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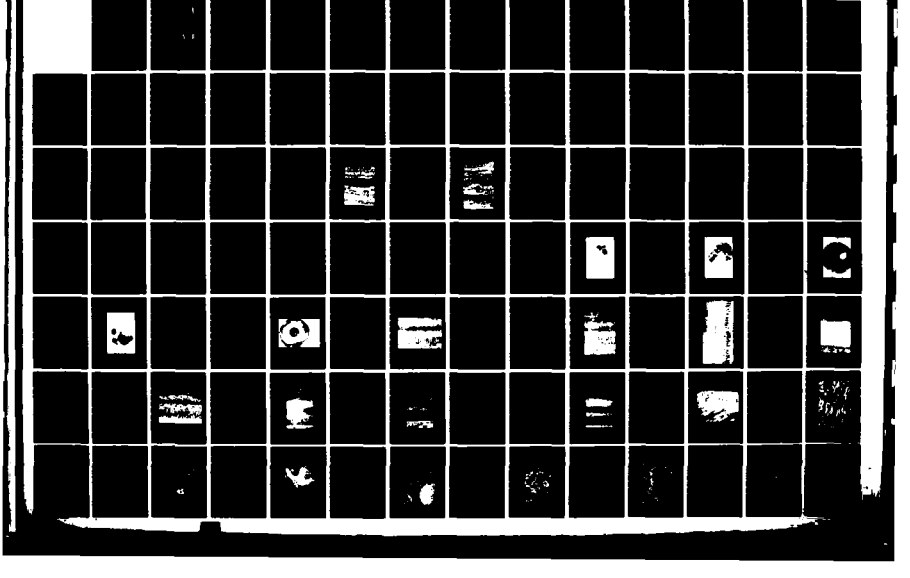

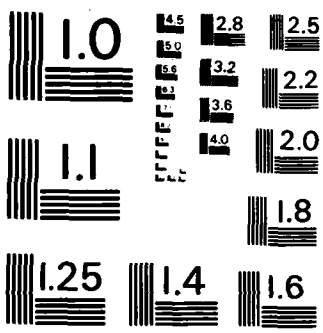

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Mechanisms of Retinal Damage from Chronic Laser Radiation

Final Report

T. Lawwill, M.D. and R.S. Crockett, Ph.D.

July 1981

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ANIMAL USE STATEMENT

In conducting the research described in this report, the investigator adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences-National Research Council.

ABSTRACT

This report covers and summarizes the results of an extensive investigation concerning retinal damage from chronic exposure to intense visible light. Conclusions are drawn from threshold studies of retinal damage. Several mechanisms by which the damage can be caused are discussed in relation to their importance for primate retina.

Three specific experiments are presented. The first is related to determining thresholds and the action spectrum for retinal damage. The development of methods of evaluating damage, both functional and anatomical, is outlined along with an extensive anatomical description of the damage based upon clinical, histopathological and ultrastructural findings. This experiment further substantiates that short wavelength light is more effective than green light which we first determined in 1976 and presented in 1978. The anatomical description of this type damage is expanded beyond that presented in earlier reports. The second experiment concludes in finding a wavelength dependent differential cone specific functional deficit as measured with pattern ERG. This damage is found separately for the blue, red and green sensitive cones depending upon the exposure wavelength. The level of damage is similar to that causing the short wavelength damage in primates. The third experiment presented is the evaluation of light damage in an isolated retinal tissue in culture. The pigment epithelium is determined to be almost equally susceptible to damage in vitro as in vivo and the same action spectrum is similar in the short and intermediate regions of the visible spectrum.

The conclusion of this report is that three major damage pathways are simultaneously active and that they exist in different proportions depending upon the intensity, wavelength, exposure time and the species of animal. One effect is rhodopsin mediated, one is modulated by specific cone pigment, and one is dependent upon chromophores which exist in essentially all retinal cells. The latter, short wavelength effect is hypothesized to be a photobiological effect having its direct site of action located in enzymes of respiration.

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I. INTRODUCTION:

Both positive and negative effects of the environmental light on the human have been known to exist for ages. The immediate effect of sunburn of the skin could not be missed even by primitive people. A longer term effect of the sun's rays, carcinogenosis, was first suspected by association and later verified as a scientific fact[1]. The fact now supports a large present day research effort into the mechanism by which sunlight modifies cell multiplication. The possible mechanisms run from direct DNA damage changing genetic code in the nucleus of the cell[2], to suppression of the immune and/or cellular genetic repair system by an as yet unknown mechanism[3]. Negative biological effects have been traced to various portions of the visible and ultraviolet spectrum, and each portion of the spectrum might be expected to produce its effect by a different combination of mechanisms. There have also been a multitude of studies of physiological and pathological effects of light upon the eye.

The effects upon the eye caused by exposure to various light sources are based on a varied combination of mechanisms each of which are wavelength, intensity and time dependent. It is well known that the cornea is subject to "sunburn", which is caused by the ultraviolet wavelengths, while the visible wavelengths reach the retina and the longer wavelength infrared energy can heat the eye, particularly the iris and lens sometimes causing lenticular changes[4].

Damage caused by light that reaches the retina of the eye are also wavelength and time-intensity dependent. Well known retinal damage has been caused by individuals staring at the sun during eclipse[5], observing an atomic fireball [6], or intentionally by photocoagulation of

the retina for medical purposes. Damage to the retina caused by light is defined as any permanent identifiable change in structure or function identifiable histologically or by other means as having its effect in the retina. The retinal damage may be cell specific and manifested by cell loss or a reduction in the number of cells or nuclei within a retinal layer. Permanent changes also may be restricted to particular organelles of the cells and manifested by structural changes i.e. of the mitochondria of a cell. Theoretically, light damage also includes reversible pathological changes in structure and/or function which are repaired in several days or weeks i.e. by re-growth of the outer segments of the visual cells.

The effect of light to be discussed in this report is that caused by 1 to 4-hour exposure of the retina to the visible portion of the spectrum, at intensities less than that necessary to raise the retinal temperature one degree centigrade. We present a detailed description and evaluation of studies of various pathological states found in the light damaged primate retina over the period of support. We present new data on the progression through these states during damage and repair. A new analytical approach to light damage, which is the study of retinal cells in tissue culture, is also described. For this the pigment epithelium was selected and studied histologically with and without melanin pigment. Finally, this report presents an analysis of three separate pathways of pathological change caused by light and relates these pathways to the exposure parameters which determine them. A new hypothesis is presented on the pathway of the damage produced by short wavelength visible light.

The analysis of past and present work leads to the thesis that at least three effects together account for the damage sustained with the parameters given. These parameters are: (1) wavelengths of visible light, (2) exposures of 30 minutes or longer, (3) intensities within one log unit of threshold for ophthalmoscopically visible lesions for the given exposure times which do not increase the temperature of the retina significantly.

The three mechanisms to be discussed are highlighted by: (1) a rhodopsin mediated effect expressing itself in rod cell and pigment epithelium pathology as described by Noell[8], (2) a cone type specific functional effect[9], and (3) a short wavelength effect first described by Ham and Mueller[10], and Lawwill[11] which involves inner as well as outer retinal layers. These three effects are modulated by different variables and occur together in different proportions in primate retinas and probably others as well. The isolation of any one effect is difficult to achieve in the primate retina. Each may have a distinctly different mechanism(s) of action but all may share similar final pathways to irreversible damage.

If isolated, the first mechanism is dependent upon visual pigment concentration in the rods. It is enhanced by long term dark adaptation preceeding the exposure. It is also enhanced by increased body (eye) temperature. The damage is first shown by the rod outer segments but it includes ultimately the pigment epithelium and all parts of the rod cell. One might postulate a series of events beginning with the excessive but physiological action on the visual pigment followed by photoproduct enhanced destructive oxidation and/or metabolic derangements leading to a final common pathway of cellular degeneration.

For the second mechanism one might think of a specific class of cones being bombarded with a level of light energy in their characteristic spectral range but above normal physiological intensity. Cone cells become "over stressed"; intra- and extracellular changes occur that are incompatible with normal cell function and viability. Thus the cells can no longer maintain their integrity, their plasma membrane is breeched and they die. At threshold, the majority of cells that die or are permanently changed are the cones responding to the incident wavelength.

In the third mechanism, all of the retina is bombarded with photons of a relatively high energy, bombs looking for places to go off. The photons are absorbed by many different molecules, including the melanin of the pigment epithelium, the molecules of DNA transcription and protein synthesis, specific molecules of the cell membranes and the enzymes of important cytoplasmic or mitochondrial functions. Possibly their absorption by molecules in the nucleus causes the death of the cells. Maybe involvement of ribosomes and/or smooth endoplasmic reticulum stops the cell's synthetic machinery. Possibly also absorption by the oxidative enzymes of the mitochondria blocks energy yield of reactions on which cell life depends. One or all of these actions may be primarily responsible for the damage caused by the short wavelength light. This third mechanism is the prominent theme of this report because it seems to be the major route of acutely induced light damage in the primate retina.

II. BACKGROUND:

A. Animal Models of Light Damage.

The most recent surge in interest in the pathological effects of light exposure on the retina was initiated by reports by Noell and co-workers in 1966[12]. Noell reported that irreversible retinal damage occurs in normal laboratory rats exposed continuously to an illuminated environment. He noted that the threshold for this damage was lowered when the animals body temperature was raised. He also noted that divided doses of light produced damage more effectively than continuous exposure of the same total length and intensity. Sensitivity to damage was greater with green light than with either red or blue light. In fact, the action spectrum of the damage paralleled the action spectrum of the ERG. The iris of pigmented rats provided significant protection when compared with albino animals. Several days or weeks of dark adaptation prior to this damaging light exposure was associated with greater damage to the same light for the same period of time than in animals which had lived in cyclic light. Later Noell et al[13,14] demonstrated that vitamin A deficiency protected rats from light damage of the severe type provided rhodopsin content was reduced by the deficiency. The conclusions drawn from this initial work were that the rod's visual pigment mediates the retina damage in the rat and that the physiological reaction of the photoreceptor cell becomes a damaging one when light is strong and prolonged.

Noell divides the effects into at least 2 types, one expressed by the death of the rod cells and the pigment epithelium and the other by a slower, partly reversible rod cell degeneration[15]. It appears that a normal light cycle protects the rat eye somewhat from the most severe effect. Noell also notes that when the receptor cells had died and disappeared in the rdy (RCS) rat, the pigment epithelium remained uneffected by light of the same intensity and duration which destroyed it in the normal rat[15].

Rudeen and O'Steen[16] have stressed the hormonal influence on susceptibility to retinal damage, another evidence that the normal physiological rhythms in part determine the effect of light upon the retina. Hollyfield[17] discovered that continuous dark exposure could also cause receptor disorganization but this observation was made on frogs. However, it adds another bit of interest to the hypothesis that any significant alteration in the normal rhythm of illumination can cause retinal damage or change its threshold to damage.

Another line of investigation has been more applied using different models. Friedman and Kuwabara[18], Tso et al[19], Dawson and Herron[20], Hochheimer et al[21], have described the retinal effects of specific ophthalmic instruments presently in clinical use. Dawson and Herron[20] began with indirect ophthalmoscopy, d[illegible]ng prolonged changes in dark adaptation in patients after examination [illegible]nstrument. Friedman and Kuwabara[18] produced severe lesions with 30 [illegible] exposures of the monkey retina to the indirect ophthalmoscope in an artificial situation where the image was held stationary. Hochheimer et al[21] produced severe retinal damage in monkeys by exposing them to an ophthalmic operating microscope for 15 minutes. These studies have given evidence of the potential importance of light

damage in ophthalmology. Lawwill et al[22] has calculated retinal exposure with the office slit lamp and Hruby lens. The possibility exists that damage thresholds can be reached with this device.

A series of studies beginning with the effect of short atomic bomb flashes up to and including 1000 sec. exposures have been made by Ham et al[23] over several years time. In general, he has utilized small area spots and ophthalmoscopic detection of the lesions to describe the threshold under various conditions of time and light wavelength. He has published evidence that the shorter wavelength blue light (441 nm) is more effective in producing these lesions[24].

The rabbit as studied by Lawwill et al[25] (supported by contract No. DADA 17-68-8105), responded to light damage differently than rats although the retinas of both species are rod dominant. More energy was required in the rabbit and the action spectrum was different. The rabbit retina was more sensitive to shorter wavelength light than to medium wavelength, and the pigment epithelium was relatively less affected. Damage was seen also in non-receptor cells of the retina. These studies on the rabbit provided the basis for the extensive analysis of damage by light in the monkey's retina. It demonstrates the particular sensitivity of the primate retina to short-wavelength (blue) light and the involvement of the inner as well as outer layers.

II. B. Overview of Possible Mechanisms.

If the receptor as well as non-receptor cells of the retina are particularly sensitive to short wavelength light one must postulate that the damaging light is absorbed by some molecule which is not a visual pigment (e.g. rhodopsin). Since short wavelength ocular transmission to the retina is blocked due to lenticular absorption below 400 nm, one may be investigating with blue light an effect several log units down from the peak sensitivity of the damaging wavelength region, or one may be at a wavelength near or at the peak. The cut-off filter of the optical media limits the transmission to the retina of the higher energy short wavelength photons. This gives an effectiveness spectrum which is an algebraic combination of the transmission spectrum and the actual action spectrum. Thus the "effectiveness" spectrum may be very different from the action spectrum which is required to identify the molecules that absorb the damaging light. Similar effectiveness spectrums have been well described for sunburn[26].

There is reason to believe that at least one of the mechanisms involves the visual pigment rhodopsin, based on Noell's work showing rat retinal damage sensitivity peaking in the center of the visual spectrum. Whether this effect is primarily a molecular change that is damaging or whether the bleaching of a large portion of the visual pigment sensitizes the cells metabolically to damage or releases a chromophore to mediate a photo-sensitive reaction is not known. There is also evidence from Harwerth and Sperling's work[9] that repeated exposure to low intensity light may damage cone cells at wavelengths which peak at the absorption maximum of the specific cone pigment.

An area not covered in this report is damage by heat. An important distinction must be drawn between heat and photochemical effects. It is a distinction between rotational and vibrational molecular vs. electronic effects. The absorption spectrum is peculiar to a particular molecule. A photon may be absorbed only when its energy matches the energy required for a possible transition between energy states of the molecule. The energy supplied by a photon is determined by its wavelength, ($E = hv$). The critical energies for photochemical reactions extend into the visible spectrum. When transition to an excited state is caused by the absorption of the photon, the change in electron orbital position may cause formation or breakage of a chemical bond. This would be a photochemical effect as opposed to the generalized effect of molecular motion expressed as a rise in temperature. Raising the basic molecular kinetic energy, heating, may enhance superimposed photochemical reactions. In general, we propose that a complex interaction occurs which includes photochemical as well as physiological metabolic processes, in which there are many energy transitions not necessarily photic even though the first, primary step is light dependent.

When the energy state of a molecule has been raised, it seeks its ground state. Return can be achieved by emitting another photon, a process called fluorescence. A state of molecular excitation may be a singlet state with no change in electron spin, a short lived state of high reactivity which can then transition into a triplet state with reversal of electron spin. The triplet state can last several seconds and is also highly chemically reactive. Sensitization to photochemical reaction can occur when the

sensitizing agent by chemical binding either increases the absorption of photons of a particular wavelength or decreases the activation energy required for a reaction. Photochemical reactions which are biologically damaging often occur through intermediates such as singlet oxygen, peroxides, and other radicals or excited molecules. In the retina, it would be most useful to know what possible sensitizing compounds or molecules are normally present or which might be produced by light exposure. Cell components which may be altered directly by these reactions include nuclear DNA, RNA, proteins, and the membranes of cellular organelles and of the cell. Most studies suggest DNA effects are most prominent with high energy photons of wavelengths less than 300 nm. Dimer formation, strand breakage and cross linking, are possible effects which may permanently alter the cell. Damaging effects on enzyme systems could be acutely devastating to the cell. Energy yielding processes carried out in the mitochondria are of particular importance and the effect of light on mitochondrial function will be elaborated upon below. Membrane damage by visible light has been reported in microorganisms[27].

II. C. Overview of Anatomical and Functional Parameters.

The primate retinal layers are made up of cells with vastly different functions which are in the most part highly specialized. Not only are the cells specialized but so are the areas of the retina i.e. macular, peripheral. These variations in structure and function give rise to differences in the effect of damaging light.

Beginning with the outer layers, the choroid is important as a supplier of nutrients and as a heat sink and energy absorber. The heat sink function is not as important in low level light damage, but the nutrient function may be. A secondary effect of a long light exposure is edema and cellular swelling and disruption of the function of the pigment epithelium. The receptor cells may become physically separated from the pigment epithelium, disturbing the metabolic support of the receptors. Gross separation as in retinal detachment is known to cause degeneration of receptors and minor separation may play a part in the damage attendant with light exposure[28].

Bruch's membrane has been found thickened in light damage[11]. The basal cell membrane of the pigment epithelium apposed to Bruch's membrane has a peculiar enfolding which is susceptible to damage. The pigment epithelial cells are pluripotential and may form phagocytic cells which migrate through the subretinal space after the intercellular tight junctions and attachment to Bruch's membrane are broken. The apical membrane of the pigment epithelial cells carries the microvilli. These extend up between the outer segments to meet the Müller cell processes coming the other way. At the base of the microvilli are the cigar shaped melanin granules pallisaded in a row at the inner surface of the cell. This is an area of concentrated light absorption, a part of a very carefully arranged black screen. The granules also may have physiological or chemical properties of

absorbing or generating toxic radicals[48] along with their function of absorbing light. In light damage this is one of the first or a prominent area of structural change[11].

The outer segments are fragile structures containing a stack of membrane discs with an exceptional phospholipid matrix of high fluidity. They are continually renewed by new membrane formation at their base where visual pigment incorporation occurs. Light damage is frequently associated with a disorganization of outer segment membranes. The inner segments on the other hand are packed with mitochondria reflecting the high metabolic needs of the cells. The mitochondria of the rods are long and slender compared to the thicker ones in the cones. Specific functional differentiation is suspected. Light damage, as will be described affects differentially the mitochondria of rods and cones.

The outer nuclear layer contains the nuclei of the rods and cones with the cone cells lining the outer edge of the layer with their more lightly stained nuclei near their wide inner segments. Sometimes one or two cone nuclei are even found in the inner segment. Cells in the outer nuclei layer show damage by pyknosis or karyorrhexis as well as karyolysis and are lost individually in light damage[11]. There is no reason to expect strong absorbing properties of this layer to visible lights as one does for the outer segment with its visual pigment and the inner segment with its stacks of mitochondria and concentrated cytochromes and flavins.

The outer plexiform layer contains the proximal processes of rods and cones making contact with the processes of the next neurons. This layer is particularly susceptible to edema or swelling of cellular processes in light damage[11]. The bipolar cell layer has several specialized types of cells. This layer has the most distal extension of

the capillaries while the choroid is the source for the nutrients to all outer layers. Therefore the level of nutrients and oxygen tension may vary significantly throughout the receptor cell regions. This is important because many photochemical reactions that are biologically harmful are oxidative ones and depend upon the concentration of oxygen. Formation of singlet oxygen which is highly reactive, is believed to be the first step in a cascade of reactions that leads to the destruction or inactivation of a biological system during light exposure.

The ganglion cell and nerve fiber layers are nearly transparent but the interface with the vitreous is prominently seen with deep blue illumination. Fiber layer defects in glaucomatous eyes can be seen in blue light with the ophthalmoscope. The latter suggests that blue light transmission is affected at this border by scatter and reflection.

A particular kind of specialization in the retina is represented by the fovea and macula. In the center of the macula, the most proximal retinal region is the outer plexiform layer, and just lateral to this the inner layers are usually thickened. A yellow carotenoid pigment derived from the diet is accumulated[29] in this region and is most concentrated in the center 1 mm decreasing rapidly over a 1-2 mm radius from the center of the fovea. This yellow pigment layer partially screens the area from short wavelength light. It has an optical density of approximately .5 at 460 nm, .2 at 515 nm and .1 at 590 nm[30]. Other specializations of interest in the macular area include a high concentration of slender type cones and almost total exclusion of rods. Between 10^{0} and 15^{0} from the fovea the highest concentration of rods exists[31]. The melanin pigment concentration in the pigment epithelium also shows a gradient with the highest concentration in the central macular area decreasing toward the periphery. All of

these differentiations may have a part in determining the type and distribution of damage by light. There may also be metabolic differences with similar circumferential distribution around the fovea[32].

In the rabbit these differentiations are arranged linearly instead of circumferentially. More of the inner retina derives its nutrition from the choroidal blood supply. The area of the most acute vision in the rabbits lies in a horizontal strip along the anatomically defined visual streak. There is no specialized accumulation of cone cells in this region. In spite of these differences light damage in the rabbit has many similar features as found in the monkey[25].

The retina is metabolically a very active organ and a great portion of this activity is located in the photoreceptors, especially in the absence of light stimulation. The neural cells are limited in their biological activity while the pigment epithelial cells perform many functions and can de-differentiate and re-specialize in some of these functions, most notably phagocytosis. In addition, they may have the ability to divide. All retinal cells are liable to denaturing events, i.e. thermal covalent bond breaking and high energy photon induced enzyme or genetic defects. The reparative powers of the retinal cells are probably very limited.

II. D. Overview of Physical Parameters.

In order for light energy to cause pathological changes it must be absorbed. The portion of energy absorbed in any tissue is dependent upon the transparency or opacity of the tissue for the incident light. The transparency is dependent upon the wavelength components of the light. Just as a red filter passes longer wavelength light and absorbs the shorter wavelength blue and green light, the cornea absorbs essentially all of the ultraviolet below 310 nm[33]. Most of the near ultraviolet light between 310 and 400 nm is absorbed in the lens[33]. Thus, one would look first in the cornea for ocular damage from short wavelengths and in the lens for longer ultraviolet wavelength (UVA) damage. If not all of the near UV light were absorbed in the cornea and lens but passed further into the eye, and other components were sensitive, one might find damage deeper in the eye.

Transmission of the short wavelength light to the retina is increased in the aphakic condition. The ocular spectral transmission curve and the transmission curve for each component of the media and cellular layers is of utmost importance in knowing where in the eye each portion of energy at each wavelength might be absorbed. Several curves have been ascertained by direct measurement and by calculation[33]. Since each eye differs and marked changes also occur with age, only a general characterization is valid. The shortest wavelengths are stopped at the corneal epithelium and at high intensity can cause damage there. The arc welders' superficial keratitis and the "eyes open sun lamp enthusiasts' keratitis" are the typical human examples. The sharp cut off "filter" of ocular transmission to the retina for short wavelengths is at about 400 nm

and is attributable to absorption in the crystalline lens. The transmission changes several log units over a narrow spectral region. This does not prevent a very small fraction of the energy at wavelengths shorter than 400 nm from reaching the retina, but more posterior elements would have to be uniquely susceptible to sustain damage from wavelengths shorter than 390 nm. However, in the aphakic eye a significantly larger portion of the energy with a wavelength longer than 310 nm is transmitted to the retina. With the lens in place, cut-off at the red end of the spectrum is not as sharp but definitely exists. Heating of the front of the eye occurs in glass blowers causing effects in the crystalline lens without obvious effects on the retina. Almost any type of radiation in reasonable quantity can affect the crystalline lens in situ causing temporary or permanent opacification. The very long wavelengths of the CO_2 laser are effectively stopped at the ocular surface. Damage to the tissue outside of heating effects, which require a great deal of energy, have not been shown to be prominent at these longer wavelengths. Therefore, for the purposes of this presentation the cut-off at the longer wavelengths is of less importance. One should note that between 400 and 700 nm, the spectral transmission curve of the normal human eye is nearly flat and percentage transmission to the retina very high[33]. The problem for the future comes down to this: What portion of the energy at each wavelength between 380 and 700 nm is absorbed by which molecules in each retinal layer and by each retinal cell or structure?

The light which damages must be rigorously qualified and measured in its quantities. Many units have been used to measure light incident upon the retina in the light damage literature. Much confusion can arise from inexact or inaccurate measurements. Comparison of studies is sometimes difficult when spectral intensity curves of sources are not provided. In general, measurements are made in physical units relatable to intensity and wavelength or in physiological units related to the production of the visual response, photometric units. Physical units are watts for power or intensity and Joules for energy or power times time. These are then related to area, cm^2, or solid angle, steradian, and may be broken down per nanometer of wavelength.

Photometric units represent a potpourri of colorful names with difficult relatability to the physical units, i.e. nits, apostilbs, lux, lumens, trolands, candelas and others. All have their origin and utility in psychophysical studies where detectability and perceived brightness are the important factors. These measures have no place in the quantitative study of retinal light damage.

If light damage were solely a function of visual pigment bleaching, photometric units would be appropriate. But, if one starts by using photometric units the measures of true importance may be obscured because the visual pigment may not be involved and must be factored out again. As well, the human visual spectral response on which these units are based is not applicable to other animals. In the case of short wavelength light damage, a blue light (457 nm) which is of equal intensity (watts/ cm^2) to a green light (514 nm) is not only more damaging in the monkey but also less bright (has lower photometric value). Thus, the best record of light falling on the retina is in watts/cm^2 with a spectral distribution curve supplied.

The research to be presented in this report covers effects of light when applied to the retina over an extended area for an extended time. It is known that the dynamics of damage can change with changes in the size of the area of exposure[34]. This is particularly true of damage related to heating which may be dependent upon the qualities of the surrounding retina as a heat sink. The local conduction of heat becomes important for exposures lasting longer than a fraction of a second, and adjacent blood flow in the choroid becomes important for longer exposures[35,36]. The problems of light damage dependent upon instantaneous or prolonged heating of tissues or instantaneous heating by a pulse of energy, are not a theme for this report. Excluded also are the mechanical effects of the shock wave associated with the ultrashort pulses of q-switched lasers.

