each portion.

High radiation doses to the rods and cones caused atrophy and
degradation. Atrophy can be characterized either by deflation of the
element or phagocytosis by the pigment layer (Figure III-18), but the
ultimate effect was degeneration (Figure III-13). Trapped gas within
the elements is capable of explosive decompression during critical-
point drying. This type of damage must be differentiated from the
phagocytosis and degeneration induced by radiation.

Calculated from the known dose, flux, and the linear energy transfer
of particles, the Poisson distribution was used to determine the mean number of particles striking the typical visual cell (Appendix 2). At the lowest doses for neon, five to ten particles were incident upon each element. Due to statistical variation in these small numbers, not every element was expected to be affected; in fact, the appearance of an undamaged element surrounded by damaged elements was common at 50-, 100-, and 500-rad doses. Conversely, at the lowest doses, a single damaged element would appear in a field of normal elements.

Data derived from scoring a large number of micrographs were plotted as a function of dose. The PI of the photoreceptors was normalized by subtracting the PI value from 30 and dividing by 30. The plot of this value, which is the survival of control characteristics, was plotted on a logarithmic scale. Best-fit curves were drawn from the plotted data to calculate the relative biological effectiveness (RBE) of neon particles to alpha particles. Graphic calculation of the PI50 dose yielded an RBE of 8.68 for neon particles compared to alpha particles. The spread of the RBE values from PI20 to PI80 was 5.56 to 10.26. Fitting an outer boundary to the data graphically gave a RBE value of 3.9. The latter figures imply that the RBE is abnormally high. When isodose ratio of effects for 50 and 500 rod were used values of 1.7 and 1.37 were obtained. Extrapolation of the neon curve agrees with the assumption that if there is any threshold dose it is less than one rad (Figure III-19).

MUS NEGRUS #G57

Ultrastructure

Figure III-20 is a diagram of a generalized mouse retina showing the major layers. Starting from the anterior side is the inner limiting
Figure III-19: Plots of biological effectiveness based on pathology index versus radiation dose for alpha and neon particles. The damage criteria was rated on a pathological index. The plotted function was obtained by normalization, subtracting the pathological index score from 30 and dividing by 30. The log percent of this value, which is the survival of control characteristics, was plotted versus the log dose.
Figure III-20: A generalized schema of mammalian retinal morphology which suggests the relation of retinal cells, cell layers, and cellular components.
GENERALIZED MAMMALIAN RETINAL MORPHOLOGY

TERMINAL BAR
ZONE ADHAERENS
ZONE OCCLUDENS

PIGMENT EPITHELIUM
CHORIOCAPILLARIS
BASEMENT MEMBRANE

VILLI

CONIC
CILIARY ROOTLET
SYNAPTIC RIBBON

RC - ROD CELL
CC - CONE CELL
H - HORIZONTAL CELL
BP - BIPOLAR CELL
MC - MULLER CELL
AC - AMICRINE CELL
GC - GANGLION CELL

OUTER SEGMENTS
INNER SEGMENTS
OUTER LIMITING "MEMBRANE"
OUTER NUCLEAR LAYER
OUTER PLEXIFORM LAYER

INNER NUCLEAR LAYER
INNER PLEXIFORM LAYER
GANGLION CELL LAYER
INNER LIMITING MEMBRANE

D.E. Philpott

Fig. III-20
membrane, the ganglion cell layer, the inner plexiform layer, the inner nuclear layer, the outer plexiform layer, the outer nuclear layer, the outer limiting membrane (a line of tight junctions), the inner segment layer, the outer segment layer, the pigment epithelium, the basement membrane, and the choroid. Figure III-21 is a light micrograph of the same layers, while Figure III-22 is a transmission electron micrograph of the retina.

**Light microscopy.** The advantage of light microscopy is the ability to rapidly scan whole sections of the retina and observe unusual phenomena. It was determined from light photomicrographs that the thickness of the outer nuclear layer and the inner–outer segment layer had in some cases decreased 40 percent, 2 months postirradiation.

**Autoradiographic studies were conducted with the light microscope.** Figure III-23 is a composite of control and radiated retina with bands of labeled discs running from the inner–outer segment boundary to the pigment epithelium. Grain distribution and density were determined as a function of time postlabeling or label activity (Figure III-24), and as a function of time postirradiation or label uptake (Figure III-27). Relative rates of inner–outer segment growth (Figure III-28) were plotted.

The autoradiographic activity was plotted as a function of time after label uptake in the control and the irradiated samples. Control retina label activity had reached a peak by the first sacrifice two days after labeling. A steady decrease in activity was observed over the two-week period that the label was followed. The activity of the last sections of the control retina had a value one-third less than
the activity of the first sections of retina (Figure III-24).

In the neon plateau, particle radiated retina label uptake was only one-third that of the control retina at the first sacrifice two days after labeling. Activity decreased in the retinas labeled three days and those labeled two months postirradiation. Over the two-week periods followed, final activity was almost one-half the initial values observed (Figure III-25).

The growth rates of the outer segment for control and irradiated retinas were found to vary ± 15 percent. The transit rate of the outer segment discs increased 15 percent compared to the control immediately postirradiation, while the transit rate two months postirradiation had decreased to a value 15 percent less than that of the control (Figure III-26). The variance for controls with this technique approaches 10 percent, which suggests that the variations were significant.121

The outer segment area exhibited a sporadic but continual decrease in samples of radiated retina. After a two-month period the outer segment area of the radiated retina was found to be 35-40 percent smaller than that of the control retina (Figure III-27). The decrease in the outer-segment area along with the decrease in the outer-segment disc transit rate suggest a potential change in cellular activity, a decrease in the volume of these layers, an indication of decreasing synthesis, and/or increased phagocytic activity.

The ratio of outer-segment length to inner-segment length depended on the time postirradiation. For the first two-week period the proportion of the outer-segment area to the outer-segment/inner-segment
Figure III-21: Light micrograph of a *Mus niger* retina exhibiting the different retinal layers (x 700). Compared to the amphibian the cells were much smaller (see Figure III-1), the retinal layers were more distinct, and the complexity was greater in the mammalian. The unstained plastic section was photographed under the phase contrast microscope.
MOUSE C57BL/6J
RETINA

INNER LIMITING MEMBRANE
GANGLION CELLS
INNER PLEXIFORM LAYER
INNER NUCLEAR LAYER
INNER NUCLEAR LAYER
INNER NUCLEAR LAYER
INNER SEGMENTS
OUTER SEGMENTS
OUTER EPITHELIUM
CHOICE

XBB 776-5480

Fig. III-21
Figure III-22: TEM of control and neon plateau irradiated *Mus nigerus* retina. Areas of loose tissue and nuclear change were found in the radiated retina. Note the decrease in the thickness of the radiated retina; see Figure III-20 for the legends.
1000 Rads Neon (Plateau)

Areas of Loose Tissue

TRANSMISSION ELECTRON MICROSCOPY
MOUSE RETINA

Fig. III-22

XBB 776-5482
Figure III-23: Autoradiographs of irradiated and control *Mus nigrus* retina. Left: the control had heavier labeling and thicker cell layers than the radiated retina. Center: Phase contrast microscope was used to highlight the retinal layers. Right: the radiated retina was photographed to emphasize the labeling.
Fig. III-23
Figure III-24: A plot of the grain density of control *Mus nigrus* retina as a function of time (two week periods). Label, tritiated phenylalanine and leucine, was given interperitoneal (IP) three days after day 0. A rapid uptake in Label was followed by a slower metabolism and excretion of the Label.
Mouse Retina Autoradiography
Protein Label Density
C57 BL/6J

