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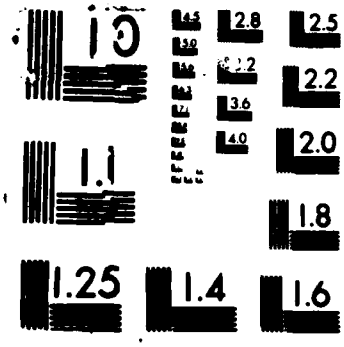
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SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION: Unclassified	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT: Approved for public release: distribution unlimited
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S)	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION: Virginia Commonwealth University Medical College of Virginia; 6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION
6c. ADDRESS (City, State, and ZIP Code): Department of Physiology and Biophysics, 1200 East Broad Street, Box 694, Richmond, Virginia 23298-0001	7b. ADDRESS (City, State, and ZIP Code)
8a. NAME OF FUNDING/SPONSORING ORGANIZATION: U.S. Army Medical Research & Development Command; 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER: DAMD17-82-C-2083
8c. ADDRESS (City, State, and ZIP Code): Fort Detrick, Frederick, MD 21701-5012	10. SOURCE OF FUNDING NUMBERS

PROGRAM ELEMENT NO.	PROJECT NO.	TASK NO.	WORK UNIT ACCESSION NO.
62777A	3E162. 777A878	BA	206

11. TITLE (Include Security Classification)

Biological Applications and Effects of Optical Masers

12. PERSONAL AUTHOR(S): William T. Ham, Jr., Harold A. Mueller, John J. Ruffolo, Jr., Stephen F. Cleary, R. Kennon Guerry and Dupont Guerry, III

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Annual	FROM 3/16/85 TO 3/15/86	1987 March	11

16. SUPPLEMENTARY NOTATION

17. COSATI CODES		
FIELD	GROUP	SUB-GROUP
06	18	
20	06	

18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)

Keywords: oxygen radicals, superoxide dismutase, catalase, photic maculopathy, UVA cataractogenisis, photic repetitive, exposure.

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

Evidence that superoxide dismutase (SOD) enhances and catalase (CAT) reduces blue light toxicity in the monkey retina when injected intravenously (i.v.) was inconclusive and it is surmized that i.v. injection is ineffective because these large enzymes have a short lifetime in the circulation and cannot penetrate the blood-retinal barrier. Repetitive eye exposures of trained monkeys to the broad spectrum 330-420 nm were terminated in February 1985 after 1,171 daily exposures in one animal and 584 in the other animal. To date, no lenticular anomalies have been noted. Both monkeys are being maintained in good health and examined with the biomicroscope at frequent intervals. Repetitive daily exposures for 21 days of 440, 475 and 533 nm light to the same retinal site in 3 monkeys have shown that photic maculopathy is cumulative on a daily basis, especially for 440 nm light. In one monkey, daily radiant exposures of 2.8 $J \cdot cm^{-2}$ resulted in the appearance of a lesion on the 20th day.

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION
☐ UNCLASSIFIED/UNLIMITED ☒ SAME AS RPT. ☐ DTIC USERS	Unclassified

22a. NAME OF RESPONSIBLE INDIVIDUAL	22b. TELEPHONE (Include Area Code)	22c. OFFICE SYMBOL
Judy Pawlus	301/663-7325	SGRD-RMI-S

DD Form 1473, JUN 86 — Previous editions are obsolete. — SECURITY CLASSIFICATION OF THIS PAGE

(Cont'd)

#19 Histologic data are presented for retinal lesions produced by 40 microsecond pulses of 647 nm laser light at a pulse repetition frequency (PRF) of 1600 Hz. Exposures were 10 s in duration at a peak power to the cornea of the monkey eye of 43 mW. The results are unusual in that the photoreceptors above the retinal pigment epithelium (RPE) are completely ablated. Possible mechanisms are discussed.