II. E. Evaluation of Light Damage.

Damage can be described anatomically or functionally. The study of functional effects helps in understanding the sequence of events that occur in retinal light damage and which may not be revealed by histology. Both temporary (reversible) and permanent (irreversible) damage occurs, and some damage may be detected on the functional level only. Sailors who stare over open water show measurable delay in dark-adaptation and raised thresholds[37] as do individuals who are examined by indirect ophthalmoscopy[20]. The histological status in these type conditions has not been determined, except for direct sungazing[38].

Visual function tests have been put to particularly good use by Sperling in his monkey studies[9,39]. He and his group have shown color specific destructive light adaptation in primates using light levels approximately three log units below those for the short wavelength light effect in the monkey experiments to be described. These effects are most convincingly shown for the "blue" cone receptors, but are also reported for the medium wavelength receptors.

The flash ERG is a well-known measure of retinal receptors and bipolar layer function. It reflects adaptational and luminance responses in a highly predictable and repeatable way[40]. It can be used to determine the gross state of function of rods or cones and, as well, gross changes in more inner layers. The a-wave reflects the outer segment response while the b-wave is dependent upon activity in the bipolar cell layer. The pattern ERG, once thought to reflect these same layers, is now believed to also reflect activity in the inner plexiform and ganglion cell layers. Both techniques combined are particularly appropriate for

the study of light damage in the retina. The VER is recorded from the scalp or skull and provides a record of the integrated response for the retina. It can be used to measure visual function for the aspect of spatial resolution. It is particularly important in evaluating lesions in the macular area since it is representative mainly of the response from the central 5^{0} of the visual field.

The most important techniques for evaluating permanent damage are the anatomical ones of light and electron microscopy. With respect to the short wavelength damage they were first put to use by Lawwill[41]. They demonstrated that light damage is much more complex pathologically than originally believed from work on the rat.

Figure 1, a and b, demonstrates the value of histology in describing and grading pathological changes from light damage. These five panels are drawn from the rabbit model. They depict the normal retina in section one and progressively more severe damage in 2-5. The lowest levels of detectable damage are shown in (a), bottom. The plentiful melanin pigment is normally located in the pigment epithelium. The outer segments are shortened and distorted. The inner segment mitochondrial area shows many small vacuoles and general cell swelling. A halo can be seen surrounding the nuclei in the outer nuclear layer. In figure 1 b top, the pigment epithelium is distorted and has lost some of its melanin to macrophages in the subretinal space. The outer segments are distorted and their ends are swollen. Some may be missing. There is thinning of the outer nuclear layer and swelling of both plexiform layers, the inner nuclear layer and of the ganglion cells. This is moderate damage graded 2+ on a scale from 0 to 4. More severe damage 3+ is shown in figure 1 b (middle). Here, the pigment epithelium appears almost necrotic. Melanin distribution is abnormal.

Legend

Figure 1 (a):

Figure 1 comprises a series of five rabbit retinas from normal to extreme light damage. The upper panel of (a) is a normal control. The lower panel shows 1.5+ damage with swollen and shortened outer segments. The inner segments have lucent areas which would correspond to swollen mitochondria seen with electron microscopy. The cells in the outer nuclear layer have nuclear halos. There are pyknotic cells in the ganglion cell layer. This figure is continued in (b). Toluidine blue stain. Upper X 150. Lower X 376.

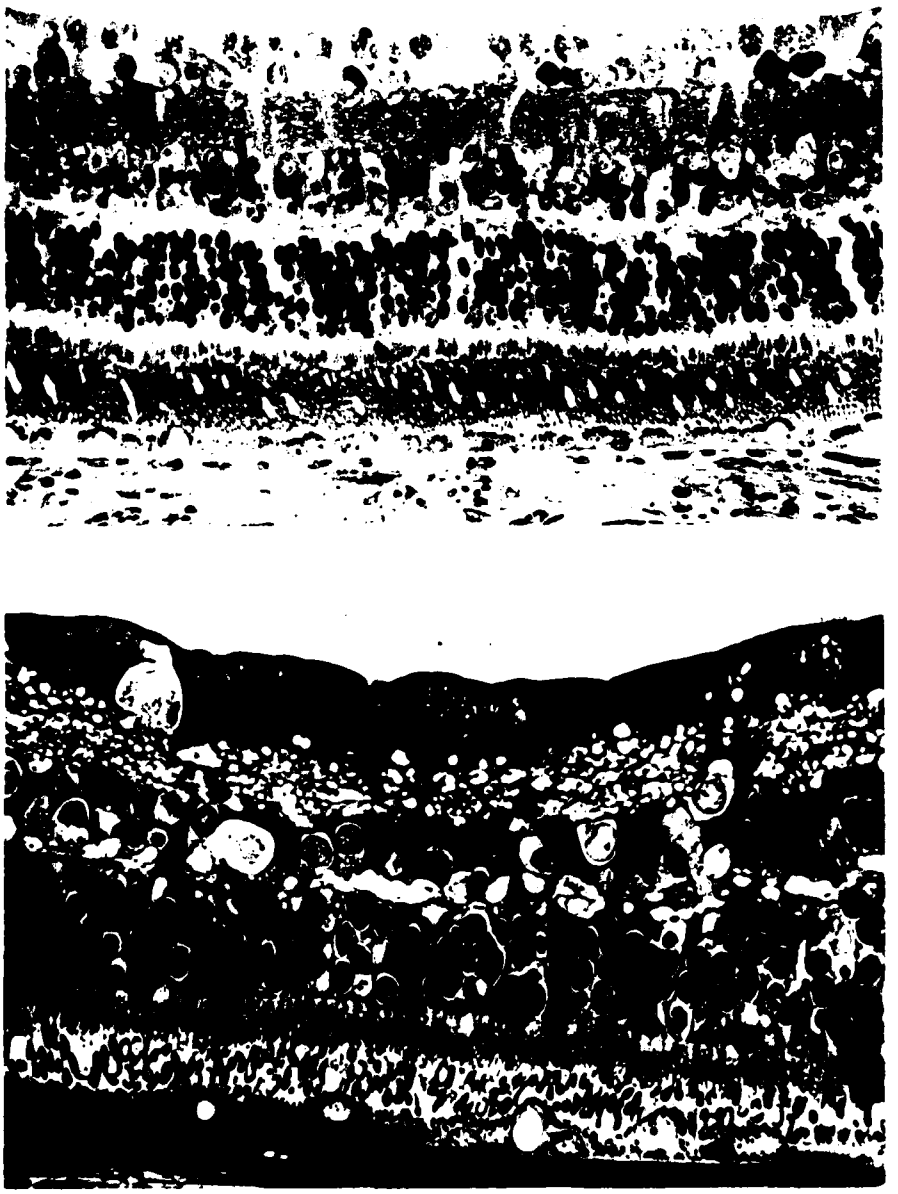

Figure 1a

Legend

Figure 1 (b):

The upper panel represents "2.5+" damage. The pigment epithelium is thinned; phagocytic cells contain much of the pigment. Pyknosis and ghosting are seen in the inner layer. The two lower panels show greater (3+ and 4+) damage. All layers are finally lost except outer nuclei and a thin pigment epithelium. Upper X 150, middle X 375, lower X 375; toluidine blue stain.

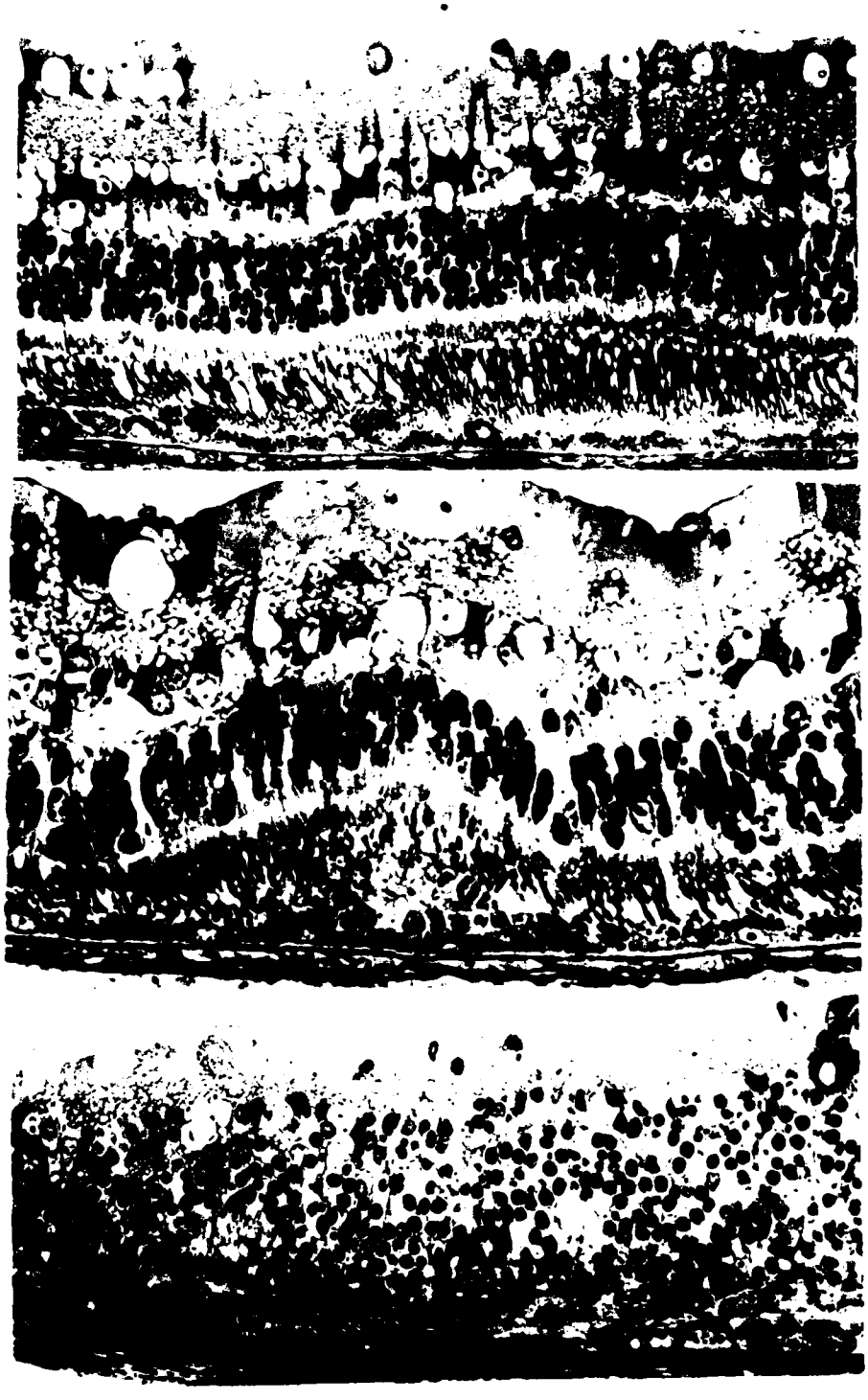

Figure 1b

Outer segments are completed disorganized. Inner segments are grossly swollen and some are missing. The outer nuclear layer is thinner. The bipolar and ganglion cells are necrotic and all their processes are enlarged. In the last section, figure 1 b bottom, severe (4+) damage is shown. All that remains are dislocated receptor nuclei, the damaged pigment epithelium, and a few cells of the inner layers.

These findings and the levels of light required to produce them contrast with previous findings on the rat and lead to the necessity for a primate model which is extensively discussed in following sections.

III. Experimental Research

A. General Introduction

In order to answer the many open questions on light damage and its mechanisms, a large scale study was launched in my laboratory to analyze light damage produced by long-term exposures in the monkey (supported by contract No. DAMD 1774C4026). All physical parameters of light exposure were rigidly controlled and accurately quantified. The exposures covered a large area of the fundus with an even field of various wavelength light. The effects were carefully measured by an array of clinical, functional and anatomical examinations. Functional measures included flash and bar-pattern electroretinography and the recording of the visual evoked cortical response. Clinical examination included indirect ophthalmoscopy and fluorescein angiography. The anatomical examinations included observation by light and electron microscopy.

The monkey was chosen as an experimental animal, being the closest possible model to the human. A four-hour exposure period was initially selected to be short enough to achieve accurate dosimetry and long enough to simulate the environment to which humans might be exposed.

Later, in order to prepare for the study of any cumulative effect that might be present from multiple repeated exposures, and to describe any time interval dependence, thresholds for one hour exposures were established. It was of practical importance to know whether light damage found from a divided dose regimen would have additive, less than additive or potentiating effects. This portion of the project was also undertaken to analyze mechanisms dependent upon visual pigment concentration and/or the metabolic state of the retina. If short exposure times

produced their damage through the visual pigment or shortlived photoproducts, one should expect a pathology similar to the rat model in Noell's "damage of the first kind".

Also, it would be convenient for technical reasons to use a shorter period of exposure than four hours if the pathology produced was the same. This was the case and therefore one hour exposures were chosen to follow the progress of damage in the latter part of this experiment.

The studies were performed on more than 100 monkey eyes followed up to eight months after exposure. They yielded unambiguous results, permitting a virtually complete description of the phenomenology of light damage in the primate.

In the following, three separate experiments will be presented with their methods, results and discussion in sequence. Experiments I and II present the work on monkeys. Experiment III is an approach to studying light damage in tissue culture of the pigment epithelium.

III. B. Experiment I: Light damage in the monkey retina.

In the description of Experiment I, I will give an account of data collected with 80 monkeys on the damaging effects of exposure to laser lights with emphasis on the cellular changes revealed by light and electronmicroscopic observations.

1. Methodology:

a. Animal Preparation:

Female rhesus or cynomolgus monkeys weighing between 3 and 4 kg were used. Prior to exposure, the animal was given 0.12 mg of atropine sulfate and 8 mg of phencyclidine HCl (Sernylan) @ intramuscularly. When the monkey was sedate, it was placed in a primate chair. Initiation of anesthesia was by means of a single 20 mg/kg intravenous dose of sodium pentobarbital. A constant intravenous infusion of sodium pentobarbital (0.22 mg/min., 2.84 mg/ml) was begun a short time later and continued throughout the exposure. The eye to be exposed was dilated with 1 drop each of atropine 1%, tropicamide 1%, and phenylephrine 10%.

b. Light Exposure:

The light was presented to the eye in Maxwellian view. The eye lid was held open by a Burian Allen contact lens electrode. This protected the cornea and kept it moist. The alignment was maintained by the experimenter's sighting through a beam splitter to allow direct visualization of the exposed fundus. The intensity of exposure was constantly monitored via a beam splitter in the light path reflecting on an Eppley thermopile. Exposures were for a period of from 15 minutes to 4 hours with a constant intensity light source covering 40 degrees solid angle (0.88 cm^2 of retina). The intensity of the beam was measured with a Gamma Scientific Model 2020a spectroradiometer calibrated with a standard of

spectral irradiance traceable to the National Bureau of Standards. The homogeneity of the field was determined with a small (2.5 mm diameter) cosine receptor by measuring the intensity at the center and edge of the field. Uniformity was maintained within 50%.

The retinal area exposed was calculated both from the angle of convergence of the incident beam and from direct measurement of the chord of the exposed section and the diameter of the eye using a freshly enucleated monkey eye. The area value used in calculating the irradiance was taken from the direct measurement. There was about 20% difference between the two results.

c. ERG recordings:

The ERG in response to a brief and intense flash was recorded binocularly prior to exposure at least twice a week until a stable pattern was achieved. After exposure, recordings were made at 24, 72, and 144 hours after the end of exposure, and 2 or 3 times per week thereafter. In brief, the animal was tranquilized, following pretreatment with atropine sulfate I.M. (0.012 mg/kg) with 8 mg phencyclidine HCl, I.M., and the pupils were dilated with tropicamide 1% and phenylephrine 10%. Modified Burian-Allen type monkey contact lens electrodes were inserted. The animal was pre-adapted in 1370 cd/m^2 white ganzfeld hemisphere. The flash stimulus was provided by a Grass PS2 photostimulator with the intensity set at 16. The flash lamp of the Grass instrument was placed inside the hemisphere to provide a ganzfeld type stimulus. Each recording session began in the dark 3 minutes after completion of the 2 minute period of light adaptation. Records were taken every 3 minutes for 27 minutes. Evaluation of the functional damage after exposure was made on the basis

of the decrease in amplitudes of the a and b waves and the persistence of this decrease after exposure. The response of the opposite eye served as a control. The damage was graded on a scale from 0 to 4+.

d. Clinical Procedures:

Ophthalmoscopy and fundus photography was performed regularly before and after exposure with the indirect ophthalmoscope and Zeiss fundus camera. A change was graded on a scale of 0 to 4+, without the grader having knowledge of the exposure level. The slightest question of edema or pigmentary change was graded as $\pm$. A definite change in appearance of the fundus no matter how transient, was graded 1+. A 4+ grading was assigned when there was extensive damage of retina and pigment epithelium. Fluorescein angiography was performed prior to exposure on each eye and again after exposure.

e. Tissue Preparation for Histology:

Following enucleation the globe was suspended by the 4 rectus muscles in a dish and covered by a solution of 3% glutaraldehyde in a 0.1 M phosphate buffer. The cornea was removed with a trephine and 4 radial cuts were made through the sclera between the rectus muscles. The zonules were cut, and the lens removed. A syringe was used to gently flush the eye with fixative, and the vitreous was cut out with scissors as it was forced through the anterior chamber opening. The whole procedure required less than 3 minutes after enucleation. These eyes were then left in fixative for 24 hours at room temperature.

After fixation the eye was placed in phosphate buffer and washed to remove the glutaraldehyde. Radial cuts were made passing through the macula to divide the globe into octants. These 8 pieces of retina were placed into tubes and labelled accordingly. The tissue was washed 3 times for 15 minutes in buffer, postfixed for one hour in 1% osmium tetroxide at $4^{o}C$, dehydrated with an alcohol series, and then infiltrated with a plastic embedding media. Each octant was then bissected radially to divide the globe into 16 pieces. Each of these pieces was cut into 4 parts with cuts running perpendicular to the bissecting one. Thus 64 pieces of retina were obtained. They were oriented in flat embedding molds and were hardened overnight at $65^{o}C$. One micron thick sections were cut from selected blocks on an ultramicrotome, placed on glass microscope slides and stained with a 1% toluidine blue-O in a phosphate buffer. After rinsing and drying a cover slip was applied, and the section viewed under oil on a Zeiss photomicroscope. Structures could be viewed that were not seen with the conventional hematoxylin-eosin stained paraffin embedded tissue. Selected blocks were then thin-sectioned, triple stained with lead citrate, uranyl acetate, and Reynold's lead citrate and processed for electron microscopy.

f. Histological Evaluation:

After viewing several thousand sections of normal and light damaged monkey retina, it was found that the same descriptive terms were continuously used. Some terms were peculiar to specific retinal cell types or even to specific intensity levels of exposure. These realizations led to the formulation of a classificatory rating system in which clusters of descriptive terms were placed on an ordinal scale of 5 steps ranging from 0 to 4. Midstep ratings expanded the scale to 9 steps.

Figure 2 is a reproduction of the scoring sheet which was completed for each retinal section. The terms appearing in Column 3 were used when minor histological changes were noted. The assignment of a 0.5 or 1.0 rating was dependent upon the degree or incidence of the effect observed. It was not unusual that a rating of 0.5 was assigned to non-exposed retinas. Very rarely, however, was a 1.0 rating given to an untreated section. Therefore, damage required grades greater than 1.0. While this grading is a somewhat subjective system of evaluation, it proved to be internally consistent and reliable on both intra- and inter-observer tests.

The rating scale is an ordinal one. Therefore, one can say only that a grade of 2 represents a greater effect than a grade of 1. Similarly, the same can be said of a grade of 1.5 compared to 1.0. However, the difference between grades 1.0 and 2.0 does not represent the same increase in effect as that by the step from 3.0 to 4.0. For this reason, only ordinal statistics are appropriate for analysis. If one treats the expanded scale (9 steps) as ranks with respect to damage, then one can compute the median rank observed for the retinal area exposed at a particular irradiance. The function relating median exposure and rank is a dose response plot, and it assumes the familiar ogival form. Since grades greater than 1.0 are a consequence of light exposure, the lowest irradiance yielding a median grade greater than 1.0 (i.e., 1.5) is taken to be the exposure threshold for a particular wavelength and cell type. Then, a plot of the threshold irradiance as a function of wavelength yields the action spectrum of damage.

Legend

Figure 2:

Score sheet for the evaluation of the histological findings. Each anatomical layer from choroid to ganglion cell layer is evaluated with respect to the presence or absence of the features entered in the row corresponding to the layer under study. If the observed features fall into only one column for a particular layer, then that layer is given the damage code appearing at the head of the column. If features are noted which occur in adjacent columns, the damage code would be the average of the two column headings.

Spec.#		Grid #	Animal #				
		Code	0-1	2	3	4	Remarks
1	PE		o vacuolization o edema o hyperchromasia o ghosting	o phagocytes o pigment shift o ↑ in lysosomes	o ↑ pigment o pigment balling o absence of pigment	o absence o ↑ phagocytes o hyperplasia o dead cells	
2	OS		o disorientation o slight swelling	o phagocytes o mild swelling	o extreme swelling o bizarre forms	o absence o ↑ phagocytes o dead cells	
3	IS		R C o o vacuolization o o edema	R C o o phagocytes o o slight swelling	R C o o extreme swelling	R C o o absence o o ↑ phagocytes o o dead cells	
4	ONL		R C o o vacuolization o o edema o o hyperchromasia	R C o o phagocytes o o ghosting o o nuc.halo o o chrom.clumping	R C o o lg.nuc.halo o o ↓ cell numbers	R C o o absence o o ↑ phagocytes o o pycnosis o o dead cells	
5	OPL		o vacuolization o edema	o phagocytes o slight swelling	o extreme swelling o ↓ cell numbers	o ↑ phagocytes o dead cells	
6	INL		o vacuolization o edema o hyperchromasia o ghosting	o phagocytes o nuc.halo o chrom.clumping	o lg.nuc.halo o ↓ cell numbers	o absence o ↑ phagocytes o pycnosis o dead cells	
7	IPL		o vacuolization o edema	o phagocytes o slight swelling	o extreme swelling	o absence o ↑ phagocytes o dead cells	
8	GCL		o vacuolization o edema o hyperchromasia	o phagocytes o ghosting o chrom.clumping o slight swelling	o extreme swelling	o ↑ phagocytes o pycnosis o dead cells	
9	Chor.		o edema, slight o ↑ inflammatory cells o ↑ size Bruchs Membrane		o vessel occlusion o ↑↑ inflammatory cells o breaks in Bruchs Membrane		
10	Ves.		o thickening of walls o ↑ inflammatory cells		o occlusion		

Figure 2

2. Results: (Experiment I)

a. Electroretinographic Changes.

The flash ERG over the course of dark adaptation was measured in over 100 eyes pre- and post exposure. Exposures intense enough to produce a functional deficit yielded an equal reduction in the a- and the b- wave, which never exceeded 50%. The time course of dark-adaptation as measured by the ERG was not affected. The degree of deficit as determined by the reduction in ERG amplitude and the period of time over which this reduction occurred, was correlated with the exposure intensity. At three days post exposure the greatest reduction in amplitude occurred, being less at shorter post exposure intervals. In most cases, with the exception of the most severely damaged eyes, significant recovery in the ERG was observed. Figure 3 shows that this recovery may be complete and that the amplitude of the ERG of the exposed eye may return to the control level.

It is important to realize that the exposed retinal area was limited to 40^{o} or 12% of the whole retinal surface. Thus the recorded ERG was mainly determined by the function of the non-exposed retina. Maximally only a fraction of the ERG could be abolished depending upon the loss of cells in the exposed region and the "shunting" of the ERG generated by the non-exposed region through the damaged area.

b. Clinical Measures.

Indirect ophthalmoscopy of exposed eyes revealed that the earliest evidence of pathology was edema, which coincided with the damaged area later seen in histology. Opacification peaked at 72 hours followed by return of transparency in approximately 5 days. It was this loss of transparency of the retina that was interpreted as edema. When

Legend

Figure 3:

The ERG (a- and b-waves) before [day 0 to 93] and after exposure of the right eye at day 92, and the left eye at day 127, Macaque. Each ERG was measured after 21 minutes of dark-adaptation. The day to day variation in response amplitude before exposure averaged 7%. Following exposure of OD to 514.5 nm for 4 hours at 73 mW/cm^2 a 20% and 30% reduction in the a- and b-wave amplitudes, respectively, was measured. The reduction was greatest from 3 to 10 days following the exposure. By 3 weeks, the ERG amplitude had almost recovered to the levels recorded in the non-exposed left eye (OS). Minimal changes were produced in the ERG of OS following exposure to 514.5 nm for 4 hours at 30 mW/cm^2.