Grain counts

Days post-irradiation

Fig. III-24

XBL 776-3517
Figure III-25: Plots of grain density in control and radiated *Mus nigrus* retina. Label was given 3 days and 58 days postirradiation. After subtraction for background the amounts of protein incorporated postirradiation were only one third of preirradiation values. The decrease over a two-week period in the controls illustrates protein turnover during normal metabolism; 60 days postirradiation the rate of metabolism appeared to be greatly reduced over the same time period.
Fig. III-25
Figure III-26: Lifetime of *Mus* *negrus* rod disc as determined by transit time. This was the period from synthesis to phagocytosis was plotted for control and neon radiated retina. The decrease in transit time, middle graph, suggests an increase in the synthetic rate and/or phagocytic rate. The reverse, an increase transit time, which suggests a decrease in synthetic and/or phagocytic rates, was found several months postirradiation.
Mouse Retina Autoradiography
Protein Transit Time
C57 BL/6J

Fig. III-26
Figure III-27: Packing density (outer segment stack volumes) was calculated by multiplying OS diameters by their heights. This value was plotted as a function of time after irradiation for control and irradiated mice. Some variation was seen in control outer segment stacks, top figure values ranged between 12-16. Immediately postirradiation values were depressed 10% or more. Two months postirradiation values were depressed 25 - 30%. Interpolation between experimental periods suggests a linear decrease in stack area during the first ten weeks postirradiation.
Outer Segment Growth

Normalized Outer Segment Stack Area

Control

16
14
12
10
8
6
4
2
0

0 5 10 15 20

Days post-irradiation

1000 rads Neon (plateau)

Fig. III-27
Figure III-28: The ratio, percent, of the inner-outer segment area that was composed of discs was measured as a function of time after radiation. Immediately postirradiation there may have been a slight growth of the outer segments at the expense of the inner segment region. This, coupled with the linear decrease in stack area, could account for the apparent increase in transit and synthetic rate at a time of decreased amino acid uptake and protein synthesis. Of significance was the finding that several months postirradiation the percent of outer segment in the inner outer segment layer was decreased; the ranges of values for ratios immediately and several months postirradiation do not overlap. Radiation appears to decrease the outer segment layer both compared to controls and compared to other retinal layers over a ten week period.
Outer Segment Growth

Ratio Outer segment length : Inner-outer segment length

Control

1000 rads Neon (plateau)

1st week PI

2nd month PI

Days post-irradiation

Fig. III-28
area was higher than that of the control. Several months later the ratio of the outer-segment area to the inner-segment/outer-segment area had decreased to a level 10 percent below the controls (Figure III-28).

Scanning electron microscopy. The inner-outer segment area of the light-sensing elements was examined after dry fracture of the retina (Appendix III). Retinae of animals sacrificed 1 to 10 weeks postirradiation at initial doses of 50, 100, and 1000 rad are shown in Figures III-29, 30, and 31. The lower magnifications offer a general orientation to retinal components, specifically the outer nuclear layer, the inner segment, and the outer segment. The pigment epithelium has been removed so that the outer segments can be seen more clearly. The higher magnifications are localized in the inner-outer segment regions of the retina and exhibit characteristic radiation-induced morphological changes. Figure III-32 is a schematic representation of the changes observed with the scanning electron microscope.

The control rods were rod-shaped, extending from the line of tight junction that separates the nuclear layer from the inner-outer segment layer to the area of the pigment epithelium. The diameter of the inner segments and the outer segments was uniform and appeared to be tightly packed as in a crystalline array. Numerous connections were observed between the inner segments, as well as between the inner segments and the numerous filamentous processes that run up from below the outer limiting membrane. Figure III-33 is a micrograph of the connections observed between inner segments. The rod outer segments had an almost fluffy, convoluted appearance in less packed areas while they maintained a convoluted appearance in tightly packed areas. In contrast to Necturus
maculosus rods, mouse rods do not exhibit grooves or fissures, and the origin of the filaments is most probably the Müller cells rather than the dendritic fingers of the inner segments.

Irradiated retina was found to exhibit a variety of changes from the structures characteristic of the normal retina. The two major indications of morphological change postirradiation are changes in the discs of the outer segment and changes in the filaments surrounding the segments. During the first week postirradiation changes were noted within the inner segment layer. These changes parallel the classic symptoms of Wallerian degeneration of neural filaments. First there is a retraction of filaments, prevalent at lower doses, and then a degeneration and granulation of the filaments. The first elements to be affected appear to be the Müller cell processes, which originate between the ganglion and inner nuclear cell layers and extend up into the inner segment layer. The granulation process is detailed in Figure III-32. The connections between adjacent inner segments (zona adherens) observed in the control retina exhibit a characteristic degenerative appearance after irradiation (Figure III-33).

By the second week postirradiation, the characteristic changes noticed included smoothing and reorientation of the discs of the outer segments. At high magnification (50 k) these changes can be observed between the control and the 10-week retina at 100- and 1000 rad (Figures III-30 and 31).

At lower doses (50 and 100 rad) some regeneration can be seen in the inner segment layer. These observations correspond to the last stages of neural regeneration where new filaments issue from the neural
Figure III-29:  SEM of control and 50-rad neon plateau irradiation

Mus nigrus retina exhibiting changes seen immediately (1 week) and long term (10 week) postirradiation. Top photos were of cross sections through the whole retina after it had been separated from the pigment epithelium. The lower photos were localized in the inner-outer segment layers. (Magnification is one third of that given on the right margin.) In the control numerous connections were seen between adjacent photoreceptors. By the first week postirradiation (x 10 or 20K) communication was lost, i.e., the loss of adjacent connections, and the IS-OS area became granular (Figure III-32). The outer segments appear unaffected (50K) after the first week but by the tenth week have lost their fluffy character and the surface has become smooth (for damage criteria see N.m. sections). Granulation of the inner segment appears reduced after ten weeks and this response to injury may be indicative of repair.
MOUSE RETINA
LIGHT SENSING ELEMENTS
50 rads NEON IRRADIATION

Post-Irradiation

CONTROL

1st WEEK

10th WEEK

1K

5K

10K

20K

50K

SCANNING ELECTRON MICROSCOPY

XBB 775-4990

Fig. III-29
Figure III-30: SEM of control and 100-rads plateau neon irradiated Mus nigrus retina. (Magnification is one third of that given on the right margin.) The sequence of events was essentially the same at 100 rads as with 50 rads (Figure III-29). Granulation occurred in the inner and outer segments and appeared grosser earlier, the first week (10K and 20K), than later. The outer segments had lost most of their fine structure by week ten (50K).
MOUSE RETINA
LIGHT SENSING ELEMENTS
100 rads NEON IRRADIATION

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SCANNING ELECTRON MICROSCOPY

Fig. III-30
Figure III-31: SEM of control and 1000-rads neon plateau irradiated *Mus nigrus* retina. (Magnification is one fourth of that given on the right margin.) The sequence of pathological events, loss of filaments and connections, granulation, and smoothing of the outer segments, occur in the same order as they were observed to occur at lower doses. The severity of effects was greater and persisted longer at the higher dose. Phagocytic action had commenced by the second month but no repair was evident by the end of the experiment, ten weeks postirradiation.
MOUSE RETINA
LIGHT SENSING ELEMENTS
1000 rads NEON IRRADIATION