Keywords: cataracts; vision

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BIOLOGICAL APPLICATIONS AND EFFECTS OF OPTICAL MASERS

Annual Report

March 16, 1985 - March 15,1986

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

March 1987

Supported by

U.S. Army Medical Research and Development Command
Fort Detrick, Frederick, Maryland 21701-5012

Contract No. DAMD17-82-C-2083

Department of Physiology and Biophysics
Medical College of Virginia
Virginia Commonwealth University
1200 East Broad Street, Box 694
Richmond, Virginia 23298-0001

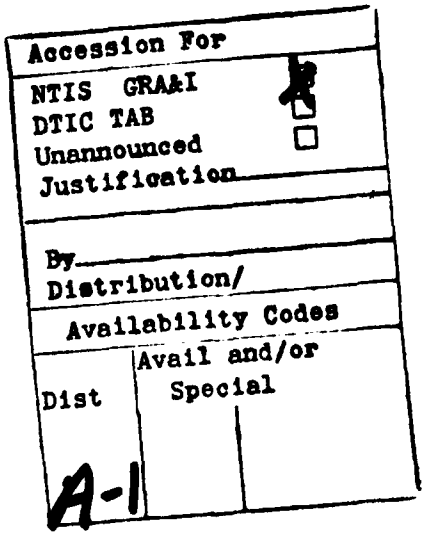

TABLE OF CONTENTS

FOREWORD

In conducting the research described 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 publication No. (NIH) 78-23, Revised 1978).

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Summary

Although there is some evidence that superoxide dismutase (SOD) enhances and catalase (CAT) reduces blue light toxicity in the monkey retina when injected intravenously, it is concluded that this mode of administration is not effective because the size of these enzymes inhibits penetration of the blood-retinal barrier and because their life-time in the circulation is very short. Repetitive eye exposures of trained monkeys to the broad spectrum 330-420 nm were terminated in February 1985 after 1,171 daily exposures in one animal and 584 in the other animal. To date, no lenticular anomalies have been noted. Both monkeys are being maintained in good health and examined with the biomicroscope at frequent intervals. Repetitive daily exposures for 21 days of 440, 475 and 533 nm light to the same retinal site in 3 monkeys have shown that photic maculopathy is cumulative on a daily basis, especially for 440 nm light. In one monkey , daily radiant exposures of 2.8 $J \cdot cm^{-2}$ resulted in the appearance of a lesion on the 20th day. Histologic data are presented for retinal lesions produced by 40 microsecond pulses of 647 nm laser light at a pulse repetition frequency (PRF) of 1600 Hz. Exposures were 10 s in duration at a peak power to the cornea of the monkey eye of 43 mW. The results are unusual in that the photoreceptors above the retinal pigment epithelium (RPE) are completely ablated. Possible mechanisms are discussed.

Conclusion of Superoxide Dismutase (SOD) and Catalase (CAT) Experiments.

In a series of recent experiments (see Annual Progress report, March 16, 1984-March 15, 1985) we attempted to detect the effects of SOD and CAT on the retinal toxicity of blue light (440 nm). These enzymes are specific for the dismutation of superoxide anion to hydrogen peroxide (H_2O_2) and oxygen and the catalysis of H_2O_2 to oxygen and water. They were injected intravenously into monkeys both before and after exposure of the retina to measured radiant exposures of 440 nm light. Our 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 pigment epithelium (RPE).

If one assumes that 440 nm light damage can break down the blood-retinal barrier there was some evidence that SOD aggravated and CAT ameliorated the retinal lesions. This could be explained on the basis that H_2O_2 is a major toxic agent in the blue light lesions. SOD would accelerate the production of H_2O_2 from superoxide anion and CAT would reduce the H_2O_2 to oxygen and water. A major portion of the light incident on the retina is absorbed by the melanin granules of the RPE and there is in vitro evidence for the photoinduced generation of H_2O_2 and hydroxyl radicals in melanin.

Judged in retrospect these experiments were inconclusive when SOD and CAT were administered intravenously. Pending further support we plan to try a more direct approach to the problem by injecting these enzymes directly into the retina via the vitreous humor.

Long Term Repetitive Exposures of Trained Monkeys to Near UV Radiation and Short Wavelength Light (330-420 nm). Lenticular Effects.

