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BIOLOGICAL APPLICATIONS AND EFFECTS OF OPTICAL MASERS

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March 16, 1982 - October 15, 1986

William T. Ham, Jr., Harold A. Mueller, John J. Ruffolo Jr., Stephan F. Cleary, R. Kennon Guerry and Dupont Guerry, III.

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The literature pertaining to retinal light damagefrom environmental and man-made optical sources prior to the last decade is reviewed with particular attention to acute and long-term chronic exposure leading to thermal and photochemical lesions of the retina. The parameters of wavelength, exposure time and power level are given as the major factors defining thermal vs photochemical damage. Light toxicity as a function of wavelength is dealt with in some detail. In particular, wavelengths ranging from 1064 nm to 325 nm in the near ultraviolet are studied using macaque monkeys. Exposures in the near UV were accomplished using aphakic monkeys (lens removed surgically). The histopathologie of blue light and near ultraviolet lesions of the retina in the macaque monkey are described. Light damage to the RPE and its possible relationship to aging and macular degeneration is investigated. Repetitive exposures to wavelengths 440, 475 and 533 nm on the same site of the macaque retina are investigated for daily exposures ranging from 50 to 10% of threshold over a period of 21 days. A radiant exposure to 440 nm light at 10% of threshold for acute damage produced a lesion after 20 daily exposures. Basic mechanisms leading to photochemical or (cont)

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19. Continued: or actinic injury to the retina are discussed in terms of oxygen free radicals and excited molecules like singlet oxygen and the body's defense mechanisms against oxygen and light toxicity are described. Monkeys exposed to 440 nm light and/or 325 nm near UV radiation while oxygenated (breathing 80/20 oxygen/nitrogen or 100% oxygen) reduced the threshold radiant exposure for retinal damage by a factor of three or more. Studies of the effects of superoxide dismutase and catalase on the blue light lesion were inconclusive but beta-carotene did protect the retina of a monkey exposed to blue light under high levels of blood oxygen tension. Threshold radiant exposure to the retinae of macaque monkeys were determined for 40 μs pulse trains at pulse repetition frequencies (PRF'S) of 100, 200, 400 and 1600 Hz for two laser wavelengths, 647 and 488 nm. Histological data were obtained on the retina of one monkey exposed to 40 μs pulses of 647 nm laser light at a PRF of 1600 Hz. A rhesus monkey trained for visual function tests was exposed to a MILES prototype GaAs laser (910 nm) on a daily basis for a period of 4 months. There was no evidence of damage to the retina or to visual function.

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1. FOREWORD

In Conducting the research desc-ibed in this report, the investigator(s) adhered to the " Guide for the Care and Use of Laboratory Animals," prepared by the committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW publicatin No. (NIH) 78-23, Revised 1978).

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2. BACKGROUND AND RESEARCH SYNOPSIS

It is only in recent times that the actinic or photochemical effects of short wavelength light on the retina have come to be recognized. Prior to 1950, thermal injury was the concept used almost exclusively to explain retinal damage resulting from excessive exposure to infrared (IR) and visible light. Birch-Hirschfeld in 1912 postulated that the visible portion of the solar spectrum was responsible for solar retinitis and/or eclipse blindness.[1] He believed that the effects of sunlight on the retina were abiotic or photochemical in nature. His opinion was contested by Verhoeff and Bell who produced what they considered incontrovertible evidence that solar retinitis was a thermal phenomenon.[2] This dictum dominated the thinking of most ophthalmologists for the next 5 decades.

During World War II, the damaging effects of sunlight on the retina became a major concern for military personnel operating in the Pacific and Mediterranean theaters. Observers spotting planes attacking from the direction of the sun sustained severe retinal lesions.[3] Smith had already reported in 1944 on actinic pigment degeneration in the macula of 150 servicemen stationed on a tropical island in the Pacific.[4] He described the retinal photopathology as similar in appearance to senile macular degeneration. Cordes described the syndrome of foveomacular retinitis, observed in 1948 among 176 naval personnel.[5] It consisted of macular edema with loss of foveal reflex. Dispute over the etiology of this disease was reviewed by Marlor et al in 1973.[6] They concluded that solar retinitis was a probable cause. Another aspect of chronic light damage to the retina during World War II occurred in the prison camps in southeast Asia where thousands of allied troops were interned under severe malnutrition or starvation conditions and daily exposure to bright sunlight.[7,8] These prisoners of war experienced a loss of central vision and in many cases macular lesions similar to those observed after sun gazing.

After the war, the ocular hazard from the nuclear fireball became a matter of concern. In Nevada, rabbits exposed to a nuclear fireball received retinal lesions out to distances of 42 miles, while monkeys exposed in an airplane to a high altitude burst, developed retinal lesions at 300 nautical miles from the fireball. We developed in the laboratory a carbon arc source to simulate the nuclear flash and used it to study retinal lesions in the rabbit.[9] This apparatus was used to treat successfully a macular angioma in a patient and represents the first photocoagulation treatment performed in this country.[10] Meanwhile, Meyer-Schwickerath in conjunction with the Zeiss Optical Company had developed the first clinical photocoagulator.[11] We purchased one of the first three coagulators to be exported from Germany and used it to treat patients. We also developed a research coagulator for short pulses using a 2500 W xenon lamp with associated optics.[12] This apparatus modified by quartz optics, filters, "hot" and "cold" mirrors etc. has been a valuable research tool throughout the years, providing a simulated solar spectrum, 10 nm bandwidths throughout the visible and near infrared spectrum and near ultraviolet wavelengths down to 300 nm.

Most of our early research before lasers became available involved short exposure times and high power levels which made it valid to assume that thermal injury was the basic mechanism involved in retinal damage. By 1966, enough data on rabbits was available, including some laser data, to plot irradiance in $W \cdot cm^{-2}$ vs exposure time in seconds for minimal or threshold retinal damage over 10 log units of time, from 30 nanoseconds (10^{-9}) to 100 seconds.[13] Plotted logarithmically, these data fell on a

straight line from 30 nanoseconds and 3 $MW \cdot cm^{-2}$ to 1 s and 10 $W \cdot cm^{-2}$. Beyond one second, the curve flattened out along the abcissa but never quite reached a plateau. The common interpretation was that the plateau represented a temperature too low to cause thermal denaturation. There was no evidence at the time to indicate a wavelength dependence. In fact, over the straight portion of the curve it was immaterial whether the radiation was infrared or visible, coherent or incoherent.

We found the threshold for retinal damage in the rhesus monkey when exposed to a simulated solar source at sea level for three minutes to be 18.9 $W \cdot cm^{-2}$, corresponding to a temperature rise of less than 3°C.[14] Most authorities on thermal injury and denaturation do not believe that such a small temperature rise can damage tissue. It is generally assumed that 10°C above ambient is the threshold for thermal damage. Accordingly, we postulated that this represented some type of thermally enhanced photochemical damage.

We were able also to show that the simulated solar spectral bandwidth 400-1400 nm was at least five times more effective than the bandwidth 700-1400 nm in producing a retinal lesion in the monkey. This led us to believe that the short wavelengths in the solar spectrum were primarily responsible for solar retinitis and that infrared radiation produced injury only when the power level was high enough to cause a retinal burn. In later and more elaborate experiments with the simulated solar source we proved that in the rhesus monkey solar retinitis and eclipse blindness were photochemical phenomena caused by the short wavelengths in the solar spectrum and that the infrared component produced negligible injury.[15]

These observations prompted us to make a definitive study of photic damage to the retina as a function of wavelength.[16] We exposed the rhesus retina to 8 monochromatic laser lines extending from 441 nm in the blue visible to 1064 nm in the near infrared. We found that the retinal sensitivity increased dramatically in the blue region of the spectrum, especially for long exposure times (1000 s). The corneal power required to produce a minimal lesion in 1000 s increased by three orders of magnitude in going from 441 nm to 1064 nm. Furthermore, the type of lesion produced by 441 nm was entirely different from that produced by 1064 nm. The latter was a retinal burn whose image diameter was smaller than the exposed site and at a temperature rise of 23°C, whereas the 441 nm lesion occured with negligble temperature rise (< 0.1°C) and the lesion appeared two days after exposure and was full size. This was clear evidence that some type or types of actinic or photochemical reaction(s) were produced by short wavelength light.

Histologically as well as morphologically, minimal burn lesions differ markedly from minimal blue light lesions.[17] When thermal lesions are examined by light microscopy at 24 to 48 hours postexposure it is found that many of the photoreceptor cells have been irreversibly damaged (pyknotic) as well as the cellular structure of the retinal pigment epithelium (RPE). Destruction is greatest at the center of the lesion, tapering off toward the periphery because maximum temperature occurs at the center of the irradiated area. Thus, a minimal burn lesion is always smaller than the irradiated area. In contrast, minimal blue light (441 nm) lesions are nearly uniform across the irradiated area and do not appear until 48 hours after exposure. Histologically, damage appears initially in the RPE at 48 hours postexposure. The RPE is inflamed and edematous, melanin pigment granules are agglutinated resulting in depigmentation, and macrophages filled with melanin granules appear in the subretinal

space.[18] The photoreceptors do not begin to show major damage until 5-6 days postexposure. By 20 to 30 days postexposure a minimal blue light lesion has healed leaving only hypopigmentation and macrophages in the subretinal space. Tests with rhesus monkeys trained to perform a visual task show that 20/20 vision is lost 5-6 days postexposure but returns in 20-30 days.[19] After 60 days the macrophages have disappeared from the subretinal space; at 90 days a slight granular and depigmented area remains in the RPE that bears a suggestive resemblance to age-related macular degeneration (AMD).

We have extended our investigation of retinal sensitivity to radiation into the near ultraviolet (UV). Aphakic monkeys (lens removed surgically from one eye) were exposed to 405, 380, 350 and 325 nm radiation from our 2500 W xenon lamp system with quartz optics. We found that the rhesus retina was 6 times more sensitive to wavelengths 350 and 325 nm than to 440 nm blue light.[20] The photochemical effects of near UV radiation are similar but more exaggerated than those of blue light damage and include, in addition to injury to the RPE cells, extensive damage to the photoreceptors, especially the cones.

The basic mechanisms promoting or causing photochemical reactions in the retina are unknown but there is good reason to believe that oxygen free radicals and reactive molecules like superoxide, hydrogen peroxide, hydroxyl radical and singlet oxygen play an important role in producing toxic effects. To test this hypothesis we exposed the retinae of anesthetized macaque monkeys under high levels of arterial blood-oxygen tension (PO_2's ranging from 100-350 mm Hg) to short wavelength light (435-445 nm) and compared the threshold for retinal damage to that determined under normal conditions. The results were definitive.[21] Threshold radiant exposure in $J \cdot cm^{-2}$ decreased exponentially with increase in PO_2. We have also shown that oxygenation reduces the threshold for near UV retinal damage by a factor of three or more. Histological analysis showed excessive damage to the RPE.[22] These experiments strengthen but do not prove the hypothesis that oxygen free radicals are involved in toxic reactions produced in the retina during light or near UV exposure. In another experiment, a monkey fed beta-carotene over a 2 year period was definitively protected from blue light retinal damage when exposed under high levels of arterial blood-oxygen tension. The protective action of beta-carotene under these conditions implies that singlet oxygen may be an important toxic factor since beta-carotene is known to be an efficient scavenger of singlet oxygen. However, it is also an effective scavenger of many other excited molecular species.

In a series of recent experiments we have attempted to detect the effects of superoxide dismutase (SOD) and catalase on the retinal toxicity of blue light (440 nm).[23] These enzymes are specific for the disautation of superoxide to hydrogen peroxide and oxygen and the catalysis of hydrogen peroxide to oxygen and water. They were injected intravenously (i.v.) into monkeys both before and after exposure of the retina to measured radiant exposures of blue light. The results were erratic and difficult to interpret and we have concluded that this method of administration is not effective because of the short half-life of these enzymes in the circulation and their inability to penetrate the blood-retinal barrier at the RPE.

Pulses of 40 microseconds duration at pulse repetition frequencies (PRF) of 100, 200, 400 and 1600 Hz at the laser wavelengths 647 and 488 nm have been investigated for minimal or threshold damage in the macaque retina. The threshold for 488 nm pulses is

always lower than the threshold for 647 nm pulses and for 1000 s exposures at 1600 Hz, the 488 nm threshold is even lower than the cw threshold for 647 nm. For each PRF the difference in threshold between the two wavelengths increases with exposure time; the difference widens as the PRF increases. Technical difficulties with the acoustic modulator attachment to the argon-krypton laser have prevented us over the past two years from investigating pulse trains at PRF's of 10 and 100 kHz and 1, 10, and 20 MHz. We hope to overcome these difficulties during the next year.

Daily exposures of the two trained monkeys to near UV radiation (330-420 nm) were terminated in February 1985. One animal with 3 mm pupillary diameter received 1171 daily exposures of 1000 s duration to 5 $mW \cdot cm^{-2}$ as measured at the cornea. The other animal with dilated pupils (>8 mm) received 584 daily exposures under identical conditions. No evidence of cataract in the exposed eye of either animal has been detected up to the present time (March 1987). These animals will be maintained in their cages and examined every 3 months.