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SCANNING ELECTRON MICROSCOPY

Fig. III-31
Figure III-32: SEM of inner segments connections occurring in control and irradiated *Mus musculus* retina. The top micrograph (higher magnification inset) depicts the appearance of normal inter-inner segment connections. After a radiation insult these tended to granulate, lower micrographs. The appearance of the connection after granulation was distinctive. It consisted of a spherical central region that had smaller radial arms connecting the body of the sphere to the cells. The properties of the formation are unknown but were found to occur at both low (50 rad) and high (1000 rad) doses.
Fig. III-32
Figure III-33: Schema of changes seen in *Mus negrus* photoreceptors after 100 and 1000 rad of neon plateau irradiation. Radiation induced perturbation, Figures 29-32, are compared. Primary changes are the granulation of the filaments and the processes originating outside of the inner-outer segment layers and the loss of the inter-inner segment connections. Changes in the discs of the outer segment commenced post-irradiation and were manifested by the tenth week. Occasionally displaced nuclei were observed in the inner outer segment layer at higher doses.
SEM VISUAL CELLS
Mouse Rod
Neon Irradiation

Fig. III-33
Figure III-34: SEM of filaments (A and B) in the inner segment of
Mus niger retina 10 weeks after a 50 rad neon plateau irradiation. The
starred growing tip of filament B appeared to be tracing a degenerated
track, dotted line, along the inner segment (IS). This process may be
similar to the Wallarian degeneration-regeneration model for neural
injury.
Figure III-35: TEM of outer nuclear layer of *Mus ngrus* retina in a control and after neon irradiation. Nuclei (n) in a 4000-rad irradiated retina exhibit gross pathological changes; these were not commonly observed at lower doses. See Figure III-22 for captions and comparisons.
1000 Rads neon

Control

4000 Rads neon

Fig. III-35
Figure III-36: Schema of changes observed with TEM after particle radiation. (Compare to Figure III-33 of scanning results.) Radiation effected synthesis, surface structures and internal cellular structures. SEM in general was found to be a more sensitive detector of changes than TEM. Changes observed with the TEM were generally pathological at doses rated by SEM criteria.
TEM VISUAL CELL
Mouse rod
Neon and Argon Irradiation
Changes observed at high doses >1000 rads

Fig. III-36
cell soma and attempt to reinfiltreate the tract left by the degenerating filaments and reestablish the original path of communication. This regeneration can be seen in the medium magnification (10 k and 20 k) the tenth week after 100 rad of neon irradiation (Figure III-30), where several new filaments appear to be infiltrating the inner segment area. Close examination of Figure III-34 suggests that the route taken by the developing filaments follows that of degenerated older filaments.

By the second month postirradiation, a general trend among surviving retinal cells is the phagocytosis of degenerated material. The tenth week retina is shown in Figures III-29 and 30; smoothing of the inner and outer segment component surfaces and the absence of the granular material found the first weeks postirradiation can be observed.

Filamentous structures appear quite sensitive to radiation; over 90 percent of the area viewed at the lower doses exhibited a characteristic degenerative appearance. Because the diameter of the mouse rod outer segment is less than 10 percent of that of the Necturus rod, the normal flux of particles to the mouse disc is less than one, and the flux through the nucleus is somewhat greater, from 5 to 10 particles.

Transmission electron microscopy. Control retinae and retinae exposed to 1000 rad of neon radiation were examined at both high and low magnification. Maps of a complete cross section were prepared and studied for damage (Figure III-35). Figure III-36 is a diagram summarizing damage. Some damage and rearrangement of the outer segment area (a common preparation artifact in membranous structure) was noticed in most of the samples, but no overt radiation and malformation
of this layer was observed. The nuclear layer of the radiated retina might have decreased in thickness and exhibited some degenerative changes. Because the absolute plane (or orientation) of the section is difficult to establish, the relative thickness of the retina was not a true indication of degeneration.

The nuclear packing in some areas appeared to be decreased, and areas of "scar" tissue and/or glial invasions are indicated in Figure III-14. Areas of "loose" tissue in the outer plexiform layer, the inner nuclear layer, and the outer plexiform layer are also apparent in the same micrograph. These areas are close to the capillary beds and thus to the perfusate, an indication of radiation damage rather than poor fixation. All of the normal structures and layers of the retina were observable in the irradiated and control retinae studied; the boundary and limiting membranes of the retina remained intact.

A comparison of the outer nuclear layers of control retinae exposed to 1000- and 4000-rad neon irradiation (material from a previous NASA study) demonstrates the reaction of the nuclei of the outer nuclear layer to higher doses, i.e., acute versus chronic degeneration (Figure III-35). Figure III-37 shows chronic changes in the nuclear layer the first week postirradiation. Several stages of nuclear degeneration can be differentiated; these stages include changes in chromatin structure and distribution, changes in shape and structure of the nuclear soma, swelling and migration of the nucleus, and degeneration of the membranes and cellular components.
Figure III-37: Portions of the outer nuclear layer and inner and outer segments of *Mus nigrus* retina after 1000 rads of neon plateau irradiation. Circular disruptions in the outer segment may be due to the structure of particle tracks. Similar lesions have been found in laser-damaged retina. The space between the columns of the cells of the outer nuclear layer may be a result of the death and the phagocytosis of a photoreceptor nuclei.
SECTION IV. DISCUSSION

THERMODYNAMIC MODEL

The elaborate patterns and structures of the retina at the supramolecular level of organization and their suitability for approximating functional responses has been the subject of much research. There is no current coherent physical theory that would adequately explain the manner in which biological structures form and maintain themselves. Nonequilibrium-thermodynamic models of structure, stability, and fluctuation have approximated a variety of conditions, criteria, and materials for operational systems. The stability of open and closed systems has been categorized starting with generalized flow and forces; it is possible to use these generalized parameters to explore instabilities in the thermodynamic branch and the formation of dissipative systems.

One approach to the complicated phenomena of membrane formation is to try to explain entropy production as it occurs during the process of syntheses and condensation of membrane components. A stable membrane configuration represents a minimum in spatial potential energy distribution. In a closed system entropy eventually proceeds to a maximum state, equilibrium, in an open system (such as a set of rod outer segment membranes) there is a measure of order, a coherent behavior, and a minimum entropy production. Glansdorff and Prigogine note that, far from thermodynamic equilibrium, the competition between homogenization of chemical components by free diffusion and spatial localization give rise to instabilities. These are due to local disturbances of chemical processes involving autocatalytic steps and ultimately lead to the appearance of stable non-uniform distributions of matter.
Membrane synthesis may be driven by the entropy production at the inner-outer segment transition.

In an effort to reach maximum entropy, the open, dissipative, system constrained by minimum entropy production forces the thermodynamic branch to become unstable. The instability is channeled into the evolution of a new structure involving coherent behavior. A new molecular order appears that corresponds to a macroscopic fluctuation stabilized by exchange of energy, photon capture and signal propagation, with the outside system. Feedback mechanisms control the amplification of instabilities and direct the appearance of the dissipative structure.

