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BIOLOGICAL APPLICATIONS AND EFFECTS OF OPTICAL MASERS

FINAL REPORT

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Research was continued on basic mechanisms involving photochemical events in mammalian retina by injecting superoxide dismutase (SOD) and catalase (CAT) into vitreous of monkey eye before and after exposure to blue light. Intravitreal injection plus exposure to blue light proved toxic to the eye and no information on oxygen radicals was obtained. The argon-krypton laser line at 488 nm was acoustically modulated at 1, 10 and 20 MHz for 1000s exposures of the monkey retina. Threshold radiant exposures at 20 MHz were 3 times lower than those at 1 MHz. The oxygen effect (high PO_2 arterial blood-oxygen) on retinal damage was investigated at 3 wavelengths (540, 640, 840 nm). Lack of an appreciable O_2 effect at 640 nm and none at 840 nm provides evidence that photocehmical toxicity is confined primarily to wavelengths below 640 nm. Threshold exposures less than 1 s to 514.5 and 488 nm light fit well with previous data at 1, 10, 100 and 1000s. The

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lesions were predominately thermal in character. Comparable data at 441 nm was not obtained due to lack of sufficient power in the He-Cd laser. Lenticular and retinal examinations of two visually trained monkeys exposed chronically on a daily basis to a near-UV spectrum (330-420 nm) were concluded in August 1988. There was no evidence that chronic exposure to wavelengths above 330 nm were cataractogenic.

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army

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X Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

X In conducting research using animals, the investigators 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 (NIH Publication No.86-23, Revised 1985).

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William T. Ham, Jr. 10/31/89

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5-1 Introduction:

Research on the "Biological Applications and Effects of Optical Masers" began on February 1, 1962 under contract number DA-49-193-MD-2241 supported by the office of the Surgeon General, U.S. Army. A review of our research previous to and leading to Contract DA-49-193-MD-2241 is given in reference 1, pages 3-4. This contract was superceded on July 1, 1972 by contract DADA17-72-C-2177 which in turn was superceded by contract DAMD17-82-C-2083. The research performed under these contracts was reported in detail in reference 1 which is a Final Report for February 1,1962 to March 15, 1982. Continuation of research on the "Biological Applications and Effects of Optical Masers" from March 16, 1982 to October 15, 1986 was reported in detail in reference 2 which is an Annual/Final report.

In these reports,[1,2] the literature pertaining to retinal light damage from environmental and man-made optical sources prior to the last two decades was reviewed with particular attention to acute and extended or long-term chronic exposure producing thermal and/or photochemical lesions in the mammalian retina. The major factors producing thermal vs photochemical injury were defined in terms of the parameters of wavelength, exposure time and power level. Light toxicity as a function of wavelength was dealt with in great detail. In particular, wavelengths ranging from 1064 nm to 325 nm were studied in the macaque retina using ND:Yag, He:Ne, argon-krypton, dye lasers, He:Cd monochromatic laser lines. Retinal exposures in the near-UV (405,380,350,325 nm) were made using aphakic monkeys (lens removed surgically) and a special optical system employing a 2500 W xenon lamp equipped with quartz optics. The photopathology of blue light and near-UV lesions in the macaque retina were investigated in detail. Photic damage to the retinal pigment epithelium (RPE) and its implications for aging and macular degeneration in the mammalium retina were studied. Repetitive exposures to wavelengths 440,475 and 533 nm on the same site of the macaque retina were investigated for daily exposures ranging from 50 to 10% of threshold over a period of 21 consecutive days. A radiant exposure to 440 nm light at 10% of threshold produced a lesion after 20 daily exposures.

A primary objective of the research program was to establish safe exposure levels to the eye of laser radiation, particularly those wavelengths used by the Armed Services. Laser wavelengths studied included the following: CO_2 10.6 micrometers, HF and DF at 2.5-3.0 micrometers, GaAs 820,830,850 and 905 nm, He:Cd at 441 and 325 nm, argon-krypton 458,488,514 and 647 nm, He:Ne 633 nm, Nd:Yag at 1064 nm and argon at 351 and 363 nm. Wavelengths in the near infrared emitted by GaAs lasers (820-910) were shown not to be an ocular hazard at levels used in the MILES prototype system or in fiber optic communication systems. A rhesus monkey trained for visual function tests was exposed to a MILES prototype GaAs laser (910 nm) on a daily basis for 4 months. There was no evidence of retinal damage or loss of visual acuity.