The significance of our research should not be overlooked. Since its inception in 1972 our research program has provided valuable biological data leading to the establishment of ocular safety standards by the Armed Services, the American National Standards Institute (ANSI Z-136 and Z-311) and other groups such as the American Industrial Hygiene Association (AIHA) and the American Conference of Governmental Industrial Hygenists (ACGIH). A primary objective was to establish safe exposure levels to the eye of laser radiation, particularly those wavelengths used by the Armed Services. Laser wavelengths investigated include the following: CO_2 10.6 μm, HF and DF at 2.5-3.0 μm, GaAs at 820, 830, 850 and 905 nm, He:Cd at 441 and 325 nm, and argon-krypton 458, 488, 514 and 647 nm, He:Ne at 633 nm, Nd:YAG at 1064 nm and argon at 351 and 363 nm. We have shown that the wavelengths in the near infrared emitted by GaAs lasers (820-910 nm) do not present an ocular hazard at the levels used in the MILES prototype system or in fiber optic communication systems.[24] Solar retinopathy and eclipse blindness,[14,15] foveomacular retinitis,[5] actinic macular pigment degeneration[4] and nutritional amblyopia from starvation plus exposure to sunlight[7,8] are all primarily photochemical phenomena resulting from either acute or chronic exposure to the short wavelengths (550-400 nm) in the solar spectrum which at sea level peaks at about 470 nm and represents the most important environmental hazard to the retina. There is convincing evidence suggesting that acute exposure to sunlight causes a photochemical type of maculopathy that can be identified as the blue light retinopathy and there are a number of reasons for believing that long-term exposure to blue light accentuates aging in the RPE, contributing to age-related macular degeneration.[23,24] For example, the depigmentation accompanying the aging process in the retina is similar to that resulting from blue light exposure. A significant feature of the blue light lesion is the loss of melanin granules suggesting that the formation of complex melanolipofuscin granules is increased. Increased photooxidation of the outer segments of the rods and cones by blue light and oxygen increases the accumulation of lipofuscin in the RPE cells, leading to the extrusion of debris onto Bruch's membrane. Thus light exposure increases the phagocytic burden of the RPE, producing more lipofuscin and melanolipofuscin granules, depigmentation, undigested cross-linked photooxidation products and drusen, the precursors of age-related macular degeneration.

After defining the action spectrum for retinal damage in the visible and near infrared spectrum,[16] we have shown that the mammalian retina is 6 times more sensitive

to near UV radiation than to blue light[20] and that the action spectrum continues to increase exponentially down to 325 nm; that near UV injury involves both the RPE and the photoreceptors; that oxygenation increases the sensitivity of the retina to both blue light and near UV radiation; that beta-carotene increases the threshold for damage at high arterial blood-oxygen tensions, thereby implying that singlet oxygen may play an important role in photochemical toxicity; that photochemical lesions are histologically different from thermal lesions and that when the lens is removed the mammaliann retina is extremely sensitive to small daily exposures to near UV radiation. Our research on the sensitivity of the retina to near UV radiation has had a profound influence on the modern practice of implanting intraocular lenses (IOL's) in patients after lens extraction for cataract. IOL's that strongly attenuate the UV are now commercially available and are being implanted in patients by ophthalmic surgeons. There is also a growing awareness among ophthalmologists that overexposure of the retina to short wavelength light and near UV radiation during surgical procedures is a major cause of those retinopathies occuring after lens extaction.[27,28] Our research has helped establish U.S. Army safety standards and laser safety standards promulgated by the American National Standard Institute (ANSI Z-136.1).[29]

3. LIGHT TOXICITY AS FUNCTION OF WAVELENGTH

The first definitive study of photic damage in the retina as a function of wavelength was performed by Ham et al[16] using the rhesus monkey as the experimental animal. We exposed the rhesus retina to 8 monochromatic laser lines extending from 1064 nm in the near infrared to 441 nm in the visible blue. Exposure times were 1, 16, 100 and 1000 s. The criterion for minimal damage was the appearance of a visible lesion as seen in the fundus camera at 48 hours postexposure. Each laser beam was optically adjusted to produce a Gaussian distribution on the retina which was 500 micrometers in diameter at the $1/e^2$ points. The action spectrum for minimal retinal damage rose exponentially toward the short wavelengths in the visible spectrum. The corneal power required to inflict a minimal lesion with 1064 nm radiation was three orders of magnitude greater than for blue light at 441 nm when the exposure time was extended to 1000 s. During irradiation the calculated maximum temperature in the retina was 23°C for the infrared beam and less than 0.1°C for the blue light beam. It was obvious that thermal injury resulted from near infrared exposure while the blue light lesion was caused by some type or types of photochemical damage. These two types of retinal lesions differ not only in the basic mechanisms producing them but lead to entirely different biological effects which are distinguishable both <u>in vivo</u> with the fundus camera and histologically with the light microscope. Ruffolo et al.[17]

In another publication, Ham et al,[30] we summarized our conclusions on retinal damage as follows: There are at least three types of radiation insult in the spectral range 400-1400 nm. These are; <u>mechanical disruption</u> of retinal structure resulting from sonic transients or, at very high irradiance levels, shock waves engendered by extremely short pulses of radiation which are absorbed in the RPE and choroid, Ham et al[31]; <u>thermal insult</u> (independent of wavelength to a first approximation) resulting from absorption of energy in the RPE and choroid sufficient to produce temperatures greater than 10°C above ambient in the RPE, neural retina and choroid; <u>actinic insult</u> from the photochemical effects of extended exposure to the short wavelengths in the visible spectrum (400-550 nm) at irradiance levels too low to produce temperatures of more than a few degrees Celsius above ambient.

Power level, wavelength and exposure time are the important parameters determining the type of damage. There is no sharp demarcation between these types of retinal injury. Non-linear phenomena associated with picosecond pulses of mode-locked laser radiation merge into thermal effects as exposure times approach the microsecond range for Q-switched laser pulses. Only power level and exposure duration determine whether the damage is mechanical or thermal in nature. Wavelength is relatively unimportant except insofar as transmittance through the ocular media and absorption by the RPE and the choroid are concerned. Rate of delivery and amount of energy absorbed are the dominant factors. As irradiance on the retina is further reduced and exposure duration extended a point is reached where thermal effects become minimal or even completely negligible and wavelength becomes the dominant factor for photochemical effects.

In another study we compared the efficiency of short vs long wavelengths of light to produce minimal retinal lesions in the rhesus monkey as detected with the fundus camera at 48 hours after exposure to cw coherent and incoherent optical sources. We compared the retinal response of seven nearly monochromatic laser lines ranging from 441 to 632.8 nm with the incoherent light from a 2500 W xenon optical source using sharp cut-off filters and 80 nm bandwidth interference filters. The three types of radiation exposure are shown in Figure 1 where the radiant exposure in $J \cdot cm^{-2}$ is plotted logarithmically along the ordinate vs wavelength in nm along the abscissa. The sharp cut filter data designated by squares represent bandwidths of 435-735, 455-735, 485-735, 515-735, 545-735, 575-735, 625-735 and 675-735 nm; the 80 nm bandwidth data represented by the X's were peaked at 450, 500, 550, 600, 650 and 700 nm; the laser wavelengths denoted by the solid circles were 441, 458, 488, 514, 580, 610 and 633 nm. All exposure times were 100 s. The incoherent spot sizes on the retina were 500 micrometers and the laser spot sizes were 500 micrometers in diameter to the $1/e^2$ points of the Gaussian distribution. These experiments demonstrate clearly the influence of wavelength on photic damage to the retina and indicate that the biological effects of coherent and incoherent light are entirely similar. The nature of the damage ranges from pure photochemical at the shortest wavelengths to pure thermal at the longest wavelengths. The intermediate wavelengths produce a mixture of thermally enhanced photochemical damage and thermal damage.

Retinal thresholds in the rhesus monkey at wavelengths beyond 600 nm have been obtained by Ham et al.[24] Using a 2500 W xenon lamp with interference filters, the threshold radiant exposures in $J \cdot cm^{-2}$ were determined for wavelengths 820 ±5 nm, 860 ±5 nm and 910 ±25 nm. Exposure times ranged from 1 to 1000 s and image diameters on the retina were 500 micrometers. No significant difference in threshold was noted for these wavelengths. In Figure 2 these near infrared thresholds are compared with similar data previously obtained for laser wavelengths 1064 nm (Nd:YAG), 647 nm (Ar-Kr) and 632.8 nm (He-Ne). Radiant exposures in $J \cdot cm^{-2}$ are plotted logarithmically against exposure times in seconds. All three lines are straight and approximately parallel, indicating a similar type or mechanism of injury for wavelengths greater than 600 nm. The type of injury is thermal in nature as verified by funduscopy and histological analysis, Ruffolo et al.[17] Histological examination at 24 and 48 hours postexposure discloses structural damage in the RPE and numerous pyknotic nuclei in the outer nuclear layer. Minimal thermal lesions are always smaller than the image diameter on the retina because temperature is maximal at the center of the irradiated image. The damage is maximal at the center of the lesion, tapering off towards the periphery. This is in sharp contrast to minimal photochemical lesions where damage is fairly uniform across the lesion with a definite border between injured and uninjured RPE cells at the

periphery and only a few pyknotic nuclei in the outer nuclear layer.

Figure 1

The radiant exposure in $J \cdot cm^{-2}$ to produce a minimal retinal lesion for a 100 s exposure is plotted against wavelength in nm for sharp-cut filters (open box) 435-735, 455-735, 485-735, 515-735, 625-735 and 675-735 nm; 80 nm bandwidth filters (X's) 450 ±40, 500 ±40, 550 ±40, 600 ±40, 650 ±40 and 700 ±40 nm; laser lines (circles) 441, 458, 488, 514, 580, 610 and 633 nm. Image diameter for laser lines was 500 µm to the $1/e^2$ points of Gaussian distribution. The sharp-cut and 80 nm filters image diameter was 500 µm across a uniform distribution as produced by a xenon optical source.

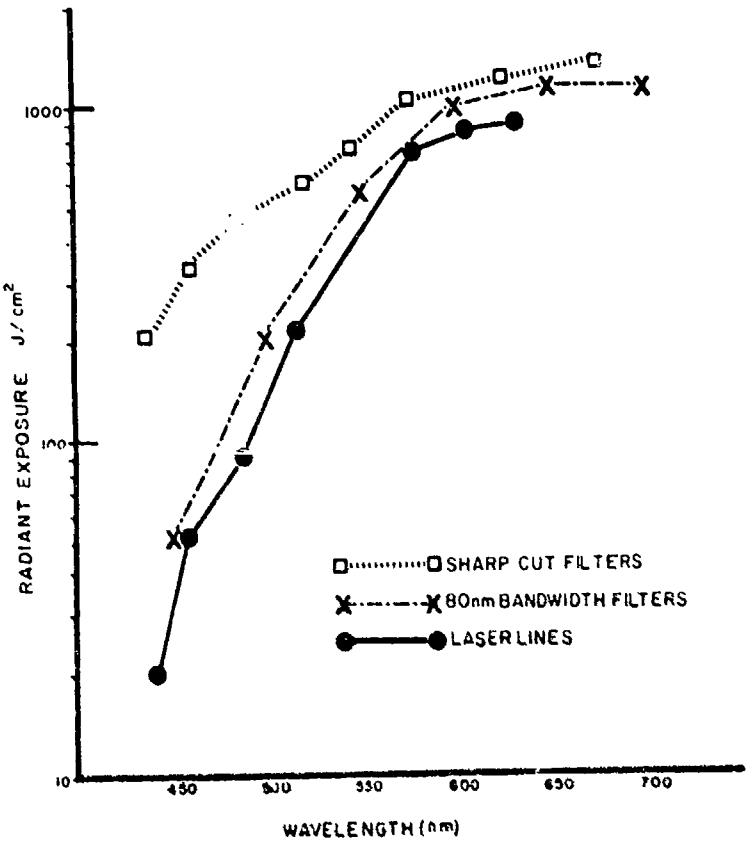
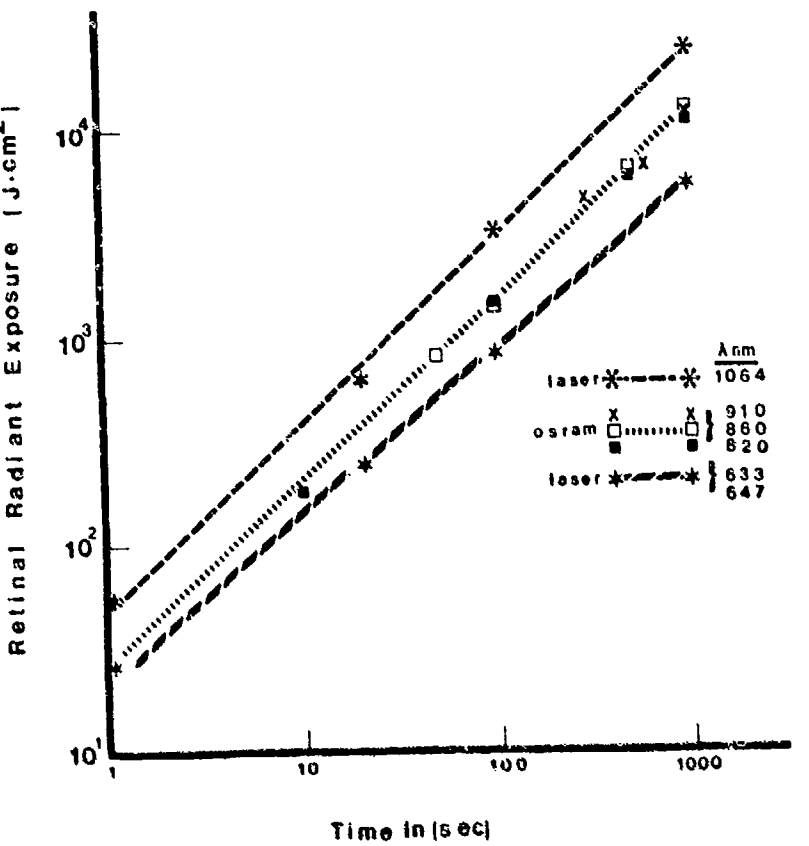


Figure 2

Comparison of near infrared thresholds at 820, 860 and 910 nm wavelengths (xenon) with previous laser data at 1064, 647 and 633 nm.