The critical point beyond which spatial differentiation may be possible corresponds to the situation where the excess entropy production vanishes. Excess entropy production may contain the contributions of several terms including diffusion and chemical reactions. If the excess entropy production is interpreted as a potential that decreases in time and corresponds to a minimum for a stable steady state, then a combination of non-zero entropy production events would meet the stability requirements. Dynamic states of matter induced by a flow of free energy in a system far from equilibrium do exist. These states are governed by supermolecular level physical chemistry, and their coherent behavior corresponds to an amplification of specific molecular properties in far from thermodynamic equilibrium conditions. A hierarchy of structures comprised of internal and external variables influence the stability of biological structures, leading to aggregation of a membranous system while obeying the laws of thermodynamics. The concept of non-equilibrium stability reconciles this process with the existence of well-defined levels of description giving impetus for successive approximation of
open (biotic) systems by biophysical models.

The discs of the rod photoreceptors have achieved a stable state, assuming that morphological stability and functional ability define stability for this system. The discs are functional for a specific period between synthesis and phagocytosis. Therefore, stability has a periodicity of several weeks. The different components are synthesized (transcription and translation) and are then assembled under conditions far from equilibrium—the basic processes whereby a disc is formed. Ideally the functioning disc would then exist in a steady state until the phagocytation process. However, simultaneously irreparable molecular degradation and information loss, entropy production, degrade performance.

In perpetuating biological systems, genetic information is maintained by entropy producing processes. Since the entropy of a nonequilibrium closed system must increase with time, a bounded system without the capacity for repair must degenerate. Biotic examples are the red blood cells (RBCs) and the rod discs. When degenerative processes are superimposed on the stable structure of discs, the lifespan of the system which does not contain the information for its own repair is limited both by the stability of formation and by the rate of information loss.

It is possible that feedback and closed loop control mechanisms are responsible for the life of the disc, directing the pigment epithelium which functions to phagocytize discs. Discs appear to remain stable as long as the boundary conditions, i.e., the outer membrane, remains inviolate. Radiation doses have the ability to alter membrane structure and probably disrupt stored information. The effect of radiation on the control mechanisms remains as elusive as the actual control mechanisms.
MEMBRANE SYNTHESIS

Membrane components include lipids with different head and tail moities, a variety of proteins, small organic carbon molecules, enzymes, coenzymes, and mucopolysaccharides. Since membranes have different functions, either their composition or their ratios of components vary to deal with specific activities. Rod disc membranes are an interesting system to study because of their exclusive function and constant synthesis, dissipative structures in a steady state.

In the situation of the rod photoreceptor, the cellular components are synthesized proximally to the nucleus and travel distally towards the outer segments and pigment epithelium (Figure IV-1 and 2). The greatest tendency for lipid bilayers is micelle (liposome) formation; bilayers usually do not form free ends, i.e., exhibit discontinuities. Lipids are synthesized in the cytoplasm of the parabaloid below the ellipsoid. The cytoplasmic milieu present at the time of micelle formation is enclosed in the micelle during aggregation. This milieu contains the different proteins present at the synthetic or aggregative sites. Probably the proteins are made and fold up by the cytoplasmic tubules, and by virtue of their three-dimensional structure, partition into the membrane driven by the protein is hydrophobic forces.24

Other membrane proteins, ionic pumps (e.g., Na⁺, K⁺, Ca²⁺), enzyme pores, isomerases, and esterases could be incorporated at different times during the transport along the inner segment. The opsin complex is probably "packaged" into a phospholipid micelle as it is transported to the outer segment.14 The final opportunity for components to be
incorporated into the membrane is later when the opsin-retinal dehyde-
rhodopsin complex is completed.

The cilia are believed to be responsible for the transport of proteins from their site of synthesis in the lower part of the inner segment up to or beyond the inner outer segment border. The cilia may play an active role in transporting components through the interior of the cilia, which is missing the central two microtubules commonly found in the center of the ring of microtubules (the 9 + 2 structure). Flagellate motion of the cilia around the periphery of the cell may act as a driving force for movement from the inner segment to the outer segment. A cilia undergoing circular motion would describe a spiral around the inner segment until it reached a constant length due to abrasion at the inner-outer segment boundary. Either mechanism or a combination of both types of transport would mix different components at the inner-outer segment boundary.

Proteins capable of acting as proton pumps (Halobacter-type proteins) or as energy transducers (cytochromes and mitochondrial enzymes) could serve various functions in the altering cytoplasmic milieu of the ellipsoid and/or supply energy from either ATP, light, or another energy "coin." Membrane components incorporated during the original lipid aggregation may start functioning when they are swollen during light adaptation.

Secondary aggregation of membrane components, the addition of components to a membrane, and restructuring a formed membrane may require activation energy for membrane rearrangement or may be a result of a far-from-equilibrium process. The activation energy may arise
from the processing of components of the intercellular milieu, it may arrive via special transport tubules as the opsin-rhodopsin complex, or it may be derived from processing light energy. Alternatively, flow dynamics from structural forms causing turbulence (microtubule termination) could supply the activation energy as well as the catalytic "seed" for the disc membrane formation.

A possible driving force for membrane synthesis could be the pressure generated by the accumulation of intercellular components below the ellipsoid. The newly synthesized opsin protein would be forced up to the outer segment through the microtubules; membrane materials would be pushed by the pressure up the ellipsoid. An added stimulus for membrane synthesis may be the pressure induced in the inner segment by light stimulation. The rod disc outer segments are shed at the initiation of the cyclic light period; light adaption induced pressure may be responsible for a favorable local thermodynamic instability for disc synthesis throughout the duration of the light adapted period.

The exact mechanism of the formation of a specific membrane remains unknown. Vinnikov found that disc membranes will not form unless the rhodopsin is available in the original membrane "primer" coating the flagellum which induces the appearance of disc membranes.240 The availability of a template may be a prerequisite, although in the laboratory the spontaneous formation of a lipid layer is possible by the use of proper boundary techniques. The advantage of a template model is that precise positioning and control of membranes would be available.

There exists a number of template models in biology: DNA,
mitochondria, and membranes are all passed intact to proceeding genera-
tions. Templates are usually complimentary, identical, or a combination
of the two. Such models are not wholly distinct from the spontaneous
generation models of far-from-equilibrium thermodynamics. Using a con-
cept of spontaneous formation on a like template, a crystalization model
would not necessarily imply a rigid matrix, but rather a pracrystalline,
or liquid crystal, state. If the membrane mycelles are mitochondria
and are incorporated in discs, then both the template and the coding
for components would be passed intact in these organelles.

A thin film flow model constrained by a cylindrical boundary
(Appendix IV) was used to generate an axially propagating system of
discs. The discs were formed from the passage of components through
a tube with a small orifice located axially in the cylinder. Glass
spheres containing deuterium and tritium for laser fusion experiments
are formed by similar processes. The existence of such systems argues
against the necessity of a template that invokes boundary conditions
to maintain form.
Figures IV-1 and IV-2: Model of photoreceptor outer segment disc synthesis followed from information coding through component synthesis to aggregation. In the lower right hand corner lies the nucleus (N) within the nuclear membrane (NM), which may be seen crossing the page diagonally. The messenger ribonucleic acid strands (mRNA) leave the nucleus through nuclear membrane pores after being synthesized from complementary deoxyribonucleic acids (DNA) templates. Endoplasmic reticulum (ER), shown joined to the nuclear membrane in two locations, is covered with ribosomes and therefore is classified as the rough endoplasmic reticulum (RER). Amino acids, enzymatically prepared and linked to transfer RNA (tRNA), are assembled into a protein chain in response to a sequence read from the mRNA strands. After passing from the nucleus the mRNA strands travel to the ribosome and are inserted, caught, between the large and small subunits. Three nucleotides are exposed and paired to a complementary sequence on the tRNA. The amino acid at the opposite end of the tRNA is transferred to the protein chain which may be preopsin, a cell membrane, enzyme or other cellular protein.