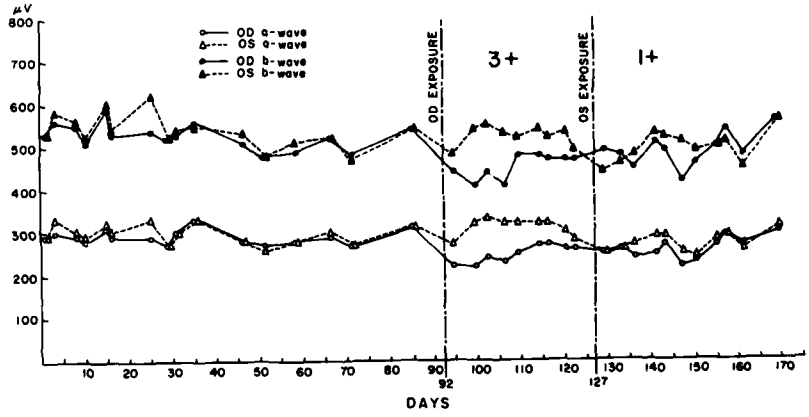
μV
800
700
600
500
400
300
200
100
OD a-wave
OS a-wave
OD b-wave
OS b-wave
OD EXPOSURE
OS EXPOSURE
3+
1+
10
20
30
40
50
60
70
80
90
92
100
110
120
130
127
140
150
160
170
DAYS

Figure 3

the edema subsided it was followed by mottling of the retinal epithelium, which in the monkey is heavily pigmented. In severe damage irregularity in the retinal surface was noted occasionally. No changes were observed in the optic disc or retinal vessels. A photograph of a normal fundus is shown in figure 4. The same fundus after a 457.9 nm exposure is shown in figure 5. One can see the ring of depigmentation which is at the outer edge of the macula as well as some decrease in pigmentation extending out toward the temporal vessels. This area first appeared edematous and later mottled or depigmented. The edema peaked about 72 hours after exposure.

Fluorescein angiography showed lucency of the retinal epithelium and baring of the choroid ranging from window defects just at the edge of the macula to complete transparency of the retina out to the temporal vessels depending upon the degree of damage. The defect persisted as long as any animals were followed. There was little evidence of discrete fluorescein leakage, but extensive late staining and/or persistent choroidal blush was usual. This occurred in the area of damage and was probably related to the loss of pigment epithelial integrity. Retinal vasculature was essentially not affected except for some pooling of dye around the larger vessels in late photographs. These findings are demonstrated in figures 6 and 7 which are a post-exposure early and later angiogram, respectively.

Legend

Figure 4:

The normal fundus of a cynomolgus monkey prior to exposure.

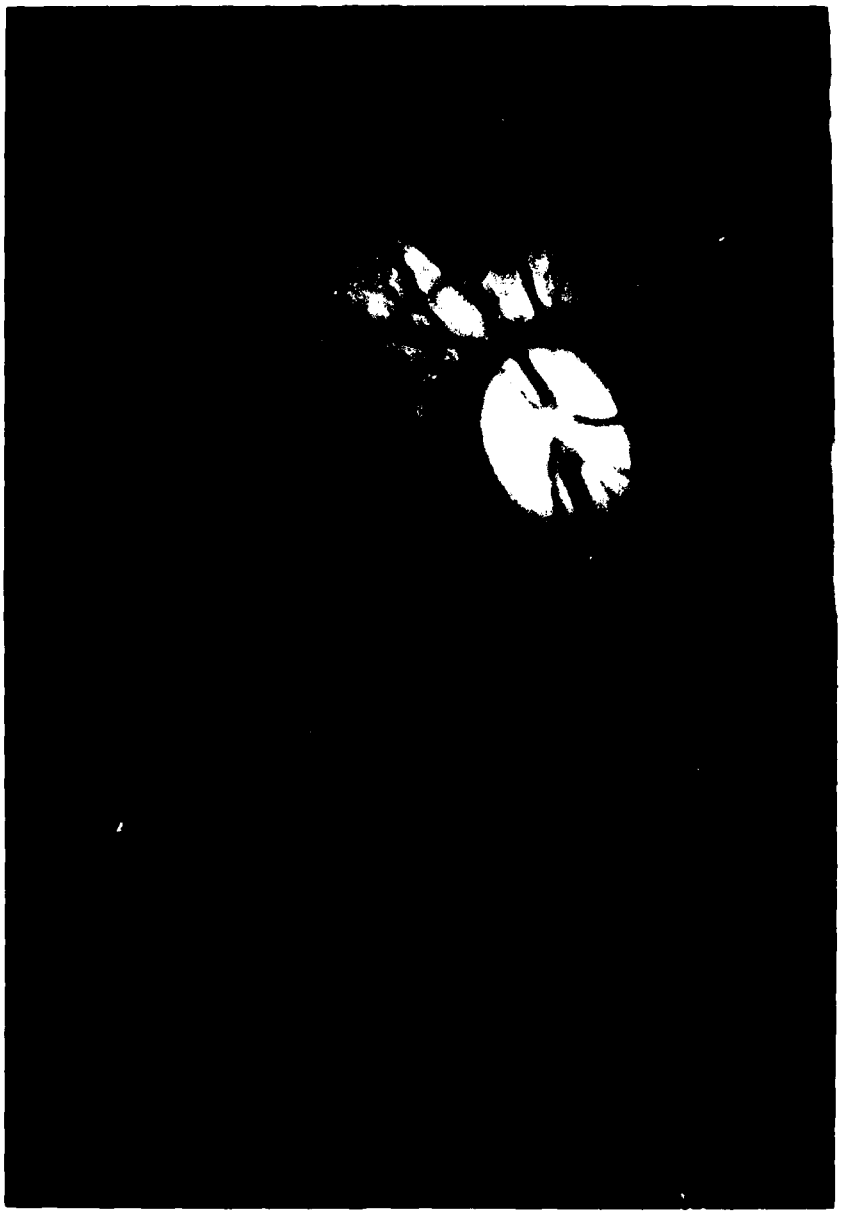

Figure 4

Legend

Figure 5: The fundus several weeks after exposure.

The same fundus as in figure 4 several weeks after an exposure to 457.9 nm light for 4 hours at an intensity twice average threshold. The macular area is lined by a ring of depigmentation, at about $3\text{-}5^{o}$ from the center. This depigmented area has histologically the most significant damage. There is some mottling of the pigment epithelium within the macular area. Pigmentation is decreased all the way out to the temporal vessels, though this is difficult to determine in this black and white photograph.

Figure 5

Legend

Figure 6:

This early-phase fluorescein angiogram was taken several weeks after an exposure to 457.9 nm light for 4 hours at a level .5 log units above threshold. The overall histological grade for this eye was 4+. It shows increased lucency of the pigment epithelium more prominent in an area between 3 and 7^{o} eccentric from the fovea and inferiorly. There is no evidence of leakage from the major vessels.

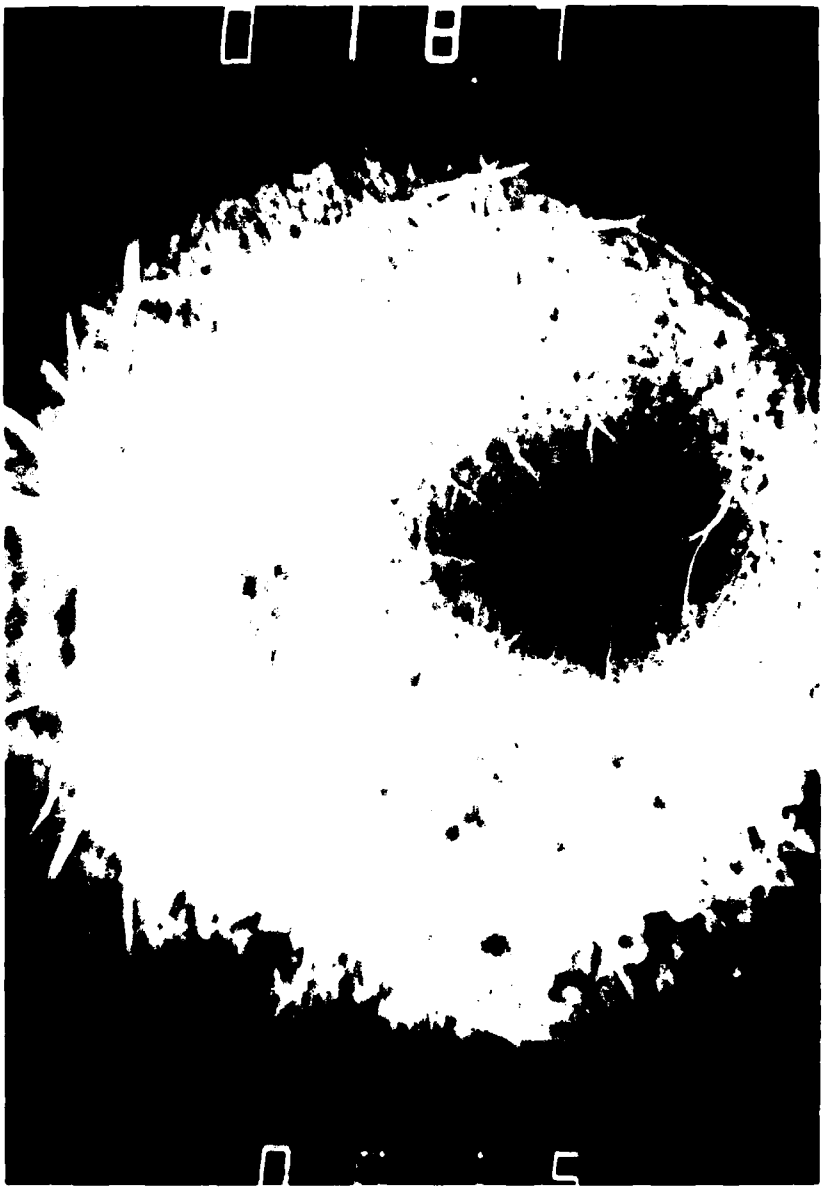

Figure 6

Legend

Figure 7:

This is a late-phase fluorescein photograph of the same eye as figure 6, showing extensive staining in the distribution of light damage. There are two potential sources for this apparent late staining. One is that the choroid which normally leaks fluorescein from its vessels has been a source of fluorescein which has come through damaged Bruch's membrane and pigment epithelium to lie in the retina. The other possibility is that this rather diffuse staining is normal staining of the choroid seen through a de-pigmented retina. Other fluorescein angiograms have shown patterns modified by the retinal vessels suggesting that at least part of the staining is in the retina proper.

Figure 7

c. Histological Changes.

1) Exposures at Various Wavelengths and for Various Durations.

Typical examples of light damage in the layers of the retina will be presented here by microphotographs of both light and electron microscopic sections. The sections are selected for the pathology they demonstrate. The degree of damage in any section may not correlate with the level of exposure for that eye because almost any level of damage may be found somewhere in a severely damaged eye. The level of overall light damage for the eye is classified by the most severe damage found in any area, though a damage grade is recorded for each layer in each section and for each section as a whole.

(a) Light Microscopy:

The most striking changes seen in severe light damage are changes in the pigment epithelial and outer segment region. This is particularly noted in sections taken several months following exposure. The changes in pigmentation as seen grossly by viewing the eye cup are illustrated in figure 8.

In the microphotograph of figure 9, the pigment epithelium is denuded and the outer segments are missing. The exposure here was intense, approximately 0.7 log units (5 times) above threshold for the 457.9 nm, four hour exposure. There are phagocytic cells in the subretinal space and severe swelling of the remaining cone inner segments. Nuclei of the rod and cone cells show pyknosis, while cell processes in the outer plexiform layer are swollen. Though the major destruction extends from Bruchs membrane to the middle of the retina, some swelling and cell loss is evident

Legend

Figure 8: A view of the posterior eye segment after exposure. This is a gross photograph of the fundus of an exposed eye with the anterior segment removed. In this case the exposure was extremely intense and the affected pale area covers the exposed retina. The lighter appearance is secondary to loss of pigment in the pigment epithelium. This eye was exposed to 457.9 nm for 4 hours at an intensity four times average threshold. The overall damage grade was 4+.

Figure 8

Legend

Figure 9:

This eye was exposed to 457.9 nm light for one hour at 0.3 log units above the average threshold intensity for this wavelength and duration. The animal was sacrificed at 4.5 months. The overall damage score was 3+. This is a section showing extreme disformation and destruction of the inner and outer segments and the loss of the pigment epithelium in this area. Note the many pigmented macrophages. There is scattered pyknosis in the outer nuclear layer which is reduced in width due to cell loss. Pyknosis of cone cells is especially prominent. Many nuclei of the inner nuclear layer are pyknotic. The outer plexiform layer is severely swollen and has hyperchromatic processes. Toluidine-Blue, X 300.

Figure 9

is evident in the inner layers. Damaged areas such as this occur in a patchy but localized distribution in a zone beginning one or two degrees from the fovea and extending concentrically out, depending on the exposure intensity. They rarely extend beyond the temporal vessels.

In eyes with the illustrated severe degree of damage, damaged areas may be immediately adjacent to areas showing little or no change. Figure 10 is such a section from the same eye as in figure 9, only slightly more eccentric. It shows preservation of pigment epithelium, even fairly normal location of the pigment in spite of overlying macrophages. The rod outer segments are fairly well intact though distorted. Cone inner segments show swelling. Some cone nuclei are fading and many rod nuclei are hyperchromatic. Condensed cytoplasm in the processes in the outer plexiform layer is noted. The two panels shown in figure 11 are from the same tissue block and are adjacent except that one panel of equal width is missing in between. This figure demonstrates the rapid transition from a damaged area on the left with abnormal outer segments to almost normal outer segments on the right.

Figure 12 shows cell changes in the inner layers one month following a one hour 514.5 nm exposure a little below the average threshold. However, this eye overall had "2.5+" damage. The sections were taken one month following exposure. There are prominent hyperchromatic nuclei in the inner nuclear layer. Processes with condensed cytoplasm are seen in the inner plexiform layer as well as in the ganglion cell layer. These changes are present in regions where the pigment epithelial cells remained intact, though depigmented. Many outer segments of both rods and cones are preserved.

Legend

Figure 10:

This eye was exposed to 457.9 nm light for one hour at 0.3 log units above the average threshold intensity for this wavelength and duration. The animal was sacrificed at 4.5 months. The overall damage score was 3+. The microphotograph shows a normal choriocapillaris. The pigment epithelium has moderate vacuolization and there are phagocytic cells in the subretinal space. Cone inner segments are weakly stained and some seem disformed and some inner rod segments are also disfigured. Heavily hyperchromatic nuclei are present throughout the outer nuclear layer. The outer plexiform layer shows dark stained processes as well as swelling. The inner layers show ghosting of cells, and swelling of cytoplasm. The receptor cells appear the most affected structure in this section. The survival of the general retinal architecture is surprising, considering the individual cell pathology. Toluidine-Blue, X 300.

Figure 10

Legend

Figure 11:

This eye was exposed to 476.5 nm light for four hours at approximately the average threshold intensity for this wavelength and time. The animal was sacrificed at five months. The two panels in this figure show the transition from a damaged area to an undamaged area. The two panels are separated by one panel width. In the left panel there is significant loss of cone cells and those cones which remain are damaged. Large vacuoles exist in the outer segment area. These appear on electron microscopy to be swollen cellular elements, either cone inner or outer segments. The rods are remarkably intact with only an occasional pyknotic nucleus with disturbed outer segments. In this case, essentially all cones appear to be affected. The pigment epithelium in contrast contains the normal distribution of pigment in the apical portion of the cells though there is marked mitochondrial vacuolization. There is significant swelling of elements in the outer plexiform and bipolar layer, as well as the ganglion cell layer. These most likely represent both mitochondria and cell processes. In the righthand panel the cells show almost no disturbance except vacuolization of the pigment epithelium and some disarray in the cone outer segments. There may be some mild swelling of the outer plexiform layer and Mueller cell swelling in the bipolar layer. Processes in the inner plexiform layer do not appear as swollen as in the left hand panel. This figure shows the typical sharp demarcation of damage even though the light intensity between these sections does not vary more than 15%. Toluidine-Blue, X 300.

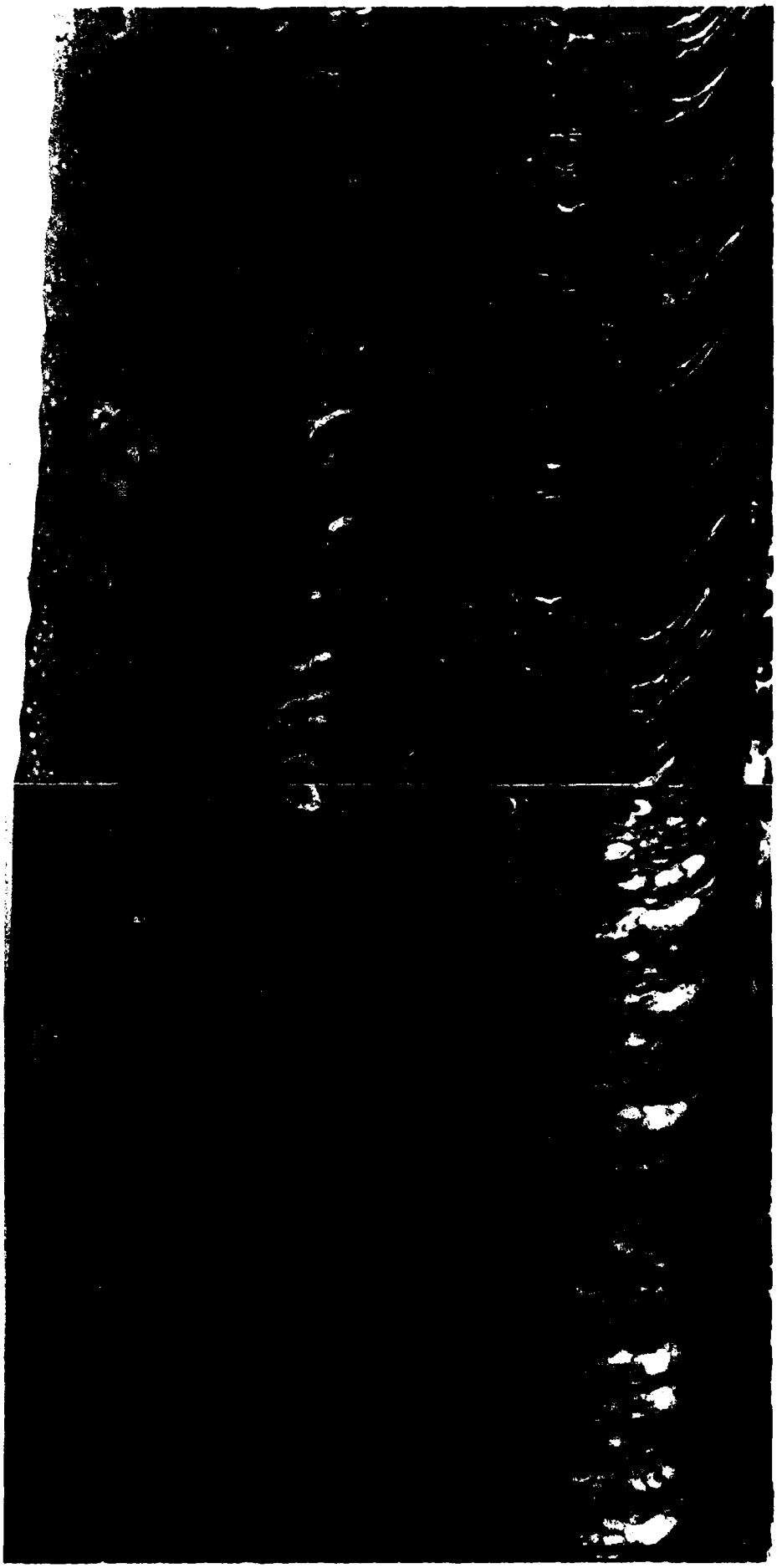

Figure 11

Legend

Figure 12:

This eye was exposed to 514.5 nm light for one hour at 0.2 log units below the average threshold intensity for this wavelength and exposure duration. The animal was sacrificed one month after exposure. The overall damage score was 2.5+. The pigment epithelium in this section has lost much of its pigment to phagocytes. Outer segments of both rods and cones seem to be affected. There are scattered pyknotic nuclei in the outer nuclear layer. Hyperchromatic processes are seen in the outer plexiform layer at the border of the inner nuclear layer.

This eye has been damaged out of proportion to the applied dosage and its rod cells have responded to a greater degree than the cones which is not usually seen. One might suspect a longer dark-adaptation preceding this exposure but no such event can be determined from the records. Toluidine-Blue, X 300.

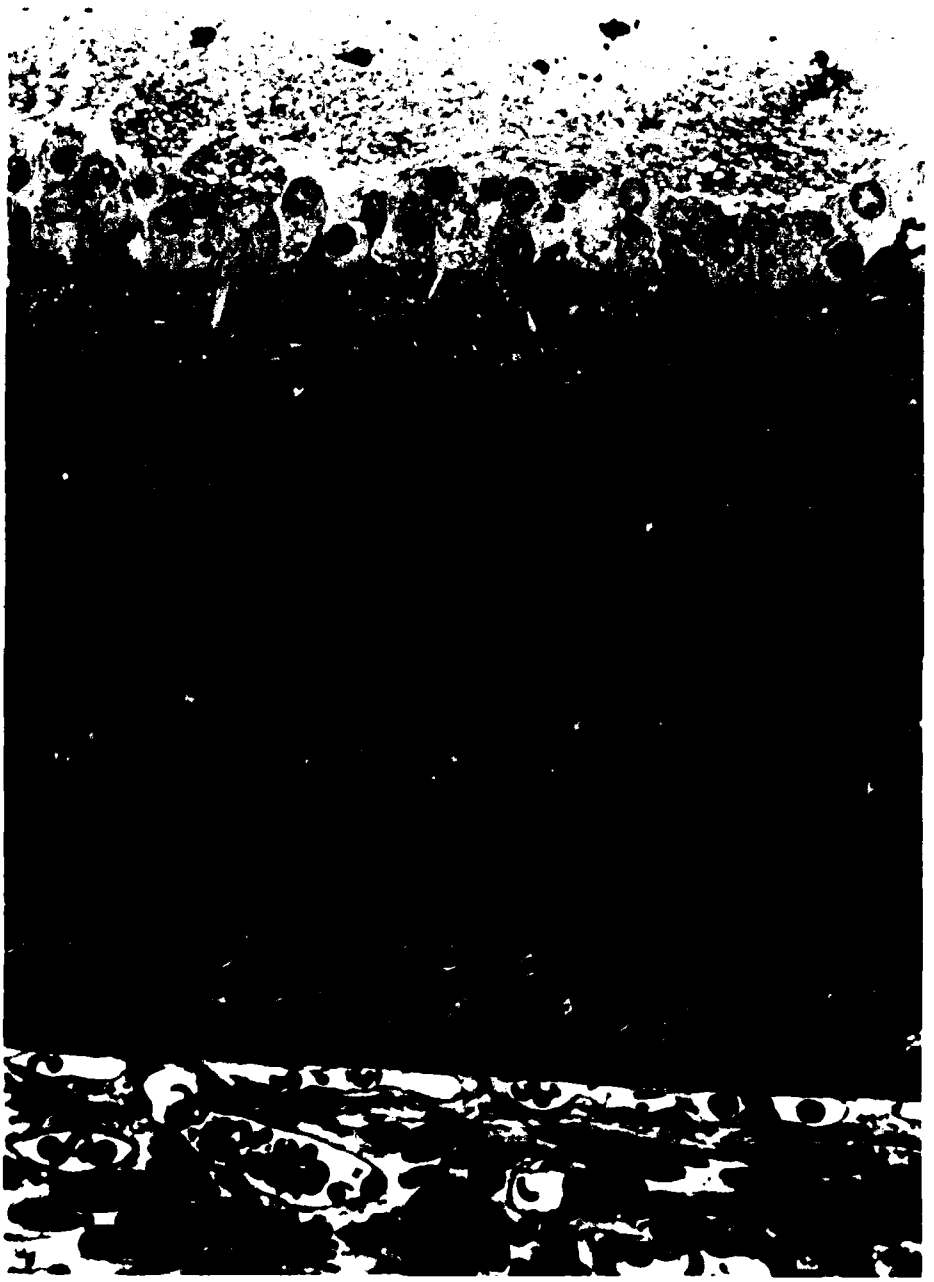

Figure 12

Overall, the damage is evenly distributed within the retinal layers except for a tendency with the 457.9 nm exposure to injure the inner layers to a greater degree than with longer wavelengths. An example of this is figure 13. The eye was exposed for four hours at threshold to 457.9 nm light and removed at 3 weeks, it showed overall "3.0+" damage. In this section, the pigment epithelium shows little disturbance. Many rod outer segments are still intact. Mitochondria and cell processes are markedly swollen throughout the retina and hyperchromatic nuclei abound. This picture would suggest that the major insult was not to the outer segments nor the pigment epithelium.

The second question addressed by light microscopy is the time of formation of these lesions as seen several weeks or months after exposure. Sections were taken one hour after exposure and 24-hours later in eyes exposed to 1.5 times average threshold of 514.5 nm light for 1 hour.

Figure 14 was taken one hour after a one hour exposure. This section (Fig. 14) shows minimal changes in the pigment epithelium. The outer segments are distorted, some are swollen. The cone inner segments, however, are markedly enlarged and sections through the myoid show condensed cytoplasm in some instances. Dark-stained processes of receptor cells course through Henle's fiber layer. Dark cells are seen in the bipolar layer and an occasional dark process in the inner plexiform layer. Figure 15 shows the foveal area of the same eye. Cell pathology in the outer layers is well demonstrated. These findings are not that much different from those at 24 hours or a month or more following exposure.

Legend

Figure 13:

The eye was exposed to 457.9 nm light for four hours at the average threshold intensity for this wavelength and duration. The animal was sacrificed at three weeks. The overall damage score was 3+. Extreme damage and necrosis is noted throughout the section. Cone nuclei and their inner segments are severely disfigured or vacuolized. A great portion of the rod nuclei is also pyknotic. There are patches of rod outer segments which appear retained. The pigment epithelium seems devoid of changes. There is extensive swelling of the plexiform layers. The inner nuclear layer shows scattered pyknosis and nuclear destruction. Toluidine-Blue, X 300.