A number of basic mechanisms underlying photochemical or actinic effects in the retina were studied, i.e. oxygen free radicals and excited molecules like singlet oxygen, photooxidation, and defense mechanisms against light toxicity. Monkeys exposed to 440 nm light and 325 nm UV radiation while oxygenated (80/20

oxygen/nitrogen or 100% oxygen) reduced the threshold radiant exposure for retinal damage by a factor of at least 3. The protective effects of superoxide dismutase (SOD) and catalase (CAT) injected intravenously both before and after light exposure were investigated. The results were erratic and difficult to interpret. It was concluded that these macromolecules could not penetrate the blood-retinal barrier when injected intravenously. Beta-carotene fed orally for 2 years protected a monkey from retinal damage when exposed to 440 nm light while highly oxygenated.

Pulses of 40 microsecond duration at pulse repetition frequencies (PRF) of 100,200,400 and 1600 Hz at laser wavelengths 647 and 488 nm were used to obtain threshold retinal lesions in the macaque monkey. Thresholds for 488 nm pulses were always lower than thresholds for 647 nm pulses. The threshold for 488 nm pulses (1600 Hz) was lower than the cw threshold for 647 nm pulses for 1000 s exposures. The difference in threshold between the two wavelengths increased with exposure time and this difference widened with increase in PRF.

A long-term study on cataractogenesis from chronic exposure to near-UV radiation was begun in 1980. It was designed to study long-term effects on the lens from daily exposures to near-UV radiation similar to that emitted by the sun at sea level. A 2500 W xenon lamp equipped with quartz optics and suitable filters and special mirrors produced a cw spectrum (330-420 nm) which grossly simulated sunlight. Two experiments were designed for two rhesus monkeys trained to sit in a chair and press a lever for food pellets when a large Landolt C changed orientation on a screen. The eye exposed to the beam through a hole in the middle of the C received 5 $mW \cdot cm^{-2}$ for 1000 s on a daily basis, 5 days per week, while the unexposed eye served as a control.The daily radiant exposure at the surface of the lens was 3.6 $J \cdot cm^{-2}$. The first monkey to be exposed had a normal pupillary diameter, approximately 3 mm. After 600 daily exposures there was no evidence of lens changes in the irradiated eye. We conjectured that perhaps the iris protected the vulnerable equatorial region of the lens from near-UV photons. To test this hypothesis a second monkey with pupils dilated to 8 mm or more by topical application of atropine was exposed to the same regimen beginning in August 1982. Both animals were kept on schedule through February 1985 at which time they had received 1171 and 584 daily exposures respectively. Examination at three month intervals with the biomicroscope disclosed no lens changes or anomalies in either animal up to August 1987.

A proposal to continue research on the "Biological Applications and Effects of Optical Masers" under Contract DAMD17-82-C-2083 was submitted to the U.S. Army Medical Research Acquisition Activity, ATTN:SGRD-RMA-BA on January 15, 1986. The proposed research was a natural "follow-up" of the research program outlined above in this introduction. This proposal received scientific approval at a meeting of the Vision and Laser Bioeffects Group of the U.S. Army Medical Research Development Advisory Committee in San Francisco on April 24-25, 1986. However, a new contract DAMD 17-C-87-7186 financing this research proposal was not funded until September 1, 1987 and salaries were not available until November 1, 1987 because of bureaucratic problems.

The research protocol contained the following 5 programs to be completed

over a two year period, Sepetember 1, 1987- August 31, 1989:

1. Continue research on the basic mechanisms causing the blue light and near-UV retinal lesions by investigating the effects of superoxide dismutase (SOD-Cu-Zn) and/or catalase (CAT), injected intravitreously into the rhesus eye, pre- and postexposure, to measured radiant exposures of 440 nm light.

2. Continue acquisition of threshold damage data to the macaque retina from exposure to 647 and 488 nm wavelengths with the argon-krypton laser acoustically modulated at frequencies 0f 10 and 100 kHz and 1, 10 and 20 MHz.

3. Investigate the oxygen effect (high arterial blood-oxygen tension PO_2) on retinal damage at three wavelengths in the visible and near infrared spectrum (540,640 and 840 nm). Is there an oxygen effect at the longer wavelengths?