Lipids (L) are formed primarily around the cytoplasmic tubules above and to the right of the RER in the model. Fatty acids are enzymatically joined to polar head groups to synthesize the membrane lipids. The biphobic nature of these molecules induce forces which, depending upon concentration and cytoplasmic composition, form micelles or vesicles. Mucopolysaccharides (Msps) are incorporated onto the extracytoplasmic membrane surface and into the membranes with other enzymes and proteins; this forms membrane micelles (MM). Within the local environment components combine to form a unique membrane composition. The three membranes in the picture, the outer segment (OS) membrane, the inner segment (IS) membrane, and the disc membrane are synthesized to serve different functions, their areas of assemblage are subsequently shown as being spatially separated.

The inner segment outer membrane (ISOM), bottom left, extends as a barrier upwards to either form a hollow calyce process, dendritic filament (DF), which extends up along the outside of the outer segment outer membrane or interfaces to join the outer segment outer membrane (OSOM). The outer segment growth and interface region are on the middle left boundary above the inner segment growth region. Calcium and/or other messengers controlled pores in the OSOM modulate the passage of signals, chemicals, nutrients, and ions. Retinal crosses the OSOM to combine with opsin on the surfaces of the discs, top right; signals derived from light, photon capture, pass in the reverse direction across the OSOM to the calyce process or into the extracellular space between the outer limiting membrane and the pigment epithelium.

Proteins (OSMP) may be transported actively via tubules or cilia or passively pushed by flagellar action into the inner-outer segment boundary layer. The presence of opsin at the IS-OS boundary in proximity to the micelles may act to seed the formation of the discs membrane while using existing membranes as a template. The pressure of membrane synthetic action creates a positive pressure which pushes the rod outer segment discs away from the inner-outer segment boundary towards the space created by the phagocytic action of the pigment epithelium.

* Figure IV-1 is not included.
Although electron microscopy is a useful technique for ultrastructure research, a major problem exists in that what is seen are artifacts due to the method of preparation rather than the actual biological structure. Biological material rapidly disintegrates because localized energy doses of $10^8$ to $10^{10}$ rads are delivered by the electron beam in exposing an electron micrograph.$^7$ Standard preparation includes first fixing the material into a rigid matrix and then extracting the water, or about 80 percent of the sample weight. Samples were infiltrated with a nonwater soluble resin. The dehydration and solvation process is very hard on membranes because of the lipid content of the bilayer, and it generally causes a loss of fine structure of the lipid bilayer.

In areas near the outer edge of the inner-outer segment boundary, (Figures III-12) a number of membranes may be distinguished. The disc membranes may be observed stacked in the outer segment area and are responsible for photon capture, signal initiation, transduction, and possibly amplification. The outer-segment limiting membrane is continuous over the outer segment discs, and functions in transmitting the impulse initiated in the disc outside of the outer segment to the inner segment. The membrane to the side of the membrane micelles is the inner segment membrane which has the function of conducting light stimulated visual impulses to the synapses which lay inferior to the nucleus. Figure IV-3 relates known structure and function in the light sensing elements.

Membrane information is often lost or undistinguishable in areas of high membrane concentration like the outer segment, and in areas of
membrane synthesis like the ellipsoid and inner-outer segment boundary. In the retinas studied, a boundary layer in which disc membrane is synthesized was situated directly below the layer of disc membranes. When an optimum cross section of the whole retina was observed, it appeared as if the ellipsoid was engaged in membrane synthesis. The portion of the paraboloid above the protein, i.e., opsin, producing ribosomal area was packed with membrane micelles which could be the source of membrane lipid components. At the border of the inner-outer segment, the layer where the membranes may grow by "invagination," several areas where the micelle membranes are continuous with the disc membrane were seen in Figures III-9, 10, and 11, and may be membrane "growth centers." Figure IV-4 details a generalized model for membrane component incorporation for membrane growth. Different species have a variety of structures and morphologies, which suggests a number of permutations on this assembly model.

Several methods to preserve membrane structure using water soluble resins now exist. These techniques may offer increased resolution of membrane structure and eventual elucidation of the membrane synthetic process. Methods of staining contractile filaments in the eye as well as in the cytoplasm are available and could be used to localize membrane synthetic forces producing structures if membrane synthesis and disc invagination occur from active contractile processes. The absence of an actin-like material may be indicative of a process of favorable thermodynamic conditions for membrane crystalization or enzymatically constructed membranes. The filaments in Figure III-10(f) may be either membrane components being incorporated into the membrane or contractile elements causing membrane invagination of component arrangements.
Figure IV-3: Schema of the structure and the functions of a rod photoreceptor involved in photon capture. The nucleus, in the outer nuclear layer below the tight junctions, codes for light sensing elements (discs) and synaptic and other cellular elements (not shown). Molecules are combined at the inner-outer segment boundary to form discs, the area between this junction and the nucleus is the inner segment. Protein, lipid, and mucopolysaccharide synthesis and assembly occur in this region.

Rhodopsin in the disc absorbs a photon and initiates a signal which is transduced to the outer segment outer membrane and transmitted through pores to the inner segment outer membrane. The signal is conducted via cable properties along the cell to the synapses in the outer plexiform layer below the nuclear layer (not shown).
LIGHT SENSING ELEMENTS
STRUCTURE & FUNCTION

Fig. IV-3

Photon absorption by rhodopsin molecule

Signal transduction to outer segment outer membrane

Ca$^{++}$ controlled pores for signal transmission to inner segment

Outer segment discs

OS Boundary where outer segment outer membrane and disc membrane are synthesised

IS Inner segment membrane

Signal conduction to synapse below tight junction

Ellipsoid membrane micelles

Microtubules for protein transport

Lipid bilayer component synthesis

Ribosomes (protein synthesis)

Tight junctions or outer limiting membrane

XBL 776-3522
Figure IV-4: Schema of the disc formation process driven by component aggregation. Side and top views of a "growth center". The simplest growth model is from a single point. An invaginating disc requires membrane production to expand surface area. The juxtaposition of the myriad of components and factors at a single point would lead to the condensation of membrane to the expanding disc membrane. The longitudinal view has microtubules transiting the micelle region and terminating at the inner outer segment boundary. This would represent an active transport model of opsins being incorporated into the membrane being formed from the materials composing the micelles.

In the top view growth is seen to proceed radially away from the growth center within the fluid lipid bilayer sheet.
Disc Formation

- Rhodopsin type complexes
- Mucopolysaccharides
- Disc
- Invaginating disc
- Vitamin A
- Microtubules
- Opsin
- Filaments
- Lipid bilayer materials and other proteins
- Lipid micelles

Cross Section

- Growth center
- Lipid bilayer sheet
- Proteins & Lipids added to membrane
- Growth

Top View

Fig. IV-4
RADIATION PATHOLOGY

The smoothing of the outer-segment outer membrane may be due to loss of the glycocalix (mucopolysaccharides) or other structural components on the membrane surface either through phagocytic activity or membrane activity. Such smoothing may also be due to internal membrane changes or may be indicative of changes inside the outer membrane, e.g., swelling due to osmotic changes.