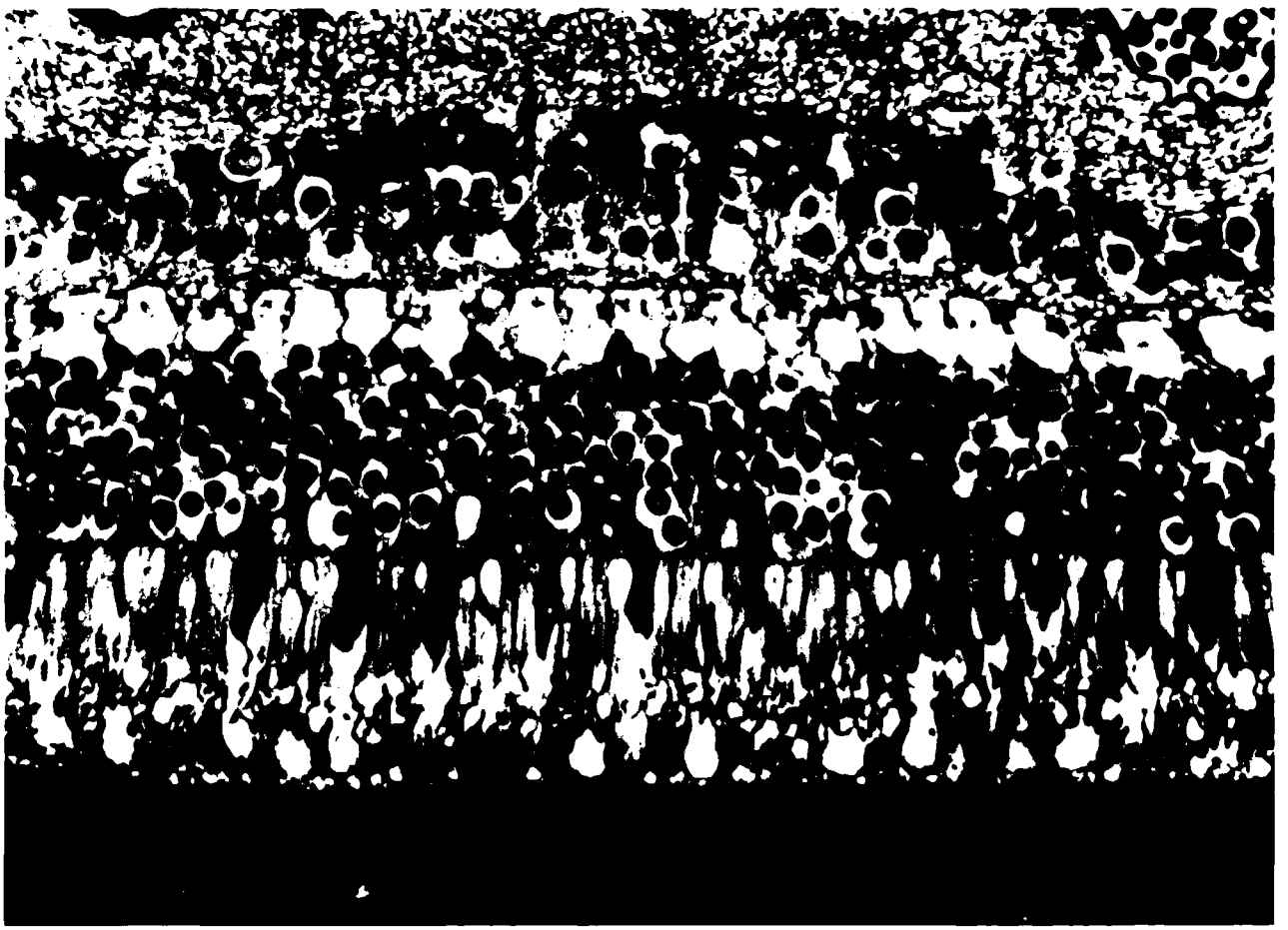

Figure 13

Legend

Figure 14: The eye was exposed 514.5 nm light for 1 hour, approximately at threshold and immediately thereafter removed.

This section was taken from the macular region. There is some disturbance of the pigment and mild vacuolization in the pigment epithelium. The outer segments seem swollen and somewhat shortened. The inner segments in this cone rich area are swollen, often darkly stained, and vacuolized in their distal half. Many nuclei of the outer nuclear layer are pyknotic. Condensed (dark) cytoplasm extends into Henle's fiber layer up to the synaptic region. There is abnormal cellular staining in the inner nuclear layer and the inner plexiform layer shows a few darkened processes. The ganglion cells are fairly well preserved. These changes seen one hour after exposure are not much different from those seen at 24-hours or several months. Toluidine-Blue, X 650.

Figure 14

Legend

Figure 15:

A section through the fovea of the same eye as in figure 14 exposed to 514.5 nm light for one hour approximately at threshold and immediately thereafter removed. The overall damage grade was 2.5+. Cellular changes are prominent in the ganglion cell layer, inner nuclear layer and very prominent in the outer plexiform layer. There are scattered, dark cone nuclei, a few cone inner segments also are hyperchromatic. No significant changes are seen in the pigment epithelium. Toluidine-Blue, X 650.

Figure 15

Figure 16 is a section from the macula, 24 hours after a one hour exposure. It shows mild swelling of mitochondria in the pigment epithelium. The cone inner segments are swollen and some nuclei in the outer nuclear layer are pyknotic or hyperchromatic. Condensed cytoplasm is seen in processes in both the outer and inner plexiform layer. These findings are not much different from the findings at one hour or at three months. The small clear vacuoles seen in several layers can be identified as swollen mitochondria by electron microscopy. While condensed cytoplasm is noted in the plexiform layers, little swelling is noted here. This might correlate the fact that retinal translucency only becomes prominent at 72 hours, seen clinically with indirect ophthalmoscopy as edema.

b. Electron Microscopy.

Electron microscopic section of light damaged eyes usually show in every retinal layer mitochondrial swelling of some degree. In many mitochondria the cristae are destroyed. Figure 17 shows typical mitochondrial destruction in the pigment epithelial cells. These pigment epithelial cells also show a partial shift of pigment away from the apical border. The rod outer segments are distorted but many are intact at this time three weeks after exposure.

The section in figure 18, shows the vacuolized mitochondria as part of the cone inner segments. Many more of the long thin rod mitochondria retain a few of their cristae and are generally better preserved than the thicker cone mitochondria. Mitochondria contained in receptor cell processes in the outer plexiform layer appear to be swollen or disorganized

Legend

Figure 16:

The macular region of this eye was exposed to 514.5 nm light for one hour at average threshold intensity for this time. The animal was sacrificed 24 hours following exposure. The overall damage grade was 2+. There is mild to moderate vacuolization of the pigment epithelium but this is difficult to evaluate without electron microscopy. The outer segments of rods and cones appear grossly intact. The inner segments of the cones are thicker than normal and a few are abnormally dark. There are scattered hyperchromic nuclei in the outer nuclear layer and some swelling. The processes of the outer plexiform layer are uniform in size. However, there are several dark staining processes noted out as far out as the synaptic region. Scattered hyperchromic cells are seen in the inner nuclear layer and the ganglion cell layer. These findings are not that different from those seen at shorter or longer survival times. Toluidine-Blue, X 650.

Figure 16

Legend

Figure 17: Pigment Epithelium: mild form of damage.

The eye was exposed to 514.5 nm light, four times at one hour each, at 24-hour intervals, at 0.2 log units less than average threshold intensity for this wavelength. The animal was sacrificed at one month following exposure. The overall damage score was 2+. This photomicrograph shows the very mildest changes noted in pigment epithelium from light damage. The mitochondria are swollen and cristae obliterated. There is a mild increase in the number of residual bodies and there are halos around some of the lysosome bodies. The basal enfolding is intact and only very slightly swollen. The normal cigar shaped pigment granules are aligned along the apical surface of the cell, though the villi are somewhat shortened. There are a few instances where the pigment granules are located more deeply in the cell. X 7,200.

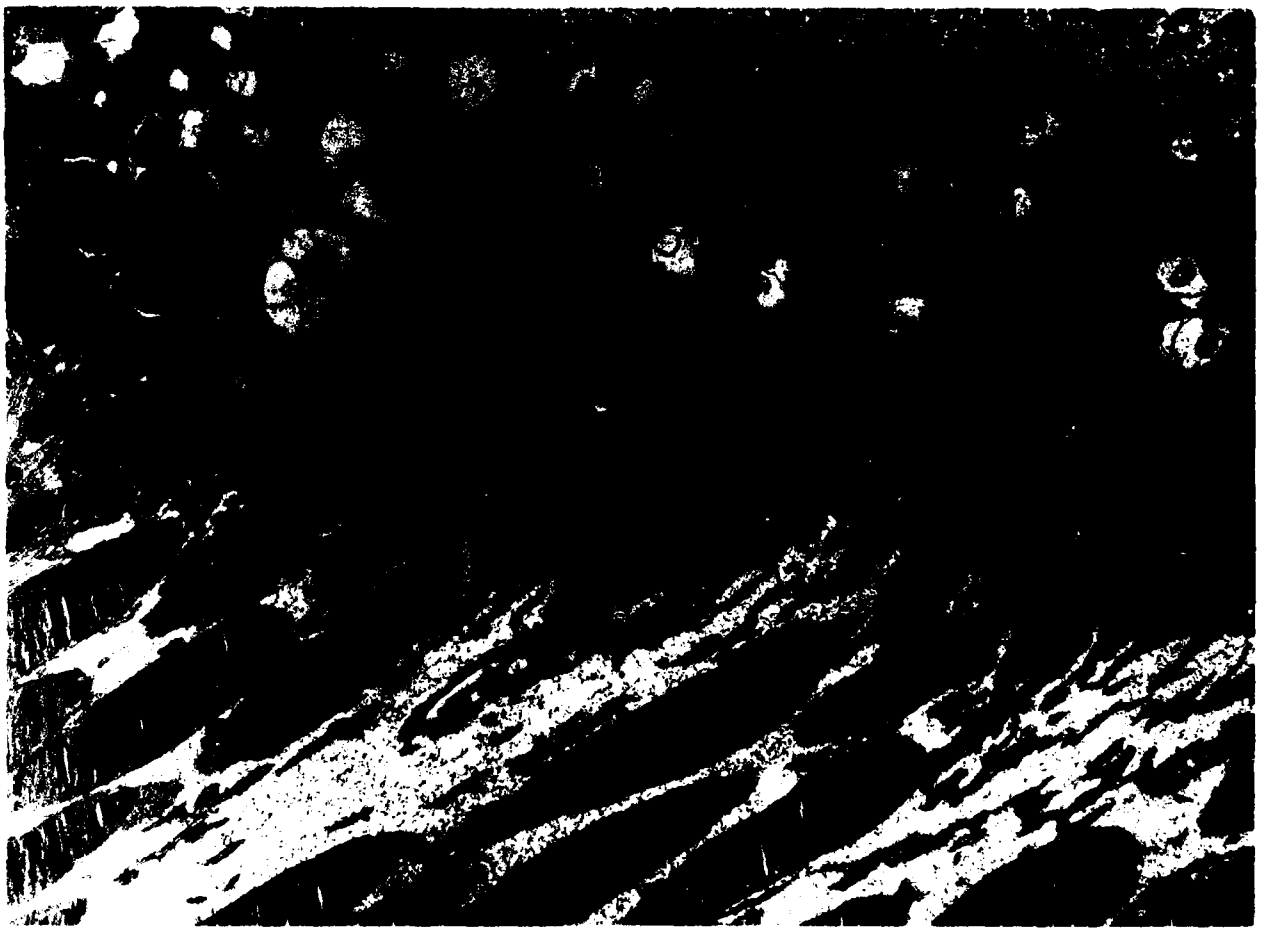

Figure 17

Legend

Figure 18: Severely Damaged Inner Segments:

The eye was exposed to 457.9 nm light for four hours at the average threshold intensity for this wavelength and duration. The animal was sacrificed at three weeks. The overall damage score was 2.75+. Extreme swelling of the mitochondria, particularly of the distal end of cone inner segments, is noted in this photograph. There is also severe disorientation of discs in the outer segments. These discs may have been assembled during the 3 week period after exposure and may have been misformed because of the severely damaged inner segments. The rod cells seem less severely affected but some show condensation of the cytoplasm. The dark staining inner segments are attached to outer segments in at least one instance. X 5,200.

Figure 18

(Figs. 19 and 20) synaptic vesicles can still be seen and the cells may continue to function. Mitochondrial swelling may or may not be associated with condensed chromatin of the cytoplasm (Fig. 20). Cellular disorganization is also evident in the nuclear region of the receptor shown by pyknosis, fading and cellular organelle swelling (Fig. 21).

Similar mitochondrial changes as in the receptor cells are found through the bipolar and inner plexiform layers. This is shown in figures 22 and 23. The synthetic machinery of these cells appears intact and possible hyperactive if one can judge by the appearance of the endoplasmic reticulum and Golgi body.

Swollen mitochondria and swollen processes are also seen in cells of the ganglion cell layer (Fig. 24), and in the nerve fiber layer (Fig. 25).

The EM finding of outer segment swelling, vesiculation of discs, swelling of cellular organelles other than mitochondria and many other changes throughout the retinal layers have been previously described by Lawwill[11] and others[24,47].

3. Discussion (Experiment I)

The following findings deserve emphasis:

1. The changes observed with electrophysiological testing and clinical observation are to some degree reversible. ERG changes generally become less within 2 weeks after exposure. Similarly, edema disappears while fundus changes produced by pigment epithelial abnormalities tend to persist.

Legend

Figure 19: Outer Plexiform Layer: Damaged Cone Pedicle.

The eye was exposed to 476.5 nm light for four hours at approximately the average threshold intensity for this wavelength and duration. The animal was sacrificed at 5 months. The overall damage score was 2.5+. This photograph shows a cone pedicle with mitochondria showing swelling and lack of cristae. No severe damage is noted in this area of an otherwise severely damaged retina. X 15,600.

Figure 19

Legend

Figure 20: Outer Plexiform Layer: Condensed Cytoplasm.

The eye was exposed to 457.9 nm light, for one hour at 0.3 log units above the average threshold intensity for this wavelength and duration. The animal was sacrificed at 4.5 months. The overall damage score was 3+. This is a photograph of a cell process in the outer plexiform layer which shows extensive condensation of cytoplasm. Moderately swollen mitochondria are present in this process. The cell membrane appears intact. X 42,380.

Figure 20

Legend

Figure 21: Mitochondrial Damage in Bipolar Cell Layer.

The eye was exposed to 514.5 nm light, four times at one hour each, at 24-hour intervals, at 0.2 log units less than average threshold intensity for this wavelength. The animal was sacrificed at one month following exposure. The overall damage score was 2+. This section demonstrates the extensive destruction of mitochondria in neural processes. They are swollen and missing their cristae. A Müller cell process with an unusued accumulation of glycogen is coursing through the section. EM X 18,000.

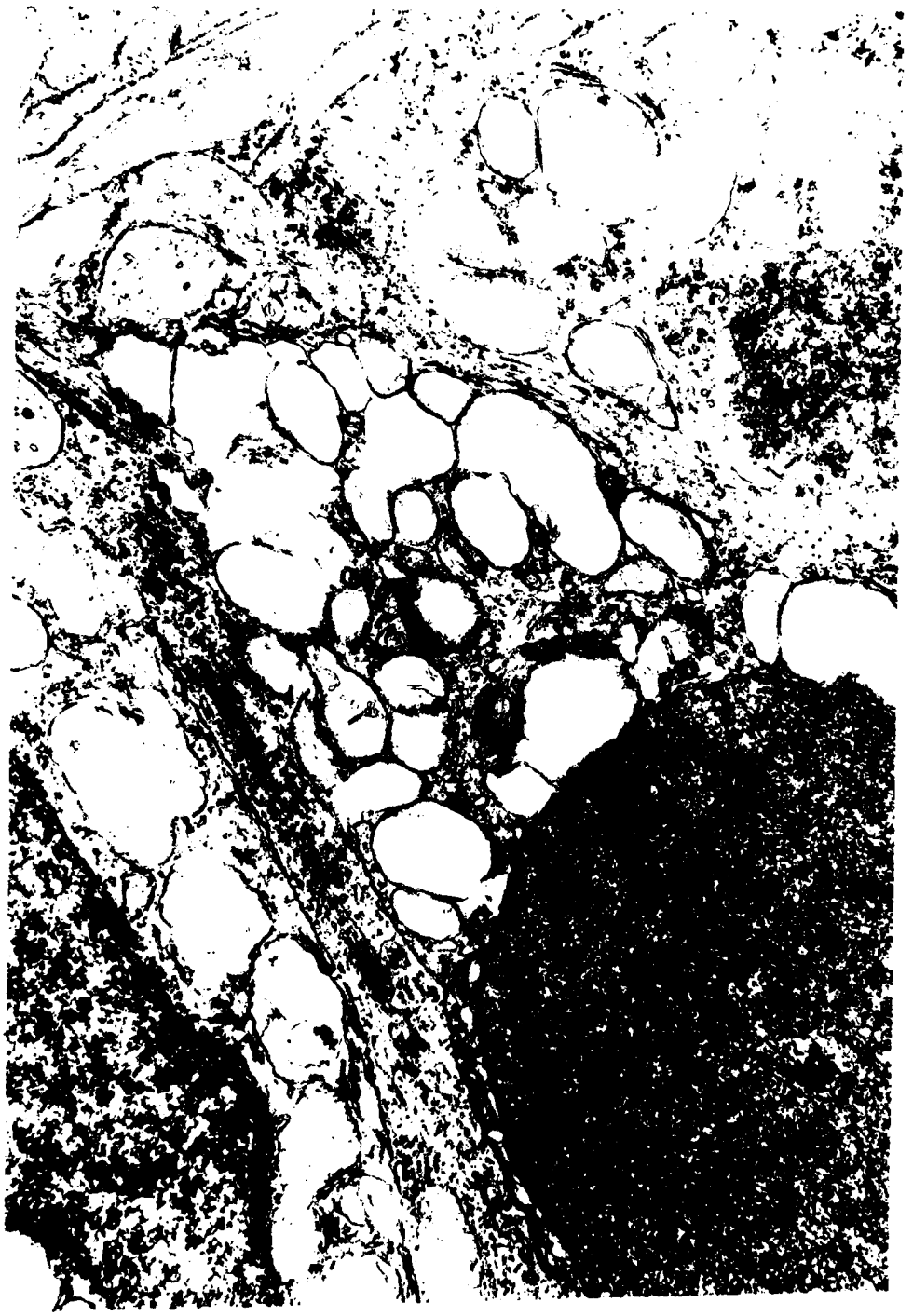

Figure 21

Legend

Figure 22: Enhancement of smooth and rough ER in bipolar cell layers.

The eye was exposed to 514.5 nm light for one hour at 0.2 log units below the average threshold intensity for this wavelength at this time. The animal was sacrificed at one month. The overall damage score was 2.5+. This section through the bipolar layer shows increased activity of both the smooth and rough ER. Most mitochondria are severely swollen with loss of cristae. Lysosomal bodies are more frequent than is normal. EM X 35,620.

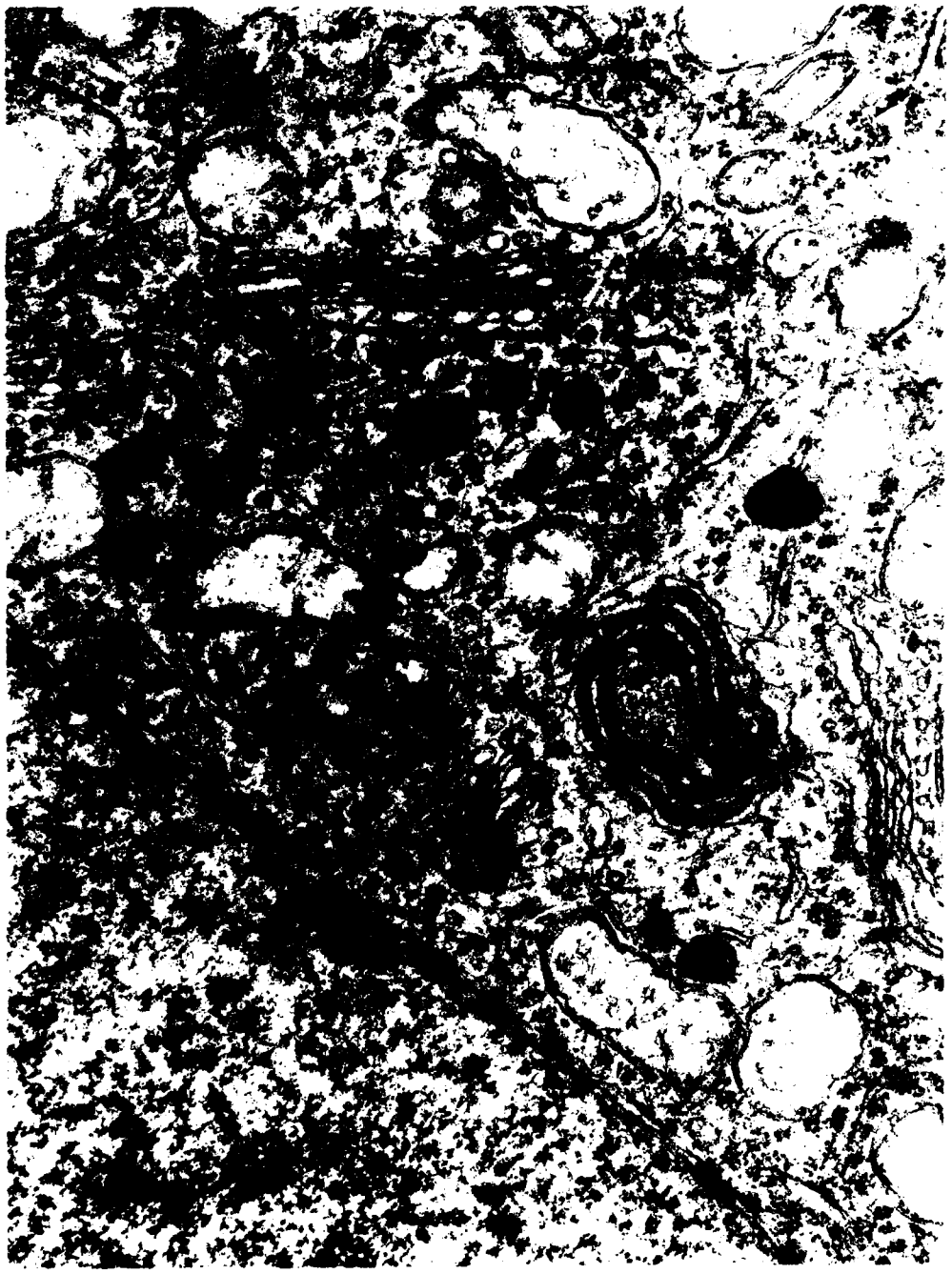

Figure 22

Legend

Figure 23: Inner Plexiform Layer

The eye was exposed to 476.5 nm light for four hours approximately at the average threshold intensity for this wavelength and duration. The animal was sacrificed at 5 months. The overall damage score was 2.5+. This photograph shows normal and swollen mitochondria, and a significantly swollen cell with evidence of metabolic hyperactivity signified by the pattern of the rough endoplasmic reticulum. X 42,380.

Figure 23

Legend

Figure 24: Ganglion Cell Layer

The eye was exposed to 476.5 nm light for four hours approximately at the average threshold intensity for this wavelength and time. The animal was sacrificed at 5 months. The overall damage score was 2.5+. The cell shown was found in the ganglion cell layer. There is swelling of the mitochondria and other intracellular organelles. The cytoplasm appears pale and somewhat empty. This is one of the milder types of damage seen in the ganglion cell layer. X 15,600.