4. An examination of threshold retinal damage produced by laser lines 441,488 and 524.5 nm at exposure times less than 1 s in duration. This constitutes an attempt to further differeniate between thermal and photochemical injury.

5. Continue at 3 month intervals, lenticular and retinal examinations of the two monkeys previously exposed over a period of years to a near-UV spectrum (330-420 nm).

The prosecution and completion of these 5 research projects is the subject of the Body of this Annual and Final Report. The research ojectives followed naturally from the previous research reported in references 1 and 2. A primary objective was to continue the investigation of basic mechanisms leading to photochemical events in the mammalian retina. The size of the macromolecules SOD and CAT and their short lifetime in the circulation precluded their passage through the retina-RPE barrier when injected intravenously. It seemed natural to attempt to overcome this problem by trying the intravitreous route. Either a therapeutic or deleterious effect would demonstrate that oxygen radicals and hydrogen peroxide were involved in retinal phototoxicity. A better understanding of the basic principles of photochemical effects in the retina might lead to therapeutic measures in clinical ophthalmology. Closely allied to these experiments was a study as to whether an oxygen effect existed at the longer wavelengths in the visible and near infrared spectrum. A positive effect (decrease in threshold upon oxygenation) would suggest that singlet oxygen might be involved.

Another important question concerned whether pulses of short duration (< 1s) involved photochemical as well as thermal effects for wavelengths below 550 nm (441,488 and 514.5 nm), especially at radiant exposures below the threshold for thermal damage. The production of free radicals and excited molecules by short pulses of blue light should produce retinal damage below the temperature required for thermal denaturation of proteins.

It was important to extend the pulse train data for wavelengths 647 and 488 nm to PRF's beyond 1600 Hz to determine whether these thresholds would approach

the cw threshold at higher frequencies. PRF's of 1,10 and 20 MHz could be produced by incorporating the acoustic modulator system with the argon-krypton laser. At lower frequencies, 10 and 100 kHz, it was doubtful whether the power output through the modulator was high enough to produce retinal injury but nevertheless an attempt should be made.

Finally, it was necessary to maintain the life span of the two trained rhesus monkeys that had been exposed to near-UV radiation until lenticular changes were observed; alternatively, if no changes were noted after a latency of several years the hypothesis that near-UV radiation is cataractogenic remains unproven and open to question for wavelengths greater than 330 nm.

6. Body:

6-1. Basic Mechanisms Producing Photochemical Retinal Lesions:

The protocol calls for 3 monkeys, one for SOD injection, one for CAT and one for SOD+CAT. Each animal under anesthesia receives 6 blue light (435-445 nm) radiant exposures of 33 $J \cdot cm^{-2}$, 100s exposure duration, 500 micrometer spot size spaced evenly across the superior paramacular area in both eyes, during a 5 day period, Monday thru Friday; one radiant exposure in each eye on Monday, Tuesday, Thursday and Friday with two exposures to each eye on Wednesday, spaced about 2 hrs. apart with intravitreal injection of control buffer and Sod or Cat midway in time between the two exposures. One eye receives an injection of the enzyme, the other (control) eye is injected with a similar volume of phosphate buffer. Light exposures are performed with the 2500 W xenon lamp equipped with quartz optics, using our well established technique for producing accurate radiant exposures to the retina. This protocol was adopted to make it possible to compare the effects of SOD, CAT and SOD+CAT immediately pre- and postinjection and 24 and 48 hrs. pre- and postinjection. At a later date the entire procedure was scheduled to be repeated using the inferior paramacular area in each eye.