The decrease in the labeling density of protein in the retina, specifically in the inner-outer segment region, suggests that protein syntheses is drastically curtailed as a result of radiation. Although the materials used for labeling were chosen to trace opsin and rhodopsin synthesis, the general decrease in labeling is probably indicative of a decrease in synthetic activity of other membrane and cytoplasmic protein entities.

Synthesis of the light-receptor membranes of rods may proceed de novo or by an infolding with the outer membrane surface being enclosed within the discs. Changes in the outer-membrane surface appearance suggest similar changes in the inner disc surface which has both proteins and mucopolysaccharides attached. Changes in opsin labeling suggest a decrease in rhodopsin formation, and therefore a decrease in the rhodopsin density on the disc-membrane outer surface. Radiation probably alters the inner and outer surfaces of the disc membranes both in proteins incorporated and mucopolysaccharides attached.

Decreases in the diameter of the outer segment are probably related to changes in the synthetic process. This decrease may be the result
of losing the underlying fine structure of the discs, either through
loss of rigidity, loss of structural material, or changes in the compo-
sition of material synthesized for the discs.

**Necturus maculosus**

In *Necturus* the vertical structure is due to both the conformation
due to disc synthesis and the extension of filaments of the inner seg-
ments. Structural changes observed may be related to the observed
filament breakage and dissociation. Retraction, degeneration, and dis-
sociation of the filaments due to damage to the cell (retraction of
neural appendages after irradiation) have been observed previously with
other nerve cells. Swelling and rotational displacement of the discs
could be contributing to this phenomenon.

The skewed nature of the vertical serrations indicate a rotation
of the individual discs, and implicate loss of adhesive properties in
the disc membrane and outer membrane. The detachment of vertical fila-
ments may be a contributing factor to disc rotation.

Although the functions of the Müller and pigment epithelium
processes are not totally delineated, functions of support, mobility,
nutrition, regeneration, and signal propagation (for Müller cells) are
implicated. Loss of these filaments is indicative of a subsequent loss
of these functions, and loss of function of light-sensing elements.
Even with the degeneration of the filaments of the inner-outer segment
regions, ultrastructural studies do not indicate a concomitant loss of
the limiting membranes of the retina, both of which are related to the
Müller cells. The diversity of functions of the support cells and
the selective loss of structure may be indicative of a correlation between the different cell structures and functions and the consequently different radiosensitivities of specific cell structures.

Deflation of the outer segment may be an active process like the phagocytic activity of the pigment layer. Loss of the adhesive properties of the disc would be a passive deflation process. Phagocytic activity may occur in response to degeneration of the light-sensing element or to hyperactivity of the pigment layer. Degeneration depends on ultimate cell death or dysfunction. Dysfunction at the synthetic metabolic level or loss of the ability to transform or utilize nutrients would cause cell death.

Mus negrus #C57

Radiation damage to the photoreceptor layers of the retina (inner and outer segments) separates into two categories. The first is degeneration of existing structures, which is demonstrated by the loss of fine structure and the loss of filamentous processes in the inner segment layer. A consequence of these events is the granulation on the cell surfaces which can be observed with the scanning electron microscope. Since the geometry of a sphere and rod in cross section is similar, the TEM would normally not be expected to differentiate the observed loss of filaments and granulation except by reconstruction through serial sectioning.

The second indication of radiation damage is the biological amplification of the affected subcellular components (DNA) observed as misinformation and deformity in the rapidly synthesized outer-segment
discs. Since transit times are nominally on the order of two weeks, the full effect of this phenomenon would not be expected until several weeks postirradiation. TEM of irradiated mouse retina indicates that the discs of the outer segments incur degenerate changes. Using SEM, fine structural changes on the surface of the outer membrane of the outer segment as well as perturbations in stacking orientation were observed.

At low doses only a small percent of the visual cells appear to be lost. Unless significant changes occur in the pigment epithelial cells and cell processes, gross changes in disc stacking would not be expected. Postsynthetic disc stacking appears to be a function of the restraining geometry of the surrounding elements.

Indications of regeneration and repair appeared after the damage and degeneration. Nucleic acid repair in the retina has been demonstrated to be initiated immediately postirradiation, with maximal repair accomplished within a few hours.\textsuperscript{257} Cellular degenerative changes appeared within a week but they were not observable within the first few days. Changes in the appearance of the outer segment were most prominent at the approximate timing of the discs synthesized immediately postirradiation reaching the distal portion of the outer segment before phagocytosis. Phagocytic changes within the layers of the retina that result in the loss of granularity appear only several weeks postirradiation and seem to precede the actual regenerative process of the neural filaments.
AUTORADIOGRAPHY

Autoradiographic data appears to be self-consistent. In general, the response of the outer segments to radiation was a decrease in area, and, by inference, volume; there was a concomitant decrease in protein synthesis and/or label uptake. Coupled to the decrease in label uptake was an increase in transit time for the disc outer segments, which is indicative of an increase in either or both synthetic rate and phagocytation rate. The increase in the ratio of outer segment length to inner segment length may be indicative of an increase in disc synthesis, occurring at the expense of disc components in the inner segment which would decrease the volume of the inner segment.

The general observation that both the outer nuclear layer and the inner–outer segment layers had decreased in size by as much as 40 percent may in part be due to the plane of sectioning, but it is also indicative of a loss of photoreceptor cells due to the loss of nuclei in the outer nuclear layer. Not only is such a cell loss difficult to quantitate but it also affects the measurement of the inner and outer segment layers, decreasing the volume of each. The use of normalization factors in computing the autoradiographic data has had the advantage of offsetting this phenomenon, but consequently increases the difficulty of interpreting data.
SECTION V. CONCLUSIONS

There are immediate and delayed effects of radiation on the retina. The immediate effects result from high doses (thousands of rads), and cause an immediate inactivation of visual function. The delayed or late pathological effects occur after a longer interval postirradiation and in general at doses of 0.1 to 1.0 percent of doses causing immediate effects. The exact mechanisms of the immediate effects remain unknown but may involve membrane permeability or functional enzyme complexes.

Delayed effects were usually observable only after biological amplification of the damage to the point where a system became dysfunctional or grossly deformed. The labeling of the amino acid precursors of the disc proteins demonstrated that radiation effected the amount of proteins synthesized and the rates of disc formation.

The effects of heavy-ion particles are observable at lower doses than are the effects of x rays. Two factors contribute to this phenomenon: the first is the high local energy deposition along particle tracks; the second is that the effectiveness of particle irradiation increases with the charge of the particle. For x rays, previous work has shown a probable threshold for morphological effects. For neon, the lowest doses used were 10 rad. The significant amount of damage observed indicates the necessity of studying the effects of still lower doses. The low-dose findings presented here may indicate lack of a threshold; the absence of a threshold may mean that each heavy ion crossing the retina may cause lasting deleterious effects. If there is a lack of threshold, cosmic-ray primaries do constitute a hazard
to humans on long-term space flights. Moreover, because the retina is part of the nervous system, we cannot disregard the effect heavy ions may have on the nervous system as a whole.*

The evaluation of the delayed effects of radiation damage to the light-sensing elements of the retina implicate not only the receptor cells, dendrites and cell-to-cell communication, but also the support cells, the Muller cells, and the pigment epithelium. Damage to these elements, as well as to the control mechanisms, at very low doses, of particle radiation (1 to 10 rad), illustrate the sensitivity of the retina due to its complexity and interassociations. It is not possible to view the radiosensitivity of the retina solely as a function of the neural cells but only as an integrative unit.