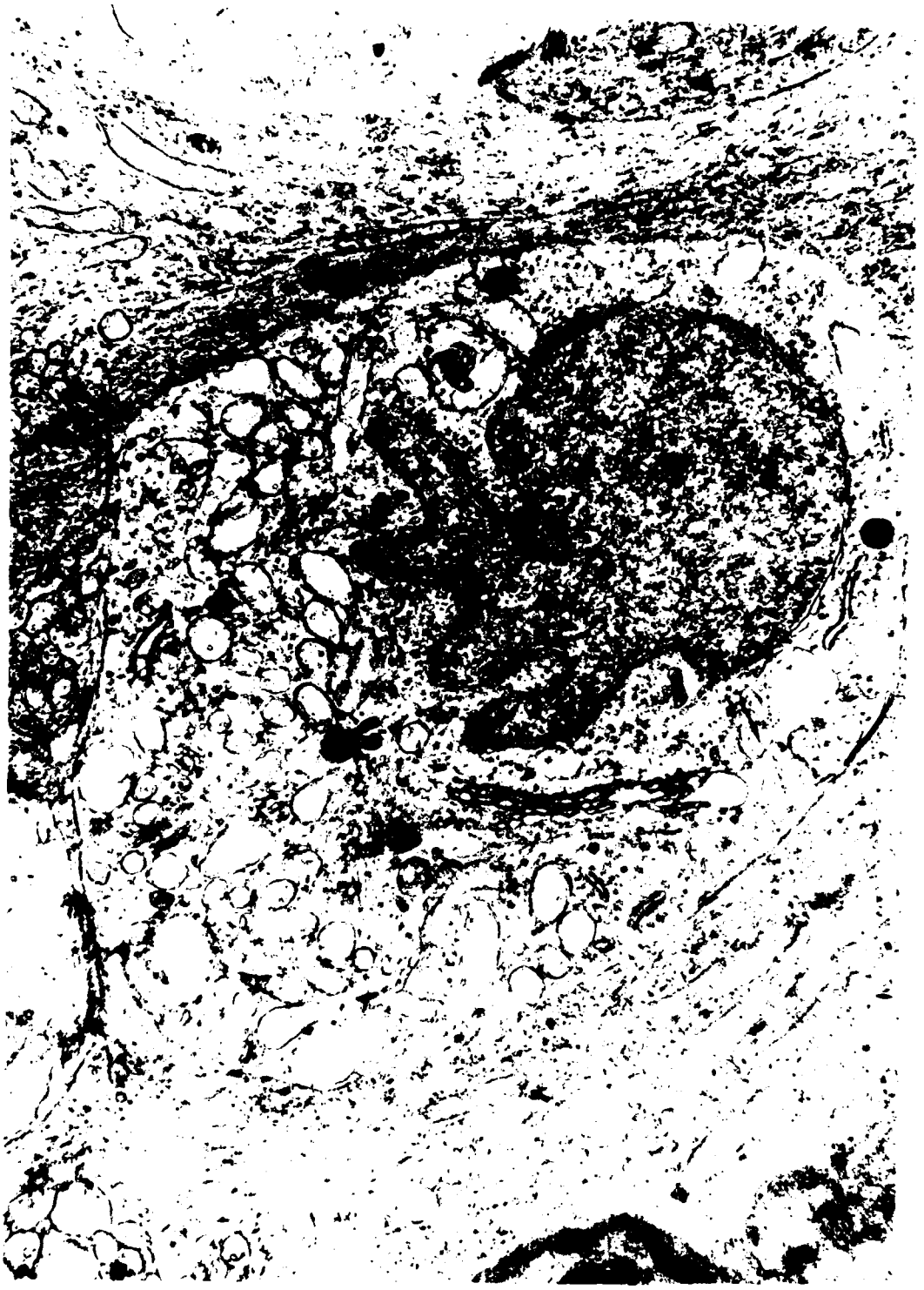

Figure 24

Legend

Figure 25: Nerve Fiber Layer

The eye was exposed to 476.5 nm light for four hours approximately at the average threshold intensity for this wavelength and duration. The animal was sacrificed at 5 months. The overall damage score was 2.5+. This cross section of the nerve fibers shows relatively normal fibers mixed with others that have swollen mitochondria and pale cytoplasm. This photograph represents a lesser grade in the variable range of observed reactions. X 50,444.

AD-A140 122 MECHANISMS OF RETINAL DAMAGE FROM CHRONIC LASER RADIATION(U) KANSAS UNIV COLL OF HEALTH SCIENCES AND HOSPITAL KANSAS CITY T LAWWILL ET AL. JUL 81 2/2

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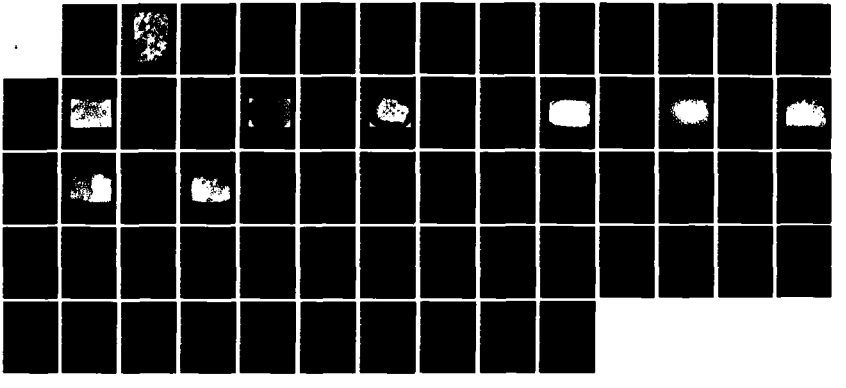
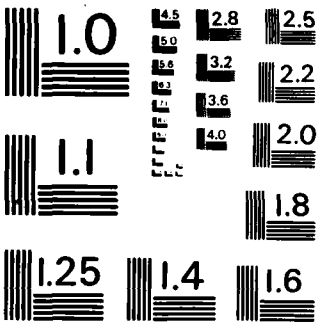




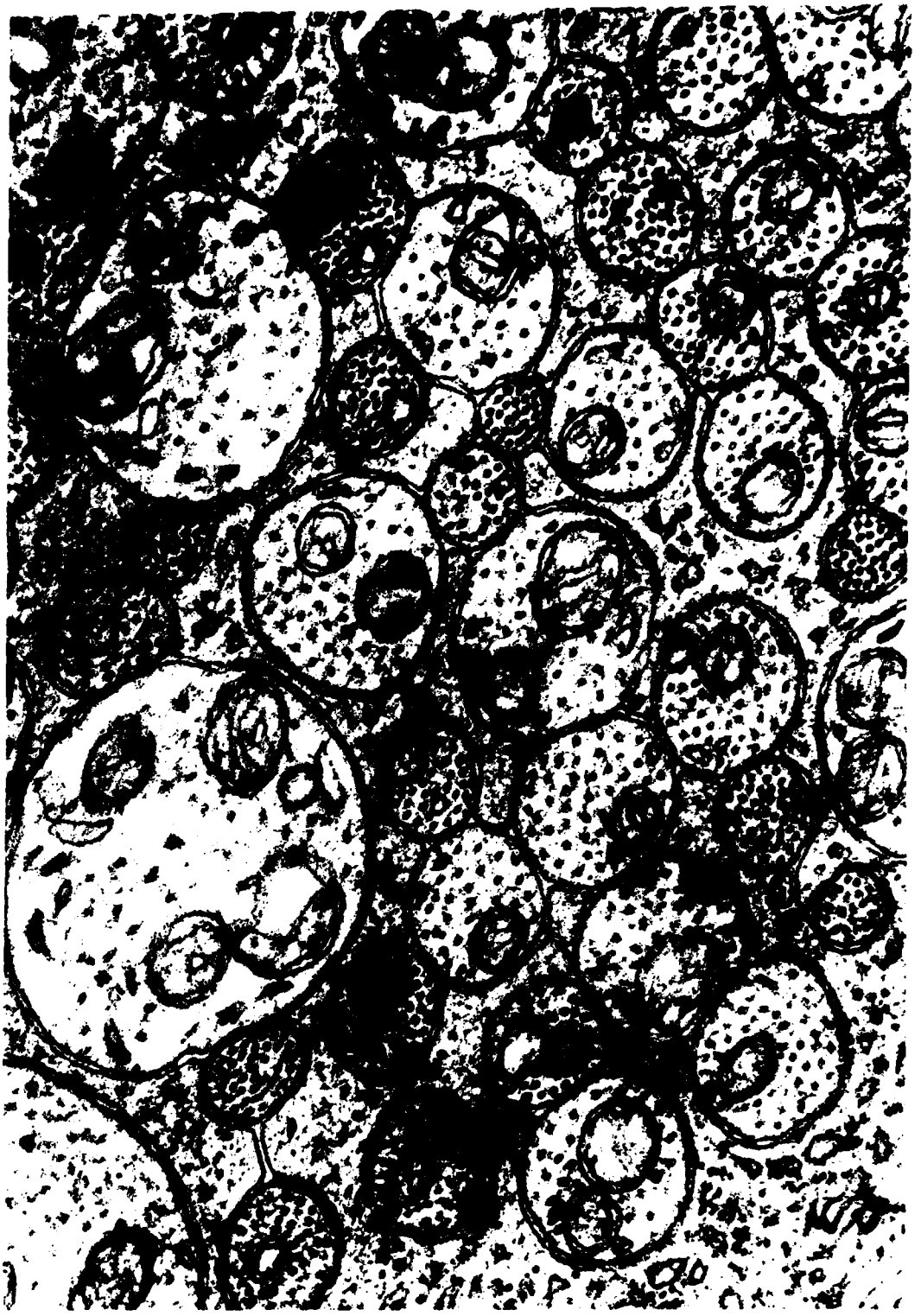


Figure 25

2. Histological changes at the light microscopic and EM levels, however, were as marked a few hours after exposure as after 3 months. At least it seems that recovery from histological change was minimal. In fact, loss of cells may have become more evident with time after exposure. Thus it is impossible to relate the functional changes to the histological abnormalities. The two may involve different pathways of damage but they may also be the expression of the same process, the one measured by function being more evenly distributed through the exposed region and affecting certain cell types preferentially and reversibly while the others seen by histology, are the extreme expression of the effect which is scattered over all layers and many cell types.

3. The most outstanding result of the histological studies is the finding that the mitochondria of all layers of the retina are the structure most sensitive to anatomical change in primate light damage. Rod mitochondria seem significantly more resistant than the mitochondria of the cones and other cells. It seems reasonable to assume that the mitochondria are a primary site of light damage under the conditions of exposure as studied. This possibility will be further explored in Final Discussion (IV).

C. Experiment II. Specific Cone Damage in the Monkey Retina.

Experiment 2 describes an elaborate attempt to determine whether cone cells are specifically susceptible to wavelength of light which are absorbed by their specific visual pigment. Following exposure to 451.9 (blue), 514.5 (green) or 590 (red) nm laser light covering 40^{o} of the posterior retina, spectral sensitivity functions were obtained by electroretinography using as the stimulus an alternating monochromatic bar pattern.

1. Methodology:

a. Animals, Exposures:

Six mature female cynomolgus monkeys (Macaca fasicularis) were obtained by the Carolyn Verhoeff animal care center. They were housed in cages with a 12 hr. day/12 hr. night cycle. Food, (Purina Monkey Chow) supplemented with fresh fruits and vegetables, was provided once each day in the late afternoon. The animals were prepared for the damaging exposure in same way as in Experiment I. Four eyes were randomly assigned to each of three argon laser treatment groups. One group received a four-hour exposure to 457.9 nm laser light at a retinal irradiance of 5 mW/cm^{-2}. The second received 514.5 nm light at 10 mW/cm^{-2}. The third group was exposed to 590 nm light at 40 mW/cm^{-2}. Irradiances for exposure were based upon the best estimates for those required to produce a just greater than threshold damage. As in Experiment I, laser light was presented in Maxwellian view providing a retinal field of 40^{o} centered on the macula. Retinal position was monitored with a hand held beam splitter. Anesthesia during exposure was the same as described in Experiment I. Heating pads were used to maintain body temperature which had a tendency to fall under barbiturate anesthesia. The eye to be exposed was held open and the cornea protected by the same contact lens electrode as used for the ERG recording.

b. Spectral Sensitivity Measurements:

ERG spectral sensitivity was determined in 12 eyes before and after laser light exposure. Phase reversal ERG's were recorded with a lock-in amplifier and voltmeter (Princeton Applied Research, 5204). The measuring system was phase-locked with the 8 Hz pattern reversal. The response in phase with the stimulus change was integrated over a 3 or 10 second period and displayed.

The bar-pattern target was a celluloid film with alternating light and dark horizontal bars with a contrast between the light and dark bars of 1.6 log units. Alternate vertical movements of the target reversed the position of the dark and light bars providing local changes in retinal illumination resulting in the ERG signals. The method was essentially that of Johnson, Riggs and Schick[42]. Eight wavelengths with 10 nm band width were provided by a grating monochromator (Bausch and Lomb 33-80-02). The wavelengths selected were: 460, 490, 520, 560, 595, 610, 640 and 685 nm. The target field of 38^{0} diameter was presented to the eye in Maxwellian view so that it was centered on the macula. Each pattern cycle of the target subtended 2.72^{0} i.e. the spatial frequency was 0.367 cycle per degree. Position of the pattern on the retina was monitored with a beam splitter.

A neutral density filter was used to adjust the amount of light entering the eye until an 0.75 uV criterion response was measured on the lock-in voltmeter. The retinal irradiance necessary to produce the ERG criterion response was measured three times during each experimental session. Four separate sessions at four day intervals were used to evaluate the ERG spectral sensitivity both before and after laser light exposure.

The animals were prepared for recording by phencyclidine HCl injected intramuscularly (1mg Kg^{-1}) to produce a "dissociated" state. The eyes were dilated and the animal was placed in a chair designed to position the subject into Maxwellian view. After endotracheal intubation with local anesthesia, the animal was immobilized with intravenous gallamine triethiodide (5.3 mg Kg^{-1}) and positive pressure ventilation was adjusted to achieve 4% end tidal CO_2. Immobilization was maintained with an i.v. infusion of gallamine in saline (2.8 mg/ml^{-1}) at a rate of 0.05 ml/min^{-1}. At the conclusion of the 2 hr. experimental session, the animal's recovery was assisted by injection of atropine sulfate (0.13 mg Kg^{-1}) and neostigmine methylsulfate (0.3 mg Kg^{-1}). It was not unusual to observe the monkey eating biscuits and fruit 30 minutes after the end of the recording session.

The use of a neuromuscular blocking agent was based upon the need to restrain the animal, insert contact lens electrodes in each eye, and eliminate eye movements. Initially phencyclidine alone was used. However, this induced a vertical nystagmus which made recording of pattern evoked responses and ERG impossible. The inhalation anesthetic, methoxyfluorane, was then tried. This agent was not suitable for a 2 hour procedure due to its nephrotoxicity. A review of the literature revealed that pentobarbitol altered components of the ERG depending upon the level of anesthesia. Nitrous oxide was not used due to the difficulty of maintaining adequate oxygenation without the availability of blood gas measuring equipment. The procedure which we finally chose allowed reliable ERG measurements while meeting the ethical standards in the humane treatment of the monkeys.

2. Results (Experiment II):

The results are presented in figure 25 as the log relative spectral sensitivity for all twelve eyes before exposure and for each of the three treatment groups after exposure. After exposure one of the eyes exposed to the 457.9 nm light and one of the eyes exposed to the 590 nm light could not generate the criterion level ERG so only three eyes represent each of these groups.

As figure 26 shows, the relative sensitivity to the 460 nm test light was most reduced after the 457.9 nm exposures. On the other hand, the great reduction in sensitivity after 514.5 nm exposure was revealed for the 520 and 560 nm pattern lights. Relative sensitivity to 610 and 640 nm test lights was most reduced after the 590 nm exposures. It is also significant that the least reduction in sensitivity to the blue portion of the spectrum was found after the 590 nm exposures. Similarly, the least reduction in sensitivity to the red test lights was found after the 457.9 nm exposures.

Figure 27 demonstrates the changes in spectral sensitivity as a difference between the pre- and post-exposure curves. This presentation clearly shows that the spectral region with the greatest decline in sensitivity corresponds to the color quality of the damaging light, i.e. blue, green or red. This is in addition to the fact that the blue 457.9 nm exposure required 1/8 or less the intensity of the 590 nm exposure to achieve a similar level of damage.

Legend

Figure 26: Spectral Sensitivity Function

The log sensitivity (see text) is plotted against the wavelength of the stimulating light. Spectral sensitivity (12 eyes) was determined before exposure (squares) and after exposure to each of the three wavelengths (590 nm - filled diamonds; 514.5 nm - triangles; 457.9 nm - circles). Exposure at 457 nm reduced the ERG sensitivity to a 460 nm test light to a greater extent than did exposure at the other wavelengths. The sensitivity to test lights from 490 to 560 nm was most affected by exposure at 514.5 nm; while the sensitivity to test lights from 595 to 685 was most effectively reduced by exposure at 590 nm.

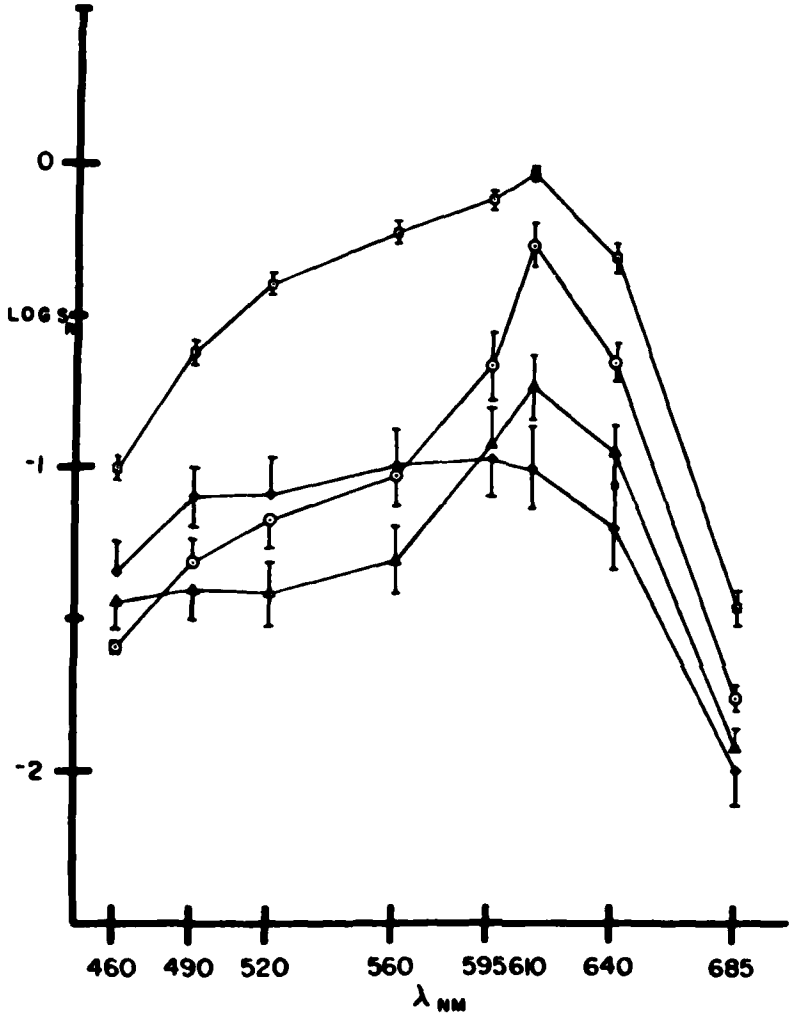


Figure 26

Legend

Figure 27: Difference spectrum of ERG sensitivity between pre- and post-exposure measurements.

The % loss in spectral sensitivity is calculated for each exposure group. This analysis shows that (1) 590 nm exposure selectively reduces sensitivity in the long wavelength region of the visible spectrum; (2) 514.5 nm exposure preferentially reduces sensitivity in the mid-range wavelengths; and (3) 457.9 nm exposure decreases sensitivity throughout the short and mid-range region with considerably less effect in the red.

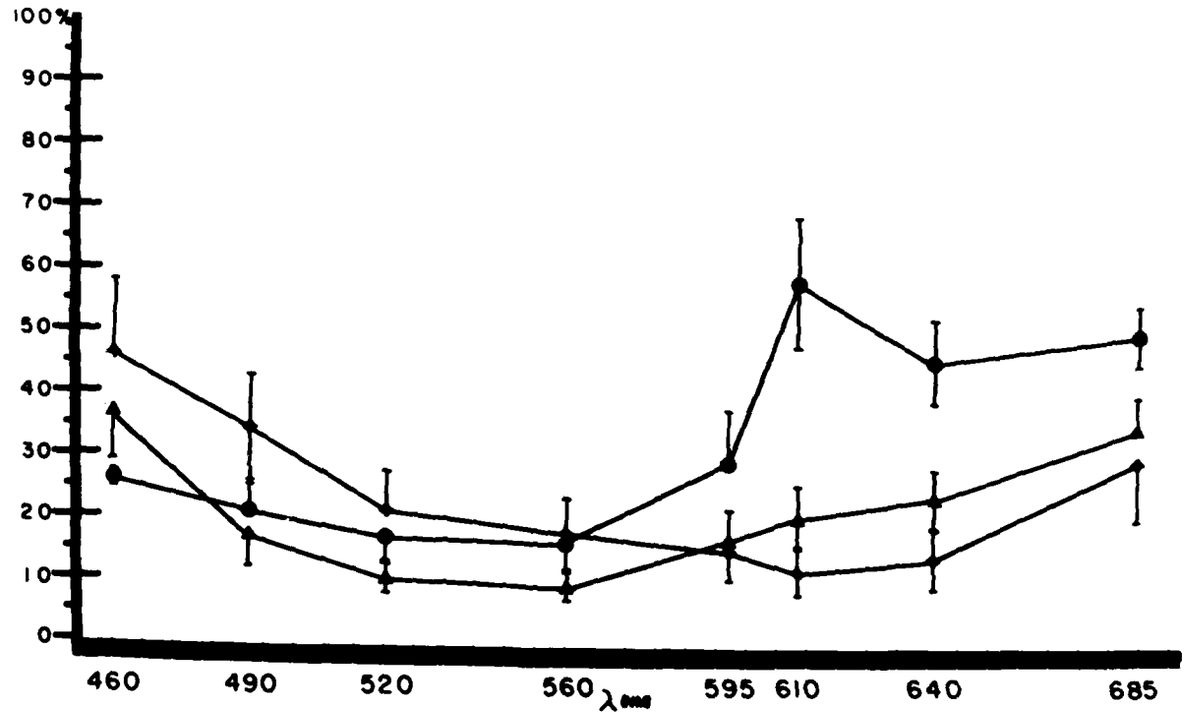


Figure 27

3. Discussion (Experiment II)

The findings of Experiment II clearly demonstrate a damage mechanism dependent individually upon the cone pigments in addition to or on top of a separate damaging effect of light which has an action spectrum peaking at the short wavelength end of the spectrum. The spectral effect most closely relates to Sperling's damage to specific cones which occurred at two to three log units lower intensity. However, at those intensities he was unable to "red blind" his animals whereas in our experiments relative red blinding did occur but only in the presence of non-specific destruction. Experiment II, then is an example of a mixture of mechanisms or pathways to damage.

D. Experiment III. Light Damage in Culture of Bovine Retinal Pigment Epithelium.

This study is an attempt to determine whether the light damage of the retinal pigment epithelium (RPE) is a primary or a secondary one. Secondary damage refers to the possibility that the pigment epithelial damage occurs in response to changes in neighboring tissue, the photoreceptor cells or the choroid. The question asked was whether light at a similar intensity as in-vivo produces a pigment epithelial effect in-vitro, and which wavelengths are most effective.

1. Methodology:

a. Collection of Cells:

Fresh cow eyes were obtained from the local slaughterhouse and transferred on ice to the laboratory. The ophthalmic artery was cannulated, and the eyes were infused with a solution consisting of protease, collagenase and trypsin. Ten minutes later the anterior segment was removed, and the retina was dissected free of the eye cup. The RPE cell layer was removed from the eye cup by a gentle flushing and aspiration action with 0.1% EDTA solution. The final effluent was aspirated, and the solution was centrifuged. Cells were washed several times with cold media, then resuspended and distributed to 20 ml petri dishes. The primary cultures were incubated in a CO_2 water jacketed incubator, and within 7 days they had grown to confluency. At this time the cells were resuspended, collected, distributed to 15 ml petri dishes and returned to the incubator. Within approximately 5 days these first order subcultures have grown to confluency (Fig. 28). The cells were maintained on Eagle's minimal essential media in a bicarbonate buffer. The medium was changed twice each week.

Legend

Figure 28: Bovine retinal epithelial cells in culture. Ten days after the first passage the cells have the polygonal morphological feature characteristic of the in-vivo RPE. The culture shown is from a cell harvest including the tapetal and non-tapetal areas. The degree of pigmentation is more sparse than that observed in the tapetal cells in-vivo. Brightfield illumination, fixed and stained with cresyl violet (X 700).

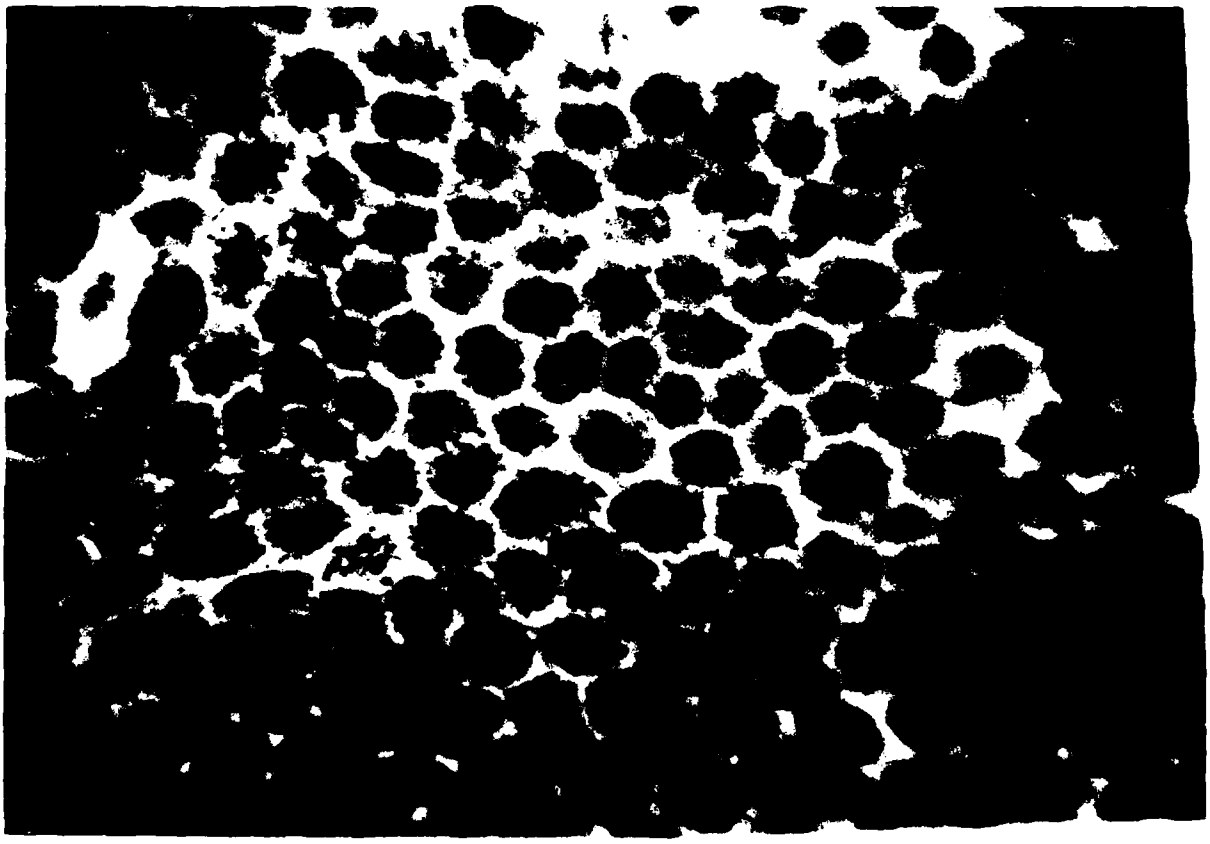


Figure 28

The cow eye is composed of tapetal and non-tapetal regions. The RPE in the tapetal area lacks melanin granules (Fig. 29) while the non-tapetal region has a pigmented RPE (Fig. 30). By dissecting these areas apart RPE cells with and without melanin were successfully obtained. This differential harvesting technique has been utilized to produce melanin-containing and melanin-free cell cultures.

b. Exposure of Cell Culture:

In one procedure confluent monolayer subcultures of mixed tapetal and non-tapetal cells were exposed to the 457.9 nm or 514.5 nm lines of the argon laser, or the 590 nm emission of the rhodamine 6-G dye laser for a period of 2 hours. During exposure the cultures were maintained at $37^{o}C$ in an atmosphere of 5% CO_2 in air at a relative humidity of 95%. Twenty-four hours after exposure the cultures were fixed in phosphate-buffered 3% glutaraldehyde and stained with 1% toluidine blue O. No precautions were taken to avoid exposure of the cultures to ambient laboratory illumination when being transported.