The first experiments began in July 1988. Both eyes (retinae) of a large rhesus monkey under anesthesia were exposed to 33 $J \cdot cm^{-2}$ of 440 nm light on Monday, Tuesday and Wednesday July 25-27. Area irradiated was approximately 500 micrometers in diameter, exposure time 100s. None of these exposures produced visible lesions until 48 hrs. postexposure. On Wednesday, one hour after the first exposure, 3.67 mg of SOD in 0.1 ml of buffer was injected into the vitreous of one eye with a 30 gauge needle. A similar 0.1 ml of buffer without enzyme was injected into the vitreous of the other (control) eye. One hr. after these injections another exposure to 440 nm light was given to each eye. At this time the ocular media of both eyes was perfectly clear. When examined the next day, the eye injected with SOD was completely opaque while the control eye was perfectly clear. It was suspected that the SOD was too concentrated. To test this possiblity the vitreous of both eyes in two monocularly aphakic monkeys were injected with SOD concentrations of 5×10^{-5} and 5×10^{-6} gms, each in 0.1 ml of buffer. Examination over a period of 2 weeks disclosed no anomalies. The ocular media in both eyes of each monocularly aphakic monkey remained clear. Encouraged by this, another monkey was subjected to the 5 day protocol, injecting 5×10^{-5} gms of SOD in 0.1 ml of buffer on Wednesday between exposures

of 33 $J \cdot cm^{-2}$ pre- and postinjection. On Thursday both eyes were clear before and after another exposure to 33 $J \cdot cm^{-2}$ but on Friday, once again the cornea of the SOD eye was too opaque to permit examination or exposure to 440 nm radiation of the retina. The ocular media and especially the cornea and lens of the SOD eye continued to get progressively worse on Saturday and Sunday. It was concluded that the combination of SOD and blue light exposure was toxic to both cornea, lens, ciliary body and iris as well as the vitreous body and the retina.

Much the same results occured when CAT was injected into the monkey vitreous with exposure to 440 nm light. The same protocol outlined above was used in the CAT experiment. A solution of 1 X 10^{-5} gm of Cat in 0.1 ml of sterile buffer was injected on a Wednesday into the vitreous of one eye while the other control eye received 0.1 ml of sterile buffer; The retina was exposed to 30 $J \cdot cm^{-2}$ 1 hr. before injection and 1 hr. after injection. The previous exposures on Monday and Tuesday did not become visible until Wednesday when the Monday exposure became visible (48 hrs. postexposure). Both eyes had clear ocular media (OM) on Wednesday at 3 hrs. postinjection. Thursday morning the ocular media of the eye receiving CAT was too cloudy to permit observation of the retina; the control eye was clear. In subsequent observations on Friday, Saturday and Sunday, clouding of the ocular media in the CAT injected eye grew progressively worse. It became increasingly obvious that the mammalian vitreous could not tolerate the injection of a protein macromolecule followed by acute exposure to short wavelength light. This, despite the fact that both SOD and CAT are endogenous in the primate system. It was never contemplated that intravitreal injections of SOD and CAT could be useful therapeutically but it was hoped that they could be used to demonstrate the production of oxygen radicals and hydrogen peroxide in the retina during exposure to short wavelength light. No further attempts were made to inject SOD and/or Cat into the mammalian vitreous.

6-2. Threshold Data For Pulse Trains at 1, 10, 20 MHz and 10, 100 kHz:

Initiation of this research program was deferred until data could be obtained for exposures less than 1 second (item 4 in research protocol) which called for 441, 488 and 514.5 laser lines. Two of these lines (488 and 514,5 nm) were obtained with the argon-krypton laser before the acoustic modulator was installed. However, difficulties with the power supply for the argon-krypton laser delayed both programs with the result that the experiments on threshold data for pulse trains did not begin until the middle of February 1989.

When the acoustic modulator was adjusted to the 488 nm line from the argon-krpton laser it was discovered that the pulse duration and energy outputs were well below those needed to accomplish the objectives of the original protocol. It became necessary to develop a method of recording the number of pulses and pulse width with each exposure and it was also necessary to increase the exposure time to 1000 s to produce a lesion in the macaque retina. Pulse width was measured on a Tektronix 585 oscilloscope; power entering the eye was measured with a Scientech calorimeter and was monitored during exposures with a Spectra Photometer/Radiometer 301. With no optics in the laser beam spot size on the retina was very small (25-50 micrometers) and difficult to detect. Also, the

beam reflected off the acoustic modulator was poorly defined.These difficulties made it necessary to use a large number of exposures to define a threshold by the interpolation technique. Some improvement in power output and beam definition was obtained by substituting a modulator crystal borrowed from Spectra-Physics Corp. It transpired that our modulator crystal was inefficient because the cement that held it to the RF generator became slightly separated. With this improvement it was possible to get threshold data on three monkeys at 20, 10 amd 1 MHz. It required 1000 s exposures to produce lesions with the available power. Attempts to produce lesions at 100 kHz were unsuccessful for lack of power even for 1000 s exposures.