These experiments were begun as far back as 1980. They were designed to test the hypothesis that small daily exposures to near UV radiation accelerate aging effects in the lens, leading eventually to senile cataract. Exposures were on a daily basis, 1000 s per day, 5 days per week. The irradiated eye received 5 $mW \cdot cm^{-2}$ of 330-420 nm radiation as provided by a 2500 W xenon lamp with quartz optics; the unirradiated eye served as a control. Exposures on one trained animal began on February 20,1980 and continued until February 15,1985, at which time exposures were terminated at a total of 1171. Frequent biomicroscopic (slit lamp) examinations failed to detect any differences between the lens in the control eye and that in the irradiated eye. Midway through this experiment the negative results led to the hypothesis that perhaps the iris protects the vulnerable equatorial region of the lens epithelium from near UV radiation. During exposure this animal had a pupillary diameter of approximately three millimeters; under the conditions of exposure (cone of UV radiation with small angle of divergence impinging on the cornea) only scattered photons would reach the equatorial region. To test this hypothesis another monkey was trained and exposed under identical conditions except that both pupils were dilated to 8 mm or greater by the topical application of atropine sulphate. Exposures began on August 12, 1982 and were terminated on Feb. 15, 1985 with negative results after 584 exposures. Both animals are being kept for long term observation of both lenses and retina.

Pitts et al[2] have shown in the rabbit that the threshold for cataract reaches a

mininum of 0.15 $J \cdot cm^{-2}$ at 300 nm and is greater than 50 $J \cdot cm^{-2}$ at 325 nm. Our results to date seem to indicate that the cataractogenic wavelengths are below 330 nm or, at least, in agreement with Pitts, the wavelengths are below 330 nm are cataractogenic only for large radiant exposure. We estimate that the anterior surface of the lens received a radiant exposure of 3.6 $J \cdot cm^{-2}$ per day in each animal. This amounts to 4,216 $J \cdot cm^{-2}$ total radiant exposure for the monkey with 3 mm pupils and 2,102 $J \cdot cm^{-2}$ for the monkey with dilated pupils.

We continue to maintain these animals in good health. They are examined every three months with the biomicroscope (slit lamp). Results are still completely negative as of the latest examination (March) 1986.

Repetitive Daily Exposures to the Same Site in the Retina of the Macaque Monkey at Wavelengths 440, 475 and 533 nm.

Greiss and Blankenstein[3] have shown in the rhesus monkey that repeated subthreshold exposures to blue light produce cumulative retinal injury which is countered by an exponential repair process. Retinae were exposed to 458 nm blue light from an argon-ion laser at a radiant exposure equivalent to one-half that required to produce a minimal lesion. At time intervals of 1, 2, 3, 4, and 6 days, the same sites were re-exposed to determine the split radiant exposure threshold which is related to the single radiant exposure threshold by an additivity constant 'A' which in turn depends upon the time interval between exposures. They found, for example, that the additivity A was 91% at 1 day, 57% at 2 days and only 23% at 6 days. The parameter A could be expressed as an exponential $A = \exp(-\Delta t/\tau)$ where Δt is the interval between exposures and $1/\tau$ is the repair rate constant, Tau (τ) which turned out to be almost exactly 4 days. While the basic mechanisms producing photochemical or actinic damage as well as the repair mechanisms are unknown, the authors have described the molecular dynamics by a rate equation which under certain assumptions can be solved for repetitive exposures when the radiant threshold exposure for a single exposure is known. This research represents a valuable addition to our very limited knowledge concerning the cumulative nature of photochemical or actinic damage to the retina.

We have investigated the cumulative or additive effect of repetitive light exposures to the same site on the macaque retina but our research protocol differed somewhat from that of Greiss and Blankenstein. Instead of using the split-dose' technique, the retinae of 3 monkeys (3 eyes) were subjected to daily radiant exposures of 100 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 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 21 and 20 repetitive exposures 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 far 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 $J \cdot cm^{-2}$ or 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.

These experiments demonstrate that photic maculopathy is cumulative on a daily basis and support the thesis that long-term exposure to low levels of short wavelength light can lead to aging of the retina and macular degeneration.[4,5]

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					

Addendum to Histologic Studies Reported in the Annual Progress Report for March 16, 1984 - March 15, 1985.

One reviewer of the draft Annual Progress Report for March 16, 1984 - March 15, 1985 requested that our Histologic Studies be supported by photographs of the results described for focal krypton laser exposures at 647 nm. These exposures to the retina of one monkey eye were 40 microsecond pulses at a pulse rate of 1600 Hz. Exposures were 10 s in duration at a peak power to the cornea of 43 mW. Previous experiments had determined the threshold at 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 photographs 1a, 1b and 1c 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 Bruchs' membrane seems intact. The other 4 exposures were much milder than the lesion shown in photogarphs 1a, 1b and 1c. Photograph 5 (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 Photographs 1a, 1b, 1c and 5.