In another study, Ham et al[20] demonstrated that the rhesus retina is more sensitive to near UV than to 441 nm light by a factor of six. We exposed the aphakic eye in 3 monkeys to wavelengths of 405, 380, 350 and 325 nm as produced by a 2500 W xenon optical source with quartz optics through 10 nm band-pass interference filters. Exposure durations were 100 and 1000 s and the retinal spot size was 500 micrometers in diameter. Retinal sensitivity to photic damage continues to increase with decreasing wavelength. The radiant exposure required to inflict a minimal retinal lesion with blue light (441 nm) was 30 $J \cdot cm^{-2}$ as compared to 5 $J \cdot cm^{-2}$ for 350 and 325 nm UV radiation. The histology of the UV lesions are discussed in Section 5. These findings support the previous forebodings of several authors as to the retinal hazard in the aphakic eye.

4. HISTOPATHOLOGY OF THE BLUE LIGHT LESION IN THE MACAQUE RETINA

The pathological effects of blue light on the retina have been controversial. Investigators differ in their opinions as to whether the initial insult involves the photoreceptors of the neural retina or the single layer of epithelial cells comprising the retinal pigment epithelium (RPE). The confusion has been augmented by the tendency to relate the blue light lesion in primates to the syndrome involving the loss or destruction of the photoreceptors in albino rats exposed to a continuous or constant white light environment. The long-term biological endpoints are completely different. In the albino rat, exposure results in a complete loss of photoreceptors with resultant blindness, Noell[32]; extended exposure of the primate retina to blue light leads to hypopigmentation of the RPE and an appearance which bears a close resemblance to the aging retina, Ham et al[18].

There is general agreement that photic damage to both photoreceptors and RPE occurs at exposure levels to blue light appreciably above those required to produce a minimal lesion. Moon et al[17], demonstrated a loss in visual acuity in rhesus monkeys exposed to levels of blue light well above threshold. Most investigators agree that damage to the outer segments (OS) is reparable with time so long as the photoreceptor cells are not irreversibly damaged; and even the loss of a few photoreceptor cells following pyknosis of a few nuclei in the outer nuclear layer (ONL) is a relatively minor injury that does not affect visual function.

The distinction as to the initial or primary site of photic injury is extremely important because it can provide an insight into the long-term chronic effects of blue light. Only by investigating minimal or subminimal lesions after extended exposure (1000 s) can the events leading up to long-term effects be discovered. Overexposure obliterates in a badly damaged matrix of tissue the subtle effects of phototoxicity. Extended exposures beyond 1,000-10,000 s are impractical in the laboratory, but repeated exposures on a daily basis to monkeys trained to sit in a chair and perform a visual task for a reward are feasible. Such experiments have been underway since 1979 in our laboratory at the Medical College of Virginia.

A rhesus monkey was exposed monocularly over a period of 30 months (608 exposures) on a daily basis (1000 s per day, 5 days per week) to a short wavelength spectrum 330-490 nm provided by a 2500 W xenon lamp with quartz optics and suitable mirrors and filters, Ham et al[34]. The corneal irradiance was 5 $mW \cdot cm^{-2}$; estimated radiant exposure to the retina was 8 $J \cdot cm^{-2}$ per daily exposure, based on the assumption that the exposed eye remained fixed on the light source. In reality, both the exposed eye and the unexposed control eye were moving constantly. Nevertheless, the image size on the

retina (1.2 mm diameter) was large enough to assure an appreciable overlap on the macular area. Periodic examinations with the fundus camera revealed no startling changes though there was definite evidence of depigmentation in the temporal, superior area of the fundus. Fluorescein angiography was normal in both eyes. Histology on this animal was negative when sacrificed after 608 exposures. These results were inconclusive, especially as there was some doubt as to whether the depigmented area was included in the histological examination. It should be noted that only 27% of the energy in the 330-490 nm spectrum penetrated the ocular media to irradiate the retina. Wavelengths shorter than 400 nm were absorbed, primarily by the lens. When the lens is removed the effects on the retina are dramatic. In another experiment a monkey whose lens had been extracted surgically was exposed to a near ultraviolet spectrum, 330-420 nm, under similar conditions to those above except that the corneal irradiance was only 66.5 microwatts·cm^{-2}. The radiant exposure to the retina was estimated to be 1.1 J·cm^{-2} per daily exposure. This animal underwent 316 daily exposures before sacrifice for histology. Early on, after 80 exposures, fluorescein angiography had disclosed multiple focal areas of depigmentation in the RPE of the superior macula. Funduscopic examination before sacrifice showed a large lesion in the superior paramacular where an edematous area had been observed previously, as well as another large lesion in the temporal macula; also numerous small depigmented areas in the superior macula and what appeared to be a retinal hole in the periphery at about ten o'clock. Histological examination confirued these <u>in vivo</u> findings. These results demonstrate the extreme sensitivity of the primate retina to repeated small exposures of near ultraviolet light. The histological findings after exposure to near ultraviolet radiation are described in section 5.

In an attempt to investigate more thoroughly the basic mechanisms leading to the blue light lesion, Ham et al[18] prepared for light microscopy and electron ultrastructural analysis approximately 3000 sections from 20 eyes in 10 rhesus monkeys exposed to blue light (441 nm) at levels slightly above threshold. Sections were taken at postexposure times of one hour, 1, 2, 5-6, 10-11, 30, 60 and 90 days. Experimental methods and procedures are given in the publication. In what follows, the histological findings are discussed in some detail.

In specimens examined one hour postexposure the neural retina, RPE and choroid appeared normal with the exception of a few pyknotic rod nuclei and a few dense cone ellipsoids. Findings were similar at one day postexposure, but at two days postexposure there were definite changes in the RPE which was edematous in about 90% of the exposed area (1 mm diam.). The most characteristic feature of the lesion was a pigmentary change caused by the agglutination of melanin granules which produced interstices in the curtain of melanin granules normally found in the apical region of the RPE. A few macrophages containing melanin granules were present in the subretinal space. This hypopigmentation of the RPE made the lesion visible funduscopically for the first time at two days postexposure. In more severe lesions the choroid was involved over the central 50% of the irradiated area. A mild choroidal response or none at all was a common finding for most of the lesions examined histologically at two days postexposure. In these two-day lesions the RPE was mildly inflamed but the outer segments (OS) of the photoreceptors were not grossly damaged. The initial lesion was localized predominatly in the RPE with widespread damage and possible necrosis of some cells.

Lesions examined at 5 and 6 days postexposure usually showed a highly inflamed RPE,

often with cellular proliferation (mitotic figures) and always with hypopigmentation. Several macrophages loaded with melanin granules were now visible in the subretinal space. For the first time the OS seemed to be disarranged and damaged. Cellular proliferation, hypopigmentation and macrophages in the subretinal space were clearly visible. The OS showed mild disarrangement.

By 10 to 11 days postexposure lesions usually showed remarkable recovery. The RPE, while hypopigmented, had returned to a single layer of cells; macrophages loaded with melanin granules persisted in the subretinal space, but the OS of the photoreceptors appeared fairly normal. At 30 days postexposure, most lesions showed a normal RPE except for hypopigmentation and the continued presence of macrophages in the subretinal space. In lesions examined at 60 days postexposure the macrophages had disappeared from the subretinal space. Except for hypopigmentation the RPE and neural retina appeared normal. The same was true at 90 days postexposure except that the hypopigmentation seemed less evident. From these observations it would appear that partial to almost complete recovery had occured by 90 days postexposure.

The characteristic features of the blue light lesion as outlined above closely resemble the clinical events leading to solar retinitis and eclipse blindness as well as to the syndrome of foveomacular retinitis reviewed by Marlor[6] and the pigment degeneratiion of the macula in 150 servicemen stationed on a tropical island in the Pacific, Smith[4]. The recovery phase after blue light exposure is similar to the clinical data on eclipse gazing reported by Penner and McNair[34], who reported recovery to pre-exposure vision in 59% of their patients at six months postexposure. Hatfield[35] reported 145 cases of solar retinopathy during the 1970 eclipse; 45% of those afflicted returned to normal vision. Tso and La Piana[36] exposed three patients scheduled for enucleation because of melanoma to direct sungazing for a period of one hour. The eyes were removed 38 to 48 hours after exposure and examined histologically. They found varying degrees of damage to the RPE including irregular pigmentation, necrotic RPE cells and edema, but the photoreceptor cells appeared normal. The vision of two patients had returned to preexposure levels before their eyes were enucleated.

Noon et al[17] were able to demonstrate that the photopathology of the blue light lesion correlated well with monocular visual acuity tests in the trained rhesus monkey as defined by the Landolt ring technique. Exposure of the fovea to paramacular threshold levels of 441 nm light (30 $J \cdot cm^{-2}$ in 1000 s) did not impair vision, but 60 $J \cdot cm^{-2}$ in the fovea produced a decline in 20/20 vision on about the fifth or sixth day postexposure; the visual acuity gradually returned to normal at 30 days postexposure. An animal exposed to 90 $J \cdot cm^{-2}$ in the fovea lost 20/20 vision permanently. When this animal was sacrificed 4 $^1/_2$ years later, histological examination disclosed plaque formation in the RPE similar to that reported by Tso and Fine[37] We have demonstrated conclusively in the rhesus monkey that solar retinitis is caused by exposure to the blue component in the solar spectrum, Ham et al[18]. All these data support the thesis of Young[24] that "some of the entities in the current nosology of retinal disease are radiation diseases provoked or aggravated by light.....". In particular, there is ample reason to postulate that exposure to blue light plays a role in the Irvine-Gass-Norton syndrome, especially after the investigation of Tso and Shih[38] regarding the pathology of macular edema following lens extraction in the rhesus monkey and the recent measurements of retinal light exposure from operation microscopes and surgical overhead lamps made by Calkins and Hochheimer[39].

5. HISTOPATHOLOGY OF THE NEAR ULTRAVIOLET LESION IN THE MACAQUE RETINA

The ocular media, especially the lens, protect the primate retina from short wavelength light and near ultraviolet (UV) radiation, but this protection is largely forfeited when the lens is removed. Since over 575,000 cataract operations were performed in the United States in 1983, Maumenee[40], the question arises as to whether blue light and near UV radiation constitute a hazard for the aphakic eye. We have shown (Section 3) that the rhesus retina is six times more sensitive to near UV radiation than to blue light. The radiant exposure required to produce a minimal lesion was approximately 5 $J \cdot cm^{-2}$ for a 100 or 1000 s exposure to 350 and 325 nm, as contrasted with 30 $J \cdot cm^{-2}$ for 441 nm blue light. Histological data is available now to demonstrate that near UV lesions differ from blue light lesions in several important respects, Ham et al[41]. The UV lesion is funduscopically visible immediately after exposure as contrasted to a latent period of 48 hours before a minimal blue light lesion appears, and the photoreceptors as well as the RPE are damaged, particularly the cone ellipsoids which appear to be especially vulnerable, probably because of absorption by the metalloflavoproteins and cytochromes in the mitochondria. Rhodopsin and the cone photopigments have strong absorption peaks in the near UV that also may account for the sensitivity of the photoreceptors. In both types of exposure (blue light and near UV) damage to the RPE plays an important and similar role.

Only one aphakic eye in a rhesus monkey was available for histological analysis. This eye was exposed to 5.5 $J \cdot cm^{-2}$ of 350 nm radiation in 100 s on a retinal spot size of about 500 micrometers. All exposures were paramacular and scheduled so that at sacrifice lesions could be examined at postexposure times of 2, 5, 10 and 30 days. It was apparent at two days postexposure that the OS of the photoreceptors were damaged, a finding in direct contrast with a blue light lesion where the photoreceptors are virtually intact. There was mild damage and some depigmentation in the RPE. The primary focus of damage was the OS of the photoreceptors and possibly just perceptible thinning of the nuclei in the outer nuclear layer (ONL). At 5 days postexposure there was obvious damage to the photoreceptors, especially the OS, and some damage to the nuclei in the ONL. The RPE was also involved with mild derangement among the melanin granules. Damage is confined mainly, however, to the photoreceptor cells of the neural retina.

In one lesion at 5 days postexposure the photoreceptors are severely damaged; within a small area the entire photoreceptor population including the ONL has disappeared. Below the damaged area and resting on Bruch's membrane is a clump of melanin granules. Toward the left of the damaged area the RPE and neural retina appear reasonably normal. It is difficult to explain this highly localized damage. Perhaps it is due to refractive errors producing focal hot spots at these short wavelengths. In any event, it demonstrates that near UV radiation is lethal to photoreceptor cells, a phenomenon which is not apparent with blue light at threshold levels.