A knife edge beam-stop was placed along a cross-sectional diameter of the laser beam and imaged on the culture plate. This technique produced a hemi-field exposure. The lasers were maintained in the TEM_{oo} mode, and thus a two-dimensional Gaussian field of irradiance was provided. The spatial distribution of irradiance was mapped using a light detector with a 1 mm aperture.

In a second procedure, groups of pigmented or non-pigmented cells were exposed for 2 hours to the broadband filtered (335-600 nm) light from a mercury-xenon lamp.

Legend

Figure 29: Bovine RPE from tapetal region of retina.

The tissue was dissected free of Bruch's membrane under an operating microscope after removal of the neural retina. The few melanin granules which are present appear as bright flecks under the phase microscope. The tissue, an "explant" was observed 10 minutes after its removal from the eye. Unfixed, phase contrast (X 525).

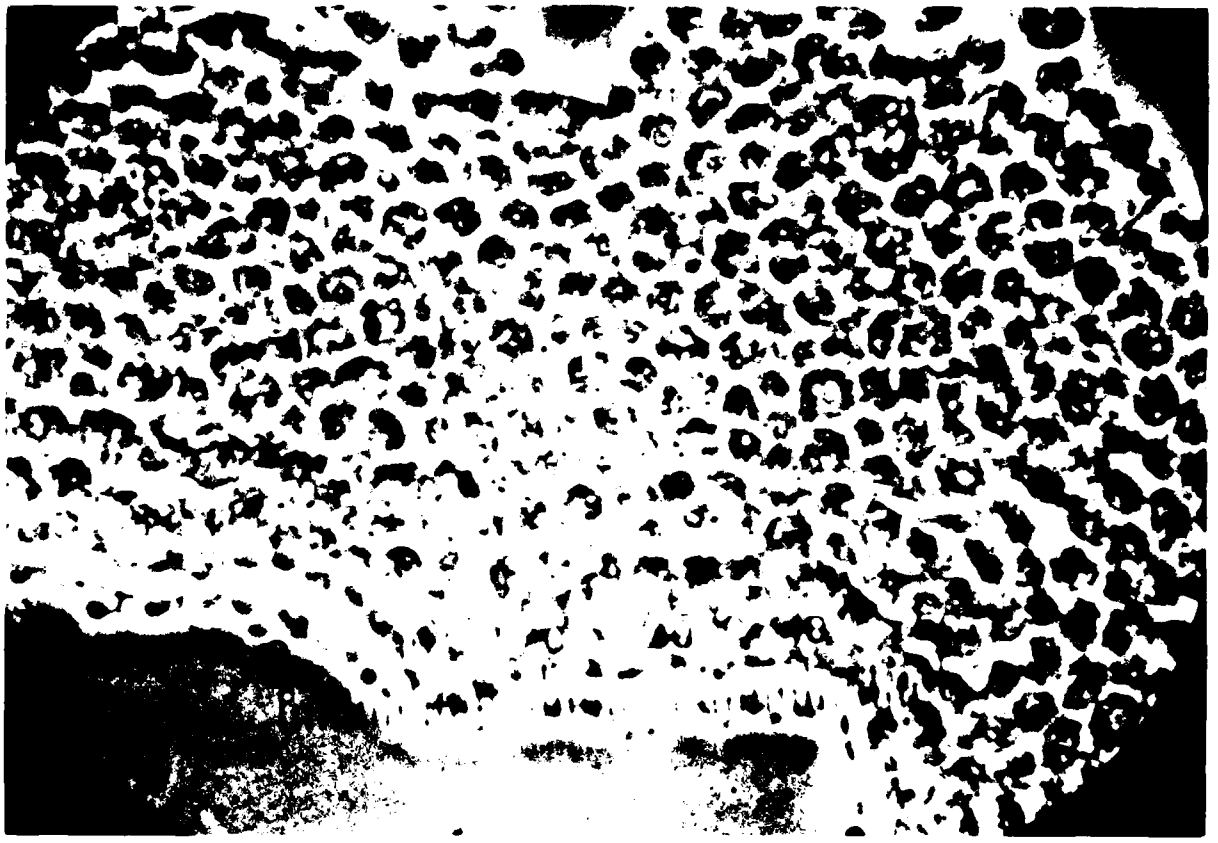


Figure 29

Legend

Figure 30: Bovine RPE explant from non-tapetal region of retina. As in figure 29, the explant was dissected free of Bruch's membrane under an operating microscope after removal of the neural retina. The cells contained a dense population of melanin granules which in sufficient quantity appear as opaque areas over the cell. Those cells with fewer granules are observed at varying shades of gray. Samples taken from more peripheral regions contain more melanin. Unfixed, phase contrast (X 525).

Figure 30

The effects of light exposure were determined by histological evaluation of stained preparation with light microscopy. Correlations between the locus of observed histopathology and exposure irradiance were made possible by the precise registration of the culture plate, both in the exposure chamber and on the microscope stage. The geometry of the irradiance field allowed each culture to serve as its own control, i.e. unexposed regions were adjacent to areas receiving a graded irradiance. Thus an entire dose-response function could be obtained from a single culture.

2. Results (Experiment III):

Exposure of RPE cell cultures to narrow band laser or broad band irradiation produced a variety pathological effects. Cell shrinkage and vacuolization were manifest in unfixed preparations observed immediately after exposure under the phase microscope. Fixed and stained preparations showed increased basophilic staining, pyknosis, and loss of cells evident by "holes" within the irradiated area. Figures 31 and 32 show representative histological findings from the control and exposed area of a tapetal RPE cell culture. Similarly, figures 33 to 35 depict cellular changes after exposure in corresponding areas of a pigmented, non-tapetal cell culture.

The threshold of the minimal damage detected histologically by the changes listed above were:

15 mW/cm^2 for irradiation with 335-600 nm

23 mW/cm^2 for irradiation with 457.9 nm

45 mW/cm^2 for irradiation with 514.5 nm

800 mW/cm^2 for irradiation with 590 nm

Legend

Figure 31:

This cell culture was derived from the tapetal region. The plate was exposed to broad band illumination; however, the region photographed was in the non-irradiated, control portion of the plate. The cells are polygonal in shape, contain no melanin, and show only the outline of cell nuclei. Intercellular contact appears to be maintained. The diagonal bands seen in this micrograph are due to striations in the plastic culture dish. Fixed, cresyl violet stain, phase contrast (X 825).

Figure 31

Legend

Figure 32:

This micrograph is of the same non-pigmented culture shown in figure 31. However, these cells were in the irradiated area. The irradiance on this region was 20 mW/cm^2 with a broad band illumination (350-600 nm). The cells have shrunk leaving thread-like processes contacting neighboring cells. The presence of vacuoles and pyknosis is evident. Fixed, cresyl violet stain, Phase contrast (X 825).

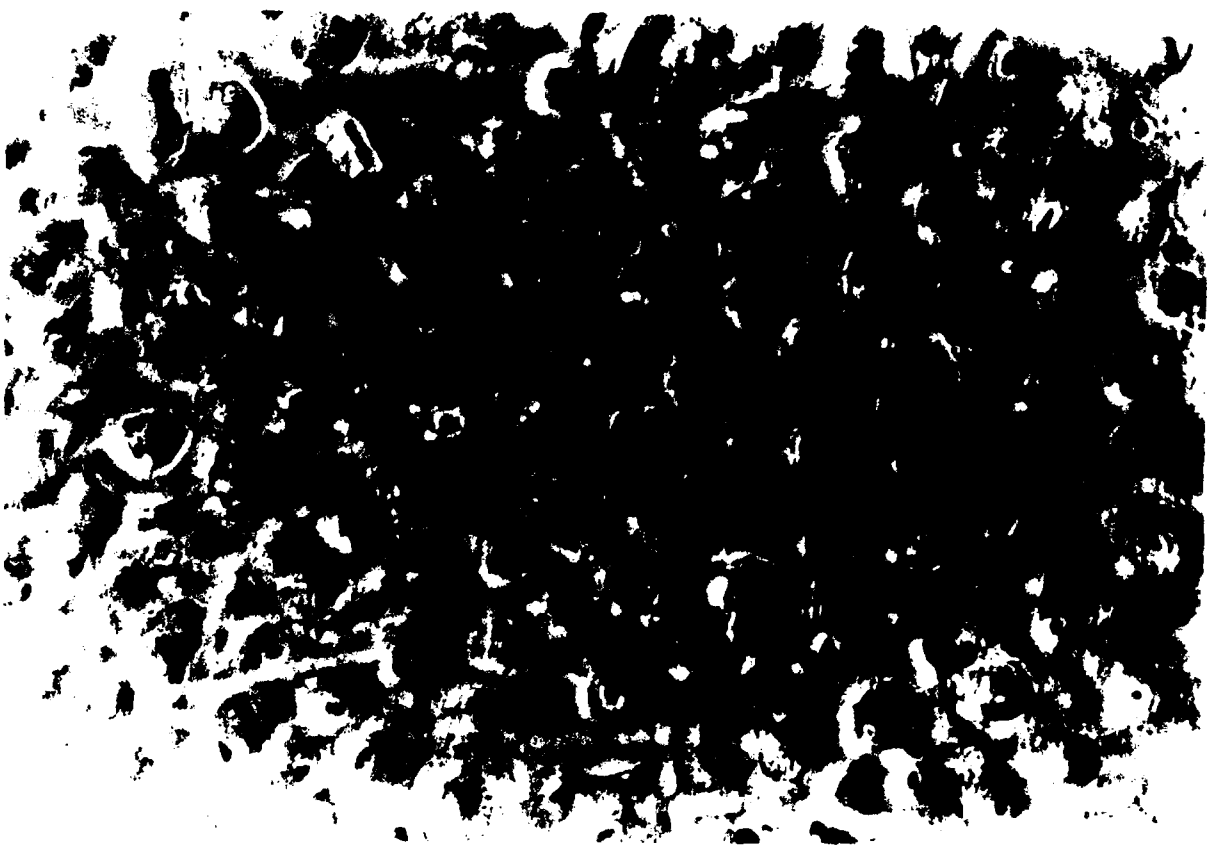


Figure 32

Legend

Figure 33:

This cell culture was derived from the non-tapetal region. The area shown here was in the non-irradiated, control portion of the plate. The cells are polygonal and in apposition to neighboring cells. The moderate degree of pigmentation in most cells is revealed by the presence of highly refractile bright flecks corresponding to aggregates of melanin granules. The dense, black structures represent a population of non-dividing heavily pigmented cells which may be choroidal melanocytes. Fixed, cresyl violet stain, phase contrast (X 400).

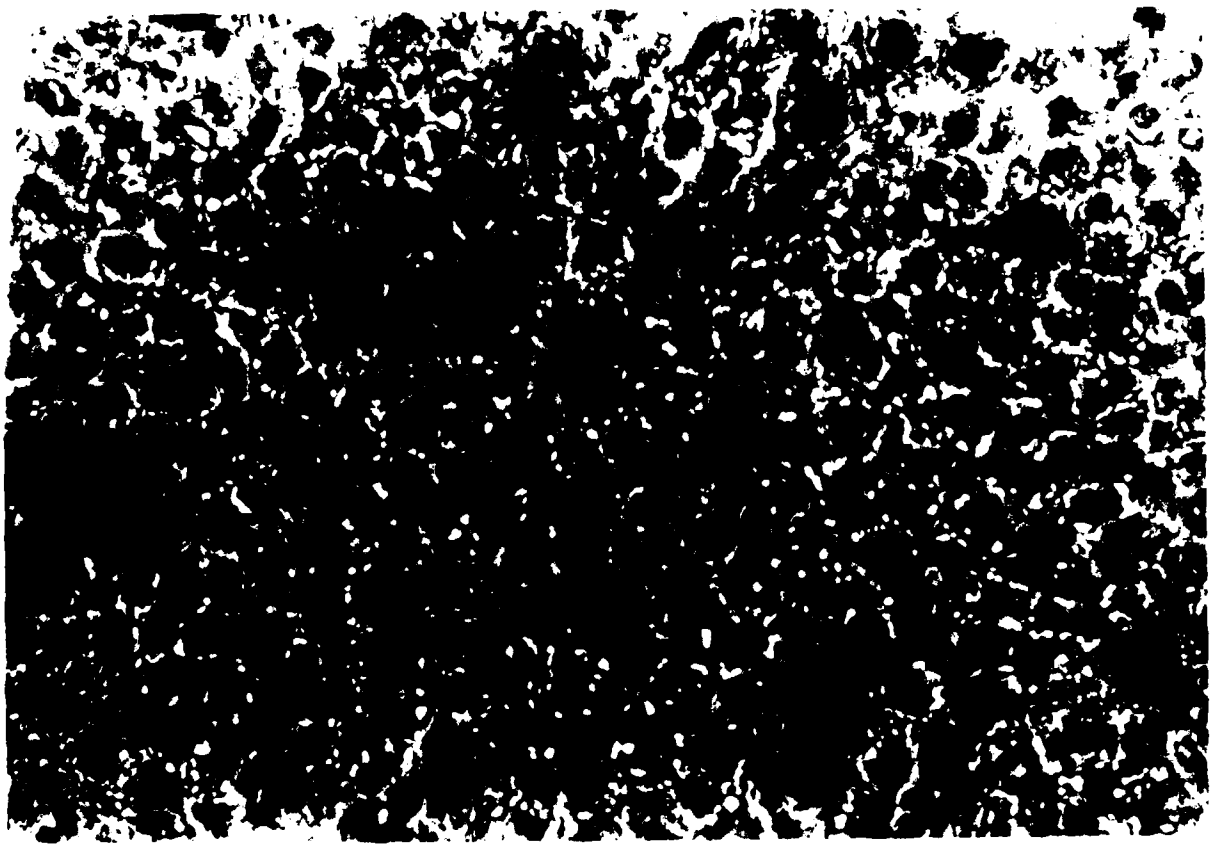


Figure 33

Legend

Figure 34:

This figure is of a different area of the same culture shown in figure 33. It shows a sharp line demarcating the exposed portions (36 mW/cm^2 broad band) on the left from the control portion on the right. Cells in the exposed region have shrunk increasing the intercellular space. The cells are pyknotic or show condensation or clumping of nuclear material. Vacuolization of cytoplasm is observed with higher resolution microscopy. Fixed, cresyl violet stain, phase contrast (X 400).

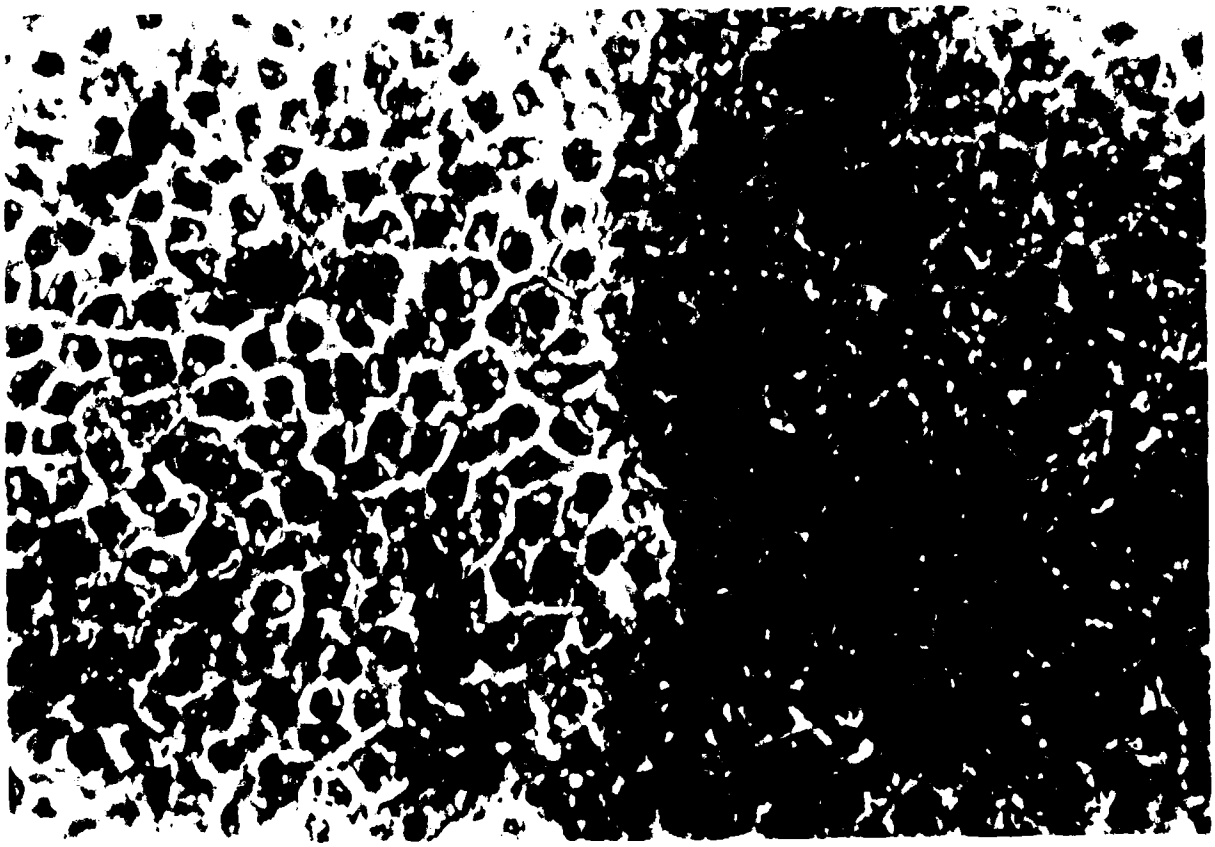


Figure 34

Legend

Figure 35:

These cells are from the same culture as presented in the two previous figures (Figs. 33 and 34). The area shown here is at the border of threshold irradiance (17 mW/cm^2 broad band). The cellular effects range from shrinkage, pyknosis, and vacuolization in the upper right corner, to decreased cytoplasmic staining and nuclear halo formation at center right, to minimal changes at lower left. Fixed, cresyl violet stain, phase contrast (X 400).

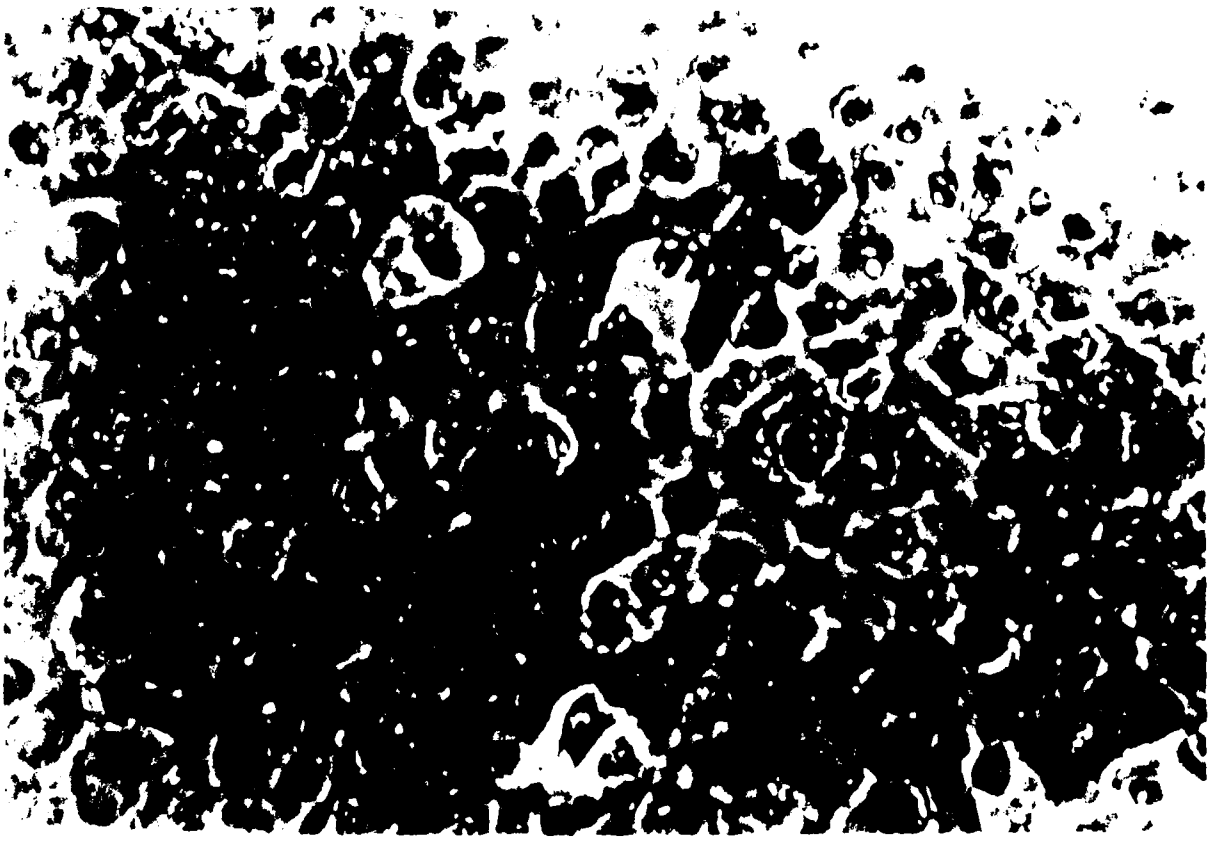


Figure 35

For comparison, the threshold of the histological damage in the macaque retina for a 4-hour exposure are given. These were:

2 mW/cm^2 for 457.9 nm

10 mW/cm^2 for 514.5 nm

30 mW/cm^2 for 590 nm

There was no difference in sensitivity related to the degree of melanin pigmentation present in the exposed cell.

3. Discussion (Experiment III)

Damage thresholds in the RPE cell culture at 457.9 nm and 514.5 nm are sufficiently similar to the in-vivo threshold to support the conclusion that similar mechanisms may mediate the effects of these wavelengths (450-520 nm) in both systems. The irradiance with these wavelengths for producing pathology is well below that required to induce thermal effects.

However, there is a great difference between the in-vivo and in-vitro system in respect to the 590 nm damage. The threshold for this wavelength is 25 times greater in the RPE culture and is sufficiently high to involve thermal processes. Exposing a monolayer of cells on a plastic substrate may significantly alter the heat diffusing characteristics of the tissue environment in comparison to the in vivo case. The 4-hour 590 nm threshold for the retina in-vivo is known to be well below the irradiance required to produce thermal damage, but this cannot be said for the in-vitro condition; in fact special measurements will have to be performed to exclude a heat effect.

The important point, however, is the following: Since damage caused at 590 nm in-vitro requires 800 mW/cm^2 vs. 30 mW/cm^2 in-vivo, this huge difference suggests that the same effect as observed in-vivo does not occur in-vitro. Thus, the pigment epithelial pathology seen in the retina at 590 nm in-vivo may be secondary to the damage to other cells. In this respect, therefore, the mechanism of light damage to the pigment epithelium in the primate would resemble that in the rat (see Introduction). In the rat, the pigment epithelium is not destroyed when light exposure occurs after the photoreceptors have been lost due to a hereditary abnormality[15]. Furthermore, in the rat, the action spectrum of light damage is not compatible with a direct effect of light upon the pigment epithelium[12]. It is assumed, therefore, that the pigment epithelial pathology is secondary to the action of light on the photoreceptors in the rat model.

In the same way, the effect of 590 nm light on the cultured cells, or better the lack of it, suggests that in the primate the pigment epithelium is secondarily involved when 590 nm light produces the damage in-vivo. It remains to be studied whether the primary site of action in this case is also the photoreceptor population. It is important to state that the in-vivo effect of 590 nm light in the monkey included damage to the pigment epithelium as it did with the other wavelengths. It is important to stress also that the comparison of the in-vivo and in-vitro conditions excludes the melanin as playing a role in pigment epithelial damage. Contrary to the assumptions by others melanin may neither enhance the damage nor protect from it.