Whether the loss of function or light-sensing ability is sufficient to cause cell death is a question requiring further exploration. Genetic pathological studies indicate that functional loss may result from any of several causes. Possible sites of dysfunction are the pigment epithelium, the structure, composition or communication of the inner-outer segment area, the nuclear layers, or the synaptic communication in the plexiform layers. The inherent complexity of the retina in both structure and function requires the cooperative effort of observation from a variety of disciplines for clarification.

Until proven otherwise, it is sound to assume that pathological changes denote physiological perturbations that are ultimately dysfunctional. Because of the great redundancy of the retinal visual apparatus and the large latitude of operations of the visual process,
minor physiological changes may go unnoticed until stressful conditions require the optimal performance from the system. To decipher the cryptic relationship of morphology and function requires a better understanding and elucidation of the biophysical processes underlying the visual response of the light-sensing elements of the retina.

* Subsequent work with low fluences, less than one particle passage per cell, of argon and iron HZE particles has shown late pathological effects to cells, within the track of the particle.
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APPENDIX I. NECTURUS MACULOSUS—LIGHT-SENSING ELEMENTS

The light microscopy of the Necturus maculosus visual cell was described by Howard. Three types of visual cells—rods, cones, and double cones—are distributed fairly evenly within the retina. Palmer found the Necturus maculosus retina to contain 110,000 receptor cells—48 percent rods, 38 percent cones, and 10 percent double cones. Rods are 12 microns in diameter and 30 microns long; cones and double cones are from 9 to 15 microns wide and 24 microns long.

A ciliary process arises from the basal body in the distal portion of the inner segment to form the backbone structure of the outer segments. Below the outer segments are clumps of mitochondria-like micelles, the ellipsoid, and clumps of glycogen deposits, the paraboloids. The rod outer segments in Necturus maculosus are cut into columns radially arranged about a solid center by a series of 22 to 31 deep longitudinal fissures. Dendritic fingers originating in the inner segment run up the lower portion of these grooves. Possible functions of these filaments include support, communication, nutrient transport, energy transport, and mobility. In some vascular retina containing a variety of support (glial) cells, these dendritic arrangements are not seen; instead, processes arising from other cells are observed. The cone cells do not contain the vertical fissures found in Necturus maculosus rod outer segments but they do have a similar palisade of dendritic fingers surrounding the outer portion.

The ciliary processes of Necturus are composed of nine triple filaments in an arrangement typical of cilia. The profissural portion
of the cilium contains nine double filaments arranged periplurally but it lacks the additional nine secondary and two central filaments present in motile cilia. The cilium extends about halfway up the rod outer segment and considerably farther in the cones.

The outer segment communicates through a system of fine cytoplasmic processes to the pigment epithelium and to the inner segment by the dendrites. Large numbers of pigment granules migrate within the matrix of the outer segment in response to light adaptation. Nutrients may also be supplied by the interdigitation of the pigment epithelium.

In the disc membrane, a number of particles are found—about 140 particles per square micron, each containing about 50 molecules of porphyropsin which is the visual pigment of Necturus. The outer segments of rods and cones are quasi-crystalline structures in the sense that many of their corresponding molecules display a high degree of mutual orientation. This structure may be important in inducing a visual response. Several types of signal transfer have been proposed for a crystalline structure, exciton migrations—the propagation of an excited state—and photoconduction. The exact mechanism remains uncertain. Although both rods and cones have photosensitive membranes, the organization of these membranes is different. The lamellae of the cones are a continuous membrane whereas those of the rods are pinched off to form discs. Cones have a somewhat different shape than rods, and their filaments are longer and rounder than those of rods. The synaptic connections of cones are larger, and the basal processes are more complex, being riddled with numerous synaptic vesicles of dendrites of cells from the inner nuclear layer.
APPENDIX II. POISSON DISTRIBUTION

Assume a function $P(X = x)$ for $x = 0, 1, 2, 3, \ldots$. If $X$ is binomially distributed then

$$P(X = x) = \binom{n}{x} p^x q^{n-x}$$ (1)

where the expectation $E(X) = np$.

Let $\lambda = np$ and $p = \lambda/n$.

Then (1) becomes

$$P(X = x) = \binom{n}{x} \left(\frac{\lambda}{n}\right)^x (1 - \frac{\lambda}{n})^{n-x}$$

$$= \frac{n(n-1)(n-2)\ldots(n-x)}{x!} \left(\frac{\lambda}{n}\right)^x (1 - \frac{\lambda}{n})^{n-x}$$

$$= \frac{(1 - \frac{1}{n})(1 - \frac{2}{n})\ldots(1 - \frac{x-1}{n})}{x!} \left(\frac{\lambda}{n}\right)^x (1 - \frac{\lambda}{n})^{n-x}$$ (2)

As $n \to \infty$

$$(1 - \frac{1}{n})(1 - \frac{2}{n})\ldots(1 - \frac{x-1}{n}) \to 1$$ (3)

and

$$(1 - \frac{\lambda}{n})^{n-x} = (1 - \frac{\lambda}{n})^n (1 - \frac{\lambda}{n})^{-x} \to (e^{-\lambda})(1) = e^{-\lambda}$$ (4)

using the result from calculus

$$\lim_{n \to \infty} (1 + \frac{u}{n})^n = e^u$$ (5)

Therefore, as $n$ becomes large and $X$ remains constant using equations (3), (4) and (5) in (2)

$$P(X = x) \approx \frac{x^x e^{-x}}{x!}$$
which is the Poisson distribution.

For the binomial distribution, if $n$ is large while the probability $p$ of occurrence of an event is close to zero, the event is called a rare event. When $\lambda = np$ is less than 5 the binomial distribution is very closely approximated by the Poisson distribution.

The calculation of target hit probability at low fluxes (dose) may be approximated by the Poisson distribution. The typical mouse rod photoreceptor nuclei is 3.5 to 4 microns, $\approx 4 \times 10^{-8}$ cm$^2$. For a flux of 1 particle/cm$^2$ the probability of interaction is very small. For $\lambda$ close to unity the probability for a hit on a target becomes large. $\lambda = np \approx 1 + n = 2.5 \times 10^7$.

This implies that at doses corresponding to 6.4 rads of $\alpha$ particles or 100 rads of neon particles would insure a single hit per cell nuclei. Several factors combine to moderate the flux required for interaction including the finite radius of the partial path, the possible penetration of a single particle of multiple targets, other possible critical cell targets, and the passage of a particle through a cell without interacting.
APPENDIX III:

The Retina in Cross Section
A Use of Dry Fracture for Scanning Electron Microscopy

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Retina was first prepared for Scanning Electron Microscopy by Critical Point Drying. Retina dried in this manner was then fractured, coated, and observed under the electron beam. The interdigitating nature of the Outer Segment layer of the retina allowed, upon fracture, the lateral aspects of these organelles responsible for photon adsorption and capture stimuli to be exposed to view. After fracture there was no evidence of mechanical damage obliterating structural detail. The next layer, the Inner Segment layer, exhibited like characteristics. In mammals dendritic and glial processes, arising from below the tight junctions separating the Nuclear layers from the Inner and Outer Segments of rods, were visible. The nuclei were clearly evident by either hemispherical depressions or bulging nuclei scattered along the nuclear layers of the anterior portion of the retina.