No data were collected using the 647 nm wavelength. Mr. Mueller was in the process of changing the mirrors and reflectors for the red wavelengths when his unfortunate incident occurred. He was attacked by two thugs on his way home from the laboratory. As reported in the last quarterly report dated September 14, 1989 his cheek bone was shattered, resulting in severe trauma and surgery. As of this writing (October) he is still severely handicapped and undergoing medical treatment.

Threshold data on 3 monkeys with the 488 nm wavelength modulated at 20, 10 and 1 MHz were as follows:

P_c W X 10^{-6}	PRF MHz	E_o $W \cdot cm^{-2}$	Exposure Time (s)	Threshold H_o $J \cdot cm^{-2}$
30.9	20	5.25	1000	5,244 ± 1020
37.5	10	6.37	1000	6,376 ± 393
92.1	1	15.6	1000	15,650 ± 1213

P_c is the power in Watts entering the eye, PRF is the frequency modulation in MHz, E_o the peak irradiance on the retina as calculated by the formula $E_o = P_c T (2 \pi \sigma^2)^{-1}$, T is the transmittance of ocular media (0.834 for 488 nm), s is the exposure duration and H_o is the radiant exposure or threshold as given in $J \cdot cm^{-2}$.

Thresholds appear to decrease as the modulation frequency increases for 488 nm light. Unfortunately, no data were obtained for 647 nm light and no data for pulse repetition frequencies 10 and 100 kHz. There is no valid way to compare pulse trains modulated at 1, 10 and 20 MHz with 40 microsecond pulses at 100-1600 Hz. For 488 nm light the threshold at 1 MHz is approximately 3 times higher than the threshold at 20 MHz. This suggests that as the modulation frequency increases it approaches the threshold for cw but no data are available for cw threshold at 488 nm for spot sizes 25-50 micrometers in diameter for extended exposure times (1-1000 s).

6-3. Investigation of Oxygen Effect on Retinal Lesions at Wavelengths in Visible and Near Infrared (540, 640 and 840 nm).

The untimely death of Dr. J.E. Millen delayed the beginning of this series

of experiments. Dr. Millen was anesthesiologist with the pulmonary Division, Medical College of Virginia and the Veterans Administration Hospital. It was necessary to enlist further aid from the Pulmonary Division. Four monkeys (4 eyes) were used in these experiments. Monkeys under anesthesia respired through an endotracheal tube with attached gas bag. They breathed ratios of oxygen/nitrogen of 20/80 (air), 80/20 and 100% oxygen. Arterial blood samples were taken before and after 30 minutes of breathing a given ratio and analyzed for PO_2 in mm of Hg. Using the value of their predetermined thresholds for 540, 640 and 840 nm, 5 exposures at each wavelength were inflicted. Starting with threshold and decreasing each succeeding exposure by approximately 10% until a level 60% below threshold was obtained. All four animals at 540 nm had an approximate reduction of threshold of 20%. At 640 nm one animal did not show a decrease and the other three showed a decrease from 5-10%. There was no change in the thresholds at 840 nm under increased oxygen tension. The xenon lamp with quartz optics provided the wavelengths (540, 640 and 840 nm) through 10 nm interference filters. Spot size on the retina was 500 micrometers.

TABLE I

Results for 540 nm:

Monkey Number	O_2/N_2 %	PO_2 in mm Hg before O_2	PO_2 in mm Hg after O_2	Threshold $J \cdot cm^{-2}$ before O_2	Threshold $J \cdot cm^{-2}$ after O_2
666	80	62.3	335	529	443
819	100	74.0	430	567	428
708	100	78.3	486	526	430
432	80	75.1	407	530	472

Results for 640 nm:

666	80	62.3	335	1210	1210
819	100	74.0	430	1211	1102
708	100	78.3	486	1208	1147
443	80	75.1	407	1206	1145

Results for 840 nm were completely negative,i.e. there was no decrease in threshold under increased oxygen tension.

6-4. Threshold Data for Exposures Less Than One Second at Wavelengths 441, 488 and 514.5 nm.