The histological data to date indicate that near UV radiation attacks both the photoreceptor cells of the neural retina and the RPE, but that the primary effect is the destruction of the photoreceptor cells, especially the cones. Presumably, radiant exposures well above threshold would result in a massive loss of photoreceptor cells and irreparable damage to the retina.

Calculations based on an estimated radiance from sun and sky of 1 $mW \cdot cm^{-2} \cdot ster^{-1}$ for the near UV, a pupillary diameter of 2.5 mm, and a transmittance for the aphakic

eye of 0.5 yield a retinal irradiance of about 8.5 microwatts·cm^{-2}. Exposure for three hours on a bright sunny day at sea level would give a radiant exposure of 92 mJ·cm^{-2} which is far below the threshold of 5 J·cm^{-2} for retinal damage in the rhesus monkey. Even if no repair processes were operative in the retina, it would require about 54 repetitive daily exposures to accumulate a radiant exposure of 5 J·cm^{-2}. While these calculations may be reassuring, it is also prudent to assume that near UV exposures may be partly cumulative. For example, the loss of a few photoreceptors daily would go unnoticed but over a period of years the depletion in photoreceptor population would lead to serious consequences. Also, it is important to realize that removal of the lens increases the retinal exposure to blue light. The tremendous preponderance of blue light over near UV radiation in the solar spectrum more than compensates for the toxicity ratio of 6 to 1 for near UV vs blue light. Transmittance of the aphakic eye to the bandwidth 400-500 nm is about 0.7. Nothing is known about possible synergistic effects of short wavelength light and near UV radiation.

6. AGING AND DEGENERATIVE EFFECTS IN THE RETINAL PIGMENT EPITHELIUM (RPE) FROM CHRONIC EXPOSURE TO LIGHT

Young[26] has proposed a theory of central retinal disease based upon long-term chronic exposure of the fovea to light. In his words, "Perhaps some of the entities in the current nosology of retinal disease are diseases provoked or aggravated by light....", and "Analysis reveals that it is possible to develop a theory which accounts for the pathogenesis of many forms of retinal degenerative disease, and provides a rational basis for prevention and treatment". He cites a large number of empirical facts which are substantiated by numerous references to the literature. It is a convincing thesis, well documented and augmented by scholarly research. Particularly impressive is the relationship between long term radiation exposure and degenerative or aging effects on the retina; these correlate well with the observations of Smith[4], Ham et al[18], and Tso and Fine[37] concerning hypopigmentation of the RPE, serous detachment of the RPE, macular edema and age-related macular degeneration (AMD).

The concept that long-term, chronic exposure to sunlight is a contributing factor to aging of the retina and age-related macular degeneration (AMD), is not new; van der Hoeve[42] in 1920 supported a similar thesis. The aging of the retina, particularly in the macular region, is intimately associated with the gradual accumulation of debris (residual bodies, lipofuscin, melanolysosomes and melanolipofuscin) in the RPE, Feeney-Burns[43], Ham[18], Young[44] has reviewed the clinical and histopathological features of AMD. In his words, "Many of the features of AMD can be attributed to the progressive deterioration of the retinal pigment epithelium. It is proposed that this deterioration arises primarily from imperfections in metabolic processes concerned with intracellular renewal-the incessant replacement of the cells' molecular constituents. In particular, inefficiency of the cells' digestive apparatus seems to play a primary role in setting off a complex sequence of events involving residual bodies, alteration of Bruch's membrane, drusen, basal laminar deposits and pigmentary disturbances. Two major characteristics of AMD which remain unexplained by this hypothesis - The central location of the lesion and the protective effect of ocular pigmentation - can be accounted for by postulating a role for the damaging effects of radiant energy in the etiology of AMD."

The Division of Risk Assessment, National Center for Devices and Radiological Health of the Food and Drug Administration sponsored a Workshop on "Long-Term Visual

Health and Optical Radiation" in September 1983. Seven working groups were established to identify and define specific long-term visual problems that could be induced by optical radiation. The Retinal Pigment Epithelial Working Group, chaired by Ham[25], specifically considered aging and its possible relationship to light damage to the retina. There is convincing evidence suggesting that both acute exposure (eclipse blindness and sun gazing) and long-term chronic exposure to sunlight causes a photochemical type of maculopathy in both humans and non-human primates that is closely related, if not identical, to the blue light lesion. Whether long-term exposure to short wavelength light and near ultraviolet radiation can be related to aging of the RPE and AMD remains questionable but there are several reasons for believing that such a relationship exists. For example, a significant feature of blue light damage in the primate retina is the loss of melanin granules, suggesting an increase in the formation of complex granules (melanolysosomes & melanolipofuscin) and an acceleration of the depigmentation process that accompanies aging. It makes sense to postulate that light exposure increases the phagocytic role of the RPE, producing more lipofuscin and complex granules with extrusion of debris onto Bruch's membrane. For example, in the rhesus monkey each retinal rod produces 80 to 90 disks per day; the OS is replaced every 9 to 13 days. Thus, each RPE cell must phagocytize and digest approximately 3000 disks per day. As Feeney[45] has emphasized, "The pigment epithelial cell must have a highly developed phagocytic-lysosomal system in order to digest these enormous amounts of exogenous material daily for 70 or more years". Again in Young's[46] words, " The senescent changes are centered on the macula, where the intensity of radiation appears to be the greatest. Accumulation of lipofuscin in the pigment epithelium begins in childhood. Several decades later, the cells are filled to overflowing with the remnants of failing molecular renewal, despite abortive attempts to clear the cytoplasm of debris by extruding it onto Bruch's membrane." While the evidence that chronic blue light exposure is a contributing agent in the aging and degeneration of the macula is not conclusive, it is suggestive enough to warrant recommending that the public wear protective yellow filters or sunglasses during exposure to bright light and near ultraviolet optical radiation. Such filters are harmless and may slow down the aging process in both the retina and the lens.

Weiter et al[47] in a study of the relationship of senile macular degeneration to ocular pigmentation conclude with the statement, "The strong association between ocular melanin and both senile macular degeneration and lipofuscin in the retinal pigment epithelium suggests a role for light damage in the eye and offers possibilities for research to prevent this important cause of blindness in our population".

A workshop of ocular safety and eye care was held at the Duke Eye Center under the auspices of the National Research Council, Committee on Vision, Wolbarsht[48]. Members of the workshop explored potential ocular hazards from radiation emitted by ophthalmic instruments currently in use. It was recommended that "in general, instruments be designed to minimize the amount of ultraviolet and infrared radiation. Especially, chronic exposure to emission in the blue end of the spectrum should be reduced as far as possible to avoid photochemical damage to the retina. This 'blue light' retinal hazard of any instrumentation may be evaluated by using guidelines as the proposed Threshold Limit Values (TLVs) of the American Conference of Governmental Industrial Hygienists", (ACGIH 1979). A table is given which estimates the retinal hazard of chronic exposure to different wavelengths relative to 435-440 nm, judged to be the most dangerous visible wavelengths.

7. REPETITIVE DAILY EXPOSURES TO THE SAME SITE IN THE RETINA OF THE MACAQUE MONKEY AT WAVELENGTHS 440, 475 AND 533 nm

We have investigated the cumulative or additive effect of repetitive light exposures to the same site on the macaque retina. The retinae of 3 monkeys (3 eyes) were subjected to daily radiant exposures of 1000 second duration and 500 micrometer spot diameter for 21 consecutive days at each of three wavelengths, 440, 475 and 533 nm. The optical source was a 2500 W xenon lamp equipped with quartz optics and 10 nm interference filters peaked at 440, 475 and 533 nm. Initially a threshold radiant exposure in $J \cdot cm^{-2}$ was determined in the other eye of each animal using the interpolation technique and the same parameters as above, i.e. exposure time, spot size and wavelength. The criterion for a threshold lesion from a single exposure was the appearance of a minimal lesion at 48 hours postexposure as seen with the fundus camera. The other eye of each animal was used for repetitive daily exposures to the same site at 50, 40, 30, 20 and 10% of threshold for a single exposure at each wavelength. In a given eye, accordingly, there were 5 retinal sites for each wavelength or a total of 15 retinal sites. All retinal sites were in the paramacular area. They consisted of a parallel, horizontal row for each wavelength, either above or below the macula and these were varied in each animal.

Results are shown in Table 1 where the daily repetitive radiant exposures in $J \cdot cm^{-2}$ are listed for each wavelength according to the number of exposures required to produce a minimal threshold lesion at 24 hours postexposure. At 440 nm, three animals underwent the repetitive exposure protocol. The 'C' monkey was removed from the experiments after 5 days because of a corneal opacity from an unfortunate blunder by the animal caretaker, but the other two monkeys received 20 and 21 repetitive exposures to 440 nm respectively. In the 'A' monkey 17 exposures to 6 $J \cdot cm^{-2}$ produced a lesion but 21 exposures to 3 $J \cdot cm^{-2}$ did not. The 'B' monkey showed threshold lesions after 14 exposures to 5.6 $J \cdot cm^{-2}$ and after 20 exposures to 2.8 $J \cdot cm^{-2}$. This demonstrates that the cumulative effect of daily exposures to subthreshold amounts of 440 nm light can damage the primate retina and suggests that some of the photochemical effects of light toxicity are irreversible even at radiant exposures well below the threshold level. The animals exposed to 475 and 533 nm light did not develop visible lesions for repetitive daily exposures at levels less than 30% of threshold of the single exposure, even though they received 21 daily exposures to approximately 19 and 60 $J \cdot cm^{-2}$ for total radiant exposures of 399 and 1260 $J \cdot cm^{-2}$ respectively. From Table 1 it can be seen that in monkey 'A', 3 exposures to 12 $J \cdot cm^{-2}$ of 440 nm light produced a lesion while 3 exposures to 47.3 $J \cdot cm^{-2}$ of 475 nm light were required to produce a lesion ; similarly, monkey 'B' required 3 exposures to 11 and 46.2 $J \cdot cm^{-2}$ respectively for these two wavelengths. Wavelength 440 nm is about 4 times more toxic than 475 nm. When 440 nm light is compared to 533 nm the toxicity ratio is about 17. However, this type of comparison is not entirely valid since different mechanisms are involved at different wavelengths. Nevertheless, these experiments illustrate the extreme sensitivity of the primate retina to the blue end of the visible spectrum.

Table 1

Radiant exposure in $J \cdot cm^{-2}$ per exposure for wavelengths 440, 475, and 533 nm vs number of exposures required to produce a minimal lesion in the macaque retina for 3 eyes in animals designated (a), (b) and (c).

No. of exposures	$J \cdot cm^{-2}$/Exp. 440 nm	$J \cdot cm^{-2}$/Exp. 475 nm		$J \cdot cm^{-2}$/Exp. 533 nm	
1	30a; 28b; 28c	94.6a	92.4b	300a	294b
2	15a; 14b; 14c				
3	12a; 11b; 11c	47.3a	46.2b		
4		38.8a	36.4b		147b
5	9a; 8.5b; 8.5c			150a	
6			27.3b		118b
7		28.4a		120a	
8					
9					
10					
11					
12					88.1b
13				90a	
14	5.6b				
15					
16					
17	6a				
18					
19					
20	2.8b				
21					

8. INVESTIGATION OF BASIC MECHANISMS LEADING TO PHOTOCHEMICAL LIGHT DAMAGE

Actinic or photochemical injury to the retina begins at wavelengths below approximately 550 nm from retinal irradiance levels too low to produce appreciable temperature rises, Ham et al[30]. The transition from thermal to actinic damage as wavelength decreases is gradual with an ill-defined mixture of both types of insult through the range 550-500 nm. Below 500 nm, photochemical effects predominate. Because chemical reactions are a strong function of temperature there is a region of thermally enhanced photochemical reactions bridging the gap between predominately thermal and predominately photochemical events.

The basic mechanisms leading to photochemical effects in the retina are not known, yet there is no scarcity of deleterious reactions in photobiology which might play a role and there is little experimental evidence to single out a specific reaction to the exclusion of others. The mammalian retina is unique among body tissues in that light is focussed directly on a group of cells which are highly oxygenated. According to Parver et al[49] the choroidal circulation accounts for 85% of all ocular blood flow. Per gram of tissue the choroid has four times the volume of blood found in the renal cortex and is structured so that a dense matrix of small blood vessels with a large surface area is immediately adjacent to the RPE and the outer layers of the neural retina. Parver has shown that the choroidal circulation plays a key role in dissipating the heat generated by the absorption of light in the RPE and the choroid. Choroidal circulation is unusual in having a very low arteriovenous oxygen differential (approximately 5%), suggesting that this high flow characteristic may serve purposes above and beyond supplying metabolites and oxygen, i.e. a heat dissipating mechanism for the macula. The presence of numerous large mitochondria in the ellipsoid of the photoreceptor cell demonstrates how dependent the retina is on oxygen. Indeed, the photoreceptor and RPE cells are among the most metabolically active cells in the body.

Either light or oxygen individually can damage cells. The retina would be subject to oxygen toxicity even without light but the combination of the two greatly enhances the probability of deleterious reactions. Thus, nature's dilemma, light is essential for vision but light is toxic; oxygen is essential for life but oxygen is also toxic. Fridovich[50] has pointed out that all respiring organisms survive by virtue of maintaining a delicate balance between their energy requirements as obtained by the reduction of oxygen to water and the toxic effects engendered in tissue by the free radicals produced during these catabolic processes.