Treatment of the retina by dry fracturing was found to be useful for both mammalian and amphibian retina. In retina damaged to prevent light stimuli response characteristic morphological changes and degeneration of retinal cells and organelles were identifiable.

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The value of the scanning electron microscope (SEM) lies in its ability to demonstrate surface properties. The surface of a cell is of extreme importance because all the materials and actions necessary for life as well as all communication and information must pass this barrier. While there are a number of methods used by electron microscopists to visualize the internal structure of tissue, there are few techniques available to show surface structure. Critical point drying combined with dry fracture reveals fine-structural, three-dimensional details not seen with other methods.
Retinal detachment is a pathological condition with subsequent loss of vision. Though uncommon in nature, retinal detachment can be induced in the laboratory to facilitate the visualization of those portions of the retina normally interdigitated with the pigment epithelium—the rods and cones. Since these elements are long, only their tips are exposed after detachment. In order to explore their lateral aspects, it is necessary to cut or fracture the retina. A slice through the retina is indiscriminate of cellular components and the technique of freeze fracture, which, while following membranes, does not necessarily follow the surface (Fig. IIA). Dry fracture is advantageous in that it follows the path of least resistance across the retina, in general, along and around the surfaces that are to be explored.

MATERIALS AND METHODS

Vascular retina was perfused with glutaraldehyde solution (1) in situ. After enucleation, the anterior of the eye was removed, and the retina was detached. Avascular retina was perfused after enucleation by cross laminar flow under the lens with a hypodermic needle supplying the perfusate on one side and an exit hole on the opposite side. A combination of glutaraldehyde fixation (2) with OsO₄ (3) postfixation was followed by ethanol dehydration to fresh TF, 113, acetone or amylacetate depending on the critical point drying (CPD) technique to be used (4). After CPD, specimens were mounted on SEM studs with silver paint. Fracturing was done on the stud under the dissecting microscope with forceps either before mounting, in order to orient the fractured surface perpendicular to the beam perpendicular to the beam or after the specimen was mounted in approximately the correct position to view the expected fracture. Specimens were coated with gold, platinum or palladium singly or in combination. SEM was done with Japan Electron Optical Laboratory (JEOL), Cambridge, Coates and Welter, and Advanced Materials Research (AMR) 1600 scanning electron microscopes.
Fractured specimens exhibited very little mechanical damage to the outer or inner segment portions of the retina. Rods of amphibians (Fig. IIIA), mice (Fig. IIIB), and dogs (Fig. IIIC), show many common characteristics.
In amphibians, the basic layers of the retinal nuclei are clearly present (Fig. A) and provide information on the distribution and organization of the retina. In mammals, the nuclear layers are likewise present as are a number of glial and supporting tissues (Fig. B). In amphibians, the filamentous elements originating in inner segments can be seen clearly surrounding the lower portions of the outer segments (Fig. C). The fine structure around the tight junctions separating the cell body from the inner segments can also be seen (Fig. D).
In mice, numerous filaments originating below the tight junction can be seen around the inner segments in Figure VA. Figures VB and VC show changes with time after the irradiation of the retina.

CONCLUSIONS

Dry fracture is an effective technique to view the lateral aspects of the inner and outer segments of the retina. Information about these structures and their interaction and communication with other retinal elements is revealed by fracturing. Additional information on other elements of the retina, including width, organization and structure, can be obtained. In general, dry fracture is capable of providing information about the retina that is not obtainable by other techniques and, in conjunction with other techniques, helps provide a more complete understanding of retinal morphology.
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APPENDIX IV. A THIN FILM FLOW MODEL CONSTRAINED
BY A CYLINDRICAL BOUNDARY

Introduction

Although the mechanisms and processes of membrane formations are unknown, the spontaneous formation of lipid or membrane vesicles suggests a number of possibilities. It seems probable that the hydrophobic and hydrophylic forces associated with the aqueous and lipid, polar and nonpolar phases are responsible for this spontaneous formation. If the driving forces for such a reaction are phase dependent then any two phase system should exhibit similar properties.

As a first approximation for the construction of a membrane producing system a two phase, liquid-gas, system was chosen. With the intention of working on a larger than life model of membrane production, a system of lesser density was required. The ability to selectively deal with one phase, manipulating it with state-of-the-art physical interactions rather than biochemical means, makes a liquid-gas system preferable to a polar-nonpolar system. In general the parameters of a two phase system are similar but the choice of phases determines the size of the model.

Materials and Methods

In order to simulate the rod outer segment, a cylindrical boundary condition was imposed on the membrane synthesis model system. A radial symmetry around a central axis was created by a tubular piece of plastic. Holes in the plastic were provided for the introduction and extraction of liquids. The axis of symmetry was parallel to the gravity vector.
in order to minimize differential gravavectic forces. Membrane propagation was from the bottom to the top. A limiting upper boundary was a one hole cork provided with a glass tube terminating in a nozzle. Cylinders of diameter from 2 to 5 cm were tried. The first system consisted of two 30 cm long plastic tubes joined end to end (Figure 1). In operation the top was occluded to cause the escape of gas from the annulus formed by the junction of the two tubes. The second system was formed by rolling a rectangular micropore filter into a cylinder and taping the joint together. The length was typically 30 cm while the diameter was variable (Figure 2).

The liquid layers were formed by introducing water saturated air into the system through a small (≈1 mm) nozzle formed by melting the end of a glass tube. The nozzle was located inferior to the water-soap liquid boundary.

Results

In operation the gas was introduced at varying rates into the system, liquid discs were propelled up the tube and the spacing between discs was controlled by varying the partial pressure along the axis of the cylinder and the size of the opening at the top. The air introduced through the nozzle was bounded by a then spherical liquid boundary. As the radius of the sphere approached that of the cylinder, the edge merged to the surface of the cylinder and a convex thin film would propagate upwards. The radius of the film varied with the amount of air induced into the bottom of the system. When the air was shut off the film assumed a concave appearance due to the force of gravity.
APP. IV - Fig. 1 & 2
In a steady state situation a flat disc could be formed by introducing sufficient pressure to offset the force of gravity and cause an upward migration of flat discs. The spacing between discs was controllable manually by varying the pressure at the top of the tube in the first case and on the outside of the cylinder in the second. It was possible to form stacks of discs of similar spacing by these methods.

Discussion

The initial success of this system suggests the feasibility of modeling a membrane synthetic system. Several improvements can be made in the model. A selective doping of layers with the ultimate elimination of the gas from between thin layers may be a way of forming a polar-nonpolar biphasic system. Doping could be accomplished by a number of means. Selective components could be introduced into the space between thin films as they propagate upwards. The use of separate nozzles to form alternating environments using different gases with different diffusion properties is possible.

An all liquid system could be constructed by sending bolusis of polar and nonpolar liquids through a common port into a bounded chamber. Whether this is a feasible approach has not been demonstrated. For the present more information is needed to approximate the thermodynamic properties responsible for the operation of this model system and the rod outer segment discs system. It is plausible that the operation of this system falls under the constraints of a non-equilibrium thermodynamic system far from equilibrium. Whether the information gained from a gas-liquid biphasic system can be applied to a polar-nonpolar biphasic system is unknown. However, the possibilities are intriguing.