During the latter months of 1987 trouble developed with the power supply of the argon-krypton laser needed to provide the 488 nm and 514.5 nm wavelengths; after considerable delay spent in trouble shooting, the problem was corrected and an optical system with a spatial filter was designed to produce image diameters of 500 micrometers at the $1/e^2$ points in the monkey retina. This corresponded to the image sizes used in our earlier research.[3,4] The expanded laser beam was scanned with a photodetector to measure the Gaussian parameters.

The original protocol called for threshold data in 6 eyes from three

monkeys. However, 6 monocular aphakic monkeys from a former study were available in the vivarium; examination of the normal eyes in these animals disclosed no anomalies in the retinae. Therefore it was decided to use these 6 eyes in 6 different animals, a more advantageous statistical design than 6 eyes in 3 monkeys and also helpful in conserving the limited supply of macaque monkeys. The criterion for minimal photic damage was the appearance of a visible lesion in the retina as seen with the fundus camera at 24 hrs. postexposure for thermal lesions and 48 hrs postexposure for photochemical lesions. The laser beam, set for the proper divergence to provide a 500 micrometer diameter image ($1/e^2$) on the retina, entered the dilated pupil (> 8 mm) of the anesthetized animal by means of a beam splitter which made it possible to align the fundus camera coaxially with the laser beam. This arrangement allowed the retina to be viewed during the exposure. Exposure times of 0.5, 0.125 and 0.016 seconds were electronically controlled. Peak retinal irradiance E_o in $W \cdot cm^{-2}$ was calculated by the formula $E_o = P_c T (2\pi\sigma^2)^{-1}$ where P_c is the power incident at the cornea in W, T is transmittance through the ocular media and $E = E_o e^{-r^2/2\sigma^2}$ is the Gaussian distribution, r being the radius in cm. Power levels at the cornea were measured with a calibrated Scientech calorimeter.

Results for 488 and 514.5 nm wavelengths are given in Table II and plotted logrithmically in Figure 1 , where radiant exposure in $J \cdot cm^{-2}$ is plotted along the ordinate vs exposure time in seconds along the abscissa. It can be seen from Figure 1 that exposures less than 1 s for wavelengths 488 and 514.5 nm fit well with previous data[4]. The approximately straight lines for exposures less than 10 s are interpreted as meaning thermal injury is the predominate mode of injury. This is supported by observations that the minimal or threshold lesions at 24 hrs. postexposure were much smaller than 500 micrometers and did not appear larger when viewed at 48 hrs. postexposure. Usually, threshold photochemical lesions correspond in size with the irradiated area whereas minimal thermal lesions are always smaller than the irradiated area because the temperature is highest at the center of the irradiated field.

When the optical system for the argon-krypton laser was converted to the He-Cd laser the 500 micrometer 441 nm wavelength was not powerful enough to produce a retinal lesion in exposure times below 1 s. After conferring with David Sliney it was decided to use the He-Cd laser without optics. This resulted in a very parallel beam which produced spot sizes on the retina that were estimated to be less than 25 micrometers. Detecting these small lesions proved to be very difficult. The technique of using three equal power exposures in a row to verify the appearance of a lesion proved helpful. The He-Cd data is too limited to be usefully compared with the thresholds for the other two wavelenghths (488 and 514.5 nm). Such small lesions defeat the purpose of trying to detect photochemical effects which require long exposure times and large image sizes on the retina.

Table II

488 nm Radiation

0.5 Second Exposure			0.125 Second Exposure			0.016 Second Exposure		
P_c W	E_o W·cm^{-2}	H_o J·cm^{-2}	P_c W	E_o W·cm^{-2}	H_o J·cm^{-2}	P_c W	E_o W·cm^{-2}	H_o J·cm^{-2}
.017			.028			.084		
.018	AVG.		.030	AVG.		.082	AVG.	
.021	15.22±2.13		.027	23.08±1.78		.088	70.17±2.37	
.016			.026			.081		
.021		AVG.	.026		AVG.	.080		AVG.
.015		7.61±1.06	.027		2.88±0.22	.083		1.12±.038
.0180±.0025			.0273±.0021			.0828±.0028		