Photochemical reactions are initiated by photons of light ($h\nu$) exciting a molecular sensitizer, (S), to form an initially excited electronic state, the singlet state, ^1S, which has a very short lifetime ($<10^{-8}$s). There are three major ways in which the molecule ^1S can dissipate its quantum of absorbed energy: by reaction with a solvent, usually water; by emission of a photon (fluorescence); or by a radiationless transition or crossing-over to a triplet or metastable state ^3S which has a much longer lifetime than ^1S and therefore has more time to react with other molecules. The triplet state ^3S is believed to be the pathway leading to most photochemical reactions. The most effective sensitizers are those which yield a long-life triplet state in high quantum yield, Foote[51]. The retina has molecular species which could serve as photosensitizers. Examples are hematoporphyrins, flavins and aromatic hydrocarbons which are distributed ubiquitously throughout mammalian tissue. These are among the many chromophores which can absorb visible or near UV radiation to become sensitizers leading to photochemical

reactions. In addition to endogenous sensitizers account must also be taken of exogenous substances which can also act as chromophores, e.g. certain drugs, foods, dyes, etc.

There are two major types of photochemical reactions designated as Type I and Type II. In Type I, the redox reactions do not involve oxygen and ^3S reacts directly with the substrate to produce cellular damage. In Type II reactions, usually called photodynamic reactions, ^3S reacts directly with molecular oxygen to produce either excited singlet oxygen, $^1O_2$, or the superoxide anion radical O_2^-. In either Type I or Type II, the net result is the production of active free radicals which can attack other molecules. According to Foote[51], the majority of Type II processes involve singlet oxygen as the primary reactive species.

Molecular oxygen has two unpaired electrons with parallel spins in the triplet ground state, $^3O_2$. To react with other molecules one electron spin requires inversion. Spin inversion is a slow process in comparison to the lifetime between molecular collisions. Because of spin restriction, $^3O_2$ is not as highly reactive a molecule as singlet oxygen. Excitation of $^3O_2$ to singlet oxygen $^1O_2$ results in a spin inversion so that $^1O_2$ becomes a very reactive molecule, especially for the lipid peroxidation of polyunsaturated fatty acids, PUFA. Singlet oxygen has a half-life in water of 3.3 microseconds, Rogers[52], and an excitation energy of 22 kcal or 0.98 eV corresponding to a photon wavelength of 1270 nm, Fridovich[50], Foote et al[53].

The reduction of oxygen to water requires the removal of 4 electrons. Oxygen is toxic, not because of its own reactivity, but because its reduction to water tends to favor (because of the spin restriction) a series of univalent single electron transfers which generate superoxide radical O_2^-, hydrogen peroxide H_2O_2 and hydroxyl radical $OH\cdot$. The latter is the most potent oxidant known. It is mainly these intermediates that cause oxygen toxicity. Most of the oxygen reduction in respiring cells proceed by pathways which are directly multivalent. Thus, cytochrome c oxidase, which accounts for most of the oxygen consumption by aerobes, produces H_2O tetravalently without the intermediate production of radicals. This enzyme represents the cell's first line of defense again oxygen toxicity. There are also flavin-containing enzymes which perform the divalent reduction of $^3O_2$ to H_2O. Such enzymes also represent part of the cell's defense against oxygen toxicity since they skip the univalent reduction of oxygen which produces superoxide anion and hydrogen peroxide.

However, recent research has shown that there are numerous spontaneous oxidations as well as enzymatic oxidations in biological systems which can generate free radicals, Freeman[54]. For example, the autooxidation of epinephrine, leucoflavin, hydroquinones and hemoglobin are known to generate superoxide anion. Mitochondria and phagocytic cells have been shown to produce O_2^-. Babior et al[55] have demonstrated that during phagocytosis granulocytes show an increased production of O_2^-. They were among the first to suggest that superoxide may be a bactericidal agent. There is no cell in the body with greater powers of phagocytosis than the RPE cell, one of whose major functions is the digestion of outer segments which have been discarded by the rods and cones. Part of the digestive process may involve the oxygen radicals and singlet oxygen. The spontaneous generation of free radicals by metabolic processes in living systems is thought to be a major cause of aging, Tappel[56], Feeney and Berman[57], Harman[58] and Cutler[59].

Whenever superoxide anion radical, O_2^-, is generated in aqueous media, hydrogen peroxide, H_2O_2, is also produced. This is because superoxide is not stable and dismutates spontaneously to $^3O_2$ and H_2O_2 . The presence in solution of both $^3O_2$ and H_2O_2 can also result in the production of the powerful hydroxyl radical OH· under special circumstances when iron salts are present, Fridovitch[40]. The OH· radical indiscriminately attacks all organic compounds while singlet oxygen preferentially attacks carbon-carbon double bonds. Singlet oxygen is the major product of Type II photodynamical reactions. Thus the combination of oxygen and light in the retina can produce a quartet of toxic poisons, i.e. O_2^-, H_2O_2, OH· and $^1O_2$.

9. PROTECTIVE MECHANISMS AGAINST PHOTOCHEMICAL LIGHT DAMAGE IN THE RETINA

It seems obvious that cells which utilize both light and oxygen must have protective mechanisms to minimize the production of toxic substances and also to scavenge effectively those whose production cannot be avoided. Those enzyme systems which reduce oxygen to water by tetravalent or divalent pathways represent one type of defense system. The recent recognition that cells, particularly the RPE cell, are constantly digesting their own cytoplasmic contituents and synthesizing new molecules to replace them represents another defense system, Young and Bok[41]. This process of molecular turnover or renewal provides the cell with a powerful tool for combating the damage caused by toxic substances. Enzymatic repair systems like the well known DNA repair enzymes constitute still another method of defense against damage from free radicals. The photoreceptor cells have developed a unique defense against the peroxidation of the PUFA'S which are the major fatty acid constituents of the outer segments. Both rods and cones renew their outer segments, rods at a daily rate of 10% while cones have a lower but appreciable turnover rate. The apical tips of the rod outer segments represent those disks which have been exposed the longest to the deleterious effects of light and oxygen and it is those tips (sometimes 100 disks or more) that are pinched off and phagocytized by the RPE.

In addition to the defense systems listed above, cells have very specific mechanisms, enzymes and antioxidants, to scavenge radicals and/or inhibit their action on susceptible structures, notably membranes. One such mechanism involves superoxide dismutase (SOD), an enzyme that catalyses by dismutation the conversion of O_2^- to H_2O_2 and $^3O_2$. Altogether, there are four different kinds of SOD. One of these is found in the cytosol of mammalian cells. It contains both copper and zinc and has a molecular weight of 32,000. This enzyme has been isolated from a wide variety of eukaryotic cells including those from humans, cows, chickens, yeast and bread mold. Another superoxide dismutase containing manganese is found in mitochondria. The other two types are bacterial in origin and do not occur in eukaryotic cells. It is interesting to note that the striking similarities in the amino acid sequences between bacterial and mitochondrial superoxide dismutases provide biochemical evidence on the evolution of unique dismutases by prokaryotes and protoeukaryotes during the period when the blue green algae transformed the earth's atmosphere from anaerobic to aerobic. Biochemically, man bears close kin to E. Coli!

While SOD does protect the cell from superoxide anion radical, in so doing it produces H_2O_2. Hydrogen peroxide is also generated by the divalent reduction of oxygen to water and by some photochemical reactions; it also is toxic to the cell and in the presence of Fe^{++} and $^3O_2$ can generate the extremely reactive hydroxyl radical. There are two classes of related enzymes, the catalases and the peroxidases, that catalyze

the divalent reduction of H_2O_2 to water and oxygen. The catalases are found predominately in liver, kidney and red blood cells. They can reduce H_2O_2 to water directly without the aid of an electron donor. Most tissues need little catalase because the circulating blood can remove and decompose the H_2O_2 excreted by those tissues. An important function of the choriocapillaris may be the removal of H_2O_2 generated in the outer retina, RPE and choroid by the combined actions of light and oxygen. Peroxidases acting on H_2O_2 require a co-substrate or hydrogen donor such as gluathione or ascorbic acid. Glutathione peroxidase is a seleno-enzyme which is widely distributed in mammalian cells, e.g. leucocytes, mammary, thyroid, salivary glands and RPE, Feeney and Berman[57]. Glutathione peroxidase is effective at low concentrations of H_2O_2 and can act also upon a wide range of hydroperoxides by converting them to harmless hydroxy fatty acids.

The dismutases, catalases and peroxidases protect the cell from superoxide anion radical and hydrogen peroxide but do little to inhibit singlet oxygen, the major product of Type II photochemical reactions. Delmelle[62] has proposed that light damage to the retina could be due in part to photosensitized reactions involving singlet oxygen. Direct proof of the photosensitized formation of singlet oxygen in aqueous media is difficult but indirect evidence definitively supports the role of singlet oxygen in many solution photooxidations. Proteins, polypeptides and individual amino acids affected are methionine, histidine, tryptophane, tyrosine and cysteine either in the free state or in peptides. There is no breaking of peptide or disulfide bonds but there is loss of conformation which can lead to inactivation of many enzymes. However, the most destructive role of singlet oxygen is the peroxidation of the polyunsaturated lipids which represent the main constituents of membrane structure. Antioxidants supply the cell's main line of defense against lipid peroxidation of membranes. Foremost among naturally occuring antioxidants is vitamin E, which is distributed throughout mammalian cells. Lipid peroxidation can be a chain reaction in membranes, Barber and Bernheim[63]. It is the nature of chain reactions that a single initiating event propagates itself to adjacent molecules in the membrane in a domino-like process which is called autooxidation. Vitamin E or alpha-tocopherol is able to intercept or terminate the autooxidation chain reaction and protect the membrane. Vitamin E is especially concentrated in the outer segments of the photoreceptors. Vitamin E is also thought to be a scavenger of singlet oxygen and to act synergistically with selenium to protect cells from oxygen damage but the inhibition of membrane autooxidation is probably the most important function of alpha-tocopherol in the retina.

Hayes[64] has assessed the effects of vitamin E deficiency on the retina in two species of monkeys over a period of 2 3/4 years. Macular degeneration developed after two years on a vitamin E deficient diet. The lesion was characterized by focal, massive disruption of photoreceptor outer segments which was attributed to lipid peroxidation of those lipoprotein structures containing highly unsaturated fatty acids. The remarkable accumulation of lipofuscin pigment in the RPE was identical to that previously described in dogs, Hayes et al[65] and demonstrated that the RPE is capable of extreme phagocytic activity and lysosomal digestion. Robison et al[66,67] and Katz et al[68] have shown that Vitamin E-deprived rat retinas show massive accumulations of lipofuscin in the RPE, disorganization of rod outer segment membranes, and loss of photoreceptor cells. The role of vitamin E in protecting the outer segments of photoreceptor cells from lipid peroxidation is unequivocally demonstrated by these experiments.

If singlet oxygen is indeed the reactive species in most Type II photodynamic effects, what protection does the cell have beyond those provided by alpha-tocopherol? One of the major protective devices in bacterial systems consists of coloured carotenoid pigments, Krinsky[67]. There is convincing experimental evidence that beta-carotene is an effective quencher of $^1O_2$ in mammalian cells. That singlet oxygen quenching is involved in the protective action of carotenes comes from the fact that the rate of $^1O_2$ quenching is a function of the number of conjugated double bonds in the polyene chain. Carotenes with nine or more conjugated double bonds are efficient quenchers, Foote[31]. Quenching of $^1O_2$ by beta-carotene is due to energy transfer with the resulting production of the triplet state of beta-carotene; the latter dissipates this energy directly to the solvent without damage and therefore can react again in cyclic fashion. The energy of the transition from the ground state to the first excited triplet state must be equal to or less than that of the singlet-triplet transition of the excited oxygen which is 22 Kcal mol^{-1} or 0.98 eV. One of the major functions of carotenoids in nature is to protect cells from harmful or lethal photodynamic effects. In addition to quenching $^1O_2$ directly, carotenoids can intercept the photosensitization reaction at an earlier stage by effectively quenching the sensitizer ^3S, thereby preventing the formation of $^1O_2$ and thus further decreasing the $^1O_2$ available for initiating photodynamic damage.

Still another potent defense against the toxic effects of oxygen and light is melanin, the major ingredient of the melanin granules situated primarily in the apical portion of the RPE where they are in close apposition to the outer segments of the photoreceptor cells; melanin is also the major constituent of the melanocytes in the choroid. Until recently, it was generally assumed that the major role or function of melanin was to shield the outer segments of the photoreceptors from scattered light and to convert absorbed photons into harmless heat. There is little doubt that this concept of the role of melanin is generally valid. However, melanin may have other functions above and beyond the mere conversion of light to heat.

Photoprotection of the skin is a major function of melanin, Pathak et al[70], McGinness et al[71], and it may well play a similar role in the RPE. One hypothesis, Proctor et al[72], is that melanin plays a protective role at low rates of energy input by a conversion to innocuous phono-vibrational modes (heat) but that at a high rate of energy transfer via photon absorption, melanin becomes cytotoxic. Melanin may be equally as protective by absorbing the energy of potentially disruptive excited state species or free radicals as in absorbing blue light or UV radiation. While at lower doses of UV or blue light, melanin may thus have a protective effect, there is some evidence that at high radiant exposures the melanin itself becomes cytotoxic. Data to support this thesis comes from the histological effects of the blue light lesion, Ham et al[18] where 48 hours after exposure the RPE undergoes an inflammatory reaction accompanied by agglutination of melanin granules and some phagocytosis of melanin granules by macrophages. Presumably, the melanin granules have undergone some type of damage from overexposure to blue light.