IV. FINAL DISCUSSION: The Three Pathways of Light Damage.

Damage of the first kind as described in the rat by Noell[8] has as its prime histological feature, the localized loss of the rod cells and of the adjacent pigment epithelium when more than 15% of the rhodopsin is bleached by light applied continuously for 2 to 6 days at normal body temperature. He typically notes a loss of all rod nuclei with the preservation of cone nuclei in the area of greatest damage. This type of damage is modulated by body temperature and requires long-term dark adaptation prior to exposure to be fully manifest. The action spectrum of this effect follows grossly the rhodopsin absorption curve and the bleaching of rhodopsin is most probably the primary change which leads to damage.

Noell[8] described a second kind of damage in the rat which occurred when the animal was very young or when an adult animal had been kept for weeks in cyclic light prior to exposure. Rhodopsin concentration in these retina was 15-30% lower than in the long term dark-adapted retinas. The outer segments also tended to be shorter than in the dark group[43]. In this case the pigment epithelium was not lost. Outer segment degeneration was a prominent feature and showed some evidence of recovery. Most probably, rhodopsin is also the mediator of the light damage of the second kind. Galagos night monkeys and hamsters required more light to produce an effect. And, when produced it resembled the second kind.

There is only scant evidence of this effect in the primate. It appears that the sensitivity of rhesus rod cells is significantly less than of the rhesus cone cells. Histological changes similar to those seen by Noell[12] in the pigment epithelium and rod cells are only seen in areas

where extensive destruction of cone cells and inner layers has occurred. The flash ERG is also much more resistant to reduction in the primate suggesting that rod function has a high survival potential in the rhesus. Therefore, it seems reasonable to conclude that the pathway to light damage initiated by rhodopsin is not significantly active in the primate under the exposure conditions studied. It follows that the rod cells of the rat (and of the nocturnal Galagos monkey) differ importantly from those of the rhesus monkey.

The second pathway to light damage is demonstrated by Sperling's experiments. Permanent destruction of blue sensitive cones and long-term inactivation of green sensitive cones were observed in primates using exposure to specific wavelengths. In these experiments rod function remained normal. In response to long-term intermittent exposure with intense spectral light, the pathological finding showed selective loss of cone cells in a mozaic pattern consistent with the distribution of specific cone types. The effect was proportional to the predicted cone density for each type for the central two degrees beyond which the proportion of damaged cones was less than predicted from the distribution of the 3 cone types. The histological sections accompanying Sperling's article show moderate to extensive change in the pigment epithelium, particularly prominent in the section three degrees from the fovea. Outer segments also appear swollen and disoriented in this section. In the electron microscopic picture the mitochondria are normal, even apparently in one cell which is pyknotic.

With psychophysical tests Sperling was able to show permanent loss of blue sensitivity and reversible loss or green sensitivity in animals exposed to these wavelengths. He was unable to "red blind" the animals with long wavelength light.

In contrast, the studies reported in this report show the combination of two effects. First, the signs of damage found to increase at short wavelengths were evident histologically throughout the spectrum. In addition, the spectral ERG was reduced across the spectrum for the exposure to every wavelength. Second, a differential effect was evident in the ERG measurements of Experiment II, especially by comparing the effects on "red" sensitivity after exposure to 457.9 nm (or 514.5 nm) with those after 590 exposure.

It is assumed, therefore, that a cone specific damage is riding on top of a less specific short wavelength effect and that the cone specific second pathway to light damage is active in single exposures of one hour duration. This second pathway is clearly overshadowed, however, by the short wavelength effect and the pathway and processes upon which this effect depends.

The short wavelength effect is the main pathway to damage in the rhesus and cynomolgus retinas during single exposures. It affects all layers of the retina. Its action spectrum for clinically observed damage does not follow the action spectrum of rhodopsin or cone pigments. Damage is greater with shorter wavelength 458 nm light than with longer wavelengths. The histological changes are found throughout the retina including cells of the inner nuclear layer and ganglion cell layers. The degree to which each layer is affected is variable, as are the changes throughout

the fundus in any given eye. Pigment epithelial involvement is present in most but not all cases. It may be absent even when extensive outer segment damage is evident.

The short wavelength effect is most easily explained by the work of SantaMaria et al[44]. They published a series of experiments of direct importance to this one of the three types of pathology. They discovered that isolated bovine retinas, when exposed to light, showed a marked reduction in oxidative respiration, whereas anerobic glycolysis was less affected. They found that liver cells and other tissues not normally exposed to light did not exhibit this inhibition by light. They determined that the inhibition was accelerated in oxygenated media and almost absent when the media was saturated with nitrogen. They determined the effect as a function of time and wavelength. The effect was evident with their intensity of light after 10 minutes exposure. It continued to become stronger for 50 minute exposures and then began leveling off. Their spectral action curve peaked between 380 and 500 nm, as close as they could determine with their methods. They note this effect to be opposite that of x-ray irradiation which affects anerobic glycolysis first. In the rat retina they noted that respiration and glycolysis were affected to the same extent.

Subsequent work on beef heart muscle mitochondria by Nimmemann[45,46], is more specific on this subject. She reports inhibition of respiration in beef heart mitochondria by light and the destruction of cytochrome a_3 as

as the possible primary site of light action. The Sorbet absorption band for these cytochromes is at the short wavelength end of the visual spectrum and could very well correlate with the histology and functional effects in this model.

The anatomical description presented here shows one group of organelles to be by far the most affected by light exposure: the mitochondria. Not only that but specific mitochondria of the "fat" type found in cones are differentially susceptible. The mitochondria located in the inner layers of the retina are in some instances more affected than the packed mitochondria in the inner segments. One might relate this to possibly a lower oxygen tension at the receptor layer than near the capillaries of the inner layers. The hypothesis that the initial damage is in the mitochondria is especially attractive, since the mitochondria contain potentially photodynamic compounds such as cytochromes and flavoproteins, some of which happen to have an absorption band in 390-440 nm region, the short-wavelength region of light damage.

I propose that the short wavelength effect as described (and the effect studied by Ham[10]) has the following mechanism: Light passing through the retina is absorbed by all mitochondria. Wavelengths between 390 and 440 nm are specifically absorbed by some components possibly a cytochrome. This destroys a specific reaction in the oxidative cycle. It causes the cell to drastically decrease its function but not die, because energy supplied by glycolysis is sufficient to maintain the integrity of the plasma membrane of the cell. Glycogen builds up; function continues at a reduced rate, and returns toward normal only in those cells which have retained sufficient functional mitochondria to repair the damage and which have

the regenerative capacity to replace mitochondria. The continuing production of distorted outer segments could be supported by a reduced energy source. The distortion could be secondary to either mechanical distortion of the cell or, more likely, abnormal synthesis and abnormal membrane assembly. The abnormal glycogen accumulation might be secondary to a decrease in the rate of oxygen consumption.

Given this mechanism it would appear that destruction of a majority of mitochondrial activity in any cell is necessary before the cell succumbs to damage. The mitochondrion may contain sufficient genetic information to reproduce itself. Repeated exposures would be expected to be additive to some extent, but this addition would be decreased by any repair that could occur between exposures. The fact that mitochondria must be reproduced places total repair on a long time scale, especially since the respiration of the cell is compromised. The meaning of this might be that cumulative short wavelength light effects may be an important factor in the retinal pathology of advanced age. At the other end of the age spectrum, we must consider that the short-wavelength effect may be enhanced by high oxygen concentration and that the infant's retina may be endangered if both phototherapy and supplemental oxygen is administered.

In discussing the type and location of histological damage seen in the monkey retina, one must address what is primary and what is secondary. Work with functional measures had led us to evaluate the histological changes especially at a period 1 to 3 months after exposure. This long after exposure, scattered damage throughout all layers of the retina is evident. Individual hyperchromaticity can be seen most prominently in

receptor cell bodies and processes. The inner and outer segments are distorted in their form. The pigment epithelium also presents pathology. However, function as measured by the ERG returns to normal in 2 to 4 weeks in many instances. Several questions exist: (1) Do these distorted inner and outer segments function normally or nearly so? (2) Do the hyperchromatic cells become this way immediately or are cells still dying three or more months following the insult. There are gaps found in the pattern of receptors to suggest that cells are missing. When were they lysed and removed? Appearances suggest that some may just fade away slowly while other cells remain fossilized. Or, are these darkened cells with condensed cytoplasm and nuclei still metabolicly surviving and even functional?

For these reasons only an arbitrary definition of primary and secondary can be given. Primary refers to a direct immediate effect of light on the cell in question from which it does not recover completely. Secondary includes effects in the same or different cells which may develop with time after exposure. The data presented on 1 hr, 24 hr. and 7 days after exposure are not significantly different from those studied at longer survival intervals up to 5 months. The comparison must be made with a clear understanding of the variability in degree of damage from eye to eye and from area to area within each eye. Already at 12 hours, there are present hyperchromatic nuclei in the outer nuclear layer and condensed cytoplasm. Cells in the inner nuclear layer and ganglion cells are also disintegrating at this time. The pattern seen is the same as that seen except for (a) the spaces left in the cellular pattern, (b) pale staining cone nuclei, and (c) enlarged and disorganized processes in the outer plexiform layer. All these (a-c) are most obvious at later times. It is concluded therefore, that the

main histological changes after a single exposure result from the direct, immediate effect of the damaging light. There are most probably "primary" effects.

Because the retina is such a complex organ, it is likely that the individual cell's sensitivities will change depending upon their environment and level of activity. Therefore studying each cell type separately or even in binary combination will only give a hint as to their susceptibility in the functioning organ. Nevertheless, such a systematic approach is warranted because of the importance of the light effect and the reputed possibility of potentiation of some hereditary retinal degenerations and/or retinal toxicity from other agents. The studies with the cultured pigment epithelium are a start of this research.

There are many unknowns in the relationships between exposure and effect for which new data are required. (1) As emphasized, there is a large difference in susceptibility across species. (2) There is a difference in the most susceptible cell type not only across species but also depending upon the intensity-time-wavelength parameters of the damaging stimulus in the same species. (3) In some models basal body (retinal) temperatures have a profound effect while in others it does not. (4) The anatomical damage in individual cells appears almost all or none. This digital response may have as its basis the efficient repair mechanisms which are continually active. Some cells die, some live, very few are in between. (5) While each animal model shows a most sensitive area of retina, it is not the same area in the different animals. The rabbit is the most striking case, where the damage occurs in an oblong area with a vertical/horizontal disparity of more than one to four. Certainly, this

is well beyond any differential in retinal illumination. (6) The area of greatest susceptibility seems to reflect the area of the greatest number of receptors, the central area in primates and the visual streak in rabbits. (7) There is little effect of choroidal and pigment epithelial melanin concentration upon the susceptibility of the retina in rabbits. In varigated fundi the strip of damaged area shows no discontinuity or change in dimension crossing over the sharp borders of change in pigmentation. (8) There must be influence from factors such as metabolic demand nutritional supply, synthetic activities, plasma membrane properties, genetic makeup, systemic or ocular abnormalities. (9) The area of retina most susceptible to one type of light damage is also that area most susceptible to chloroquine in the primate. The susceptibility therefore does not necessarily follow the patterns of greatest illumination. The retina can be made even more susceptible by toxins or deficiencies be they externally or internally (genetically) modulated. (10) Leaving a frog in darkness can also lead to retinal degeneration. This might suggest that any sudden change in the biological rhythm can cause a deleterious effect on this highly sensitive tissue, the retina.

These and many other questions are open to future research. I am confident that most can be answered within the foreseeable future and that the time is not far off to apply the experimental work to the human. I hope that the work described in this report will facilitate reaching this goal.

V. SUMMARY:

This document presents information which is important in present day research and understanding of damage by light to the retina. It presents a description of three distinct pathophysiological mechanisms of light damage which combine in various proportions in different animal models. The pathology in each species is dependent upon a unique mixture of the three mechanisms. The proportion of damage represented by each mechanism is dependent upon the characteristics of the species and the physical parameters of intensity, wavelength and duration of the damaging light.

The first mechanism is visual pigment, rhodopsin specific. It is seen in the rat and some other nocturnal animals whose rod cells are several orders of magnitude more sensitive to damage than primate rods, and more sensitive than their own cones. This mechanism is almost non-existent in the primate. It is as though the rods of rhesus and cynomolgus monkeys are for some unknown reason specifically resistant to light damage, and are only affected when all the cells around them have already been severely damaged.

The second mechanism is cone pigment specific and occurs in the most pure form in primates when long-term, repeated, low intensity spectral exposures cause damage. It is also seen in combination with the third mechanism, short wavelength light effect, as shown in this thesis. Long term functional loss of specific cone populations (red sensitive, green sensitive) following spectral exposure is demonstrated by ERG chromatic sensitivity. This mechanism is overshadowed in the primate by the short wavelength light damage which is considerably less specific.

The third mechanism, the one which is responsible for light damage in the primate in single exposures, has an action spectrum peaking

in the short wavelength (blue) end of the visual spectrum. It leads to damage in all layers of the retina, from the pigment epithelium through the nerve fiber layer. For this particular effect, this report presents a definitive, testable hypothesis as to its mechanism. The hypothesis is that the effect is caused by a direct action of light on the mitochondria in the different retinal layers which inactivates the respirating enzymes, other effects being secondary to or additive with this major mechanism.

Three experiments are presented as well as a detailed account of work by the author over the last ten years. The first experiment on more than 80 monkeys establishes the action spectrum of light damage in the primate retina for 1 to 4 hour single exposures. Its results provide an all inclusive description of the histopathological features of this effect and how they vary with the physical exposure parameters. The progress of these changes is followed for periods ranging from one hour to 5 months after exposure. Measurements include a clinical (ophthalmoscopic and fluorescein angiographic) description and grading of the damage and flash ERG recordings. The clinical and histological descriptions depict a great variability in the severity of damage as well as of its patchy location in specific layers and retinal regions. The general trend shows the damage to be most concentrated in a concentric ring between 3^{o} and 5^{o} from the fovea and to be located histologically throughout all layers of the retina and pigment epithelium.

In the second experiment, the chromatic bar pattern ERG was utilized to determine chromatic sensitivity before and after narrow band light exposures. It demonstrated specific cone type susceptibility overshadowed by the short wavelength (blue) light effect.

The third experiment exposes pigmented and non-pigmented pigment epithelial cells in culture. There is no difference in damage threshold between the pigmented and non-pigmented cells. The striking difference is between the action spectrum in-vivo and in-vitro. The damage thresholds at 457.9 and 514.5 nm are relatively similar, but for 590 nm the sensitivity is 25 times less in culture (800 mW/cm^2). The pigment epithelium's participation in the in-vivo 590 nm damage (30 mW/cm^2) appears to be secondary to effects of the light on adjacent tissues.

REFERENCES:

1. Parrish, John A., Anderson, R. Roy, Urbach, Frederick and Pitts, Donald: UV-A: Biological Effects of Ultraviolet Radiation with Emphasis on Human Responses to Longwave Ultraviolet. Plenum Press, New York, 1978, p. 2.

2. Giese, A.C.: Ultraviolet action spectra in perspective: With special reference to mutation. Photochem. Photobiol. 8:527-546, 1968.

3. Kripke, M.L. and Fisher, M.D.: Immunologic parameters of ultraviolet carcinogenesis. J. Natl. Cancer Inst. 57:211-215, 1976.

4. Wolbarsht, M.L., Yamanashi, B.S. and Orr, M.A.: The origin of cataracts in the lens from infrared radiation. Report 7-20-1977: 62772A 3E62772A813.00.013, Duke Univ. Eye Center, Durham, NC, Contract DAMD 17-74-C-4133 US Army Med. Rsh. and Dev. Command, Washington, D.C., 1977.

5. Penner, R. and McNair, J.N.: Eclipse blindness. Am. J. Ophth. 61:1452, 1966.

6. Chisum, G.T.: Flashblindness recovery following exposure to constant energy adaptive flashes. Aerospace Med. 44:407-413, 1973.

7. Clarke, A.M., Geeraets, W.J. and Ham, W.T., Jr. An equilibrium thermal model for retinal injury from optical sources. Appl. Optics. 8:1051-1054, 1969.

8. Noell, Werner, K.: There are Different Kinds of Retinal Light Damage in the Rat. In THE EFFECTS OF CONSTANT LIGHT ON VISUAL PROCESSES, Eds. Theodore P. Williams and B.N. Baker, Florida State Univerisity, Tallahassee, FL, Plenum Press, New York, 1980.

9. Harwerth, R.S., Sperling, H.G.: Effects of intense visible radiation on the increment-threshold spectral sensitivity of the rhesus monkey eye. Vis. Res. 15:1193-1204, 1975.

10. Ham, W.T. and Mueller, H.A.: Retinal sensitivity to damage from short wavelength light. Nature. 260:153-155, 1976.

11. Lawwill, T., Crockett, R.S. and Currier, G.: Retinal damage secondary to chronic light exposure thresholds and mechanisms. Documenta Ophth. 44:379-402, 1977a.

12. Noell, W.K., Walker, V.S., Kang, B.S., Berman, S.: Retinal damage by light in rats. Investigative Ophthal. 5(5):450-473, 1966.

13. Noell, W.K., Delmelle, M.C., Albrecht, R.: Vitamin A deficiency effect on retina: Dependence on light. Science. 172:72-76, 1971.

14. Noell, W.K., Albrecht, R.: Irreversible effects of visible light on the retina: Role of Vitamin A. Science. 172:76-80, 1971.

15. Noell, Werner K.: Possible mechanisms of photoreceptor damage by lights in mammalian eyes. Vis. Res. 20:1163-1172, 1980.

16. Rudeen, P.K., O'Steen, W.K.: The effects of the pineal gland on light-induced retinal photoreceptor damage. Exp. Eye Res. 28:37-44, 1979.

17. Hollyfield, Joe G., Rayborn, Mary E. and Medford, Donna: Damaging effects of constant light and darkness on the retina of the frog. In The Effects of Constant Light on Visual Processes, Ed. Theodore P. Williams and B.N. Baker, Plenum Press, New York, 1980.

18. Friedman, E. and Kuwabara, T.: The retinal pigment epithelium. IV. The damaging effects of radiant energy. Arch. Ophthal. 80:265-279, 1968.

19. TSO, M.O.M., Fine, B.S., and Zimmerman, L.E.: Photic maculopathy produced by the indirect ophthalmoscope. Amer. Journal of Ophthalmology. 73(5):686-699, 1972.

20. Dawson, W.W. and Herron, W.L.: Retinal illumination during indirect ophthalmoscopy: Subsequent dark adaptation. Investigative Ophthalmology. 9:89-96, 1970.

21. Hochheimer, B.F., D'Anna, S.A. and Calkins, J.L.: Retinal damage from light. Am. J. Ophthal. 88:1039-1044, 1979.

22. Lawwill, Theodore, Crockett, R.S. and Currier G.: The Nature of Chronic Light Damage to the Retina. In The Effects Of Constant Light On Visual Processes. Eds. Theodore P. Williams and B.N. Baker, Plenum Press, New York, 1980.

23. Ham, W.T., Mueller, H.A., Ruffolo, J.J., Clark, A.M.: Sensitivity of the retina to radiation damage as a function of wavelength. Photochem. Photobiol. 29:735-743, 1979.

24. Ham, W.T., Ruffolo, J.R., Jr., Mueller, H.A., Clarke, A.M. and Moon, M.E.: Histologic analysis of photochemical lesions produced in rhesus retina by short-wavelength light. Inves. Ophthal. Vis. Sci. 17:1029-1035, 1978.

25. Lawwill, T., Sharp, F. and Speed, N.: Study of ocular effects of chronic exposure to laser radiation. Report No. III. Army Contract DADA17-68-C-8105, April, 1968 to August 1973a.

26. Everett, M.A., Sayre, R.M. and Olson, R.L.: Physiologic response of human skin to ultraviolet light. In The Biologic Effects of Ultraviolet Radiation (with Emphasis on the Skin) Ed. F. Urbach, Pergamon, Oxford, pp. 181-186, 1969.

27. Hollaender, A.: Effect of long ultraviolet and short visible radiation (3500 to 2900 A) on Escherichia coli. J. Bacteriol. 46:531-541, 1943.

28. Machemer, Robert: Experimental retinal detachment in the owl monkey: II. Histology of retina and pigment epithelium. Am. J. of Ophthalmol. Vol. 66, pp. 396-410, Sept. 1968.

29. Wyszecki, G. and Stiles, W.S.: Color Science. John Wiley & Sons Inc., New York, pp. 217-219, 1967.

30. Wyszecki, G. and Stiles, W.S.: Color Science. John Wiley & Sons Inc., New York, p. 218. 1967.

31. Osterberg, G.: Topography of the layer of rods and cones in the human retina. Acta. Ophthalmologica Sup. 6, 1935.

32. Shiono, Takashi, Hayasaka, Seiji, Hara, Satoshi and Mizuno, Katsuyoshi: Topographic distribution of lysosomal enzymes in human ocular fundus. ARVO Abstract, p. 186, No. 31, 1980.

33. Boettner, E.A., Wolter, J.R.: Transmission of the ocular media. Invest. Ophthal. 1:776-783, 1962.

34. Sliney, David and Wolbarsht, Myron: Safety with lasers and other optical sources. Plenum Press, New York, p. 131, 1980.

35. Beatrice, E.S., Frisch, G.D.: Retinal laser damage thresholds as a function of image diameter. Arch. Environ. Health. 2:322-326, 1973.

36. Sliney, David and Wolbarsht, Myron: Safety with Lasers and Other Optical Sources. Plenum Press, New York, p. 129, 1980.

37. Marlor, R.L., Blais, B.R., Preston, F.R., Boyden, D.G.: Foveomacular retinitis, an important problem in military medicine: Epidemiology. Invest. Ophthal. 12:5-16, 1973.

38. Tso, M.O.M. and LaPiana, F.G.: The human fovea after sun gazing. Trans. Am. Acad. Ophth. and Otolaryng. 79:788-795, 1975.

39. Sperling, H.G., Johnson, Clement and Harwerth, R.G.: Differential spectral photic damage to primate cones. Vis. Res. 20:1117-1126, 1980.

40. Armington, John A.: The Electroretinogram. Academic Press, Inc., New York, 1974.

41. Lawwill, T., Reese, E., Sharp, F. and Cox, W.: Study of ocular effects of chronic exposure to laser radiation. Report No. 1 Army Contract DADA17-68-C-8105. April-December, 1968.

42. Johnson, E. Parker, Riggs, Lorin A., Schick, Amy M.L.: Photopic retinal potential evoked by phase alternation of a barred pattern. In Clinical Electroretinography Proceedings of the third international symposium. Eds. Hermann Burian and Jerry Hart Jacobson. Pergamon Press, New York, 1966.

43. Organisciak, Daniel T. and Noell, Werner K.: The rod outer segment phospholipid/ opsia ratio of rats maintained in constant dark or cyclic light. Invest. Ophthal. Visual Sci. 16:188-190, 1977.

44. SantaMaria, L.: Natural photodynamic sensitivity in retina and cancer cells. In Radiation Research, Proceedings of the Third International Congress of Radiation Research, Ed. G. Silini, North Holland Pub. Co., Amsterdam, 1967.

45. Ninnemann, H., Butler, W.L., Epel, B.L.: Inhibition of respiration and destruction of cytochrome A3 by light in mitochondria and cytochrome oxidase from beef heart. BBA. 205:507-512, 1970.

46. Ninnemann, H.: Photoinhibition of isolated complexes I, II, and III of Beef Heart Mitochondria. Febs. Letters. 39:353-358, 1974.

47. Sykes, Stephen M., Robison, Jr., W. Gerald, Waxler, Morris and Kuwabara, Toichiro: Damage to the monkey retina by broad-spectrum fluorescent light. Investigative Ophthalmol. & Vis. Sci. Vol. 20/4, pp. 425-434, April, 1981.

48. Cope, Freeman W., Sever, Raymond J., and Polis, David B.: Reversible free radical generation in melanin granules of the eye by visible light. Arch. Biochem. Biophys. 100:171-177, 1963.

APPENDIX

Preface: The material supplied in this Appendix is composed of the scientific content of Report Number V, Mechanisms of Retinal Damage from Chronic Laser Radiation: Thresholds and Mechanisms, Contract Number DAMD 1774C4026 which was submitted in June, 1980 from the University of Louisville.

The effect upon the retina of exposure to large fields of bright visible light has been evaluated. The thresholds for permanent retinal damage for 4, 1, and 0.25 hour exposures in rhesus or cynomolgus monkeys have been established for laser lines of 514.5 nm, 488 nm, 457.9 nm, and 590 nm. In addition, the effect of distributing the four hour exposures in one hour doses separated by intervals of one day or one week has been studied. The damage has been evaluated by ophthalmoscopy, electroretinography, fluorescein angiography and light and electron microscopy. The shortest wavelength light (457.9 nm) is more effective in causing damage, particularly histological damage, which is spread throughout the fundus and throughout the retinal layers. There appears to be more than one mechanism for retinal damage in chronic light exposure, and at least one mechanism is not dependent solely upon the visual pigment and the pigment epithelium. The results from our studies suggest that with relatively low intensity, long duration exposures there is a lack of reciprocity between exposure duration and irradiance level. There appears to be a saturation effect in which during exposures of increasing duration little additional damage occurs. A similar effect occurs when the interval between exposures is increased beyond a critical duration, i.e. the threshold for damage from one hour exposures separated by seven day intervals is not appreciably different from a single one hour exposure, whereas with shorter intervals between exposures the effect is additive. Thresholds of permanent damage appear to be within one or two log units of light levels encountered in the normal visual environment.