1a, 1b, and 1c 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 rotatating disc into 4[illegible] microsecond pulses at a pulse repetition frequency of 1600 Hz, exposure time 1[illegible] 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.

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

Publications, Recent and in Press.

1. Guerry, R.K., Ham, W.T.Jr. and Mueller, H.A. Light toxicity in the posterior segment. "Clinical Ophthalmology," edited bt T.D. Duane, Chapter 37, pp 1-17, Harper and Row, Publ. Philadelphia, PA (1985).

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3. Ham, W.T.Jr. Allen, R.G., Feeney-Burns, L., Marmor, M.F., Parver, L.M., Procter, P.H., Sliney, D.H. and Wolbrasht, M.L. The Retinal Pigment Epithelial Working Group. The involvement of the retinal pigment epithelium (RPE). Chap. in "Optical Radiation and Visual Health", edited by M. Waxler and V.M. Hitchins, CRC Press, Baca Raton, FL (1987).

4. Ham, W.T.Jr., Mueller, H.A. and Guerry, R.K. Light Damage. Chap. in "Cell and Developmental Biology of the Eye", Vol. VI, edited by J.B. Sheffield and S.R. Hilfer. Springer-Verlag (1987 in press).

5. Ham, W.T.Jr. and Mueller, H.A. The photopathology and nature of the blue light and near UV retinal lesions produced by lasers and other optical sources. Chap. in Vol. 4. "Laser Applications in Medicine and Biology", edited by M.L. Wolbarsht, Plenum Publ. Corp. NY 10011 (in press 1987)

Additional Activities

May 6-10, 1985: Dr. Ham attended the annual ARVO meeting at Sarasota, FL and delivered a paper entitled " Effects of superoxide dismutase (CuZn SOD) and/or catalase on retinal light damage." The abstract is pulished in Invest. Ophthalmol. & Vis. Sci., Suppl. 26, 130 (1985).

May 20-22, 1985: Dr. Ham attended the annual meeting of the American Ophthalmological Society as the guest of Dr. DuPont Guerry III, who presided as president of the society.

June 4-7, 1985: Dr. Ham attended an International Laser Seminar in Interlaken, Switzerland and participated in a panel discussion on "Basic mechanisms of laser effects."

August 19, 1985: David Sliney, Myron Wolbarsht and Paul Erikson (Denmark) visited our laboratory.

Sept. 16-Dec. 15, 1985: This quarter was devoted primarily to analysing and assessing data to prepare a proposal for competing continuation of research support for the period July 16, 1986 - July 15, 1988. This proposal was submitted to the U.S. Army

Medical Research Acquistion Activity, ATTN: SGRD-RMA-BA on January 15,1986. Copies of this propasal were sent to the Commander, U.S. Army Medical Research and Development Command. LAIR, SGRD-ULZ-RC, Presidio of San Francisco, CA.

October 19, 1985: Dr. R.K. Guerry delivered a lecture entitled "Light Damage" at the 10th Symposium on Ocular and Visual Delvelopment at the Eye Institute, Pennslyvania College of Optometry, Philadelphia, PA. This lecture has been published (see 8. References).

During the year March 16, 1985 - March 15, 1986, Dr. Ham reviewed small grant proposals for NIH-NEI, research proposals for the Lighting Research Institute (LRI) and peer review of manuscripts for the following scientific journals: Cancer Research, Applied Optics, Current Eye Research, Vision Research, Proceedings of the National Academy of Sciences and the American Journal of Ophthalmology.

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1. Korytowski, W., Pilas, B., Sarna, T. and Kalyanaraman, B. Photoinduced generation of hydrogen peroxide and hydroxyl radicals in melanins. Photochem and photobiol., 45, 185-190 (1987).

2. Pitts, D.G., Cullen, A.P. and Hacker, P.D. Ocular effect of UV radiaation from 295-360 nm. Invest. Ophthalmol. & Vis. sci. 16, 932-939 (1977).

3. Greiss, G.A. and Blankenstein, M.F. Additivity and repair of actinic retinal lesions. Invest. Ophthalmol. & Vis. Sci. 20, 803-807 (1981).

4. Young, R.W. A theory of central retinal disease. In "Future Directions in Ophthalmic Research", edited by M.L. Sears, Yale Univer. Press, New Haven, CT (1981).

5. Young, R.W. Solar Radiation and age related macular degeneration. Survey of Ophthalmology, (in press 1987).

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