Feeney and Berman[37] suggest that biochemical damage to the RPE by light and/or oxygen should be re-examined in view of the free radical character of melanin and its possible role as an electron-transfer agent. Melanin is a heterogeneous random polymer comprising several different monomers coupled by various bond types into an amorphous substance containing stable free radicals and semiconductor properties that ensure efficient electron-transfer for redox systems. Recent research has shown that melanin

is a complex substance with a number of interesting features. Cope et al[73] demonstrated by electron spin resonance measurements that the melanin granules of the mammalian eye generate free radicals when irradiated with visible light. Melanin was for a long period considered to be an inert substance, but Gan et al[74] present data to demonstrate that melanin functions as an efficient electron-transfer agent in redox systems. Blois and associates[75] conclude that melanin is a highly irregular, three dimensional polymer whose optical absorption spectroscopy in the UV and visible regions of the spectrum reveals a lack of structure. This is in accord with Wolbrasht et al[76] who believe that melanin has little biological significance other than its absorption of light. However, Menon and Haberman[77] have presented data to indicate that the protective effect of melanin is not entirely due to the absorption of light. They suggest that more attention be paid to the protective and deleterious effects of melanin and that pigment biologists keep an open mind for other possible biological effects which are not presently recognized.

Felix et al[78] have reported that there is rapid scavenging of oxygen by melanin in the presence of light with saturation of the electron spin resonances of free radicals and reduction of the scavenged oxygen to hydrogen peroxide, accompanied by some production of superoxide. Chedekel et al[79] provide evidence that the absorption of light by pheomelanin (polymeric pigments found in the hair of red-headed individuals) in aerated aqueous media produces superoxide and hydroxyl radicals as well as solvated electrons. The action spectrum for superoxide production is greatest in the UV spectral region but continues well into the visible wavelengths.

Another interesting property of melanin is its ability to bind metals and certain drugs. This could be a mixed blessing-either a storehouse for needed materials or for toxic substances that poison the RPE. Sarna et al[80] found several specific types of metal binding sites on melanin. The interaction of Cu^{2+} with melanin was studied in some detail. Other metals which bind to melanin include Mn^{2+}, Ni^{2+} and Zn^{2+}. Since melanin binds metals very tightly, these authors compared the binding strength with that of ethylenediaminetetraacetic acid (EDTA). Melanin has sites that bind metals more tightly than EDTA and some that bind them less tightly. Lindquist[81] has reviewed the literature on the affinity of drugs for melanin. Chloroquine, quinine and antibiotics of the streptomycin group are rapidly localized in the melanin-containing tissues of the eye. Adrenaline, dopamine and noradrenaline bind reversibly to melanin while tyrosine and DOPA lack melanin affinity; this affinity appears to be an important factor in drug-induced lesions. The authors recommend that new drugs should be tested for melanin affinity before clinical use.

Mainster[82] cites melanin phagocytosis and pigment clumping in photic and senile maculopathies as examples of the part played by melanin in the transfer of free radicals in the RPE, and Feeney[83] points out that the RPE of the eyes of elderly humans often contain complex granules consisting of both melanin and lipofuscin, suggesting an interrelated biological history for these two substances. Feeney studied the history of melanin and lipofuscin granules in 30 human RPE's, spanning a lifetime of 90 years. Her data indicate that RPE melanin undergoes autophagic remodeling and degradation during the human lifespan. She postulates that melanin plays a key role in protecting cells from light-generated free radicals and suggests that the loss or degeneration of melanin can lead to senile changes in the RPE. As mentioned earlier, Young[44] also attributes a major role to melanin as a protective agent against AMD.

In view of the many interesting properties of melanin as listed above, i.e., semi-conductor, electron-transfer, scavenger of singlet oxygen and free radicals, superoxide and other free radical production when irradiated with light, affinity for drugs and metals, liason with aging phenomena involving lipofuscin and drusen, it seems plausible to propose that melanin plays both a protective and a cytotoxic role in retinal photopathology. The association of pigmentary disturbances with specific disease symptomology such as deafness, inflammatory lesions, neurological disorders, or pigment retinopathies, suggests an active rather than a passive role for melanin in biological systems, McGinness & Proctor[64], Proctor & McGinness[65], Barr et al[66], Proctor[67].

Thus, nature has evolved an impressive array of defense mechanisms to protect the retina (and also the lens) from the toxic effects of light and oxygen. Modern science and medicine, however, have doubled the life span of man, thereby subjecting the eye to aging effects like cataract and macular degeneration. There can be little doubt that long-term, chronic exposure to light and oxygen accelerates the aging process, despite the body's defense mechanisms. It is interesting to note that rhesus monkeys, like man, are also subject to retinal degeneration, El-Mofty et al[68] and Bellhorn et al[69]. It is possible to overwhelm the retina's protective mechanisms as is done when albino rats, Noell[52], are exposed continuously to light. Under these conditions of continuous bleach, the photoreceptor cells are destroyed first, followed by damage to the RPE. This is not a surprising result considering the lack of both a protective pigment and a nocturnal environment. The same result can be achieved in diurnal pigmented primates, Sykes et al[70], but only by exposing the eye continuously for 12 hours to bright light levels through a dilated pupil. The blue light lesion, Ham et al[18], is another example of overwhelming the defense mechanisms of the retina but the damage is highly localized and first appears in the RPE and not in the photoreceptors and the biological endpoint is a depigmented RPE which bears a close resemblance to the early stages of age-related macular degeneration. The retina is also extremely sensitive to near UV radiation. Here the damage appears in both the photoreceptors and the RPE. Retinal defense mechanisms against near UV radiation are probably minimal because the ocular media of the normal eye with intact lens transmits very little UV radiation.

10. THE EFFECT OF OXYGEN ON LIGHT TOXICITY.

Although definitive proof is lacking, there is suggestive evidence that oxygen free radicals and reactive molecules, superoxide (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH\cdot$) and singlet oxygen ($^1O_2$) play an important role in photochemical damage to the retina. To further test this hypothesis, Ham et al[21], exposed macaque monkeys under oxygenation to blue light (435-445 nm) peaked at 440 nm and compared the threshold for retinal damage to that determined under normal conditions (breathing air). Monkeys under anesthesia respired through an endotracheal tube with attached gas bag. They breathed various ratios of oxygen/nitrogen ranging from 20/80 (air) to 80/20 and 100% oxygen. Arterial blood samples were taken before and after 30 minutes of breathing a specific mixture and analyzed for (PO_2) in mm of Hg. The decrease in radiant exposure ($J\cdot cm^{-2}$) was exponential with increase in PO_2. An empirical equation, $H = 39.3 \exp(-.0049\ PO_2)$ was established from the data on 8 eyes in 4 monkeys breathing various mixtures of oxygen/nitrogen;e.g. at a PO_2 of 270 mm of Hg, the threshold was 10.5 $J\cdot cm^{-2}$ as contrasted with 30 $J\cdot cm^{-2}$ for monkeys breathing 20/80 (air). Histopathology disclosed more severe damage to the RPE than that observed under normal conditions, Ruffolo et al[22]. RPE cells were swollen and distorted at 24 hours

postexposure rather than at 48 hours as normally found for threshold blue light lesions.

Continuing our research, Ham et al[91], we exposed the aphakic eye (lens surgically removed) of a rhesus monkey to 325 nm ultraviolet radiation while elevating the arterial blood oxygen level to various PO_2's (398, 389, 278 and 139 mm of Hg). Radiant exposures for threshold damage were reduced from 5.5 $J \cdot cm^{-2}$ at normal PO_2's (75-100 mm Hg) to less than 2 $J \cdot cm^{-2}$ for PO_2's greater than 300 mm Hg. The actual threshold was not determined but estimated to be less than 1 $J \cdot cm^{-2}$. The histological appearance of these lesions was dramatic. There was severe damage to cone ellipsoids and in some histologic specimens the effect of oxygen plus near ultraviolet radiation was devastating. These findings certainly demonstrate that increased arterial blood-oxygen tension increases the sensitivity of the primate retina to radiation damage but they do not prove that oxygen radicals and reactive molecules are involved. The oxygen effect, while suggestive, does not exclude many other reactions from taking place. Meanwhile, lack of understanding of the basic mechanisms underlying photochemical light damage should not obscure the practical and cliniical significance of the oxygen effect.

The protective features of beta-carotene have been shown in one rhesus monkey at PO_2 levels of 226 and 316 mm Hg. The radiant exposure needed to produce a minimal blue light lesion was increased by 60 and 44% respectively. This experiment can be interpreted as presumptive evidence for singlet oxygen toxicity in the retina but definite proof is lacking since beta-carotene can desensitize other excited or reactive molecules as well as singlet oxygen. Other experiments with the steroid methylprednisolone and the enzymes superoxide dismutase (SOD) and catalase were inconclusive, so that solid proof that oxygen radicals are responsible for photochemical light toxicity is still lacking.

11. PULSE TRAIN STUDIES OF 40 MICROSECOND PULSES AT 647 AND 488 nm WAVELENGTHS FOR PRF'S OF 100, 200, 400 and 1600 Hz.

We have submitted for publication a study of 40 microsecond pulses at two wavelengths (647 and 488 nm) produced by the argon-krypton laser[92]. A rotating disk with holes in the periphery produced PRF's of 100, 200, 400 and 1600 Hz. The average thresholds as determined by exposures to 4 different macaque monkeys are given in Tables 2 and 3. Table 2 provides data for 647 nm; Table 3 for 488 nm. Peak power, P_c, was measured with a Scientech calorimeter. Pulse width was measured to the 1/e points on a Tektronix 585 oscilloscope. These thresholds were difficult to obtain because of the small size of the lesion produced by the laser beam operating in the TEM_{oo} mode. Sometimes it required as many as 160 exposures to define a threshold by interpolation. All data points are the average of four thresholds determined in four individual monkey retinae. Due to the extremely small size of the lesion it was necessary to place three exposures of equal intensity in a row to verify the threshold.

The data in Tables 2 and 3 are represented graphically in Figures 3, 4, 5 and 6 where corneal power P_c in W necessary to produce a threshold lesion is plotted against exposure time in seconds in a log-log plot. Each figure represents a specific PRF beginning with 100 Hz for Fig. 3, 200 Hz for Fig. 4, 400 Hz for Fig. 5 and 1600 Hz for Fig. 6. The 647 nm data are the X plots, the 488 nm data the circle plots; also shown on each figure are the threshold data for 647 nm in the CW TEM_{oo} mode.

In every case the threshold for 488 nm pulses is lower than the threshold for 647 nm pulses and for 1000 s exposures at 1600 Hz, the 488 nm threshold is even lower than the CW threshold for 647 nm. For each pulse repetition frequency the difference in threshold between the two wavelengths increases as the exposure time increases. The difference in the thresholds widens as the PRF increases. The sharp bend in the graphs of 488 nm wavelength at 100 s exposure time can be interpreted as a basically photochemical effect at the longer exposure times as opposed to a probable mixture of thermally enhanced photochemical effects at the shorter exposure times. At still longer exposure times we would expect the 647 nm pulse curve to asymptotically approach the CW threshold for 647 nm , especially as the PRF was increased into the MHz region. Unfortunately we do not have any data for 488 nm wavelength in the CW mode.

Figure 3

Thresholds of 647 nm krypton vs 488 nm argon, 40 microsecond pulses at 100 Hz PRF. Corneal power (P_c) in Watts (W) necessary to produce a threshold lesion is plotted against exposure time in seconds. No optics in beam.(TEM_{oo} mode).

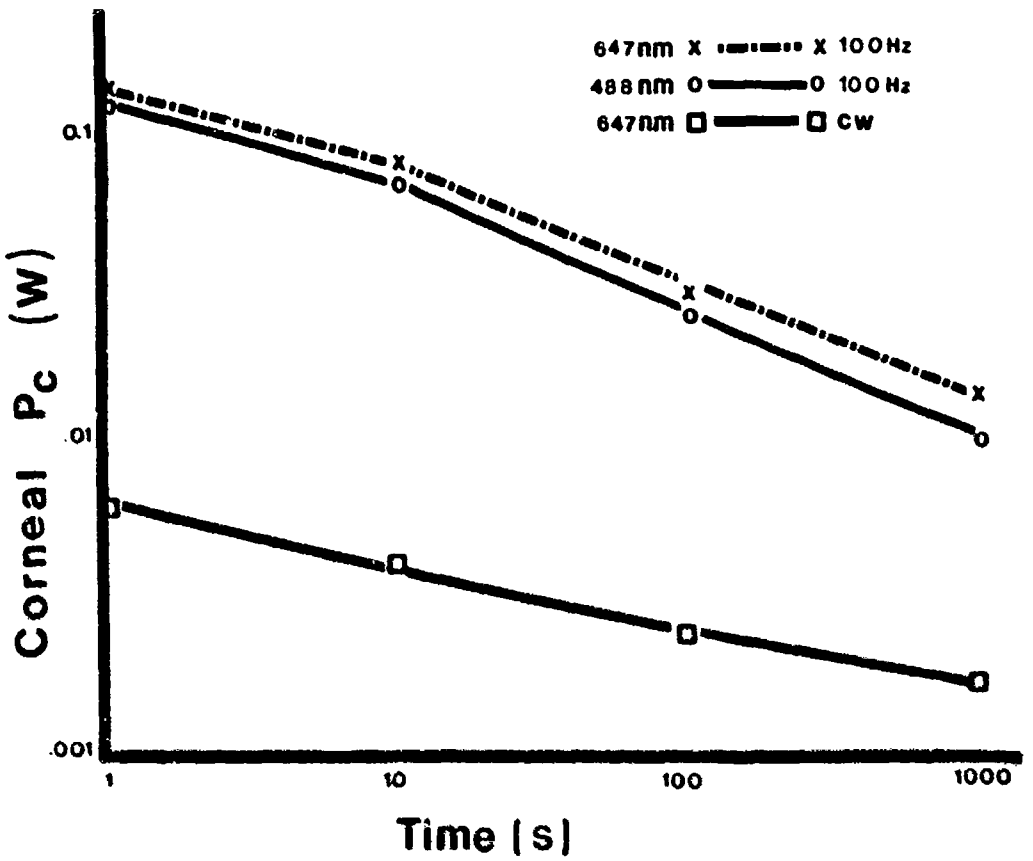


Figure 4

Thresholds of 647 nm krypton vs 488 nm argon, 40 microsecond pulses at 200 Hz PRF. Corneal power (P_c) in Watts (W) necessary to produce a threshold lesion is plotted against exposure time in seconds. No optics in beam. (TEM_{oo} mode).