METHODS

Exposure Procedure:

The experimental animals are female cynomolgus monkeys weighing between 3 and 4 kg. Prior to exposure, the animal is given 0.12 mg of atropine sulfate and 8 mg of phencyclidine HCl intramuscularly. When the monkey is sedate, it is placed in a primate chair. Initiation of anesthesia is by means of a 20 mg/kg intravenous dose of sodium pentobarbital. A constant intravenous infusion of sodium pentobarbital (0.22 mg/min., 2.84 mg/ml) is begun and is continued throughout the four hour exposure. The eye to be exposed is dilated with one drop each of atropine 1%, tropicamide 1%, and phenylephrine 10%. The light is presented to the eye in Maxwellian view. The eye lid is held open by a Burian Allen contact lens electrode. This protects the cornea and keeps it moist. The alignment is maintained by the experimenter's sighting through a beamsplitter to allow direct visualization of the exposed fundus. The intensity of exposure is constantly monitored via a beamsplitter in the light path reflecting on an Eppley thermopile. Exposures are for a period of from 14 minutes to 4 hours with a constant intensity light source covering 50.1 degrees solid angle (0.88 cm^2 of retina). The intensity of the beam is measured with a Gamma Scientific Model 2020a spectroradiometer calibrated with a standard of spectral irradiance traceable to the National Bureau of Standards. The homogeneity of the field is checked with a small (2.5 mm diameter) cosine receptor by measuring the intensity at the center and edge of the field. The uniformity is maintained with 50%.

The retinal area exposed is calculated both from the angle of convergence of the incident beam and from direct measurement of the chord

of the exposed section and the diameter of the eye using a freshly enucleated monkey eye. The area value used in calculating the irradiance is taken from the direct measurement. There is about a 20% difference between the two results.

We evaluate retinal damage induced by light by four measures: Electroretinogram, (dark adapted, pan-retinal flash and light adapted, localized pattern), funduscopy and fluorescein angiography, light and electron microscopy. Our methods of evaluation have been reported (Lawwill, 1972, 1973b, 1976, and 1977a,b).

RESULTS

Initially our subject was the rhesus monkey. Approximately 100 eyes were exposed to delineate the action spectrum of damage for a 4 hour continuous exposure. In 1978 due to the increased cost of this specimen and more importantly, the lack of availability, we elected to change to the cynomolgus monkey. This change necessitated redefining the model. This has been accomplished. Table I lists physical measurements made on the globe size of the two specimens. These data were used to calculate the retinal irradiance of exposure. When damage thresholds for the two specimens are based upon retinal irradiance (uncorrected for ocular media absorption) the values obtained are the same.

The largest portion of our effort has been directed to determining the action spectrum of damage as a function of several parameters. These factors are summarized in Table IIa and IIb. Our goal in this series of experiments is to (1) determine the degree of reciprocity between exposure duration and irradiance and (2) to determine the effect of distributing a four hour exposure over four one hour periods separated by various intervals. These studies are in progress. At the present we have determined that the threshold of damage at 516.5 nm with an exposure duration of 4 hours, 1 hour, or 0.25 hour is 10, 20 and 90 mW/cm^2, respectively (Figures 1, 2, 3).

To determine the effect of repeated exposures we began a series of experiments in which a total exposure of 4 hours duration was divided into 4 one hour exposures. The set of intervals between exposures chosen was 1 day or 7 days. The respective damage thresholds at 24 hour intervals was found to be 10 mW/cm^2. Data is still being taken to complete the threshold determination at 7 day intervals but it appears to fall in the neighborhood of

20-30 mW/cm^2. It was the preliminary determination of this threshold at 20-30 mW/cm^2 level that led us to interrupt the 7 day series and work on the single one hour threshold. Since the two are so similar, there appears to be little additivity of effort when near threshold exposures of one hour's duration are repeated at weekly intervals.

	d(cm)	a(cm)	$S(cm^2)$
Rhesus	1.9	1.6	2.54
Cynomolgus	1.8	1.0	0.89

d - diameter of globe

a - diameter of circle described by intersection of illuminating cone of light with posterior pole

$$S = 2 \frac{d^2}{4} [1-\cos(\arcsin \frac{a}{d})]$$

DURATION (hr)

(nm)	4	1	0.25
457.9	8	4	-
514.5	25	13	12
590	13	-	-
488	11	-	-
476	4	-	-

Table IIa. Matrix showing number of eyes exposed in single session under conditions given. Cells above the dashed line will be completed first. N>13/cell is required to adequately define the damage vs. intensity relationship.

INTERVAL (da)

(nm)	1	3	7
457.9	-	-	2
514.5	9	2	3
590	-	-	-
488	-	-	2
476	-	1	2

Table IIb. Matrix showing number eyes exposed in four, one-hour sessions under the conditions indicated. Cells above the dashed line will be completed first. N>13/cell is required to adequately define the damage vs. intensity relationship.

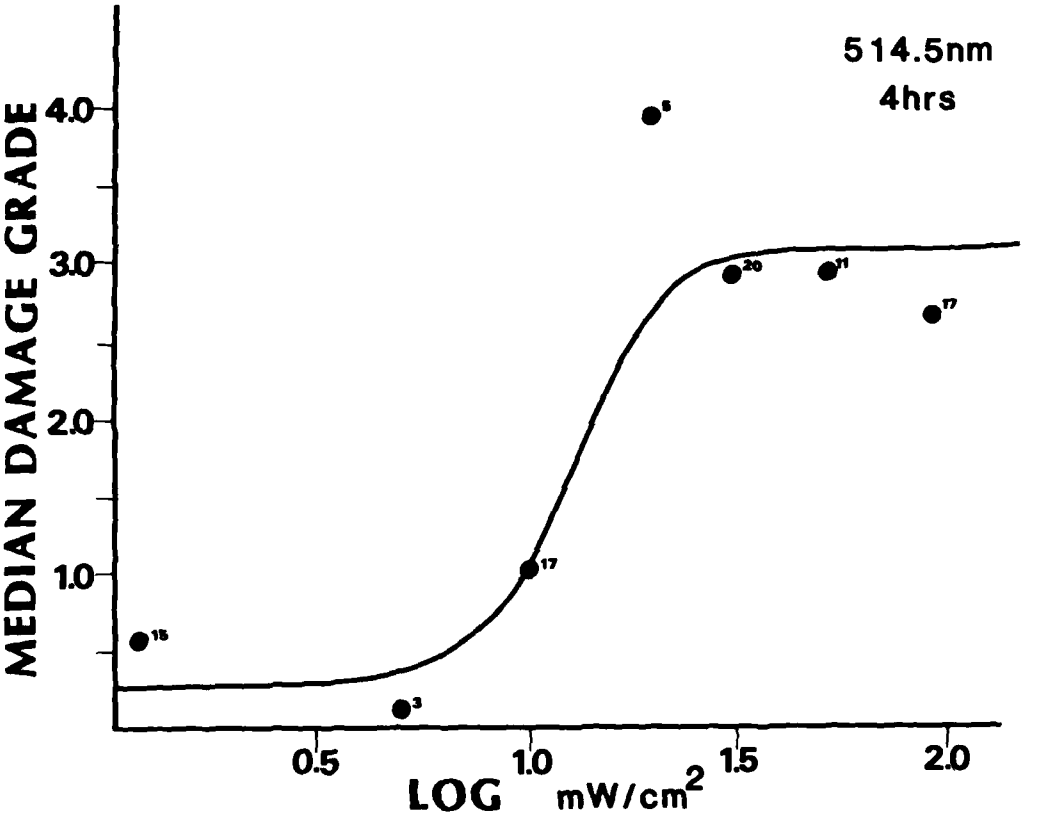

514.5nm
4hrs
MEDIAN DAMAGE GRADE
4.0
3.0
2.0
1.0
0.5
1.0
1.5
2.0
LOG mW/cm2
15
3
17
6
20
21
17

FIGURE 1

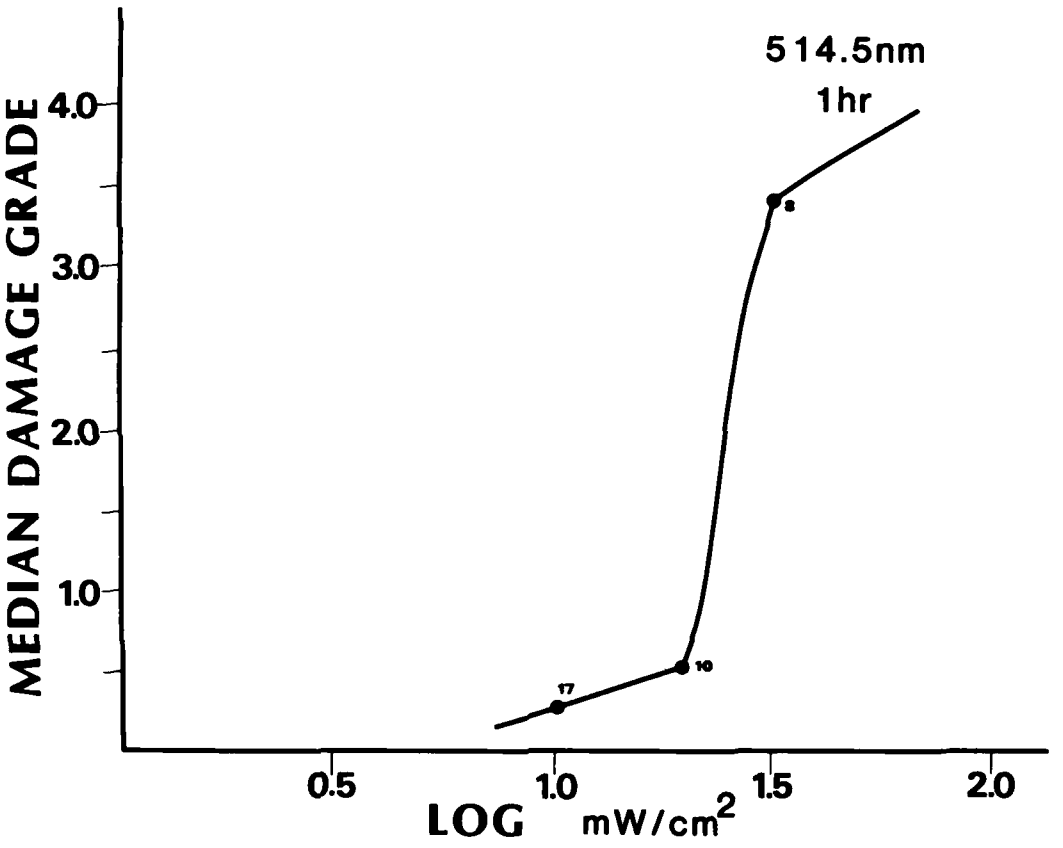

514.5nm
1hr
MEDIAN DAMAGE GRADE
4.0
3.0
2.0
1.0
0.5
1.0
1.5
2.0
LOG mW/cm2
17
10
8

FIGURE 2

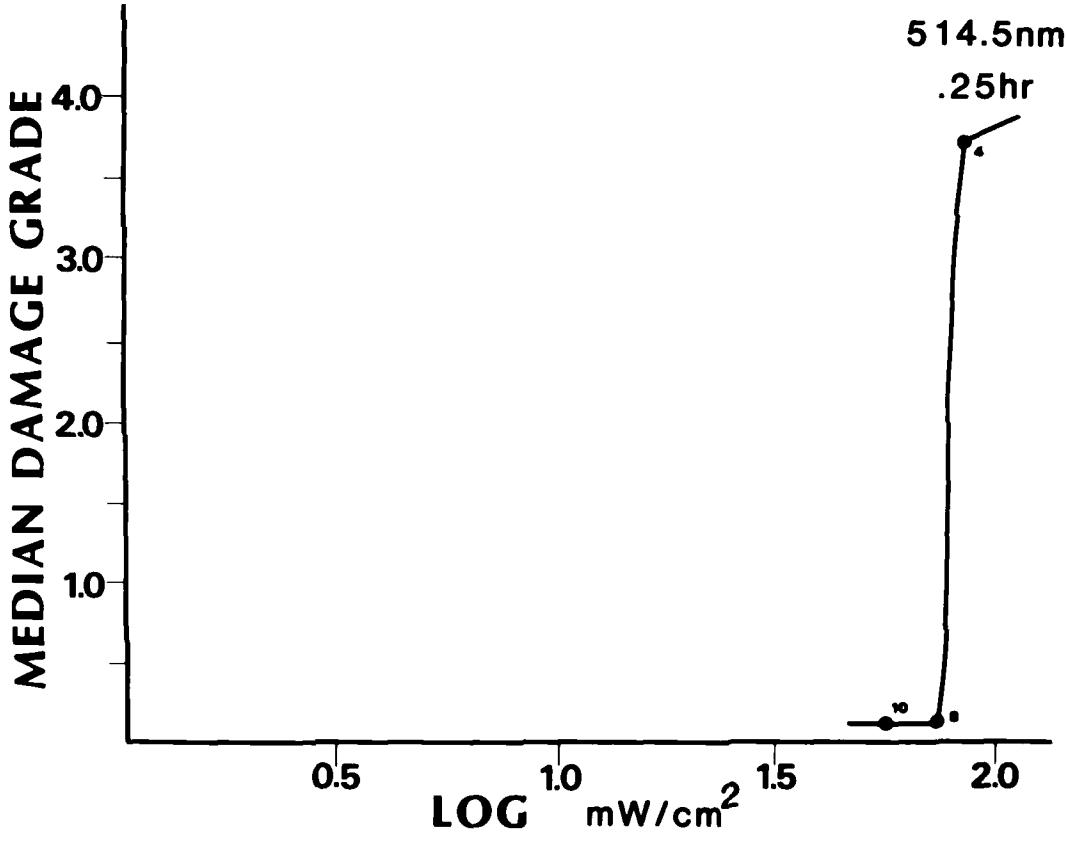

514.5nm
.25hr
MEDIAN DAMAGE GRADE
4.0
3.0
2.0
1.0
0.5
1.0
1.5
2.0
LOG mW/cm2

FIGURE 3

DISCUSSION

This line of research has led to several interesting and unexpected conclusions. When this work was first begun, the available evidence in the literature suggested that the damaging effects of light on the retina were mediated by a visual pigment and that the primary site of effect was the photoreceptor outer segment and pigment epithelium. We soon determined that the action spectrum of damage did not fit a visual pigment absorption curve. The threshold for damage at 457.9 nm was one log unit below that at 514.5 nm. We also found that while the cells most consistently affected were photoreceptors and pigment epithelium other retinal cell types were also damaged. In the range within one log unit above damage threshold all cellular elements were susceptible to being damaged.

The shape of the irradiance-duration reciprocity function was unexpected. The 0.25 cm (900 sec.) threshold at 90 mW/cm^2 is only eight times the 4 hour threshold rather than 16 times as the reciprocity hypothesis would predict. However, the 0.25 hour threshold is approximately 4.5 times the one hour threshold. Our conclusion is that beyond one hour of exposure little additional damage is produced. This conclusion is supported by the observation that the damage threshold for 4 distributed one hour exposures is 8 mW/cm^2 at 24 hour intervals and 20-30 mW/cm^2 at 7 day intervals. Given the levels of irradiance required to produce just detectable amounts of damage, with our evaluation procedure, it appears that the insult occurs by the first hour with a threshold of from 10-30 mW/cm^2. Additional exposures produce little additional effect. These data complement the findings of Ham, et al, (1979) which show reciprocity for 441.6 and 514.5 nm exposures from 10 to 1000 second duration. Our results extend their relationship to 3600 seconds; but beyond that period proportionally more energy (Joules)

is required for exposures longer than 3600 seconds. Our thresholds for comparable exposure conditions are lower than Ham et al, (1979) by about 0.2 log units. However, their criteria for damage is a funduscopically visible lesion. Our threshold determination reflects a contribution from functional and histopathological measures and should be slightly more sensitive.

Since the action spectrum of damage in our model doesn't correlate with the absorption spectrum of the visual pigment, what is absorbing the light? Some investigators (Ham, et al, 1979) have noted a rough correspondence between the action spectrum of damage and the absorption spectrum of melanin as well as the involvement of the retinal pigment epithelium at low levels of exposure. A melanin hypothesis is difficult to support however. While the role of species pigmentation on light damage is unsettled (Reuter and Hobbelen, 1977; Rapp and Williams, 1980; Howell and Williams, 1980) there is no evidence to show that the albino is less sensitive to light damage than is a pigmented species. We have noted in our rabbit studies that in animals with varigated pigmentation of the fundus, the pattern of damage is not correlated with the pattern of pigmentation. In addition, we have noted in monkeys exposed to near threshold levels of light that there are areas of retina in which the retinal pigment epithelium appears normal in the face of disrupted and disorganized outer segments.

We will address the pigmentation issue directly in the near future utilizing our bovine retinal pigment epithelium tissue culture model. We have been successful in differentially harvesting pigmented and non<pigmented retinal epithelium from the cow eye. Pigmented cells lie outside while non-pigmented cells lie over the tapetal area of the bovine fundus. We will determine the threshold for damage in each of these cell types. Assuming

we find no other significant difference between these types other than pigmentation, the study should be decisive in evaluating the merit of the melanin hypothesis. The validity of the bovine retinal pigment epithelium culture model is presently under study. The threshold of damage as crudely measured by cell death is in the range of the irradiance effective in vivo. Whether the type of effect of the cellular level is similar has not been determined.

We have adopted several strategies to reveal the involvement of other chromophores in the light damage process. Other researchers (Harwerth and Sperling, 1975) have noted selective receptor damage in the monkey retina. While our histological results have not shown that such changes occur, we are continuing to develop other more sensitive tests of these effects. The use of the pattern evoked spectral electroretinogram will provide this kind of information. Our results to date suggest that a differential cone effect does occur. However, we have analyzed the dates from only 457.9 and 514.5 nm exposures. It is necessary to expose of 590 nm to confirm the hypothesis of selective cone damage. If we find a reduced spectral sensitivity in the same spectral range as the exposing wavelength, this would provide strong support that sub-population of cones are being affected.

We have begun a project, partially funded from other sources, which will allow us to measure in vivo, visual pigment regeneration kinetics biochemical constituents following light exposure. A fundus reflectometer with digital photon-counting system is under construction. It will be combined with the exposure optics to make spectral reflectance measures during and after exposure. These results will provide information on the metabolism of photochemicals in the retina; and if a normal chromophore is produced as a by-product of exposure and is present in sufficient quantity, we should be able to detect it.

SUMMARY

Our research in light damage is evolving. Emphasis has been placed on careful measurement of the physical variables, parametric manipulation of these variables and a multifactorial assessment of the effects produced. This constitutes what could be termed a descriptive stage of research. This information is essential to the classification and organization of information. It gives us an answer to the question of "what" it is we are studying. These data are essential in planning the next phase of investigation in which the emphasis will be to explain the phenomenon of light damage. In this phase we will determine what biochemical entity is absorbing the light energy and the mechanism by which cellular function is altered leading to a pathological state. With an understanding of this process, investigation will move into the final phase of determining a means of manipulating or controlling the system to whatever advantage we choose.

RECOMMENDATIONS

In light of the findings of this study, I would recommend continued investigation into the chronic effects of light on the retina - thresholds and mechanisms. The importance to the military is great because those levels of light which cause damage to the retina are close to everyday environmental levels, and in the military situation uncomfortably high levels might be endured to complete a mission when the conditions would not be endured in the normal civilian situation.

Some areas which need further examination: (1) the increased sensitivity at the blue end of the spectrum; (2) the cumulative effect of repeated exposures; and (3) the mixture of mechanisms which control the several types of damage in different animals and which have different action spectra as well as thresholds.

BIBLIOGRAPHY

Ham, W.T., Jr., Mueller, H.A., Ruffalo, J.J., Jr. and Clarke, A.M. Sensitivity of the retina to radiation damage as a function of wavelength. Photochemistry and Photobiology 29:735-743, 1979.

Harwerth, R.S. and Sperling, H.G. Effects of intense visible radiation on the increment-threshold spectral sensitivity of the rhesus monkey eye. Vision Res. 15:1193-1204, 1975.

Howell, W.L. and Williams, T.P. Melanosomes of the retinal pigment epithelium: Distribution and correlation with light-damage. Supplement to Invest. Ophthalmol. and Visual Science 15:190, April 1980.

Lawwill, T. The ERG and its correlation with damage caused by chronic exposure to light. Documenta Ophthalmologica Proceedings Series 2:65-76, August 1972, Xth ISCERG Symposium, Los Angeles. Jerome T. Pearlman (editor).

Lawwill, T. Effects of prolonged exposure of rabbit retina to low-intensity light. Invest. Ophthalmol. 12:45-51, 1973a.

Lawwill, T., Sharp, F. and Speed, N. Study of ocular effects of chronic exposure to laser radiation. Report #III, Army Contract DADA17-68-8105, 1973b.

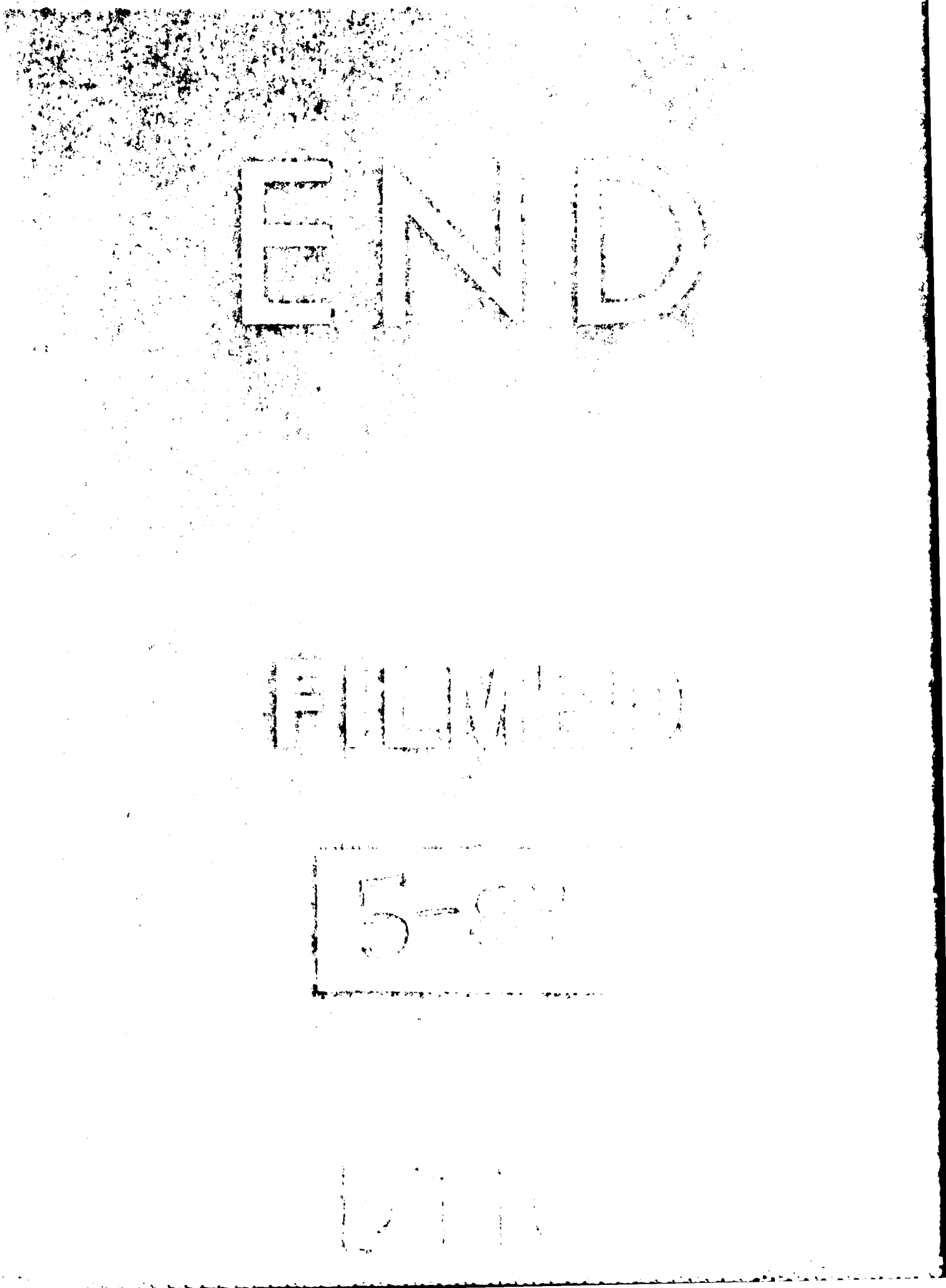
Lawwill, T., Crockett, S. and Currier, G.J. Mechanisms of retinal damage from chronic laser radiation. Report #II, Army Contract DAMD17-74-C-4026, 1976.

Lawwill, T., Crockett, S. and Currier, G.J. Functional and histological measures of retinal damage in chronic light exposure. Documenta Ophthalmologica Proceedings Series 15:285-295, 1977, XVth ISCERG symposium, Ghent. Jules Francois and Alfred De Rouck (editors), June 1977a.

Lawwill, T., Crockett, S. and Currier, G.J. Retinal damage secondary to chronic light exposure, thresholds and mechanisms. Documenta Ophthalmologica 44(2):379-402, 1977b.

Rapp, L.M. and Williams, T.P. The role of ocular pigmentation in protecting against light-induced damage to the rat retina. Supplement to Invest. Ophthalmol. and Visual Science 189: April 1980.

Reuter, J.H. and Hobbelen, J.F. The effect of continuous light exposure on the retina in albino and pigmented rats. Physiology and Behavior 18:939-944, 1977.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