514.5 nm Radition

P_c W	E_o W·cm^{-2}	H_o J·cm^{-2}	P_c W	E_o W·cm^{-2}	H_o J·cm^{-2}	P_c W	E_o W·cm^{-2}	H_o J·cm^{-2}
.023			.033			.091		
.025	AVG.		.033	AVG.		.104	AVG.	
.021	20.65±1.33		.036	30.22±1.15		.102	88.88±4.29	
.023		AVG.	.035		AVG	.101		AVG.
.023		10.3±0.67	.035		3.78±0.14	.104		1.42±0.07
.025			.033			.100		
.0233±.0015			.0341±.0013			.1003±.0048		

Threshold retinal data for 6 eyes from 6 monkeys for wavelengths 488 nm and 514.5 nm at exposure times of 0.5, 0.125 and 0.016 s. P_c is the power entering cornea in W, E_o is retinal irradiance in W·cm^{-2} and H_o is retinal radiant exposure in J·cm^{-2}, E_o is calculated from the formula $E_o = P_c T (2\pi\sigma^2)^{-1}$ where T is transmittance through ocular media (0.83 for 488 nm; 0.87 for 514.5 nm) and image size on the retina is 500 μm to the $1/e^2$ points of the Gaussian distribution where $E = E_o \exp.(r^2/2\sigma^2)^{-1}$.

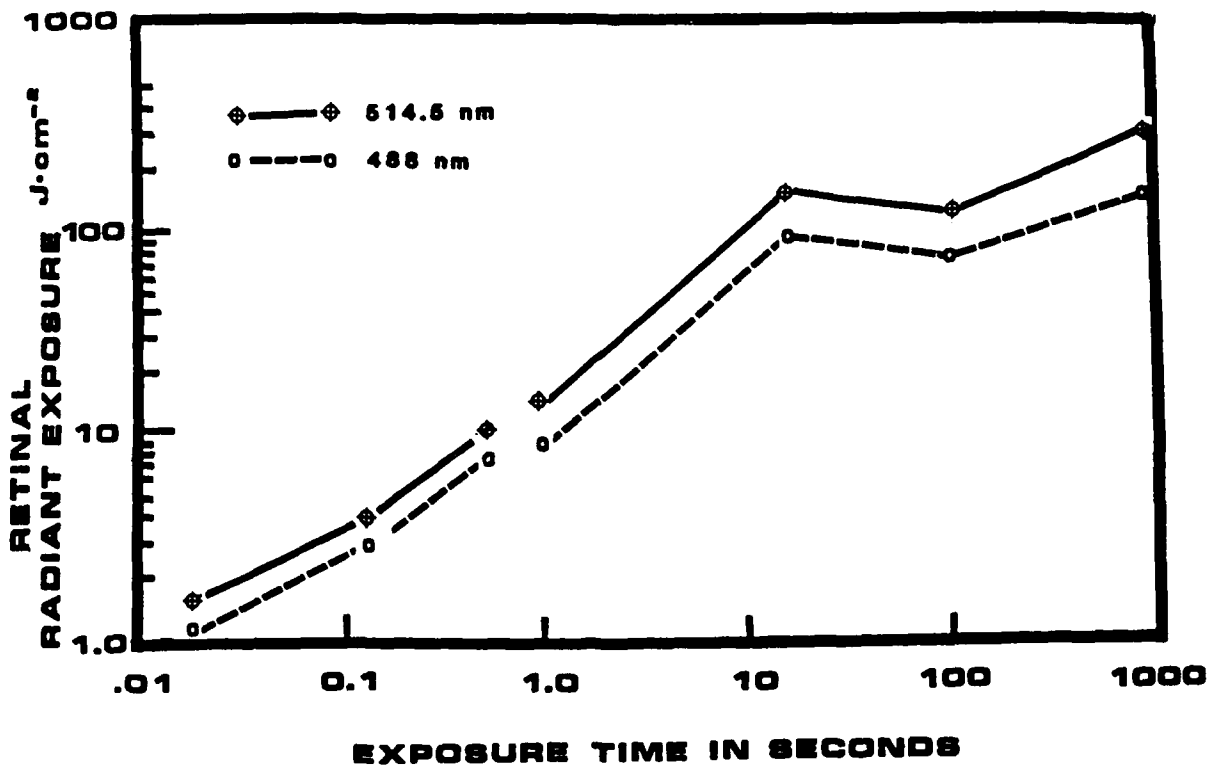
1000
100
10
1.0
RETINAL RADIANT EXPOSURE J·cm⁻²
514.5 nm
488 nm
.01
0.1
1.0
10
100
1000
EXPOSURE TIME IN SECONDS

Figure 1

6-5. Complete and Terminate Investigation of Cataractogenesis in Two Visually Trained Monkeys Exposed Chronically to Near-UV Radiation.