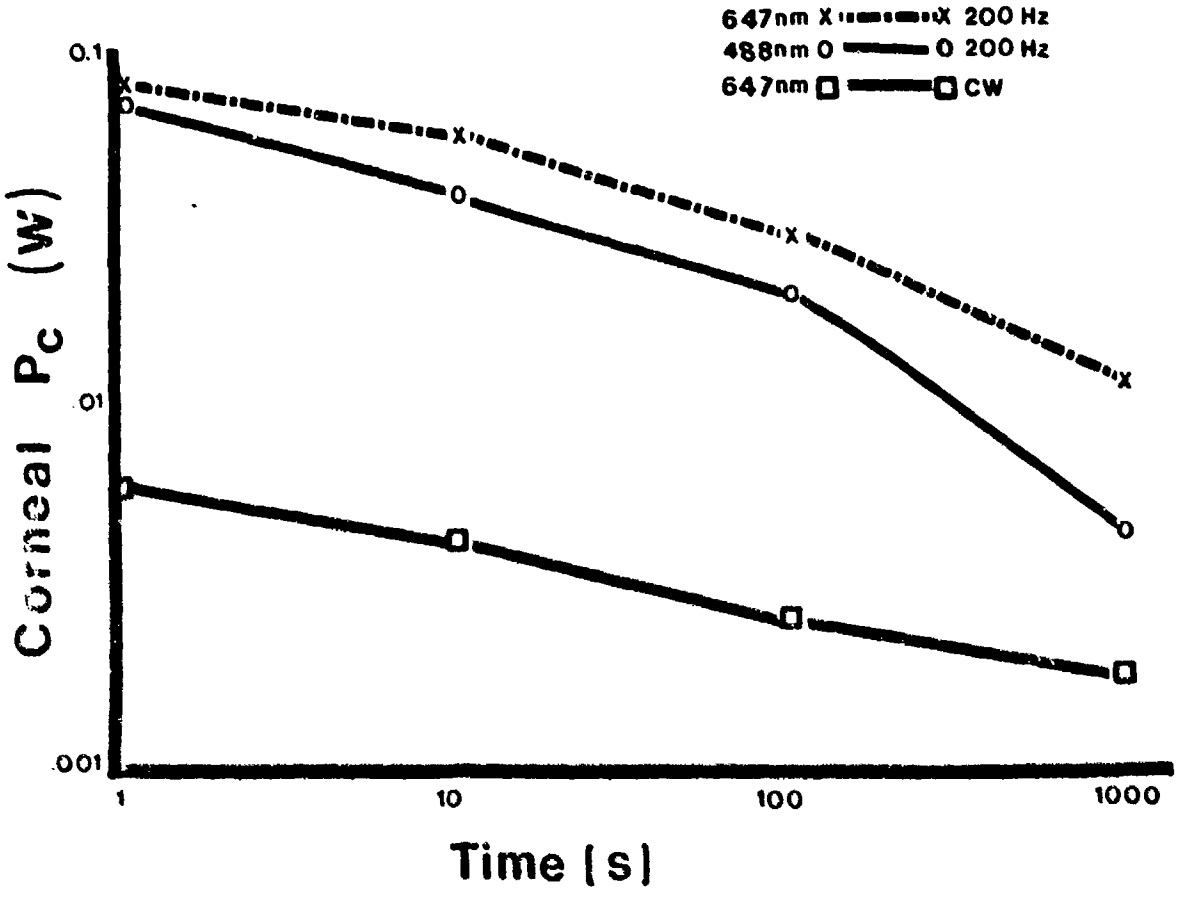


Figure 5

Thresholds of 647 nm krypton vs 488 nm argon, 40 microsecond pulses at 400 Hz PRF. Corneal power (P_c) in Watts (W) necessary to produce a threshold lesion is plotted against exposure time in seconds. No optics in beam. (TEM_{oo} mode).

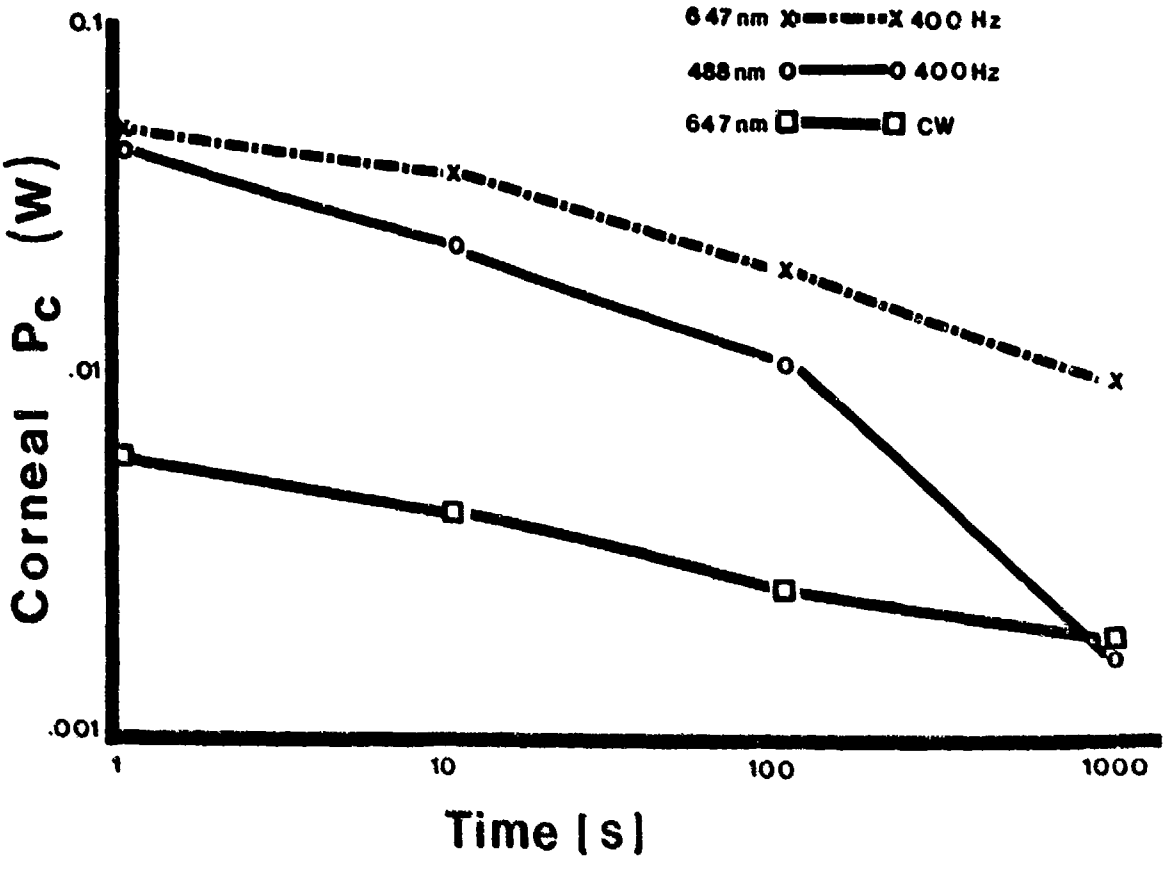
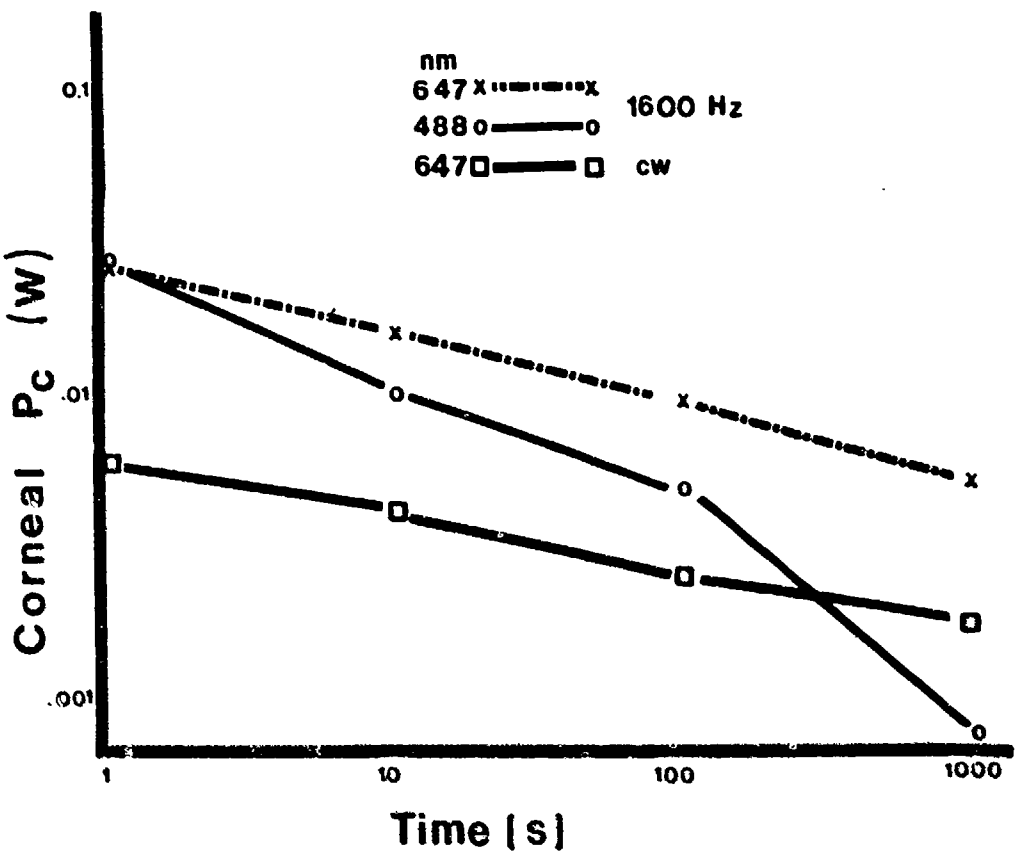


Figure 6

Thresholds of 647 nm krypton vs 488 nm argon, 40 microsecond pulses at 1600 Hz PRF. Corneal power (P_c) in Watts (W) necessary to produce a threshold lesion is plotted against exposure time in seconds. No optics in beam. (TEM_{oo} mode).

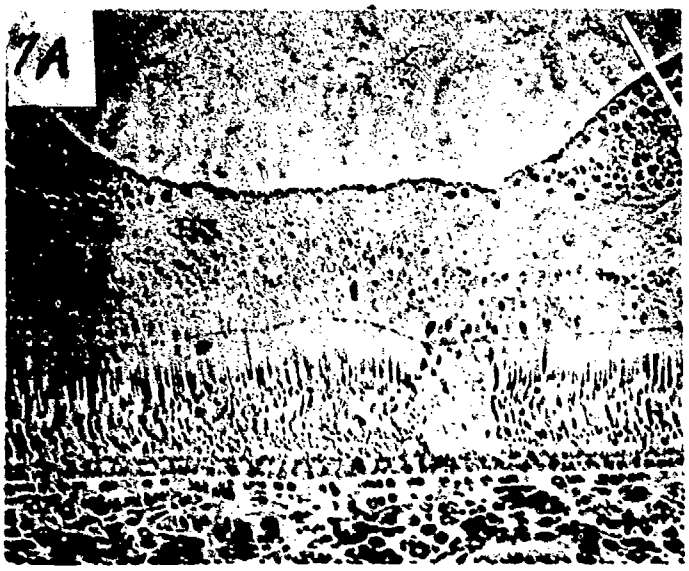


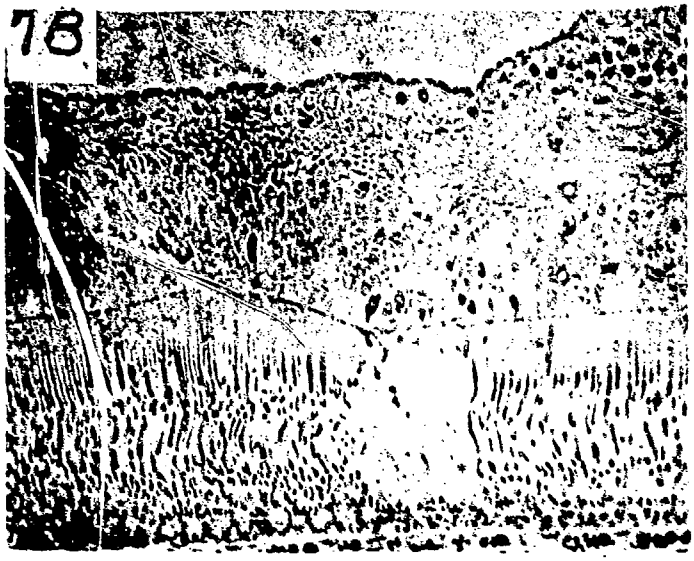
Retinal lesions produced by 40 microsecond pulses of 647 nm red light at a PRF of 1600 Hz were examined histologiclly in one monkey eye. Exposures were 10 s in duration at a peak power to the cornea of 43 mW. Previous experiments had determined the threshold as 16 mW to the cornea. Nine exposures were placed in two rows across the macular area. They were clearly visible in the fundus camera and estimated to be 25 to 50 micrometers in diameter. Five of these lesions were detected histologically. The severest lesion is shown in Figures 7A, 7B and 7C which are 200, 300 and 480 magnifications respectively. The lesion is about 4 retinal pigment epithelial (RPE) cells across. The photoreceptor cells above these 4 RPE cells are completely ablated and there are pyknotic nuclei in the outer nuclear layer (ONL). There is damage and depigmentation of the RPE but Bruch's membrane seems intact. The other 4 exposures were much milder than the lesion shown in Figures 7A, 7B and 7C. Figure 8 (480X) shows one of these mild lesions. Only the cone ellipsoids and nuclei immediately adjacent to the border between the ONL and the inner segments of the photoreceptors are involved and there is no evidence of damage to the underlying RPE. The cone ellipsoids appear to be particularly vulnerable. In our experience this type of damage does not correspond to either thermal or photochemical injury as we have noted it in the past. Peak powers are not high enough, nor exposure time short enough to postulate non-linear damage from sonic transients. We suspect that marked absorption takes place in the cone ellipsoid mitochondria and that singlet oxygen may also be involved but this is mere speculation. In future experiments we plan to investigate the role of oxygenation (elevated PO_2) while exposing the retina to 647 nm laser light. A positive effect (enhancement of retinal sensitivity) would suggest that singlet oxygen was involved.

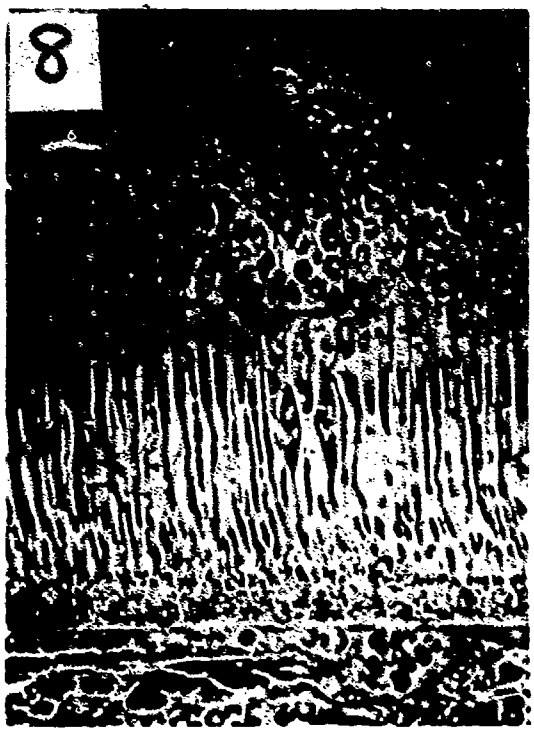
Legend for Figures 7A, 7B, 7C and 8.

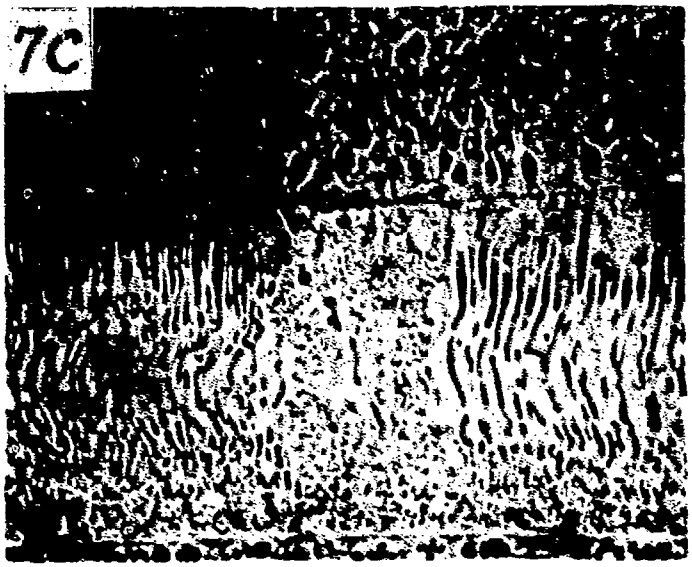
7A, 7B, and 7C are photographs of the same lesion at magnifications of 200, 300 and 480 respectively. The histology is phase-contrast, unstained. The exposure details are as follows: Krypton laser beam (647 nm) chopped by a rotating disc into 40 microsecond pulses at a pulse repetition frequency of 1600 Hz, exposure time 10 s, peak power at cornea 43 mW, estimated spot size on retina about 40-50 micrometers in diameter to the $1/e^2$ points. The $1/e^2$ beam diameter at the cornea < 1mm.

Figure 8. Photo of a mild lesion. Histology and exposure details identical to above.









Threshold Data for 40 Microsecond Pulses of Argon-Krypton 647 nm Laser Radiation. No Optics in Beam.

Radiant exposures H_o per pulse and H_o Total in $J \cdot cm^{-2}$ for a minimal lesion in the monkey retina are given for 40 microsecond pulses at pulse repetition frequencies (PRF) of 100, 200, 400 and 1600 pulses per second for exposure durations ranging from 1 to 1000 s. E_o in $W \cdot cm^{-2}$ on the retina is calculated on the asssumption that the laser beam produced 25 micrometer lesions at the 1/e points of the Gaussian distribution, $E=E_o \exp(-r^2/2\sigma^2)$ according to the formula $E_o=P_cT/2\pi\sigma^2$ where P_c in Watts is the power entering the cornea as measured, T is the transmission through the ocular media (0.93 for 647 nm) and σ is the Gaussian parameter corresponding to a radius r of 12.5 micrometers. Each data point represents the average of the threshold in 4 different monkeys. It required approximately 160 exposures to interpolate for one threshold in each monkey.

Exposure Time s	Number Pulses	P_c W	E_o $W \cdot cm^{-2}$	H_o/Pulse $J \cdot cm^{-2}$	H_o Additive $J \cdot cm^{-2}$
1	1×10^2	.140	2.65×10^4	1.060	[illegible]6
1	2×10^2	.082	1.55×10^4	0.620	124
1	4×10^2	.052	0.98×10^4	0.395	158
1	16×10^2	.027	0.51×10^4	0.205	328
10	1×10^3	.080	1.52×10^4	0.607	606
10	2×10^3	.059	1.12×10^4	0.447	895
10	4×10^3	.039	0.74×10^4	0.295	1,183
10	16×10^3	.016	0.32×10^4	0.125	2,000
10^2	1×10^4	.029	5.50×10^3	0.219	2,199
10^2	2×10^4	.031	5.87×10^3	0.235	4,700
10^2	4×10^4	.021	3.98×10^3	0.159	6,369
10^2	16×10^4	.0095	1.80×10^3	0.072	11,525
10^3	1×10^5	.014	2.65×10^3	0.106	10,615
10^3	2×10^5	.012	2.27×10^3	0.091	18,670
10^3	4×10^5	.010	1.89×10^3	0.076	30,329
10^3	16×10^5	.0052	0.99×10^3	0.039	63,084

Table 2

Threshold Data for 40 Microsecond Pulses of Argon-Krypton 488 nm Laser Radiation. No Optics In Beam.

Radiant exposures H_o per pulse and H_o total in $J \cdot cm^{-2}$ for a minimal lesion in the monkey retina are given for 40 microsecond pulses at pulse repetition frequencies (PRF) of 100, 200, 400 and 1600 pulses per second for exposure durations ranging from 1 to 1000 s. E_o in $W \cdot cm^{-2}$ on the retina is calculated on the assumption that the laser beam produced 25 micrometer lesions at the 1/e points of the Gaussian distribution, $E=E_o\exp(-r^2/2\sigma^2)$ according to the formula $E_o=P_cT/2\pi\sigma^2$ where P_c in Watts is the power entering the cornea as measured, T is the transmission through the ocular media (0.834 for 488 nm) and σ is the Gaussian parameter corresponding to a radius r of 12.5 micrometers. Each data point represents the average of the threshold in four different monkeys. It required approximately 140 exposures to interpolate for one threshold in each monkey.

Exposure Time s	Number Pulses	P_c W	E_o $W \cdot cm^{-2}$	H_o/Pulse $J \cdot cm^{-2}$	H_o Additive $J \cdot cm^{-2}$
1	1×10^2	.127	2.16×10^4	.864	86.4
1	2×10^2	.072	1.27×10^4	.511	102
1	4×10^2	.045	7.66×10^3	.306	122
1	16×10^2	.029	4.94×10^3	.197	316
10	1×10^3	.069	1.17×10^4	.467	467
10	2×10^3	.040	6.81×10^3	.272	545
10	$4\times10_3$	.024	4.00×10^3	.160	640
10	16×10^3	.010	1.70×10^3	.068	1,089
10^2	1×10^4	.026	4.4×10^3	.176	1,763
10^2	2×10^4	.022	3.7×10^3	.150	2,996
10^2	4×10^4	.011	1.9×10^3	.075	2,985
10^2	16×10^4	.0048	8.2×10^2	.033	5,250
10^3	1×10^5	.0103	1.75×10^3	.070	7,012
10^3	2×10^5	.0044	7.49×10^2	.030	5,990
10^3	4×10^5	.0016	2.54×10^2	.010	4,221
10^3	16×10^5	.0008	1.29×10^2	.005	8,278

Table 3

12. EXPOSURE OF A VISUALLY TRAINED MONKEY TO A MILES PROTOTYPE GaAs LASER

The military establishment, particularly the Army, is concerned about the potential ocular hazards of GaAs laser radiation. The Army has developed a training protocol in the field (MILES) that employs GaAs lasers mounted on rifles, tanks, etc. to simulate live ammunition. The laser transmitter is a GaAs laser operating at 910 nm. The output is a modulated pulse train at an average PRF of 1632 Hz with a pulse duration of 60 ns.

A monkey trained for visual function tests was exposed to a GaAs Miles prototype laser (furnished by the U.S. Army R&D Command) emitting a 30 nm bandwidth peaked at 910 nm. The beam was collimated to almost parallel and reflected off a "hot mirror" to produce a semi-rectangular beam 2 X 1.5 cm at the cornea of the exposed eye. The animal looked through the "hot mirror" to the screen that the testing image (Landolt ring) was focussed on. Irradiance at the cornea was 293 microwatts·cm^{-2} at a PRF of 1632 Hz. The pupillary diameters of both eyes were approximately 5 mm. The beam produced an image on the retina estimated to be < 50 micrometers in size. Both eyes, exposed and unexposed, were tested monocularly for visual acuity, spectral sensitivity, and latency of response on a daily basis, 5 days/week. The GaAs laser was switched on for 1000 s during the testing period which normally was 30 min.

The monkey selected for these exposures had a long history of visual testing. Normal baselines on visual acuity, spectral sensitivity and latency of response were avalable over a period of years for comparison to any changes brought about by the GaAs laser radiation. These baselines were kept on a daily basis during the exposure regime that consisted of 81 sessions over a 4 month period and for 3 months after laser exposures ceased. Thereafter, similar baselines were tested on a weekly basis for more than one year. No significant changes were noted in either the control eye (unexposed) or the exposed eye up to the time of sacrifice for histological analysis. There was no histological evidence of damage from the GaAs laser exposures. This experiment provided further evidence that the MILES program is not an ocular hazard to troops in the field.

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15. ABBREVIATIONS AND SYMBOLS IN THIS WORK

AMD : age-relatedd macular degeneration
mm : millimeter
nm : nanometer
μm : micrometer
s : second
ms : milliseecond
μs : microsecond
ns : nanosecond
PRF : pulse repetition frequency
RPE : retinal pigment epithelium
ONL : outer nuclear layer
OS : outer segments
Hz : Hertz
He : helium
Ne : neon
Ar : argon
Kr : krypton
Hg : mercury
Ga : gallium
As : arsenide
PO_2 : oxygen pressure (tension).
O_2 : oxygen molecule
N_2 : nitrogen molecule
$^3O_2$: moleccular oxygen in ground state
$^1O_2$: Molecular oxygen in excited singlet state
O_2^- : superoxide anion radical of molecular oxygen
S : molecular snsitizer
^1S : singlet excited state of molecular sensitizer
^3S : excited triplet state od molecular sensitizer
OH· : hydroxyl radical
UV : ultraviolet
SOD : superoxide dismutase
CAT : catalase
h : Planck's Constant
ν : frequency
CW : continuous wave
TEM_{oo} : Transverse electromagnetic wave-fundamental mode

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