As explained in the introduction exposure of these two visually trained monkeys to 330-420 nm radiation was terminated in February 1985. These animals were examined with the biomicroscope and the fundus camera at three month intervals until August 1988. No anomalies were found in either the lenses or the retinae of both exposed and control eyes. Rather than sacrifice these animals for further in vitro examination it was decided to use them in the retinal research program since rhesus monkeys are extremely expensive and difficult to obtain and the animmals had intact retinae as well as lenses.

The conclusion is that the spectrum used in these long-term chronic exposures (330-420 nm) was not cataractogenic for the rhesus monkey. However, It must be emphasized that most of the energy in this spectrum was above 350 nm and only 30% was between 330-350 nm. Pitts[5] et al have published threshold levels of exposure for cataract in the rabbit which show maximum sensitivity at 300 nm; at 325 nm the radiant exposure required to produce a threshold cataract was more than 300 times greater than at 300 nm. Thus for wavelengths above 330 nm the mammalian lens may not be very sensitive to radiation cataract. Also, the period of latency is much longer in the monkey than in the rabbit. The monkeys were about 2 years old when exposures started in 1980. Now at age 10, they are about halfway through their lifespan.

7. Conclusions:

It is clearly evident that intravitreal injection is not a suitable route to introduce SOD and/or CAT to the mammalian retina. In themselvesthese macromolecules do not seem to be toxic; after all, both of them are endogenous to the mammalian system. Irradiation with short wavelength light renders them toxic. Unfortunately, these experiments contribute little to the hypothesis that oxygen radicals play a part in phototoxicity of the retina during and after exposure to short wavelength light. Another route to penetration of the RPE-retinal barrier by these enzymes is via lyposomes. Hopefully other investigators will try this route. The hypothesis that oxygen radicals and excited molecules (singlet oxygen and others) play a major role in phototoxicity of the retina during and after exposure to short wavelength light remains unproven but highly suspect.

The very limited data on pulse modulation frequencies of 1, 10 and 20 MHz for 488 light indicate that threshold radiant exposures for retinal injury decrease as the frequency increases from 1 MHz to 20 MHz. The threshold at 1 MHz is three times higher than that at 20 MHz. No data are available for a cw threshold at 488 nm for 25-50 micrometer spot sizes. Presumably, as the frequency increases beyond 20 MHz, the threshold would approach the cw threshold but no data are available to prove this. There is no significant difference between 10 MHz and the 20 MHz thresholds which suggests that the radiant exposures for threshold damage is already approaching a plateau for frequencies above 10 MHz.

The lack of an appreciable oxygen effect at 640 nm and the complete absence of an effect at 840 nm is strong evidence that phototoxicity to the retina is confined to wavelengths below 640 nm.

Exposures to the retina less than 1 s in duration for wavelengths 488 and 514.5 nm fit well with previous data[4] as shown in Figure I. Exposures less than 10 s in duration produce predominately thermal lesions though photochemical events may be present but do not manifest themselves before thermal denaturation takes over. Estimates of retinal temperatures for 488 and 514.5 nm are well above 10°C (above ambient) for exposure times less than 10 s[4]. The data for 441 nm are difficult to interpret because of the small spot size on the retina. The appearance of the lesions suggest a thermal mechanism of injury. the spot size been 500 micrometers in diameter like the data for 488 and 514.5 nm photochemical effects might have been manifested. This experiment should be repeated with a He-Cd laser capable of producing sufficient power to produce a retinal lesion for spot sizes 500 micrometers in diameter.

The lack of any evidence for cataractogenesis in the two rhesus monkeys exposed to near-UV radiation (330-420 nm) over a period of years does not support the current hypothesis that near-UV radiation is cataractogenic, at least for wavelengths greater than 330 nm.

8. References:

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ABBREVIATIONS AND SYMBOLS

μ : micron
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
PO_2 : oxygen pressure (tension).
O_2 : oxygen molecule
UV : ultraviolet
SOD : superoxide dismutase
CAT : catalase
cw : continuous wave